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SPECIAL ISSUE

SECOND WORLD CONGRESS ON HIGH ALTITUDE
MEDICE AND PHYSIOLOGY
September 24 - 27, 1996
Cusco - Perú

...L INSTITUTO DE INVESTIGACIONES DE LA ALTURA, UNIVERSIDAD PERUANA CAYETANO HEREDIA, LIMA

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HEMATOLOGICAL, CARDIOVASCULAR AND RESPIRATORY PHYSIOLOGY AND PHYSIOPATHOLOGY

INTERMITTENT EXPOSURE TO HYPOBARIA OVERCOMES THE SUPPRESSING EFFECT OF TRANSFUSION POLYCYTHEMIA ON ERYTHROPOIETIN SECRETION

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RESUMEN: La exposición intermitente a hipoxia rebasa el efecto superior de la policitemia de transfusión sobre la secreción de eritropoyetina. Hemos comunicado previamente que la policitemia post-transfusional (TP) en ratones suprime la secreción de eritropoyetina (EPO) en respuesta a la exposición a hipobaria cuando el nivel de la policitemia, evaluada a partir del valor hematocrito, es lo suficientemente alto. Este efecto, sin embargo, no es aparente en ratones con policitemia inducida por exposición crónica a hipoxia hipobárica (PH), en los que la producción de EPO puede ser tan elevada como la observada en ratones normocitémicos. Como PH fue inducida mediante exposición intermitente a hipobaria, el presente estudio fue realizado con el objeto de explorar el efecto de la exposición diaria sobre la secreción de EPO en ratones y ratas TP, en los que la policitemia fue inducida mediante dos inyecciones consecutivas de eritrocitos homólogos. Los animales fueron expuestos a hipobaria en una cámara de altura simulada en forma continua o intermitente (16h/d). Se obtuvieron muestras de sangre a distintos tiempos de exposición para la determinación de la concentración de EPO inmunoreactiva (piEPO) mediante RLA. Los valores de piEPO fueron considerados como representativos de la tasa de producción de EPO. TP anuló el incremento de piEPO que se observa durante la exposición a hipobaria cuando fue continua o durante las primeras 34 exposiciones intermitentes. El efecto supresor de TP fue atenuado durante las exposiciones siguientes pese al mantenimiento del estado policitémico. Estos hallazgos sugieren que el mecanismo que controla la expresión del gene para EPO podría ser condicionado por la exposición intermitente a hipobaria de manera tal que se volvería insensible al efecto supresor de la policitemia sobre la producción de EPO oxígeno-dependiente.

Palabras Claves : Eritropoyetina, Hipoxia, Hipobaria, Policitemia.

RÉSUMÉ: L'exposition intermittente à l'hypobarie l'emporte sur l'effet suppresseur de la polyglobulie de transfusion sur la sécrétion d'érythropoïétine.

Nous avons préalablement montré que chez les souris, la polyglobulie de transfusion (TP) supprime la sécrétion d'érythropoiétine (EPO) en réponse à l'exposition à l'hypobarie, quand la valeur de l'hématocrite est suffisamment élevée. Cet effet n'est cependant pas observé chez les souris dont la polyglobulie a été induite par hypoxie (HP). Dans ces conditions, la sécrétion d'EPO peut être aussi importante que celle observée chez les souris normoglobuliques. La HP ayant été induite par exposition intermittente à l'air hypoxique, la présente étude a été réalisée dans le but d'explorer l'effet des expositions journalières à l'hypobarie sur la sécrétion d'EPO chez les souris et les rats à TP. Cette polyglobulie a été induite au moyen de deux injections consécutives d' unités homologues de globules rouges. Les animaux ont été exposés de façon continue ou intermittente (16h/j) à de l'air hypoxique dans une chambee de décompression. A partir du sang prélevé par intervalles on a mesuré la concentration plasmatique d'EPO immunoréactive (piEPO) par RIA. Les valeurs d'EPO ont été considérées comme taux de production d'EPO. La TP a inhibé la production d'EPO liée à l'hypoxie pendant l'exposition continue, et pendant les 3-4 premières expositions intermittentes. L'effet suppresseur de la TP sur la production d'EPO a diminué au cours des expositions intermittentes qui ont suivi. Ces résultats suggèrent que le mécanisme contrôlant la transcription du gène de l'EPO peut être conditionné par l'exposition intermittente à l'hypobarie, de telle sorte qu'il le rend insensible à l'effet suppresseur de la polyglobulie dans la production hormonale.

Mots-clés : Erythropoiétine, Hypoxie, Hypobarie, Polyglobulie.

SUMMARY: We have previously shown that transfusion polycythemia (TP) in mice suppresses erythropoietin (EPO) secretion in response to exposure to hypobaria when the hematocrit value is high enough. This effect, however, is not observed in mice with hypoxia-induced polycythemia (HP). In this condition, EPO secretion can be as high as that observed in normocythemic mice. Since HP was induced by intermittent exposure to hypobaric air, the present study was undertaken to explore the effect of daily exposures to hypobaria on EPO secretion in TP mice and rats, in which polycythemia was induced by two consecutive injections of packed homologous red cells. Animals were exposed continuously or intermittently (16 h/d) to hypobaric air in a decompression chamber. Blood was obtained at intervals and plasma immunoreactive EPO concentration (piEPO) determined by RLA. EPO values were taken as EPO production rates. TP blunted hypoxia-dependent EPO production during both continuous and the 3-4 first intermittent exposures. The suppressing effect of TP on EPO

production decreased during the next intermittent exposures. These findings suggest that the mechanism controlling EPO gene transcription could be conditioned by intermittent exposure to hypobaria in such a way that makes it insensitive to the suppressing effect of polycythemia on hormone production.

KEY WORDS: Erythropoietin, Hypoxia, Hypobaria, Polycythemia

INTRODUCTION

It has been repeatedly demonstrated (1-3) that erythropoietin (EPO) production in response to acute exposure to hypobaria (hypoxemic hypoxia) is very much higher in post-hypoxia (pH) than in hypertransfused (HT) polycythemic mice at equal levels of polycythemia. This finding, which does not fit with the feedback control theory of regulation of EPO production (4), has not been still adequately explained. However, it seems evident that the way in which polycythemia is an induced (chronic exposure to intermittent hypobaria vs. hypertransfusion) play an important role.

PH mice are usually made polycythemic by exposing them to about 456 hPA for 16-18 h/d for 2-3 weeks with return to sea level conditions for 6-8 h/d. This intermittent regimen of exposure to hypoxia determines that the EPO-synthesis machinery (EPO gene expression) and/or the regulatory mechanisms that controls it is turned on and off every day during a 2-3 week-period. This type of peak stimulation could be responsible, at least partially, for the unexpected high EPO production seen in PH than in HT mice during hypoxemic hypoxia.

Transfusion-polycythemia inhibits EPO production in response to acute exposure to hypobaria in a hematocrit-related fashion (1). The present investigation was thus designed to explore EPO production in response to continuous or discontinuous exposure to hypobaria in TP animals in an attempt to clarify the role of intermittent hypoxemic hypoxia on the genesis of the EPO-hypersecretory state seen in the PH mouse in several experimental conditions.

MATERIALS AND METHODS

Adult female mice and wistar rats were used in the experiments. Animals were made polycythemic by injecting them with 0.8 ml (mice) or 2.5 ml/100 g b. wt (rats) of a homologous red cell suspension (Hct = 0.80) on two consecutive days. Exposure to hypobaria was always started on the 4th day after the second transfusion. At this time, piEPO was very low and red cell production has almost disappeared. Hypoxemic hypoxia was induced by placing animals into a simulated altitude chamber, either continuously or intermittently (16 h/day). The pressure inside the chamber was maintained at 456 hPA. EPO production was estimated by determination of piEPO by a sensitive RIA (5). Results from animals with hematocrits below 0.58 were not considered. Results are presented as the arithmetic mean ± SEM. They were analyzed by one-way ANOVA and Newman-Keuls'test.

RESULTS AND DISCUSSION

No evidence exists that the clearance rate of EPO is subjected to physiologic regulation and, therefore, it can be assurned that piEPO directly reflects the EPO secretion rate. In addition, EPO is not stored in endocrine EPO-synthesizing cells and thus the rate of secretion is directly dependent on the rate of synthesis. Our results, expressed in terms of piEPO can thus be taken as representative of the EPO secretion rate.

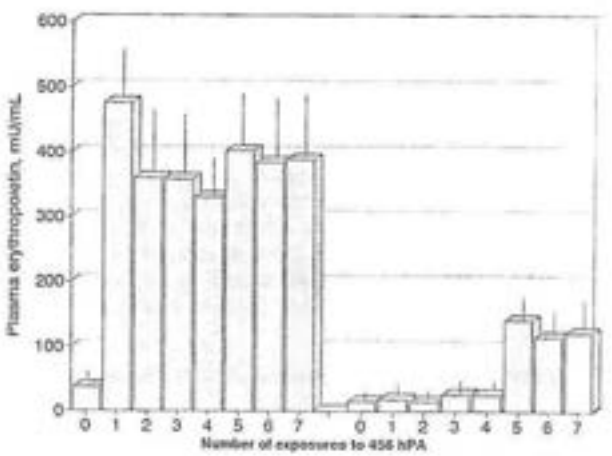

Figure 1. Effect of intermittent exposure to hypobaria on plasma erythropoietin concentration in both normocythemic and transfused polycythemic mice. Groups of 8 normocythemic and polycythemic mice were exposed to 456 hPA in a hypobaric chamber. Each exposure lasted 16 h. Number of exposures are shown in the abscissa. Bars and lines on top of bars indicate mean ± SEM. Normocythemic mice values are shown on the left side of the figure. Values for polycythemic mice are shown on the right side.

To explore EPO production in both normocythemic (N) and transfused-polycythermic (HT) mice in response to intermittent exposure to hypobaria, N and HT mice were exposed to 456 hPA for 16 h/d for 7 days. Each consecutive day of the period, 8 mice from each group were selected at random,

anesthetized with ether, and exsanguinated by heart puncture. Hematocrits and piEPO were measured. Figure 1 shows piEPO in both groups of experimental mice as a function of the number of exposures to hypobaria. It rose in N mice to a level about 10 times the normal, nonhypoxic level during the first exposure, with a slight, non-significant decrease during the remaining exposures. This pattern of EPO response was opposed to that found in HT mice, in which the corresponding normal, nonhypoxic level of piEPO did not change during the first 4 exposures. Interestingly, piEPO titer rose to a level about 7 times higher than that of control, non-exposed HT mice. This occurred in spite of the persistence of the polycythemic state.

Analysis of the above results indicates that intermittent exposure to hypobaria counteracts the inhibitory effect of transfusion-polycythemia on EPO production when it is applied for enough time. The next experiment, therefore, was designed to know whether continuous exposure to hypobaria induces the same pattern of EPO response in HT animals. If this were really the case, then humans or animals living permanently at high altitude would secrete continuously increasing amounts of EPO with the concomitant increase in the erythrocyte production rate and the volume of the red cell mass. This does not occur in most high altitude inhabitants, in which high altitude polycythemia is, in general, a stationary condition and not a progressive process.

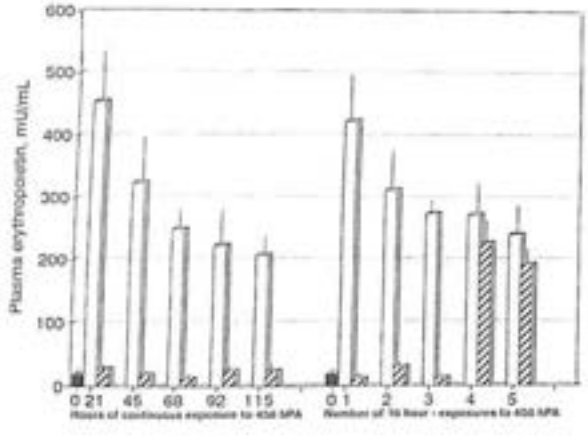

Figure 2. Effect of continuous or intermittent exposure to hypobaria on plasma erythropoietin concentration in both normocythemic and transfused polycythemic rats. Groups of 5 normocythemic and 5 polycythernic rats each were exposed to 456 hPA in a hypobaric chamber. Both types of animals were exposed either continuously (left side of the figure) or intermittently (right side of the figure). Each exposure to intermittent hypobaria lasted 16 h. Bars and lines on top of bars indicate mean ± SEM. Black bars = control, non-exposed rats; dotted bars = normocythemic, exposed rats, dashed bars = polycythemic, exposed rats.

N and HT rats were exposed to 456 hPA for 16 h/d for 5 days (intermittent hypoxia) or continuously for 115 h. Each consecutive day of the period (intermittent exposure group) or after 21, 45, 68, 92 and 115 h of continuous exposure, 5 rats from each group were selected at random and treated as explained above. Figure 2 shows piEPO in N and HT rats as function of either the time of continuous exposure or the number of exposures to hypobaria. N rats showed similar panerns of responses to both types of exposures, with a rapid rise in piEPO followed by a progressive decay with either time or nurnber of exposures. The responses of HT rats differed from those of N rats and could be summarized as follows: l) transfusion-induced polycythemia blunted the increased EPO production which occurred in N rats continuously exposed to hypobaria, and 2) transfusion-induced polycythemia blunted the increased EPO production which occurred in N rats every time they were exposed to hypobaria for 16 h. Blunting was, however, only effective during the first 3 exposures. It did not occur from the 4th exposure on, in spite of the persistence of a high degree of polycythemia in the animals.

The results presented herein confirm previously reported studies that indicate that transfusion-induced polycythemia depresses hypoxia-dependent EPO production (1, ó). These results, however, were obtained from experiments in which polycythemic animals were exposed to short periods of hypoxia (4-48 h) while in the present ones the exposure time was extended to 5-7 days.

Such prolonged exposition to hypobaria revealed that transfusion-induced polycythemia blunted the hypoxia-induced EPO production when the hypoxic stimulus was applied continuously. When it was applied intermittently, the blunting effect was only evident during the 24 exposures, disappearing thereafter.

Unfortunately, we do not have a valid explanation for the observed difference between continuous and intermittent exposure to hypobaria on EPO production in HT animals. EPO is synthesized by endocrine cells through the expression of an EPO gene apparently in response to transcription-regulating factors. The system, that include oxygen sensing by adequate receptors/sensors, intracellular signals, and regulation of genes expression, could be conditioned by intermittent exposure to hypobaria in such a way that makes it insensitive to

the suppressing effect of high cellular oxygen tension on hormone production. It is evident that we need to learn more about the mechanisms by which oxygen is sensed, the transduction of the hypoxic signal and the resulting impact of this signaling process on the regulation of the EPO gene in order to understand the meaning of the results presented.

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PENTOXIFYLLINE AND ENALAPRIL AND ITS EFFECTS ON POLYCYTHEMIA INDUCED BY HYPOBARIC HYPOXIA IN MICE.

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RESUMEN: Pentoxifilina y Enalapril y sus Efectos Sobre la Policitemia Inducida por Hipoxia Hipobárica en Ratones.

Se estudió 70 ratones Swiss macho de 3-4 meses de edad, para evaluar los efectos profilácticos y terapéuticos del enalapril y la pentoxifilina en la policitemia inducida por hipoxia. Se dividió a los animales en dos grupos: grupo pentoxifilina (n=39) y grupo enalapril (n=31). Cada uno fue adicionalmente dividido en un grupo profiláctico que recibió el medicamento antes de la exposición a hipoxia hipobárica intermitente (IHH) y en un grupo terapéutico que recibió el medicamento luego de la exposición a IHH. Cada subgrupo tuvo su respectivo control. La exposición a IHH fue realizada a través de una cámara hipobárica que simulaba una altura equivalente a 4,500m, 22 horas por día. Se midió semanalmente peso y hematocrito. La evolución del peso corporal en el tiempo no mostró diferencias sustanciales entre los animales tratados y los controles en los grupos profilácticos y terapéuticos, tanto en los grupos pentoxifilina como enalapril. Hubo una disminución significativa del hematocrito a los 36 y 47 días del inicio de la profilaxis en el grupo pentoxifilina. En el grupo profiláctico enalapril los hematocritos fueron significativamente menores en los animales tratados. Concluimos que ambas drogas son efectivas cuando son usadas profilácticamente antes de la exposición a IHH. Se sugiere que las drogas podrían ejercer su efecto a través de un bloqueo parcial de la producción de eritropoyetina (EPO).

Palabras Claves: Pentoxifilina, Enalapril, Policitemia, Hipoxia, Ratones.

RÉSUMÉ: Effets de la pentoxifilline et de l'enalapril sur la polycytémie induite par hypoxie hypobare chez les souris.

L'étude a porté sur 70 souris Swiss mâles de 3-4 mois dans le but d'évaluer les effets prophylactiques et thérapeutiques de la pentoxifiline et de l'enalapril sur la polycytémie induite par hypoxie. Les souris ont été divisées en deux groupes : le groupe pentoxifiline (n=39) et le groupe enalapril (n=31), chacun d'eux subdivisé ensuite en un groupe prophylactique qui a reçu le médicament avant l'exposition à l'hypoxie hypobare intermittente (IHH), et en un groupe thérapeutique qui l'a reçu après l'exposition à l'IHH. Chaque sous-groupe a été soumis à un contrôle. L'exposition à l'IHH s'est effectuée au moyen d'une chambre hypobare simulant une altitude équivalente à 4 500 m, 22 heures par jour. Le poids et l'hématocrite ont été mesurés une fois par semaine. L'évolution dans le temps du poids corporel n'a pas montré de différences substantielles entre les souris traitées et les animaux de contrôle des groupes prophylactiques et thérapeutiques, aussi bien dans le groupe pentoxifiline que dans le groupe enalapril. Une diminution significative de l'hématocrite est apparue le 36e et le 47e jour après le début de la prophylaxie dans le groupe pentoxifiline. Dans le groupe enalapril les hématocrites ont été significativement inférieurs chez les souris traitées. Nous en concluons que les deux substances sont effectives lorsqu'elles sont utilisées de façon prophylactique avant l'exposition à l'IHH. On suggère que celles-ci pourraient exercer leur effet par blocage partiel de la production d'érythropoïétine (EPO).

Mots-clés : Pentoxifiline, Enalapril, Polycytémie, Hypoxie, Souris.

SUMMARY : To assess the prophylactic and therapeutic effects of enalapril and pentoxifylline on hypoxia-induced polycythemia in laboratory mice, 70 Swiss male mice of 3 to 4 months of age were studied. They were divided in 2 groups: pentoxifylline group (n=39) and enalapril group (n=31). Each group was further divided in a prophylactic group which received the drug before exposure to intermittent hypobaric hypoxia (IHH) and a therapeutic group which received the drug after exposure to IHH. Each subgroup had their respective control. Exposure to IHH was performed through a hypobaric chamber which simulated an altitude equivalent to 4 500 m above sea level, 22 hours a day. Weight and hematocrit were measured weekly. Time-course of body weight did not show substantial differences between treated and control animals in prophylactic and therapeutic groups in both pentoxifylline and enalapril groups. There was a significant hematocrit decrease 36 and 47 days after the beginning of prophylaxis in pentoxifylline group. In enalapril prophylactic group hematocrits were significantly lower in treated animals. We conclude that both drugs are effective when they are used prophylactically before exposure to IHH. It is suggested that the drugs might exert their effects through a partial blockade of erythropoietin (EPO) production.

Key Words: Pentoxifylline, Enalapril, Polycythemia, Hypoxia, Mice.

INTRODUCTION

Hypoxic polycythemia has traditionally been considered as an adaptive mechanism. Many studies have shown that, in humans and non-genotypically adapted mammals, chronic exposure to high altitude hypoxia may lead to excessive erythrocytosis (1-3), that, along with other clinical and laboratory features, is known as chronic mountain sickness (CMS). There is epidemiological evidence showing that aging at high altitude is a risk factor for the development of chronic mountain sickness, and it is associated with an increased blood red cell mass (4,5). It has been shown that clinical symptoms of CMS dramatically improve after descent to low altitudes or after isovolemic hemodilution (6,7). As blood volume remained unchanged in these studies, it seems that it is the decreased red blood cell mass which accounts for the clinical improvement. Therapeutic strategies aimed to decrease excessive erytrocytosis seem attractive approaches to CMS. Besides descent to low altitude and hemodilution, there have been attempts to decrease excessive erythrocytosis, in both humans and animal models

exposed to chronic hypoxia. Unfortunately, the development of an effective, acceptable therapy for CMS is still a remaining problem. One study performed in males with CMS and treated with medroxyprogesterone showed an unacceptable frequency of decreased libido (8). Likewise, pharmacologic blockade of erythropoiesis has been attempted with several drugs, including the α_1-adrenergic antagonist prazosin, the methylxanthines theophylline and pentoxifylline, and angiotensin converting enzyme inhibitors (ACEI) like enalapril (9,10).

In particular, erythrocytosis associated to renal diseases and to congestive cardiac failure has been assessed. Methylxanthines,particularly theophylline significantly reduced hemoglobin and hematocrit in post-renal transplanted patients who had developed polycythemia (11). It has also been reported that pentoxifylline reduces blood viscosity (12). In addition, enalapril has also been used in the treatment of post transplantation polycythemia with better results than those obtained with theophylline (13,14), and it also reduced the levels of EPO in patients with congestive cardiac failure (15) and the hemoglobin concentration in patients with renal diseases (16).

At our laboratory, the pharmocological blockade of the polycythemia induced by hypobaric hypoxia has been tested in two experimental studies in animals and in one study in humans. Yzaguirre showed a decrease in the hematocrit and a disminushed iron -59- labeled uptake with prazosin (9). Also, Huicho et al. tested two drugs: enalapril and theophylline. Enalapril decreased hematocrit two weeks after beginning of treatment, while theophylline had no effect in hematocrit compared with control group (10). Vargas et al. administered enalapril to 8 men and 6 postmenopause women with CMS and showed a decreased hematocrit, and an improvement of signs and symptoms of CMS (17).

CMS patients who move to lower altitudes show a substantial symptomatic relief, but there is recurrence of symptoms when they go back to high altitude. Thus the prevention and correction of polycythemia in CMS patients would be a more desirable aim than just the correction of preexisting polycythemia. We are not aware of drug studies showing a clear prophylactic effect on polycythemia. Thus we were prompted to evaluate both the prophylactic and therapeutic effect of enalapril and pentoxifylline on hypoxia-induced polycythemia in laboratory mice.

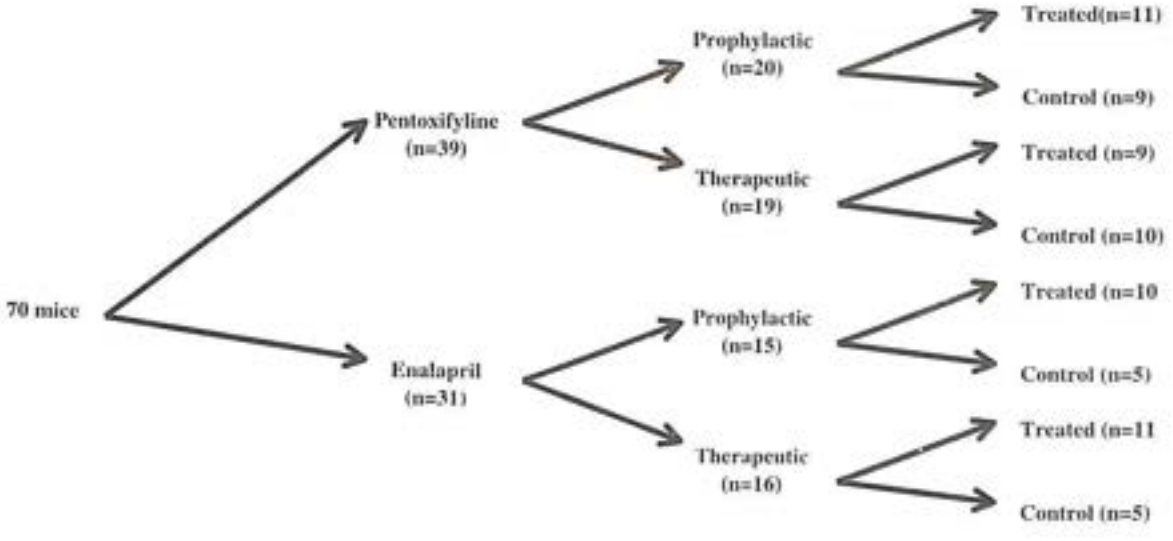
MATERIALS AND METHODS

Animals. 70 Swiss male mice of 3 to 4 months of age were divided in 2 groups:

Pentoxifylline Group (n=39). It was divided in a prophylactic group which began the drug treatment three weeks before exposure to intermittent hypobaric hypoxia (IHH) and continued until the end of the study, and a therapeutic group which began the treatment three weeks after exposure to IHH. Both groups had their respective controls.

Enalapril Group (n=31). It was divided in a prophylactic group which began the drug treatment four weeks before exposure to IHH and continued until the end of the study, and a therapeutic group which began the treatment five weeks after exposure to IHH. Both groups had their respective controls. A summary of the general, prophylactic and therapeutic experimental protocols are shown as diagram (figure 1).

Figure 1. Experimental Model



Drugs. Pentoxifylline 1,43 g/kg/day and enalapril 2,8 mg/kg/day were orally administered *ad libitum* in drinking water. Control groups received drinking water. The dose administered was calculated considering the mouse metabolic rate and on the base of the therapeutic dosis used in humans for each drug (18).

Exposure to IHH. It was performed in a hypobaric chamber equivalent to 4,500 meters above sea level, during 22 hours a day, as it was described before (19).

Measure of weight and hematocrit. They were measured weekly. Hematocrits were measured through the microhematocrit method.

Statistics. Unpaired *t* test was used for comparison of treated and control groups. $P<0,05$ was considered significant.

RESULTS.

Weight. Time-course of body weight did not show substantial differences between treated and control animals in prophylactic and therapeutic groups in both pentoxifylline and enalapril groups. (Tables 1 and 2).

Table 1. Body weight (g) in mice treated or not with pentoxifylline.

Days	0 (basal)	5	12	19	25	36	40	47
Prophylactic group*	SL	SL	SL	SL	IHH	IHH	IHH	IHH
Controls	34,2±1,5	34,0±1,6	34,3±1,6	34,4±1,2	32,6±1,3	29,1±2,2	28,8±2,4	26,7±1,9
Treated	36,4±2,9	35,7±2,9	34,7±3,5	35,5±3,0	28,8±2,2	28,8±2,1	27,3±2,5	26,1±2,4
p	0,09	0,14	0,73	0,31	0,00002	0,08	0,14	0,7
Therapeutic Group**	SL	IHH	IHH	IHH	IHH	IHH	IHH	IHH
Controls	35,8±3,7	34,4±3,0	34,0±2,8	31,8±3,4	30,7±2,8	29,3±1,3	28,3±1,0	27,6±0,8
Treated	38,2±3,6	34,9±3,1	35,0±3,3	34,9±3,7	31,3±3,4	26,3±3,2	26,6±3,3	29,5±3,3
p	0,21	0,74	0,49	0,09	0,22	0,67	0,49	0,77

Values are mean ± SD, SL = sea level, IHH = intermitent hypobaric hypoxia.
*Received the drug since day 1.
**Received the drug since day 21.

Table 2. Body weight (g) in mice treated or not with enalapril.

Days	0 (basal)	8	22	27	38	51	59	67
Prophylactic group*	SL	SL	SL	SL	IHH	IHH	IHH	IHH
Controls	31,7±1,2	34,5±0,9	35,8±1,6	35,9±1,2	30,0±0,8	27,3±2,0	26,8±2,4	28,3±1,7
Treated	34,1±2,6	36,8±3,4	37,9±3,2	37,3±3,8	30,1±3,9	27,5±2,7	28,8±4,1	29,4±3,5
p	0,038	0,08	0,14	0,35	0,94	0,91	0,29	0,47
Days	0 (basal)	8	22	29	38	44	51	59
Therapeutic Group**	SL	IHH	IHH	IHH	IHH	IHH	IHH	IHH
Controls	31,2±1,0	28,1±1,1	29,6±0,4	30,3±0,4	29,7±0,5	26,3±1,1	31,0±1,3	32,0±1,0
Treated	30,7±0,9	29,2±1,0	30,1±2,3	30,9±2,4	27,7±2,2	29,1±2,2	33,1±2,8	31,9±2,5
p	0,65	0,35	0,54	0,47	0,022	0,008	0,064	0,92

Values are mean ± SD, SL = sea level, IHH = intermittent hypobaric hypoxia.
*Received the drug since day 1. **Received the drug since day 34.

Hematocrit. There was a significant decrease 36 and 47 days after the beginning of prophylaxis in pentoxifylline group (Table 3 and figure 2). In enalapril prophylactic group hematocrits were all lower in treated animals as compared with control ones, except at 51th day of the study (Table 4 and figure 3).

Table 3. Hematocrit (%) in mice treated or not with pentoxifylline.

Days	0 (basal)	5	12	19	25	36	40	47
Prophylactic group*	SL	SL	SL	SL	IHH	IHH	IHH	IHH
Controls	49,8±3,3	48,1±2,9	48,0±2,1	46,7±1,8	52,1±4,1	60,7±3,7	61,3±4,5	64,8±1,3
Treated	48,2±1,9	46,8±2,9	47,3±2,1	45,2±1,2	57,0±4,3	56,4±4,9	58,2±5,2	60,4±4,5
p	0,26	0,36	0,51	0,07	0,97	0,035	0,29	0,022
Therapeutic Group**	SL	IHH	IHH	IHH	IHH	IHH	IHH	IHH
Controls	46,7±1,3	56,0±1,3	57,1±1,6	59,6±1,9	60,1±1,4	60,0±3,1	56,4±1,4	56,9±1,4
Treated	47,8±2,2	56,7±1,7	55,9±2,0	56,9±2,0	59,8±2,9	64,2±4,5	58,5±2,5	56,4±1,2
p	0,22	0,7	0,19	0,01	0,77	0,10	0,14	0,66

Values are mean ± SD, SL = sea level, IHH = intermitent hypobaric hypoxia.
*Received the drug since day 1.
**Received the drug since day 21.

Table 4. Hematocrit (%) in mice treated or not with enalapril.

Days	0 (basal)	8	22	27	38	51	59	67
Prophylactic group*	SL	SL	SL	SL	IHH	IHH	IHH	IHH
Controls	47,8±1,3	47,0±0,6	48,8±1,5	47,8±2,6	62,4±1,9	59,8±2,6	62,2±1,9	61,0±2,4
Treated	46,1±1,8	44,0±2,2	43,2±1,7	43,3±1,8	59,7±2,1	57,5±2,2	57,8±4,3	55,7±3,7
p	0,47	0,003	0,0002	0,02	0,05	0,17	0,025	0,024
Days	0 (basal)	8	22	29	38	44	51	59
Therapeutic Group**	SL	IHH	IHH	IHH	IHH	IHH	IHH	IHH
Controls	47,3±1,5	61,4±2,8	59,4±0,5	61,6±2,4	61,7±1,7	61,8±2,8	57,2±1,2	58,8±2,4
Treated	47,6±3,0	60,1±3,2	58,1±2,5	60,1±1,6	63,0±2,3	61,0±2,2	55,8±2,0	58,5±2,0
p	0,81	0,47	0,14	0,3	0,29	0,6	0,13	0,81

Values are mean ± SD, SL = sea leve, IHH = intermittent hypobaric hypoxia.
*Received the drug since day 1.
**Received the drug since day 34.

Therapeutic groups did not show significant differences in hematocrit values.

Figure 2: Pentoxifylline
Hematocrit. Prophylactic Group

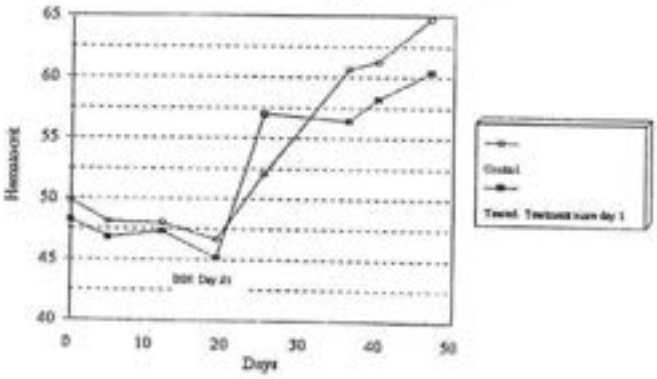
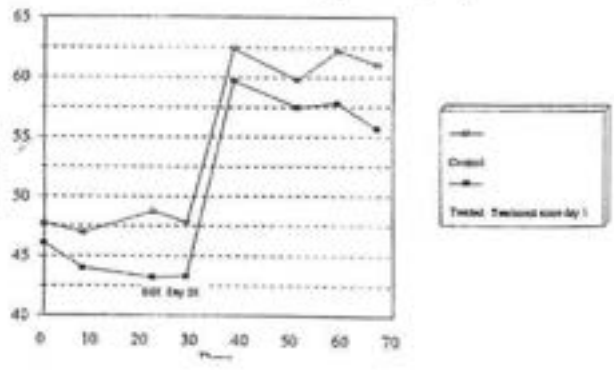


Figure 3: Enalapril
Hematocrit. Prophylactic group



DISCUSSION.

Our study showed, on a prophylactic base, a decreased level of hypoxic polycythemia in animals treated with pentoxifylline and enalapril. Therapeutic groups did not show differences in hematocrit values when compared with their respective controls. We must acknowledge that a definite conclusion on the clinical relevance of the role of these drugs in CMS associated polycythemia needs appropriate trials in humans. Also, a longer follow-up period seems necessary to ascertain whether or not the polycythemic response reaches a plateau.

Also, additional experimental studies addressed to elucidate the underlying mechanism of action of pentoxyfilline and enalapril in attenuation of hypoxic polycythemia are warranted. It has been shown, in conditions of exposure to hypobaric hypoxia, that serum erythropoietin reaches a maximum concentration within 6-24 h in rodents and within 48h in humans (20). In our study, the drugs attenuated the erythropoietic response when they were administered prior to hypoxic stress. It is a possibility that the drugs may have partially blocked erythropoietin production, well before EPO can exert their maximum effect on erythroid target cells.

Adenosine is a cellular messenger whose levels increase in hypoxia. Increased levels of adenosine lead to inforced EPO production. In our hypoxic model, it is likely that pentoxifylline may have inhibited erythropoiesis by means of a renal adenosine stimulus. We will briefly summarize some studies addressed to elucidate the cellular mechanisms of EPO production in hypoxia. We think that such an approach may help to the development of potential strategies of intervention in different phases of the erythropoietic response. In hypoxic conditions the increased degradation of ATP results in the formation of adenosine and other nucleosides (21), and also increases EPO production (22). Two types of adenosine receptor have been proposed.: A_1 receptors which inhibit adenilatecyclase, activated by nanomolar concentrations of adenosine, and A_2 receptors which stimulate adenilatecyclase, activated by micromolar concentrations of adenosine (23). Under hypoxic conditions micromolar concentrations stimulate A_2 receptors resulting in an increase of the levels of AMP_c, second messengers in the renal production of EPO (24). Pentoxifylline antagonizes the A_2 receptor, so it would produce a reduction in the levels of AMP_c and EPO.

At the target organ level, the relationship between the renin-angiotensin system and EPO would be angiotensin-II by means of a vasoconstrictive action in renal tissue thus yielding to tissue hypoxia, which is the stimulus for EPO production (25). It is known that enalapril increases renal blood flow (26), which in turn increases oxygen supply to renal tissue, and that would produce a reduction of renal EPO release. However, we must acknowledge that recent studies, especially in post renal transplanted polycythemia, point out the independence between erythropoeitin levels and the effect of ACEI on the hematocrit (14,16,27), so the exact enalapril mechanism remains unknown.

From our study it can be concluded that a prophylactic approach seems more promising for attenuation of the erythropoietic response to hypobaric hypoxia. Pharmacological intervention of different steps of erythopoietic process, at renal level and at early phases of erythroid target cells, may prove useful for identification of other useful alternatives to treatment of CMS.

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THE EFFECT OF HYPOXIA ON BETA-ADRENORECEPTORS IN LYMPHOCYTES OF HIGHLANDERS.

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RESUMEN: El efecto de la hipoxia sobre los receptores beta-adrenérgicos en linfocitos de residentes de altura

Para evaluar el posible rol de la desensibilización de los receptores beta-adrenérgicos (β-AR) en la génesis de la hipertensión pulmonar de altura (HPAH), estudiamos residentes de altura en el Centro Nacional Kyrgyz de cardiología y medicina interna en Bishkek (760 m). Se demostró que estos sujetos sanos representaban un grupo heterogéneo de 3 subpoblaciones: 1) no respondedores; 2) hiper- respondedores; 3) con HPAH moderada. En estos tres subgrupos se determinó las diferencias de la respuesta de los β-AR a la hipoxia. En los no respondedores la B_{max} en normoxia estaba disminuida en comparación con los normo-respondedores y la hipoxia la incrementaba hasta el nivel de los no respondedores. En los residentes de altura con HPAH moderada la B_{max} estaba inicialmente alta e incrementó más en condiciones de hipoxia. Los estudios de activación de adenilato-ciclasa (cAMP) en condiciones hipóxicas en los linfocitos sanguíneos demostraron que el nivel de cAMP tanto basal como estimulado por agonista estaban reducidos en los tres grupos de residentes de altura, lo que revelaba un desacoplamiento de los β-AR con la proteína G y el desarrollo de desensibilización de los receptores.

Palabras claves: Hipertensión arterial pulmonar de altura; Residentes de altura; Linfocitos humanos; Receptores beta-adrenérgicos; Adenilato-ciclasa; Desensibilización.

RÉSUMÉEffet de l'hypoxie sur les récepteurs adrénergiques bêta des lymphocytes des habitants de haute montagne.

Pour évaluer le rôle possible de la désensibilisation des récepteurs adrénergiques bêta (ß-AR) dans la genèse de l'hypertension pulmonaire d'altitude (HPAH), nous avons réalisé une étude des habitants de haute montagne au Centre National Kyrgyz de cardiologie et de médecine interne de Bishkek (760 m d'altitude). Il a été démontré que ces sujets sains représentaient un groupe hétérogène de 3 sous-populations 1) non-répondants; 2) hyper-répondants; 3) avec HPAH modérée. Dans ces trois sous-groupes ont été déterminées les différences entre les réponses des ß-AR à l'hypoxie. Chez les non-répondants la B_{max} en normoxie était déprimée en comparaison avec les normo-répondants et l'hypoxie la faisait croitre jusqu'au niveau des non-répondants. Chez les montagnards à HPAH modérée la B_{max} était initialement élevée et elle augmenta encore dans des conditions hypoxiques. Les études d'activation d'adénylate-cyclase (cAMP) dans des conditions hypoxiques ont démontré que dans les lymphocytes du sang le niveau de cAMP, aussi bien basal que stimulé par agonistes, était déprimé dans les trois groupes, révélant un désaccouplement des ß-AR et de la protéine G, et le développement de la désensibilisation des récepteurs.

Mots-clés : Hypertension artérielle pulmonaire de haute altitude, Habitants des hautes montagnes, Lymphocytes humains, Récepteurs adrénergiques bêta, Adénylate-cyclase, Désensibilisation.

SUMMARY: In order to assess the possible role of ß-AR desensitization in the genesis of high-altitude pulmonary hypertension (HPAH), we conducted investigations of healthy highlanders in the Kyrgyz National Center of cardiology and internal medicine in Bishkek (760 m above sea level). It was shown that these conditionally healthy highlanders represented the heterogeneous group consisting of 3 subpopulations: 1) nonresponders; 2) hyperresponders; 3) with moderate HPAH. In these three groups the discrepancies of the ß-AR response to hypoxia were determined. In nonresponders hypoxia did not change the amount of ß-AR (B_{max}). In hyperresponders under normoxia BMax was decreased compared to normoresponders and hypoxia increased it up to the level of nonresponders. In highlanders with moderate HPAH BMax was initially high and it further increased under hypoxic conditions. Our studies of adenylate cyclase activation under hypoxic conditions demonstrated that basal and agoniststimulated cAMP level in blood lymphocytes were reduced in all three groups of highlanders, that revealed the uncoupling of , ß-AR with Gs-protein and the development of receptor desensitization.

Key Words : High altitude pulmonary arterial hypertension; Highlanders; Human lymphocytes; ß-adrenoreceptors; Adenylate cyclase; Desensitization.

INTRODUCTION

It is known that sensitivity to hypoxia in various animals and in humans is very variable. Concentration of norepinephrine in blood is increased when people rise at altitude 5400-6300 m above the sea level (1). It was demonstrated that in healthy lowlanders after 8 days of residence at 4350 m above sea level the blood norepinephrine level was increased and amount of ß-AR on lymphocytes decreased (2). 3-5 hours after returning to the lowland the receptor amount comes back to the initial level. Thus adrenoreceptors could play an essential role in adaptation to hypoxia. Moreover, hypernorepinephrinemia which associated with high-altitude hypoxia (3), results in desensitization of ß-AR. Infringements in the adrenoreceptor state, possibly, lead to disorders of organism adaptation to high altitude and to diseases, associated with the loss of adaptation to hypoxia. However, in the majority of investigations conducted in this field only the effect of hypoxia on adult lowlanders was studied.

At the same time, there are a lot of evidences that high altitude diseases, such as Monge's disease, high-altitude pulmonary arterial hypertension

(HPAH), chronic mountain sickness, affect mainly native residents of high altitude. People with hyperreactivity of pulmonary vessels to hypoxia are found more often among the residents of high altitude than among the lowlanders (4). Long-term studies of Kyrgyz Institute of cardiology have shown that development of severe HPAH with right ventricular hypertrophy (HRV) occurred more frequently among the highlanders than in the residents coming to high altitude at mature age. The same investigations demonstrated that, on the other hand, the most of native highlanders had higher stability to the development of high altitude sickness (5). For 7 years we have conducted our studies of ß-AR state in the highlanders native to Pamir and Tien-Shan, permanent residents of the altitudes of 3000-4200 M above the sea level.

We demonstrated that development of HAPH in highlanders correlated with desensitization of their ß-AR (6). In this study we investigate the correlation of the responses of ß-AR and pulmonary arterial blood pressure to hypoxia in healthy highlanders. Our study demonstrated that resistance of ß-AR to desensitization in hypoxia correlated with resistance of the organism to HAPH.

MATERIALS AND METHODS

Subjects studied

For investigation of ß-AR desensitization role in the genesis of HAPH with RVH of the heart, we studied healthy highlanders (without symptoms of RVH and HAPH, which were defined by the indirect methods) in Bishkek (760 m above the sea level). Mean pulmonary arterial pressure (PAP) was measured by the invasive method at the rest and after the inhalation of the hypoxic gas-mixture (10% 02). The thickness of the front wall of the right ventriculum was examined by the 2D-echocardiography.

According to PAP response to hypoxia these healthy highlanders were divided into three groups:

1. nonresponders
2. hyperresponders
3. hyperresponders with moderate HPAH.

Blood samples were collected before and after hypoxia and the state of ß-AR in the lymphocytes of peripheral blood was analyzed. According to the data of Brodde et al. (7) the state of ß-AR on lymphocytes reflected the state of ß-AR in right atrium of human heart.

Preparations of cells

Blood samples were drawn from highlanders into plastic tubes containing heparin. Lymphocytes were separated from whole blood samples according to method of Boum et al. (8). Blood samples were diluted with phosphate buffered saline (PBS) to twice the volume and layered over FicollVeragrafin (specif1c gravity 1,077). The tubes were centrifuged at 425g for 30 min. The layer of the lymphocytes was harvested and washed twice with PBS and once with 20 mM HEPES in MI99 (DMEM) containing 1 mg/ml BSA. The final cell suspension was diluted in DMEM. The vialbility of the cells were tested by excluding of trypan blue.

Radioligand binding

ß-AR were quantitated using ligand [^{3}H]-dihydroalprenplol ([^{3}H] DHA). The assays were performed in polypropylene test tubes. Incubations were performed in a total volume of 0,5 ml of DMEH containing 3-5x10^6 cells. Non-specific binding was determined by performing incubations in the presence of 1 mM propranolol. The reactions were terminated by adding three volumes of ice-cold 10 mM Tris buffer and samples were filtered (GF-C filters, Whatman). The receptor-bound ligand radioactivity on the filters was counted in liquid scintillation counter

Adenylate cyclase activity

ß-Adrenergic-stimulated adenylate cyclase activity was assayed by method of Solomon and reflected the rate of generation of [^{32}P] cAMP from alpha[^{32}P] ATP (9). Lymphocytes were incubated in balanced salt solution containing 1 mM isobutyl-methyl-xanthine (IBMX). The mixture was incubated for 10 min at 30°C. Incubations were terminated by addition of 0,2 ml 0,1 N HC1. A radioactivity of the samples was measured by method of Cherenkov.

Materials

[^{3}H] - dihydroalprenolol was purchased from Amersham (England), and Ficoll 400 from Pharmacia (Sweden). All other compounds were obtained from Sigma Chemical Co. (USA).

RESULTS.

According to PAP response to hypoxia we isolated 3 subpopulations of healthy highlanders: 1) nonresponders, 2) hyperresponders, 3) hyperresponders with moderate HPAH. Nonresponders had low PAP at the rest (12,8 ±1,6 mmHg), which moderately increased under hypoxia (21,6±2,9 mmHg). Hyperresponders also had low PAP at the rest (6,7±1,9 mm Hg), which increases after hypoxia (34,8±2,7 mm Hg). Highlanders with moderate HPAH had PAP 20,7±1,1 mmHg, which sharply increased, when we gave the hypoxia (Table 1).

The differences between groups of highlanders in ß-AR response to hypoxia were examined. The ß-receptor density on lymphocytes of nonresponders in normoxia and under hypoxia was not significantly different (Fig. 1). In hyperresponders Bmax was much lower in normoxic conditions but under hypoxia increased to the level of nonresponders (3,7±1,4 and 6,9±1,8 fmol/10^6 cells, respectively). In highlanders with moderate HPAH BMax was initially high and it was further increased under hypoxia (9,5±1,4 and 12,1 ± 1,0 fmol/ 10^6 cells).

After 5 years we investigated , ß-AR density in lymphocytes and their response to hypoxia in the same subjects (3 nonresponders and 6 hyperresponders). We demonstrated that Bmax did not change both in hyperresponders and nonresponders (4.0±1.8 and 7.5±2.7 fmol/ 10^6 cells). When we incubated these lymphocytes in hypoxic conditions (hypoxia in vitro) for 48 hours we found that lymphocytes of nonresponders did not change their density (6.9±1.3 fmol/10^6 cells), where as lymphocytes of hyperresponders dramatically changed Bmax (4.0±1.8 to 7.7±1.1 fmol/10^6 cells).

Table 1. Parameters of the small circle haemodynamic, ß-AR and AC activity in highlanders

Group	Test	TWR V	PAP (mmHg)	B_{max}	Basal activity	AC activity Iso	AC activity Forsk
Non	N	36±2	12,8±1,6	6,8±2,3	7,9±0,8	10,4±1,1**	10,2±1,3**
	H		21,6±2,9*	7,2±1,9	7,1±0,8	11,2±1,6**	14,1±1,6**
Hyper	N	36±3	16,7±1,9	3,7±1,4	7,4±0,4	10,2±1,3**	12,1±1,3**
	H		34,8±2,7*	6,9±1,8***	7,5±1,1	10,1±1,8**	14,3±1,1**
With Mode-	N	36±3	20,7±1,1	9,5±1,4	20,2±1,9	23,5±1,5**	27,5±2,1**
rateHPAH	H		36,2±2,9*	12,1±1,0***	23,2±1,5	23,2±2,5**	26,4±3,0**

*- p < 0,0001 normoxia against hypoxia; ** -p < 0,001 basal activity against stimulated; *** - p < 0,005 B^{max} under normoxia against hypoxia.

Concentration of B_{max} was measured in fmol/10^6cells,Iso and Forsk 10^{-5}-10^{-6}M.

N - normoxia; H - hypoxia

The activation of adenylate cyclase (AC)

AC basal activity was not significantly different in both non- and hyperresponders before and after hypoxic test (Table 1). Isoproterenol stimulation in normoxic conditions led to almost identical increase of AC activity in both groups, but under hypoxia only in nonresponders the activation was slightly increased, whereas in hyperresponders percentage of activation did not change authentically.

Stimulation of AC was similar in hyper- and nonresponders . We have demonstrated above that B_{max} was two times lower in hyperresponders compared to nonresponders, so these data revealed hyperreactivity of ß-AR in hyperresponders. Hypoxia did not change isoproterenol- stimulated activation of AC . In highlanders with moderate HPAH basal activity of AC was significantly higher compared to non-and hyperresponders. Hypoxia blocked effect of isoproterenol and forskolin on AC. These data revealed desensitization of ß-AR with development of HPAH.

Our measurements of cAMP level in lymphocytes after hypoxia in vitro demonstrated that hypoxia decreased the basal and agonist-stimulated cAMP levels in hyper- and normoresponders (Table 2).

Figure 1

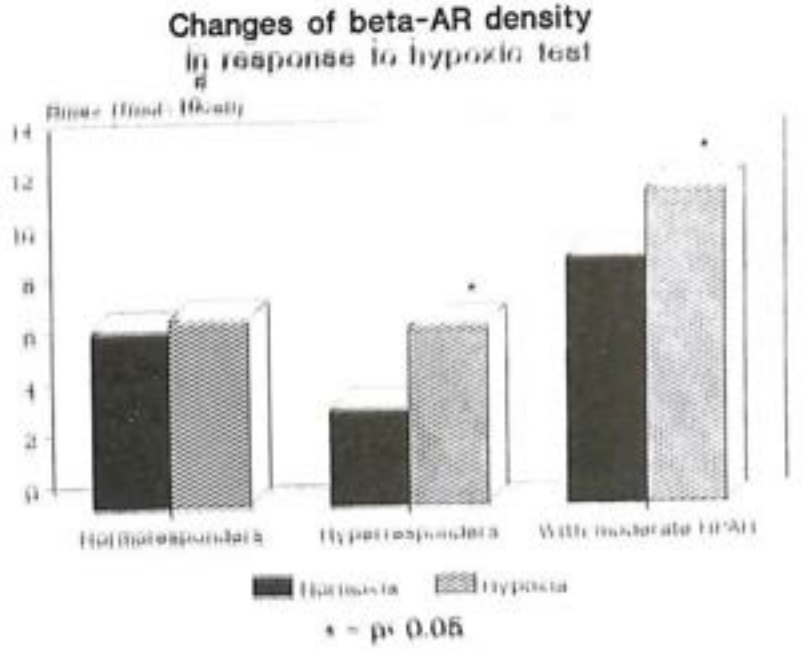

Table 3. cAMP concentration (pmol/in ml cells in highlanders

Group	Test	Basal activity	AC activity	
			Iso 10^{-5}M	Forsk 10^{-5}M
Normoresponders	Norm	7,2±0,8	8,1±0,4**	8,4±0,3**
	Hyp	3,2±0,2	3,1±1,0,1***	8,4±0,4**
Hyperresponders	Norm	5,7±0,3	6,3±0,3**	6,6±0,2**
	Hyp	3,0±0,06	3,6±0,04***	5,7±0,4**

Where *- the discrepancies between control and hypoxia are authentic with p<0,05; ** - p<0,05 basal activity against stimulated; *** - non-significant.

DISCUSSION

It is known that at mammals hypoxia increased blood norepinephrine levels and it could result in desensitization of ß-AR (1). At the same time, preservation of normal sympathetic reactivity is the important factor of the individual survival in extremal environment. Our data demonstrated the heterogenic responses of human organism to hypoxia and would allow us to forecast the clinical prognosis of chronic hypoxia on developing HPAH. It is known that ß-AR will stimulate vasorelaxation of the systemic and pulmonary vessels and decrease arterial blood pressure. Thus the functional disability of ß-AR will correlate with decreased vasorelaxation and increase in arterial blood pressure.

By dividing highlanders into three subgroups, we found out the correlations between the ß-AR and PAP responses to hypoxia. In nonresponders hypoxia moderately increased PAP and did not change ß-AR density. In hyperresponders hypoxia increased ß-AR density and PAP. In highlanders with moderate HPAH Bmax was initially higher and hypoxia increased it furthermore. When we investigated the functional activity of ß-AR by isoproterenol dependent activation of AC we found that hypoxia did not change the rate of activation of AC both in hyper- and normoresponders. However, in hyperresponders under hypoxia it was almost 2-fold increase of ß-AR amount which was not accompanied by proportional increase in AC activity. These data proved that hypoxia recruited part of ß-AR which were still not coupled with Gs protein and AC (10). In nonresponders hypoxia did not affect both ß-AR amount and their activity. In highlanders with moderate HPAH isoproterenol moderately activated AC in normoxia and did not stimulate it in hypoxia. Thus, in this group the ß-AR desensitization becomes obvious already in normoxia and hypoxia enhances it furthermore.

High basal activity and disappearance of

isoproterenol dependent activation of AC revealed that AC activity in this group of subjects was uncoupled from ß-AR and had have different mechanisms of regulation (11). It is known that elevation of intracellular calcium, activation of PKC could activate AC by ß-AR independent pathways (12).

Interestingly, that lymphocytes from hyperresponders could upregulate ß-AR density in response to hypoxia not only in vivo but in vitro. Nonresponder's lymphocytes did not change Bmax to hypoxia in vitro. Early we had shown that hypoxia upregulated different proteins in lymphocytes and this effect was mediated by calcium (13). When added calcium scavenger EGTA ablated the effect of hypoxia on protein expression (14). We demonstrated that hyperresponders and highlanders with moderate HPAH exhibited hyperactivity of their calcium channels and increased intracellular calcium levels (15). We suggest that increased intracellular calcium might upregulate expression of ß-AR and hyperactivation of AC in highlanders with moderate HPAH.

Our data suggest that healthy highlanders are heterogeneous by their response to hypoxia. The difference in response of pulmonary arterial blood pressure to hypoxia is correlated with the response of ß-AR to hypoxia. In hyperresponders hypoxia upregulates ß-AR density which does not enhance signal transduction because of partial desensitization of ß-AR. In the group with moderate HPAH most of ß-AR are desensitized and AC activity is not regulated by AR. Thus, the resistance to development of HPAH is correlated with the resistance of ß-AR to desensitization in hypoxia, and its ability to setivate AC and conduct vasodilatation of pulmonary arteries.

CONCLUSIONS

1. According to the response of pulmonary arterial pressure and ß-adrenoceptors to hypoxia, healthy highlanders appeared to represent three subgroups: nonresponders, hyperresponders, highlanders with moderate HPAH.
2. Hyperresponders and highlanders with moderate HAPH have signs of desensitization of ß-AR which correlates with hyperresponse of pulmonary arterial blood pressure to hypoxia. Resistance of highlanders to the development of HPAH is correlated with the resistance of their ß-AR to hypoxia-induced desensitization.

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NEOCYTOLYSIS IN THE ADAPTATION OF RED CELL MASS ON DESCENT FROM ALTITUDE

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RSUMEN: Neocitolisis en la Adaptación de la Masa de Eritrocitos al Descenso de la Altura

Los individuos aclimatizados que descienden de la altura deben adaptarse rápidamente a la policitemia, que es un exceso en la masa de eritrocitos y en el volumen sanguíneo en relación al nuevo ambiente. Creemos que esto se acompana por neocitólisis, que consiste en la destrucción selectiva de los eritrocitos más jóvenes, un proceso fisiológico que descubrimos recientemente a través de estudios en astronautas. Al entrar en microgravedad, la sangre de un astronauta se distribuye centralmente causando policitemia aguda. Luego se produce una disminución reproducible de 10% en la masa de eritrocitos en los primeros días de permanencia en el espacio. La producción de eritrocitos no disminuye en estos primeros días y la supervivencia de los eritrocitos marcados más viejos es normal. La conclusión evidente es que se produce neocitólisis. Estudios realizados hace 45 años en el Perú y en los Himalayas demuestran claramente que hay hemólisis al descender de la altura. Hay un 10% de disminución en la masa de eritrocitos durante los primeros días de permanencia a nivel del mar, además hay un incremento en la bilirrubina sérica y en la urobilina fecal, todo lo que ocurre antes que haya cualquier caída significativa en la producción de eritrocitos. Estudios realizados en sujetos aclimatizados que desciendan de la altura podrán demostrar directamente la neocitólisis,-a través del marcado selectivo de grupos de eritrocitos de diferentes edades. Estos estudios aclararán los mecanismos subyacentes de la neocitólisis, si niveles sub-umbral de eritropoyetina afectan o no la expresión de moléculas de adhesión, así como las interacciones entre fagocitos. La neocitólisis puede tener implicancias amplias en la medicina clínica, incluyendo el aspecto de los regímenes de dosificación de eritropoyetina.

Palabras claves: Eritrocitos, Altura, Adaptación, Neocitólisis, Policitemia

RÉSUMÉ: Néocytolyse dans l'adaptation de la masse d'érythrocytes chez les voyageurs descendant de régions de grande altitude.

Les individus acclimatés qui descendent des hauteurs doivent s'adapter rapidement à la polyglobulie, un excès de la masse d'érythrocytes et du volume sanguin en rapport avec le nouveau milieu. Nous pensons qu'elle est accompagnée de néocytolyse, destruction sélective des érythrocytes les plus jeunes, processus physiologique que nous avons récemment découvert grâce à des études sur les astronautes. En entrant en micro-gravitation, le sang d'un astronaute se distribue centralement, provoquant une polyglobulie aiguë. Survient ensuite une diminution reproduisible de 10 % de la masse d'érythrocytes au début du séjour dans l'espace. Il n'y a pas de diminution de la production d'érythrocytes au cours des premiers jours et la survie des érythrocytes marqués les plus vieux est normale. La conclusion évidente est qu'il se produit une néocytolyse. Des études réalisées il y a 45 ans au Pérou et dans l'Himalaya démontrent clairement qu'il y a hémolyse lorsqu'on descend des hauteurs. La diminution de la masse d'érythrocytes est de 10 % durant les premiers jours de séjour au niveau de la mer, accompagnée d'une élévation de la bilirubine sérique et de l'urobiline fécale, réactions observées avant que ne se produise une chute significative de la production d'érythrocytes. Des études effectuées chez des sujets acclimatés qui descendent des hauteurs pourront démontrer directement la néocytolyse, par le marquage sélectif de groupes d'érythrocytes d'âges différents. Ces études élucideront les mécanismes sous-jacents de la néocytolyse, si les niveaux d'érythropoïétine inférieurs au seuil affectent ou non l'expression de molécules d'adhésion, ainsi que les interactions entre phagocytes. La néocytolyse peut avoir d'amples implications en médecine clinique, y compris l'aspect des régimes de dosification de l'érythropoïétine.

Mots-clés : Erythrocytes, Altitude, Adaptation, Néocytolyse, Polyglobulie.

SUMMARY: .Acclimated individuals descending from high altitude must rapidly adapt to plethora, an excess in red cell mass and blood volume for their new environment. We believe that this is accomplished by neocytolysis, the selective destruction of the youngest red blood cells, a physiologic process we recently discovered through studies on astronauts. On entering microgravity, an astronaut's blood distributes centrally causing acute plethora. There ensues a reproducible 10% decline in red ceil mass in the first several days in space. Red cell production does not decline in these first days, and survival of labeled older red cells is normal. The inescapable conclusion is that neocytolysis ensues.

Studies done 45 years ago in Peru and in the Himalayas clearly demonstrate hemolysis on descent from high altitude. There is a 10% decline in red cell mass in the first several days at sea level, an increase in serum bilirubin and stool urobilin, all occurring before there is any significant fall in red cell production. We preview studies on acclimated subjects descending from high altitude which will directly demonstrate neocytolysis by selectively labeling red cell cohorts of different ages. These studies will elucidate underlying mechanisms of neocytolysis, whether sub-threshold erythropoietin levels affect surface adhesion molecule expression and red cell-phagocyte interactions. Neocytolysis may have broad implications to clinical medicine, including to current dosing regimens of erythropoietin.

Key words: Blood red cels, Hygh-altitude, Adaptation, neocytolisis, Polycythemia.

INTRODUCTION.

Current dogma in hematology holds that red blood cell mass (RBCM) is controlled entirely at the level of red cell production under the influence of the hormone erythropoietin (EPO). EPO works at the level of primitive red cell progenitors, stimulating proliferation of cells committed to an erythroid

maturation pathway, preventing apoptosis of primitive erythroid colony-forming units (1). The actions of EPO are believed to be limited to early progenitor cells, not on more mature normoblasts and erythrocytes. Red cells released into the blood survive 120 days and dogma holds that there are no physiologic mechanisms to shorten red cell survival.

A decrease in RBCM (anemia) is much more commonly observed than plethora. Studies of physiologic responses in normal and anemic individuals gave rise to the principles stated above. There has been relative neglect of physiologic processes that come into play in circumstances of plethora. Through studies of the unusual environment of spaceflight, we became aware of the process of neocytolysis, the selective hemolysis of young red blood cells. In this paper, we concentrate mainly on how this process is manifest when individuals acclimated to high altitude descend to sea level, and on how studies of such descending individuals can shed further light on neocytolysis. Proving and better understanding this process may have broad implications in physiology and clinical medicine.

Spaceflight anemia. It has been known for decades that astronauts returning from space consistently experience a decline in RBCM of about 10% after spaceflights of 8 to 10 days or more. The mechanism responsible for the anemia has finally been clarified by our group's studies on SLS-1 and SLS-2 (2-3). On entering microgravity, the blood that is normally held in the extremities by gravity suddenly shifts centrally. There ensues a very rapid decline in plasma volume (due to a third "spacing" caused by factors not yet defined) and a decrease in EPO secretion. There are two reasons why a hemolytic mechanism must be invoked to explain the fall in RBCM. First, the fall is too rapid to be explained entirely by decreased red cell production. Second, ferrokinetic studies demonstrate that there is little if any decrease in red cell production during the first several days in space. This is just as predicted from our understanding of EPO action; circumstances effecting a decrease in red cell production transpire after a delay of several days (1).

While hemolysis seemed certain, it was disconcerting that ^{51}Cr-labeled red blood cells were repeatedly found to have normal survival in space. This became understandable when we considered that red cells were labeled with ^{51}Cr twelve or more days before launch. The only way to reconcile all observations is to conclude that on entering microgravity, there is selective hemolysis of the youngest red blood cells, a process we call neocytolysis (3,4) (see figure).

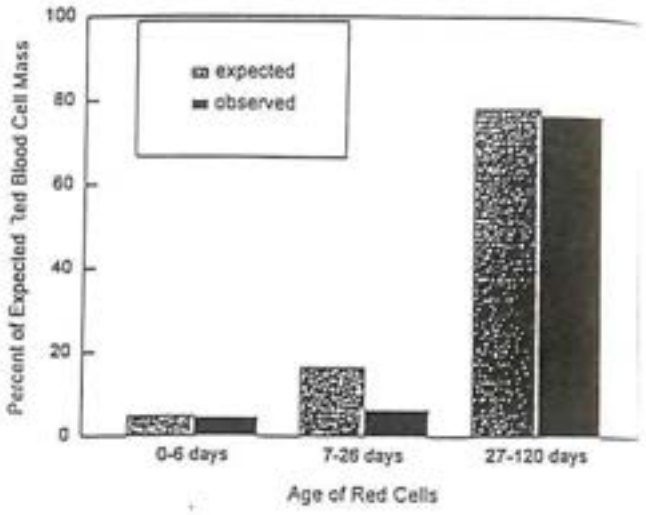

Figure. Red Cell Cohorts Measured After Fourteen Days in Space Cells 0-6 days old were labeled with ^{59}Fe on the third day in space. Cells 7-26 days old were unlabeled. These consisted mainly of red cells less than 12 days old at the time of launch. Cells 26-120 days old were labeled with ^{51}Cr. Based on data from SLS-2 astronauts (3).

Prior studies of descent from altitude. Adaptation to the hypoxic environment of high altitude produces polycythemia. Acclimated individuals rapidly descending to sea level find themselves in a situation very similar to astronauts entering microgravity, experiencing plethora maladaptive to their new environment. The changes that occur with descent were studied in Peru in 1950 (5). Merino found that hemoglobin declined by 10 to 18% in the first 10 days after descent. Serum indirect bilirubin and fecal urobilinogen increased markedly with peaks at 6 to 8 days after descent. He concluded that a "very distinct hemolytic process occurred in all cases."

Huff amplified these observations by studying 11 acclimated natives descending from high altitude in Peru to sea level (6). A fall of 9% in RBCM occurred in 8 to 10 days. Ferrokinetic studies, bone marrow examinations and reticulocyte counts again demonstrated no significant decline in red cell production during the first several days of the RBCM decline, but there was a substantial fall in red cell production later on. Pace's observations on ten men living in the Himalayas and returning to sea level echoed the conclusion that a hemolytic mechanism was necessary to explain the early, rapid fall in RBCM (7).

Thus, observations made on descent from high

altitude complement those made on astronauts entering microgravity and provide further evidence that some type of hemolytic mechanism comes into play in adapting to plethora. Confusing the issue was a study which showed that red cells of rats had normal survival on descent from altitude (8). Just as in the astronauts, the rat red cells were labeled with ^{51}Cr several days before descent. Neocytolysis, hemolysis selectively affecting the youngest red blood cells, was not considered by the investigators of descent from altitude but it would have reconciled all observations.

Proposed mechanism of neocytolysis. Newly-released red blood cells interact intimately with reticuloendothelial phagocytes particularly in the spleen. Inclusions such as Howell-Jolly bodies are pitted and culled, and red cell membrane phospholipid is conditioned. Surface adhesion molecules have been found to be important in red cell maturation (9). Among circulating red cells, adhesion molecules are richest on the youngest cells. It seems likely that neocytolysis is mediated through changes in red cell-reticuloendothelial cell interaction resulting from changes in surface adhesion molecule expression.

EPO is the main regulator of RBCM by up-regulating red cell production in times of need, and there is reason to believe that EPO remains the main regulator of RBCM in adaptation to plethora. A fall in EPO levels below a critical threshold may initiate neocytolysis. Neocytolysis occurs in just the situations where EPO levels are suppressed. How EPO effects changes in adhesion molecule expression remains to be elucidated.

Neocytolysis can be viewed as an example of a general emerging physiologic paradigm, an extension of apoptosis to non-nucleated cells. The body maintains homeostasis and is able to adapt to environmental changes both by regulating cell production and cell death. Cells require lineage-specific growth factors for their birth, their proliferation, and for their survival. Planned studies on descent from altitude. With collaborators at the Institute of Altitude Studies, Universidad Cayetano Heredia in Lima, we are poised to begin studies designed to definitively prove the existence of neocytolysis and to elucidate the underlying mechanism. Individuals acclimated to 14,500 feet will have studies of RBCM, red cell survival and heme turnover in Cerro de Pasco, Peru. Cohorts of red cells of differing ages will be differentially labeled using ^{51}Cr, ^{13}C and ^{15}N, allowing direct determination of which red cells are later hemolyzed. Subjects will be transported to sea level where we will repeat measurements of RBCM, red cell survival and heme turnover. We expect to directly show that only the youngest red cells, less than 7 to 12 days old, will hemolyze. Some subjects will receive daily subcutaneous EPO injections on descent. We expect this to abrogate neocytolysis and prove the role of low EPO levels in initiating the process.

Wider implications. These studies have implications far beyond the unusual situations of descent from high altitude or spaceflight. As one example, neocytolysis should occur in athletes who try to enhance performance by "blood doping" administering supraphysiologic autologous red cell transfusions. Whenever new physiologic processes are defined, perturbations at various steps are soon appreciated which lead to disease. An example might be the congenital hemolytic anemia due to deficiency of pyruvate kinase where it is known that young red cells are selectively destroyed (10). It is likely that understanding and manipulating neocytolysis would allow fresh pathophysiologic thinking and novel therapeutic approaches to a variety of hematologic disorders, such as polycythemias.

The anemia of renal disease is another situation where EPO levels are low, and levels may be low enough in some patients to precipitate neocytolysis. Neocytolysis could contribute to the documented hemolytic component in some patients with the anemia of renal disease. Our theory predicts that currently widely used EPO dosing regimens of three intravenous boluses weekly are highly inefficient because peak levels would stimulate progenitors toward erythroid maturation but neocytolysis may occur at the EPO nadir. This may explain recent empiric observations that much lower doses of EPO are therapeutically effective when given daily subcutaneously (11). Subcutaneous regimens could result in substantial health dollar savings.

In summary our studies on the decline of RBCM with spaceflight led to the inescapable conclusion that adaptation occurred by the selective hemolysis of young red cells, a process we call neocytolysis. We theorized that this should occur as well when individuals acclimated to high altitude descend to sea level. We were surprised to uncover forgotten and ignored data from 45 years ago demonstrating hemolysis on descent. While neocytolysis was not considered by these investigators, it would best explain their data and would be the most efficient way for the body to adapt to acute plethora. Planned studies in Peru will prove and clarify the mechanisms underlying neocytolysis, including the possible role of sub-threshold EPO suppression. Understanding this process will permit a fresh look at hematologic disorders and their therapy.

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ERYTHROPOIETIN SECRETION DURING HYPOBARIC HYPOXIA. MODULATION OF THE RESPONSE BY THE LEVEL OF ERYTHROPOIESIS

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RESUMEN La eritropoyetina (EPO) es una hormona glucoproteica que forma parte de un mecanismo de retroalimentación negativa involucrado en el control de la eritropoyesis. La concentración de EPO en plasma (pEPO) en los mamíferos depende del balance existente entre el aporte de oxígeno (0_2) a los tejidos y la demanda del gas por ellos. Sin embargo, observaciones clínicas y experimentales indican que otros factores, además del 0_2, estarían también involucrados en la modulación de la síntesis de la hormona. Por lo tanto, modelos experimentales murinos fueron desarrollados en nuestro laboratorio para investigar la relación posible entre la producción de EPO 0_2-dependiente y el nivel de la eritropoyesis. Esta fue estimulada mediante administración de rh-EPO o inhibida mediante inyección de ciclofosfamida. Cuando ratones así tratados, junto a ratones controles, fueron expuestos durante 6 horas a hipoxia hipobárica para inducción de hipoxemia, pEPO fue 208 % y 33 % del valor normal en los animales con eritropoyesis deprimida o estimulada, respectivamente. El catabolismo de EPO no fue afectado por ninguno de los tratamientos. Estos resultados sugieren que la síntesis de EPO en los mamíferos no sólo guarda relación con el balance entre oferta y demanda de 0_2 tisulares (estímulo principal) sino también con la actividad eritropoyética de la médula ósea (acción moduladora).

Palabras Claves : Eritropoyetina, Hipoxia, Hipobaria

RÉSUMÉ: Sécrétion d'érythropoïétine au cours de l'hypoxie hypobare. Modulation de la réponse par le niveau d'érythropoïèse.

L'érythropoïétine (EPO) est une hormone glycoprotéique faisant partie d'un mécanisme de rétroalimentation impliqué dans le contrôle de la production de globules rouges. Les niveaux plasmatiques d'EPO (pEPO) des mammifères sont liés à l'apport d'oxygène (O_2) aux tissus en fonction de leurs besoins. Les observations cliniques et expérimentales indiquent cependant que d'autres facteurs en plus de O_2 peuvent également être impliqués dans la modulation de la synthèse d'EPO. Des modèles expérimentaux de souris ont donc été développés dans notre laboratoire afin de rechercher une éventuelle relation entre la production d'EPO dépendante d'O_2 et le niveau d'érythropoïèse. Celle-ci a été stimulée par rh-EPO ou inhibée par cyclophosphamide. Les souris ainsi traitées et un groupe de souris normales ont été soumises à l'hypoxie hypobare pendant 6 heures. Chez les souris hypoxiques les valeurs de pEPO ont atteint 208 % par rapport à la normale chez les souris à l'érythropoïèse déprimée et 33 % chez celles où elle a été stimulée. Le catabolisme de l'EPO n'a pas été affecté par les traitements. On suggère donc que la synthèse de l'EPO chez les mammifères est liée non seulement à l'apport d'oxygène aux tissus selon leurs besoins (stimulus principal), mais aussi à l'activité érythroïde de la moëlle osseuse (action modulatrice).

Mots-clés : Erythropoïétine, Hypoxie, Hypobarie.

SUMMARY: Erythropoietin (EPO) is a glycoprotein hormone that is part of a feedback mechanism involved in the control of red cell production. Plasma EPO levels (PEPO) of mammals are related to the oxygen (0_2) supply to tissues relative to their 0_2 needs. However, clinical and experimental observations indicate that factors other than 0_2 could be involved also in the modulation of EPO synthesis. Therefore, experimental mouse models were developed in our laboratory to investigate the possible relationship between 0_2 dependent EPO production and the level of erythropoiesis. Erythropoiesis was either stimulated by rh-EPO or depressed by cyclophosphamide. When mice so treated, as well as normal mice, were made hypoxemic by a 6 hour-exposure to hypobaria, pEPO was 208 % of normal and 33 % of normal in mice with depressed or stimulated erythropoiesis, respectively. EPO catabolism was not affected by treatments. It is thus suggested that EPO synthesis in mammals is not only related to the 0_2 supply to tissues relative to their 0_2 needs (main stimulus) but also to the erythroid activity of the bone marrow (modulatory action).

Key Words : Erythropoietin, Hypoxia, Hypobaria

Oxygen (0_2) transport within the body is often divided into a convective portion, comprising bulk transport of 0_2 to the capillaries, and a diffusive portion, consisting of off-loading of 0_2 within the capillaries and diffusion ultimately to cytochrome aa3 within the mitochondrion (1). Convective 0_2 transport (COT) is the product of blood flow and arterial 0_2 content (CaO_2). CaO_2 is a function of hemoglobin (Hb) concentration, oxyHB binding properties, and arterial $P0_2$ (PaO_2) The circulating red cell mass (RCM) is an organ that collaborates in COT by providing a HB mass that binds 0_2 is a reversible form. The organ is composed of red blood cells (RBC), which have a finite life span and lack the ability for self-renewal. The RCM is maintained at an optimal size for its function by adjustments in the rate of erythropoiesis since the red cell life span is a biological constant.

Erythropoiesis is thus a vital process that is able to adjust to different physiological and pathological conditions. Consequently, the level of erythropoiesis will decrease if the RCM is artificially increased by transfusion, or will increase under conditions that induce hypoxia, as defined by a diminished 0_2-carrying capacity of blood (anemia), by a decreased arterial $P0_2$ (hypoxemia), or by an increased HB 0_2-affinity at sea level.

Physiologic adjustments of the rate of erythropoiesis and RCM are mediated by erythropoietin (EPO), a glycoprotein which acts as a specific growth factor for erythroid progenitor cells in the bone marrow (2). The hormone is thus a part of a feedback mechanism involved in the control of erythropoiesis. It is mainly secreted by renal endocrine cells in inverse correlation with COT through the expression of an EPO gene apparently in response to both contitutive and hypoxia-induced transcription-regulating factors (3).

According to the almost 40-year old hypothesis of Fried et al (4) that EPO synthesis depends on the convective 0_2 supply to tissues relative to their 0_2 needs, it seems evident that the EPO production rate (EPO-PR) is negatively correlated to 0_2 availability, namely tissue $P0_2$. A structure, possibly a hemoprotein, has thus been proposed that senses the 0_2 tension and initiates a signal that turns on the expression of the EPO gene (5). According to this model, a kidney 0_2 sensor measures intersticial $P0_2$ and modulates EPO-PR by the kidney, which in turn adjusts the rate of erythropoiesis to meet the demand for 0_2-carrying cells.

Although Fried's hypothesis has received strong experimental and clinical support, the following evidences militates against its inherent simplicity, suggesting that factors other than 0_2 could modulate EPO synthesis:

1) Fried et al (6) reported that plasma EPO levels (pEPO) of WWw mice, which have a mild, congenital anemia, a decreased response to EPO, and a defect in their multipotential hematopoietic stem cells (HSC), were higher that those of comparably anemic non-mutants (+++). This difference was not longer present 7 days after transplanting marrow cells into WWv mice. At this time, the response of WWw mice to EPO was comparable to that of +++; yet the colonizing ability of their HSC was still defective. From these data, the authors suggested that EPO-PR at any level of anemia is modified by the ability of the hematopoietic cells to respond to EPO.

2) Barceló and Bozini (7) presented evidence that pEPO during continuous exposure to hypobaria in mice with marrow aplasia induced by whole body X-irradiation or 5-fluorouracil injection were higher than in control mice similarly exposed. These finding gave support to the hypothesis that a relationship exists between EPO-PR and the erythroid responsiveness to EPO.

3) Birgegard et al (8) measured pEPO in 23 patients before, during and after intensive cytostatic treatment courses for acute leukemia or before bone marrow transplantation. A marked increase was seen in all patients, starting 1 or 2 days after initiation of treatment. A peak was reached after about 7 days, after which pEPO fell rapidly, even in patients who were anemic at that time. In 13 of the patients there was no fall in HB levels that could explain the increase in pEPO. The increase was too large to be explained by an altered EPO metabolism or marrow utilization. Authors suggested the existence of a mechanism other than anemia for EPO-PR stimulation.

4) Piroso, Erslev and Caro (9) performed serial pEPO measurements in 6 patients with acute leukemia treated by intensive chemotherapy. In all cases pEPO increased after the onset of treatment, although the HB concentration remained at stable values. Subsequently pEPO gradually returned to baseline levels at the time of bone marrow recovery. It was concluded that this inapropriate increase in pEPO could be related to a direct or indirect effect of a suppressed marrow on sites of EPO production or catabolism.

5) Jelkmann and Wiedemann (10) compared pEPO in nonrenal anemic patients with erythrocytic hypoplasia or active erythropoiesis. In both groups, a negative correlation was determined between the blood HB concentration and the logarithm of pEPO. However, the two regression lines were not identical, and pEPO was significantly higher for the degree of anemia in the patients with erythroid hypoplasia. This data support the idea that, independent of the 0_2 offer, the proliferating erythrocytic progenitors lower pEPO by negative feedback.

In the five described studies pEPO was unexpectedly higher in humans or animals with demonstrated or assumed poor erythroid response to EPO. It was also assumed that pEPO reflects EPO-PR.EPO concentration in the plasma compartment, however, depends on the balance between EPO formation and EPO disappearance rates. Consequently, the above findings could be attributed to changes in either EPO synthesis or EPO catabolism, or both. To clarify this point, studies were performed in our laboratory (11, 12] to estimate both pEPO (during stimulation of hormone production by hypobaric hypoxemia] and plasma EPO half-life in mice in which the rate of erythropoiesis (RBSPR) was either

increased by rh-EPO administration or depressed by cyclophosphamide (CP) treatment. Two mouse models were thus developed in which the 0_2-carrying capacity of blood, pEPO, blood viscosity, 0_2 supply/demand ratio - factors that alter EPO production - and the kinetics of plasma EPO were within normal values in spite of intense stimulation or depression of erythropoiesis. Any observed difference in pEPO in response to the hypoxemic stimulus between experimental and control mice should therefore be attributable to the increased EPO-PR. As expected from previously reported results (13), hypoxiadependent EPO-PR was inversely related to the level of erythropoiesis occurring in the animals during exposure to hypobaric hypoxia, EPO-PR being 208% of normal in mice with CP-induced depression of erythropoiesis and 33% of normal in those with EPO-induced enhancement of erythropoiesis.

No evidences exist on the nature of the operating mechanism. However, data suggest that a functional link could exists between the EPO-responsive cells in the bone marrow and the EPO-synthesizing cells that could modulate the hypoxiadependent expression of the EPO gene. If this is really the case, then the EPO-PR in mammals will be not only related to the 0_2 supply to the tissues relative to their 0_2 needs (main stimulus) but also to the erythroid activity of the marrow (modulatory action).

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NEUROCHEMICAL EFFECTS OF LONG-TERM HYPOXIA ON NORADRENERGIC NEURONS IN NUCLEUS TRACTUS SOLITARIUS OF THE RAT

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RESUMEN: Efectos Neuroquímicos de la Hipoxia Prolongada en las Neuronas Noradrenérgicas del Núcleo del Tracto Solitario de la Rata

Para determinar los efectos de la hipoxia prolongada sobre la actividad catecolaminérgica en el núcleo del tracto solitario (NTS), se sometió a ratas machos a hipoxia normobárica (10% O_2 en nitrógeno) por 3 días, 1,2 o 3 semanas. La ventilación aumentó gradualmente durante la exposición hipóxica alcanzando una meseta luego de 10 días de hipoxia. La hipoxia prolongada estimuló el recambio de norepinefrina en las neuronas noradrenérgicas localizadas en el NTS caudal al obex, la región a la cual se proyectan fibras carotídeas aferentes. La quimiodenervación bilateral abolió los cambios centrales en la actividad noradrenérgica. La actividad y la cantidad de tirosina hidroxilasa (TH), la enzima limitante de la síntesis de catecolaminas, y el nivel de codificación de mARN para TH aumentaron en el NTS caudal en respuesta a la hipoxia prolongada, indicando inducción de nuevas moléculas de proteína TH. Estos cambios neuroquímicos ocurrieron solamente luego de hipoxia prolongada, sugiriendo que las neuronas noradrenérgicas están implicadas en la vía cental de quimioreceptores durante la hipoxia sostenida, pero que no son esenciales para las respuestas reguladoras a la hipoxia aguda. La norepinefrina liberada bajo condiciones de hipoxia prolongada podría jugar un rol neuromodulador en la aclimatación ventilatoria.

Palabras claves: Tronco encefálico, Células A2, Vía quimiorefleja, Tirosina hidroxilasa.

RÉSUMÉ: Effets neurochimiques de l'hypoxie prolongée sur les neurones noradrénergiques du noyau du tractus solitaire du rat.

Afin de déterminer les effets de l'hypoxie prolongée sur l'activité des catécholamines du noyau du tractus solitaire (NTS), des rats mâles adultes ont été soumis à une hypoxie normobare (10% O_2 dans l'azote) pendant 3 jours, puis 1, 2 ou 3 semaines. La ventilation respiratoire a augmenté graduellement pendant l'exposition hypoxique, jusqu'à atteindre un palier au bout de 10 jours. L'hypoxie prolongée a stimulé le remplacement de la norépinéphrine des neurones noradrénergiques localisés dans le NTS caudal par rapport à l'obex, région où se projettent les afférents carotidiens chimiosensoriels. La chimioénervation bilatérale a aboli les changements centraux au cours de l'activité noradrénergique. On a noté une augmentation de l'activité et de la quantité de l'enzyme limitative de la biosynthèse des catécholamines tyrosine hydroxylase (TH) et du niveau de mRNA pour la TH dans le NTS caudal, en réponse à l'hypoxie prolongée, indiquant l'induction de nouvelles molécules de la protéine TH. Ces changements neurochimiques ne se produisent qu'après une hypoxie prolongée, suggérant que les neurones noradrénergiques sont impliqués dans le trajet des chimiorécepteurs centraux pendant l'hypoxie soutenue, mais qu'ils ne sont pas essentiels pour les réponses régulatrices à l'hypoxie aiguë. La norépinéphrine libérée dans des conditions d'hypoxie prolongée joue un rôle neuromodulateur dans l'acclimatation respiratoire.

Mots-clés : Tronc encéphalique, Cellules A2, Trajet des chimiorécepteurs, Tyrosine hydroxylase.

SUMMARY: In order to determine the effects of long-term hypoxia on catecholamine activity in the nucleus tractus solitarius (NTS), male rats were subjected to normobaric hypoxia (10% O_2 in nitrogen) lasting for 3 days, 1, 2 or 3 weeks. Ventilation increased gradually during the hypoxic exposure before reaching a plateau after 10 days of hypoxia. Long-term hypoxia stimulated the norepinephrine turnover in noradrenergic neurons located in the NTS caudal to the obex, the discrete region to which the chemosensory carotid afferents project. Bilateral chemodenervation abolished the central changes in noradrenergic activity. The activity and quantity of tyrosine hydroxylase (TH), the rate-limiting enzyme of catecholamine biosynthesis, and the level of mRNA coding for TH were increased in the caudal NTS in response to long-term hypoxia, indicating induction of new molecules of TH protein. These neurochemical changes occurred after long-term hypoxia only, suggesting that noradrenergic neurons are involved in the central chemoreceptor pathway during sustained hypoxia but are not essential for regulatory responses to acute hypoxia. Norepinephrine released under long-term hypoxia could play a neuromodulatory role in ventilatory acclimatization.

Key words: Brainstem, A2 Cell Group, Chemoreflex Pathway, Tyrosine Hydroxylase.

Various physiological adaptive responses take place in face of reduced oxygen concentration or pressure in inspired air. Among them, hyperventilation is probably the most obvious. When prolonging hypoxia, there is a progressive increase in hyperventilation even though the hypoxic stimulus remains at a constant level. Hyperventilation then reaches a plateau after a more or less long time depending on species. This phenomenon is known as ventilatory acclimatization to hypoxia. The mechanism underlying ventilatory acclimatization is still undefined, although it depends on peripheral arterial chemoreceptors (21).

Low blood pressure in oxygen is primarily sensed by peripheral arterial chemoreceptors. Stimulation of the carotid body chemoreceptors elicits an increase in firing rate of chemosensory neurons whose fibers course in the carotid sinus nerve and terminate in the caudal part of the nucleus tractus solitarius (NTS) within the brainstem (5, 6). The NTS contains respiratory neurons whose activity is modulated by central and peripheral afferents in response to environmental conditions. Thus, the NTS has been considered a structure of prime importance for the integration of chemosensory stimuli and control of respiration in response to afferent inputs. In the area to which the chemosensory afferents project, is located a cluster of noradrenergic neurons that constitute the A2 cell group (7). Norepinephrine in the NTS can depress the bulbar respiratory neurons (3).

The aim of our studies was to determine the effects of long-term hypoxia on catecholamine activity in the NTS during exposure to long-term hypoxia by investigating changes in norepinephrine turnover, content and activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis. In order to determine if the observed changes could be found at the gene level, in situ hybridization was employed to investigate the influence of long-term hypoxia on the level of mRNA coding for TH.

METHODS

Animals and hypoxia

Experiments were carried out on male Sprague-Dawley rats (IFFA Credo, L'Arbresle, France), a species that demonstrates human-like ventilatory acclimatization to hypoxia (13). The animals were placed for 3, 7, 14 or 21 days in a normobaric Plexiglas chamber. The chamber was supplied with a gas mixture consisting of 10% 02/90% N_2. The chamber air was recirculated in a continuous circuit. Incorporated into the circuit were a chilled tank and soda lime to trap expired water vapour and to absorb carbon dioxide, respectively. The CO_2 concentration inside the chamber was less than 0.1%. Control groups of normoxic rats were kept in normoxia and sacrificed at the same time as their respective hypoxic counterparts.

Chemodenervation was performed by cutting both carotid sinus nerves between the apical pole of the carotid body and the point of branching with the glossopharyngeal nerves. In these conditions no chemosensory reinnervation is possible. The rats were allowed to recover from anesthesia and surgery for one week before to be subjected to hypoxia. Sham-operated animals were used as controls.

Neurochemistry

Noradrenergic activity in the brainstem was assessed using different experimental approaches based on the assessment of catecholamine turnover by pharmacological blockade of their biosynthesis (22), *in vivo* and *in vitro* measurements of TH activity, the rate-limiting enzyme in catecholamine biosynthesis (23), assays of TH content (17, 18, 19), and *in situ* hybridization of mRNA coding for TH protein (4).

Estimation of catecholamine turnover

•-Methyl-para-tyrosine injected intraperitoneally at 250 mg/kg b.w., 2.5 hours before sacrifice allowed the estimation of norepinephrine turnover by blocking the catecholamine biosenthesis. Each experimental group was divided into two half groups: one receiving α-methyl-para-tyrosine, the other receiving the same volume of vehicle (0.9% NaCl). The norepinephrine content was measured in the NTS of saline-treated and α-methyl-para-tyrosine-treated animals. After injection of α-methyl-para-tyrosine, the decline of norepinephrine is exponential. The slope of norepinephrine decrease was calculated and multiplied by the mean of norepinephrine content of saline-treated rats to ahtain the turnover rate.

Estimation of tyrosine hydroxylase activity

In vivo activity of TH was estimated by measuring L-DOPA accumulation after the inhibition of L-amino acid decarboxylase by NSD 1015 (3-hydroxybenzylhydrazine dihydrochloride). Rats were injected intraperitoneally with either NSD 1015 (100 mg/kg b.w.) or the same volume of vehicle (0.9% saline) 20 min before sacrifice. Tyrosine hydroxylation rate was estimated by subtracting the content of DOPA in the structures of saline-treated rats from the content of DOPA in the NTS of NSD-treated rats. DOPA and norepinephrine were assayed by high performance liquid chromatography coupled with electrochemical detection.

Tissue dissection for biochemical analyses

The brain was rapidly removed, frozen on dry ice and stored at -80 °C. The brainstem was cut into serial frontal slices of 48(), µm in thickness. The noradrenergic cell group A2 was punched out according to the dissection procedure described by Palkovits and Brownstein (5). The A2 cell group was subdivided into two parts, respectively caudal and rostral to the calamus scriptorius (22).

In situ hybridization

Frozen horizontal sections (15 µm thick) of NTS were cut serially on a cryostat (Leitz). In situ hybridization was carried out using a labelled 35S probe complementary to the rat TH mRNA (4). The TH protein was located on adjacent sections by immunocytochemistry and revealed by autoradiography (9).

Ventilatory measurements

Ventilation was measured every 3 days for a period of 18 days in animals exposed to hypoxia (10% O_2 in nitrogen). The tidal volume and respiratory frequency were measured by plethysmography using the barometric method (2). In brief, the spirogram of each rat was obtained from a differential transducer (Validyne) interfaced between the chamber flushed with humidified hypoxic air and a reference box of same size. The tidal and minute volumes, and the respiratory frequency were measured and the product of these two parameters gave the minute volume.

RESULTS

The minute volume increased gradually during the first 7 to 14 days of hypoxic exposure, and thereafter, stabilized, thus indicating that ventilatory acclimatization was achieved.

The turnover rate of norepinephrine in the caudal portion of the A2 cell group was increased about 5 fold after 2 weeks of hypoxia. In striking contrast, the norepinephrine turnover remained unaffected in the rostral portion of A2. In order to compare the influence of alteration in barosensory activity, a second group of rats was given daily for 2 weeks the hypotensive drug dihydralazine (20 mg/kg b.w.). The pharmacological treatment induced a selective increase in norepinephrine turnover in the rostral portion of A2 (+104 ±12% above control level) whereas the caudal portion was unaffected. Bilateral transection of the carotid sinus nerves carried out one week before the hypoxic exposure abolished the hypoxia-induced changes in norepinephrine turnover observed in the caudal NTS.

Regarding the influence of hypoxia on catecholamine biosynthesis, the in vivo activity of TH was found to be enhanced after 1 week of hypoxia (±60 ± 8% above control level), although it was unaffected by shorter hypoxic exposures (3 days). Long term hypoxia elicited a delayed increase in TH content (±36 ± 4% above control level) that was apparent after 2 weeks of exposure.

In situ hybridization of the THmRNA in the NTS revealed that hypoxia lasting for 2 weeks elicited a marked increase in THmRNA expression within the caudal NTS. Hypoxia elicited both an increase in the number of grains per cell and a rostral extension of the labeled area. The TH immunoreactivity in the caudal NTS was also increased but more rostrally than THmRNA.

DISCUSSION

Our main finding was that long-term hypoxia induced the stimulation of noradrenergic A2 neurons in the caudal NTS, the primary site of projection of peripheral chemosensory afferents within the brainstem (5, 6).

The changes in noradrenergic activity cannot be explained by a reduced availability in oxygen for A2 neurons. Indeed, increased capillary density in the brain, increased cerebral blood flow and polycythemia are common features observed in response to long-term hypoxia, that may maintain the tissue oxygen concentration close to basal level (8, 10). In contrast, the activation of A2 noradrenergic neurons appear clearly dependent on the integrity of chemosensory afferents since the prior bilateral chemodenervation abolished the changes in norepinephrine turnover induced by longterm hypoxia (22). In the rat, the carotid bodies are the major peripheral arterial chemoreceptors whereas functional aortic chemoreceptors are absent (16).

Hypoxia failed to stimulate the norepinephrine turnover or to increase the TH content in the rostral part of A2 cell group (18, 22). This area corresponds to the site of projection of barosensory nerve fibers in the rat (6). In contrast, chronic hypotension induced by dihydralazine induced a selective increase in

noradrenergic activity in the rostral A2 subset while the caudal A2 subset remained unaltered (19, 22). Taken together, the data provide evidence for the functional heterogeneity of A2 neurons according to their location, caudal or rostral to the obex. The caudal cells are part of the chemoreceptor pathway, whereas the rostral cells are influenced by barosensory inputs.

From a neurochemical point of view, stimulation of noradrenergic neurons by hypoxia is accompanied by a sustained release of norepinephrine (20, 22). The neuronal norepinephrine stores can be replenished, first by an increase in the TH activity (23) and then by an increase in the content of TH protein (18). Our data also showed that the changes in TH content did not result from alterations of the catabolism of the protein but from increased expression of the gene coding for biosynthesis of TH. Indeed, the TH mRNA expression was strikingly enhanced in the caudal NTS of long-term hypoxic rats (4). Thus, the data reveal an hypoxia-induced plasticity of noradrenergic neurons at the gene level in the caudal NTS.

Acute hypoxia increases the firing rate in chemosensory afferent fibers. During sustained hypoxia, the carotid body afferent discharge is increased resulting in a parallel increase in ventilatory output (1, 12). These functional changes are associated with a progressive increase in catecholamine activity in the carotid body as reflected by gradual increases in dopamine and norepinephrine turnover, and in TH content and activity (15, 17). The increased carotid chemoreceptor afferent input during sustained hypoxia could be expected to influence gradually the neuronal activity in the NTS. However, in contrast to the carotid body, the norepinephrine activity in the NTS was largely delayed as the early noradrenergic alterations in this area appeared only after 1 week of exposure (23). This finding shows that norepinephrine is not the first-order neurotransmitter involved in the immediate physiological responses to hypoxia. In fact, glutamatergic neurons are possible candidates to play the role of primary first-order neurons involved in the integration of chemosensory inputs within the NTS. Glutamate is released in the caudal NTS in response to acute hypoxia (11, 24). Instead, norepinephrine might act as a neuromodulator rather involved in the processes of acclimatization to hypoxia. In this context, it is worthwhile to mention that norepinephrine injected locally in the caudal NTS can depress the central respiratory neurons, leading to a decreased discharge of phrenic nerve fibers (3). Within the NTS are located the neurons of the dorsal respiratory group which are adjacent to the A2 noradrenergic neurons. The anatomical vicinity of both types of neurons and the ability of norepinephrine to alter the activity of respiratory neurons led to the suggestion that activation of A2 noradrenergic neurons induced by long-term hypoxia might participate in the stabilization of the hypoxic stimulation of respiratory neurons in the NTS (20). This hypothesis is further supported by the time coincidence between the occurrence of ventilatory acclimatization and noradrenergic activation. Both respiratory and neurochemical changes were indeed observed between 1 and 2 weeks of hypoxia. In addition, a significant correlation was found between the level of minute ventilation after ventilatory acclimatization and the amount of TH protein (20). This strengthens the suggestion of a functional relationship between A2 neurons and ventilatory acclimatization to hypoxia (20).

In conclusion, noradrenergic neurons in the caudal NTS are involved in the central chemoreceptor pathway during sustained hypoxia but are not essential for regulatory responses to acute hypoxia. Caudal A2 neurons could contribute to the ventilatory acclimatization to hypoxia through the inhibitory effects of norepinephrine on the dorsal respiratory group.

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II

EXERCISE, SPORTS AND TRAINING AT HIGH ALTITUDE

EXERCISE TRAINING AT HIGH ALTITUDE: HOW DO THE RESPIRATORY MUSCLES RESPOND?

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RESUMEN: Ejercicios de entrenamiento en altura: Cómo responden los músculos respiratorios?

Al igual que todos los músculos esqueléticos de vertebrados, los músculos ventilatorios (VM) de humanos pueden mejorar adaptativamnete en su capacidad funcional. Puesto que estos cambios ocurren consistentemente en respuesta a protocolos específicos de entrenamiento de resistencia y fuerza para los VM, se podría asumir que se aplican los mismos principios de entrenamiento de músculos esqueléticos a los músculos ventilatorios. Sin embargo, es aparente que las adaptaciones de los VM requieren ejercicios muy fuertes (y tal vez un tipo de entrenamiento específico), puesto que el ejercicio de entrenamiento crónico de animales totales no parece tener un efecto significativo o siquiera predecible sobre los aspectos estructurales o funcionales de los VM. En este artículo se revisa los resultados ambiguos de varios programas de ejercicio de entrenamiento regular con respecto a los músculos respiratorios de animales. También se revisa estudios de entrenamiento de altura, dirigidos a incrementar la fuerza de estímulo necesario para las adaptaciones de los VM, estudios que tampoco revelan cambios funcionales de los VM. Finalmente, puesto que parece haber una relación pobre entre poder de los VM y entrenamiento incluso con incremento de altitud, se plantea la pregunta de si el diseño evolutivo de los VM es primariamente para "respiración". Proponemos una hipótesis alternativa, que la ventilación no ha sido seleccionada como la tarea primaria de los VM. Más bien, son tareas no ventilatorias las que plantean una mayor demanda que la ventilación misma sobre los "músculos ventilatorios".

Palabras claves: Entrenamiento de resistencia, Altitud, Plasticidad muscular

RÉSUMÉ: Exercices d'entraînement à grande altitude : Comment répondent les muscles respiratoires?

Comme tous les muscles squelettiques des vertébrés, les muscles de la ventilation respiratoire (VM) des humains peuvent s'adapter et améliorer ainsi leur capacité fonctionnelle. Ces changements survenant invariablement en réponse à des protocoles spécifiques d'entraînement de force et d'endurance des VM, on pourrait penser que les mêmes principes d'entraînement des muscles squelettiques s'appliquent aux muscles respiratoires. Cependant, il apparaît que les adaptations des VM nécessitent des exercices de stimulation très intenses (et peut-être un type d'entraînement spécifique), étant donné que l'exercice d'endurance prolongé des animaux vivants ne semble pas avoir d'effet significatif ni même prévisible sur les aspects structuraux ou fonctionnels des VM. Dans cet article ont été révisés les résultats ambigus de plusieurs programmes d'exercice régulier d'endurance concernant les muscles respiratoires d'animaux. De même ont été reconsidérées les études d'entraînement à l'endurance à grande altitude visant à augmenter la force du stimulus nécessaire aux adaptations des VM, études qui ne révèlent pas davantage de changements fonctionnels des VM. Finalement, puisqu'il ne semble y avoir qu'un faible rapport entre puissance des VM et endurance, même lorsque l'altitude augmente, on se pose la question de savoir si les VM ont été conçus en premier lieu pour la "respiration". Nous proposons une autre hypothèse : que la ventilation respiratoire n'a pas été sélectionnée comme étant la tâche primordiale des VM et que ce sont au contraire les tâches non respiratoires, plus que la respiration en elle-même, qui exigent davantage des "muscles respiratoires".

Mots-clés : Entraînement d'endurance, Altitude, Plasticité musculaire.

SUMMARY: La Stayo et al.: Exercise training at high altitude: how do the respiratory muscles respond?

Like all vertebrate skeletal muscles, the ventilatory muscles (VM) of humans can adaptively improve in their functional capacities. Since these changes occur consistently in response to specific VM strenght and endurance training protocols, one would assume the same principles of skeletal muscle training can be applied to the VM. It is apparent, however, that VM adaptations require a very strong exercise stimulus (and perhaps a VM training specific stimulus) as chronic whole animal endurance exercise do not appear to have significant or even predictable effect effect on the structural or functional aspects of the VM. This manuscript reviews the equivocal results regarding animals' respiratory muscles to various programs of regular endurance exercise. It also reviews endurance training studies at high altitude, predicted to increase the strenght of the stimulus for VM adaptations, which again fails to elicit VM

functional changes. Finally, since there seems to be a poor relationship between VM power and endurance with increases in (even at altitude) the question is raised as to whether the evolutionary design of the VM is primarily for "breathing". We propose an alternative hypothesis that ventilation has not been selected as the primary task of the VM. Rather, non-breathing tasks put a larger demand on the "ventilatory muscles" than does ventilation per se.

Key words: Endurance training, Altitude, Muscle plasticity

INTRODUCTION

Like all vertebrate skeletal muscles, the ventilatory muscles (VM) of humans can adaptively improve in their functional capacities. These changes occur consistently in response to specific VM strength and endurance training protocols. There is less evidence, however, that functional or structural adaptations occur as a consequence of increased ventilatory demands concomitant with whole animal, i.e., chronic endurance training. Even when the magnitude of the challenge to the VM is increased dramatically, as with endurance training at altitude, there may be adequate VM functional capacity to meet this demand. Therefore, the question remains as to what type and magnitude of whole animal chronic endurance training would produce a phenotypic shift in the VM similar to that seen in skeletal muscle exposed to similar stressors. That is, since chronic endurance training combined with high altitude conditions is a strong stimulus for adaptive skeletal muscle changes (see papers in this issue), yet VM remains unchanged, what stressors are needed for VM to adaptively respond ?.

SKELETAL MUSCLE PLASTICITY

Virtually every structural aspect of muscle can change given the appropriate stimulus. In general, these transformations have been documented in a number of different muscles and species and include (but are not limited to) changes to architecture (1,2), fiber type (3-5), mitochondrial distribution, capillary density (6), etc.

Functional changes are causally linked to these structural changes (7). For instance, the capillary density and mitochondrial content increase in response to chronic whole body endurance training as does VO_2max (6). The adaptive response of skeletal muscle may be even greater in magnitude when training is performed at altitude (8,9). Studies using muscle biopsies have shown that endurance exercise performed at high altitude can affect muscle structure. Desplanches et al (10) compared 3 weeks (2 hr/day) of cycle ergometry exercise in subjects who first trained in severe hypoxia (5500 m) and then 14 months later trained in normoxia. The effect of high altitude significantly increased the average muscle fiber area, capillary to fiber ratio and total mitochondrial volume, but the same level of training at sea level produced no significant morphometric changes. Citrate synthase also has been shown to significantly increases in muscles trained in hypoxia (2300 m) as compared to muscles trained in a normoxic setting (11).

ARE THE VENTILATORY MUSCLES PLASTIC ?

If the VM share the same phenotypic plasticity as other skeletal muscle, chronic exposure to an exercise bout which results in significant muscle loading should be a powerful stimulus for the production of specific functional as well as structural adaptations to occur. Therefore, one might expect some structural and functional adaptations to the VM with endurance type exercise. Regarding VM, however, chronic whole animal endurance exercise (even in hypoxic settings) does not appear to have a significant or even predictable effect on the structural or functional aspects of the VM. Numerous investigators have examined the adaptive responses of animals' respiratory muscles to various programs of regular endurance exercise. however, the results are equivocal. Guinea pigs trained by endurance running for 6 weeks showed no significant effect on muscle mitochondrial content and capillarity in the diaphragm (12). Likewise, a number of earlier studies failed at demonstrating training effects on the diaphragm induced by whole body exercise (13-15). Several reports, however, demonstrate increases in the activity of marker enzymes of oxidative metabolism in the diaphragm of rats subjected to various types of endurance training (16-20). Powers et al. (16-19) and Uribe et al. (20) have reported increases in aerobic marker enzymes in the rat diaphragm following various intensities of chronic endurance training. Taken collectively, these animal studies demonstrate some functional phenotypic plasticity of the ventilatory muscles. It has been hypothesized, however, that exercise intensity must be very high indeed to induce structural adaptations in the diaphragm (16,19,20).

Some studies on humans suggest that VM endurance can be improved with swimming or running exercises (21,22). Following three

months of swim training, Clanton et al (22) observed a significant increase in inspiratory muscle endurance nearly equal to that observed in a group of swimmers who underwent the same swim training, but in addition performed inspiratory muscle training. Likewise, Robinson and Kjeldgaard (21) linked a 16% increase in ventilatory muscle endurance to a similar increase in running performance following training in a group of previously sedentary humans. Neither of these studies reported VO_2max before and after training. Moreover, swimmers' specialty (sprint versus endurance) were not reported making it difficult to judge the magnitude of the training effect. Therefore, swim- or run-training performed at sufficient intensities may improve the structural and functional capacity of the chest wall muscles. The possible explanation for an adaptive effect with human swimming, and in the quadruped/animal treadmill running regimes, is that the chest wall muscles (which includes to some degree the VM) are recruited for propulsion, not just ventilation. Although bipedal running does not recruit the chest wall musculature to the same degree, it is conceivable that substantial running efforts (as may be seen in previously sedentary subjects who are submitted to a novel running program) may involve ample upper extremity movement so as to stimulate the VM more than when the VM are used solely for ventilation.

It is apparent that some modes of endurance exercise can formidably stress the VM. Coast and Weise (23) reported a significant decrease in MIP at the mouth following a progressive load cycle ergometry test to exhaustion. In the same study, however, following the VO_2max test, a group of elite cross-country skiers (mean VO_2max = 71.8 ± 3.8 mlO_2 kgl min^{-1}) "protected" their MIP. During a competitive classical triathlon, Hill et al (24) observed a non-significant decline in maximum inspiratory pressure measured at the mouth following the swim leg and a significant decrease in MIP following the bike and run legs respectively. Loke et al. (25) also measured a 16 % decrease in MIP in four subjects after completing a marathon. From these studies it seems that the parameters setting MIP are malleable as a function of certain types of repetitive exercise stress, but the effects appear to be mode and intensity of exercise specific.

In contrast to whole-body endurance training, specific respiratory tasks that emphasize both VM strength and endurance unambiguously provide a powerful stimulus sufficient to induce adaptive changes. In the laboratory, specific VM training protocols have resulted in significant improvements in ventilatory capacity tasks (26-29). In a now classic study, Leith and Bradley (26) studied respiratory mechanics before and after a 5 week training program that included maximum static inspiratory and expiratory maneuvers and "ventilating to exhaustion" 30-45 minutes/day, 5 days /week. In applying accepted principles of skeletal muscle training to the VM (and seeing significant improvements in VM strength and endurance) it has been concluded that appropriate VM training programs can promote adaptive changes.

It is apparent that VM adaptations require a very strong exercise stimulus (and perhaps a VM training specific stimulus). The reason for this may be that the main VM muscle, the diaphragm, is extremely well equipped to perform continuous work and very specific high intensity exercises are needed to induce changes. The mitochondrial content and capillary supply of the diaphragm, in a considerable number of mammalian species analyzed, consistently surpasses that of locomotor muscles (diaphragm being second only to the heart with respect to these characteristics; (30,31). Some would suggest that VM structure is "overbuilt" for breathing tasks and does not contribute to limiting aerobic performance in chronic whole body endurance activities (32). That is, are the VM of animals built with a fundamental balance between structure and function and do they uphold the concept of optimal design, or symmorphosis (33).

THE CONTRIBUTION OF THE VENTILATORY MUSCLES TO AEROBIC PERFORMANCE AT ALTITUDE

Since aerobic performance and VO_2max at altitude are primarily constrained by availability of environmental oxygen (34,35) one would expect the stressors on the VM to be greater at altitude and hence provide an ideal stimulus for VM adaptive changes to occur. This hypothesis was tested in human subjects (at a moderately high altitude) by examining peak inspiratory flow and its contribution to oxygen demand (36). In this study peak inspiratory flow performance was compared between trained (Vo_2max> 65 ml O_2 kg-l min^{-1}) and untrained (VO_2max< 45 ml O_2 kg^{-1} min^{-1}) subjects, at a moderately high altitude (2100 m). Despite great differences in Vo2max and exercise habits and abilities between the two groups of subjects, their respective inspiratory muscle performances were indistinguishable (figure 1). Consequently, there was no evidence

that regular and high-intensity chronic whole body endurance training at moderate altitude elicits adaptations of either airways or inspiratory muscles. It is as interesting to note that when the data from the trained and untrained subjects are extrapolated, the inspiratory capacities of these subjects (even without any adaptation) could potentially support ventilation sufficient for a Vo2max of 85 ml O_2 kg^{-1} min^{-1}, which is the highest recorded value in humans (37). Therefore, the VM would seem to be "overbuilt" in most individuals, with only those of very rare elite endurance athletes conforming to the concept of ' symmorphosis.'

Obviously the VM stressors need to be very high for potential adaptations to occur with endurance exercise.

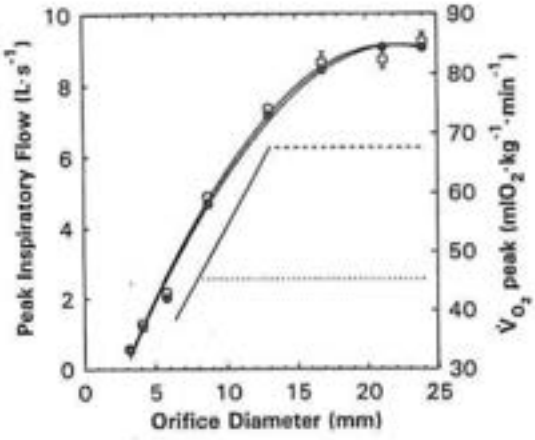

Figure 1. Highly trained cyclists, VO_2max = 68 ml O_2 kg^{-1} min^{-1} (filled symbols) and their sedentary controls, VO_2max = 45 ml O_2 kg^{-1} min^{-1} (open symbols) have nearly identical peak flows when breathing through inspiratory resistors. However, when the two groups of subjects are asked to cycle at workloads corresponding to their respective VO_2max both can do so while breathing through a resistor that caused a reduction in peak flow (trained: dashed line; sedentary, dotted line). Further, the sedentary subjects can maintain their Vo2max while breathing through much smaller orifices (#8mm) than can the trained subjects (≈13mm). In all subjects, peak Vos declined as a function of smaller orifice diameters, indicating peak VO_2 was ventilation limited. When this regression line is extrapolated, it predicts that the inspiratory resistor necessary to cause a decline in peak flow, would result in a decrease in Vo_2 only if VO_{2max} exceeded 85 ml O_2 kg^{-1} $.min^{-1}$, nearly equal to the highest usually reported in humans. (From 32. Used with permission from Respiratory Physiology).

Since VM adaptations did not occur with endurance exercise at moderate altitude, one way of increasing the VM demand greatly is to train at an even higher altitude, thereby providing a more powerful ventilatory challenge and critical test of VM functional plasticity in humans. Therefore, we measured VM power and endurance in a group of high altitude residents of La Paz, Bolivia (3600 m) prior to and immediately following an endurance training protocol designed to increase Vo_2max (see Favier et al., 1995b). Specifically, we tested the hypothesis that the static (isometric) and dynamic (miometric) properties of the human ventilatory muscles, as defined by the slope of the maximum inspiratory pressure-flow curve (MIPF) through graded resistors, the maximum sustainable ventilatory capacity (MSVC) and the maximum 12 seconds ventilation (MVV_{12}) will respond adaptively to six weeks of hypoxic endurance (cycle ergometry) training.

A group of 18 young men, residents of La Paz, Bolivia (3600m) were assigned to either an endurance training (ET) or control (C) group. The endurance training program consisted of cycling for 30 min/day, 5 day/wk, for 6 wk at an external power output initially set to elicit 70 % of their individual VO_2max. Our hypothesis predicted that endurance training at high altitude should provoke detectable functional adaptations of the ventilatory muscles. However, this study failed to demonstrate this result. Peak inspiratory pressures, flows, MVV_{12} and MSVC, were not different before vs. after nor comparing post-training values with those of control, untrained, subjects (figure 2).

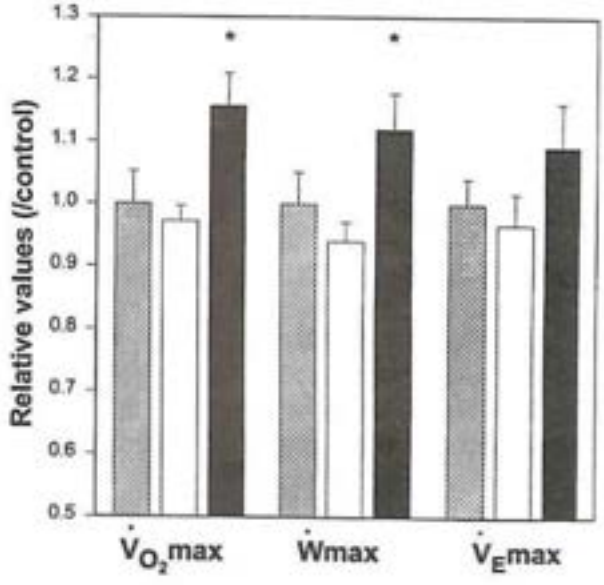

Figure 2. When healthy, but untrained, subjects trained on the cycle ergometer at 3,400 meters in

La Paz, Bolivia, they experienced a significant increase in VO_2max, and the maximum workload (Wmax) comparing before training (empty bars) with after training (filled bars), while maximum ventilation ($V_E$max) increased insignificantly. Untrained control subjects are shown in hatched bars.

Despite the fact that VO_2max did significantly increase, that increase was not accompanied by significant changes in either $V_E$max, nor in any of our measures of ventilatory muscle performance. Thus, these results suggest accepting the null hypothesis that VM power and endurance do not track increases in VO_2max in humans, even when exercise training is carried out in environmental hypobaric hypoxia. These results coincide with another study which assessed the effect endurance training at altitude on maximal inspiratory pressure (38). Like the results in the Bolivia study, VO_2max increased after training, but the maximal inspiratory pressure and inspiratory muscle fatigue did not. From these studies, which theoretically increased the stimulus for VM muscle adaptation via a hypoxic condition, we have to conclude that sufficient VM structural and functional capacity was present prior to the endurance training bout to accommodate increases in VO2max. In other words, this implies that the inspiratory muscles do not limit performance and there apparently is "excess ventilatory muscle architecture" in all but the most elite endurance athletes.

ARE THE VENTILATORY MUSCLES PRIMARILY FOR VENTILATION?: AN ALTERNATIVE HYPOTHESIS

There seems to be a poor relationship between VM power and endurance with VO_{2max} suggesting that the evolutionary design constraints for these muscles may not be the breathing task (40). As an alternative hypothesis, we have proposed that ventilation, even maximum sustainable ventilation, has not been selected as the primary task of the VM. If non-breathing tasks (e.g. trunk tasks such as lifting, coughing, yawning, sneezing, or posture) put a larger demand on (i.e., require greater recruitment of) the 'ventilatory muscles' than does ventilation (even during hyperpnea of exercise in hypoxia), these tasks should promote measurable changes in VM capacity.

To test this alternative hypothesis, that the VM respond primarily to non-respiratory tasks such as upper body exertion, we measured inspiratory power and maximum ventilation in 6 healthy young males prior to and immediately following a 6 week training program (consisting of working with free weight and calisthenics) (41). Resistance exercises were designed to work on the trunk muscles without increasing ventilation. Four subjects did no training and functioned as controls. Subjects in the trained group experienced an increase in VM inspiratory power, as determined by pressure-flow curves, and the VM capacity both acutely (MVV12, 12% increase, p=0.0014) as well as MSMV (12% increase, p=0.0023) (figure 3). These preliminary results suggest that the ventilatory muscles are very responsive to non-respiratory tasks, more so than to the normal ventilatory demands of chronic whole animal endurance exercise.

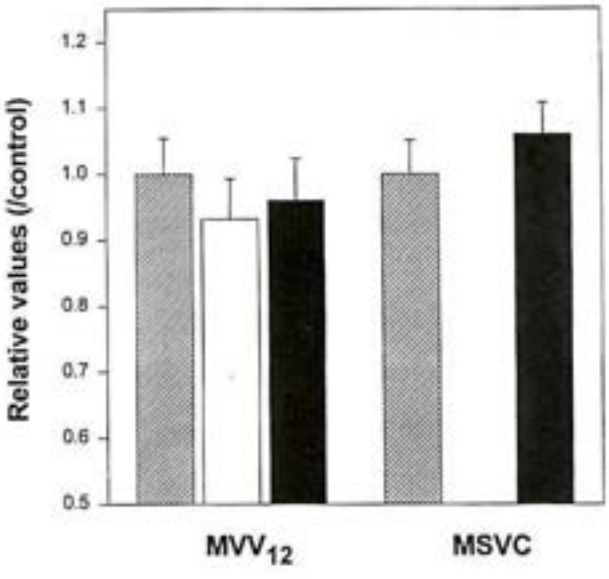

Figure 3. Despite significant increases in VO_2max and work output, training for 6 weeks at 3,400 meters had no significant effect on maximum voluntary ventilation in 12s (MVV_{12}) nor on the maximum sustained ventilatory capacity (MSVC). (These data are from 36).

The fact that ventilatory parameters do not predictably change with chronic, whole animal endurance training (even in severe hypoxic settings) may be evidence that this type of training is quite variable. As well, the stressors to the VM are not consistently adequate enough to promote VM changes with endurance training. This, coupled with an apparent excess of VM structure, suggests specific, high-intensity VM stressors are needed to produce a phenotypic shift in the VM. Certainly, VM specific exercises (like inspiratory and expiratory maneuvers and ventilating to exhaustion over a 5 week training period) provide ample stimulation, but perhaps non-respiratory tasks (such as upper body resistance exercises) can also be considered when attempting to

adaptively improve the functional capacity of the VM. Acknowledgments: This work was supported by NSF IBN 17527 and NIH - MBRS GM 821510 to SLL. We are grateful for the collaborative support and lucid insights of David Leith and for continued support from Hanz Hoppeler.

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PERMANENT AND INTERMITTENT HYPOXIA AS RESPONSE MODIFIERS OF SKELETAL MUSCLE TISSUE WITH EXERCISE TRAINING

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RESUMEN: Hipoxia Permanente e Intermitente Como Modificadores de Tejido Muscular Esquelético En Ejercicio de Entrenamiento

Es una creencia todavía ampliamente sostenida que el músculo esquelético de animales y humanos expuestos a hipoxia crónica tiene una capacidad oxidativa y una capilaridad incrementadas. Sin embargo, el análisis de biopsias de humanos tanto antes como luego de ascensos reales o simulados a los Himalayas, ha demostrado consistentemente una disminución en la capacidad oxidativa y una capilaridad sin cambios. Más aun, en biopsias de vastus lateralis de indígenas de altura de Los Himalayas y Los Andes, el contenido mitocondrial estaba marcadamente reducido en comparación con el de nativos de nivel del mar emparejados por edad y estado de entrenamiento. En conjunto, estos estudios indican que la exposición permanente a hipoxia severa (producida por vivir a alturas por encima de 3500 m) producen una disminución en la capacidad oxidativa así como en el rendimiento aeróbico. Si se produce hipoxia severa solamente durante las sesiones de entrenamiento, entonces las mejoras resultantes son comparables a las producidas por entrenamiento por un período similar bajo condiciones normóxicas. Adicionalmente, se observa que el volumen del músculo esquelético y la concentración de mioglobina aumentan con el entrenamiento en hipoxia pero no en normoxia. Teniendo en cuenta estos hallazgos, los atletas deberían limitar su exposición hipóxica al período mínimo compatible con la inducción de una respuesta eritropoyética. Si se desea una ganancia en la masa muscular oxidativa y en la concentración de mioglobina, deberían lograrse por sesiones de entrenamiento bajo condiciones de hipoxia severa.. Los mecanismos celulares responsables de la respuesta del tejio muscular esquelético a la hipoxia no se conocen actualmente.

Palabras claves: Altitud, Humano, Ejercicio de entrenamiento, Estructura muscular, Capilar

Hypoxie permanente et intermittente en tant que modificateurs de la réponse du tissu musculaire squelettique aux exercices d'entraînement.

Selon une croyance encore très répandue, le tissu musculaire squelettique des animaux et des humains exposés à l'hypoxie chronique aurait une capacité oxydative renforcée et une capillarité accrue. L'analyse des biopsies faites sur des sujets après des ascensions réelles ou simulées dans les montagnes de l'Himalaya a démontré de manière constante une diminution de la capacité oxydative musculaire et une capillarité inchangée. En outre, des biopsies du *vastus lateralis* provenant de populations natives de l'Himalaya et des Andes ont révélé que le contenu mitochondrial était nettement diminué comparé à celui des natifs du niveau de la mer, appariés en fonction de l'âge et du degré d'entraînement. Ces études indiquent que dans l'ensemble l'exposition permanente à l'hypoxie sévère (due au fait de vivre à plus de 3500 m d'altitude) entraîne une diminution de la capacité oxydative musculaire ainsi que du rendement aérobique du travail. Si l'hypoxie sévère ne se produit qu'au cours de séances limitées d'exercices d'endurance, les améliorations obtenues sont comparables à celles résultant d'un entraînement de même durée dans des conditions normoxiques. On observe en outre une augmentation du volume du muscle squelettique et de la concentration de myoglobine avec l'entraînement en hypoxie, mais pas en normoxie. Compte tenu de ces découvertes, les athlètes d'endurance devraient limiter leur exposition à l'hypoxie à la durée minimum nécessaire à l'induction d'une réponse de l'érythropoïétine. La recherche d'un gain de la masse musculaire oxydative et de la concentration de myoglobine devrait se faire au cours de séances d'entraînement se déroulant dans des conditions hypoxiques sévères. Les mécanismes cellulaires responsables de la réponse du tissu musculaire squelettique à l'hypoxie ne sont pas connus actuellement.

Mots-clés : Altitude, Humain, Exercice, Entraînement, Structure, Muscle, Mitochondries, Capillarité, VO_{2max}, Hypoxie.

ABSTRACT It is still a widely held belief that the skeletal muscles of animals and humans exposed to chronic hypoxia have an enhanced oxidative capacity and increased capillarity. However, analysis of biopsies from subjects both prior to and after real or simulated ascents to the Himalayas have consistently shown a decrease in muscle oxidative capacity and an unchanged capillarity. Furthermore, in vastus lateralis biopsies derived from highland populations indigenous to the Himalayas and Andes, the mitochondrial content was markedly reduced in comparison with that of lowlanders matched for age and training status. Combined, these studies indicate that permanent exposure to severe hypoxia (incurred by living at altitudes above 3500m) elicits a decrease in muscle oxidative capacity as well as in the aerobic work performance. If severe hypoxia is incurred only during the constrained limits of endurance training sessions, then the resulting improvements are comparable to those elicited by training for a similar period under normoxic conditions. Additionally, it is observed that skeletal muscle volume and myoglobin concentration increase with training in hypoxia but not in normoxia. Bearing these findings in mind, endurance athletes should limit their hypoxia exposure to the minimum period commensurate with induction of an erythropoietin response. If a gain in oxidative muscle mass and myoglobin concentration are desired, then they could be achieved by training sessions conducted under severe hypoxic conditons. The cellular mechanisms responsible for the response of skeletal muscle tissue to hypoxia are currently not known.

Key words: High altitude, Human, Exercise training, Muscle, Structure, Mitochondria, Capillary, VO_2 max, Hypoxia

Background

Since the late fifties and early sixties, there has been seemingly incontrovertible experimental evidence that continuous exposure to a hypoxic environment, such as is incurred by living at high altitude, leads to characteristic modifications in skeletal muscle tissue. Valdivia (1958) demonstrated a significantly increased capillarization of skeletal muscles harvested from guinea pigs native to high altitudes as compared to those derived from sea-level controls. Reynafarje (1962) found higher myoglobin and cytochrome reductase concentrations in sartorius muscle biopsies obtained from subjects living permanently at 4400m above sea level (in the mining city of Cerro de Pasco) than in controls living at sea-level (Lima). These milestone findings had a profound influence on people's ideas concerning the effect of local hypoxia on muscle structure and function. And when, a decade later, it was demonstrated that continuous endurance exercise training leads to an increase in muscle oxidative capacity (Holloszy et al 1970; Hoppeler et al. 1973), as well as increasing its muscle capillarity (Andersen 1975), these changes were accordingly attributed to (a putative) local tissue hypoxia experienced during training sessions. In 1983, Hochachka condensed what was then the generally accepted view of the mechanisms responsible for high altitude acclimatization into what he called an "interpretative hypothesis". He proposed that an organism living at high altitude was faced with the problem of "maintaining an acceptably high scope for aerobic metabolism in the face of the reduced oxygen availability of the atmosphere". This was envisaged as being achieved (a) by increasing the capacity of oxygen transfer to the tissues and (b) by augmenting the capacity for oxidative metabolism at the periphery by an increase in mitochondrial enzyme activity. By these means, the increase in capillary density, decrease in diffusion distance, increased capacity for facilitated diffusion and enhanced oxidative capacity of muscles could all be explained within a coherent conceptual framework. This interpretation of the "classical" high-altitude results also suggested that exposure to hypoxia, such as that experienced in high-altitude training camps, was beneficial for the capacity of muscle tissue to transfer and utilize oxygen. However, an ever-increasing body of experimental evidence now throws serious doubt upon the tenability of Hochachka's hypothesis, at least as far as humans are concerned.

Lowlanders exposed to continuous severe hypoxia

During the late eighties and early nineties, a number of studies became available reporting results of skeletal muscle changes observed as a consequence of prolonged exposure to severe hypoxia at altitudes above 5000m for several weeks, as a consequence of experiments conducted during simulated (Green et al.1989) or real (Hoppeler et al.1990; Howald et al.1990) attempts to climb Mount Everest. The general consensus among these studies is that muscle tissue oxidative capacity is reduced (by about 20-30%), rather than increased after high-altitude exposure. Such a change is, moreover, accompanied by an approximately 10% decrease of muscle cross-sectional area and a concomitant reduction in fiber size of similar magnitude (see Hoppeler et al.1990). Consequently, subjects returning from an Everest expedition have a smaller muscle mass with a decreased oxidative capacity. This massive reduction in total peripheral oxidative capacity is believed to be partially responsible for the reduction in VO_2 max registered after high-altitude exposure (Hoppeler et al.1990). It could also be demonstrated that the increase in capillary density (i.e. the number of capillaries per mm^2 of muscle fiber) observed after high-altitude exposure is not a consequence of capillary neoformation but rather of the reduced muscle fiber size, the absolute extent of the capillary network remaining essentially unchanged. Nonetheless, the decrease in fiber size combined with the increased myoglobin concentration (Reynafarje 1962; Terrados et al.1990) will most likely improve the oxygen transfer conditions from erythrocytes to muscle mitochondria. Since high-altitude training camps are usually situated at a moderate altitude (between 1800 and 2500m), rather than at the extreme altitudes necessary for successful attempts of climbing the highest peaks, one would not expect the "negative" muscle changes incurred by athletes training at these altitudes to be as dramatic as those observed in "extreme" climbers; but that high-altitude training is "good" for your muscles can no longer be regarded as a valid tenet.

Permanent high-altitude residents

As Hochachka's "interpretative hypothesis" was based primarily on data obtained from highaltitude-adapted animals, such as llamas, we wished to further explore the effects of permanent high-altitude residency on human muscle structure. One set of studies was carried out on Tibetans, inhabitants of the Tibetan highland for many generations, (Kayser et al. 1991), and another on permanent high-altitude residents of multi-ethnic background in La Paz, Bolivia

(3600m). In both populations, muscle tissue oxidative capacity, measured by the volume density of mitochondria as well as by the activities of oxidative enzymes, was significantly lower (approximately 20%) than that in lowlanders matched for age, socioeconomic status and training background; both high-altitude populations also had a lower capillary-to-fiber ratio. Interestingly, Tibetans tended to have a reduced fiber size, which was not observed in Bolivians, and their capillary density was consequently higher. Recent data pertaining to lowland-born Tibetans (Kayser et al. 1996) strongly suggest that these structural modifications are inherited. Taken together, the data on high-altitude populations indicate that permanent, severe hypoxia leads to a significant reduction in the aerobic work capacity of muscle tissue. Athletes residing at these altitudes will thus be at a considerable disadvantage when competing under sea-level conditions. It is interesting to note that inhabitants of the Ethiopian highland (1800-2800m) appear to perform exceedingly well in long-term endurance events. This population has, to my knowledge, not been extensively studied, and it therefore remains open to speculation whether the Ethiopians can profit from the somewhat lower elevation of their natural environment or whether they have a better genetic predisposition. This apparent paradox certainly merits further investigation.

Training in severe normobaric hypoxia

In order to put Hochachka's "interpretative hypothesis" further to the test, we reasoned that whilst permanent hypoxia was obviously detrimental to anabolic events in muscle, acute exposure might in fact serve as an excellent stimulus for improving its aerobic work capacity. It is, indeed, widely recognized that a decrease in ambient oxygen partial pressure has a negative influence on cultured cell growth. We therefore devised an experimental protocol in which subjects were exposed to severe normobaric hypoxia (equivalent to an altitude above 4000m) by decreasing oxygen concentration in the inhaled air during training sessions. Our results indicate that when an endurance training regime of comparable intensity is carried out under normoxic and severe (intermittent) hypoxic conditions, similar, but not identical effects are elicited in skeletal muscle structure. Subjects trained in both environments had similar increases in capillary supply and mitochondrial volume density, but those exposed to hypoxia had additionally a 10% increase in muscle and fiber cross-sectional area. It may be worth mentioning here that such a response is not achieved by endurance training in previously untrained males (Hoppeler et al. 1985), and that the increase in muscle crosssectional area is more than half of that achieved after a similar number of strength-training sessions (Luthi et al. 1986). Since such a gain in muscle cross-sectional area after endurance training has a potential significance for athleticism, we have since repeated this study using a larger number of subjects, and the data thereby gleaned serve only to corroborate the findings delineated above (Geiser and Hoppeler, unpublished results).

Conclusions for athletic training

Data pertaining to the changes in muscle structure elicited by exposure to high-altitudes need to be considered in the light of other relevant performance-determining physiological variables notably modifications in the hormonal status. Additionally, one has to consider whether the competition, an athlete trains for, is held at high- or low-altitude, and finally, one has to keep track of "confounding variables", such as heat tolerance, high-altitude sleep disorders etc. when using altitude or hypoxia as an ergogenic aid.

Competition at high altitudes

Most exercise scientists would agree that it is usually a good strategy for athletes to train at the altitude at which the competition is to be held, acclimatization over a period of 3 weeks being sufficient. If for financial or logistic reasons, an adequate altitude-training for the necessary duration is not practicable, then it can be replaced by training sessions under hypoxic conditions. We used this method to advantage in preparing the Swiss Mountainbike team for the World Championships at Vail in 1994. Competitors were asked to undergo three strenuous training sessions per week under hypoxic conditions (30min, approx. 80% of VO_2 max) during the 3 week period prior to the race, with their race bikes mounted on rollers. This afforded participants with the opportunity of becoming used to working hard under hypoxic (race-) conditions. Although this experiment was not supervised closely from a scientific point of view, we nonetheless gained the impression that the reported benefit considered in the light of the excellent results achieved, was attributable to an enhanced facility for adjusting respiratory and leg muscle work to hypoxic conditions.

Competition at sea level

It is still a matter of dispute whether high-altitude training is of benefit for a competition taking place at sea level. Recent data by Levine and Stray-Gundersen (manuscript) suggest that the best strategy for an athlete relying heavily on his aerobic work capacity during competition (i.e.

activity exceeding a 2 minutes duration) is to live at moderate altitude (approximately 2000m) and train at low altitudes. The sojourn at moderate altitude permits an augmentation of VO_2 max by increasing the mass of circulating erythrocytes; the training at low altitude enables the athlete to sustain high levels of training intensity, which is of technical advantage and results in an increased VO_2 at the maximal possible steady state.

As an alternative to living at high altitude, athletes can, of course, also sleep in a house with a reduced oxygen atmosphere at sea level (a so-called "Finnish House"). All these training paradigms must necessarily take into account the potentially negative effects related to highaltitude sojourn, such as sleep disorders or different temperature and humidity conditions (discussed by Roach in this issue). One might additionally consider the use of severe hypoxia during training sessions as a means of increasing the physiological effects of the exercise stimulus; this should allow a gain in muscle mass during aerobic training. The potential benefits of utilizing hypoxia in this way during preparatory off-season training would appear to be patent. And yet, few teams, if any, employ hypoxia as an ergogenic aid. In the context of its assets, it would be interesting to ascertain whether acute hypoxia applied during strength-training sessions would induce an augmented skeletal muscle response to high intensity loads.

Acknowledgments

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ACUTE HIGH ALTITUDE DISEASES

T.H. RAVENHILL, MOUNTAIN SICKNESS, AND PERUVIAN CONTRIBUTIONS TO HIGH-ALTITUDE PULMONARY EDEMA

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RESUMEN: T.H. Ravenhill, Mal de Montaña y las Contribuciones Peruanas al Edema Pulmonar de Altura

Thomas Holmes Ravenhill (1881-1952) fue una figura clave en la historia del mal de montaña pero la importancia de sus contribuciones sólo se ha apreciado recientemente. El fue quien brindó las primeras descripciones convincentes del edema pulmonar de altura y del edema cerebral de altura, y su clasificación del mal de montaña es la que utilizamos actualmente. Sin embargo, luego de su publicación en 1913, su artículo fue prácticamente olvidado durante 50 años. Mientras tanto, el edema pulmonar de altura fue independientemente descrito con exactitud y detalle en el Perú, pero desafortunadamente la información no fue conocida fuera de América del Sur. Fue recién a partir del viaje de Herbert N. Hultgren, M.D. a La Oroya en 1953 que el mundo de habla no hispana tomó conocimiento de estos importantes avances.

Palabras claves: Mal de montaña; Historia; Edema pulmonar de altura

RÉSUMÉ: T.H. Ravenhill, Mal des Montagnes et contributions péruviennes à l'oedème pulmonaire de haute altitude.

Thomas Holmes Ravenhill (1881-1952) est une figure clé de l'histoire du mal des montagnes, mais l'importance de ses contributions n'a été reconnue que récemment. C'est lui qui fit les premières descriptions réellement convaincantes de l'oedème pulmonaire et de l'oedème cérébral de haute altitude et sa classification du mal des montagnes est celle qui est utilisée de nos jours. Cependant, après sa publication en 1913, son article fut pratiquement oublié pendant 50 ans. Pendant ce temps, et indépendamment, une description exacte et détaillée de l'oedème pulmonaire de haute altitude fut faite au Pérou. Malheureusement l'information ne fut pas divulguée hors d'Amérique du Sud. Ce n'est qu'à partir du voyage d'Herbert N. Hultgren, M.D. à La Oroya en 1959 que l'avancement important des connaissances dans ce domaine parvint au monde non hispanophone.

Mots-clés : Mal des Montagnes, Histoire, Oedème pulmonaire de haute altitude.

SUMMARY: Thomas Holmes Ravenhill (1881-1952) was a key figure in the history of mountain sickness but the importance of his contributions has only recently been appreciated. He gave the first convincing clinical descriptions of high-altitude pulmonary edema and high-altitude cerebral edema, and his classification of mountain sickness is the one we use today. However after its publication in 1913, his paper dropped out of sight and was essentially forgotten for 50 years. In the meantime, high-altitude pulmonary edema was independently described with accuracy and detail in Peru but unfortunately the information was not known outside South America. It was not until Herbert N. Hultgren, M.D. visited Oroya in 1959 that the non-Spanish speaking world were made aware of these important advances.

Key Words : Mountain sicness, History, High altitude pulmonary edema

THOMAS HOLMES RAVENHILL (1881-1952)

T.H. Ravenhill was a key figure in the history of mountain sickness but the significance of his contributions has only recently been fully appreciated. He was born in Birmingham, England where his father was a surgeon and obtained his medical degree at the University of Birmingham in 1905 (1). In 1909 he went to the Poderosa and Collahuasi mines in north Chile for two years as medical officer or "Surgeon" as the position was then called. These mines are in a remote part of north Chile close to the Bolivian border and Ravenhill gave their altitudes as 15,400-16,200 ft (4690-4940 m) though these were slight

overestimates. It is not clear what prompted Ravenhill to go to the mines but there were very strong links between Britain and the Chilean mining industry at that time. Furthermore, the mines were growing fast because the railway link between Ollague (on the main Antofagasta-La Paz line) and Collahuasi had just been completed.

As a results of his period there, Ravenhill wrote a landmark paper "Some Experiences of Mountain Sickness in the Andes" (2). The paper contains the classification of mountain sickness that we still use, and also vivid, accurate descriptions of what we now know as acute mountain sickness, high-altitude pulmonary edema, and high-altitude cerebral edema.

Ravenhill used the term "puna" for mountain sickness because that was the local word. His classification of mountain sickness was as follows:

A) Acute mountain sickness (puna of a normal type)

B) Two divergent types of the disease

1. High-altitude pulmonary edema (puna of a cardiac type)
2. High-altitude cerebral edema (puna of a nervous type)

This is the classification that we use today. The term "divergent" is a particularly happy one because it is difficult to think of a better term. It is somewhat misleading to refer to highaltitude pulmonary edema and high-altitude cerebral edema as complications of acute mountain sickness because they occasionally occur in its absence.

Acute mountain sickness. Ravenhill's clinical description of acute mountain sickness is accurate and vivid. Here is part of it.

> It is a curious fact that the symptoms of puna do not usually evince themselves at once. The majority of newcomers have expressed themselves as being quite well on first arrival. As a rule, towards the evening the patient begins to feel rather slack and disinclined for exertion. He goes to bed, but has a restless and troubled night, and wakes up next morning with a severe frontal headache. There may be vomiting, frequently there is a sense of oppression in the chest, but there is rarely any respiratory distress or alteration in the normal rate of breathing so long as the patient is at rest. The patient may feel slightly giddy on rising from bed, and any attempt at exertion increases the headache, which is nearly always confined to the frontal region.

Of course there have been many descriptions of acute mountain sickness before Ravenhill's but very few of them ring as true as his.

High-altitude pulmonary edema. Ravenhill describes three cases of "puna of a cardiac type" and here is part of the description of the first case.

He seemed in good health on arrival, and said that he felt quite well, but nevertheless he kept quiet, ate sparingly, and went to bed early. He woke next morning feeling ill, with symptoms of the normal type of puna.

As the day drew on he began to feel very ill indeed. In the afternoon his pulse rate was 144, respirations 40. Later in the evening he became very cyanosed, had acute dyspnea, and evident air hunger, all the extraordinary muscles of respiration being called into play. The heart sounds were very faint, the pulse irregular and of small tension. He seemed to present a typical picture of a failing heart. This condition persisted during the night; he coughed up with difficulty. He vomited at intervals. This condition persisted during the night; he had several inhalations of oxygen; strychnine and digitalis also were given. Towards morning he recovered slightly, and as there was luckily a train going down to Antofagasta in the early morning, he was sent straight down.

I heard that when he got down to 12,000 ft. [3660 m] he was considerably better, and at 7,000 ft. [2130 m] he was nearly well. It seemed to me that he would have died had he stayed in the altitudes for another day.

The third case described by Ravenhill was interesting because it was of a young man, aged 23, who had lived in the mining areas for some months but had descended to sea level for some weeks and then developed puna of the cardiac type after returning to high altitude. This "re-ascent" high altitude pulmonary edema has been described many times since.

One of the remarkable features of Ravenhill's paper was how it disappeared from sight and was essentially forgotten before it was discovered some 50 years later. It was not until 1964 that William H. Hall, M.D. was doing an extensive literature search and brought the paper to general notice. In the meantime, however, high-altitude pulmonary edema was described independently in Peru but again these descriptions were unknown outside South America until 1960, just before Ravenhill's description was rediscovered.

Peruvian Contributions to High-Altitude Pulmonary Edema

One of the first articles in the Peruvian literature to refer to pulmonary edema at high altitude was-that by Alberto Hurtado in 1937. This was a small booklet (*Aspectos Fisiólogicos y Patológicos de la Vida en la Altura*) (3) which now is extremely difficult to find outside Peru. On page 29 he states (in English translation):

> There is undoubtedly a type of Soroche [mountain sickness] which is quite rare and infrequent, and is characterized by the presence of intense congestion and edema of the lung. Possibly in these cases there is a prior cardiac condition of myocardial disease, and the acute and severe episode on arrival at altitude can be best classified as a state of circulatory insufficiency rather than a true high altitude sickness.

Hurtado then went on to describe a 48 year old man who was a native of Junin and of indigent race, and who had resided at altitude for the last 29 years. He went down to Lima for three days but on returning to Oroya by train he became very dyspneic, had a severe headache and some mental confusion. Examination showed orthopnea, intense cyanosis, and there were crepitations at the bases of the lungs. The sputum contained blood. The patient descended to Lima and improved slowly. When he returned to high altitude after two months there were signs of circulatory insufficiency including dyspnea, congestion of the lung bases, and edema of the extremities. Thus this patient is not typical of high-altitude pulmonary edema because the continuation of signs of circulatory insufficiency suggest that there was underlying cardiac disease.

However a few years later a number of clinical descriptions in Peru leave no doubt that high-altitude pulmonary edema was clearly recognized there. The first descriptions came from the Chulec General Hospital in Oroya (3750 m) where Leoncio Lizárraga Morla (4) described 7 cases of high-altitude pulmonary edema seen between July 1951 and August 1952. Previously Einar A. Lundberg, Chief of Medicine at Chulec General Hospital, had presented 6 cases to the Asociación Médica de Yauli but these cases were not published.

Lizárraga described his cases in detail with summaries of their clinical history, physical examination, hematology, chest radiography, electrocardiography, treatment and outcome. He described the condition both in individuals making their first ascent to high altitude, and also in people who had been at high altitude for some time, had descended to sea level for a few days, and then returned to high altitude. The patients typically had severe dyspnea and rales in their lungs. The chest radiograph showed increased shadowing but the heart size was normal. The electrocardiogram showed right axis deviation and sometimes P waves were prominent suggesting cor pulmonale. Most cases responded well to rest, though oxygen and digitalis were sometimes administered.

Following Lizárraga's classical paper, Arturo Bardález Vega (5-7) described 12 additional cases in Morococha (4500 m) which he saw between August 1953 and November 1955. Again the descriptions were detailed along with the clinical history, physical examination, chest radiology, hematology, electrocardiography, and outcome. Bardález stated that a frequent complication was bronchitis or bronchopneumonia and he recommended the administration of penicillin or some other chemotherapeutic agent.

It is remarkable that the detailed studies by Lizárraga and Bardález were essentially unknown outside South America. The only report in the English-speaking literature was a brief "Foreign Letter" *in the Journal of the America Medical Association* (8). However this was generally overlooked, partly because of its brevity and partly because the readers were not prepared for this new disease.

Introduction of the Peruvian Studies to North America

In February and March of 1959 Herbert Nils Hultgren (1917-), a cardiologist at Stanford University School of Medicine and a mountain climber, visited Chulec General Hospital and was astonished to find that high-altitude pulmonary edema was a well-known condition which had been accurately described on many occasions. When he returned to California he wrote an account with Dr. Warren Spickard under the title "Medical Experiences in Peru" which was published in the *Stanford Medical Bulletin* in March 1960 (9). After some general remarks about Peru, and Lima in particular, they stated, "During the two weeks spent at the Chulec General Hospital, the authors had the opportunity to review the clinical records of 41 patients who had experienced acute pulmonary edema shortly after their arrival on the hill." The clinical features were described and were much as reported by Lizárraga (4) and Bardález (5-7). Hultgren and Spickard pointed out that the mechanism of the disease was unknown. However the normal cardiac size and absence of murmurs or gallop sounds suggested that left ventricular failure was absent. In addition there was evidence of physiological pulmonary hypertension and right

ventricular hypertrophy. This partly came from electrocardiographic studies refereed to above, but they also cited the work of Rotta *et al.* (10) who had recently demonstrated pulmonary hypertension in 4 native residents of Morococha.

In retrospect this important paper by Hultgren and Spickard did not make the impact that might have been expected. Partly this was because the *Stanford Medical Bulletin* is not widely read, and also the authors implied that a fuller account of the patients with high-altitude pulmonary edema would be published later. This is regrettable because this classical paper was the first full account of high-altitude pulmonary edema published in English. Not only did the authors review a large series of cases and clearly report the salient clinical and investigative findings, but they also gave useful insights into possible mechanisms of the disease.

In the event, the most influential publication in the rediscovery of high-altitude pulmonary edema in the English-speaking literature was by Charles Snead Houston (1913-). In January 1959 he saw a 21 year old college student who had begun a cross-country ski trip from Aspen, Colorado about 4 days before and had become so ill with severe dyspnea, weakness and cough that he had to be evacuated to the nearest hospital. On examination there was cyanosis, marked orthopnea and dyspnea, and both lungs were filled with coarse to medium rales. A chest radiograph showed mottled infiltration especially on the right side. The diagnosis was initially unclear and the case was first reported as "Pneumonia or Heart Failure?" (11). However later the diagnosis was changed and the case was written up for the *New England Journal of Medicine* under the title "Acute Pulmonary Edema of High Altitude" and published in the September 8, 1960 issue (12). The pathogenesis was uncertain and the conclusion was that, "The condition is attributed to the combined stresses of cold, exertion and the anoxia occurring at 12,000 feet." The Peruvian studies were not cited although the text included the statement, "More recently, Hultgren examined the records of a large number of patients with acute pulmonary congestion believed to be due to acute exposure to high altitude." However the *New England Journal of Medicine* is widely read throughout the world and Houston's article brought the condition of high-altitude pulmonary edema into great prominence.

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WATER BALANCE AND ACUTE MOUNTAIN SICKNESS BEFORE AND AFTER ARRIVAL AT HIGH ALTITUDE:4,350 M

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RESUMEN: Balance Hídrico y Mal de Montaña Agudo Antes y Después de la Llegada a Alturas de 4.350 m

Este estudio es el primero que intenta medir el balance hídrico y sus componentes en altura, usando agua marcada y dilución de bromuro, y relacionando los resultados con mal de montaña agudo (AMS). Se midió ingesta de agua, excreción total de agua y excreción de agua en heces y orina en un intervalo de tiempo de 4 días, antes y luego de un período de de 4 días de permanencia a 4.350 m. Se midió el agua corporal total y el agua extracelular al inicio y al final de ambos intervalos. Hubo una relación estrecha entre la ingesta energética y la ingesta de agua y la relación no cambió con la permanencia en la altura. Los sujetos que desarrollaron AMS redujeron consecuentemente su ingesta energética y su ingesta de agua. El incremento en el agua corporal total (TBW) en sujetos afectados por AMS se acompañó de una reducción en la pérdida total de agua. Ellos no mostraron un débito urinario incrementado compensador de la pérdida evaporativa reducida de agua que se describe en altura. Los sujetos mostraron un incremento significativo de TBW luego de 4 días en altura. Los afectados con AMS mostraron las variaciones más grandes en agua extracelular (ECW) en relación al TBW. En conclusión la retención de líquido en relación al AMS es independiente de un cambio en los requerimientos hídricos debidos a la exposición a la altura. Los sujetos que desarrollaron AMS fueron los que mostraron un pasaje de líquido de por lo menos 1 l del compartimiento intracelular al extracelular o del extracelular al intracelular.

Palabras claves: Ingesta hídrica, Pérdida hídrica, Balance hídrico, Agua corporal total, Agua extracelular.

La présente étude est la première tentative de mesure du bilan hydrique et de ses composants en altitude au moyen d'eau marquée et d'une dilution de bromure, et d'une mise en relation des résultats avec le mal des montagnes aigu (AMS). L'ingestion d'eau, l'élimination totale d'eau et l'élimination d'eau par l'urine et les selles ont été mesurées dans un intervalle de temps de 4 jours, immédiatement avant et après une période de 4 jours de séjour à 4 350 m. L'eau corporelle totale et l'eau extracellulaire ont été mesurées au début et à la fin des deux périodes. Une étroite relation entre l'ingestion énergétique et l'ingestion d'eau a été observée, sans changement après passage en altitude. Les sujets affectés par l'AMS réduisirent en conséquence leur ingestion énergétique et hydrique. L'augmentation du poids corporel total (TBW) chez les sujets affectés s'est accompagnée d'une réduction de la perte totale d'eau. Une augmentation de l'excrétion urinaire compensant une perte par évaporation réduite du fait de l'altitude n'a pas été observée. Ce sont les sujets atteints d'AMS qui ont montré les plus grandes variations de l'eau extracellulaire (ECW) en rapport avec le TBW. En conclusion, la rétention de liquides liée à l'AMS est indépendante d'un changement des besoins hydriques dû à l'altitude. Chez les sujets affectés par l'AMS on a constaté le passage d'au moins 1 litre d'eau du compartiment intracellulaire à l'extracellulaire, ou de l'extracellulaire à l'intracellulaire.

Mots-clés : Ingestion hydrique, Perte hydrique, Bilan hydrique, Eau corporelle totale, Eau extracellulaire.

ABSTRACT

The present study is a first attempt to measure water balance and its components at altitude, using labeled water and bromide dilution, and relating the results with acute mountain sickness (AMS). Water intake, total water output and water output in urine and feces were measured over a 4-day interval before and a subsequent 4-day interval after transport to 4,350 m. Total body water and extracellular water were measured at the start and at the end of the two intervals. There was a close relationship between energy intake and water intake and the relation was unchanged by the altitude intervention. Subjects developing AMS reduced energy intake and water intake correspondingly. The increase in TBW in subjects developing AMS was accompanied by a reduction in total water loss. They did not show the increased urine output, compensating for the reduced evaporative water loss at altitude. Subjects showed a significant increase in TBW after 4 days at altitude. Subjects with AMS showed the biggest shifts in ECW relative to TBW. In conclusion, fluid retention in relation to AMS is independent of a change in water requirements due to altitude exposure. Subjects developing AMS were those showing a fluid shift of at least 1 l from the intracellular to the extracellular compartment or from the extracellular to the intracellular compartment.

Index terms: Water Intake, Water loss, Water balance, Total body water, Extracelular water

INTRODUCTION

One of the problems of high altitude is the maintenance of water balance. Water availability is low when the only water source is by melting snow. Theoretically water requirement is increased due to increased insensible water loss at low ambient water vapor pressure (Ferrus et al 1984). Consolazio et al (1968) measured consistently negative fluid balances during 28-day high-altitude exposure to 4,300 m. One of the speculative explanations was an increase in respiratory water loss due to

the increased pulmonary ventilation and the decreased humidity at high altitude. However, there is insufficient data to support this statement.

The present study allowed the measurement of insensible water loss at high altitude by simultaneously measuring total water turnover and fluid loss in urine and feces. Water intake, total water output and water output in urine and feces were measured over a 4-day interval before and a subsequent 4-day interval after transport to 4,350 m. Total body water and extracellular water were measured at the start and at the end of the two intervals.

METHODS

Subjects were three women and seven men, aged 30±8 (SD) and with a body mass index of 21.1±2.0 kg/m^2 (Table 1). They gave their informed consent to participate in the study and the protocol was approved by the Ethical committee of Necker Hospital, Paris. The observation started with baseline measurements over 4 days at sea level (Paris, France). On the 5th day subjects were transported by car and helicopter to a field laboratory on Mont Blanc in the French Alps (observatoire Vallot, 4,350 m) to be observed for another 4 days (day 6-9, Fig. 1). The activity level of the subjects was low. They spent most of the time in reading and household activities and underwent two short exercise tests at sea level and at altitude. Mean ambient temperature, pressure and relative humidity in the laboratory in Paris and in observatoire Vallot on Mont Blanc were, respectively, 31±3 °C and 21±3 °C, 761±6 mm Hg and 452±1 mm Hg, and 58±4% and 48±2%. Measurements of water balance comprised food and water intake, water loss, and changes in body composition over the two obervation intervals.

Table 1. Subject characteristics

Subj No.	sex	age (y)	height (m)	body weight (kg)	body fat (%)
1	m	29	1.80	70.0	12
2	m	30	1.82	67.5	12
3	m	26	1.92	84.0	11
4	f	26	1.61	52.2	17
5	f	34	1.63	62.5	20
6	f	22	1.56	41.5	12
7	m	23	1.76	58.0	9
8	m	24	1.78	73.0	5
9	m	36	1.82	72.0	14
10	m	47	1.66	59.4	11
mean		30	1.74	64.0	12
SD		8	0.11	12.0	4

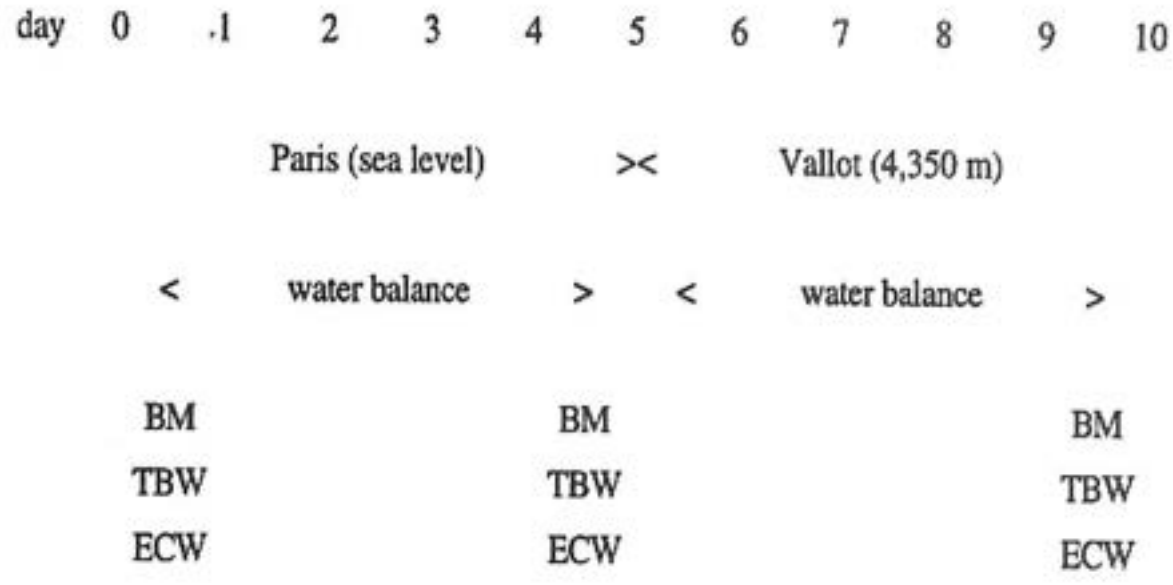

Fig. 1. The protocol. Body mass (BM), total body water (TBW), and extracellular water (ECW) were measured before and after two 4-day observation intervals of water balance, the first at sea level (day 1-4) and the second at 4,350 m (day 6-9). Vallot, Observatoire Vallot, French Alps.

Food and water intake was measured with a dietary record. Subjects recorded their food and fluid intake in a diary, including brand names and cooking recipes where appropriate. Food items were weighed with a table scale in most cases and volumes were measured with a graduated container. During the stay in the field laboratory the diaries were examined with the subject to clarify and eliminate inconsistencies. The energy and water content of the intake was then derived from food tables (Stichting Nederlands Voedingsstoffenbestand)

Total water loss was measured with labeled water (^2H_2O). The observation covered two times 4 days, day 1-4 at sea level and day 6-9 at altitude (Fig. 1). Subjects were given a weighed dose of 75 ml water with a measured enrichment of 4.36276 APE ^2H so that baseline levels were increased to ~100 ppm. Urine samples for isotope measurement were collected before dosing at night and from the second voiding every morning afterwards until the end of the observation interval. Isotope abundancies in the urine samples were measured with an isotope-ratio mass spectrometer (VG Isogas, Aqua Sira). Water loss was calculated from deuterium elimination as described before (Westerterp et al 1992). Subjects drank lowland water at altitude, allowing the assumption that background 2H levels do not change as has been observed before (Westerterp et al 1992).

To determine water loss in urine and feces, subjects collected total urine and total feces for all days. Urine was collected in a calibrated container to measure volume at each voiding and sampled for isotope analysis if appropriate. Feces were collected in preweighed bags, weighed, and a sample was collected in an airtight container for the measurement of moisture content by freeze drying in the laboratory.

Body mass, total body water, and extracellular water were measured at the start and end of the two 4-day intervals. Body mass (BM) was measured on rising, after emptying of the bladder, on a scale ±0.1 kg (Seca). Total body water (TBW) was calculated as the deuterium dilution space, from the deuterium enrichment of the second voiding minus the deuterium concentration of the sample before dose administration (Van Marken Lichtenbelt et al 1994a). The deuterium dilution space was divided by 1.04 to correct for isotope exchange with non-aqueous hydrogen of body solids

(Schoeller at al 1980). Extracellular water (ECW) was determined by bromide dilution. A known amount of sodium bromide (60 mg Br/L estimated TBW) was mixed with the ^2H_2O solution and thus administered simultaneously with the deuterium solution. Venous blood samples were obtained at 1030 PM, before bromide intake, and at 0800 AM the next morning, within one h before or after the second urine voiding for the measurement of TBW

Bromide concentration was determined in serum ultrafiltrate with HPLC, and ECW was calculated from corrected bromide space as described before (Van Marken Lichtenbelt et al 1994b).

Acclimatization to altitude was evaluated by a standardized scoring system of AMS of 4 symptoms: headache, digestive signs, fatigue, and insomnia (The Lake Louise consensus, 1992). The symptoms were scored on a 3-point scale each, 4 times a day with 4-hour intervals.

RESULTS

Four subjects, two women and two men, developed AMS after transport to 4,350 m. They had a mean AMS score of 17.0 (range, 16-18), 14.3 (8-19), 5.0 (3-7), and 4.5 (0- 11) on day 1 to 4 of the 4-day interval at altitude, respectively. The corresponding score for those who did not develop AMS was 2.5 (0-9), 2.0 (0-7), 0.5 (0-1), and 0.5 (0- 1). Results on food intake and the measured components of water balance will be presented for the group with AMS (n=4) and the group without AMS (n=6) separately (Fig. 2).

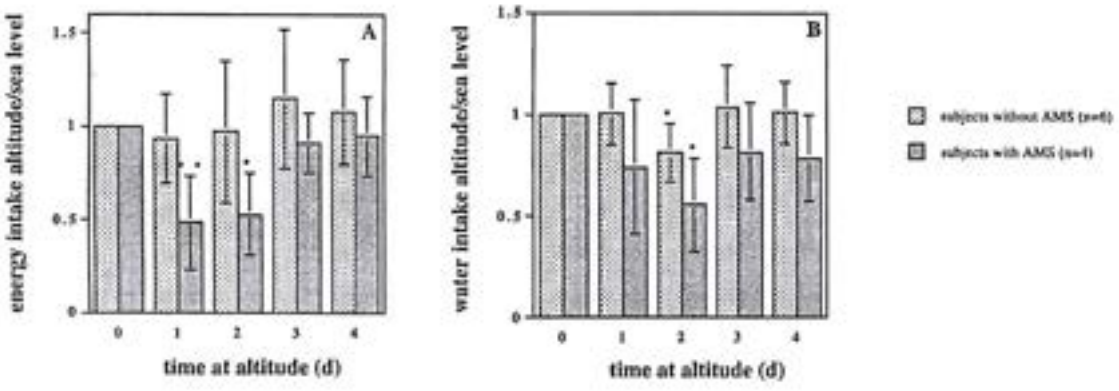

Fig 2. Energy intake (A) and water intake (B) on day 1 to 4 after arrival at 4,350 m, expressed as a multiple of the 4-day mean value over the preceding interval at sea level. Value was significantly different from value over preceding interval at sea level (*P<0.05, **P<0.01). AMS, acute mountain sickness.

Comparing energy intake of the subsequent days at altitude with the 4-day mean energy intake at sea level, all subjects with AMS showed a reduction of intake at altitude. Energy intake was 48±25%, 53±22, 91±16, and 95±21 % of the mean sea level value on the subsequent days at altitude. Corresponding values in the other six subjects were 93±24%, 97±38%, 115±37%, and 108±28%. Water intake was more variable but showed the same trend. Water intake in subjects with AMS was 75±33%, 56±23%, 82±24%, and 79±21% of the mean sea level value on the subsequent days at altitude. Corresponding values in the other six subjects were 100±15%, 81±14%, 104±20%, and 101±15%. Comparing mean energy intake and mean water intake over the two 4-day intervals there was a significant relation at sea level ($r = 0.83$, $p < 0.01$) and at altitude ($r = 0.84$, $p < 0.01$), with no difference between slope and intercept of the two regression lines (Fig. 3, co-variance analysis).

Mean total water loss at altitude in subjects with AMS (nrs 3,4,5,10) was significantly reduced to 74±21 % of the sea level value ($p = 0.05$), while the change in subjects without AMS to 92±15% was not significant.

Urine production, as a component of water loss, in subjects with AMS was 83±27%, 97±36%, 99±34%, and 68±22% of the mean sea level value on the subsequent days at altitude. Corresponding values in the other six subjects were 200±58%, 165±52%, 179±34%, and 142±50%, i.e. significantly increased on the first day ($p <0.01$), second day ($p <0.01$), and

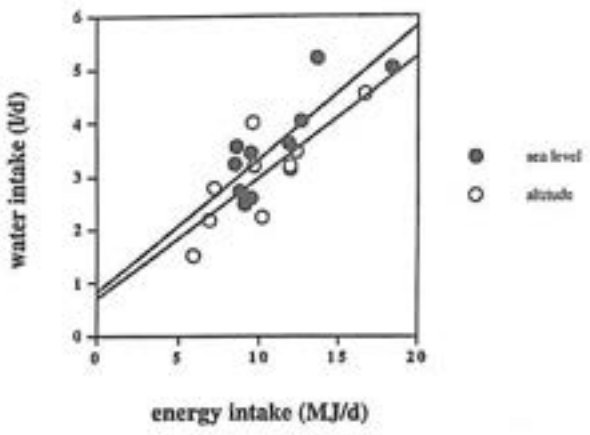

Fig. 3. Mean water intake as a function of mean energy intake in ten subjects over 4-day intervals at sea level and at altitude with the calculated linear regression line.

third day ($p < 0.001$) at altitude. Water loss in feces was at altitude decreased in all subjects with AMS. The decrease was due do a decrease of mean fecal mass from 0.25±0.14 kg/d at sea level to 0.13±0.08 kg/d at altitude and a decrease in mean fecal hydration from 79±5% to 68±11%. Water loss in feces at altitude was unchanged in subjects without AMS.

The mean fecal mass was 0.19±0.06 kg/d at sea level and 0.19±0.10 kg/d at altitude, and the corresponding values for mean fecal hydration were 62±17% and 67±18%. Evaporative water loss, calculated as total water loss minus urine water and fecal water, was decreased at altitude. In absolute figures it went down from 2.5±0.8 l/d to 1.3±0.5 l/d ($p < 0.001$). Expressed as a proportion of total water loss it went down from 57±14% to 38±11% ($p < 0.01$). Changes in evaporative water loss were not different for subjects with AMS and subjects without AMS.

Mean changes in TBW and ECW were not different for subjects without and with AMS. There was a significant increase in TBW of 1.2±1.0 l between the mean of the two observations at sea level and after 4 days at altitude ($p<0.01$). The increase in ECW of 0.5±1.2 l was not significant. However, subjects with AMS showed the biggest shifts in ECW (Fig. 4). Three subjects gained more than 1.5 l ECW (nrs: 3, 4, 10) and one subject lost 1.0 l ECW (nr 5), while the mean change in ECW in the subjects without AMS was -0.1±0.5 l (range +0.5 to -0.8 l).

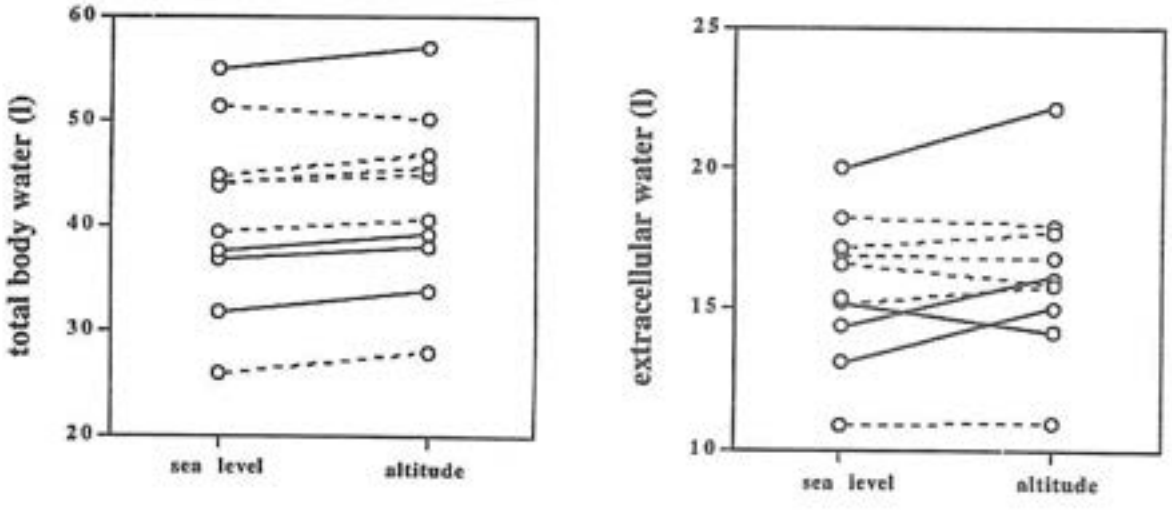

Fig. 4. Change in total body water and extracellular water from sea level to altitude for subjects without acute mountain sickness (data points connected with continuous lines) and subjects developing acute mountain sickness (data points connected with dotted lines).

DISCUSSION

The present study is a first attempt to measure water balance and its components at altitude, using labeled water and bromide dilution, and relating the results with AMS. There was a close relationship between energy intake and water intake and the relation was unchanged by the altitude intervention. Subjects developing AMS reduced energy intake and water intake correspondingly because nausea and loss of appetite are part of the clinical syndrome of AMS. The increase in TBW in subjects developing AMS was accompanied by a reduction in total water loss. They did not show the increased urine output, compensating for the reduced evaporative water loss at altitude.

AMS is associated with rapid ascent and with fluid retention (Bärtsch et al 1991).

Fluid retention seemed to be independent of a change in water requirements due to altitude exposure. The relation between energy intake and water intake was not affected by the ascent, not in subjects without AMS nor in subjects with AMS. Apparently altitude exposure does not affect fluid intake independent from energy (food) intake. Water requirement is not increased. The increase in evaporative water loss due to decreased barometric pressure is counterbalanced by additional clothing reducing cutaneous water efflux as suggested by Hoyt et al (1994). Evaporative water loss in the present study even went down from 2.5±0.8 l/d to 1.3±0.5 l/d ($p < 0.001$) or from 57±14% to 38_11% ($p < 0.01$) of total water loss. The decrease could be an effect of the high temperature during the interval at sea level (31±3 °C) though temperature at altitude also was relatively high. Subjects stayed nearly all the time in a comfortable hut at room temperatue (21±3 °C). The difference of 10 °C between sea level and altitude was probably even less than subjects normally experience in altitude studies. Thus, altitude exposure in the presence of sufficient food and water is a problem of how to increase water loss. This study shows for the first time that total water loss was reduced in subjects with AMS because they did not increase diuresis at the reduced evaporative water loss.

Subjects showed a significant increase in TBW after 4 days at altitude. Subjects with AMS showed the biggest shifts in ECW relative to TBW. Hannon et al. (1969) observed a shift from ECW to intracellular water (ICW = TBW - ECW) while TBW was unchanged in subjects exposed to 4,300 m, under sedentary conditions and no change in environmental temperature (25 °C), by transporting soldiers from sea level to a laboratory trailer at altitude. Krzywicki et al. (1971) observed a decrease in TBW and ICW after 6 days exposure to 4,300 m with 'normal' exercise, while ECW was increased. In both studies mentioned, subjects had minimal symptoms of AMS. Whatever happens with TBW at altitude exposure, hypohydration (Krzywicki et al 1971), euhydration (Hannon et al 1969), or hyperhydration (present study), the present study showed that subjects with AMS showed the biggest shifts in ECW relative to TBW. This is in accordance with a study of Carson et al. (1969) also showing that maximum severity of AMS went together with the greatest fluid shifts. This can be a relative gain (present study 3 subjects) or relative loss in ECW (present study 1 subject).

In conclusion, fluid retention in relation to AMS is independent of a change in water requirements due to altitude exposure. Subjects developing AMS were those showing a fluid shift of at least 1l from the intracellular to the extracellular compartment or from the extracellular to the intracellular compartment.

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THE HIGH ALTITUDE MEDICAL STUDIES IN JAPAN

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RESUMEN: Los Estudios Médicos Sobre Altura en Japón

Los estudios médicos japoneses sobre altura empezaron con la expedición de Manaslu en 1953. desde entonces, entre muchas expediciones de Japón a los Himalayas, 34 de ellas han reportado trabajos médicos. Ocho reportes fueron destacados entre estas 34 expediciones, y especialmente dos de ellas se distinguieron: la *Japanese Alpine Club Mt. Everest Expedition*, 1970 (JMEE'70) y la *Kyoto University Medical Research Expedition to Xixabangma*, 1990 (KUMREX'90). Se presenta una introducción de algunos artículos de KUMREX'90. La *Sociedad Japonesa de Medicina de Montaña (JSMM)* se estableció en 1981 y se publica cada año los reportes de las Symposia Japoneses sobre Medicina de Montaña. El JJMM vol. 16, último número, se publicó en Diciembre de 1996. Entre los numerosos especialistas e investigadores de altitud, los Dres. Matsubayashi, Saito, Masuyama y Horii han realizado remarcables contribuciones y se presentan aquí algunos de sus trabajos. Desde 1966, se ha reportado muchos casos de AMS severo (HAPE/HACE) en Japón, si bien no hay muchas montañas por encima de 3,000 m. El Dr. Kobayashi y sus colaboradores han reportado investigaciones sobre muchos casos de HAPE y de alta susceptibilidad de los casos a la altura. Además de la medicina de montaña, se están realizando también trabajos sobre fisiología en altura. El Dr. Asano de la Universidad de Tsukuba es el líder en este campo en Japón.

Palabras claves : JJMM, Expedición Medicina

RÉSUMÉ: Les études médicales de haute altitude au Japon.

Historique et travaux récents sur la recherche médicale de haute altitude au Japon : Les études médicales japonaises dans ce domaine ont commencé avec l'expédition Manaslu en 1953. Depuis lors, 34 expéditions japonaises à l'Himalaya ont rendu compte de travaux de recherche médicale. Huit de ces reportages sont à signaler et deux se distinguent particulièrement : ceux de la *Japanese Alpine Club Mt Everest Expedition*, 1970 (JMEE 70) et de la *Kyoto University Medical Research Expedition to Xixabangma*, 1990 (KUMREX 90). Une introduction à quelques articles sur la KUMREX est proposée. La *Japanese Society for Mountain Medecine* (JSMM) a été créée en 1981 et la revue *Japanese Journal of Mountain Medecine* (JJMM) - comptes rendus de la réunion annuelle du symposium japonais de médecine de montagne - est publiée chaque année. Le dernier numéro du JJMM, vol 16 a paru en décembre 1996.

Parmi les nombreux spécialistes de haute montagne et chercheurs d'altitude, les Drs. Matsubayashi, Saito, Masuyama et Horii ont réalisé des travaux remarquables dont quelques-uns sont présentés ici.

Bien qu'au Japon il y ait peu de montagnes dépassant 3 000 m, de nombreux cas graves de AMS (HAPE/HACE) ont été signalés depuis 1966. Le Dr. Kobayashi et ses collègues ont rendu compte de leurs recherches sur de nombreux cas de HAPE et de la très grande susceptibilité des sujets affectés à la haute altitude. En plus de la médecine d'expédition, l'investigation physiologique comprend l'altitude simulée que l'on réalise dans de nombreuses universités. Le Dr. Asano de l'Université Tsukuba est à la tête des recherches dans ce domaine au Japon.

Mots-clés : JJMM, Oedème pulmonaire d'altitude, Médecine d'expédition.

SUMMARY: Japanese high altitude medical study began at Manaslu Expedition in 1953. Since then, among many expeditions to Himalaya from Japan, 34 of them have repor ted medical research works. Eight reports were remarkable among these 34 expeditions, and especially two of them were distinguished. These two reports were *Japanese Alpine Club Mt.Everest* Expedition, 1970 (JMEE'70) and *Kyoto University Medical Research Expedition to Xixabangma*, 1990 (KUMREX'90). Abstract of some papers on KUMREX'90 are introduced here. The Japanese Society of *Mountain Medicine(JSMM)* was established in 1981, and the *Japanese Journal of Mountain Medicine*(JJMM), proceedings of the annual meeting of the Japanese Symposium on Mountain Medicine is being published every year. JJMM vol.16, the latest issue, was published in Dec. 1996. Among many Japanese medical researcher-mountain climbers, Dr. Matsubayashi, Dr. Saito, Dr. Masuyama and Dr. Horii did remarkable works and some of their main works are introduced here. Since 1966, many cases of severe AMS(HAPE/HACE) have been reported in Japan though there are not many mountains higher than 3,000m. Dr. Kobayashi and his colleagues have reported researches on many HAPE cases and high susceptibility to high altitude of the cases. Besides expedition medicine, physiological research works on simulated altitude are also carring out at several universities. *Dr. Asano* of Tsukuba University is the leader of this field in Japan.

Key Words: JJMM, High Altitude pulmonary edema, Expedition Medicine.

1. INTRODUCTION

In Japan, there are many mountaineers, hikers, climbers and trekkers going up not only low mountains within Japan but high mountains all over the world, so consequently, the number of victims of acute mountain sickness(AMS) or even high altitude pulmonary edema(HAPE) and/ or high altitude cerebral edema(HACE) is not a few even on the mountains in Japan, though there are few mountains over 3,000m height. Accordingly, we have many works on

mountain medicine. However, the most of them are not well known in the world, because the rate of numbers of these papers published in foreign medical journals has been low.

In 1983, Dr.Houston kindly offered me the opportunity to introduce our works to people outside of Japan at the Third International Hypoxia Symposium held at Banff, CANADA1). And Dr.Monge and Dr.León-Velarde gave me the second chance to introduce our recent works on high altitude medical researches in Japan at the Second World Congress on High Altitude Medicine and Physiology, 1996 held in Cusco, PERU.

2. JAPANESE SOCIETY OF MOUNTAIN MEDICINE

The Japanese Society of Mountain Medicine(JSMM) was established in 1981. The Japanese Symposium on Mountain Medicine has been held annually and its proceedings,"The Japanese Journal of Mountain Medicine(JJMM)_ is being published every year. This journal, written in Japanese with English abstracts, contains most of our works on mountain medicine. As for example, the contents of the latest issue of JJMM,vol.16 published in Dec.1996 are introduced here,

S.ISOMURA: Special Lecture; International Travel and Health

M.HORII: Memory of the Late Dr.John Sutton

J.R.SUTTON: Lessons from Operation Everest II

F.OKUDA: Prediction of Acute Mountain Sickness by Uric pH Measurement

S.ONODERA: Relationship Between Rating of Perceived Expedition and Heart Rate on Hiking or Climbing of Middle and High Age

M.NAKASHIMA: Recurrent Symptoms of Pneumothorax with Up and Down at Moderate Altitude (A Case Report)

Y.MATSUZAWA: Ventilatory Responce to Sustained Hypoxia in Subjects with Previous Histories of High-Altitude Pulmonary Edema

T.KIZAKI: Swimming Training Improves Immune Responce

K.MATSUBA: Trial of Disinfection of Drinking Water by Sodium Hypochlorite Solution during Nyainqentanglha(7,046m) Expedition

M.TAGAWA: A Report on a HRA Pheriche Clinic during Autumn in 1995

T.KOBAYASHI: Effect of 0_2 Breathing on Changes of Arterial Oxygen Saturation and Breath-Holding Time (BHT) during High Altitude Trekking

H.OKAMOTO: Enzymatic Adaptation of Skeletal Muscles after Hypobaric Training in Rats.

M.YAMAMOTO: Exercise Physiology of Climbing 8,000m Peak without Bottled Oxygen: Fitness, Acclimatization, and Work Capacity

M.CHIDA: The Effects of Nitric Oxide Donor on the Change of Oxygen Saturation at High Altitude

R.OGIWARA: Risk Management for High Altitude Disease in Travel Agencies Which are in Charge of High Altitude Trip and Tours in Japan

M.NAKASHIMA: Report of The Second World Congress on High Altitude Medicine and Physiology 1996

For more informations, contact with the secretariate of JSMM.

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3. JAPANESE HAPE, THE WORKS OF SHINSHU UNIVERSITY GROUP

The highest peak in Japan is Mt.Fuji(3,776m). More than 100,000 people go up this mountain every summer season, but the incidence of severe AMS is very few.

Supposedly, Mt.Fuji is a single peak and easy to approach so the most of the climbers can get to top and to down before AMS symptoms appear. On the contrary, in case of traverse of Japan Alps, mountaineers should stay at altitudes higher than 2,400m for several days, and some people hypersensitive to altitude often suffer from AMS or even HAPE. The first case of Japanese HAPE was reported by Arai et al in 1966[2]. Since then, more than 80 cases have been reported. The altitude of onset of Japanese HAPE is as low as 2,680m-3,190m. Among those reports on Japanese HAPE, the clinical works of Kobayashi and his colleagues of

Shinshu University are excellent. The summary of deal works on their own 46 cases are as follows(3, 4);

1. Features of physical examinations on admission:
   a) Hematological and biochemical analysis; increased; Ht, WBC,Creatine kinase decreased; Plt, Serum protein and Serum Fe.
   b) Arterial gas analysis: Ph; 7.48±0.04, P_aCO_2 ; 31±tl2.7 torr, P_aO_2 ; 44.6±4.0 torr
   c) Broncho-alveolar lavage: increased; IL1-ß,IL-6,IL-8,TNF- • and ICAM-1
   d) Chest X-ray and brain CT films: Characteristic findings of pulmonary edema.
2. Common physical characteristics of Japanese HAPE patients:
   a) Greater increase in pulmonary vascular resistance index
   b) Higher pulmonary arterial pressure level during hypoxia
   c) Blunted hypoxic ventilatory drive
   d) High frequency of increase of human leukocyte antigen DR-6

They concluded that these patients might have genetic predisposition to hypoxic hypersensitivity(5, 6, 7) .They also have published several other HAPE-related reports (3, 9)

Furthermore, Ueda and others of Shinshu University group organized Matsumoto Inter-national Symposium on High-altitude Medical Science with splendid proceedin~ses (10, ll) twice in 1987 and 1991.

4. THE HISTORY OF JAPANESE MEDICAL RESEARCHES RELATED TO EXPEDITONS

The Japanese high altitude medical study began at Manaslu Expedition by Japanese Alpine Club in 1953. Since then, among many expeditions to Himalaya from Japan, 34 of them(Table 1 and Table 2) have rerported medical research works. The following 8 reports out of these 34 expeditons were remarkable and especially two of them were distinguished. These two reports were Japanese Alpine Club Mt.Everest Expedition,1970(JMEE'70) and Kyoto University Medical Research Expedition to Xixabangma,1990(KUMREX'90).

1953-56; Manaslu(8,125) (Nepal) (12) Tatsunuma,Sumiyoshi,Tokunaga.

1970; Mt.Everest(8,848) (Nepal) (13) Sumiyosi,Nakashima,Hirotani, Ohmori. This was the first Japanese expedition equipped with large scale medical research team. Some results of this physiological, hemato-logical and biochemical research works were presented at The 7th International Congress of Biometeorology at Nordwijk, Netherland in 1971.

1976; Pik Kommnizma(7,595) (Pamir) (14) Asano. Asano observed very high incidence of high altitude retinal haemorage (HARH) among climbers of this expedition. He took retinophotograms of climbers at the base-camp before and after the ascent. He found 15 HARH cases out of 16(94%) climbers after the ascent.

1980; Ganesh Himal V(6,750) (Nepal) (15) Nagao,Shinohara,Sasaki, Imaizumi, et al.

1984; Kangchenjunga(8,595) (Nepal) (16) Masuyama. 1988; Mt.Everest(8,848) (Nepal-Tibet) (17 Saito, Masuyama,Suzuki, Horii,Hirata, Nukariya,Mizukoshi.

1990; Xixabangma(8,012) (Tibet) Saito, Matsubayashi, Nakashima, et al. This was the genuine large scale medical research expedition of Kyoto Univeraity.The medical reports of this expedition was distingushed. Abstracts of some papers are introduced separatedly later in 6.

1991 Cho Oyu(8,201) (Tibet) (l9) Horii.

5 THE FOUR DISTINGUISHED JAPANESE MEDICAL RERSEARCHER - CLIMBERS

The names of the most distinguished 4 medical doctors in Japan and their main mountaineering records and research works are as follows;

(✣ :Summit-climb, ✻The first ascent, ★ :Party leader)

SAITO, Atsuo M.D. (Kyoto University)

1962:Saltoro Kangri ✣✻ (7,742) (Karakorum);Elecrocardiogram (20)

He recorded electrocardiograms of climbers at the base camp.

This was the first EKG record took at 5,200m above sea level.

1973:Yalung Kang ✣✻ (8,420) (Nepal); Elecrocardiogram (2l)

1980:Everest ★ (8,8423) (Tibet); Elecrocardiogram (22)

1984:Naimonanyi ★ 7,695) (Tibet); HAPE,Respiratory Function (23)

1988:Everest ★ (Tibet-Nepal); EKG, EEG, HARH, Respiratory, etc. (17, 18)

1990:Xixabangma ★ ✣ (8,012) (Tibet); EKG, EEG, HARH, Hormons,etc.

1992:Namcha Barwa ★ (7,762) (Tibet);

MATSUBAYASHI, Kozo M.D. (Kyoto University)

1982:Gang Ben Cheng t ✣✻ (7,281) (Tibet); Urine Catecholamines (24)

1984:Naimonanyi ✣✻ (7,695) (Tibet); HAPE, Respiratory Function (23)

1985:Masakong ★ ✣ ✻ (7,200) (Bhutan); Cerebral Blood Flow.etc. (25, 26)

1990:Xixabangma ✣ (8,012) (Tibet); EKG, EEG, HARH, Hormons, etc.

MASUYAMA,Shigeru M.D. (Chiba University)

1984:Kangchenjunga(8,595);Ventilation control (16)

1986:Kunlun, Peak7, 167 ✣ ; Ventilatory Chemosensitivity (27)

1988:Everest; EKG, EEG, HARH, Respiratory, Hormons (28) 29)

He observed hypoxic ventilatory response of highlanders (Sherpa) and concluded that,

1. It is not always blunted.
2. They keep good ventilation both at rest and at exercise or acute hypoxic exposure.
3. Even highlanders, hypoxic ventilatory responce curve decreases by age, but good ventilation and oxygenation are still kept.

HORII, Masako M.D. (Kanagawa Canser Center Hospital)

1980:Kedarnath Dome (_,831); Circulatory Physiology of Women (30)

1988:Everest (Tibet-Nepal); EKG, EEG, HARH, Respiratory, Hormons (3l)

1991:Cho Oyu (8,201) (Tibet); High Altitude Physiology of Aged (19)

She found that VO_{2max} of 4 Japanese middle-aged(52-55 years old) Mt.Cho Oyu(8,201m) summit-climbers was between 52.6and 63.9 ml/kg/min, almost same level of excellent marathon runners. She also found that mean SaO_2 of summit-climbers was always higher than that of non-summit climbers significantly at any altitude.

6. MEDICAL STUDIES OF HIMALAYAN EXPEDITIONS OF KYOTO UNIVERSITY

Kyoto University is a really outstanding university in Japan, which dispatched total 28 scientific expeditions to Himalaya and other high mountains.Among them, 6 expeditions shown in Table 3 have performed distinguished medical works, and the last one, Kyoto University Medical Research Expedition to Xixabangma, 1990 (KUMREX'90) was the first and the best genuine large scale medical research expedition in Japanese history of mountaineering. Some of their works were presented on the 7th International Hypoxia Symposium, Lake Louise, in 1991. (Hypoxia and Mountain Medicine, J.R.Sutton,G.Coates and C.S.Houston ed. Queen City Printers Inc. Burlinzton. Vt. 1992)

Table 1. CHRONICLE OF JAPANESE EXPEDITIONS TO HIGH MOUNTAINS WITH MEDICAL RESEARCH(1953-1981)

(BEFORE ESTABLISHMENT OF JAPANESE SOCIETY OF MOUNTAIN MEDICINE)

YEAR	MOUNTAIN(m)	AREA	RESEARCH MEMBER
1953-56	Manaslu(8,125)*	Nepal	Tatsunuma,et al.
1962	Saltoro Kangli*(7,654)	Karakorum	Saito,Hayashi.
1966	Aconcagua(6,969)	Peru	Takasi,Hara,et al.
1970	Everest(8,842)*	Nepal	Nakashima,Ohmori,et al.
1973	Yalung Kang(8,420)	Nepal	Saito.
1976	Pik Kommnizma*(7,595)	Pamir	Asano,Hara.
1977	Tharkott(6,099)	India	Hara,et al.
1979	Huascaran(6,767)	Peru	Hara,et al.
1980	Ganesh Himal V*(6,750)	Nepal	Nagao,et al.
1980	Kedarnath(6,940)	India	Horii.
1980	Everest*(8,842)	Nepal	Saito,et al.
1981	Bogoda(5,445)	China	Nose,et al.
1981	Everest(8,842)	Nepal	Hayashida.et al.
1981	Japanese Society of Mountain Medicne(JSMM) Established		

*Remarkable Research

ADACHI,Minami (32)measured hematocrit(Ht) and erythropoietin. The mean Ht at 6,920m was 65.5 ± 3.0%(47-70%). Erythropoietin increased with ascent prior to increase of Ht, then decreased acutely with progress of acclimatization, and increased again with further gain of altitude.

DEMIZU,Akira (33) observed high altitude insomnia of climbers through the incidence of day-time sleepiness. It was observed 2.7% at 5,-6.000m, 12% at 6,-7,000 m then 28% at 7,-8.000m(summit:8,008m). Oxygen saturation measured by pulsoximeter(SpO_2) was also observed. Mean SpO_2 in night was 98% at sea level, 77% at 3,650m, 73% at 5,640m then 59% at 7,430m respectively. Arterial gas analysis at base camp(5.020m) was as follows:

On arrival(Apr/22):	PaO2:27-43torr,	PaCO2;18-32torr,	pH;7.48-7.61
On leaving(May/28):	41-46	17-31	7.40-7.58

ENDO,Katsuaki (34)recorded EEG of climbers. He stored it on magnetic tape and brought back to Kyoto. The event related potentianls and auditory evoked potentials in human brain were analysed by means of a computer. Both potentials decreased at high altitude. It is very interesting finding that, the event related potentials of climbers with high altitude retinal hemorrage(HARH) was oviously lower than that of climbers without HARH.

HIRATA,Kazuo (35) observed left ventricular function and cardiac wall motion potentials using centerline method by means of 2-Dimension Echocardiography, which was brought up to the base camp(5,020m). He observed depression of septal movement which reflected increased right ventricular load, induced by pulmonary hypertention came from emvironmental hypoxia. On the other hand, the movement of left ventricular posterior wall increased for the compensation of depressed septum movement.

JIN-NOUCHI,Yosuke (36) observed lower left ventricular function among Himalayan highlanders comparing with Japanese by means of echocardiography.

MATSUBAYASHI, Kozo (37) reported the discrepancy between brain oxygen and glucose metabolism after coming back from high altitude climbing by means of positron emission computed tomograghy(PET). It was observed that brain blood flow was decreased and brain oxygen uptake was increased but glucose metabolism was unchanged. He supposed this phenomenon might suggest that human blood

flow, oxygen uptake and glucose metabolism increased at altitude.

MATSUBAYASHI, Kiyoaki (38) brought two young Japanese monkeys up to camp 5,640m and measured several physiological indicators, such as, body weight, respiratory rate, puls rate, expiratory gas analysis, EEG, RBC, Ht, Erythropoietin, T3, T4 and Cortisol. The results from monkeys were almost same as human samples.

NAKASHIMA,Michiro (39, 40), the author, observed the incidence of HARH at the base camp(5,020m) by means of retinocamera. He found very high HARH incidence among Himalayan newcomers. On arrival to base camp, 7 out of 9 newcomers(78%), and on leaving from base camp, 8 of 9 newcomers who had climbed above 6,000m(89%) were suffered from severe HARH. On the contrary, in case of Himalayan experiences, not only the incidence was low(1 out of 8(13%) on arrival, and 7 out of 19 summit climbers(37%) on leaving), but all HARH findings of them were mild. It is interesting enough that, inspite of such high incidence of HARH, none of all cases complained any symptoms.

SETO,Siro (41) messured platelet factor 4(PF4), ß-thromboglobulin (ß-TG), thromboxan B2(TXB2) and 6-keto prostaglandin F1Q (6-PGF1a), and found all but 6-PGF1Q inceased. These results suggested increased blood coaglation tendency, but comparing with high rate of this tendency the real incidence of intravascular blood coagulation at high altitude was relatively not so high, especially among Japanese climbers, so, he supposed that there might be some unknown counter factors to protect blood coagulation at high altitude.

SUGIE.Tomoharu (42) brought gastrofiberscope up to the base camp and found high incidence of acute gastric mucosal lesion among expedition members. He concluded that hypoxia might be one of the major factors of gastric hemorrhage, which is not uncommon among high altitude climbers.

7. RESEARCH WORKS ON SIMULATED HIGH ALTITUDE

Besides these great deal of medical researches on expeditions, several experimental training to get acclimatization by means of hypobaric chamber prior to the expedition have also been investigated at some universities. According to Asano and his colleagues of Tsukuba University (43), it was mentioned that the Himalayan candidate examinees could get good acclimatization and easier and faster Himalayan climb after this sort of simulated altitude training.

8. DISCUSSION

There is an anecdote. About 200 years ago, an American natural scientist was invited to Japan as the teacher of natural science. He taught his students mathematics, chemistry, physics, botany and so on from the begining. One day he suddenly realized that his studens knew all about natural sciences but English language. They had already learned natural sciences through Dutch. The lesson of this anecdote is "It is unavoidable for someone to be considered as ignorant, if he has no means to express his knowledge."

The volume of medical documents written in English and published in foreign journals by Japanese researchers is not so few, but most of the documents have been written only in Japanese and published in Japanese journals within Japan. How huge is the accumulation of researches on mountain medicine, expedition medicine and high altitude physiology, as long as written in Japanese only, it is no use for the progress of medical sciences of the world. All of Japanese medical investigators should take this lesson to heart.

9. OVERVIEW

Japanese Society of Mountain Medicine was established in 1981, which is publishing Japanese Journal on Mountain Medicine with English abstracts every year. The latest issue, vol.16 was published in Dec.1996.

We have many cases of AMS or even HAPE and/or HACE in Japan at altitude above 2,700m. Why at relatively not so high altitude Kobayashi and his colleagues of Shinshu University have been investigating the genetic hypersensitivity predisposition to altitude of Japanese HAPE patients. Shinshu University group also has organized splendid international symposiums on high altitude medicine twice.

Until now, 34 Japanese expeditions reported medical researches, and 8 of them were remarkable.

Table 2. CHRONICLE OF JAPANESE EXPEDITIONS TO HIGH MOUNTAINS WITH MEDICAL RESEARCH(1982-1994)

(AFTER ESTABLISHMENT OF JAPANESE SOCIETY OF MOUNTAIN MEDICINE)

YEAR	MOUNTAIN(m)	AREA	RESEARCH MEMBER
1982	Gang Ben Cheng*(7,281)	Tibet	Matsubayashi,et al.
1983	Jichudrake(7,012)	Bhutan	Hashimoto,et al.
1983-84	Everest-Lhotse(8,511)	Nepal	Asaji,Song,Kobayashi.
1984	Kanschenjunga*(8,595)	Nepal	Masuyama,et al.
1984	Naimonanyi(7,694)	Tibet	Saito,Matsubayashi,et al
1984	Bhagirathill	India	Kawai,et al.
1985	Masakong(7,200)	Bhutan	Matsubayashi.
1986	Nianquingtanggula	Tibet	Katayama,Sakai,Kasai.
1986	Kuniun,P.7,167	China	Masuyama.
1988	Everest*	Nepal-Tibet	Saito,Suzuki,Nukariya, Masuyama.
1988	Yan(6,230)	India	Honjo,et al.
1989	K.P.6,666	Karakorum	Nito,et al.
1990	Xixabansma*(8,012)	Tibet	Nakashima,et al.
1990	Cho Oyu(8,201)	Tibet	Doi,et al.
1991	Cho Oyu*(8,201)	Tibet	Horii.
1992	Gasherbrum I (8,068)	Karakorum	Kamio.
1992	Crown(7,265)	China	Shimada.
1993	Damavand(5,611)	Iran	Noguchi.
1993-94	Everest	Nepal	Sumiyosi.
1994	Bhagirathi(6,856)	India	Nakashima.
1994	Lobche Peak(6,145)	Nepal	Kashimura.

*Remarkable Research

The names and main works of following 4 medical doctores, Saito and Matsubayashi (Kyoto University), Masuyama (Chiba Univ.) and Horii (Yokohama) are introduced as the distinguished mountain climber-medical researchers.

The best and the largest medical research expedition from Japan was Kyoto Univeraity Medical Research Expedition to Xixabangma, 1990(KUiZREX'90). Some of the papers, mainly presented on the 7th International Hypoxia Symposium, Lake Louise, in 1991, were introduced.

Besides medical studies on expedition, some succesful trials to get acclimatization using hypobaric chamber have been reported by Asano et al.

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Table 3. HIMALAYAN EXPEDITIONS OF KYOTO UNIVERSITY WITH REMARKABLE MEDICAL RESEARCHES

1962 Saltoro Kangli(7,742), Karakorum
Saito,Hayashi;
Cardiograghic Observations at 5.200m above sea level
1973 Yalung Kang(8,420) Nepal
Saito,Takasi;
EKG.Blood Pressure. Breath Holdinq Time.
1982 Gang Ben Cheng(7,281) Tibet
Matsubayashi;
Urine Catecholamines and their Metabolites at Altitude
1984 Naimonanyi(7,694) Tibet
Saito, Matsubayashi;
High Altitude Pulmonary Edema(HAPE)
Respiratory Function During Long Term Sojourn Above 4,700m
1985 Masakong(7,200) Bhutan
Matsubayashi;
Cerebral Blood Flow and Metabolism Before and After High Altitude SoJourn
1990 Xixabansma(8,012) Tibet
Adachi;
Sequential Analysis of Erythropoietic Drive
Demizu;
High Altitude Insomnia and Continuous Pulsoxymetry
Endo;
Event Related Potentials and Auditory Evoked Potentials
Hirata;
Left Ventricular Function and Wall Motion Examined by 2-D Echocardiograshy
Jin-nouchi;
Characteristics of Cardiovascular Physiology
Matsubayashi,Ko.;
Discrepancy Between Brain Oxygen and Glucose Metabolism
After High Altitude Climbing
Matsubayashi,Ki.;
Hematological and Physiolosical Study of Monkey
Nakashima;
High Altitude Retinal Haemorrhase(HARH)
Seto;
Platelet Activation Is Present or Not at High Altitude
Sugie;
Acute Gastric Mucosal Lesion at the High Altitude

IV

MOLECULAR PHYSIOLOGY AND O_2 SENSING

FUNDAMENTAL LESSONS FROM BLUNTED CHEMOSENSORY RESPONSE OF CAROTID BODY TO HYPOXIA

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RESUMEN: Respuesta Quimiosensorial Atenuada del Cuerpo Carotídeo a la Hipoxia: Lecciones: Fundamentales

Las células del cuerpo carotideo están conectads sinápticamnete con fibras aferentes individuales, y la despolarización de la célula resulta en incrementos de la $[Ca^{2+}]_i$, de la neurosecreción y de la descarga neural. Se supone que la supresión de la corriente de K^+ por un bajo PO_2 despolariza las células del cuerpo carotideo. Por tanto, una respuesta quimiosensorial atenuada puede brindar una clave para la percepción de O_2 por las celulas insensibles al O_2. Sin embargo la literatura demostró que la depresión de los canales de K^+-O_2 por un bajo PO_2 era normal, si bien las células no se despolarizaban. Además, las fibras sensoriales con respuesta hipóxica atenuada mostraban una respuesta CO_2/H^+ normal o supranormal. Consiguientemente, deberian manifestar una depresión de corriente de K^+ normal o supranormal con estimulos CO_2/H^+ incrementados.

RÉSUMÉ: Réponse chimiosensorielle atténuée du corps carotidien à l'hypoxie : leçons fondamentales.

Les cellules du corps carotidien sont reliées synaptiquement à des fibres afférentes individuelles et la dépolarisation de la cellule entraine l'élévation de $[Ca^{2+}]_i$, de la neurosécrétion et de la décharge neurale. La suppression du courant de K^+ par un PO_2 faible est supposée dépolariser les cellules du corps carotidien. Une réponse chimiosensorielle atténuée peut donc fournir une clé à la perception d'O_2 par les cellules insensibles à l'O_2. Cependant, la littérature montre que la dépression des canaux de K^+ - O_2 par un PO_2 faible était normale, bien que les cellules ne se soient pas dépolarisées. D'autre part, les fibres sensorielles à réponse hypoxique atténuée montraient une réponse CO_2/H^+ normale ou supra-normale. En conséquence, elles devraient manifester une dépression de courant de K^+ normale ou supra-normale avec des stimuli CO_2/H^+ accrus.

Mots-clés : Réponse CO_2/H^+, Accroissement de $[Ca^{2+}]_i$, Canaux de membrane K^+-O_2, Dépolarisation de membrane, Réponse neurale, Réponse à l'O_2.

SUMMARY: Glomus cells are synaptically connected with single afferent fiber, and depolarization of the cell should result in increases of $[Ca^{2+}]_i$, neurosecretion and neural discharge. Suppression of K^+ current by low PO_2 is supposed to depolarize the glomus cells. Therefore, a blunted chemosensory response can provide a clue to O_2 sensing by the cells being insensitive to O_2 But literature showed that K^+-O_2 depression by low PO_2 was normal, although the cells did not depolarize. Also, the sensory fiber with blunted hypoxic response showed a normal or supernormal CO_2/H^+ response. Accordingly, they should manifest normal or supernormal K^+ current depression with raised CO_2/H^+ stimuli.

Key words: CO_2/H^+ response, : $[Ca_2^+]$ rise, K^+-O_2 membrane channels, membrane depolarization, neurosecretion, neural response, O_2 response

INTRODUCTION

Blunted response of carotid chemosensory discharge to hypoxia occurs in nature (1-8), a phenomenon which can provide a clue to fundamental mechanisms of O_2 sensing. Since O_2 sensing is based upon K^+ - O_2 channels of carotid body typeI glomus cells (9-11), blunting of

chemosensory discharge should be associated with the appropriate cellular responses. Also, the same fiber of carotid body responds to hypoxia and a rise in CO_2/H^+ (12) as the same K^+ channel responds to both the stimuli (13). These ideas are presented in the following postulated model, cell to chemosensory discharge (Fig. 1). Hypoxia and CO_2/H^+ cause K^+ current depression, depolarizing the glomus cell and opening the voltage gated Ca^{2+} channels. Extracellular Ca^{2+}, whose concentration is 10^4-10^7 greater than intracellular Ca^{2+}, then enters which activates neurosecretion and neural discharge. This is the main mechanism which has been postulated. There is also mechanism for mitochondrial depolarization which can release intracellular Ca^{2+} Also, a rise of $[H^+]i$ can increase $[Ca^{2+}]i$ by way of $[Na^+]$ rise first and then by exchange with $[Ca^{2+}]e$. K^+ current will be the focus of the discussion that follows revealing some gaps in our knowledge.

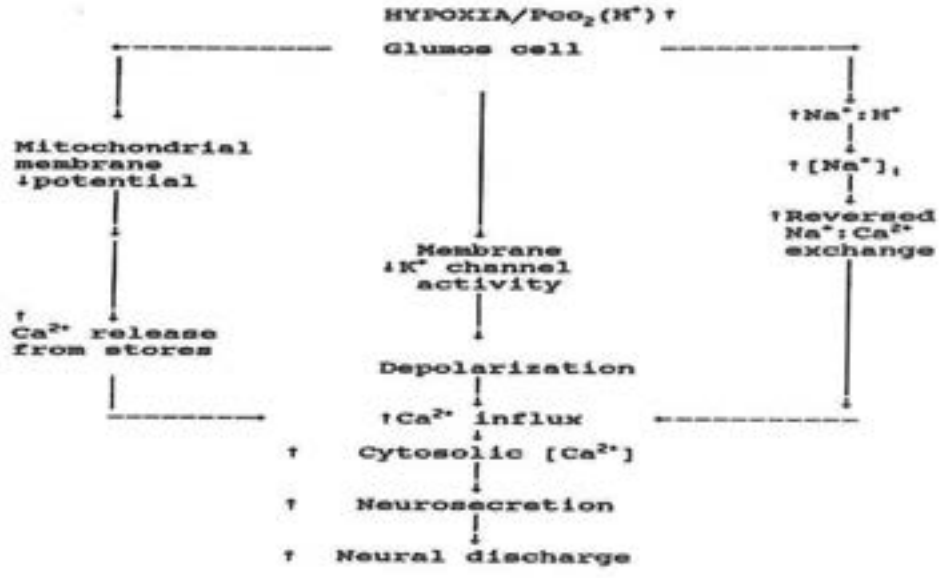

Figure 1. The postulated mechanisms for hypoxic and CO_2/H^+ stimuli in glomus cells. Note that K+ current is common to both stimuli.

HYPERTROPHY OF GOMUS CELLS AND INCREASES DOPAMINE CONTENT DURING CHRONIC HYPOXIA

Chronic exposure to hypoxia is followed by hypertrophy of glomus cells, and presumably stay that way as long chronic hypoxia persists (14,15). So is the dopamine content of the cell. However, chemosensory discharge increases first (16-18), and then becomes blunted (14). What makes the sensory response blunted is the question. We will see that K^+ channels that are sensitive to O_2 remain intact, although glomus membrane depolarize (5). many questions can be asked. What was the oxygen of the cells; what are the pathways by which the cells become hypertrophied, and like?.

EFFECT OF CHRONIC HYPOXIA: CHEMOSENSORY DISCHARGE AUGMENTS HYPOXIC RESPONSE

It is well known that chronic hypoxia causes hypertrophy of type I glomus cell of the carotid body (14,15). It is also well accepted that O_2 sensitive K+ conductance is depressed by low PO_2 (9-11). This K^+ current, however, is similarly depressed in cells which are exposed to chronic hypoxia (11). But the cells from the hypoxic rats being larger, K^+ channel density was decreased at all activating test potentials. This would tend to support the observations that chronic hypoxia initially increased hypoxic sensitivity of chemosensory discharge (16-18). This increastd discharge rate is illustrated in Fig. 2.

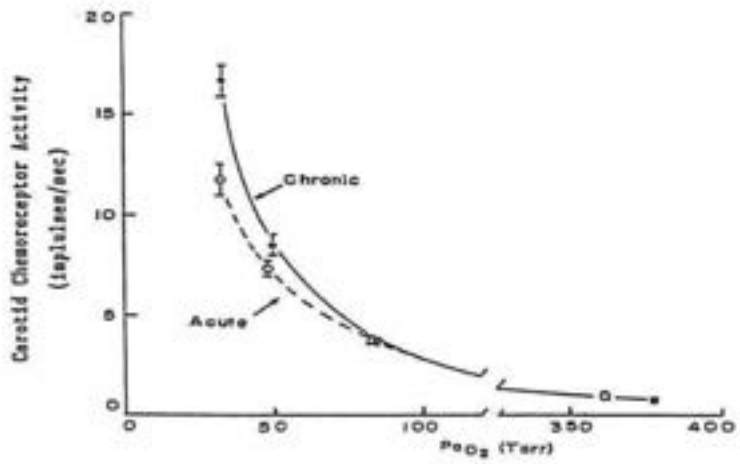

Figure 2. Cat carotid chemosensory response to hypoxia. Responses from normal (acute) and chronically hypoxic cats are compared (from Barnard et al., 1987. Ref. 16).

BLUNTED HYPOXIC RESPONSE IN CHRONIC HYPOXIA

On the other hand, prolonged hypoxia in humans (1,2) can give rise to blunted respiratory response to hypoxia. Now it is found in animals also (3,4), and glomus cell from the carotid of these animals can provide a clue as to O_2 sensing if K^+-O_2 channels is the basis of it. That is, inhibition of K^+ channels by hypoxia leads to depolarization and increased excitability of glomus cells sufficient to activate voltage-gated Ca^+ channels and neurotransmitter release and neural discharge. However, experimenting with such glomus cells from ventilatory response to hypoxia Wyatt et al. (5) found that K^+-O_2 channels were intact (see Fig. 3) but the cells failed to depolarize. That also means, that these cells would not show a rise of $[Ca^{2+}]i$ and neurotransmitter release. These experiments have not been done. However, these showed low K+ channel density, and should have shown increased sensory response to hypoxia. Instead, these cells did not depolarize and would show a chemosensory response to hypoxia.

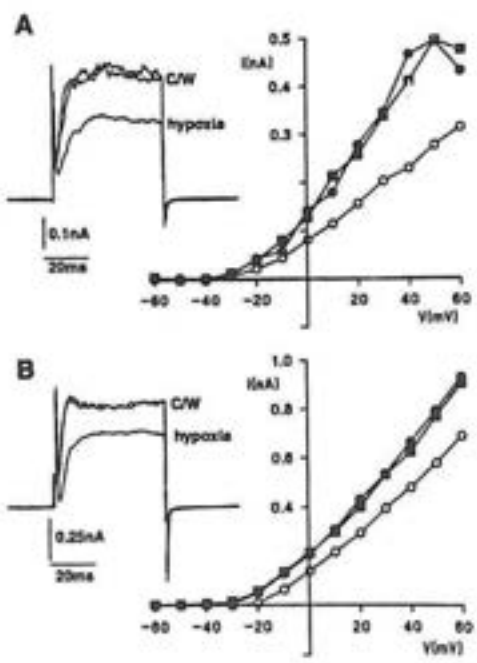

Figure 3. Effect of acute hypoxia on whole-cell K^+ currents in type I cells isolated from normoxically reared and chronically hypoxically reared neonatal rats, as measured by using the perforated-patch technique. (A) I-V relationships obtained from a type I cell isolated from a normoxically reared neonatal rat before (•), during (O), and after (•) exchange of normoxic perfusate to a hypoxic perfusate (PO_2 between 12 and 20 mmHg). (Inset) Example traces obtained from the same cell under the same three conditions (C, control; W, wash; test potential,

+20 mV). (B) Experiment identical to that in A, but in this case the K^+ currents were obtained from a hypoxically reared neonatal rat. In A and B the holding potential was - 70 mV.

Response to $C0_2/H^+$

According to the model of cellular chemoreception, (see Fig. 1) K^+channel is common to both O_2 and CO_2/H^+. Thus, when K^+-O_2 channel is insensitive, CO_2/H^+ effect should also desensitize. This experiment also has not been done.

Effects of chronic hyperoxia: blunted hypoxic but supernormal CO^2/H^+

Another testing model is the carotid body of chronically hyperoxic cats in which both ventilation and carotid chemosensory discharge are insensitive to hypoxia but it is sensitive to hypercapnia (6-8). Fig. 4 shows an illustration. Hypoxia (A) failed to elicit a response, whereas (B) initial hypercapnia manifested a huge response with an overshoot.

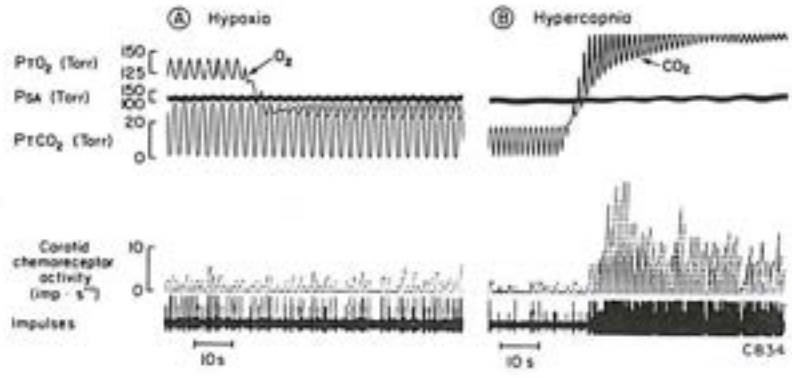

Figure 4 Blunted hypoxic chemosensory response (A) and normal Co2 response (B) of carotid body of cats which has been exposed to prolonged hyperoxia

Blunting of hypoxia but not CO_2 response of chemosensory discharge by inhibitor oxidative phosphorilation.

The blunting of chemosensory response to hypoxia but not to CO_2/H^+ occurred in oligomycin treatment of carotid body (19,20). This mimicked the foregoing responses to chronic hyperoxia.

In summary, carotid chemosensory fiber while showing a normal or super normal response to CO_2/H^+ can manifest a blunted response to hypoxia. This blunting is seen in the glomus membrane depolarization being insensitive to hypoxia whereas K^+-O_2 channel appears normal. Co_2/O_2 response of these cells is not known but K^+ channel depression and membrane depolarization are expected as the rise in sensory discharge.

In perspective, these cells with blunted hypoxic response should not show a response to $[Ca^{2+}]i$ and nourosocrotion.

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BIOCHEMISTRY OF ACUTE AND CHRONIC HYPOXIA

EVOLUTION OF HYPOXIA TOLERANCE: DIVING PINNIPED MODEL AND HUMAN HYPOBARIC HYPOXIA MODEL

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RESUMEN: Evolución de la Tolerancia a la Hipoxia: el Modelo de Pinnipedo Buceador y el Modelo Humano de Hipoxia Hipobárica

Para los fisiólogos, el término "adaptación" usualmente se refiere a cualquier rasgo considerado ventajoso; los biólogos evolutivos restringen el término para los rasgos que se originan y se mantienen por selección. Según su definición, muchos rasgos fisiológicos pueden meramente reflejar herencia transmitida por generaciones. Al considerar la evolución de la tolerancia a la disponibilidad reducida de oxigeno, hemos estudiado los pinnípedos, en los que hay dos grupos dominantes, phocids y otariids que varian en sus capacidades de buceo, y generaciones humanas expuestas por varios tiempos generacionales a hipoxia hipobárica. Los principios básicos de la evolución de los sistemas fisiológicos complejos emergieron primero del análisis de la respuesta de buceo. Luego analizamos las respuestas del ser humano a la hipoxia hipobárica en tres grupos: nativos de nivel del mar, nativos Andinos (Quechuas) y nativos de los Himalayas (Sherpas). Como en el ejemplo de los pinnípedos, encontramos caracteres fisiológicos 'conservadores' y 'adaptables' implicados en las respuestas humanas a la hipoxia. Los caracteres 'conservadores'son claramente dominantes y son muy numerosos para delincarlos en detalle; tres ejemplos son la afinidad de la hemoglobina, la organización muscular en diferentes tipos de fibras, y la preferencia casi exclusiva que tiene el cerebro por la glucosa como un combustible. Es muy notable que también encontramos evidencia de caracteres 'adaptables' a todos los niveles de organización examinados. Al nivel corporal total en Quechuas y Sherpas, encontramos (i)que las máximas capacidades de ejercicio aeróbicas y anaeróbicas tenían "desensibilización", (ii) que el efecto agudo de la hipoxia (compensador del déficit de energia debido a la carencia de oxigeno, i.e. el efecto Pasteur) esperado de los nativos de nivel del mar estaba atenuado, y (iii) que los efectos de la aclimatación estaban también atenuados. La conducta bioquímica de los músculos esqueléticos fue congruente con un menor uso de la glicólisis anaeróbica como fuente de energia, resultando así en un un mejor rendimiento de ATP por mol de carbono utilizado. Las adapuaciones del corazón también parecieron depender de ajustes de eficiencia estoiquiométrica, mejorando el rendimiento de ATP por mol de oxigeno consumido (usando de preferencia la glucosa en lugar de los ácidos grasos). La mayoría de las adaptaciones bioquímicas y fisiológicas que notamos (ya sea como respuestas agudas o como respuestas de aclimatación) fueron similares en Sherpas y Quechuas. Estos dos grupos no han compartido un ancestro común por más o menos 1/3 de la historia de nuestra especie, de manera que asumimos tentativamente que sus rasgos fisiológicos similares se originaron independientemente como adaptaciones de defensa a la hipoxia en dos tiempos y lugares diferentes de nuestra historia. Al igual que en la evolución de las delicadas capacidades para el manejo del oxigeno cada vez más escaso en los animales buceadores, la evolución de la tolerancia humana a la hipoxia puede ser descrita en términos de cómo dos categorías de caracteres fisiológicos (conservadores vs adaptables) son ensamblados en diferentes linajes humanos y cómo cambia el ensamblaje a través de generaciones. Evidencias más recientes que indican que nuestra especie ha evolucionado bajo condiciones 'más frías', 'más secas' y 'de más altitud', sugieren que estas adaptaciones pueden representar la condición fisiológica 'ancestral' para los humanos.

Palabras claves: Hipoxia, Hipobaria, Fisiología evolutiva, Fisiología del buceo, Mamíferos marinos, Adaptación a la altura

RÉSUMÉ: Evolution de la tolérance à l'hypoxie : le modèle du pinnipède plongeur et le modèle humain d'hypoxie hypobare.

Pour les physiologistes, le terme "adaptation" se réfère généralement à n'importe quel caractère considéré avantageux. Les biologistes évolutifs restreignent le terme aux caractères qui apparaissent et subsistent par sélection. Selon leur définition, de nombreux caractères physiologiques peuvent être le simple reflet de l'héritage transmis à travers les générations. Considérant l'évolution de la tolérance à une disponibilité réduite en oxygène, nous avons étudié les pinnipèdes dont les deux groupes dominants, les phocides et les otarides, présentent des capacités variables de plongée, et les lignées humaines exposées à l'hypoxie hypobare pendant des périodes générationnelles variables. Les principes de base de l'évolution des systèmes physiologiques complexes ont surgi d'abord de l'analyse de la réponse de plongée. Nous avons analysé ensuite les réponses de trois groupes humains à l'hypoxie hypobare : natifs du niveau de la mer, natifs des Andes (Quechuas) et natifs des montagnes de l'Himalaya (Sherpas). Comme chez les pinnipèdes, nous rencontrons des caractères physiologiques "conservateurs" et "adaptables" intervenant dans les réponses de l'être humain à l'hypoxie. Les caractères "conservateurs" dominent nettement et sont trop nombreux pour que l'on puisse les définir de façon détaillée; trois exemples en sont l'affinité de l'hémoglobine avec l'oxygène, l'organisation musculaire dans différents types de fibres et la préférence quasi exclusive du cerveau pour le glucose comme combustible. De même, la présence de caractères "adaptables" à tous les niveaux

d'organisation examinés est tout à fait remarquable. Au niveau corporel total nous avons trouvé chez les Quechuas et les Sherpas, (1) que les capacités maximales d'exercice aérobique et anaérobique étaient "down-regulated"; (2) que l'effet aigu de l'hypoxie (compensant le déficit d'énergie dû au manque d'oxygène, p. ex. l'effet Pasteur) auquel on pouvait s'attendre chez les natifs du niveau de la mer était atténué; (3) que les effets de l'acclimatation étaient également atténués. Le comportement biochimique des muscles squelettiques était congruente à la moindre utilisation de la glycolyse anaérobique comme source d'énergie, dont le résultat était un meilleur rendement de l'ATP par mole de charbon utilisé. Les adaptations du coeur paraissaient dépendre également d'ajustements de l'efficience stoechiométrique, améliorant le rendement de l'ATP par mole d'oxygène consommé (en utilisant le glucose de préférence aux acides gras). La majorité des adaptations biochimiques et physiologiques observées (que ce soit comme réponses aiguës ou réponses d'acclimatation) ont été les mêmes chez les Sherpas et les Quechuas. Ces deux groupes n'ayant pas partagé d'ancêtre commun pendant environ un tiers de l'histoire de notre espèce, nous présumons que leurs caractères physiologiques similaires sont apparus de façon indépendante, comme des adaptations de défense face à l'hypoxie, à des époques et dans des lieux différents. De même que dans l'évolution des très fines capacités d'utilisation de l'oxygène s'affaiblissant de plus en plus chez les animaux plongeurs, l'évolution de la tolérance humaine à l'hypoxie peut être décrite de la façon suivante : comment deux catégories de caractères physiologiques (conservateurs vs adaptables) sont assemblés en différentes lignées humaines et comment l'assemblage change à travers les générations. Les preuves les plus récentes indiquant que notre espèce a évolué dans des conditions "de froid plus intense", "de plus grande sécheresse" ou "d'altitude plus élevée", suggèrent que ces adaptations peuvent représenter la condition physiologique "ancestrale" pour l'être humain.

Mots-clés : Hypoxie, Hypobarie, Physiologie évolutive, Physiologie de plongée, Mammifères marins, Adaptation à l'altitude.

SUMMARY: To physiologists, the term 'adaptation' usually refers to any trait which is considered advantageous; evolutionary biologists restrict the definition to traits arising and maintained under selection. By their definition, many physiological traits may merely reflect inheritance passed on through lineage. In considering the evolution of tolerance to reduced oxygen availability, we studied the pinnipeds where the two dominant groups, phocids and otariids, varying in diving capacities, and human lineages exposed for varying generational time periods to hypobaric hypoxia. Basic principles of evolution of complex physiological systems first emerged from analysis of the diving response. We then analyzed human responses to hypobaric hypoxia in three different lineages: lowlanders, Andean natives (Quechuas), and Himalayan natives (Sherpas). As in the pinniped example, we found 'conservative' and 'adaptable' physiological characters involved in human responses to hypoxia. Conservative characters are clearly dominant and are too numerous to outline in detail; three examples are hemoglobin oxygen affinities, muscle organization into different fiber types, and the brain's almost exclusive preference for glucose as a fuel. Most notably, we also found evidence for 'adaptable' characters at all levels of organization examined. At the whole body level in Quechuas and Sherpas, we found (i) that maximum aerobic and anaerobic exercise capacities were down-regulated, (ii) that the acute effect of hypoxia (making up the energy deficit due to oxygen lack; i.e., the Pasteur effect) expected from lowlanders was blunted, and (iii) that acclimation effects were also attenuated. The biochemical behaviour of skeletal muscles was consistent with lowered reliance on anaerobic glycolytic contributions to energy supply, thus improved yield of ATP per mole of carbon fuel utilized. Heart adaptations also seemed to rely upon stoichiometric effficiency adjustments, improving the yield of ATP per mole of oxygen consumed (by using glucose in preference to fatty acids). Most of the biochemical and physiological adaptations we noted (both as acute and acclimation responses) were similar in Sherpas and Quechuas. These two lineages have not shared a common ancestor for about 1/3 of our species history, so we tentatively assume that their similar physiological traits arose independently as hypoxia defense adaptations in two different times and places in our history. As in the evolution of exquisitve capacities for management of oxygen down to vanishing low levels in diving animals, the evolution of human hypoxia tolerance can be described in terms of how two (consesative vs adaptable) categories of physiological characters are assembled in different human lineages and how the assembly changes through generational time. More recent evidence indicating that our species evolved under 'colder, drier, and higher' conditions, suggests that these adaptations may represent the 'ancestral' physiological condition for humans.

Key Words: Hypoxia, Hypobaria, Evolutionary physiology, Diving physiology, Marine mammals, Altitude adaptation

INTRODUCTION

High altitude physiologists commonly assume that the functional responses to hypobaric hypoxia in humans represent hypoxia defense 'adaptations'. To the field of physiology an 'adaptation' - or an 'adaptive' trait - is rather loosely defined as any character whose structure or function improves chances of survival. While admitting that many such traits so identifed by physiology are 'true' adaptations, evolutionary biologists are somewhat more demanding in their criteria. By their definition, a trait can correctly be termed adaptive (i) if it arises by natural selection or (ii) if it is maintained by natural selection - or (iii) if both conditions are realized. By these criteria, many so-called physiological adaptations of humans might simply be ancestral or plesiomorphic traits, inherited and passed on in phylogeny. Since the precise combinations of such active and relatively passive processes may vary, the evolution of complex physiological systems might proceed along complex unexpected trajectories, rather than being directionally selection-driven down simple linear phylogenetic pathways. Hence, human hypoxia defense adaptations can be properly evaluated only in the context of their phylogeny. In considering the evolution of hypobaric hypoxia defense adaptations in humans what seemed to be required was a framework - or rules - by which complex physiological systems evolve. Our first insight into such a framwork arose from studies of the evolution of the diving response in marine mammals, so this is where we shall start.

The Diving Response in Marine Mammals

Although biologists have been intrigued by diving mammals and birds for well over a century, the physiological and metabolic mechanisms now known to permit an air breathing animal to operate successfully deep into the water column were first exposed in the 1930s and 1940s through the pioneering work of Scholander, Irving, and their colleagues (Scholander, 1940; 1963). The basis of their work provided the fundamental foundations of diving physiology, which are now known to include three key physiological 'reflexes': (i) apnea, (ii) bradycardia, and (iii) vasoconstriction and thus hypoperfusion of most peripheral tissues. Scholander referred to these physiological reflexes in combination as the 'diving response', and, in simulated diving under controlled laboratory conditions, he imagined the marine mammal reducing itself to a 'heart, lung, brain machine'. The metabolic representation of this response included the gradual development of oxygen limiting conditions in hypoperfused (ischemic) tissues, with attendant accumulation of end products of anaerobic metabolism (especially lactate and H' ions).

Scholander and many students following in his path observed that the key features of the diving response were evident in many different kinds of animals. Diving bradycardia was often used as a kind of index or indicator of the diving response and it was so seemingly universal among the vertebrates that Scholander (1963) referred to it as the 'master switch of life'. In his day, this 'master switch', or the diving response, was viewed as an obvious 'physiological adaptation' to diving, even if there was little indication as to how the response evolved through any particular lineage. At this time, little attention was paid to the criteria of evolutionary biology: that to be defined as adaptive a character either had to have arisen by natural selection or to be maintained by selective forces.

The advent of modern field study technologies, especially of microprocessor-assisted monitoring of aquatic animals while diving voluntarily in their natural environment (Guppy *et al.* 1986; Hill *et al.* 1987), has confirmed over the last two decades the validity and plasticity of the overall 'diving response' first elucidated in the 1930s and 1940s and has extended the list of key components. For the large seals (the champion divers of the marine mammal world, capable of diving for up to 2 hours at a time to depths of up to 1.5 km!), the major functional characteristics for such sensational diving capabilities include:

(1) apnea, with exhalation upon initiation of diving (for minimizing buoyancy and other pressure-related problems)

(2) bradycardia (in 1: 1 proportion with changes in cardiac output),

(3) peripheral vasoconstriction and hypoperfusion (in order to conserve oxygen for the central nervous system (CNS) and heart),

(4) hypometabolism of (vasoconstricted) ischemic tissues (also in order to conserve oxygen and plasma borne fuels for the CNS and heart).

(5) an enhanced oxygen carrying capacity (enlarged blood volume, expanded red blood cell mass within the blood volume - i.e. higher hematocrit (Hct), higher hemoglobin concentration ([Hb]) in red blood cells, and possibly higher myoglobin concentration ([Mb]) in muscles and heart), and

(6) an enlarged spleen (for regulating the Hct so that a very high 96 of RBCs need not be circulated under all physiological conditions). Additionally

(7) it should be noted that, for really outstanding diving, all of the above characteristics (i) are incorporated with a large body weight (in order to maximize the amount of oxygen that can be carried while minimizing mass-specific energy demands during diving by allometric effects), and (ii) are coupled with slow swimming speed while at sea (to minimize the cost of locomotion while maximizing submergence. hence foraging,. time).

The evidence for these overall patterns arises from studies of several phocid species (for example, see Kooyman et al. 1980; Kooyman, 1985; Guppy et al. 1986; Qvist et al. 1986; Castellini et al. 1992; Hindell et al. 1992; Thompson and Fedak, 1992; Reed et al. 1994; Hochachka et al. 1995; Hurford et al. 1995; Guyton et aL 1995; Butler et al. 1995).

The seals or phocids are one major branch of the pinnipedia; the other major branch, the otariids (sea lions and fur seals) are not as capable divers. When examined closely, however, essentially all of the above traits are also observable in otariids - but not all as amply expressed (see Hochachka and Mottishaw, 1996). Are all these traits 'adaptations' as commonly assumed by physiologists in this area?

Tracing the Evolution of Diving Capacity

To answer this question, what is required is a more

quantitative comparison of diving strategies within the phocids and otariids. When we tried to do this, we encountered two unexpected 'problems'. The first was that numerous physiological characteristics of diving animals - *instead of systematically varying with diving capacities* - were similar in all diving species for which we could find data. For example, values of maximum diving bradycardia (with concurrent peripheral vasoconstrictions) show no consistent phylogenetic patterns or relationship with diving duration. Since even some humans can turn down their heart to below 6 beats per min (Arnold, 1985), it perhaps should not be surprising that most divers can depress heart rate to a few beats per min (the 'floor board' for this function?). This could mean that heart rate is too crude a measure of circulatory control during diving and recovery (and its uniform control is consistent with the basic reflex being nearly universally present in some form in all vertebrates). Or, it could mean that heart rate control is 'conservative' or 'constrained' in evolution due to roles in so many different biological settings that any adaptational changes for diving are too modest to detect with the crude physiological criteria thus far utilized.

Interestingly, a similar situation seems to hold for another physiological character diving hypometabolism which we initially expected to vary with dive time. In earlier studies, we (Guppy et al. 1986; Hochachka and Guppy, 1987; Hochachka and Foreman; 1993) and others (Le Boeuf et al. 1989, 1992; Costa, 1991, 1993; Hindell et al. 1992) explicitly or implicitly assumed that the impressive diving performance of large seals depended in large part on an 'energy conserving' physiology and diving strategy. Central to this was some concept of diving hypometabolism. Subsequent research has uncovered two potential underlying mechanisms: (i) One hypothesis is that hypoperfusion (vasoconstriction) of nonworking peripheral muscles and other tissues is the proximate cause of hypometabolism, with reduction in tissue metabolic rate being a direct function of the reduction in oxygen delivery, a relationship also observed in terrestrial mammals (Hochachka, 1992; Guyton et al. 1995; Hochachka *et al.* 1995a). (ii) An alternate postulate is that regional hypothermia contributes to low metabolic rates, with metabolic suppression being a function of tissue cooling (Hill *et al.* 1987; Andrews *et al.* 1994). While these two mechanisms are not mutually exclusive, it was at first thought that the physiological characteristic of hypometabolism would be largely restricted to the large seals, or at least to phocids. However, recent careful experimental studies with sea lions (animals trained to remain submerged and relatively inactive for defined time periods) indicate that the metabolic rate declines as a direct function of diving duration; the metabolic rate for seven minute diving periods (water temperature at about 15°C) falls to about 50% of resting metabolic rate (RMR). Since the times involved are so short, it is unlikely that regional hypothermia plays a significant role in this metabolic suppression (Hurley, 1996). We interpret this to mean that activation of the diving response automatically leads to hypoperfusion of some tissues/organs and subsequently to diving hypometabolism. On its own, then, this physiological character, like bradycardia, would appear to be general among pinnipeds and thus could not be expected to vary (and indeed did not vary) in any systematic way with diving capacity.

The second unexpected 'problem' we encountered was purely practical. Ideally, for tracing the evolution of the diving response in these lineages, one would like to be able to compare all of the above metabolic and physiological characters in numerous phocid and otariid species. However, in reality, detailed information on complex characters such as tissue specific regional hypoperfusion is available for only a few species, so for multiple species comparisons (between phocids and phocids vs otariids), our analysis had to be restricted to only a few data sets: (i) body weight, (ii) spleen weight, and (iii) whole body hemoglobin (defined as the content of Hb in the entire blood volume of the organism).

In terms of diving capacity, the data for phocids were particularly clear. Thus, body weight influences the total onboard oxygen supplies as well as mass-specific energy demands; thus it would be expected - and was found - to vary with diving capacity. The spleen acts as a SCUBA tank (oxygenated red blood cell storage) (Ovist et al. 1986; Hurford et al. 1995). The spleen is controlled by a catecholamine-based regulatory circuitry which also controls several other metabolic and physiological functions during diving-recovery cycles (Hurford et al. 1995; Hochachka et al. 1995a; Lacombe and Jones, 1991). We anticipated and found that spleen weight also varies with diving abilities. Finally, whole body hemoglobin is a direct measure of oxygen carrying capacity; since maximum diving duration is presumably set by some complex balance between oxygen availability and oxygen demand, this too was expected and was found to respond to selection based on diving behaviour (see Hochachka and Mottishaw, 1996, for further discussion of these data).

Diving and Phylogeny

Taken together, the characters we analyzed reflect most of the known components of the diving response and they clearly fell into two kinds of categories - conservative vs adaptable. Included in the former are a number of diving 'characters' showing little variation in phocids and otariids, such as diving apnea, bradycardia, and regulated redistribution of cardiac output. The universality of such diving response traits, the fact that they can be elicited in terrestrial mammals including man (Arnold, 1985), suggests the possibility that their occurrence in diving animals is less 'an adaptation' for diving than it is a plesiomorphic or ancestral trait that simply preadapted air breathing vertebrates for dealing with a variety of stressful situations, including diving.

In contrast, the physiological characters which do vary, not only differ qualitatively between the pinniped lineages in the expected direction, but even more quantitatively correlate with dive time in phocids. Although our analyses do not expose causes (they expose correlations), these traits are the probable 'adaptations' accounting for the differences in diving capabilities observed in pinnipeds. Our analysis thus is consistent with the hypothesis that increased expressions of any of at least three factors - (i) body weight, (ii) spleen weight (independent of body weight) and (iii) whole body Hb (also independent of body weight) - are adaptations for extending diving duration in Phocids.

Interestingly, none of the above diving traits correlated as well with diving capacity in the sea lions and fur seals. While the apparent lack of significant correlations within otariids may be a simple artifact of the available data, three other possibilities are (i) that we did not have enough data for otariid species to reach the statistical power required, (as is probably the case in body weight vs. dive time), (ii) that variation in otariid maximum diving duration is not large enough for our relatively insensitive diving response characters to decipher any adaptive trends, (otariids are more closely related than phocids and may be less variable as a consequence), or (iii) that the evolution of the otariids has been 'driven' by factors other than requirements for long duration diving (such as reproductive requirements (Costa, 1991, 1993)).

Emerging Principles of Evolution of Physiological Systems

From the quantitative analysis of the variability of the diving response in pinnipeds (Hochachka and Mottishaw, 1997; Mottishaw, 1997; Mottishaw and Hochachka, 1997) three principles of evolution of the diving response emerged which may be generally applicable to the evolution of complex physiological systems.

1. A number of physiological/biochemical characters considered necessary in diving animals are conserved in all pinnipeds; these traits, which are necessarily similar in phocids and otariids, include diving apnea, bradycardia, tissue hypoperfusion, and hypometabolism of hypoperfused tissues. At this stage in our understanding of diving physiology and biochemistry, we are unable to detect any correlation between these characters and diving capacity.

2. A number of physiological/biochemical characters are more malleable and are clearly correlated with long duration diving and prolonged foraging at sea. These characters are more lineage specific, and, for the phocids include body weight, spleen weight, and whole body oxygen carrying capacity. Within the phocids, the larger these are, the greater the diving capacity (defined as diving duration). Since the relationships between diving capacity and spleen weight or between diving capacity and whole body oxygen carrying capacities are evident even when corrected for body weight, it is reasonable to suggest that the two traits - large spleens and large whole body oxygen carrying capacities extend diving duration. That is, in contrast to conserved traits such as bradycardia, these characters (and presumably other similar ones, such as tissue specific metabolic organization (Hochachka and Foreman, 1993)) have evolved to enable prolonged dive times. We conclude that increased spleen size and O_2 carrying capacity are likely to be physiological adaptations for increased diving duration.

3. The evolutionary physiology of the diving response thus can be described in terms of the degree of development of adaptable vs conservative categories of diving characters: i.e., in terms of how these patterns change through time and how the patterns are lineage specific.

These principles supply us with a framwork for evaluating the evolution of hypoxia tolerance in humans and may allow evaluation of so-called 'true' physiological adaptations against hypoxia.

Human Response to Hypobaric Hypoxia

To this readership, the basic outlines of how our species defends against hypobaric hypoxia are well known and are clearly dependent upon the time available for the response. For convenience, we can categorize acute human hypoxia defense responses into several categories (see Winslow and Monge, 1987, for literature in this area):

1. increased ventilation mediated through the 0_2 sensing system in the carotid body (see Lahiri, 1984; 1996),
2. increased pulmonary vasoconstriction mediated through the 0_2 sensor in the pulmonary vasculature (Oelz et al, 1990; Anand and Chandrashekhar, 1990),
3. increased heart rate possibly with some redistribution of cardiac output (Richalet, 1990)*
4. increased perfusion especially of heart and brain, pH or modulator-mediated adjustment in Hb affinity for 0_2, and increased 0_2 extraction (see Winslow and Monge, 1987; Monge and Leon-Velarde, 1991),
5. peripheral tissue oxygen limitation during exercise, a reduction in $V0_2$max (more notable in endurance trained athletes) and consequent augmentation of anaerobic metabolic pathways (higher plasma and muscle [lactate] values for any given exercise intensity - a special version of the Pasteur effect (see Hochachka et al, 1991; 1992)), and
6. increased catecholamine release and involvement in the regulation of several of the above processes (Richalet, 1990: 1997).

Taken together these adjustments momentarily compensate for the reduced availability of 0_2 in the inspired air. If the hypobaric hypoxia is not too severe, these mechanisms can fully compensate for reduced 0_2 supply. The cutoff point seems to be at about 3500 meters (see Richalet, 1990, for example), above which the compensation is incomplete and most unacclimated humans suffer various consquences (physiological defenses collapse into pathophysiology). That is why, from a purely physiological point of view, there is a need for developing backup acclimation (or acclimatization) defenses. Again, these are now well described in the literature and for lowland lineages can be summarized as follows:

1. hypoxic ventilatory response further exaggerated, implying an increased 0_2 affinity of the 0_2 sensing system in the carotid body (Lahiri, 1984; Biscard and Forster, 1996),
2. hypoxic pulmonary vasoconstriction maintained (Heath and Williams, 1981; Oelz et al, 1990) and may be further exaggerated, mediated by the pulmonary endothelium 0_2 sensor in an attempt to redistribute perfusion of the lung most advantageously,
3. heart rate, especially the maximum rate during exercise (Richalet, 1990), dampened as erythropoiesis increases the hematocrit and 0_2 carrying capacity of the blood (mediated by an 0_2 sensing system primarily in the kidney (Winslow and Monge, 1987)),
4. perfusion of heart and brain dampened to a new steady state also possible because of increased hematocrit (Severinhaus et al, 1966; Huang et al, 1987; Krasney et al, 1990; Richalet, 1990),
5. improved perfusion and oxygen supply to peripheral tissues, a partial recovery of $V0_2$max, and thus (Hochachka et al, 1991) reduced reliance during exercise on anaerobic augmentation of tissue energy production (lowering of [lactate] levels for a given level of exercise), and
6. a maintained catecholamine response (Richalet, 1990; 1997) involved in the regulation of several of the above processes.

Again, taken together these acclimation processes go a long way towards re-establishing physiological and metabolic homeostasis under hypobaric hypoxia. When, if ever, they are complete is a poorly explored area of research. Some kinds of acclimation response may continue on indefinitely. Monge's disease - a situation in which the hematocrit continues to rise throughout the lifetime of the individual - may be such a process gone somewhat out of control and thus referred to by Monge as a 'maladaptation' (see Monge and Leon-Velarde, 1991; Heath and Williams, 1981).

For lowlander Caucasians, the above acute and acclimatory processes constitute the only available defense arsenals against hypoxia. However, similar studies of highlander lineages (Quechuas and Aymaras from the Andes; Sherpas and Tibetans from the Himalayas) indicate some differences in both acute and acclimation responses. These indicate that over generational time, each of the general steps in the above hypoxia defense responses - from hypoxia sensing, through signal transduction pathways, to integrated metabolic9and physiological responses - apparently can be further adjusted. For hypoxia tolerance in the human species, we do not have enough data of the type described for the diving model to do a quantitative

analysis of evolutionary pathways within our species. Nevertheless, when we extend the timeline of hypoxia defense through generations, we begin to see that (as in the diving model) many physiological traits involved in hypoxia tolerance are highly conservative while others are more malleable.

Most Physiological and Metabolic Characters in Humans Are Conservative

For example, the brain and CNS of low altitude and high altitude natives express the same absolute preference for glucose as a carbon and energy source. What is more, regional brain specializations and hence regional brain differences in glucose metabolic rates show the same patterns in both high and low altitude humans (see Hochachka et al, 1995b, 1996a,b). Thus as 'physiological characters' both glucose preference and regional metabolic organization are highly conservative. Because we are dealing with comparisons within a single species (structures, functions, and control circuitries are necessarily built on the same biological plan), it is perhaps not surprising that most physiological and metabolic characters associated with hypoxia tolerance in humans display conservative aspects. Thus these deserve no further emphasis at this time. Instead, it is the malleable or 'adaptable' physiological and metabolic 'characters' that attract our greater interest.

Malleable or 'Adaptable' Traits in Human Hypoxia Tolerance

In contrast to the above kinds of features, relatively malleable traits are exposed when we compare the acute and acclimatory responses of Andean and Himalayan lineages to those of Caucasian lowlanders. Such comparative studies of highlander lineages indicate that

1. the hypoxic ventilatory drive is notably blunted, especially in Andean natives (Strohl and Beall, 1997), implying adjustments in the 0_2 affinity of the carotid body 0_2 sensing mechanism and in its regulation (Lahiri, 1984; 1996),
2. the hypoxic pulmonary vasoconstriction may be reduced (Heath and Williams, 1981), implying adjustments in the pulmonary vasculature 02 sensing system (Anand and Chandrasheckhar, 1992),
3. the lung diffusion capacity may be expanded to compensate for the hypobaric hypoxia of the environment (especially evident in Quechuas of the Andes (see Winslow and Monge, 1987),
4. a requirement for increased erythropoiesis may remain (in Andean natives) or may be de-emphasized (in Sherpas and Tibetans (Beall and Goldstein, 1990), but energy balance in different metabolic states (Hochachka et al, 1991) is maintained (presumably through perfusion adjustments in peripheral tissues and in part because of reduced oxidative capacities coordinated with reduced 0_2 demands),
5. heart rates during rest and exercise are lower, in part because the catecholamine response is blunted (see below) and in part because of increased preference for glucose as a fuel for the heart (Hochachka et al, 1996a) and reliance on carbohydrate during skeletal muscle work (Hochachka et al, 1991), thus improving the yield of ATP per mol of 0_2.
6. enzymic capacities of aerobic and anaerobic metabolic pathways in muscles are down-regulated (relative preponderance of slow twitch fibers) so that overall $V0_2max$ and anaerobic energy contributions are both relatively low (the higher the altitude, the lower the [lactate] for any given exercise level despite the hypoxia - a situation termed the 'lactate paradox' in the literature (Hochachka et al, 1991; 1992),
7. the overall performance strategy appears to be 'to go slowly but efficiently', and
8. the catecholamine response to hypoxia is relativley blunted even compared to hypoxia acclimated lowlanders (Richalet, 1997).

Are these true biological adaptations? Or are they merely inherited (ancestral) physiological responses in different lineages to the same environmental problem of hypobaric hypoxia?

Satisfying 'Adaptation' Criteria of Evolutionary Biologists

To adequately answer this question we need to recall the different ways in which this term is used by physiologists and by evolutionary biologists. As mentioned above, to physiologists a trait is frequently termed an 'adaptation' if its function improves chances of survival. Evolutionary biology is more demanding in its definition; in this discipline, a trait is termed an adaptation only (i) if it arises under natural selection, and/or (ii) if it is maintained by natural selection. Within the human species demonstrating these two properties (i.e. satisfying these two criteria) is no easy matter. Hence, indirect evidence is required to demonstrate

whether or not a complex physiological system such as 'hypoxia tolerance' is an adaptation. One such line of evidence involves demonstrating that the same or a similar physiological suite of traits arises in response to a given selective force more than once within the phylogenetic group under analysis. Based on the above analysis, the complex physiological system we need to evaluate can be summarized as 5 loosely linked functions:

1. Blunted hypoxic ventilatory response (carotid body 02 sensor)
2. Blunted hypoxic pulmonary vasoconstictor response (pulmonary vasculature 02 sensor),
3. Dampened heart rate and heart work (in part probably due to increased preference for carbohydrate carbon and energy source, and hence inccreased yield of ATP per mol 02 and in part due to increased hematocrit),
4. Decreased reliance upon anaerobic metabolic pathways in peripheral tissues (so called 'lactate' paradox), relative preponderance of slow twitch (ST) fibers in skeletal muscles with low oxidative capacities of all fiber types, and
5. Blunted catecholamine response to hypoxia, indicative of efficacy of above metabolic and physiological adjustments.

That these physiological traits are indeed at least loosely 'linked' is indicated by the fact that most of them are also found in humans adapted for endurance performance (see Hochachka, 1991). In endurance trained athletes these series of traits appear as high performance versions of those found in high altitude natives. Although the high performance features probably are a part of the explanation for greater percentage effects of hypoxia on $V0_2max$, an endurance athlete could be viewed as a high performance version of indigenous highlanders (such as Quechuas and Sherpas) and vice versa. Put another way, the biochemical and physiological organization of both indigenous highlanders and endurance athletes differ strikingly from the homologous organization in 'burst performance' individuals (where FT fibers form a larger percentage of skeletal muscle, exercise-induced plasma lactate concentrations can reach very high levels, and cardiovascular adjustments play as important a role in recovery from performance as they do during performance per se).

If these 5 loosely linked components compromise our species' 'solution' to the environmental 'problem' of hypobaric hypoxia, the question arises of whether the same 'solution' has arisen more than once in our species history this would be good evidence for evolutionary adaptation. A search for such evidence requires insight into the evolutionary pathways of our species. To this end, we constructed a simplified 'phylogenetic tree' for the human species from an indepth summary of human genetics and evolution by Cavalli-Sforza et al (1994). The main groups whose responses to hypobaric hypoxia to date have been extensively studied are (i) lowland Caucasians and Asians, (ii) Sherpas and Tibetans of the Himalayan plateau, and (iii) Quechuas and Aymara of the Andean range. If we assume that our species age is approximately 100,000 years, then a close examination of such information is highly instructive. First, it suggests that the last time Caucasians, Sherpas and Quechuas shared a common ancestor was over 50,000 years ago. Second, the last time the Himalayan highlanders (Sherpas and Tibetans) and the Andean highlanders (Quechuas and Aymaras) shared a common ancestor was in the range of 30,000 years ago - or about 1000-1500 generations ago. Third, despite this distant divergence of the latter two lineages, their metabolic and physiological responses to hypobaric hypoxia are similar. Fourth, many other lineages (including intermediate branches in the 'phylogenetic' tree of the human family) are known not to show these characteristics.

These phylogenetic data are consistent with two possible scenarios: (i) One possibility is that, with only modest differences, many similar metabolic and physiological 'solution' arose independently in these two lineages. If so, these comparisons satisfy at least one of the criteria of evolutionary biology and strongly support the conclusion that the above suite of physiological characters are defense adaptations against hypobaric hypoxia. (ii) A second possibility is that the above suite of physiological and metabolic traits represent the 'ancestral' condition, a view consistent with recent indications that the origins of our species occurred under conditions that were getting colder, drier, and higher (see Vrba et al, 1995). Over some 5000 generations of our species history, this condition was 'retained' in high altitude groups (Sherpas, Tibetans, Quechuas, Aymaras) and in groups selected for endurance performance. In the former, the ancestral physiological condition was 'low-capacity', fine-tuned for 'go slow but effficiently' under hypobaric hypoxia. In the latter groups, the ancestral physiological condition in relative terms became a 'high capacity' one, high capacity energy supply pathways being fine-tuned for the high capacity energy demand of sustained performance. In this scenario, the ancestral organization of our physiology was inherently very dependent upon 'aerobic' pathways, with relatively minor

development of, or reliance on, anaerobic metabolic systems.

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OXYGEN SENSING IN THE ORCHESTRATION OF HYPOXIC METABOLIC ARREST .

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RESUMEN: La Precpción del Oxígeno en la Organización del Paro Metabólico Hipóxico

La base metabólica del mecanismo sensor de oxígeno ha sido estudiada utilizando hepatocitos aislados de la tortuga pintada occidental, un vertebrado anaerobio facultativo. Como parte de una respuesta sistémica coordinada a la hipoxia, estas células suprimen activamente la síntesis de ATP en sincronía con la demanda de ATP por parte de todos los consumidores principales (Na^+ -K^+ ATPasa, Ca^{2+} ATPasa, recambio de proteínas, síntesis de urea, liberación de glucosa y gluconeogénesis). El resultado es una supresión en 10 veces de la tasa metabólica, impuesta sobre una redistribución de la demanda de ATP entre los procesos celulares. Esta reorganización metabólica dependiente de oxígeno está estrechamente controlada, es rápida en su inicio, ocurre sin perturbación de las concentraciones de adenilato o del potencial de membrana, implica la supresión y expresión de genes dependientes de oxígeno y mantiene una nueva tasa, inferior de flujo a través de vías bioquímicas específicas hasta la reoxigenación. El efecto neto ahorra dramáticamente sustrato fermentable, limita las tasas de acumulación de deshechos metabólicos y extiende profundamente el tiempo de supervivencia en anoxia. Se ha explorado roles directos de mecanismos receptivos de oxígeno en el control de las tasas de flujo para dos eventos celulares significativos luego de supresión metabólica hipoxica. El recambio proteico, que es el proceso celular energéticamente más costoso en normoxia mostró una modulación dependiente de oxígeno de bandas proteicas específicas cuya expresión podía ser predeciblemente manipulada por Co^{2+}, Ni^{2+} y CO. Esto apoya la teoría de un rol para el mecanismo receptor de oxígeno hem-proteína en el control de la expresión hipóxica de genews al ingresar en paro metabólico. Segundo, estudios no invasivos que usan electrodo de autoreferencia selectivo de Ca^{2+}demuestran que hay una supresión selectiva del 75% en el flujo transmembrana de Ca^{2+} que es oxígeno-concordante y que exhibe un KmO_2 aparente de 145 µM. La supresión del flujo de Ca^{2+} era dependiente de proteína kinasa y no era repetible bajo inhibición anaeróbica de la transferencia de electrones por KCN. Estos resultados sugieren que las respuestas hipóxicas de diferentes procesos confluyen para formar una reorquestación metabólica y molecular coordinada de la función celular que permite la supervivencia prolongada sin oxígeno. parte integral de ello es el potencial evidente de los mecanismos receptivos de oxígeno para señalar y coordinar los cambios de flujo a través de vías complejas, energéticamente costosas.

Palabras claves: Oxígeno, Paro metabólico, Respuesta hipóxica.

RÉSUMÉ: La perception de l''oxygène dans lórganization de lárrêt métabolique hypoxique

La base métabolique et moléculaire du mécanisme capteur d'oxygène a été étudié en utilisant des hépatocytes extraits de la tortue tachetée occidentale, un vertébré anaérobie facultatif. Comme élément d'une réponse systémique en relation avec l'hypoxie, ces cellules suppriment activement la synthèse de l'ATP en synchronisation avec la demande d'ATP de la part de tous les consommateurs principaux d'énergie (Na^+/K^+ ATPase, Ca^{2+} ATFase, remplacement de protéines, synthèse de l'urée, libération de glucose et néoglucogenèse). Le résultat est une suppression en 10 fois du taux métabolique, se surimposant à une redistribution de la demande d'ATP entre les processus cellulaires. Cette réorganisation métabolique dépendante de l'oxygène est étroitement contrôlée; elle est rapide à son début, se déroule sans perturbation des concentrations d'adénilate ou du potentiel de membrane; elle implique la suppression et l'expression de gènes dépendants de l'oxygène et soutient un nouveau taux, inférieur, de flux à travers des voies biochimiques spécifiques jusqu'à la réoxygénation. L'effet net économise considérablement le substrat fermentable, limite les taux d'accumulation des déchets métaboliques et allonge énormément le taux de survie en anoxie. Le rôle direct de mécanismes récepteurs d'oxygène dans le contrôle des taux de flux pour deux événements cellulaires significatifs après suppression métabolique hypoxique a été exploré. Le remplacement protéique, le processus cellulaire qui requiert le plus d'énergie en normoxie, a montré une modulation dépendante de l'oxygène de bandes protéiques spécifiques dont l'expression pouvait être manipulée de façon prévisible par Co^{2+}, Ni^{2+} et CO. Cela appuie la théorie du rôle du mécanisme récepteur d'oxygène heme-protéine dans le contrôle de l'expression hypoxique de gènes en entrant en arrêt métabolique. D'autre part, des études non invasives utilisant une électrode d'autoréférence sélective de Ca^{2+} démontrent qu'il y a une suppression de 75 % du flux transmembranaire de Ca^{2+}, qui est oxygéno-concordant et présente un KmO_2 de 145µM. La suppression du flux de Ca^{2+} était dépendant de la protéine kinase-c et ne pouvait être répétée sous inhibition aérobique du transfert d'électrons par KCN. Ces résultats suggèrent que les réponses hypoxiques des différents processus cellulaires se rejoignent pour former une réorchestration métabolique et moléculaire coordonnée de la fonction cellulaire, permettant la survie prolongée sans oxygène. Le potentiel évident des mécanismes récepteurs d'oxygène fait partie intégrante de ce processus pour signaler et coordonner les changements de flux à travers des voies complexes, très coûteuses du point de vue énergétique.

Mots-clés : Oxygène, Arrêt métabolique, Réponse hypoxique

SUMMARY: The metabolic and molecular basis of oxygen sensing has been probed using isolated hepatocytes from the vertebrate facultative anaerobe, the western painted turtle. As part of a coordinated systemic response to hypoxia, these cells actively suppress ATP synthesis in synchrony with ATP demand from all major energy sinks (Na^+/K^+ ATPase, Ca^{2+}ATPase, protein turnover, urea synthesis, glucose release and gluconeogenesis). The result is a 10-fold suppression in metabolic rate superimposed over a re-partitioning of ATP-demand among cellular processes. This oxygen-dependent metabolic re-organization is tightly controlled, being rapid in onset, occurs without perturbation to adenylate concentrations or membrane potential, it involves the oxygen-dependent suppression and expression of specific genes and it sustains a new, lower rate of flux through specific biochemical pathways until re-oxygenation. The net effect dramatically spares fermentable substrate, limits rates of metabolic waste accumulation and profoundly extends survival time in anoxia.

Direct roles for oxygen-receptive mechanisms in the control of flux rates have been explored for two energetically significant

cellular events during the onset of hypoxic metabolic suppression. Protein turnover, the most energetically costly cell process in normoxia, exhibited oxygen-dependent modulation of specific protein bands whose expression could be predictably manipulated by Co^{2+}, Ni^{2+}, and CO. This supports a role for heme-protein based oxygen receptor mechanism in the control of hypoxic gene expression on entering metabolic arrest. Secondly, noninvasive studies using a Ca^{2+}-selective self-referencing electrode demonstrate that there is a 75% suppression in transmembrane Ca^{2+}-flux that is oxygen conforming, exhibiting an apparent KmO_2 of 145μM. The suppression of Ca2+-flux was protein kinase-c dependent, and was not repeatable under aerobic inhibition of electron transfer be KCN.

These results suggest that the hypoxic response of different cellular processes coalesce to form a coordinated metabolic and molecular re-orchestration of cell function that enables longterm survival without oxygen. Integral to this is the clear potential for oxygen-receptive mechanisms to signal, and coordinate, flux changes through complex, energetically expensive pathways.

Key Words: Oxygen, Metabolic arrest, Hypoxic response

INTRODUCTION.

Although emerging studies indicate that there is a genetically-linked tolerance to hypoxia among high altitude human populations, generally, we are not a species that tolerates any degree of oxygen limitation at all well. Chronic exposure to high altitude hypoxia is both debilitating and potentially lethal, so for these reasons, biomedical research is particularly driven to determine how the oxygen signal is perceived at the organ-to-cell level, how resulting physiological changes come about, and how clinical strategies can be developed for the prevention and treatment of hypoxic pathologies. The research base is therefore directed towards understanding and sustaining a system that is failing during mild-to-moderate oxygen lack.

This review addresses the question of hypoxic survival by examining how naturally evolved hypoxia-tolerant systems deal with chronic exposure to anoxia. Hypoxia tolerance is particularly well developed in the Western painted turtle, a vertebrate facultative anaerobe that is capable of surviving systemic anoxia for up to 2 weeks at 25°C, extending upwards to 6 months at temperatures <10°C. This extreme capacity for anoxic survival is based on an oxygen-sensitive re-organization of cellular metabolism, superirnposed upon a cell biology that is geared towards the long-term provision of energy via glycolysis, and a capacity to deal with the accumulation of toxic metabolic end-products (lactate and protons). In keeping with the questions central to high altitude biomedical research, this review presents studies conducted on hepatocytes isolated from this species which were used to understand how cellular metabolism is re-orchestrated in anoxia, at what advantage to the survival of the organism and the role of the oxygen signal in coordinating cellular events among complex energy-demanding processes.

Orchestrating Coordinated Adjustments Between Cellular Processes.

Four general elements comprise cellular metabolism: i) the generation of reducing power, that drives *ii*) ATP synthesis; *iii*) a selectively permeable membrane that generates the potential energy for driving metabolic processes and *iv*) lipid, protein and nucleic acid biosynthesis. Due to the inter-relatedness of each of these processes, physiological responses to environmental change requires coordination between all of these components; the failure of one link in the chain ultimately results in the failure of the loop.

From single cells to whole organisms, metabolic responses to hypoxia can be broadly categorized into one of two adaptive responses (1). The response associated with oxygen regulation maintains the provision of energy via a Pasteur effect (enhanced rate of glycolytic flux) to support cell processes at rates approaching those found during oxidatively supported metabolism. Finite stores of fermentable substrate, and the accumulation of metabolic wastes (lactate, H^+), together, set time-restrictions on the success of oxygen regulation as a useful means for surviving oxygen lack. In contrast, the oxygen conforming response minimizes rates of energy supply coordinately with energy demand to invoke a controlled suppression of metabolic rate below resting levels. Figure 1 compares the oxygen-regulating (generally anoxia intolerant) versus conforming strategies (anoxia tolerant) for dealing with hypoxia. The trade-offs are clear and define remarkably different time limits for tissue function and survival without oxygen. In oxygen regulators, depletion of available substrate, and the rapid accumulation of toxic metabolic endproducts, leads to failure of the Na^+/K^+ ATPase, membrane depolarization, Ca^{2+} release from internal stores followed by capacitative Ca^{2+} entry over the cell membrane, and cell death (2). Although survival time is dependent on the severity of hypoxia, oxygen regulating systems will typically cope with severe hypoxia over a time frame metered in minutes. The oxygen conforming response, on the other hand, favors cell survival at the expense of metabolic scope. ATP demand and supply are coordinately down-regulated requiring specialized adaptations in cell physiology to cope with attenuated energy turnover. Principle among these

are the capacity to maintain membrane polarity, stability of macromolecules, and remain capable of detecting changes in oxygen availability (reviewed in ref. 3). The combined effect results in a survival time in severe hypoxia that is extended from months, outwards to years in some cases.

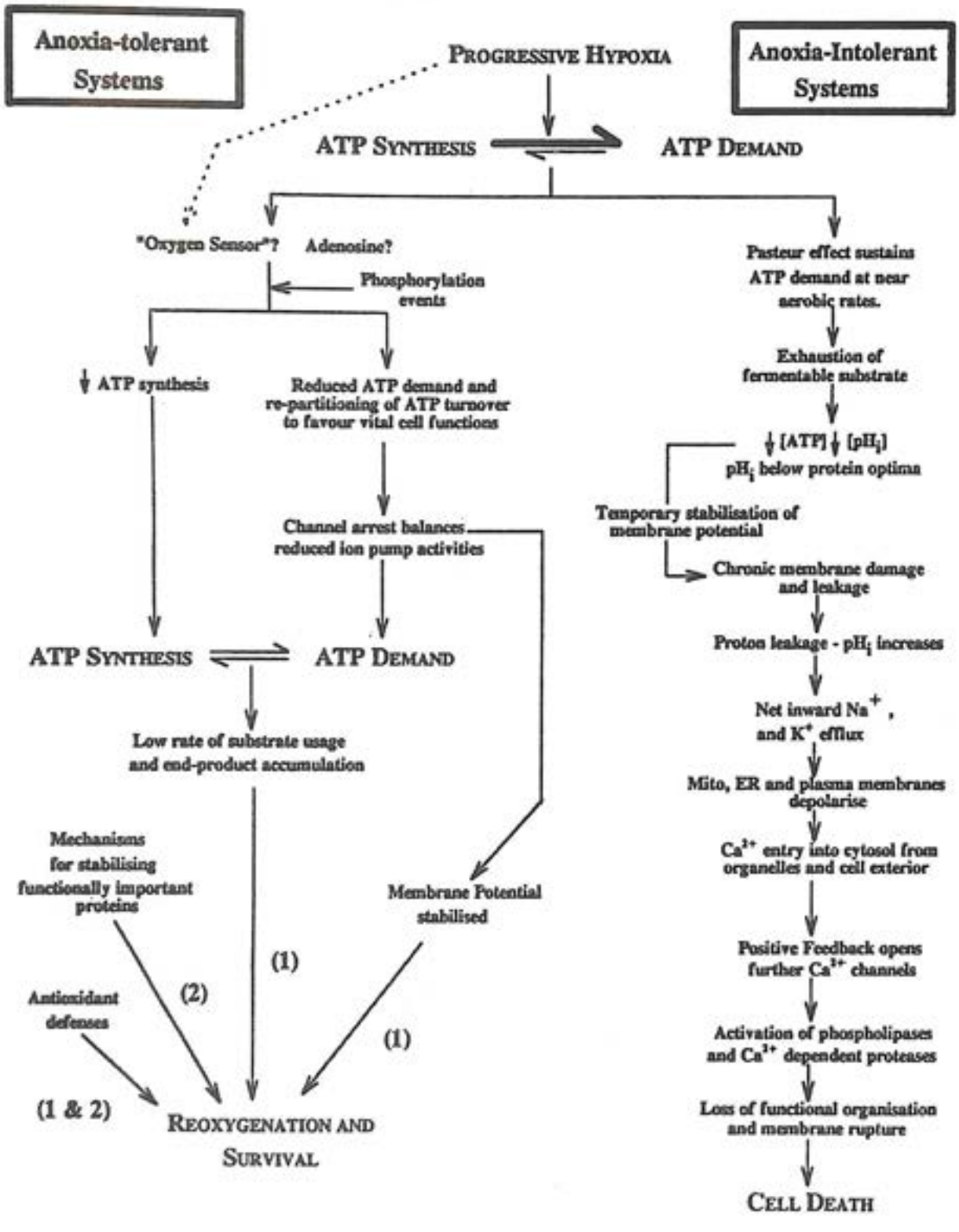

Figure 1. The pathway of acute cellular damage invoked by severe hypoxia in anoxia in-tolerant (typically oxygen-regulating) tissues, and the mechanism of avoidance in anoxia-tolerant (oxygen conforming), tissues. Anoxia-tolerant paths marked (1) denote adaptive metabolic readjustments that occur early in the transition towards hypoxia. Paths marked (2) denote probable genotypic features (ie these are physiologically inherent to the species). Figure derived from references 1-3, 10 and 13.

Oxygen regulation and oxygen conforrning responses deal with hypoxia in strikingly different ways, yet lack of oxygen is the primary signal for the induction, and presumably control, of each response. The question posed here asks: ***How does the oxygen signal coordinate all principle components of metabolism together towards an effective strategy of hypoxia survival?***

Metabolic Suppression in Response to Hypoxia, Studied in Cultured Isolated Turtle Hepatocytes.

Naturally evolved tolerance to hypoxia comprises a series of more-or-less conserved physiological characteristics that are common among species that occupy periodically hypoxic or anoxic habitats. The systemic response to hypoxia is typically oxygen conforming and involves a coordinated suppression of metabolism below standard metabolic rates. This suppression necessarily involves coordination between every aspect of cellular function residing on both ATP synthesis and demand sides of energy turnover. It therefore constitutes a useful system to probe the relationship between an oxygen signal and the metabolic, cellular, and molecular adjustments that are necessary for the periodic and long-term survival of anoxia.

We studied the cellular basis for anoxia-tolerance using hepatocytes isolated from the Western Painted Turtle (*Chrysemys picta bellfi*). As a component of the systemic metabolic suppression during hypoxia, these cells will actively suppress ATP synthesis synchronously with demand from major ATP sinks (ion pumping, protein turnover, urea synthesis, glucose release), even when isolated and maintained in a culture environment. This presented a unique opportunity to examine the cellular and molecular basis of metabolic suppression and in particular the relationship of the cessation in cellular function to the disappearance of oxygen itself. To address this issue fully, it was irnportant to understand which cellular processes constituted the major energetic drains, and to what extent their suppression contributed to the overall metabolic suppression in anoxia. The first step was therefore to tease apart the ATP synthesis and ATP demand sides of energy turnover to construct an energy budget for these cells in normoxia and after 10 hours of severe anoxia.

ATP synthesis in anoxia Figure (2) demonstrates the ATP synthesis component of ATP turnover during metabolic suppression in turtle hepatocyte primary cultures. Three important features are characteristic: i) there is a 90% fall in metabolic rate (mirrored by a 70% fall in rnicrocalorimetric heat flux), ii) there is little change in the adenylate energy charge, iii) the onset of metabolic suppression is rapid and is fully reversible on reinstatement of a normoxic environment (4,5). Flux through pathways of carbohydrate metabolism change profoundly. Rates of glucose release fall by 60%, remaining attenuated, but continuous, throughout anoxia. Gluconeogenic rates were practically immeasurable constituting an insignificant component of both ATP synthesis and demand in anoxia. Energy production in support of remaining anoxic ATP demand in anoxia relies solely on glycolysis and is fueled by near molar concentrations of on-board glycogen. Experimentally deplete glycogen stores or block glycolytic flux and ATP concentrations, remaining heat flux, and total cell viability all rapidly diminish. Given that optimally minimized, but *sustained*, ATP turnover is essential for maintaining cell viability, what is the response of the major ATP consuming components of ATP turnover?

ATP Demand in Anoxia. The maintenance of ATP concentrations throughout the period of anoxia suggests that the stoichiometry of ATP turnover never gets too far out of balance. Figure (3) demonstrates the re-partitioning of ATP demand among the most energetically expensive cell functions as oxygen availability dissipates. In normoxia, protein turnover (the compounded cost of protein synthesis and protein degradation) accounts for the majority of ATP demand (about 45%). Na^+/K^+ATPase accounts for the majority of the remainder at about 25%. In anoxia, there is a striking re-partitioning of ATP demand among the major energy consuming processes that is superimposed on a 70 to 90% suppression in energy demand for each individual pathway examined. As noted in figre 2, glycolysis supports an anaerobic ATP turnover of 6.5 µmol $ATP.g^{-1}.min^{-1}$, a 10-fold suppression in ATP turnover from normoxic standard metabolic rates. Within anoxic total ATP turnover, energy demand by Na^+/K^+ ATPase accownts for 75% (6). Protein synthesis and degradation are both coordinately suppressed (protein content of the cells does not change) and accownt for the majority of remaining anoxic metabolism (7,8,9). The emerging pattern suggests that normoxic cells expend the majority of energy metabolism on anabolic processes such as protein turnover whereas in anoxia, there is a re-organization of energy expenditive that favors the maintenance of the cellular membrane potential at a proportionate increase in the overall proportion of ion pumping activity. The postulated mechanism behind the maintenance of the membrane potential despite an overall suppression in ion pump activity

(The channel arrest theory) has been reviewed elsewhere (10).

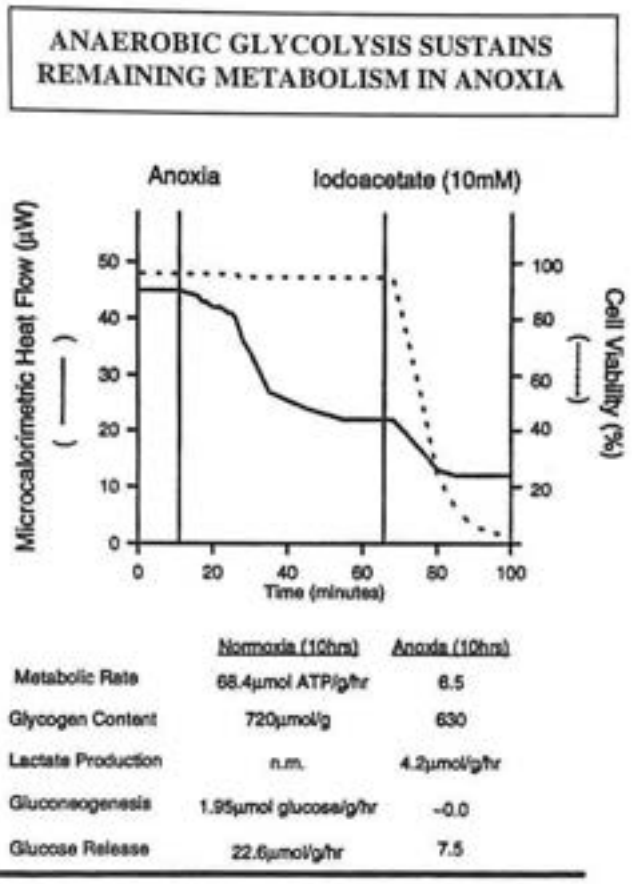

Figure 2. Scheme denoting characteristics of carbohydrate metabolism in support of ATP synthesis in normoxia and over 10h anoxia in isolated turtle hepatocytes. Microcalorimetric heatflow (solid line) falls 70% in anoxia with biochemical determinations of metabolic rate reflecting a 10-fold reduction in ATP turnover rates. Addition of the glycolytic inhibitor, iodoacetate, results in the death of the tissue emphasizing the significance of energy supply via glycolysis and the functional importance of remaining sources of ATP demand in anoxia. Note high internal stores of glycogen in support of glycolytic flux. Figure derived from references 4 & 5

Evidence that an Oxygen Signal Controls Complex ATP Consuming Processes.

The coordinated suppression of ATP turnover, and associated re-partitioning of ATP demand among cellular processes, incorporates nothing short of a complete biochemical and molecular re-arrangement to achieve a metabolically dormant state in hand with a stable cell structure. The crucial issue centers around the signal: do cells possess the capacity to sense changes in oxygen availability and respond in an adaptive manner? To investigate this issue we examined the oxygen-sensing characteristics of protein turnover and transmembrane Ca^{2+}-flux as examples of two complex, energetically expensive, metabolic pathways. As both processes represent the sum of multiple components, each tests the capacity of the oxygen signal to coordinate tightly coupled flux changes between a number of related cell functions.

I) Oxygen Sensing and Protein Turnover.

Protein turnover (protein expression and degradation) accounts for about half of normoxic and about one third of anoxic ATP demand. Aside from defining the functional and phenotypic characteristics of cells, it also maintains the relative health of the functional protein pool by ensuring the removal of damaged and dysfunctional molecules. Suppression of protein turnover is therefore perilous as it diminishes the capacity for cells to adaptively express new functional protein translates, and remove old ones (3). Through the induction of genes, appearance of mRNA, translation of proteins, modification of protein structure, proteolytic turnover of the protein, and the ATP synthesis that is required to support this process, the overall scheme of protein turnover offers a rigorous test of the oxygen signal to coordinate changes among cellular processes.

To tease out the oxygen signal and receptor mechanism, we employed a similar strategy to that employed in the erythropoietin field which aimed to modulate the responsiveness of oxygen dependent cell processes independently of oxygen availability. We could functionally achieve metabolic anoxia despite the presence of oxygen by out-competing oxygen at cytochrome-c oxidase using KCN. By assuming a transduction mechanism based ferro-heme binding of oxygen, we could modulate the conformational state of the putative heme protein by substitution of Co^{2+} or Ni^{2+} ions in the central Fe^{2+} position (induced de-oxygenated conformation) by incubation with carbon monoxide (induces a K_m-dependent oxygenated conformation) (11,12). With this approach, our hypothesis specifically tested the notion that the oxygen signal was transduced through the change in conformation of a heme protein oxygen receptor, linked through an unidentified intracellular signaling pathway.

Our results indicated that a heme protein was indeed involved in transducing an oxygen signal to elevate expression of 5 unidentified protein bands, and decreased expression of a further 5 distinct protein bands (9). This clearly suggests a role for oxygen in the modulation of protein expression at both levels of synthesis and degradation. Furthermore, it highlights a role for oxygen in the fine-tuning of cellular characteristics in the transition towards hypoxia: despite a 10-fold suppression in rates of ATP-dependent protein turnover, the phenotype of the protein pool was subject to oxygen-specific modulation via adaptive expression (or suppression) of sets of hypoxia-sensitive genes (9,13).

A broad body of literature now identifies a clear link between a ferro heme oxygen receptor mechanism and changes in the relative availability of oxygen to mediate expression of the up-stream transcriptional regulator, Hypoxia Inducible Factor-1. HIF-1 is a basic-helix-loop-helix PAS heterodimer consisting of a novel 120kd α-subunit and a 94kd , ß-subunit that is identical to the aryl hydrocarbon nuclear translocator (ARNT), a transcriptional regulator in the dioxin response (14). In hypoxia, HIF-1 regulates increased expression of the hepatic hematopoiesis factor, erythropoietin (12), vascular growth factors (VEGF and PDGF, (15)) and certain specific glycolytic enzyme isoforms in a wide variety of tissues, specifically, phosphofructokinase (PFK-L and C), phosphoglycerate kinase (PGK-A), aldolase (ALD-A and C) pyruvate kinase (PK-A), and lactate dehydrogenase (LDH-A) (15,16). Hypoxia also abrogates receptor-mediated control over the expression of the gluconeogenic rate limiting enzyme, phosphoenolpyruvate carboxykinase (PEPCK, (17)). Recent evidence further implicates a similar oxygen sensing mechanism in the control over glucose transporter expression for the isoforms GLUT 1 and 3 (expressed in hypoxia) and GLUT 2 (suppressed in hypoxia) (16,18). From a metabolic point of view, this list underscores a clear role for oxygen in the control of glucose metabolism and in particular, the expression of rate limiting isozymes for glucose transport, glycolysis and gluconeogenesis as significant control points. A most important feature of the oxygen signal transduction pathway is its capacity for modular control over defined regulatory pathways for the same gene. In liver for instance, glucagon regulates PEPCK expression through a cAMP-dependent protein kinase pathway, unless a significant transition towards hypoxia occurs where control passes to a ferroheme protein/HIF-1 regulatory pathway (17). Clearly, the intracellular signaling pathway from ferro heme protein to the induction of hypoxia-sensitive genes must juxtapose tonic regulatory pathways but how might this occur? A recently proposed hypothesis based on work conducted on carotid body and hepatoma cell lines suggests that a candidate heme-protein/REDOX based oxygen sensor may be NAD(P)H oxidase (19,20). The signal is transduced by reduction of the ferro-heme NAD(P)H oxidase subunit resulting in production of hydrogen peroxide. H202 subsequently mediates the reduction of glutathione groups and the production of cGMP by reduction of the soluble guanylate cyclase heme-subunit. Although not necessarily a unifying theory of oxygen sensing among different tissue types, this pathway for oxygen transduction is intriguing because it unites the central principles and actions of oxygen sensing mechanisms in general: i) the oxygen sensor is located as far out in the oxygen gradient as possible (ie in the plasma membrane), ii) it possesses a high $K_m.O_2$, potentially signaling changes in oxygen availability early in the transition to, and from, hypoxia, iii) by initiating a REDOX potential it juxtaposes intracellular signaling mechanisms responsible for controlling multiple cell functions including channel opening probabilities and gene expression. As a candidate oxygen sensing pathway, this surely represents an ideal system on which to base further investigations into oxygen sensing mechanisms and consequences.

ii) Oxygen Sensing and Ca^{2+} Flux.

Ca^{2+} acts as a second messenger in a wide variety of cell processes stemming from fertilization, through

hormone responses, to cell death. As a result, alterations in intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) form the basis of a signaling mechanism that can potentially coordinate a number of interdependent, Ca2+--sensitive cell processes together. This signaling mechanism must be tightly regulated as uncontrolled increases in cytosolic $[Ca^{2+}]i$ also lie at the root of events that lead to cell death (fig 1). Clearly, the success with which tissues survive hypoxia rests greatly on the ability to maintain the cell membrane potential and, in particular, control Ca^{2+}-fluxes between cellular compartments, the cytosol and the extracellular environment.

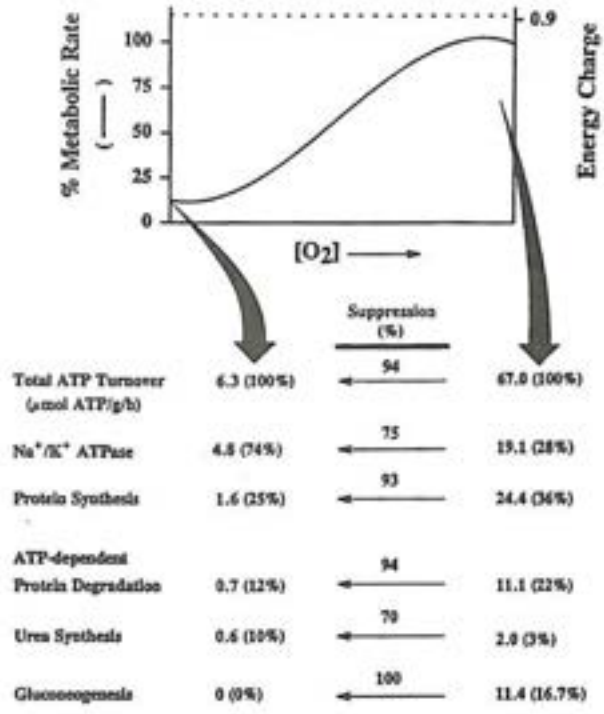

Figure 3. An energy budget for the major ATP demanding cell processes in normoxia and after 10h anoxia in isolated turtle hepatocytes. Graph at uppermost emphasizes an oxygen-dependent fall in metabolic rate (solid line) with little perceptible change in ATP concentrations or the cellular energy charge (dotted line). Normoxic ATP demand, demonstrated on the right side of the figure, largely sustains rates of protein turnover. After a 10-fold fall in metabolic rates, and energy demand by individual biochemical pathways, anoxic metabolism largely supports energy demand by ion pumping. Figure derived from references 4-9.

Continuing to use turtle hepatocytes as a model, we probed the relationship among oxygen availability, protein kinase-C activity, and adenosine purinoceptor activation in the control of turtle hepatocyte transmembrane Ca^{2+}-flux (the net balance between plasma membrane Ca^{2+} ATPase activitv. Na^{+}/Ca^{2+}-exchanger activity and Ca^{2+}-channel influx), measured with a non-invasive, Ca^{2+}-selective, self-referencing electrode (21). This technique enables non-invasive detection of extracellular Ca^{2+}-fluxes from single cultured cells by oscillating a Ca^{2+}-selective microelectrode (tip diameter of about 3 ,um) back and forth through the boundary layer next to the cell membrane. By referencing together the Ca^{2+}-specific signals obtained at each maximun of the arc described by the electrode movement, a $\Delta\mu V$ difference is obtained that can be applied to the Fick equation to determine directional Ca^{2+}-flux (figure 4). With this approach, we found that progressive hypoxia was associated with a reversible, oxygen-dependent suppression of Ca^{2+}efflux with an apparent KmO_2 of 145,μM (fig 5). As the technique only resolves slow-time course events (ie on the order of seconds) and the efflux was largely inhibited by Ca^{2+} ATPase blockers, the nature of the Ca^{2+}-efflux is likely to be Ca^{2+}ATPase activity (22). The high KmO_2 suggests that the suppression of Ca^{2+}-efflux is achieved through a signaling mechanism capable of detecting changes in oxygen availability over concentration ranges that are two orders of magnitude above the KmO_2 of the electron transfer system in liver [0.7μM 02 for tightly coupled isolated liver mitochondria (23)]. In support of this was the failure of KCN, administered over concentration ranges that achieve rapid inhibition of oxygen consumption in turtle hepatocytes, to diminish Ca^{2+}-flux when oxygen was present in the

medium (figure 6).

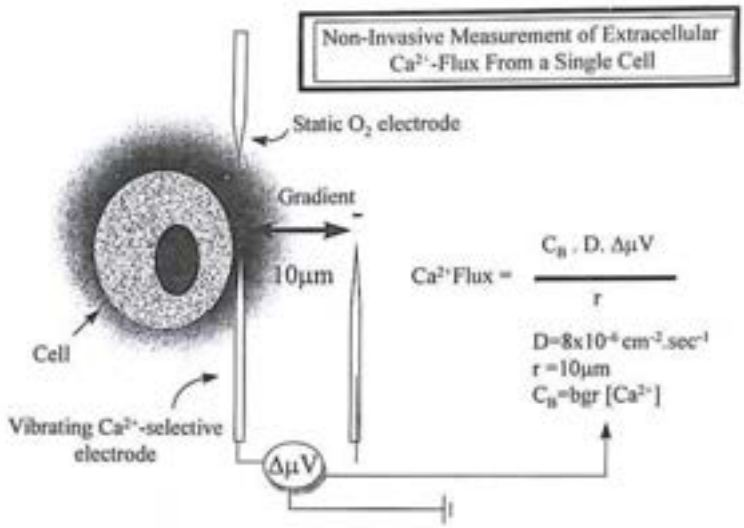

Figure 4. Operational principle of the Ca^{2+}-selective self-referencing microelectrode. Patch clamp style electrodes (tip diameter of 3 µm) were rendered selective for Ca^{2+} by placing a 25 ,µm column of a Ca^{2+}-selective ionophore in the tip. The electrode was then oscillated over a distance of 10pm, at a rate of 0.3 Hz, through the extracellular Ca^{2+} gradient at a point perpendicular to the equatorial centerline of the cell. The signals obtained at each maxima of the oscillation were referenced to one another to obtain a Ca^{2+}-specific $\Delta\mu V$ difference over the amplitude of oscillation. This could then be used in the Fick equation to calculate a directional value of Ca^{2+}flux where. D= diffusion coefficient for Ca^{2+} in physiological saline; r= amplitude of oscillation, C_b= background molar concentration of Ca^{2+} in the medium. As the tip of the electrode never contacts the cell surface, and there is no electrical field generated by the electrode, the technique is non-invasive. In the experiments described here, a static polarographic microelectrode was also maintained close to the cell to monitor local oxygen concentrations.

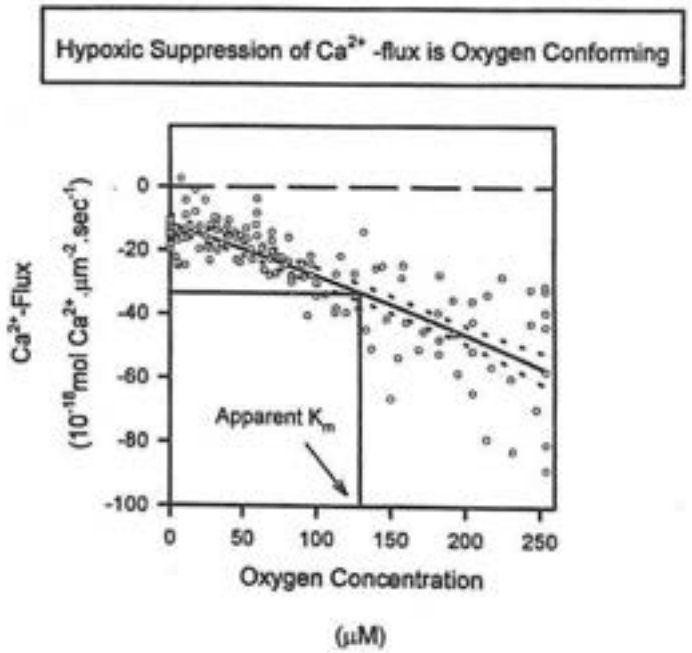

Figure 5. Oxygen conformity of transmembrane Ca^{2+}-flux during a transition towards hypoxia demonstrates an apparent K_mO2 of 145µM (sold line). 95% confidence limits in the fit of the line to the data points are also shown (broken line). The self-referencing Ca^{2+}-selective electrode was oscillated over a distance of 10, µm at a rate of 0.3Hz. Figure derived from reference 22.

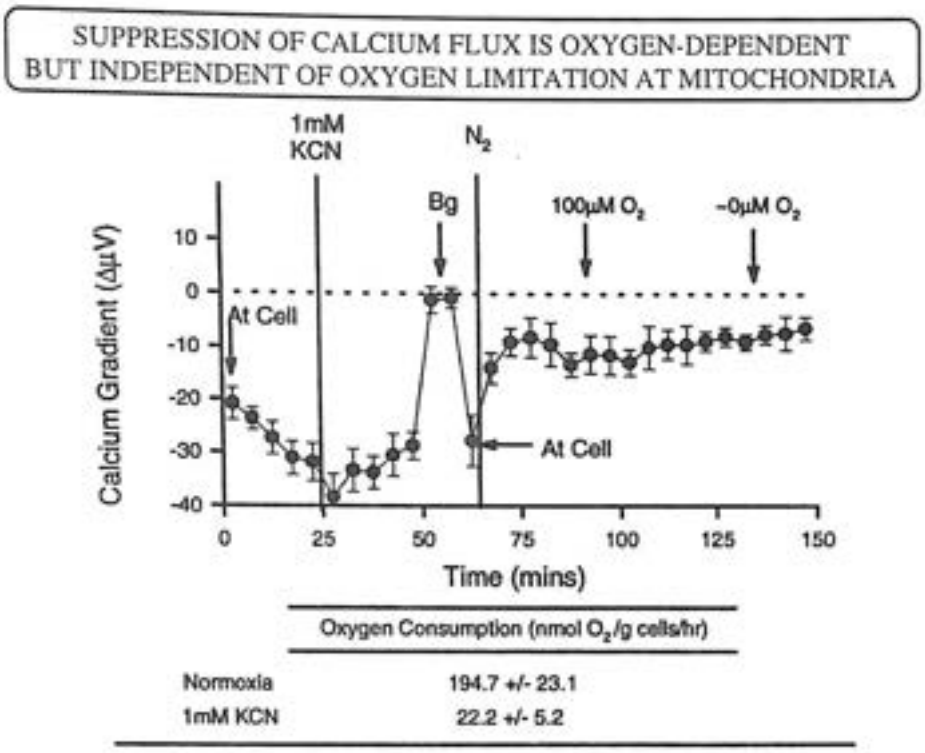

Figure 6. The suppression of Ca^{2+}-flux was oxygen dependent, but independent of inhibition of mitochondrial electron transfer. The electrode was moved to within a micron of the cell membrane ("At Cell") and a $\Delta\mu V$ signal output collected for 25 minutes. lmM KCN was then added to the medium and the signal output collected for a further 25 minutes. Note that aerobic addition of lmM KCN causes a 10-fold reduction in rates of oxygen consumption. The electrode was then moved out of the cellular Ca^{2+}-gradient to a background position (Bg) to ensure that the signal output was zero in the absence of a Ca^{2+}-gradient. Movement of the electrode back to the same measurement spot next to the cell retrieved the original signal magnitude, despite KCN blockage of cellular oxygen consumption. At 60 minutes into the experiment, dissolved oxygen was displaced by infusion of a N_2-pressure-head over the surface of the medium, causing the same, high apparent K_mO_2, oxygen-dependent suppression of Ca^{2+}-flux noted in figure 5. This occurred irrespective of the preceding inhibition of mitochondrial electron transfer by KCN. Figure depicts the change in the $\Delta\mu V$ signal difference in normoxia, with each plotted point being the mean +/-standard deviation of 100 raw data points collected

The oxygen signal was transduced by a pathway that involved the activation of PK-C as anaerobic inhibition of PK-C eliminated the controlled suppression of Ca^{2+}-efflux, causing a marked Ca^{2+}-influx followed by cell swelling and rupture. From the literature, the role PK-C might play in coordinating the suppression of Ca^{2+}-efflux during hypoxia, is vague. Aerobic experiments have detailed a role for PK-C in the opening of capacitatively coupled Ca^{2+}-channels in the plasma membrane after release of internal Ca^{2+} pools by thapsigargin (24,25). This fits with the case in rat hepatocytes where PK-C activation by TPA has been observed to prolong the time course of cytosolic Ca^{2+} re-sequestration into cellular compartments during glucagon-induced Ca^{2+} cycling (26). In glioma cells, PK-C activation by TPA results in an increase in $[Ca^{2+}]i$ that stems, in part, from an activation of plasma membrane Ca^{2+} channels (25). In normoxia, there is clearly a precedent to suggest PK-C activation opens capacitatively coupled Ca^{2+}-channels.

In our studies with turtle hepatocytes, we identified both an aerobic, TPA-induced, reduction in Ca^{2+}-efflux and also an absolute requirement for PK-C activation in the controlled suppression of Ca^{2+}-efflux towards a new steady-state during the hypoxic transition (22). Addition of the PK-C inhibitor, sphingosine, during anoxia led to a rapid Ca^{2+}-influx followed by cell rupture (figure 7A). Given the clear role of PK-C activation in the opening of Ca^{2+} channels, we cannot exclude the possibility that the observed suppression of Ca^{2+}-efflux was associated with an elevated, inwardly directed Ca^{2+}-flux component. When compounded, the absolute requirement for PK-C activation, the ensuing sustained pattern of hypoxic Ca^{2+}-flux, and the prolonged survivability of these cells during

anoxia, all serve to underscore this as a regulatory event rather than a precursor to the uncontrolled capacitative increase in $[Ca^{2+}]i$, as would be associated with hypoxic cell death.

Receptor-mediated Ca^{2+} entry in hepatocytes is under the control of two distinct, agonist activated pathways that exhibit different cation selectivities and modes of control (27). The first operates through an agonist-sensitive (vasopressin or thapsigargin), inositol $(1,4,5)P_3$-generating pathway to mobilize the intracellular Ca^{2+}-pool followed by a Ca^{2+}-specific influx over the plasma membrane by capacitative coupling. This pathway is distinct as it will not conduct Mn^{2+}. The second mechanism is less selective for Ca^{2+} over Mn^{2+} and requires continuous hormonereceptor binding to a G-protein complex with the subsequent activation of Ca^{2+} channel conductance (27,28). In turtle hepatocytes, it was important to assess the capacity for the oxygen signal to interact with existing receptor-mediated pathways of Ca^{2+}-mobilisation. We examined the adenosine-purinoceptor-mediated pathway because the production of extracellular adenosine during periods of metabolic stress has the general effect of reducing energy demand and increasing energy supply, making this an important regulator of pathways associated with the survival of anoxia (29). In hepatocytes, specific aerobic effects of adenosine include a suppression of protein synthesis (30) and gluconeogenesis (31), activation of glycogen phosphorylase and enhanced rates of glycogenolysis (32) and urea synthesis (33). All events are associated with increases in $[Ca^{2+}]i$ with activation of glycogenolysis and urea synthesis demonstrating an absolute dependence for the Ca^{2+} signal. Adenosine binding to the purinoceptor signals increases in $[Ca^{2+}]i$ by activating the $Ins(1,4,5)P_3$-mobilised Ca^{2+} pool with subsequent capacitative entry of Ca^{2+} over the plasma membrane.

In turtle hepatocytes, adenosine activated Ca^{2+}-effflux almost two-fold over normoxic controls (Figure 7B). The response was abrogated in the presence of 10,uM of the specific A, subclass-purinoceptor antagonist, 8-PT. As adenosine is well characterized to transiently increase $[Ca^{2+}]i$ in hepatocytes, we interpret the increase in apparent Ca^{2+} effflux as an elevation in outwardly directed Ca^{2+}-ATPase activity to compensate capacitative Ca^{2+} entry. When repeated under anoxic conditions, neither adenosine nor 8-PT administration altered the characteristic suppression of Ca^{2+}-effflux, suggesting a capacity for the oxygen signaling pathway to behave as a modulator of existing regulatory pathways of Ca^{2+}-efflux during severe oxygen lack. Although far from pin-pointing a clear-cut oxygen sensing mechanism, the oxygen-dependent suppression of transmembrane Ca^{2+}-flux in turtle hepatocytes (the net effect of channel. pump and transporter activity) indicates that processes associated with Ca^{2+} homeostasis are under tight control as oxygen availability diminishes.

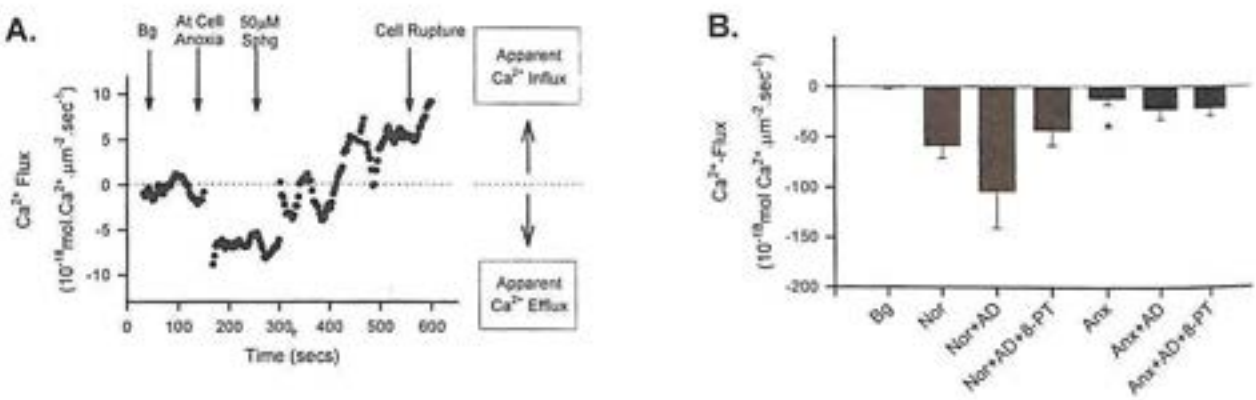

Figure 7(A) Effect of protein kinase-C inhibition by 50>M sphingosine during the anoxic suppression of transmembrane Ca^{2+}-flux. Normoxic PK-C activation using a phorbol ester leads to a reduction in the magnitude of transmembrane Ca^{2+}-flux, which is readily reversible with sphingosine. In anoxia, however, inhibition of PK-C leads to a rapid Ca^{2+} influx followed by cell rupture. **(B)** Normoxic administration of 100>M adenosine doubles transmembrane Ca^{2+}-flux which is readily inhibited by 10µM 8-PT, antagonist to the adenosine Al receptor. In anoxia, neither adenosine nor 8-PT abrogate the oxygen-dependent suppression in Ca^{2+}flux suggesting that the oxygen-dependent pathway possesses dominant control in hypoxia. Abbreviations: Bg. background recording position 200µm from cell surface: Sphg, sphingosine; Nor, normoxia; Ax, anoxia; AD. adenosine; 8-PT, 8-phenylthcophylline.

CONCLUSION.

How effective is the oxygen signal as a coordinator of metabolism? From the work described above, we now have an emergent picture of anoxic survival in turtle hepatocytes that centers around the shut-down of individual processes associated with ATP synthesis and demand, an oxygen-regulated change in protein expression profiles, preservation of the cellular membrane potential, and tight control over Ca^{2+} by oxygen and second messenger modulation of transmembrane Ca^{2+}-fluxes. This presents a clear demonstration of the interaction of the oxygen signal with each aspect of metabolism namely, reducing power (increased glycolytic flux being associated with elevated $NAD^+/NADH$, for example), ATP synthesis, biosynthesis and ion-flux. Perhaps most significantly, the studies described here, and elsewhere (13), suggest that critical, ATP-demanding cell processes can be regulated by oxygen before oxygen availability becomes limiting to aerobic function. Intrinsic to this is a capacity for the oxygen signal to over-ride or modulate other mechanisms involved in the regulation of the same cell process during the hypoxic transition.

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EARLY CARDIAC MYOCYTE RESPONSES TO HYPOXIA, ISCHEMIA, AND REOXYGENATION

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RESUMEN: Respuestas Tempranas de Miocitos Cardíacos a Hipoxia, Isquemia y Reoxigenación

La isquemia miocárdica es la causa más prevalente de morbilidad y mortalidad en las poblaciones occidentales. Las múltiples condiciones asociadas a la isquemia comparten la propiedad común de contracción reducida, anormal o perdida debido a una entrega de oxígeno disminuida a la región del miocardio. En todas las condiciones el disbalance entre aporte y demanda de oxígeno puede ser transitorio, relacionado a periodos de ejercicio físico y/o estrés mental. Como consecuencia, los tejidos que experimentan episodios isquémicos repetidos sufrirán daño tanto por la isquemia (hipoxia, ácido láctico) como por la reperfusión subsecuente (estrés oxidativo). La isquemia miocárdica es acompañada comúnmente por hipertrofia que implica crecimiento cardiaco incrementado y expresión genética alterada. Al incrementar el espesor de la pared del ventrículo izquierdo y las distancias de difusión del oxigeno, la hipertrofia exacerba la isquemia y lleva a una patología progresiva y autoperpetuante.

En estudios con modelos de hipoxia simulada hemos empezado a develar los eventos moleculares que determinan las respuestas de los miocitos cardiacos al estrés redox. Este trabajo identificó una red única de señales intracelulares, iniciadas por situaciones de hipoxia e hiperoxia, que promueven ya sea modificaciones adaptativa/defensivas o muerte celular por apoptosis. Los eventos tempranos de señalización en respuesta a la hipoxia severa incluyen cambios en el metabolismo lípido, en el manejo del calcio, en el pH intracelular y en la actividad de proteina kinasa C. Estos cambios culminan en la inducción de los genes de respuesta inmediata al estrés temprano, incluyendo *c-fos y c-jun*, que codifican información para la transcripción del factor AP1. Los blancos periféricos de las respuestas tempranas incluyen ó-actina, péptido atrial natriurético y genes de enzimas glicolíticas. La hipoxia crónica implica desensibilización de cAMP, protelna kinasa A y apoptosis incrementada. La reoxigenación causó una activación transitoria débil de la proteina kinasa activada por mitógeno, una activación fuerte y sostenida de la cascada de proteina kinasa activada por estrés, inducción de los blancos periféricos incluyendo AP1, supresión de actividad Sp1 y una oleada adicional de apoptosis.

Palabras claves: Insuficiencia cardiaca congestiva, Enfermedad de arterias coronarias, Proteina kinasa, Expresión genética, Angina, Miocardio en hibernación, Segundos mensajeros

RÉSUMÉ: Réponses précoces des myocytes cardiaques à l'hypoxie, l'ischémie et la réoxygénation.

L'ischémie du myocarde est la cause la plus fréquente de morbidité et de mortalité parmi les populations occidentales. Les multiples conditions associées à l'ischémie ont pour propriété commune la contraction réduite, anormale ou annulée, du fait d'un apport réduit d'oxygène à la région du myocarde. Quelles que soient les conditions, le déséquilibre entre l'apport et la demande d'oxygène peut être transitoire, en rapport avec des périodes d'exercice physique et/ou de stress mental. La conséquence de ce déséquilibre est que les tissus subissant des épisodes ischémiques répétés seront endommagés, autant du fait de l'ischémie (hypoxie, acide lactique) que de la reperfusion subséquente (stress oxydatif). L'ischémie du myocarde s'accompagne généralement d'une hypertrophie impliquant une augmentation de la croissance cardiaque et une altération de l'expression génétique. En accroissant l'épaisseur de la paroi du ventricule gauche et les distances de diffusion de l'oxygène, l'hypertrophie exacerbe l'ischémie et conduit à une pathologie progressive et s'auto-perpétuant.

Dans des études faites à partir de modèles d'hypoxie simulée, nous avons commencé à démêler les événements moléculaires qui déterminaient les réponses des myocytes cardiaques au stress réduction-oxydation. Ce travail a permis d'identifier un réseau unique de signaux intracellulaires, déclenchés par des situations d'hypoxie et d'hyperoxie et qui favorisent soit des modifications adaptatives / défensives, soit la mort des cellules par apoptose. Les événements précoces de signalisation en réponse à l'hypoxie sévère comportent des changements dans le métabolisme des lipides, le contrôle du calcium, le pH intracellulaire et l'activité de la protéine kinase C. Ces changements aboutissent à l'induction des gènes de réponse immédiate au stress précoce, comprenant *c-fos* et *c-jun* qui codifient l'information pour la transcription du facteur AP1. Les cibles périphériques des réponses précoces comprennent la α-actine, le peptide atrial natriurétique et les gènes d'enzymes glycolytiques. L'hypoxie chronique implique la désensibilisation du cAMP, de la protéine kinase A et une apoptose augmentée. La réoxygénation provoque une faible activation transitoire de la protéine kinase activée par mitogènes, une activation forte et soutenue de la cascade de protéine kinase activée par stress, l'induction des cibles périphériques incluant l'AP1, la suppression de l'activité de Sp1 et une vague supplémentaire d'apoptose.

Mots-clés : Insuffisance cardiaque congestive, Maladie des artères coronaires, Protéine kinase, Expression génétique, Angine, Myocarde en hibernation, Seconds messagers.

SUMMARY: Myocardial ischemia is the most prevalent cause of morbidity and mortality in Western populations. The multiple ischemia-associated conditions share the common property of reduced, abnormal, or lost contraction due to impaired oxygen delivery to a region of the myocardium. In all conditions the imbalance between oxygen supply and demand may be transitory, related to periods of physical exertion and/or mental stress. As a consequence, tissue experiencing repetitive ischemic episodes will suffer damage both from the ischemia (hypoxia, lactic acid), and from subsequent reperfusion (oxidative stress). Myocardial ischemia is commonly accompanied by hypertrophy involving increased cardiac growth and altered gene expression. By increasing left ventricle wall thickness and oxygen diffusion distances, hypertrophy exacerbates the ischemia and leads to a progressive and self-perpetuating pathology.

In studies using models of simulated ischemia we have begun to unravel the molecular events that determine the responses of cardiac myocytes to redox stress. This work identified a unique network of intracellular signals, initiated by hypoxic and hyperoxic stresses, that promotes either adaptive/defensive modifications, or cell death through apoptosis. Early signaling events in response to severe hypoxia include changes in lipid metabolism, calcium handling, intracellular pH, and protein kinase C activity. These culminate in the induction of immediate-early stress response genes including c-fos and cjun which code for transcription factor API. Downstream targets for the early responses include skeletal a-actin, atrial natriuretic peptide, and glycolytic enzyme genes. Chronic hypoxia involves down regulation of cAMP, protein kinase A, and enhanced apoptosis. Reoxygenation caused a weak transient activation of mitogen activated protein kinase, strong sustained activation of the stress activated protein kinase cascade, induction of downstream targets including API, suppression of Spl activity, and an additional wave of apoptosis.

Key Words: Congestive heart failure, Coronary artery disease, Protein kinase, Gene expression, Angina, Hibernating myocardium, Second messengers.

INTRODUCTION

Pathophysiology

Heart disease is the most common cause of death in Western populations (reviewed in (Mangano, 1990)). The clinical syndrome of cardiac failure follows a complex pathway of pathophysiologic interactions that result in progressively deteriorating cardiac pump function causing inadequate perfusion of body tissues and/or congestion. Despite multiple approaches to therapy including polypharmacology and surgery, the prognosis of congestive heart failure (CHF) remains poor. The overall 5- year survival for congestive heart failure is 50%, and patients with New York Association class IV have a dismal 1-year survival rate of less than 50% (Mangano, 1990).

Hypertension and coronary artery disease (CAD) are the most frequent underlying causes of myocardial failure (Mangano, 1990; Swan, 1990; Lamas, 1993; Chobanian, 1992; Birkenhager, 1991). With the general availability of effective antihypertensive therapies, the contribution of CAD has become more prominent (Lamas, 1993). CAD, which involves the progressive narrowing of coronary arteries by the atherosclerotic process, initiates a cascade of responses in the myocardium and vasculature. A simplified scheme of events is depicted in Fig 1. Unfortunately, for many patients the first indication of CAD is sudden complete vessel blockage due to a combination of thrombosis and endovascular disruption, resulting in myocardial infarction (MI) (Swan, 1990; Krayenbuehl and Hess, 1992; Yeung et al., 1992). The stage is set for MI by the progressive narrowing and thrombogenicity of the vascular surfaces during the early phases of CAD. In a larger number of patients, chest pain due to myocardial ischemia is the presenting complaint, as coronary reserve and blood flow are gradually restricted (Carbajal and Deedwania, 1991). Clinical and molecular events at the earliest stages of CAD are poorly understood because asymptomatic patients do not normally seek medical attention, and because until quite recently there were no good animal models of CAD. However, all forms of ischemia cause abnormal contractility and decreased cardiac output; these in turn trigger compensatory mechanisms as described below (Homans et al., 1986; Wilde et al., 1990; Kawai et al., 1990; Bolli et al., 1989; Galinanes et al., 1993; Baker et al., 1988). Ischemia also causes muscle loss through myocyte cell death, either through infarction (which can involve more than 50% of the left ventricle), or gradually, through less well understood mechanisms, in reponse to chronic hypoxia and cycles of ischemia and reperfusion (Gottlieb et al., 1994; Tanaka et al., 1994; Ivey et al., 1995; Williams et al., 1994; Swan, 1990). Because cardiac myocytes do not regenerate, this loss is permanent.

A number of secondary factors contribute to CHF progression and clinical deterioration (for reviews see (Lamas, 1993; Francis and Chu, 1995; Marian and Roberts, 1995; Nadal-Ginard and Mahdavi, 1993; Glennon et al., 1995; Morgan and baker, 1991; lee and lindpainter, 1993; Neyses and Pelzer, 1995; van Bilsen and Chien, 1993; Yamazaki et al., 1994; Schwartz et al., 1995; Scwartz, 1995)). Decreased cardiac output resulting from muscle loss or malfunction causes a drop in systemic blood pressure, triggering a cascade of compensatory mechanisms designed to raise the blood pressure and/or improve cardiac output.

Enhanced production of atrial natriuretic factor (ANF) is one of the earliest features of heart disease (Kovacic-Milivojevic and Gardner, 1992). As the name implies, this peptide promotes diuresis, and its production is probably regulated by baroreceptors in and around the cardiac atria. Ischemia-associated hypoxia stimulates the release of vasoconstrictor peptides including endothelin-l (ET-1) (Wadsworth, 1994; Watanabe et al., 1990; Malek et al., 1993; Bodi et al., 1995). The renin-angiotensin-aldosterone system is activated by reduced cardiac output, resulting in further vasoconstriction with increased renal and other systemic perfusion pressures (lee and lindpainter, 1993; Yamazaki et al., 1994). The result is increased cardiac work load and oxygen demand, further exacerbating the ischemia. Reduced cardiac output activates neurohormonal systems and increases plasma catecholamine levels. These

hormones stimulate cardiac muscle contraction and put even more strain on oxygen requirements.

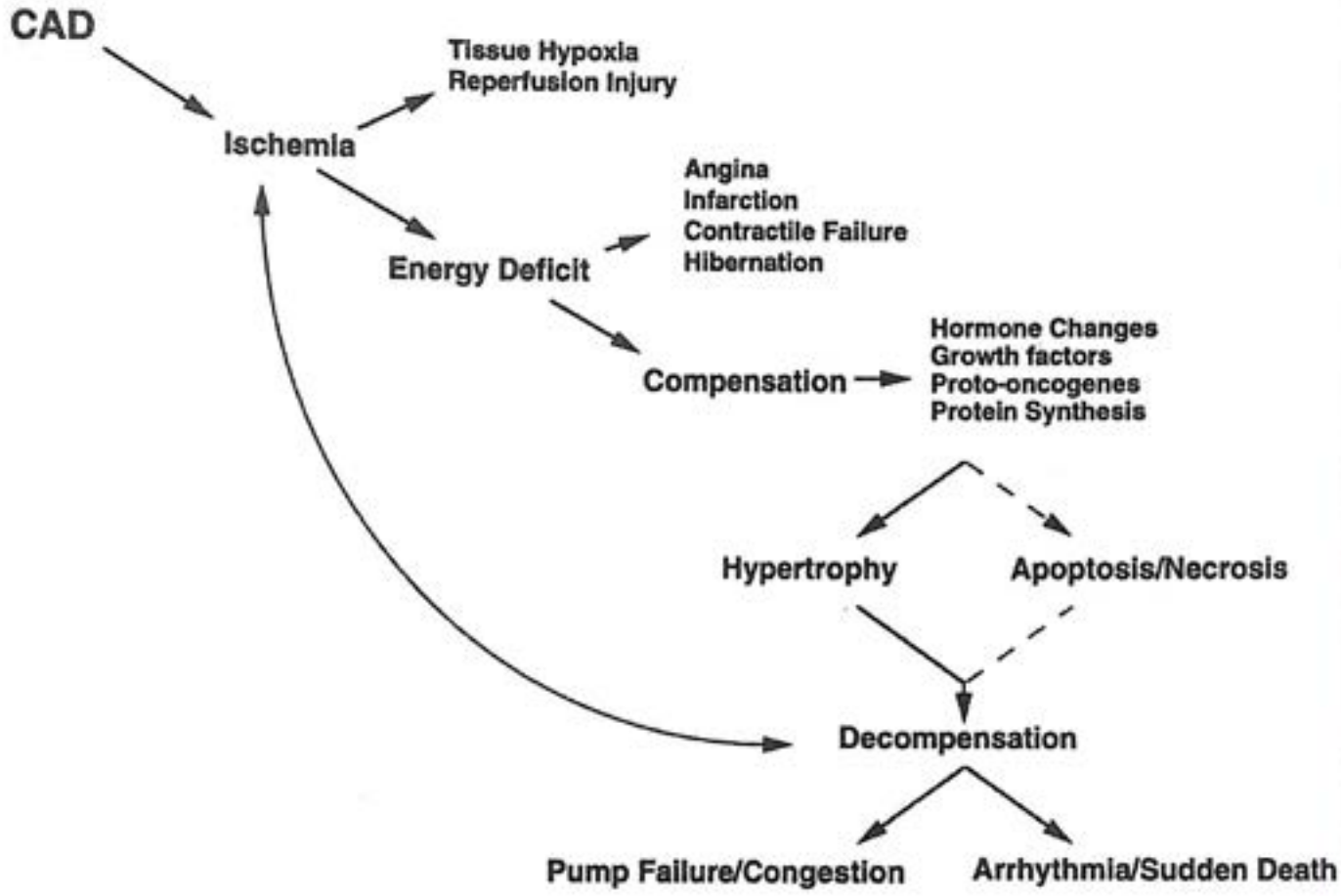

Fig. 1. Stages in the Progression of Ischemic Heart Disease

In addition to perpetuating oxygen deficits, the chronic elevation of catecholamines, ET-1, and AT-II may also play a role in the development of myocardial hypertrophy. Myocardial hypertrophy is a principal adaptive response of the heart to many kinds of stress, and frequently accompanies ischemic heart disease. Hypertrophic cardiomyopathy (HCM) (Marian and Roberts, 1995; Schwartz et al., 1995; Scwartz, 1995) and CAD associated cardiomyopathy may share common initiating signals and developmental pathways (see references listed above for reviews). In both cases, the initiating stimulus appears to be circulatory insufficiency caused by reduced contractility. Although the mechanisms for translating a contractile deficit into a growth response are presently unknown, the end result is a dramatic stimulation of cellular biosynthetic pathways, increased myocyte mass, and extensive thickening of the left ventricular wall. During hypertrophy, certain myofilament genes are selectively activated, new sarcomeres are assembled, and individual cardiac myocytes enlarge and change shape. There are changes in the balance of muscle and non-muscle proteins, changes in ion transport channels, and changes in myocardial vascularity. Hypertrophy is a major component of so-called "myocardial remodeling", which refers to changes in the overall composition, architecture, and functioning of the heart following myocardial infarction and during the progression of congestive heart failure. In recent years, many of the molecular signals and targets of the hypertrophic process have been identified, and these regulatory pathways constitute possible new targets for therapy.

To the degree that adaptive mechanisms are successful, a period of adequate compensation follows in which the combination of neurohumoral support and increased cardiac mass produce an improvement in cardiac output.

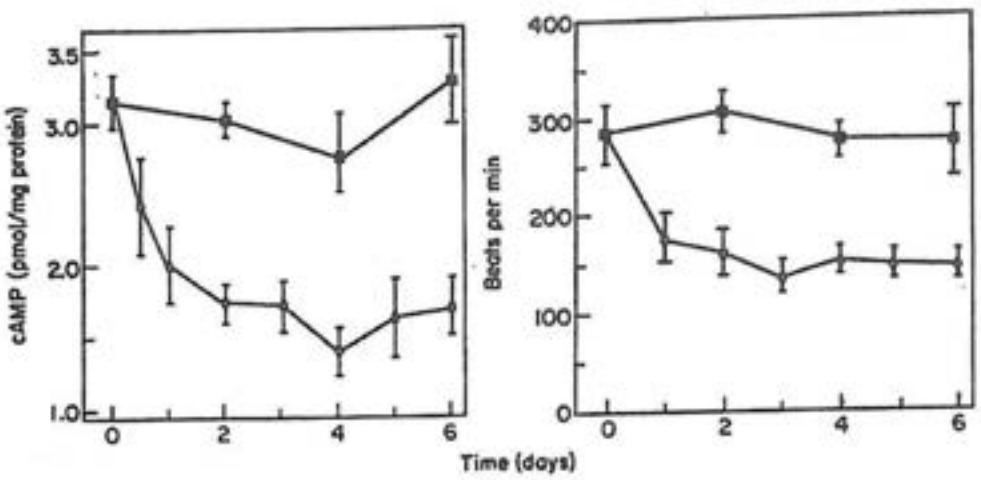

Fig. 2. Changes in cAMP and Contractility of Heart Cells under Chronic Hypoxia.

Heart cell cultures were exposed to atmospheric oxygen, pO_2=160mmHg, or hypoxia, pO_2=48mmHg five to seven days after isolation as described in ref (Webster and Bishopric, 1992). Cyclic AMP measurements are the result of duplicate determinations from six separate experiments. Contraction and motion characteristics were measured using a computerized motion analysis edge detection system as described previously (Webster and Bishopric, 1992). During this period, the remodelling process is accompanied by several membrane-associated changes that may critically affect cardiac function. cAMP production is depressed (Feldman et al., 1987; Neumann et al., 1988; Chen et al., 1991), ,β-adrenoreceptors are down-regulated (Gwathmey et al., 1987; Barnett, 1991), sarcoplasmic reticulum calcium ATPase (SERCA) expression and function are depressed, and sodium-calcium exchanger activity increases (reviewed in (Arai et al., 1994; Wankerl and Schwartz, 1995)). Together, these changes reduce inotropic responsiveness, depress the response to sympathoadrenal stimulation, impair diastole (relaxation), and increase the probability of arrhythmia. The ability of further hypertrophy to strengthen the heart is limited, and eventually the system begins to collapse; the underlying ischemia still persists, blood pressure may remain high, and the hypertrophied muscle itself creates physical and bioenergetic problems. Ultimately, adaptive resources are exhausted. Death may result either from arrhythmia or gradual pump failure, with approximately equal probability.

Pharmacology

Heart failure therapy is targeted at both the heart and the vasculature. Approaches to prevent, treat, or reverse CAD include lifestyle modulations, drug combinations, angioplasty, and surgery. These have been reviewed recently and extensively elsewhere (Gibbons and Dzau, 1996; Swynghedauw and Camm, 1994; Wickelgren, 1996; Wickelgren, 1996; Mcnamara, 1995). Goals of current therapy are to improve function and prevent further damage. Since contractile insufficiency due to muscle damage is a principal causes of death in CHF, effective treatment must not only improve organ function by increasing contractile output and efficiency, but must ultimately stem further myocardial tissue loss.

A number of pharmacological approaches have been used to improve cardiac functions, with varied but limited success. The only inotropic agent widely used for chronic CHF therapy is digitalis. It has been used for at least 2 centuries, and works by inhibiting Na^+/Ca^{2+} exchange, thereby increasing intracellular calcium (reviewed in (Taylor, 1996; Gheorghiade, 1996). Digitalis is mildly inotropic *in situ*, with a very narrow therapeutic index. Recent multiphase trials (DIG) demonstrated the positive impact of digitalis therapy on quality of life and no significant effect on mortality. Angiotensin converting enzyme (ACE) inhibitors and nitrates work at least in part by dilating peripheral blood vessels, reducing blood pressure and relieving cardiac work load (reviewed in (Swynghedauw and Camm, 1994)). Calcium channel antagonists and 5-adrenoreceptor blockers (5-blockers) may help reduce arrhythmias and control blood pressure. Chronic blocker therapy may exert additional beneficial actions, possibly related to the preservation of active adrenoreceptors, although these effects have not been fully explained (Barnett, 1991; Feurstein and Ruffolo, 1996; Poole-Wilson, 1996). Combined therapy using ACE-I.

digoxin and diuretics is standard practice; unfortunately, the overall impact on mortality is minimal (see (Taylor, 1996) for reviews). To date, only ACE inhibitors have been convincingly demonstrated to have beneficial effects on both the quality of life and mortality in congestive heart failure. Calcium channel antagonists have no beneficial effects in CHF, although some agents in this class appear to be safe for use in the treatment of CHF-associated hypertension and angina (Poole-Wilson, 1996). Some promising newer agents with ß-blocking functions may eventually be incorporated into the standard treatment regimens (Taylor, 1996).

Apart from digoxin, success with positive inotropic drugs has been limited. Two approaches to inotropic therapy are relevant in the context of this discussion. The phosphodiesterase-3 inhibitors (PDIs), of which amrinone and milrinone (Sterling Pharmaceuticals) are prototypes, improved contractility and peripheral vascular resistance in short-term clinical trials, but significantly increased mortality during chronic use (Wetzel and Hauel, 1988; Monrad et al., 1986; Cottney et al., 1990; Packer, 1991). These drugs act by selectively repressing cardiac and smooth muscle phosphodiesterase type III activity. The suppression of this enzyme raises cAMP levels, resulting in increased cAMPdependent protein kinase A (PK-A) and myosin light chain kinase (MLC-K) activity . The immediately beneficial consequences of PDIs are thus (1) increased contractility due to PKA-mediated phosphorylation of cardiac calcium channel proteins and increased intracellular calcium, and (2) peripheral vasodilation due to phosphorylation of smooth muscle MLC, which causes smooth muscle relaxation.

The disappointing outcome of clinical trials of these agents, despite their promising short term results, has alerted investigators to the complexity of heart failure pathophysiology, as well as to the dangers inherent in increasing bioenergetic expenditure in this setting. A newer class of inotropic agent works by enhancing the sensitivity of myofilament proteins to available intracellular calcium (Ruegg and Morano, 1989; Keane et al., 1990; Hajjar and Gwathmey, 1991) These drugs include pimobendan (Boehringer Ingleheim), EMD 53998 (E. Merck-Darmstadt), and levosimendan (Orion Pharmaceuticals Inc.) all of which have differing amounts of intrinsic PDI activity in addition to calcium sensitizing properties. Calcium sensitizers may have a unique ability to strengthen the heart in a bioenergetically favorable manner (Lee and Allen, 1990; Ferroni et al., 1990; Lee et al., 1989; Kubo and et al, 1992; Haikala et al., 1992) without significantly increasing intracellular calcium or cAMP. However, their clinical efficacy has yet to be established.

Further progress in heart disease research will require the identification of new molecular targets and may involve gene therapy techniques (reviewed in (Prentice and Webster, 1995)). By analyzing models of heart disease at the cellular and molecular levels, we are beginning to understand the signaling pathways, genes, and proteins that determine the responses of cardiac myocytes and vascular cells to extracellular stresses, including pressure and tension changes, ischemia, reperfusion, and hypertrophy. One unifying feature of the responses to these stimuli is the modulation of the activities of protein kinases. In the remaining part of this communication we will review recent work demonstrating critical roles for three separate protein kinase pathways in the transmission of myocardial stress responses.

Models of Heart Disease

Although there is a wealth of information on the clinical aspects of both acute and chronic ischemia, the molecular control mechanisms and cellular responses to ischemia are poorly understood. This is due in part to the limited availability of animal models of heart failure and the technical and ethical limitations of performing molecular analyses on whole animals and humans. In our molecular approach to analyze signaling pathways and gene expression we have developed cellular models of ischemia using isolated cardiac myocytes. We present here three models that represent different ischemia syndromes (1) a model of chronic myocardial hypoxia (Webster and Bishopric, 1992) with parallels to chronic ischemia (Carbajal and Deedwania, 1991) and hibernating myocardium (Braunwald and Rutherford, 1986; Rahimtoola, 1989) where the key change involves a depression of protein kinase A activity; (2) a model of acute cardiac arrest involving severe hypoxia, glucose depletion, and contractile failure (Webster et al., 1993; Webster et al., 1994; Webster et al., 1993), which is accompanied by increased protein kinase C activity and nuclear accumulation of Fos and Jun family proteins; and (3) a model of ischemia and reperfusion in which myocytes are subjected to cycles of hypoxia and reoxygenation, causing a strong activation of stress activated protein kinases (SAPK/JNKs) (Laderoute et al., 1996; Laderoute and Webster, 1997).

(1) Chronic Model of Myocardial Hypoxia

Contractile insufficiency and malfunction are integral features of myocardial ischemia and end stage heart failure. At least four separate mechanisms have been proposed to account for these: (1) decreased sensitivity of myofilament contractile proteins to calcium resulting from waste metabolite build up, in particular increased acidosis, inorganic phosphate, and adenosine in the ischemic tissue (Allen and Orchard, 1987; Allen et al., 1989; Ruegg and Morano, 1989), (2) failure of the action potential and calcium transient due to depletion of energy reserves (Stern et al., 1988), (3) changes in the turnover rates of high energy phosphates (Bittl et al., 1987; Weiss et al., 1989; Marshall, 1988), and (4) depressed adrenergic responsiveness due to changes in adenine cyclase regulation, receptor activity and regulatory proteins Gs and/or Gi. While each of these factors may regulate contractility within a particular set of conditions, many studies have found poor correlation between loss of contractility and changes in any of these parameters, suggesting that other regulatory mechanisms and oxygen sensors must exist (Matthews et al., 1986; Downing and Chen, 1990; Lodge and Gelband, 1988; Smith et al., 1982).

In our studies, to simulate conditions of chronic low flow hypoxia in vitro, cardiac myocytes were isolated from neonatal rat hearts, cultured by standard procedures (Webster and Bishopric, 1992), and exposed to a PO_2 of approximately 5 mm Hg with 5% CO_2 at 37°C in a self contained environmental chamber (described in (Webster and Bishopric, 1992)). The chamber is equipped with continuously recording microscope, oxygen electrode, and pH electrode. To prevent metabolic buildup or substrate depletion, a high volume of buffered medium was added to the cells initially (12ml per 60mm plate), and replenished with pre-equilibrated, deoxygenated medium every 8-10h. Under these conditions the lactate concentration never exceeded 3mM, glucose concentration remained > 5 mM, and pH was maintained at 7.0 ± 0.2. Contraction frequency and motion characteristics were determined by periodic microscopic examination of cultures with a 40x phase objective and CCTV camera (Hitachi). Images were stored on videocassette and analyzed by computerized motion edge analysis. Our methods for calcium, gene expression, cAMP, and ATP analyses have been described previously (Webster and Bishopric, 1992). Under these conditions the cardiac myocyte cultures could be maintained for several weeks without visual deterioration or significant leakage of creatine kinase. These conditions are similar to chronic ischemia or hibernating myocardium in that cell viability is preserved in the face of diminished oxygen supply.

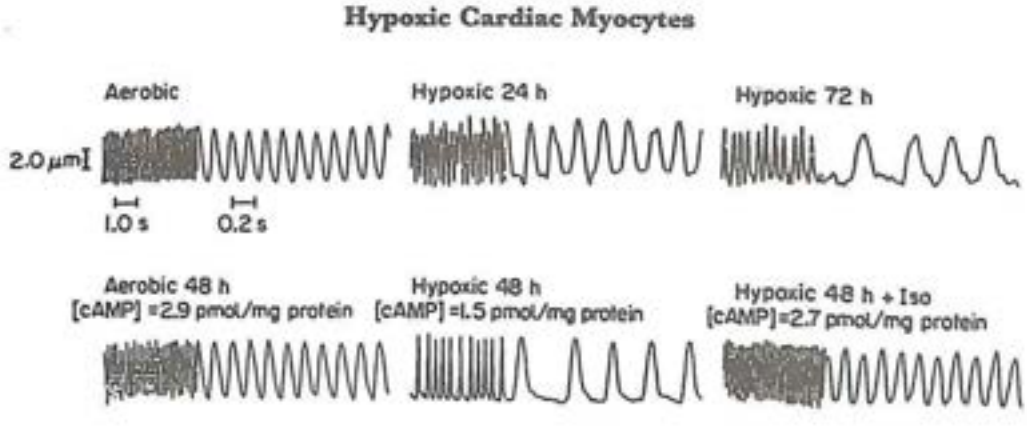

Fig. 3. Motion Characteristics and Effect of Isoproterenol

Analyses were made as described above in Fig 2, traces are typical of myocyte membrane motions at the different time points during hypoxic exposure. Routinely, areas of maximum motion were selected for analysis in both aerobic and hypoxic cultures. Concentrations of cAMP indicated on the Figure were measured in parallel duplicate plates before and after addition of isoproterenol. Note irregular motion in hypoxic cultures.

Under these conditions of simulated low flow ischemia, we monitored and correlated changes in contractility, ATP, cAMP, calcium flux, and gene expression at intervals over a 5 day period. The changes in ATP were small owing to the replacement of phosphorylation by glycolysis; glycolysis increased by 8 fold within hours of exposure to hypoxia, and remained elevated for the duration of the hypoxic period. Changes in the other parameters are shown in Figs 2 -4. The principal effects of chronic hypoxia were (1) a > 50% fall in intramyocyte cAMP (Fig. 2a); (2) a gradual decrease in the beating rate, (Fig. 2b); (3) slower sequestration of calcium during muscle relaxation (diastole) resulting in a longer, irregular shaped transient (Fig. 3); reversibility of these effects by isoproterenol treatment (Fig. 4); (4) changes in the expression of PK-A regulated gene promoters, and (5) induction of glycolytic enzyme genes (Fig 5). All of these parameters appeared to be causally related through depressed PK-A activity. The effects of chronic hypoxia on contractility and calcium transients were rapidly reversed by adding the ~adrenergic agonist, isoproterenol, which activates adenyl cyclase and re-established aerobic cAMP levels. Hypoxic induction of transfected cAMP-dependent promoters was reversed by adding cAMP inducers such as forskolin or phosphodiesterase inhibitors (Fig. 5). These results indicate that in the chronically hypoxic myocyte, cAMP levels drop gradually, with corresponding changes in signal transduction and gene expression. This can be envisioned as part of an adaptive-survival response that preserves energy through PK-A-mediated depression of contractility and induction of anaerobic metabolism. That these effects promote cell survival under hypoxia was indicated by the lethality of phosphodiesterase inhibitors and cAMP agonists in hypoxic but not normoxic myocytes (see ref. (Webster and Bishopric, 1992)). Thus, depressed cAMP may be an important adaptation for cardiac myocytes to survive extended periods of low flow hypoxia, as may be the case in hibernating myocardium (Cohen et al., 1988; Flameng et al., 1981; Spirito and Maron, 1990; Krayenbuehl and Hess, 1992).

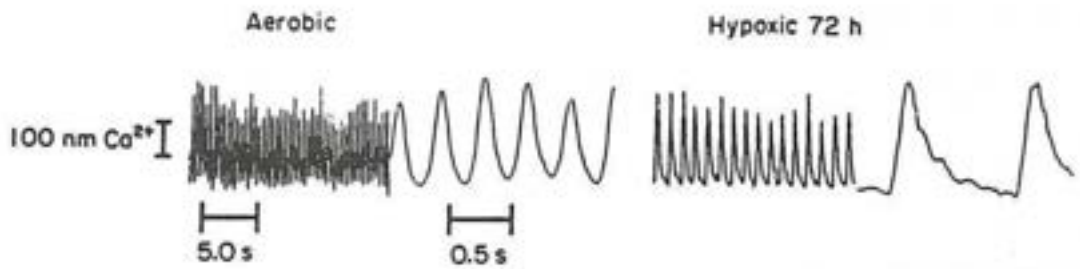

Fig. 4. Hypoxia Mediated Effects on Calcium Flux

Calcium flux was measured using an ACAS 470 Interactive Laser Cytometer (Meridian Instruments, Inc., Okemos, MI) after incorporation of the calcium selective fluorescent dye indo1 (Webster and Bishopric, 1992) and calibration as recommended by the manufacturer. Cells were grown in cover-slip diches (Nunc, Inc., Naperville, Il) and loaded with 20pLg/ml of indo-l for 2h immediately prior to analysis. Indo- 1 loading efficiency was not affected by oxygen conditions. After loading, dishes were sealed in the prevailing environment and transfered to the cytometer stage. The compartment was maintained at 37°C and flushed continuously with the appropriate gas mixture. Multiple point scans were taken on cells incubated under aerobic or hypoxic atmospheres. Traces show representative point scans with two time scales on a 72h aerobic culture and after 72h under hypoxia.

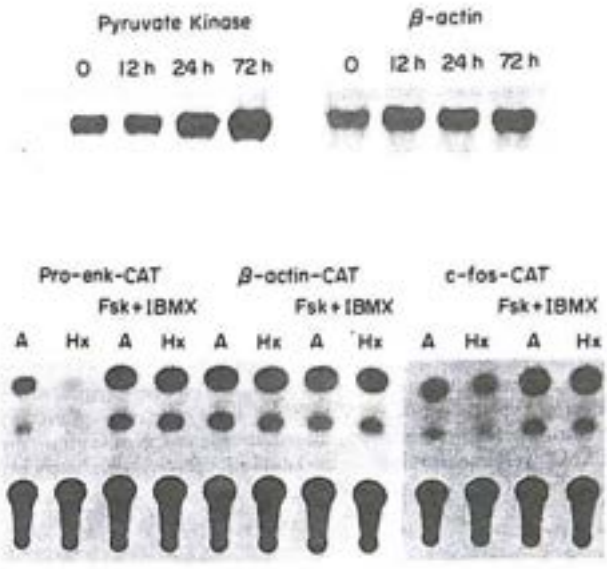

Fig. 5. Effects of Hypoxia on Gene Expression in Isolated Cardiac Myocytes.

Top panel: total RNA was isolated from hypoxic (pO_2=4mm Hg) cultures of cardiac myocytes at the times indicated, blotted and probed as described in reference (Webster and Bishopric, 1992). Bottom panels: transient expression of the transfected promoters linked to the CAT gene was assayed using equal amounts of protein also as described in reference (Webster and Bishopric, 1992).

(2) Acute model of cardiac arrest

Acute, severe ischemia results when a coronary artery or capillary becomes completely occluded. This condition causes rapid contractile failure of the affected tissue and is accompanied by hypoxia, nutrient depletion, and waste metabolite build up, especially inorganic phosphate and lactic acid. Severe ischemia, when prolonged more than a few minutes, results in infarction of the affected region, and protracted global myocardial dysfunction.

To simulate acute ischemia in vitro, spontaneously contracting, confluent cultures of cardiac myocytes were incubated under a PO_2 of <4 mm Hg with a shallow covering of medium (minimal essential medium (GIBCO) with 5% fetal calf serum). The medium contained 3-4 mM glucose, in the low physiological range. As in the chronic hypoxia model (Webster and Bishopric, 1992), glycolysis is induced; a key feature of this model is progressive glucose depletion, as with ischemic tissue *in situ* (Allen and Orchard, 1987). Under these conditions, glucose was consumed at a rate of 2.3 +/- 0.4 llmol/min./10^6cells, about 8 times faster than glucose consumption by parallel aerobic cultures (Webster et al., 1993). There was no significant change in medium pH during the relatively brief period of exposure to hypoxia used in these experiments.

Under these conditions of simulated acute severe ischemia, contractility began to decrease immediately after the cultures became hypoxic, and continued gradually to complete contractile failure at 3 to 4 hours (Fig 6 a&b). Changes in contractility were apparent before glucose was depleted, and before there was any significant change in ATP (see Fig 7). Contractile failure involved a progressive decrease in the amplitude of contraction without significant change in the beating frequency (Fig 5a). These effects on contractility have been described previously in isolated cardiac cells and tissues and in whole hearts subjected to hypoxia or ischemia (Allen and Orchard, 1987; Stern et al., 1988; Matthews et al., 1986; Downing and Chen, 1990). Contractile failure was reversible under the conditions described here; recovery was complete within lh when the cells were reoxygenated within 4h of hypoxia (n=6). Although a number of factors may contribute to the loss in contractility (Stern et al., 1988; Allen and Orchard, 1987), the combination of hypoxia and low glucose was critical because neither condition alone mediated the effect. It seems probable that an "emergency" response is triggered when the cellular energy reserve is threatened by blockade of both oxidative and glycolytic energy pathways. Reducing contraction is an obvious means to conserve energy, and other

cellular and biochemical energy conserving measures are probably implemented at the same time. The receptors and signaling pathways that promote these responses are not known, but the synergy between oxygen and glucose starvation suggests that cross-talk exists between the two stress response pathways.

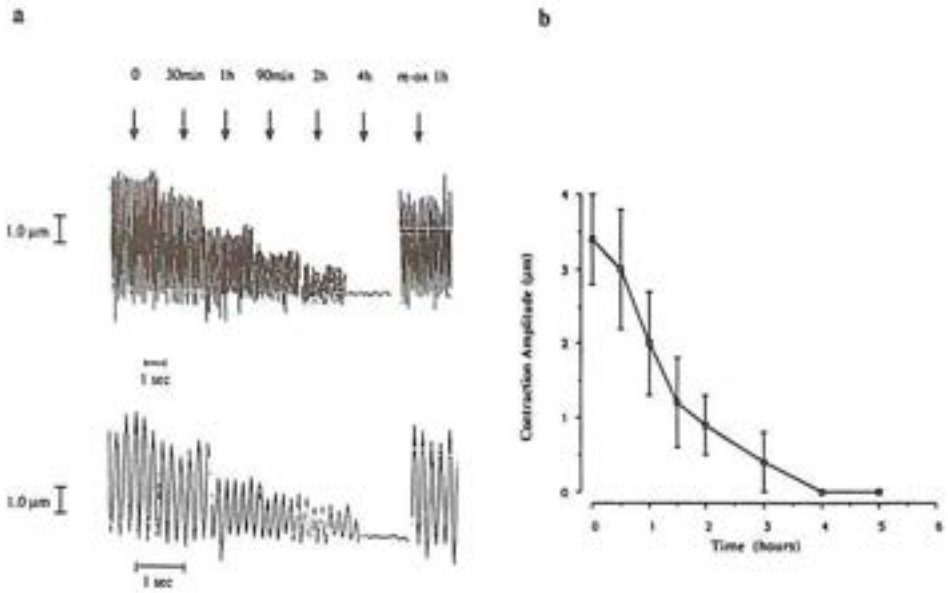

Fig. 6. Loss of contractility of cardiac myocytes during exposure to hypoxia Cardiac myocytes were exposed to hypoxia and low glucose as described in Reference (Webster et al., 1993). (a) shows the contractility changes recorded from a single myocyte and is representative of a typical response. (b) includes measurements from four experiments with four seperate myocyte preparations. A decline in contractility was apparent within the first 30 min of exposure to hypoxia and complete failure occurred within 4 h. There was no apparent decrease in the frequency of contraction until failure.

As one approach to identify possible components of this stress response, we analyzed changes in immediate early gene expression and protein kinase activity during the period of contractile failure. mRNA transcript levels of *c-fos* and *c-jun*, encoding components of the transcription factor APl,were elevated by 5-10 fold within the first hour of hypoxia (Webster et al., 1993). *c-fos* levels were maximal after 2h and began to decrease significantly after 4h, whereas *jun* transcript levels were sustained for at least 6 hours. All transcript levels returned to basal or lower within 12h. Intense Fos and Jun immunoreactivity was seen in the nuclei of cells subjected to hypoxia for 2 - 4 h, comparable to that associated with phorbol ester treatment. Severe, acute hypoxia thus induces rapid accumulation of Fos and Jun proteins in the nuclei of hypoxic cells, coincident with contractile failure

To investigate the signal transduction pathway for this response we tested the effects of selective kinase inhibitors on the hypoxia-mediated induction of fos and jun transcripts (Webster et al., 1993). The induction of both *c-fos* and cjun mRNAs was repressed by the protein kinase A-selective inhibitor KT5720 (Kamiya Biomedical), and essentially eliminated by the protein kinase C inhibitor staurosporine. It is likely that PK-A inhibition had its major effect on basal rather than on hypoxia-induced PKA activity. The potent inhibition of both *c-fos* and *c-jun* inductions by staurosporine suggests that protein kinase activation, including activation of PK-C, plays a role in the hypoxia mediated induction of these proto-oncogenes.

PK-C activation may have a number of effects on the cell in addition to protooncogene induction, including phosphorylation of calcium, potassium, and sodium channels, and phosphorylation of thin-filament regulatory proteins that control calcium sensitivity. Activation of PK-C could thus result in decreased responsiveness to calcium and hence decreased contractility (Gwathmey et al., 1987; Gwathmey and Hajjar, 1990). In our studies, the intracellular resting calcium concentration was 236 nM ± 5 under aerobic conditions, and 275 nM ± 14 following contractile failure under acute hypoxia, but this increase in intracellular calcium was associated with depressed rather than increased

contractility. Contractile failure was also accompanied by enhanced potassium efflux through a glibencamide-insensitive channel, as measured in rhubidium14l-loaded cells (data not shown). These results are consistent with a role for PK-C activation, possibly in combination with decreased intracellular pH, in the development of contractile failure in this model.

Taken together, these results suggest that conditions that simulate severe ischemia are accompanied by contractile failure, activated protein kinase C, strong transient inductions of *fos* and *Jun* transcription, and rapid accumulation of Fos and Jun (AP-1 complex) proteins in the myocyte nuclei. Contractile failure and induction of immediate early genes may be components of a stress response to hypoxia and low glucose. AP-1 has been implicated in many ischemia-associated stress responses (Gunn et al., 1990; Safirstein et al., 1990; Schiaffonati et al., 1990; Brand et al., 1992; Kindy et al., 1991) and probably has numerous target genes under its regulation. We have previously shown that AP-1 regulates the expression of the muscle specific skeletal a-actin gene (Bishopric et al., 1992), and others have demonstrated a similar activation of the atrial natriuretic peptide (ANP) gene (Schiaffonati et al., 1990; Sen et al., 1990) by AP-1. Both skeletal a-actin and ANP genes are activated during myocardial hypertrophy (Parker and Schneider, 1991). Therefore there may be a molecular link between ischemia and hypertrophy through the hypoxia-mediated activation of PKC, induction of AP-1, and the targeting of these to positive regulatory elements in the skeletal a-actin and ANP gene promoters.

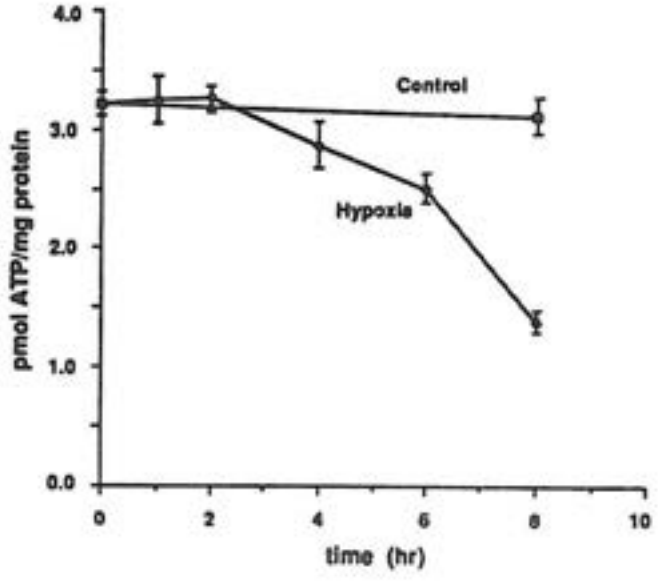

Fig.7. ATP levels during Hypoxia. ATP was measured in duplicate plates at each point as described in Reference (Webster et al., 1993). Bars represent S.E.M. (n=3).

(3) Model of ischemia and reperfusion

Intermittent myocardial ischemia imposes two extremes of redox stress on cardiac tissues. Hypoxic stress occurs during the ischemic phase, as discussed above, and oxidative stress results during reoxygenation, when the ischemic tissue is reperfused. During the ischemic phase, hypoxia triggers metabolic and ionic changes (Allen et al., 1989; Macleaod, 1989; Cascio et al., 1992), changes in cyclic nucleotide levels, protein kinase activities, and immediate early stress genes (described above and in references(Webster et al., 1989; Feldman et al., 1987; Webster et al., 1993; Neumann et al., 1988; Webster and Bishopric, 1992)). In addition, sustained hypoxia is associated with a decline in intracellular redox buffers (Werns and Lucchesi, 1990; Cowan et al., 1993). Restoration of blood flow induces the formation of reactive oxygen intermediates (ROIs) and suboxidative cell injury, exacerbated by the loss of redox buffering capacity. This reperfusion damage, including myocyte death due to both necrosis and apoptosis, can be limited by pre-exposure to antioxidants or to antibodies that block leukocyte adhesion (Gottlieb et al., 1994; Ma et al., 1993). ROIs damage cells directly by oxidizing cellular

components, and indirectly by activating inflammation(Satriano et al., 1993; Ivey et al., 1995; Galinanes et al., 1993). The relative contribution of reperfusion damage to the progression of ischemic heart disease has not been determined. Likewise, the signal transduction pathways for redox stress response in cardiac myocytes have not been defined.

Several recent reports indicate that signal pathways involving the stress- and mitogen-activated protein kinases (JNK/SAPK and MAPK/ERKs) may have important roles in the myocardial response to redox

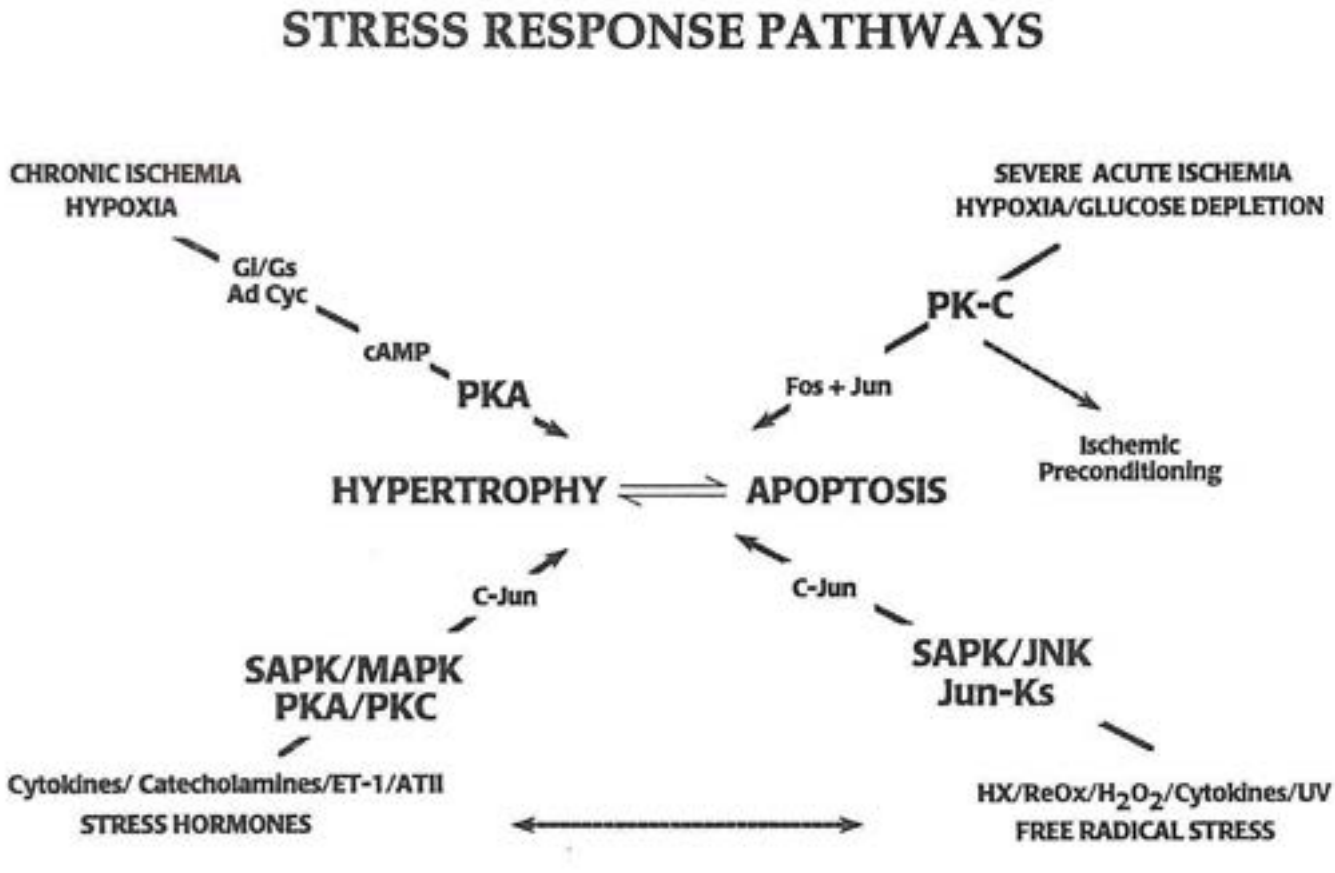

Fig. 8. Summary of Stress Responses and Second Messengers that Contribute to the progression of Myocardial Hypertrophy.

stress and hypertrophy. These pathways consist of multiple serine, threonine and tyrosine kinases that relay growth and stress-related signals from extracellular stimuli to the cell nucleus (reviewed in (Cano and Mahadevan, 1995; Karin, 1995; Sanchez et al., 1994; Kyriakis et al., 1994)). JNK/SAPKs were originally identified as serine/threonine kinases which phosphorylate the amino terminal transactivation domain of the transcription factor c-Jun. MAPKs/ERKs are defined as mitogen activated protein kinases, or extracellular signal regulated kinases (designated here as MAPKs). Activation of these pathways through plasma membrane or cytoplasmic receptors results in the translocation of a terminal kinase to the cell nucleus. Once in the nucleus, the kinase modifies specific target proteins by phosphorylation, leading to changes in gene expression and other cell functions. Although there is cross-talk between the JNK/SAPK and MAPK pathways, they are activated by different extracellular stimuli, are subject to different regulatory mechanisms, and involve different intermediate kinases and terminal target proteins. Unlike MAPKs, the JNK/SAPKs are characteristically activated by survival-threatening stresses, such as protein synthesis inhibitors, inflammatory cytokines, ultraviolet irradiation, osmolarity changes, sodium arsenite, okadaic acid, muscarinic receptor stimulation, and heat shock (reviewed in (Cano and Mahadevan, 1995; Moriguchi et al., 1995)). A number of stimuli induce both pathways, albeit with different potencies and kinetics, and both pathways can be induced by activated Ras (Westwick et al., 1994). MAPK and JNK/SAPK signaling pathways are

probably involved in coordinating growth and/or repair responses in the nucleus, cytoplasm, and cytoskeleton and may in some instances be involved in the initiation of apoptosis (Bagrodia et al., 1995; Xia et al., 1995; Johnson et al., 1996; Verheij et al., 1996).

The activation of JNK/SAPKs appears to be closely linked with redox events. The pathway is potently activated by treatment of cells with irradiation, H_20_2, and cytokines (Devary et al., 1992; Guyton et al., 1996; Verheij et al., 1996; Moriguchi et al., 1995; Lo. et al., 1996). In an experimental model of ischemia and reperfusion in the kidney the JNK/SAPK pathway was activated in a manner that correlated with ATP depletion (Pombo et al., 1994; Morooka et al., 1995). Modest and transient activations of cardiac myocyte MAPKs have been described in response to hypoxia and reoxygenation in neonatal rat, and release from metabolic inhibition in chick (Seko et al., 1996; Yao et al., 1995), but the initiating signals were not identified. Strong inductions of JNK/SAPK have been reported in ischemia-reperfusion models of perffused rat hearts (Knight and Buxton, 1996; Bogoyevitch et al., 1996) and our laboratory recently reported similar strong (10 -20 fold) inductions of JNK/SAPK in an in vitro model of hypoxia and reoxygenation (Laderoute et al., 1996; Laderoute and Webster, 1997). In these latter studies we demonstrated that the inductions were quenched by adding antioxidants and correlated with depressed intracellular levels of reduced glutathione. Therefore the JNK/SAPK cascade appears to be a component of ischemia and reperfusion at least in these experimental models. It is intriguing that transcription factor c-Jun is a target of the several different kinases that are activated during both acute hypoxia and hypoxia-reoxygenation. It seems likely that these signals form an early initiation event that results in hypertrophy (Webster et al., 1993; Glennon et al., 1995; Laderoute and Webster, 1997).

ABSTRACT

The salient features of this review are summarized in Figure 5, in which we compare the component kinases and protooncogenes of the signaling pathways initiated by redox and sympathetic stimuli. All these stimuli are likely to be involved in ischemic heart disease. Similar or related kinase pathways may be activated by two other hypertrophy mediators, angiotensin II and endothelin 1 (Bogoyevitch et al., 1993; Bodi et al., 1995; Ito et al., 1991; Paul and Ganten, 1992;). Contractility and hypertrophy are two critical parameters that may determine short and long term survival of the cardiac system in the face of ischemic stress. To preserve cell integrity there must be a balance between bioenergetic input and output; if ATP is used more rapidly than it is generated the cell will not survive (Hochachka, 1986; Hochachka et al., 1996a; Hochachka et al., 1996b). Therefore one of the immediate adaptive responses to ischemia is to modify contractility in conformity with the reduced ATP generating capacity. "Hibernating myocardium" may represent a clinical example of this type of contractile adaptation; this term refers to an area of underperfused, non-contracting heart muscle that recovers activity when the oxygen supply is restored. In this situation, reduced contraction may preserve myocyte survival for an extended time period under chronic ischemia. Our model of chronic hypoxia implicates cAMP as a possible mediator of this condition (Webster and Bishopric, 1992). Another example of reversible contractile failure occurs in the acute severe hypoxia model. In this model of low-flow ischemia, contractile failure is associated with sustained ATP levels and prolonged cell survival (Webster et al., 1993). and is reversible on replenishment of oxygen or glucose. The oxygen sensor(s) that initiate contractile failure in this situation not been identified. While reduced contractile activity may be an adequate short term solution to ischemia for the affected myocytes, the resulting depressed cardiac output may be inadequate to sustain necessary functions. Circulatory co.npromise brings about the secondary activation of neurohormonal systems whose net effects are to(l) increase cardiac output and (2) induce long-term trophic responses in the cardiac muscle.

While many components of the stress response pathways are still unidentified, including -the oxygen sensors, it is clear that protein kinases A, and C, and the JNK/SAPK pathways are important intermediates in the regulation of both contractility and hypertrophy in response to ischemic stress. It has been recognized for some time that sympathoadrenal stimulation through norepi-nephrine release regulates both contractility and hypertrophy (Bishopric et al., 1989; Bishopric and Kedes, 1991; Bishopric et al., 1992; Neyses and Pelzer, 1995; Glennon et al., 1995). Protein kinase C and A and associated alterations of IP_3 and calcium handling are likely to be directly involved in regulating contractility (Feldman et al., 1987; Gwathmey et al., 1987; Hajjar and Gwathmey, 1991; Gwathmey and Hajjar, 1990; Webster et al., 1993). Contractility is altered directly by changes in the calcium transient, as well as by changes in the phosphorylation state of specific proteins that

modify ion transport and myofiber reactivity (Garvey et al., 1988; Lee and Allen, 1990; Ruegg and Morano, 1989; Hajjar and Gwathmey, 1991; Sen et al., 1990). The latter include phospholambam, L-type calcium entry channel, SR proteins, and troponins (Garvey et al., 1988; Arai et al., 1994; Wankerl and Schwartz, 1995). Activation of AP- 1 by norephinephrine and other factors may be a critical initial event in the coordinated trophic response that results in myocardial hypertrophy. AP- 1 transactivates several hypertrophy-associated genes, including skeletal a-actin (Bishopric et al., 1992). Therefore a simplified schema for the induction of ischemia-associated myocardial hypertrophy contains five steps: (1) onset of a contractile deficit (2) secondary neurohumoral activation (3) activation of intracellular protein kinases A and C by neurohumoral factors, including norepinephrine and angiotensin II (4) induction and nuclear accumulation of immediate-early gene products, including transcription factor AP1 and (5) transcriptional activation of hypertrophy associated genes, including skeletal a-actin and ANP.

Our results demonstrate that hypoxia activates the same protein kinases and protooncogenes as those activated by sympathoadrenal stimulation. From this, it can be proposed that hypoxia induces hypertrophy through these same signal transduction pathways. In the same way, cycles of hypoxia and reperfusion may also induce hypertrophy via redox-activated pathways; in this case the activation of PKC during hypoxia and of JNK/SAPK during reoxygenation may each contribute to the induction of hypertrophy. The hypertrophy that accompanies ischemic cardiomyopathy *in vivo* may thus be the end result of signals from three different redox stress response pathways in addition to those mediated by sympathoadrenergic stimulation.

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ROLES OF HYPOXIC STRESS PROTEINS IN SOLID TUMORS PRIMING FOR REOXYGENATION?

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RESUMEN: Rol de la Proteinas de Estrés en los Tumores Sólidos: Preparación para la Reoxigenación?

Existen actualmente evidencias de que la hipoxia juega un rol central en la progresión maligna de una amplia variedad de tumores sólidos. Por ejemplo, los microambientes hipóxicos de muchos tumores humanos afectan la capacidad de respuesta terapéutica y pueden también contribuir a las tasas incrementadas de supervivencia de las células más malignas luego de la reoxigenación. La naturaleza detallada de estos fenotipos de resistencia dependientes de hipoxia todavía no están claros, pero pueden incluir un grupo de proteinas diversas que son inducidas en respuesta a los estímulos hipóxicos. Este reporte revisa la evidencia que implica a tres proteinas de estrés hipóxico importantes (*p53*, hemooxigenasa-1 y metalotioneina-IIA) en la protección contra la apoptosis, estrés oxidativo y drogas antineoplásicas luego de la hipoxia/reoxigenación.

Palabras Claves: Hipoxia, Proteinas de estrés hipóxico, Proteinas reguladas por oxígeno, Hemo-oxigenasa, Metalotioneina, Microambiente tumoral.

RÉSUMÉ: Rôle des protéines de stress dans les tumeurs solides. Préparation pour la réoxygénation ?

Il apparaît maintenant évident que l'hypoxie joue un rôle important dans la progression maligne d'une grande variété de tumeurs solides. Un exemple en sont les micro-environnements hypoxiques de nombreuses tumeurs humaines qui affectent la capacité de réponse thérapeutique et peuvent contribuer également à l'élévation des taux de survie des cellules les plus malignes après réoxygénation. La nature détaillée de ces phénotypes de résistance dépendants de l'hypoxie n'est pas encore très claire mais elle peut impliquer un groupe de protéines variées qui sont induites en réponse aux stimuli hypoxiques. Ce rapport examine l'intervention manifeste de 3 protéines importantes de stress hypoxique (*p53*, hémo-oxygénase -1 et métallothionéine-IIA) dans la protection contre l'apoptose, le stress oxydatif et les drogues antinéoplasiques, après hypoxie/réoxygénation.

Mots-clés : Hypoxie, Protéines de stress hypoxique, Protéines régulées par l'oxygène, Hémo-oxygénase, Métallothionéine, Micro-environnement tumoral.

SUMMARY: It is now apparent that hypoxia has a central role in the malignant progression of a wide range of solid tumors. For example, hypoxic microenvironments within many human tumors directly affect therapeutic responsiveness and may also contribute to increased survival rates of the more malignant cells following reoxygenation. The detailed nature of these hypoxiadependent resistance phenotypes remains unclear, but it may involve a set of diverse proteins that are induced in response to hypoxic insults. This report reviews the evidence implicating three important hypoxic stress proteins (*p53*, heme oxygenase-I, and metallothionein-IIA) in protection against apoptosis, oxidative stress, and antineoplastic drugs following hypoxia/ reoxygenation.

Key Words Hypoxia, Hypoxic stress proteins, Oxygen regulated proteins, Heme oxygenase, Metallothionein, Tumor microenvironment.

HYPOXIA IN HUMAN TUMORS

Hypoxia is known to have roles in a number of physiological and pathophysiological processes, including erythroid development, angiogenesis, wound repair, fibrosis, ischemia, and neoplasia. A growing body of evidence suggests that many human tumors contain a significant fraction of hypoxic cells which can directly affect therapeutic responsiveness and possibly malignant progression. The mean oxygen concentrations within most normal tissues exceed 40 mm Hg, while those of many malignant tumors, including breast, cervical, and squamous cell carcinomas, contain regions of very low oxygen concentrations, with PO_2 levels reaching as low as 0-10 mm Hg (Gatenby et al., 1985; Sutherland et al., 1996; Vaupel et al., 1991; Vaupel and Hockel 1995) . These regions are characterized as either chronic or transient (Hockel et al., 1996a; Overgaard and Horsman 1996). Chronic hypoxia is thought to arise from the inability of aberrant vascular networks to deliver an adequate blood supply (Rak et al., 1995a), while microregions of transient hypoxia are associated with deregulated constriction of the tumor blood vessels (Overgaard and Horsman, 1996). *In vitro* and experimental tumor studies showed that hypoxia and subsequent reoxygenation appear to affect on malignant progression in terms of the development of metastasis and resistance to therapy (see Hockel et al., 1996a for a review). The existence of hypoxic microenvironments within these tumors is believed to be correlated with a poorer prognosis independent of treatment, compared with the case for well oxygenated tumors.

Recent observations confirm the clinical relevance of tumor oxygenation. In one study of patients with advanced carcinoma of the cervix who were treated with radiotherapy with or without chemotherapy, patients with a median PO_2 • 10 mm Hg had a 50%

survival of only 8 months, whereas patients with a median PO_2 >10 mm Hg had a 50% survival rate of more than 3 years after treatment (Hockel et al. 1993). Subsequent studies by Hockel's group, using a larger patient cohort and longer follow-up (including a subgroup of patients who underwent primary surgery), confirmed the earlier studies. Specifically, the data indicated that the poorer outcome of patients with hypoxic tumors was mainly due to regional failures with and without distant metastases, regardless of whether surgery or radiation was applied as primary treatment (Hockel et al., 1996b).

The underlying phenotypic alterations involve hypoxia-associated increases in radio- and chemotherapeutic resistance (Coleman, 1988; Overgaard Horsman, 1996; Sakata et al., 1991; Shen et al., 1989; Vaupel Hockel, 1995), gene amplification (Rice et al., 1986), genomic instability (Anderson and Stoler, 1993), increased metastatic variants (Brizel et al., 1996; Ginis and Faller, 1996), and diminished apoptotic potential (Graeber et al., 1996). The precise mechanisms of these hypoxia-associated phenotypes are not well delineated, but some may involve one or more of a set of hypoxia-related stress proteins, termed oxygen regulated proteins or ORPs, that can be upregulated during periods of significant depression in total cellular protein synthesis and cell cycle arrest (Giaccia, 1996; Graeber et al., 1996; Heacock and Sutherland, 1986; Heacock and Sutherland, 1990) .

Oxygen Regulated Proteins (ORPs)

Our previous studies have shown that many cancer and normal mammalian cells respond to hypoxia by increasing the synthesis of ORPs. Figure 1 shows autoradiograms of (35 S-methionine/cysteine) labeled proteins from aerobic and hypoxic A431 human squamous cell carcinoma cultures in two-dimensional SDS-polyacrylamide denaturing gels. Maximum inductions of ORP synthesis and optimal cell survival are observed under conditions of <0.1% O_2 (4-12 h). This experiment not only demonstrates the enhanced synthesis of proteins with masses and isoelectric points similar to those of the originally documented ORPs (260, 150, 100, 80, and 33 kDa) (Heacock and Sutherland, 1990) but also reveals other prominent ORPs, including 52, 57, and 60. Although the inductions of many ORPs can be cell specific, this pattern is typical of human and rodent cell cultures. It should be also noted that this method detects only a small fraction of the approximately 50,000 different cellular protein species and, therefore, likely represents a small fraction of the actual number of proteins induced by hypoxia. Our group is actively involved in studies designed to deterrnine the identities and roles of some of these ubiquitous ORPs represented in Fig. 1. To date, we have determined that ORPs 100 and 80 are identical to glucose regulated proteins (GRPs) 78 and 94, respectively (Roll et al., 1991); ORP 33 is heme oxygenase (HO-1) (Murphy et al., 1991); and ORP 7 is metallothionein IIA (MT-IIA) (Murphy et al., 1994).

Other documented ORPs include erythropoietin, vascular endothelial growth factor (VEGF), interleukin-6, platelet derived growth factor, endothelin 1, transforming growth factor ß, DT-diaphorase, Ɣ-glutamylcysteine synthetase, xanthine oxidase, ornithine decarboxylase, adenylate kinase-3, and the glycolytic enzymes (Bodi et al., 1995; O'Rourke et al., 1996; Sutherland et al., 1996; Yan et al., 1995). Furthermore, a wide range of transcription factors, including hypoxia inducible factor-1 (HIF-1), ciun, cFos, pS3 (Graeber et al., 1994), C/EBP$_ß$, HSF-1, AP-1, NF-$_κ$B, ATF-2, and ATF-4, are associated with hypoxia and anoxia responses in both normal and cancer cells (Estes et al., 1995; Graeber et al., 1994; Laderoute et al., 1996; Sutherland et al., 1996). Transcriptional and/or posttranslational modifications of these factors may occur in response to hypoxia, but in many cases the significance of the changes is not clear.

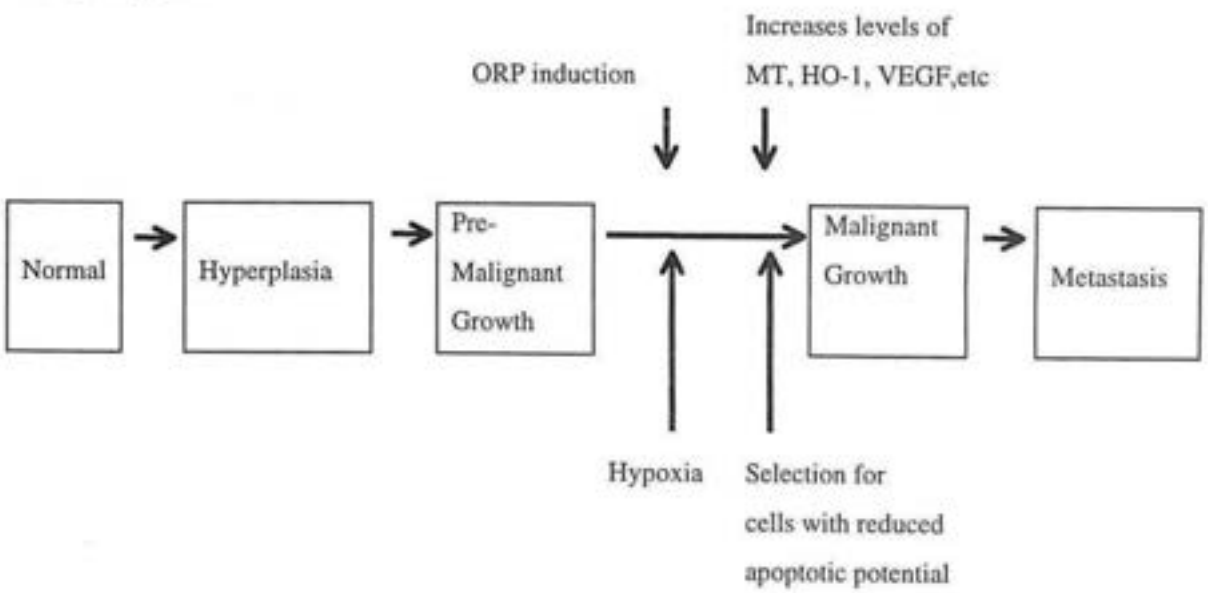
ORPs and Malignant Phenotypes

Recent research has demonstrated fundamental contributions of hypoxia-induced proteins to processes that favor malignant progression (Giaccia, 1996; Rak et al., 1995b). For example, VEGF, which mediates tumor angiogenesis (Plate et al., 1992; Shweiki et al., 1992, 1995) correlates directly with areas of hypoxia present in *in vitro* tumor models (Waleh et al., 1995) and in solid tumors (Plate et al., 1992; Shweiki et al., 1992). These studies support the theory that VEGF isoforms generated in hypoxic microregions of tumors stimulate host endothelial cells to assemble new vasculature, thus driving tumor growth by a feedback loop. Interestingly, the hypoxia-associated induction of VEGF may be also positively modulated by mutant ras oncogenes (Mazure et al., 1996; Rak et al., 1995a).

Figure 1. Two-dimensional electrophoretic patterns of Triton-XlOOsoluble polypeptides from aerobic (AIR; A) and hypoxic (HX; B) A431 cells. Approximately 7 x 10^5 cpm of sample was loaded for the first dimension. The x-axis represents pI range (pH 4.8 to 7.5), and the y-axis represents molecular weight (x1000 Da).

ORPs and Reoxygenation

We suggest that hypoxia also influences malignant progression through effects of specific ORPs on cellular survival and function during subsequent reoxygenation. This review focuses on three such proteins: *p53*, HO-l, and MT-IIA. Studies by our group (Murphy et al., 1993, 1994; Sutherland et al., 1996) and by Dr. Amotto Giaccia's laboratonS (Giaccia, 1996; Graeber et al., 1996) suggest the involvement of these ORPs in cell survival responses of tumor cells experiencing hypoxia and reoxygenation episodes. Specifically, pS3 has been implicated in apoptotic selection following hypoxia, and we postulate that the hypoxia-induced levels of HO-1 and MT-IIA proteins act as anti-oxidants during reoxygenation events. The following schematic summarizes our current understanding of how hypoxia, in particular induction of the abovementioned ORPs, may enhance malignant growth.



As adapted from Giaccia (1996).

p53

The *p53* gene encodes a tumor suppressor protein that is believed to be an important mediator of cell cycle control and a genetically encoded program of cell death, or apoptosis (Yonish-Rouach, 1991). It is believed that p53, acting as a transcription factor (Prives al., 1994), activates expression of proteins to induce cell cycle arrest or apoptosis in response to DNA damaging agents and oxidative stress (Arrowsmith and Morin, 1996; Donehower and Bradley, 1993; Lee and Berstein, 1995; Vogelstein and Kinzler, 1992). Other studies now indicate that hypoxic stress also induces the accumulation and activity of this protein (Graeber et al., 1994). This induction by low oxygen conditions suggests a potential model for hypoxia in tumorigenesis (Giaccia, 1996). The hypothesis is based, in part, on the fact that mutations of p53 occur in diverse tumor types and affect protein binding to DNA (Kinzer and Vogelstein, 1996). Most tumors, however, are initiated by genes other than *p53* and go through clonal evolution resulting in expansion. As tumorigenesis progresses, a tumor will possess both wild-type and mutant p53 cells. At this stage, many tumors will have outgrown their blood supply, the result being microregions of hypoxia or anoxia where *p53* activation occurs. Although VEGF expression is also activated by hypoxia, resulting in angiogenesis, the tumor eventually reaches a stage where both chronic and transient hypoxia (see above) will occur. As these regions experience hypoxia/reoxygenation cycles, cells harboring wild-type *p53* initiate apoptosis, while cells with mutant *p53* will begin to multiply uncontrollably.

It is believed that these cells harboring the mutant *p53* gene expand their mutant genomes through gene amplification and gross chromosomal abnormalities and utimately become metastatic (Kinzer and Vogelstein, 1996). This model has been partially confirmed in both tissue culture and animal studies. Giaccia's group (Graeber et al., 1996) demonstrated that EIA/Ha-ras transformed mouse embryonic fibroblast cells (MEFs) possessing the null genotype for *p53* exhibited greatly diminished apoptosis rates following hypoxia (0.02% O_2 for 48 h) as compared with their wild-type counterparts (Fig. 3). Studies using mixed cultures of wild-type and mutant *p53* cells (1000: 1 ratio) exposed to multiple rounds of hypoxia also demonstrated that transient hypoxia can select for apoptosis-resistant cells for example, the mutant cells had overtaken the wild-type cells following only 7 rounds of hypoxia/reoxygenation. *In vivo* evidence of this process was also obtained using tumors grown from these wild-type and null *p53* cells (Graeber et al., 1996). Specifically, apoptotic regions of tumors derived from either *p53* wild-type or null MEFs were correlated with hypoxic regions within the same tumors. However, the incidence of apoptosis was 3- to 4-fold greater in hypoxic regions of *p53* wild-type tumors than in hypoxic regions of *p53*-deficient tumors, whereas no difference was observed in aerobic regions from the same tumors.

ORPs and Redox Control

Cells have developed very intricate strategies to maintain an intracellular reduced state in response to not only an oxidizing extracellular environment but also oxidants from normal metabolism, reductive biosynthesis, and many environmental stresses. The cell apparently utilizes fluctuations of redox levels (or ratios) to control protein activity in a manner similar to posttranslational phosphorylation. It is this redox control that is believed to be an important determinant in the regulation of many animal and plant processes, including transcriptional regulation, cell division, meiosis, DNA replication, cell division, and protein assembly and repair (Buchanan et al., 1994; Powis et al., 1995). The main defenses, or modulators of redox, include three groups: (1) the glutathione (GSH) and thioredoxin redox buffer systems that maintain protein thiol homeostatasis (Meister, 1991; Powis et al., 1995); (2) superoxide dismutase, catalase, and glutathione peroxidase, which are involved in superoxide anion and H_2O_2 metabolism; and (3) protein disulfide isomerase, which regulates protein folding. A wide variety of stresses and toxins can readily result in an intracellular oxidized state that gives rise to the formation of reactive oxygen species and thus leads to lipid peroxidation, DNA crosslinking, and formation of disulfide bonds in proteins (Powis et al., 1995). In this report we focus on hypoxia with subsequent reoxygenation, an event common in many solid tumors experiencing both transient and chronic ischemia-like insults.

Hypoxia is believed to result in severely compromised oxidative defense systems upon subsequent reoxygenation. It is believed that the generation of reactive oxygen radicals upon reperfusion is responsible for tissue damage in pathophysiological processes such as stroke or ischemic infarctions of the heart. Recent studies by our group and others have begun to unravel cellular strategies of cancer cells that allow them to survive oxidative stress associated with reoxygenation. We hypothesize that it is the hypoxia/reoxygenation-resistant cells within transient and chronic hypoxic

micro-regions of a solid tumor that contribute to malignant progression. The effect of hypoxia on the redox state of human cancer cells has been determined by direct measurement of the principal cellular antioxidant thiol, GSH, in HT29 colonic carcinoma cells (O'Dwyer et al., 1994) and squamous cell carcinoma SiHa cultures (Laderoute et al., 1996). Both studies showed that total GSH content was depleted to approximately 20-30% of the level found in aerobic control cells. These reductions are thought to be caused, in part, by the inhibition of GSH-synthesizing enzymes. Interestingly, hpoxia/reoxygenation apparently has no effect on the steady state levels of either thioredoxin or protein disulfide isomerase (B. Murphy, unpublished observations).

Our group has previously identifled two ORPs that are strongly implicated in cellular protection against oxidative stress. We suggest that both MT-IIA and HO-1 function as antioxidants which may partially replace the depleted GSH buffering system and therefore offer hypoxic tumor cells protection during reperfusion. Furthermore, it is likely that inductions of the two ORPs result in increased resistance to chemotherapy in reoxygenated cells and may also have roles in cell cycle regulation.

Heme Oxygenase-1 (HO-1)

Heme oxygenase catalyzes the rate-limiting step in the oxidative degradation of heme to biliverdin (Tenhunen et al., 1968), which is enzymatically reduced to bilirubin by biliverdin reductase (Tenhunen et al., 1969). There are two documented isoforms of the enzyme, HO-1 and HO-2 (Maines,1988). HO-2 is a constitutive protein found in the central nervous system, while HO-1 is the inducible form that is found in most animal tissues. Transcription of HO-1 is induced by its substrate, heme, as well as by heavy metals, cytokines, endotoxin, hormones, and oxidative and hypoxic stresses (Lee et al., 1996a; Murphy et al., 1991). The functional significance of HO-1 induction following oxidative stress is only now becoming apparent. For example, within the last five years, a number of groups have reported that increased HO-1 provided cellular protection against heme-mediated oxidant injury. Nath et al. (1992) observed that prior induction of HO- 1 with hemoglobin prevented kidney failure and reduced mortality in the rat. Furthermore, tin protoporphyrin, a competitive inhibitor of heme oxygenase, exacerbated kidney dysfunction. Others have reported similar protective effects of HO-1 against hememediated oxidant injury in a rat model of endotoxic shock and lung injury (Otterbein et al., 1995) and in cultured endothelial cells (Abraham et al., 1995). More recently, Lee et al. (1996a) provided strong evidence indicating that overexpression of HO-1 in human pulmonary epithelial cells (A549 cells stably transfected with rat HO-1 cDNA) facilitated cellular protection against non-heme-mediated oxidant insults, (e.g., hyperoxia), as determined by comparing their reaction with that of wild-type cells. This is consistent with other studies suggesting that oxidative stresses result in elevated expression of HO-1 (Applegate et al., 1991; Choi et al., 1995; Lee et al., 1996b; Vile et al., 1994). Lee's group further showed that the overexpression of HO-1 in the A549 culture model may be associated with cell growth arrest, which may facilitate cellular protection against non-heme-mediated oxidant insults.

The precise mechanism of cellular protection by HO-1 remains unclear. However, an examination of the products of the HO enzyme and subsequent reactions in the heme degradation pathway may offer some insights. For example, it has been reported that regulation of the enzymatic rates of the HO reaction (e.g., by oxidative stress, antisense molecules, methemoglobin, and tin mesoporphyrin) directly regulates ferritin protein levels as a result of iron release-from heme (Eisentein et al., 1991; Vile et al., 1994). Ferritin constitutes the major storage site for nonmetabolized intracellular iron and therefore plays a critical role in regulating the availability of iron to catalyze reactions such as the Fenton reaction and the peroxidation of lipids (Vile et al., 1994). It is believed that the increased ferritin concentrations resulting from increased HO activities result in a sequestration of iron and thus impact on free radical reactions, reducing the oxidant burden on the cell (Balla et al., 1992). Indeed, previous studies have implicated ferritin in the protection of rat kidney, and cultured aortic and skin fibroblast cells from oxidant-induced damage (see Vile et al., 1994). Therefore, ferritin, regulated by the response of HO to inducers such as hypoxic and oxidative stresses, probably acts as an important stress-inducible antioxidant.

Another interesting by-product of heme degradation by HO- 1 is bilirubin, which is the product of the biliverdin reductase reaction. This end product of heme metabolism has generally been considered a potentially cytotoxic, lipid-soluble waste product that must be excreted. However, it now appears that bilirubin, at micromolar concentrations *in vitro*, displays potent antioxidant properties (Stocker et al., 1987).

Specifically, bilirubin has been demonstrated to scavenge peroxyl radicals as efficiently as a-tocopherol, which is regarded as the best antioxidant of lipid peroxidation. Interestingly, carbon monoxide (CO) is also an end-product of the HO reaction. In fact, HO is believed to be the sole intracellular source of CO. This small molecule is postulated to behave as a messenger molecule much like nitric oxide (NO), perhaps by activating guanylyl cyclase (Marks et al., 1991; Verma et al., 1993). CO has been demonstrated to inhibit platelet aggregation and cause relaxation of femoral, carotid, and rat coronary and aortic smooth muscle (see Marks et al, 1991, for a review) as well as function as a neurotransmitter (Verma et al., 1993). More recent studies suggest that HO may modulate intracellular signaling by NO through CO effects on cyclic GMP, a second messenger for NO. In particular, Maulik and coworkers (1996) used rat hearts to demonstrate that inducers of NO (L-arginine) and inhibitors of HO (protoporphyrins) reduced free radical formation (malonaldehyde) and increased specific myocardial functions, as compared with levels in untreated hearts, following ischemic insults of 30 min. Although NO can behave as an oxidant under certain conditions, in vitro studies by Maulik' s group demonstrated that NO reduced the reactive oxygen species produced by myoglobin and oxoferrylmyoglobin, which are present in high concentrations during the reperfusion of the ischemic heart. Our laboratory is presently developing a similar tumor model to study the effects of HO-derived CO on cell survival in transient hypoxia and thus in malignant progression.

Metallothioneine-IIA

Metallothioneins are a family of ubiquitous low molecular weight proteins (6 -7 kDa) enriched in cysteine residues. The metallothionein (MT) family consists of two major isoforms (MT-I and -II) and a brain-specific isoform, MT-III. There are at least 12 distinct genes of the MT-I and -II family, of which 6 or 7 are believed to be functional. The expression and regulation of four of these proteins, MT-IA, MT-IB, MT- 1G, and MTIIA, have been extensively studied (Samson et al.. 1995: Skroch et al.. 1993). All four isoforms are constitutivelv expressed in most cultured human cells, with MT-IIA accounting for at least 50% of the total cellular MT protein (Skroch et al., 1993), and it is this isoform which is responsive to a wide variety of inducers. MTs have well-established regulatory roles in metal ion homeostasis and in the detoxification of heavy metals (Leyshon-Sorland et al., 1993a). Other inducers include hormones (glucocorticoids and progesterone), cytokines (interferon-α, interleukin-I, and tumor necrosis factor), phorbrol ester tumor promoters, and environmental stresses such as hypoxia, oxidative stress, UV irradiation, and DNA damaging agents (e.g., cisplatin) (Lazo and Pitt, 1995; Leyshon-Sorland et al., 1993b; Murphy et al., 1994; Skroch et al., 1993).

Because MT is the major intracellular protein thiol- and zinc-binding protein, it may assume an important regulatory role in Zn homeostasis and thus affect the activity of Zn dependent proteins, including transcription factors TF-IIA and SP-1 (Woo et al., 1996). It is also possible that the metal response transcription factors (MRFs) are regulated by their target genes, since preliminary reports showed two of these proteins to contain zinc finger domains of the cysteine-histidine type (Inouye et al., 1994; Radtke et al., 1996; Woo et al., 1996). MT may also contribute copper to key antioxidants, including Zn/Cu superoxide dismutase, and therefore have an antioxidant role. The nucleophilic nature of the protein also implies a direct antioxidant role. For example, purified MT protein was reported to act as a scavenger of free hydroxyl radicals (Thornalley and Vasak, 1985) and has been shown to be approximately 39-fold more effective at protection of DNA from hydroxyl radical attack than glutathione cysteine (Abel and de Ruiter, 1989). Furthermore, a high nuclear content of MT in V79 Chinese hamster cells was shown to confer protection from hydroxyl radical attack (Chubatsu and Menrghini,1993). This ORP also protects against the toxic effects of tert-butyl hydroperoxide (Schwartz et al., 1994), NO (Schwartz et al., 1995), and some electrophilic mutagens (Kaina et al., 1990; Kelley et al., 1988; Lohrer AND Robson, 1989; Schwartz et al., 1995).

MT is of particular interest in the study of malignant progression primarily because of its suspected involvement in protection against anticancer drugs, radiation, oxidative stress, and apoptosis (Deng et al., 1996; Kaina et al., 1990; Lazo and Pitt, 1995; Lohrer and Robson, 1989; Sato et al., 1995; Schwartz et al., 1995). Overexpression of this thiol-rich protein has been implicated in increased resistance to electrophilic and alkylating antineoplastic agents including cisplatin, doxorubicin, melphalan, bleomycin, N-methyl-N-nitrosurea (MNU), and N-methyl-N'nitro-N-nitrosoguanidine (MNNG). It has also been suggested that MT plays a physiological role in the cellular proliferative (and malignant) phenotype of human colonic and breast cancer cells (Nagel and Vallee, 1995). Clinical studies reinforce

these f1ndings, since high expression of MT correlate with poor prognosis in human breast cancer (Goulding et al., 1995) and with poorly vascularized, malignant human lung tumors (Koomagi et al., 1995).

Our ongoing research appears to confirm the importance of MT in the protection of tumor cells against the deleterious effects of hypoxia/reoxygenation and subsequent drug exposure. For example, our finding that hypoxia increases hMT-IIA expression in A431 cells suggested that increased protein expression in hypoxic microenvironments could cause transient drug resistance. We investigated the ability of hypoxia to cause cisplatin resistance by comparing the clonogenic survival of aerobic and reoxygenated A431 SC cells exposed to various doses of cisplatin for 1 h at 37°C before plating. Hypoxic pretreatment caused an 18-fold increase in survival at the highest cisplatin dose at which survival could be measured (16 Ag/ml; 53 llM). It is worth emphasizing that this cisplatin resistance was a transient phenomenon that cannot be directly compared with the greater degree of resistance associated with prior selection in the presence of the drug (Kelley et al., 1988). Furthermore, it is likely that the survival differences would be greater after an in vivo-like reoxygenation, where the hypoxic cells would not experience the sudden enormous change in oxygen exposure (an approximately 200-fold increase) associated with our normal hypoxic protocol (0.01% O_2 for 8 to 14 h).

To establish a stronger link, we initiated studies using transgenic MT knockout mice. We examined the effect of the loss of MT expression on the cytotoxicity of the drug by using homologous embryonic fibroblast cells from transgenic mice with targeted disruptions of MT-1 and MT-II genes (MT -/-; null) (Kondo et al., 1995). Our initial studies focused on the transformed (SV40 large tumor antigen) knockout and wild-type cells (MT +/+). This type of transformation eliminates functional *p*53 function. Clonogenic survival assays showed that the cisplatin resistance of hypoxic MT +/+ cells was 400- and 2400-fold greater than that of hypoxic and aerobic null cells, respectively (B. Murphy et al., unpublished results). In sum, these data support the hypothesis that endogenous and induced MT levels affect the sensitivity of mammalian cells to clinically important anticancer drugs. We are presently studying primary homozygous null and wild-type cells to investigate the role of *p*53-dependent and -independent apoptosis pathways in cisplatin-mediated cytotoxicity following hypoxia reoxygenation. Preliminary studies also indicate a protective role for MT, in *p*53 wild-type cells, against oxidative stress followinz ischemia-like events.

Summary.

In summary, the evidence reviewed here implicates specific hypoxic stress proteins in the process of malignant progression. Specifically, three ORPs-p53, HO-1, and MT-IIA_appear to be intimately involved in the development of phenotypes associated with increased resistance to apoptosis, oxidative stress, and antineoplastic drugs in response to hypoxia/reoxygenation insults. Future research is needed to confirm these postulates and to further delineate the precise mechanisms of action. Ongoing studies using improved cDNA difference libraries and protocols will almost certainly identify other important proteins associated with hypoxic microenvironments of solid tumors.

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THE REGULATION OF ERYTHROPOIETIN AND OTHER GENES BY HYPOXIA: THE SEARCH FOR UNIVERSAL MECHANISMS

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RESUMEN: La Regulación de la Eritropoyetina y Otros Genes por la Hipoxia: la Búsqueda de Mecanismos Universales

En los mamíferos, la eritropoyesis es controlada por la hormona eritropoyetina, cuyos niveles son dictados por el aporte tisular de oxígeno. El control de la producción de eritropoyetina en células especializadas es ejercida principalmente a través de una secuencia reforzadora crítica y la transcripción del factor-1 inducible por hipoxia (HIF1). El hallazgo de que el reforzador de la eritropoyetina era inducible por hipoxia en la mayor parte de células sino en todas, llevó a la búsqueda de otros genes regulados por este mecanismo. La evidencia de tal regulación proviene de un examen de la expresión de genes en cultivo celular y ha utilizado tres enfoques diferentes. Primero, que como para la eritropoyetina había una inducción de expresión genética por la hipoxia, por ciertos metales de transición y por quelantes de hierro. Segundo, las secuencias de acción-cis que conferían inducción hipóxica a estos genes tenían marcada similaridad con la secuencia crítica en el reforzador de eritropoyetina y podían unirse al complejo transcripcional HIF-1. Tercero, había inducción reducida o ausente de la expresión de genes particulares en las células deficientes en HIF-1. Tal evidencia ha sido obtenida para la regulación hipóxica de muchos genes, incluyendo eritropoyetina, factores de crecimiento angiogénicos, enzimas glicolíticas, transportadores de glucosa, tirosina hidroxilasa, endotelina y sintetasa inducible de óxido nítrico. Se están realizando investigaciones adicionales sobre el mecanismo sensor de oxígeno subyacente y sobre el rol de este sistema de respuesta in vivo.

Palabras claves: Hipoxia, Oxígeno, Eritropoyetina, Angiogénesis

RÉSUMÉ: La régulation de l'érythropoïétine et autres gènes par hypoxie : recherche de mécanismes universels.

Chez les mammifères, l'érythropoïèse est contrôlée par l'hormone érythropoiétine dont les niveaux sont fonction de l'apport tissulaire d'oxygène. Le contrôle de la production d'érythropoiétine dans des cellules spécialisées s'exerce principalement par l'intermédiaire d'une séquence critique de renfort et la transcription du facteur -1 inductible par hypoxie (HIF-1). La découverte du fait que le renforçateur de l'érythropoiétine était inductible par hypoxie, dans la plus grande partie des cellules si ce n'est dans toutes, a conduit à rechercher d'autres gènes régulés par ce mécanisme. L'évidence d'une telle régulation dérive d'un examen de l'expression de gènes dans une culture de cellules, utilisant trois approches différentes. On a observé, premièrement, que comme pour l'érythropoiétine il y avait une induction de l'expression génétique par hypoxie, certains métaux de transition et par chélateurs du fer; deuxièmement, que les séquences d'action *cis* permettant l'induction hypoxique de ces gènes montraient une nette similitude avec la séquence critique du renforçateur de l'érythropoiétine et pouvaient s'unir au complexe transcriptionnel HIF-1; troisièmement, que l'induction était réduite ou absente de l'expression des gènes particuliers dans les cellules déficientes en HIF-1. Une telle évidence a été obtenue pour la régulation hypoxique de nombreux gènes, y compris l'érythropoiétine, les facteurs de croissance angiogéniques, les enzymes glycolytiques, les transporteurs de glucose, la tyroxine hydroxylase, l'endothéline et la synthétase inductible d'oxyde nitrique. Des investigations supplémentaires sont en cours concernant le mécanisme senseur d'oxygène qui sous-tend cette réponse, ainsi que sur le rôle de ce système *in vivo*.

Mots-clés : Hypoxie, Oxygène, Erythropoïétine, Angiogenèse.

SUMMARY: The regulation of erytropoietin and other genes by hypoxia: the search for universal mechanisms

Oxygen is of fundamental importance for living organisms. In higher organisms it plays a vital role in energy provision by acting as the terminal acceptor in the electron transport chain. It also has an essential role in many critical biosynthetic reactions. Despite performing these vital functions, oxygen can exert toxic effects because of the formation of reactive oxygen species. Achieving the appropriate delivery of oxygen to tissues and cells, adjusting metabolism accordingly and minimising the toxicity of oxygen is crucial for living organisms. In order for this to occur, organisms must be able to sense oxygen and respond to changes in oxygen tension by altering the expression of particular genes.

In single cellular organisms, oxygen is obtained from the environment by simple diffusion. In larger, multicellular organisms, complex organs are necessary to supply oxygen to tissues. In mammals oxygen is absorbed by the lungs into the blood. Oxygen has a relatively poor aqueous solubility. To achieve sufficient oxygen supply to the tissues, oxygen carriage in the blood is considerably enhanced by the transport of oxygen liganded to haemoglobin, packaged within red blood cells. A complex vasculature enables the delivery of this oxygenated blood to the tissues. In order for this supply to be optimally maintained, precise distribution of the vasculature to the lungs and other tissues is essential, as is the control of ventilation and red blood cell production.

Key words : Hypoxia, Erytropoictin, Angrogenesis

The regulation of erythropoicin by oxygen

Erythropoiesis is controlled primarily by the hormone erythropoietin whose levels are dictated by tissue oxygen supply. The levels of erythropoietin are altered primarily by changes in gene transcription. Studies of the mechanism of this regulation have been considerably advanced by the demonstration of erythropoietin production in cell culture by the hepatoblastomacell lines, Hep3B and HepG2 (Goldberg et al., 1987). Such experiments demonstrated the capacity for erythropoietin production and altered gene transcription in response to hypoxia in a single cell type. The use of such cells has also permitted an analysis of the cis-acting DNA elements which confer hypoxic regulation to the erythropoietin gene. Several groups independently established the critical role of an enhancer element in the 3'-

flanking region of the erythropoietin gene (Beck et al., 1991; Pugh et al., 1991; Semenza et al., 1991). A 26 base pair sequence has been isolated which could convey hypoxically inducible expression when placed near to heterologous promoters and when re-iterated.

The oxygen sensor

The nature of the oxygen sensor which mediates the regulation of erythropoietin is of great interest. Metabolic inhibitors such as cyanide do not mimic the effect of hypoxia (Tan and Ratcliffe, 1991). These experiments have led to the hypothesis that it is oxygen itself which is being sensed rather than some consequence of hypoxic metabolic compromise such as altered phosphorylation potential, or a non-specific cellular stress response.

A very interesting observation in erythropoietin production is the stimulation of erythropoietin levels by cobaltous ions. This has been observed in humans, experimental animals and in the isolated kidney (Goldwasser et al., 1958; Fisher and Birdwell, 1961; Goldbergetal., 1988). Goldberg and colleagues have analysed these observations in more detail utilising Hep3B cells. They showed that cobalt, nickel and manganese ions could all stimulate the production of erythropoietin by normoxic cells in a dose dependent manner but that other transition metals were without effect (Goldberg et al., 1988). This stimulation was of a similar magnitude to that achieved by exposure to 1% hypoxia and was not additive to it. This led them to hypothesise that the oxygen sensor was a haemoprotein which, analogous to haemoglobin, formed a 'deoxy' confomation in the absence of oxygen. They proposed that the transition metal ions cobalt, nickel and manganese could substitute for ferrous ion in the porphyrin ring of haem. These metalloporphyrins would be unable to bind oxygen and the sensing haemoprotein would be locked in the 'deoxy' conformation.

They provided further evidence for the involvement of a haemoprotein in the sensing process. Carbon monoxide is relatively inert chemically but has a particular affinity for haem. The toxicity of carbon monoxide relates in large part to high affinity binding to haemoglobin 'locking' it in its 'oxy' conformation, thereby,decreasing oxygen affinity and reducing tissue oxygen delivery. The exposure of Hep3B cells to 10% carbon monoxide inhibited the hypoxia-induced increase in erythropoietin. Although these results provide strong circumstantial evidence for a haemoprotein sensor, alternative explanations can be provided for the experimental observations. The inhibitory effects of compounds may not be specific. There are many essential haemoproteins in cells, some of which may play a critical role in oxygen sensing but not via the liganding of molecular oxygen.

Semenza and colleagues have demonstrated an increase in erythropoietin mRNA following exposure of Hep3B cells to the iron chelator desferrioxamine (Wang and Semenza, 1993a). It is more difficult to accommodate this important observation in a simple model of a haem based sensing molecule. Haem iron is not chelatable but examples exist among non-haem ferroprotein sensors of labile iron atoms being susceptible to chelation such as FNR and the iron response element binding protein. Whatever the explanation for the action of these agents on erythropoietin expression, they provide an important set of characteristic pharmacological responses. This pattern of response can be used to examine the existence of the same or similar mechanisms of sensing and signal transduction in the regulation of other genes by oxygen.

Hypoxia-inducible factor-1

The nature of the transcription factor (s) which mediate this transcriptional response to hypoxia has been elucidated by Semenza and Wang. Initially they demonstrated that nuclear extracts from Hep3B cells contained DNA binding activity induced by hypoxia which bound specifically to the cis-acting 3' hypoxia inducible enhancer element from the erythropoietin gene (Semenza et al., 1991). They termed this DNA-bindingactivity hypoxia-induciblefactor-1 (HIF-1). Other stimuli such as cobaltous ions, known to mimic the hypoxic induction of erythropoietin mRNA, also produced an inducible DNA-binding activity. Recently, Wang and Semenza have purified this DNA-bindingactivity and shown that HIF- 1 exists in a heterodimeric form of the 120 kDa HIF-1 α factor complexed with a 91-94 kDa HIF-1β subunit. The HIF Iα cDNA encoded a predicted 826 amino acid protein of 93 kDa which possessed substantial homology to the Drosophila melanogaster basic-helix-loop-helix transcription factors period (Per) and single-minded (Sim) and to the mammalian aryl hydrocarbon receptor (AHR) and aryl hydrocarbon receptor nuclear translocator (ARNT) which all possess a 150 amino acid domain known as the PAS domain (PerARNT--AHR-Sim). The HIF-1β cDNA encoded a 789 amino acid protein with a calculated mass of 87 kDa. The sequence was identical to the previously described aryl hydrocarbon receptor nuclear

translocator (ARNT). This molecule is essential for the transcriptional response to certain aryl hydrocarbons which has been termed the xenobiotic response (for review see (Hankinson, 1995)).

The widespread nature of the system controlling erythropoietin

It had been believed that this mechanism of transcriptional control of the erythropoietin gene by hypoxia would be restricted to those specialised cells which produced erythropoietin. However, Maxwell and colleagues demonstrated that reporter genes containing the hypoxically responsive cis-acting element from the erythropoietin gene could be induced by hypoxia in a variety of different ce]l types (Maxwell et al., 1993). Cells which do not produceerythropoietin and which are not derived from liver or kidney were all able to show hypoxic induction of these transiently transfected reporter constructs. Again, the characteristic responses of the erythropoietin gene to treatment with cobalt was preserved. No induction was seen with metabolic inhibitors such as cyanide (Maxwell et al., 1993). Heat shock induction is also known to operate widely in cells when exposed to noxious stimuli, including anoxia. This generalised mechanism of hypoxic sensing did not appear to be a manifestation of the heat shock response; heat shock did not activatethe erythropoietin enhancer, the 1% hypoxia used did not activate heat shock proteins and cyclohexirnide blocked the erythropoietin enhancer mediated response in contrast to its known inducing effect upon the heat shock response. These observations suggested that many, perhaps all, cells shared similar mechanisms for oxygen sensing, signal transduction and transcriptional activation. Furthermore, it appeared likely that other genes would be regulated by hypoxia via this system.

It was demonstrated subsequently that the HIF- 1 DNA-bindingactivity was also present in a variety of non-erythropoietin producing cells, indeed in all cell types tested (Wang and Semenza, 1993b). These observations, also described by Beck and colleagues (Beck et al., 1993), supported the concept that this system of hypoxic gene regulation was widely present and might regulate the expression of other hypoxically inducible genes.

The regulation of other genes by hypoxia

Glycolytic enzymes

The critical role of oxygen as the terminal electron acceptor in the electron transport chain means that energy provision is closely linked to oxygen supply. In hypoxic conditions reduction in aerobic respiration is associated with an increase in glycolytic flux to provide an increase energy supply. In the short term, increases in glycolysis are mediated by changes in glycolytic flux via alterations in the concentrations of allosteric modulators or enzyme phosphorylation. However, over longer time periods changes in the levels of glycolytic enzymes may be achieved by changes in gene expression mediated by hormonal or other influences (for review see (Pilkis and Granner, 1992)).

Evidence for the regulation of glycolytic enzyme expression by hypoxia has been provided by Webster and colleagues (Webster, 1987; Webster et al., 1990). They observed an increase in gene transcription in response to 2% hypoxia in skeletal myoblasts. The enzymes lactate dehydrogenase, pyruvate kinase, triosephosphate dehydrogenase and aldolase all showed an increase in gene transcription in nuclear run on assays. Furthermore, a reciprocal dcrease was seen in the abundance of mitochondrialy encoded respiratory enzymes. These observations supported the existence of a mechanism for the co-ordinated regulation of glycolytic enzyme expression by oxygen availability, perhaps via common trans-acting factors and similar cis-elements.

The widespread operation of the system controlling erythropoietin regulation raised the question of the role of this system in non-erythropoietin producing cells. The hypoxic regulation of glycolytic enzymes was thus an attractive candidate. The serendipitous observation that a neomycin resistance marker under the control of the phosphoglycerate kinase (PGK) promoter showed hypoxic inducibility led to an examination of the hypoxic regulation of PGK expression. Hypoxic induction of PGK and LDH-A by hypoxia was demonstrated in several tissue culture lines (Firth et al., 1994). This regulation shared several important sirnilarities with the regulation of erythropoietin. The induction could be mimicked by cobaltous ions and by the iron chelator desferrioxamine but cyanide had no effect. Similar observations were made with lactatedehydrogenaseA (Firth et al., 1994; Firth et al., 1995)and subsequently with Aldolase A, Pyruvate kinase M and phospofructokinase (Semenza et al., 1994; Ebert et al., 1995b). The analysis of the PGK and LDH-A promoters utilising the transient transfection of deleted promoter-reporter constructs defined short cis-acting sequences which conveyed hypoxic inducibility. Furthernore, the critical 18 base pair

element in the PGK enhancer/promoter shared substantial homology with the erythropoietin 3'-enhancer including a 9 bp region which was identicalexcept for a G to C substitution (Firth et al., 1994). Electrophoretic mobility shift assays utilising oligonucleotides from these promoters produced hypoxically inducible protein binding with similar characteristics to the HIF-1 complex and which could cross compete with oligonucleotides derived from the erythropoietin 3'-enhancer (Firth et al., 1994; Semenza et al., 1994).

Glucose transporters

In order to respond to an increased rate of glycolysis cells require an increased provision of glucose. Glucose is transported across cell membranes by the process of facilitated diffusion mediated by the glucose transporter farnily. Indeed for one of these transporters, Glut-l, there is evidence of increased expression in response to hypoxia (Loike et al., 1992). However, mitochondrial inhibitors can also increase Glut-1 mRNA (Shetty et al., 1992) perhaps suggesting that induction by hypoxìa is due to the hypoxic compromise of metabolism and is distinct from the mechanism underlying erythropoietin regulation. Recently however, the demonstration of the induction of Glut- 1 and Glut-3 mRNA by hypoxia was shown to share similarities with erythropoietin regulation. The induction could be mimicked by cobalt and by iron chelators (Ebert et al., 1995b). An enhancer lying 5' to the mouse Glut-1 gene was found to convey responses both to hypoxia and to mitochondrial inhibitors. The response to hypoxia was conveyed by sequence containing a HIF-1-like binding site whilst response to mitochondrial inhibitors was mediated by a serum response element lying 100 bp 5' (Ebert et al., 1995a). Thus it appears that rather than demonstrating a different mechanism of oxygen sensing, the regulation of Glut-1 by hypoxia and mitochondrial inhibitors is mediated by two different sensing systems, one of which resembles that involved in erythropoietin regulation.

Gluconeogenic enzymes

In the liver there is metabolic zonation of enzymes, notably a preferential periportal distribution of gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and increased expression of enzymes favouring glycolysis such as glucokinase in the perivenous zone (Jungermann, 1995). There is evidence to suggest that this zonation may be mediated, at least in part, by oxygenation. Increased oxygenation results in the stimulation of gluconeogenesis by hepatocytes whilst hypoxia favours enhanced glycolysis. The glucagon stimulated expression of one critical gluconeogenicenzyme PEPCK is increased when hepatocytes are exposed to arterial oxygen tensions (Hellkamp et al., 1991). This modulation by oxygen is lost if the cells are pretreated with cobaltous ions (Kietzmann et al., 1992) and carbon monoxide blocks the effect of hypoxia (Kietzmann et al., 1993). This supports the involvement of a similar oxygen sensing system to that which regulates erythropoietin.

Angiogenic growth factors

Higher organisms such as mammals have developed a complex vasculature to supply oxygen and other nutrients to their cell. The development of the vascular system appears to be under the control of many important influences one of which is likely to be hypoxia. Angiogenesis, the formation of new blood vessels from existing vasculature, appears to play a role in physiological processes such as placentation, the development of the corpus luteum and in wound healing. Excessive angiogenesis is associated with pathological states including proliferative retinopathies, atherosclerotic plaques, tumour growth and metastasis.

There are several observations that suggest that oxygen provision is an important determinant of vascular supply and of angiogenesis. The limited stores of oxygen compared with other metabolic substrates makes oxygen an attractive candidate for the signalling of vascular insufficiency. The metabolic requirements of a tissue are closely linked to its vascular supply. The capillary length density, a measure of vascular supply, of a wide range of muscles from different species and sites shows a close linear relationship with mitochondrial volume density, a marker of oxidative capacity (Adair et al., 1990). Further evidence for an important role of oxygen availability in determining vascular growth is also seen in pathological conditions. In premature infants exposed to high concentrations of inspired oxygen the retinal vasculature is attenuated. Upon return to normal oxygen, there is a profuse growth of disorganised, leaky vasculature with the subsequent development of retrolental fibroplasia.

Vascular Endothelial Growth Factor (VEGF) appears to be a particularly selective and potent angiogenic factor. Recent work has provided evidence suggesting an important role for VEGF in

physiological and pathological angiogenesis and that hypoxia is an important stimulus for expression of this gene (Shweiki et al., 1992). We have shown that hypoxic regulation of this and other angiogenic growth factors shares important similarities with erythropoietin regulation (Gleadle et al., 1995a), in particular stimulation by cobalt and iron chelation. Indeed, more recently, others have demonstrated the existence of cis-acting sequences in the VEGF gene which can confer hypoxic regulation. Levy and colleagues demonstrated a 12-fold increase in VEGF mRNA in hypoxically-stimulated PC12 cells (Levy et al., 1995). They went on to identify a 28-base pair element in the 5' promoter with sequence and binding similarities to the HIF-1 binding site within the erythropoietin 3'-enhancer (Levy et al., 1995).

This region is highly conserved in the human gene and was similarly identified by Liu and colleagues as an elementthat was necessary and sufficient to increase transcription in response to hypoxia (Liu et al., 1995). At odds with these results is another study which has defined hypoxia responsive elements at other sites in the 5' and 3' untranslated region of the VEGF gene which do not share homology with a HIF- 1 site (Minchenko et al., 1994).

Tyrosine hydroxylase

Tyrosine hydroxylase is the rate limiting step in the synthesis of catecholamines. Exposure of rats to 10% oxygen resulted in a significant increase in tyrosine hydroxylase mRNA in the carotid body Type I cells (Czyzyk-Krzeska et al., 1992). This increase appears to be mediated in part by an increase in gene transcription. The deletional analysis of the tyrosine hydroxylase promoter has demonstrated a sequence 2W150 from the start site which can confer hypoxic regulation to reporter genes. This region contains HIF-1 like and AP-1 recognition sequences, both of which show hypoxically regulated protein binding (Norris and Millhorn, 1995).

Nitric oxide synthase

The availability of oxygen and other nutrients to tissues is dictated both by the anatomy of the vascular bed and by vascular tone. One of the most important regulators of blood flow appears to be endothelially derived nitric oxide (NO). Some evidence has been provided for modulation of NO synthases by oxygen. Hypoxic exposure produced a fall in endothelial NO synthase mRNA levels in vascular endothelial cells that appeared to be mediated through a reduction in gene transcription and in mRNA stability (McQuillan et al., 1994). Melilio and colleagues examined the induction of ioducible NO synthase by the L-tryptophan metabolite Picolinic acid in interferon treated murine macrophages (Melilloet al., 1995). The cis-acting element responsible for this induction was analysed in transient transfection assays and contained a region with substantial homology to the hypoxia responsive enhancer from the erythropoietin gene, including an exact 13 bp match. Electrophoretic mobility shift assays demonstrated inducible binding to this sequence by both picolinic acid and by hypoxia. Hypoxia was then showed to induce inducible NO synthase mRNA. These observations are of interest not just because of the likelihood of HIF-1 dependent hypoxic regulation of inducible NO Synthase but also because picolinic acid can function as an iron chelator and this might underly its mechanism of action.

Endothelins

The endothelins are a family of widely expressed peptides which are among the most potent endogenous vasoconstrictors. Hypoxia is a potent inducer of endothelin-1 mRNA in human umbilical vein endothelial cells (HUVEC) (Kourembanas et al., 1991). This regulation also shares important similarities with the hypoxic regulation of erythropoietin mRNA. The hypoxic increase can be abrogated by carbon monoxide (Kourembanas et al., 1993) and can be mimicked by the transition metals nickel and manganese and to a less erextent cobalt (Bodi et al., 1995).

The regulation of genes by hypoxia in other organisms

In lower organisms, including yeast and bacteria, complex mechanisms exist for the sensing of oxygen and the modulation of gene expression. Direct homologies with the system involving the transcription factor HIF- 1 have not been demonstrated yet. In an attempt to demonstrate the existence of the mechanism of oxygen sensing which controls erythropoietin in lower organisms, Nagao et al examined for the presence of hypoxically induced DNA binding in embryonic drosophila cells (Nagao et al., 1996). They were able to demonstrate induction of binding to HIF- 1 recognition sequences by acomplex with similarities to mammatian HIF-1. Furthermore, they showed hypoxic regulation of the PGK gene. These observations support the idea that this

mechanism of oxygen sensing may be conserved in other organisms.

Two main lines of evidence have thus contributed to the idea that the system controlling erythropoietin in response to hypoxia also controls other genes. The demonstration of functionally active cis-acting sequences able to regulate the transcription of many genes in response to hypoxia together with responsiveness of these genes to stimulation with cobaltous ions and iron chelators. More recently a furtherline of evidence has been provided (Wood et al., 1996). Cells exist which are deficient for one component of HIF-1. They are unable to form a functional HIF-1 complex in response to hypoxia. In contrast to the wild type cells from which they were derived, they show marked impairment in the hypoxic induction of glycolytic enzymes such as Phosphoglycerate kinase, glucose transporters such as Glut-1 and angiogenic factors such as VEGF(Wood et al., 1996). These results provide further strong support for the existence of a widespread mechanism of oxygen sensing which acts via the transcription factor HIF-1 and regulates the expression of many genes in response to hypoxia.

Outstanding questions

The system which regulates erythropoietin and these other genes has largely been defined in cell culture. It remains to be determined whether these in vitro observations have relevance in vivo. One approach enabling a study of the effects of HIF-1 deficiency in vivo is by the xenotransplantation of the cells lacking ARNT introduced subcutaneously into nude mice. Preliminary experiments suggest significant differences in the growth characteristics, vascularity and VEGF expression of tumours formed from the c4, ARNT deficient, cells when compared with wild type.

The molecular basis of oxygen sensing still requires definition. As alredy discussed a haem based sensor accommadates many of the observations underlying erythropoietin production. A precedent for a haemoprotein oxygen sensor is provided by the Nif protein in Rhizobium meliloti. However, even if a haemoprotein does play a critical role in oxygen sensing it may not be via the liganding of oxygen and undergoing a conformational change. Several lines of evidence are difficult to accommodate in this simple model of a liganding haemoprotein sensor. The effect of iron chelators in inducing erythropoietin, HIF-1 and other hypoxically regulated genes suggests that chelatable iron is closely involved with the mechanism of oxygen sensing. Haem iron is not chelatable. In some bacteria the FNR protein appears to function as an oxygen sensor. It contains an iron-sulphur cluster and iron chelation produces an aerobic pattern of gene expression. This effect illustrates one way in which iron chelation could interact with an oxygen sensing molecule. Altemative models of oxygen sensing rely upon the redox properties of oxygen. It is envisaged that oxygen is rate limiting in the production of reduced oxygen species such as superoxide and hydrogen peroxide. Observations of the effects of catalase and hydrogen peroxide on erythropoietin expression by Fandrey and colleagues provide some support for such a mechanism (Fandrey et al., 1994) as does the inhibition of hypoxic induction of erythropoietin and other genes by iodonium compounds (Gleadle et al., 1995b). The in vitro redox sensitivity of HIF-1 has been demonstrated suggesting a mechanism by which this might occur (Wang et al., 1995).

The mode of regulation of the transcriptional complex HIF-1 is not clear. Potential mechanisms of regulation include protein phosphorylation, redox alterations and direct ligand binding. Using chimeric fusion genes containing HIF-lα, we have recently been able to confer oxygen regulated activity to heterologous transcription factors. This strategy should provide information about the sequences of HIF-lα which are capable of conferring regulated transactivation permitting insights into the likely mechanism of activation.

The system regulating erythropoietin appears to play an important role in the regulation of genes involved in oxygen supply and consumption. The regulation of other genes involved in other processes such as development and with protective functions seems highly plausible. The increasing realisation of the widespread nature of this system and similar systems in more primitive organisms, emphasises the interest in defining such genes and in the molecular mechanisms underlying oxygen sensing.

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ADAPTATION TO HIGH ALTITUDE: EFFECTS OF SMALL CHANGES IN THE REGULATORY BEHAVIOR OF THE ANDEAN CHICKEN HEMOGLOBIN.

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RESUMEN: Adaptación a la Altura: Efectos de Pequeños Cambios en la Conducta Reguladora de Hemoglobina de Gallinas Andinas

Se ha descubierto en los Andes Peruanos un grupo de gallinas de altura (*Gallus gallus*) con alta afinidad de la hemoglobina por el oxígeno. Hemos estudiado el posible mecanismo molecular subyacente de esta adptación a la altura. Se ha postulado pequeños cambios en la concentración intracelular de inositol pentafosfato (IPP), el principal efector alostérico de la hemoglobina en los eritrocitos de aves. Hemos estudiado la sangre de gallinas andinas y de nivel del mar. Las afinidades de suspensiones frescas de eritrocitos están significativamente incrementadas en gallinas andinas comparadas con las de nivel del mar. Los valores de los coeficientes de Hill a saturación 50% (n50) son mayores para suspensiones de gallinas que para las de mamíferos. Esto podría sugerir la existencia de un proceso de agregación molecular dentro de las células deoxigenadas, altamente concentradas de ambos tipos de aves. Para las soluciones "desnudas" de hemolizados de ambos tipos de gallinas, las afinidades son idénticas enel buffer libre de fosfato, indicando que las afinidades intrínsecas de las Hbs de gallinas andinas y de nivel del mar son las mismas. Con la adición de inositol hexafosfato (IHP), un fuerte efector alostérico de hemoglobinas de aves, observamos un incremento pequeño pero significativo (aproximadamente 20%) en la afinidad de los hemolisados de altura en relación a los de nivel del mar. Nuestros resultados también sugieren que la diferencia en la afinidad entre sangres de altura y de nivel del mar puede deberse a un proceso adaptativo posiblemente relacionado con una ligera disminución en la concentración y/o actividad del principal efector celular IPP, más bien que a una anormalidad estructural de la hemoglobina.

Palabras claves: Adaptación a la altura, Hipoxia, Hemoglobina, Unión al oxígeno, Eritrocitos, Sangre de Gallinas.

RÉSUMÉ: Adaptation à l'altitude : effet de légères variations du comportement régulateur de l'hémoglobine des poules andines.

Un groupe de poules natives des zones d'altitude (*Gallus gallus*) dont l'hémoglobine présente une très grande affinité pour l'oxygène a été découverte dans les Andes du Pérou. On a étudié le mécanisme moléculaire qui pourrait être à l'origine de leur adaptation à l'altitude et on a émis l'hypothèse du rôle de légères variations de la concentration intracellulaire d'inositol pentaphosphate (IPP), principal effecteur allostérique de l'hémoglobine des érythrocytes des oiseaux. On a étudié le sang des poules andines et celui des poules du niveau de la mer. L'affinité pour l'oxygène de l'hémoglobine des suspensions d'érythrocytes (RBC) augmente de façon significative chez les poules andines, en comparaison avec celles du niveau de la mer. Pour les RBC des poules, les valeurs des coefficients de Hill à 50 % de saturation (n_{50}) sont supérieures à celles des RBC des mammifères. Cela peut suggérer l'existence d'un processus d'agrégation moléculaire à l'intérieur des cellules désoxygénées et fortement concentrées des deux types de poules. Pour les solutions d'hémolysat purifié et dépouillé de ces deux types de poules, les affinités sont identiques en *buffer* libre de phosphate, indiquant que les affinités intrinsèques des Hb sont les mêmes dans les Andes et au niveau de la mer. Après adjonction d'IPP, puissant effecteur allostérique des hémoglobines des mammifères, on observe une légère mais significative augmentation (environ 20 %) de l'affinité des hémolysats d'altitude comparés à ceux du niveau de la mer. Les résultats obtenus suggèrent également que la différence d'affinité entre le sang d'altitude et celui du niveau de la mer pourrait être dû à un processus adaptatif qui pourrait lui-même être lié à une légère augmentation de la concentration et/ou de l'activité du principal effecteur IPP, plutôt qu'à une anormalité structurelle de l'hémoglobine.

Mots-clés : Adaptation à l'altitude, Hypoxie, Hémoglobine, Phosphates organiques, Affinité de l'oxygène, Erythrocytes, Poules.

SUMMARY: A strain of high altitude chickens (*Gallus gallus*) displaying a high oxygen affinity hemoglobin has been discovered in the Peruvian Andes. We addressed the question of the molecular mechanism possibly at the origin of the high altitude adaptation of these chickens. The role of small changes of the intracellular concentration of inositol pentaphosphate (IPP), the main allosteric effector of hemoglobin in avian erythrocytes, has been postulated. We have studied the blood from andean and sea-level born chickens. The oxygen affinities of fresh red blood cells (RBC) suspensions are significantly increased in andean compared to sea-level chicken blood. The values of the Hill coefficients at half-saturation (n50) are higher for chicken RBC than those observed for mammalian RBC. This may suggest the existence of a molecular aggregation process inside the deoxygenated, highly concentrated cells of both avian types. For the stripped purified hemolysate solutions of the two types of chickens, the oxygen affinities are identical in phosphate-free buffer, indicating that the intrinsic oxygen affinities of andean and sea-level Hbs are the same. Upon addition of inositol hexaphosphate (IHP), a strong allosteric effector of avian hemoglobins, we observed a small but significant increase (about 20%) of the oxygen affinity of the andean relatively to sea-level hemolysates. Our results also suggest that the difference in affinity between the sea-level and andean blood may be due to an adaptative process possibly related to a slight decrease in the concentration and/or of the activity of the main cellular effector IPP, rather than to a structural abnormality of the hemoglobin.

KEY WORDS: High Altitude Adaptation, Hypoxia, Hemoglobin, Organophosphates, Oxygen Binding, Red Blood Cells, Chicken Blood.

INTRODUCTION

A strain of chickens (*Gallus gallus*) adapted to high altitude hypoxia and displaying a high oxygen affinity hemoglobin (Hb) has been discovered in the Peruvian Andes (1,2).

These birds have proved to be extremely resistant to common avian diseases and to life at high altitudes. In contrast, sea-level chickens, transported to high altitudes, are particularly sensitive to the low oxygen pressure in the high altitude atmosphere and suffer of chronic mountain sickness and low fertility.

The needs for oxygen at high altitude may be fullfilled by several adaptative mechanisms such as hyperventilation, insuring a low viscosity blood flow, varying density of the capillaries at the periphery etc... The oxygenation characteristics of hemoglobin also play an important role; they depend on the intrinsic oxygen affinity of the molecule and of its interactions with heterotropic cofactors such as pH, chloride anions and organic phosphate compounds such as 2,3, DPG. This compound is present in most mammalian adult species while inositol pentaphosphate (IPP) is the corresponding effector in avian species. Upon binding to hemoglobin these cofactors lower the affinity for oxygen thence contribute to increase the oxygen release to the tissues. In the present paper we addressed the question of which molecular mechanism could be responsible for the adaptation to high altitude. The possible role of small changes in the activity of inositol pentaphosphate (IPP) on the oxygenhemoglobin equilibrium has been postulated (2).In this work the allosteric effector used was inositol-hexaphosphate (IHP) whose hemoglobin binding properties are similar to those of IPP.(3).

Oxygen binding properties of Chicken fresh RBC suspensions

	P_{50} (torr)	n_{50}
Andean "Puno" Chicken RBC :	**38.8**	**3.1**
"Hybrid" Chicken RBC (andean chicken living at Lima since 6 years) :	**45.7**	**3.0**
Sea level (Lima) Chicken RBC :	**56.8**	**3.1**
Human Adult RBC :	**30.0**	**2.4**

Experimental conditions :
pH 7.4, NaCl 0.14 M, bis-Tris 0.05 M, 41°C.

Table 1

MATERIALS AND METHODS

We have studied the oxygen binding properties of blood from andean (from Puno, 4 000 m) and sea-level (from Lima) born chickens. We have also studied the blood of chickens born at Puno and living in Lima for 6 years and referred to as "hybrid" RBC's .

Oxygen equilibrium curves were recorded by a continuous method using the Hemox Analyzer system (TCS, Huntington Valley, PA,USA): simultaneous recordings of the PO_2 were made with a Clark type electrode and the light transmission at 560 nm in a 1 cm optical cuvete (4). P_{50} and n_{50} values were calculated by linear regression from the Hill equation for oxygen saturation levels between 40% and 60%.

For the fresh red blood cells (RBC) suspensions, the experimental conditions were: pH 7.4, NaCl 0.14 M, bis-Tris buffer 0.05 M at 41°C.

For stripped hemolysate, the experimental conditions were: pH 7.2, NaCl 0.1 M, bis-Tris buffer 0.05 M at 41°C(1).

The organophosphate-effect on the oxygen affinity of hemoglobin was estimated after

addition of 1mM of inositol hexaphosphate (IHP) as $\Delta \log P_{50} \pm IHP$.

The DNA sequence of the high altitude chicken b chain globin has been verified. A 600bp DNA fragment containing the ß69 codon was amplified by PCR and sequenced.

RESULTS

Oxygen equilibrium curves for the fresh red blood cells suspensions

The oxygen affinities of RBC suspensions were significantly increased in adean compared to sea-level chicken blood: P_{50} were 38.8 and 56.8 mmHg respectively, at 41°C, pH 7.4, NaCl 0.14M (Table 1 and Figure 1). For the "hybrid"chicken RBC, the P_{50} value was intermediary (45.7 mmHg) syggesting a time dependent decrease of the oxygen affinity.

The value of the Hill coefficient at P_{50} (n_{50}) was increased to 3.1 instead of 2.5 in mammalian RBC (Figure 2). This suggests an aggregation process inside the cells of these different types of avian RBC suspensions. This is confirmed by the lower n_{50} values found for hemoglobin solutions studied in the obsence of organophosphates (Table 2)(5).

Oxygen equilibrium curves for stripped hemolysate

For the stripped purified hemolysate solutions of the two types of chicken, the oxygen affinities were identical in phosphate-free bufer : P_{50} = 5.0 mmHg at 41°C, pH 7.2, NaCl 0.1M (Table 2). These results indicate that the intrinsic oxygen affinities of andean and sea-level Hbs are the same. Upon addition of inositol hexaphosphate to stripped hemolysates, we observed a small but significant increase (about 20%) of the oxygen affinity of the andean relatively to sea-level hemolysates: P_{50} were 52 and 62 mmHg respectively at 41°C with ImM IHP (Table II).

DNA sequence

A point mutation was previously described in the Andean chicken ß chain sequence resulting in the replacement of the amino acid residue ß 69 Serine by a Threonine. We have confirmed the existence of this substitution.(6) This structural change cannot be related to the high oxygen affinity of the andean relative to the sea-level blood as this position does not correspond to a known organophosphate binding site (7).

Oxygen binding properties of Chicken stripped hemolysate

	P_{50} (torr)	n_{50}
Andean Chicken hemolysate :		
NaCl 0.1M	**5.0**	**2.0**
+ IHP 1mM	**52.0**	**2.6**
Δ log P_{50} ±IHP 1mM :	***1.0***	
Sea level Chicken hemolysate :		
NaCl 0.1M	**5.0**	**2.0**
+ IHP 1mM	**62.0**	**2.6**
Δ log P_{50} ±IHP 1mM :	***1.1***	
***Human Adult hemoglobin* (Hb A) :**		
NaCl 0.1M	**12.8**	**2.1**
+ IHP 1mM	**96.1**	**2.1**
Δ log P_{50} ±IHP 1mM :	***0.9***	

Other experimental conditions :
pH 7.2, bis-Tris 0.05 M, [Heme] = 60 µM, 41°C.

Table 2

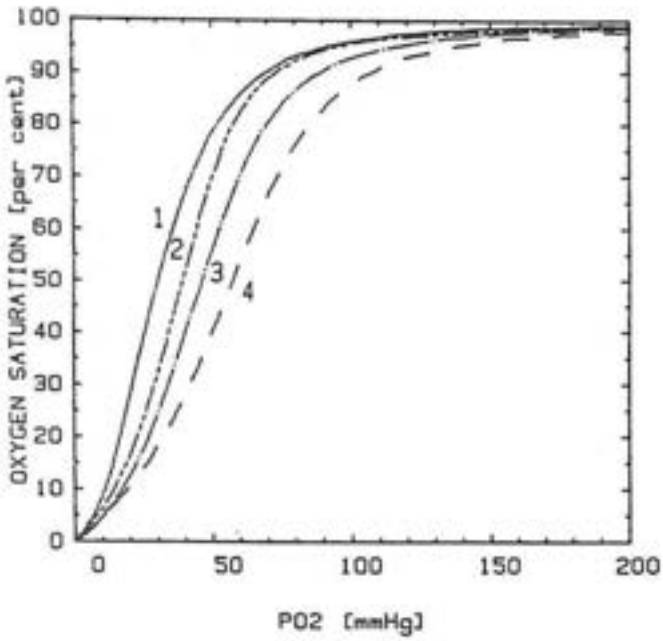

Figure 1. Oxygen Equilibrium Curves of Red Blood Cells suspensions: 1) normal human RBC's; 2) andean chicken RBC's; 3) "hybrid" (partially deadaptated) chicken RBC's; 4) sea-level chicken RBC's. Experimental conditions were: pH 7.4, NaCl 0.14M, bis-Tris buffer 0.05M at 41ºC.

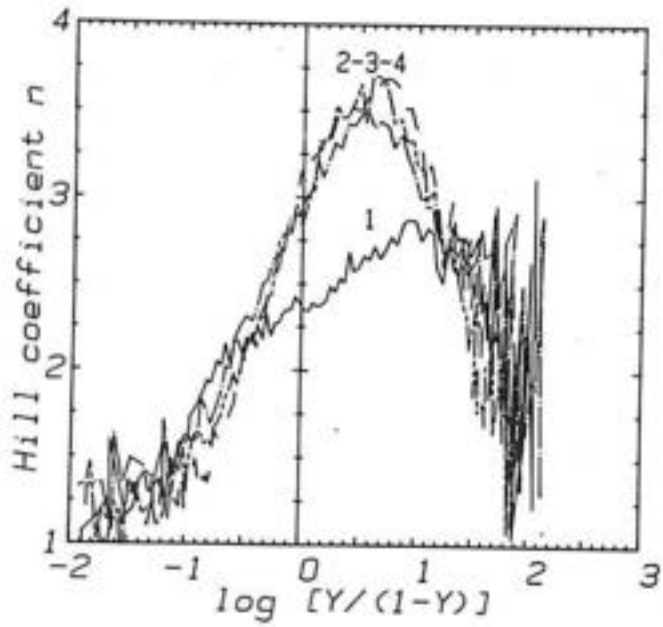

Figure 2

Figure 2. Cooperativity Curves of Red Blood Cells suspensions versus log[Y/(1-Y)] (corresponding to the first derivative of those illustrated in Figure 1): 1) normal human RBC's; 2) andean chicken RBC's; 3) "hybrid" (partially deadaptated) chicken RBC's; 4) sea-level chicken RBC's. Experimental conditions as in Figure 1.

DISCUSSION AND CONCLUSIONS

Our results therefore suggest that the difference in oxygen affinity (P_{50}) between the sea-level and high altitude andean chicken blood may be due to an adaptative mechanism: it may be related to a modification of the concentration and/or of the activity of the main cellular effector IPP rather than to a structural abnormality. Similar mechanisms have been reported in the past in other species (7,8,9). The slow reversibility of the blood oxygen affinity towards the sea-level values observed in the "hybrid" chicken group does not favor either a genetic origin. We have also performed preliminary measurements of the

intra-erythrocytic phosphates content of these red cells by ^{31}P-NMR analyses (Pr. Canioni et al., Bordeaux, France)(8). Up to now, it did not revealed important changes in the intracellular components of these red cells.(9) It will be also important to consider that birds have nucleated erythrocytes which may account for more complicated metabolic changes; such as hydration, pH, ionic activity; induced by intracellular hypoxia. We cannot exclude either a change on the intracellular hemoglobin concentration. All these phenomena, alone or associated, could be responsible for the observed so-called-adaptation to hypoxia.

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THE BRAIN AT HIGH ALTITUDE: CLINICAL RESEARCH AND MOLECULAR PHYSIOLOGY

ALTITUDE HYPOXIA EFFECTS ON BRAIN

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Cuzco, Perú, Sept 26, 1996

RESUMEN: Efectos de la Hipoxia de Altura en el Cerebro

La vasodilatación cerebral hipóxica es mediada por una vía compleja a través de células gliales ubicadas entre neuronas y arteriolas cerebrales. Esta vía supone la liberación de K^+ y adenosina en el líquido extracelular (ECF). La acidosis láctica es menos importante de lo que se había asumido previamente. Durante las primeras horas en altura, el flujo sanguíneo cerebral (CBF) incrementa 30-60% y luego de algunos días cae a valores casi normales. La magnitud de este incremento transitorio depende de la altitud, de la sensibilidad vascular cerebral y de la sensibilidad ventilatoria individual tanto al O_2 como al CO_2. La reducción posterior del flujo es un problema no bien comprendido, pero es explicado al menos en parte por incrementos de la P_aO_2, del pH del líquido cefalorraquídeo (CSF pH) y por una caída de P_aCO_2, pero no por la policitemia ni por la sensibilidad reducida de los vasos cerebrales a la hipoxia. Los efectos tardíos de la hipoxia incluyen reducción de base tampón en sangre y CSF, sensibilidad creciente de quimiorreceptores periféricos, hematocrito creciente y tal vez expresión de VEGF en macrófagos. Ninguno de los factores conocidos como reguladores del CBF (incluyendo la policitemia) pueden explicar por completo la evidencia de que el CBF sea normal o subnormal en nativos de altura y en sujetos completamente aclimatados, en comparación con sujetos normales de nivel del mar. Se ha reportado un $CMRO_2$ cerebral reducido en humanos nativos de altura, lo que podría ayudar a explicar el bajo CBF. El mal de montaña agudo (AMS) y el edema cerebral de altura (HACE) pueden acompañarse por un alto CBF, pues el P_aO_2 es menor, pero AMS y HACE no pueden ser causados por un CBF alto, ya que la hipercapnia no puede producir enfermedad sintomática, a pesar de un flujo mayor. La injuria cerebral sutil observada en montañistas puede estar relacionada con vasoconstricción hipocápnica y con una marcada caída en el PO_2. La injuria capilar de altura puede deberse a la citoquina VEGF liberada en el cerebro hipóxico por los macrófagos que inician la angiogénesis.

Palabras claves: Altitud; Hipoxia; Cerebro; Flujo sanguíneo

RÉSUMÉ: Effets de l'hypoxie d'altitude sur le cerveau.

La vasodilatation cérébrale hypoxique s'effectue par une voie complexe, par l'intermédiaire de cellules gliales situées entre les neurones et les artérioles cérébrales. Cette voie suppose la libération de K^+ et d'adénosine dans le liquide extracellulaire (ECF). L'acidose lactique est moins importante que ce que l'on supposait auparavant. Au cours des premières heures en altitude le flux sanguin cérébral (CBF) augmente de 30 à 60 % et retombe à des valeurs presque normales au bout de quelques heures. L'ampleur de cette élévation passagère dépend de l'altitude, de la sensibilité vasculaire cérébrale et de la sensibilité respiratoire individuelle à l'O_2 et au CO_2. La réduction ultérieure du flux est un phénomène imparfaitement compris, mais qui s'explique au moins en partie par des augmentations de la P_aO_2, du pH du liquide céphalorachidien (CSF pH) et par une chute de la P_aCO_2, mais pas par une polyglobulie ni par une sensibilité réduite des vaisseaux cérébraux à l'hypoxie. Les effets tardifs de l'hypoxie incluent la réduction de la base tampon dans le sang et le CSF, la sensibilité croissante des chimiorécepteurs périphériques, l'hématocrite en augmentation et éventuellement l'expression de VEGF en macrophages. Aucun des facteurs connus comme étant des régulateurs du CBF (y compris la polyglobulie) ne peuvent expliquer intégralement l'évidence d'un CBF normal ou subnormal chez les natifs des régions de grande altitude et chez les sujets complètement acclimatés, en comparaison avec des sujets normaux du niveau de la mer. Un $CMRO_2$ cérébral réduit a été signalé chez des natifs de hautes régions, ce qui pourrait aider à expliquer le CBF déprimé. Le mal des montagnes aigu (AMS) et l'oedème cérébral de grande altitude (HACE) peuvent être accompagnés d'un CBF élevé car la P_aO_2 est moindre, mais la cause de l'AMS ou du HACE ne peut être un CBF élevé, l'hypercapnie ne pouvant produire une maladie symptomatique, malgré un flux plus élevé. La légère atteinte cérébrale observée chez les alpinistes peut être liée à la vasoconstriction hypocapnique et à une chute marquée de PO_2. L'atteinte capillaire d'altitude pourrait être due à la cytokine VEGF libérée dans le cerveau hypoxique par les macrophages commençant l'angiogenèse.

Mots-clés : Altitude, Hypoxie, Cerveau, Flux sanguin.

SUMMARY: Hypoxic cerebral vasodilation is mediated by a complex pathway through glial cells positioned between neurones and cerebral artenoles, and involving both K^+ and adenosine release into ECF. Lactic acidosis is of less importance than had been assumed. During the first hours at altitude, CBF rises 30-60% and then after some days, falls to nearly normal values. The magnitude ofthis transient rise

depends on the altitude and the individual cerebral vascular and ventilatorz sensitivities to both 0_2 and CO_2 The subsequent reduction of flow remains poorly understood, but is at least partly explained by rises of PaO_2, CSF pH, and fall of $PaCO_2$, but neither by polycythemia, nor reduced sensitivity of cerebral vessels to hypoxia Later effects of hypoxia include reduced blood and CSF buffer base increasing peripheral chemo receptor sensitivity, rising Hct and perhaps macrophage expression of VEGF None of the known factorsregulating CBF (including polycythemia) can fully account for the evidence that in several studies, natives of high altitude, and those fully acclimatized, have been found to have normal or subnormal CBF (compared with sea level normals) Reduced brain $CMRO_2$ has been reported in humans native to high altitude, which could help explain the low CBF AMS and HACE may be accompanied by high CBF, because PaO_2 is lower. but AMS and HACE cannot be caused by high CBF since hypercapnia fails to cause symptomatic illness, despite higher flow Subtle brain injury seen in mountaineers may be related to hypocapnic vasoconstriction and a marked alkaline Bohr downshift in capillary PO_2. Capillary inJury at high altitude may result from the cytokine VEGF released in hypoxic brain by macrophages initiating angiogenesis.

HYPOXIA AND CBF

Cerebral blood (CBF) rises 30-50% inmediately at altitudes such as 4000-6000M. but falls to near normal; after a few days at altitude The reduction is not fully explained by improved SaO_2, Hct or CSF pifl CBF is affected by ventilatory responses to hypoxia and CO_2. With neuronal activation, O_2 consumption and local CBF rises within 1-2 CBF: is regulated by local metabolism at the level of capillaries and precapillary sphincters (1). but pressure u pstream from alterioles is regulated by the larger conducting arteries with autonomic innervation which reduce lumen diameter when arterial pressure rises (2, 3). Flow is independent of mean systemic arterial pressure between approximately 60 and 150 mm Hg, (4). Both during and following severe hypoxia autoregulation may be disrupted (5, 6).

Blood flow in individual capillaries in brain is intermittent, resulting in 6-12 per min ±30% oscillations in tissue Po2 (7-9). Vasodilators increase the number of capillanes perfused at any moment (10). Average cortex PO_2 is about 9 mm Hg as determined with recessed. calibrated gold-plated microoelectrodes (11). Neuronal mitochondrial cytochrome is normally not fully saturated with O_2, such that the redox state is not fully oxygenated, and some 'anaerobic metabolism defined as lactate excretion is normal.

CBF rises in proportion to the severity of hypoxia, but with extreme variability between individuals and species, primarily due to the effects of hypoxic hypervelitilation on $PaCO_2$. While studies in humans at altitude have found rises of the order of 30-60% during the initial hours or days, flow was shown to be increased by as much as 250% in awake sheep at PaO_2 = 40 mm Hg (12) and more than 4 fold in rats at PaO_2 = 24 mm Hg (13)

CO_2 is usually kept constant when testing CBF sensitivity to hypoxia. In 9 healthy male volunteers, (14) a step reduction of PaO_2 to 34.6±1.6 mm Hg (SE) (66% SaO_2) increased CBF about 70% (from 0.45 to 0.77 ml-gm $^{-1}$-min^{-1}) accompanied by a 27% rise in glucose consumption (CMR_{glu}) and a 4 fold rise in cerebral lactate production (CMR_{lac})$CMRO_2$ was maintained constant by the Pasteur effect(ADP controlled glycolysis) In fetal lambs made hypoxic by acute maternal isocapnic hypoxemia, CBF was an approximately linear function of fetal SaO_2 down to nearly zero at which point flow was increased to about 250% of control (15) Isocapnic hypoxia CBF in normal men at 3810m altitude (16)(Figure 1) rose 45% at 66% SaO_2. Hemodilution increases cerebral blood flow in polycythemic patients and in subjects with high normal Hct (17).

MEDIATORS OF HYPOXIC CEREBRAL VASODILATION

Cerebral arterioles are dilated by low PO_2 (14) and low O_2 content (anemia) (17). Increased local neuronal activity (18), hypercapnia (19), increasing vascular smooth muscle ECF [H] (20), [K] (21), adenosine (22) intravascular NO (nitric oxide) generated in endothelia (23) and a variety of autocoids and cytokines (4). Hypoxia may have other still unknown direct vasodilating mechanisms. Cerebral vasodilation in both hypoxia and with neuronal activity is mediated by glial cells which "connect" neurones to the nearest arteriolar smooth muscle cells. Neuronal K^+ is their putative input signal while adenosine generated by glia, K^+ (and possibly NO) may serve as the vasodilators at the sphincter surface.

CBF CHANGES DURING ACCLIMATIZATION

During acclimatization of normals at 3810m altitude, Severinghaus et al (24) reported a rise of 24% after 6-12 hrs with mean PaO_2=43 5 mm Hg, $PaCO_2$=35.0, pH_a=7 45, and pH_{CSF}=7 32 (n=4) CBF fell to 13% above sea level control values at 3-5 days as PaO_2 rose to 51.2 mm Hg, $PaCO_2$ falling to 29 7 mm Hg After 10 mill of 30% O_2 (acute normoxia), CBF fell to sea level control values on both occasions, while $PaCO_2$ remained low at 35.1 mm Hg at 6-12 hrs, and 30.9 mm Hg at 3-5 days.

After3-5 days at altitude, during acute,normoxia, when CO_2 was increased to 35 mm Hg tor 10 min, CBF rose to 33.8% above sea level control. This greatly increased response to a $PaCO_2$ of 35 is a result of the fall of ECF HCO_3-during acclimatization and its eff'ect on arteriolar ECF pH. At sea level, a reduction of $PaCO_2$ to 35 mm Hg would have reduced CBF by about 10%.

Jensen et al (25) in 19 subjects, ascending from 150 to 3,475 m, found CBF was 24% increased at 24 h and 4% increased at 6 days. In nine subjects, ascending from 3,200 to 4,785-5,430 m, CBF increased 53% above estimated sea-level values In 13 young male soldiers transported to 3700m altitude, Roy et al (26) found that CB F was 40% above control at 12-36 hrs of hypoxia, and diminished to 4% above control after 4 days. Gradual fall of CBF with time in CillOlliC hypoxia has also been reported by others (27-29)

However, not all subiects show initial vasodila..on. Those with vigorous carotid chemoreceptor responses may hyperventilate enough to block the hypoxic vasodilation (27,30). In view of the evidence that flow falls with time at altitude, one might expect the sensitivity of CBF to an acute hypoxic challenge to decrease in the course ot' altitude acclimatization. However, it apparently does not. During 5 days at 3810m in 6 normal adults, Jensen et al (16, 31) found a 34% rise of the hypoxic CBF sensitivity (Figure 1). They concluded that the observed fall of CBF with time at altitude cannot be attributed to adaptation ofthe vascular sensitivity to hypoxia.

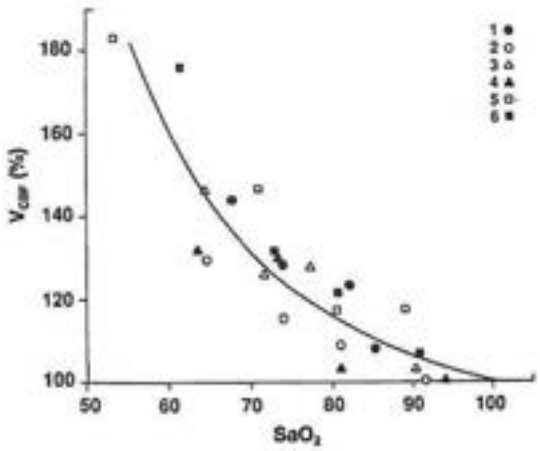

Fig. 1. CBFv% (% of control CBF velocity by TCD) in response to 5 min steps to 4 levels of isocapnic step hypoxia in 5-6_ normal subjects after 3-4 days at 3810m altitude (16). The hyperbolic empiric relationship was:

$$CBFv\% = 100(1 + X[(60/(Sa0_2-40))- 1]).$$

Factor X was found to average 0.35±0.11 at sea level and 0.46±0.08 after 5 days at altitude X may be interpreted as the fractional increase in CBFv induced by 5 min of hypoxia at 70% SaO_2.

Factors which might contribute to the slow reduction of CBF at altitude include: 1) gradual improvement of arterial PO_2; 2) gradual rise of CSF pH, as the carotid chemosensitivity increases, driving ventilation up and PCo_2 down; 3) an upward shift of P_{50} facilitating unloading of O_2 in tissue; 4) increased Hct; 5) postulated increased sympathetic cerebral arterial tone (32); 6) some remodeling of the microcirculation or the length of the critical diffusion paths between capillaries and cytochrome; and 7) a decrease in $CMRO_2$. Krasney et al (29, 33) demonstratéd in 1985 that the gradual fall of CBF with time at altitude did not occur over the course of 4 days isocapnic hypoxia in sheep if $PaCO_2$ and PaO_2 were kept constant, thus ruling out any short term adaptation of the hypoxic vasodilation of cerebral arteriolar smooth muscle. Manohar et al (34) were unable to identify any factor responsible for the gradual loss at high altitude of hypoxic cerebral vasodilation. Acute hypoxia at 3500m (simulated) altitude (PaO_2=49 mm Hg) increased CBF in control calves from 75 to 101 ml $100g^{-1}$ min^{-1}. After 7-8 weeks of hypoxia, while still in the hypobaric chamber, CBF averaged 69 ml $100g^{-1}$ min^{-1}. With acute normoxia CBF was 79 ml $100g^{-1}$ min^{-1} (n s). $PaCO_2$ was 40 mm Hg in control calves, 35.5 in chronic hypoxia and 38 in acute normoxia. Hct did not rise and there was no right shift of P_{50}.

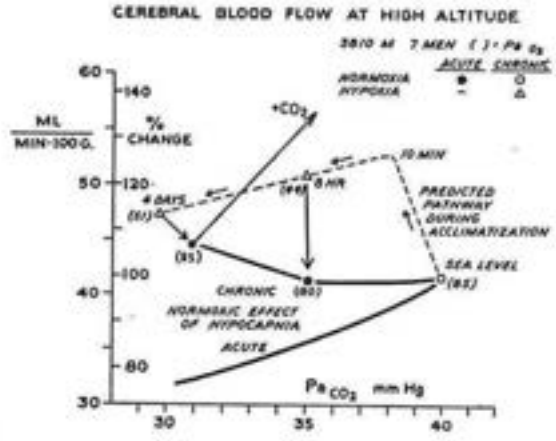

Fig. 2 Time course of CBF in newcomers at 381 Om altitude, showing the acute responses to normoxia and hypercapnia, demonstrating the gradual fall of CBF with time, and the resetting of hypercapnic response to a lower Pco_2 (24).

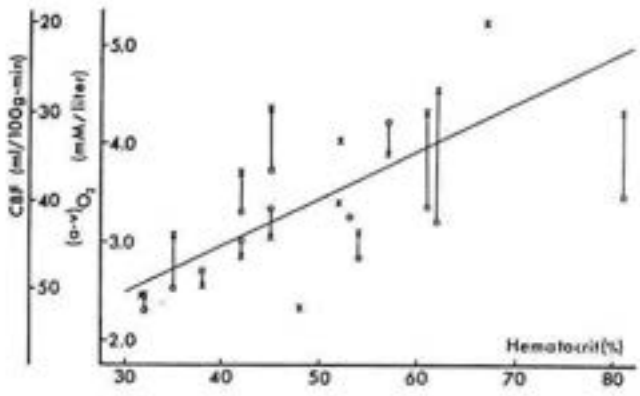

Fig. 3. CBF of natives ofthe Bolivian altiplano was found to be an inverse linear function of Hct, with normal flow corresponding to the sea level normal relationship to Hct. The relationship may be approximated by: CBF = [65-0.5Hct](ml-1Oog^{-1}min^{-1}). Circles: Acute hyperoxia at altitude. P_iO_2>I50 mm Hg; x: Ambient hypoxia at 3700m (La Paz), P_iO_2= 90 mm Hg (64).

CHRONIC HYPOXIA AT ALTITUDE

Natives of high altitude may have low cerebral metabolic rates (by as much as 20%), which could be responsible for lower CBF (35) In 8 normal adult natives of 4300M altitude (Cerro de Pasco, Peru), with a mean Hct=57.8±6.3% and mean PaO_2=43.6±2.4 mm Hg, Milledre and Sorensen (36) found that breathing 100% 0_2 increased the arterial-internal jugular 0_2 content difference from 7.89±1.01 to 9.58±1.17 ml dl^{-1}. representing an 18% decrease of CBF with hyperoxia. The study demonstrated the presence of a lifelong vasodilation due to ambient hypoxia Yet their mean (a-v) O_2 content difference while breathing ambient air was greater than that of sea level normals, suggesting a sub-normal CBF.

In the relationship of CBF to Hct, no significant difference has been detected between sea level natives studied at sea level and altitude natives studied at altitude (Figure 3) Marc-Vergnes (37) repo. ted sub-normal CB F in 16 natives ofthe Bolivian altiplano (40 compared with his normal of 50 ml 100g^{-1} min^{-1} in sea level natives). Putting these observations together suggests that, while natives at altitude always retain evidence of hypoxic vasodilation, flow is anomalously low considering their chronic hypoxia, and cannot be fully explained by the known controls, hypoxia, ECF pH, Hct etc. Several animal studies have not supported the finding of a return to normal of CBF in chronic hypoxia (34, 38-40)

AMS AND HACE: ROLE OF CBF?

Subjects with AMS are suspected to have a poor ventilatory response to hypoxia and thus to be more hypoxic and have a higher Pco_2, both of which should result in higher CBF (41) Three related questions have been addressed: 1) Are subjects with AMS more hypoxic and/or hypercapnic than their healthier associates? 2) If so i s their CBF higher? and 3) Does the high flow cause or exacerbate the AMS (31, 42, 43)?

At 4559M, overall CBFv increased 48% in subjects who developed AMS and 27% in subjects without symptoms (44). In 12 normal subjects, increases in CBF at 3475m were similar in subjects with or without AMS In six, CBF was measured before and after therapeutic intervention (25). At 2h, CBF increased 22% above pretreatment values in three subjects given 1.5 g acetazolamide, while three subjects given placebo showed no change. Overall, the results indicated that increases in CBF were similar in subjects with or without AMS while acetazolamide-provoked increases of CBF in AMS subjects caused no acute change in symptoms. The authors concluded that high CBF cannot be directly implicated in the pathogenesis of AMS.

ROLE OF LOW Pco_2

Maher et al (45) tested whether prevention of hypocapnia and alkalosis would ameliorate the symptoms of acute mountain sickness (AMS). Five subjects were exposed to simulated high altitude for 4d with 3 8% CO_2 added to the chamber to

maintain normocapnia. Four other subjects were exposed for 4 d to hypobaric hypoxia without $C0_2$ supplementation. and became hypocapnic. Barometric pressure was lower in the group with added CO_2 so that alveolar oxygen tensions (55-60 mm Hg) would not be different. The severity of symptoms was clearly greater in normocapnic than in hypocapnic subjects. The control hypocapnic subjects presumably had more alkaline pHa, thus a left shifted ODC resulting in better lung O_2 uptake, higher SaO_2 and a lower CBF (assumed) compared with the experimental CO_2-supplemented normocapnic group. Bartsch et al (46) randomly allocated twenty mountaineers with AMS at 4559 m to 3 treatment group 1) with 33% 0_2 . 2) with 3% CO_2. in air and 3) an air control. 33_% O_2 significantly relieved symptorms of AMS, and reduced CBF but $C0_2$ addition did not significantly ameliorate AMS, despite the rise of Pco_2, ventilation and alveolar Po_2.

In order to determine the role of CO_2, Yang et al (47) exposed chronically instrumented ewes to 96 h of hypoxia (PaO_2=40 mm Hg) in an environmental chamber One group of 12 was permitted to become hypocapnic ($PaCO_2$= 27 mm Hg) while the other group of 9 was kept eucapnic ($PaCO_2$ = 37 mm Hg). AMS, estimated from food and water intakes and behavior, occurred in 9 of 12 with hypocapnia and 9 of 9 with normocapnia. Intracranial pressure and the pressure gradient betwenit and sagittal sinus increased only in AMS sheep. CBF was high in all, but greater in the normocapnic animals. Brain edema occurred only in AMS sheep.

To test whether high CBF alone could cause cerebral edema, S.P.Yang and Krasney (48) kept sheep for 4 days in elevated CO, (52-55 mm Hg) CBF remained about twice normal, and $CMRO_2$ was increased both during exposure and in the post hypercapnic period. They observed no symptoms like those of AMS or HACE although brain water rose slightly fionl 79.8±0.24 to 80.3±0.2% ($p<0.05$) High CBF is unlikely to be the root cause of HACE.

ROLE OF ANGIOGENESIS IN HACE?

Retinal petechial hemorrhages found in many climbers at extreme altitude suggest a pathologic process involving cerebral circulation which may be assumed to exist throughout the brain This may be a result of the first step in the process called angiogenesis (49). Tissue hypoxia is the initiating stimulus of angiogenesis, a multistep process which (e. g. in tumors) ultimately leads to growth of new capillaries into the hypoxic tissue. A variety of protein cytokines are expressed both by the hypoxic cells and by macrophages attracted to those cells. The principal initial cytokine is VEGF (vascular endothelial growth factor, formerly termed VPF, vascular permeability factor). VEGF attacks and dissolves capillary basement membranes, permitting plasma and red cell leakage, as a precursor to growth of endothelia toward the hypoxic region. In preliminary experiments (unreported), F. P.Xu at UCSF has demonstrated transient rises in mRNA for VEGF and in VEGF protein during the first 1-3 days of steady severe hypoxemia in rats and rabbits. Dexamethasone has been found to be a highly potent inhibitor of angiogenesis, suggesting that perhaps its well established ability to prevent and treat AMS and HACE might be related to inhibiting capillary leakage initiated by VEGF in the earliest stage of angiogenesis.

Acetazolamide

Acetazolamide (AZ) has been used by mountaineers for many years, facilitating sleep at altitude and increasing cerebral 0_2 delivery Oral administration of I g of AZ to 8 normoxic subjects studied at sea level caused an acute 38% increase in CBF (50, 51). During the subsequent prolonged oral treatment with I g of AZ daily, CBF returned to normal within 2 days, The alveolar CO_2 tension decreased gradually to 70% of the control value. Based on this, the authors speculated that little of the benefit of AZ at altitude is due to increased CBF, that the beneficial effects are more due to increased ventilation raising PaO_2 affording a significant increase of the arterial oxygen content. However, in these normoxic studies, some of the flow reduction with time can be attributed to the 30% fall of $PaCO_2$. In the absence of hypoxia these studies may not be applicable to altitude effects.

Understanding the various effects of AZ on CBF during acclimatization at altitude is complicated by the slow rise of $PaCO_2$ during blood transit from lung to brain arterioles. The uncatalyzed time constant of rbc HCO_3 dehydration to dissolved CO_2 in blood is about 7 s. Due to its stimulation of ventilation, $PaCO_2$ and end capillary $PaCO_2$ in lung fall. Cerebral arteriolar PCO_2 may thereby be reduced by AZ, while PaO_2 is increased, both of which should reduce CBF. For example, Huang et al (52) utilized Doppler ultrasound in 8 volunteers to determine whether the usual AZ dose (250 mg three times daily) would increase CBF velocities in internal carotid and vertebral arteries. Although AZ decreased pHa, $PaCO_2$, and $P_{ET}CO_2$,, both during normoxia and subacute hypoxia, they saw no effect on either baseline CBF or the CBF responses to acute hypoxia or hypercapnia. Kjaliquist and Siesjo (53) used fast freeze sampling of rat brain to show

that brain HCO_3^- was increased about 9 mM kg^{-1} by AZ The rise of tissue. Pco_2 (~4mm Hg) could only have increased brain HCO_3^- by 0.7 mM- kg^{-1}.

One potential mechanism of AZ vasodilation was thought to be a blockade of the Bohr effect of metabolic CO_2 in tissue capillaries acting via pH to increase blood P O_2 and thus facilitate unloading of O_2. This possibility was excluded by Cotev et al (54) using brain surface PCO_2, pH and PO_2 electrodes When 25 mM kg^{-1} AZ in dogs increased CBF by 69% as estimated from arterial to saggital sinus blood O_2 content difference, cortex surface PO_2 increased by 16-20 mn Hg. They noted that surface pH fell from 7.22 to 7.12 within a few minutes, accompanied by a small rise of surface PCO_2 from 45 to 48 mm Hg, insuficient to explain the acid shift. In view of the rise of tissue HCO_3^- produced by AZ (53) they speculated that they were observing carbonic acidosis as if brain metabolism generates carbonic acid (i.e. H^+ and HCO_3^-), not gaseous $C0_2$, as the first products of decarboxylation. This was later confirmed directly in brain tissue homogenates (55). The isocapnic acidosis produced by AZ was confirmed by Bickler et al using flat cortical surface PCO_2 and pH electrodes (56). Following IV injection of 25 $mMkg^{-1}$ of AZ, ventilation was adjusted to hold brain surface PCO_2 constant. Brain surface pH fell approximately 0.1 pH within 3 min and brain oxygenation monitored by NADH fluorescence rose, even when animals were ventilated with 100% O_2 before giving AZ (57). The cortical surface location of the electrodes suggested that AZ rapidly penetrated the blood brain barrier.

Brain intracellular pH measured with MRS of phosphate does not fall after AZ administration according to Vorstrup et al (sensitivity of the method is limited to ±0.06 pH)(58). Cells respond to the rise in H_2CO_3 by more rapid export of H^+ than of $HC0_3^-$. Undissociated H_2CO_3 is fredy diffusible out of cells. During hypoxia at high altitude the overall effect of prolonged AZ treatment may be equivalent to a descent of several hundred metres It is probable that the increase of ventilation and oxygenation induced by use of AZ at high altitude obscures its inherent vasodilation, such that flouw would fall with time at altitude faster without than in the presence of AZ.

BRAIN PATHOLOGY AT EXTREME ALTITUDE

There is little evidence that hypoxia directly injures brain in climbers at extreme altitudes, but it is less clear whether in some way blood flow may be linked to brain injury. Hornbein and associates (59) performed neuropsychological and physiologic testing on 35 mountaineers before and 1 to 30 days after ascent to altitudes between 5488 and 8848 m, and on 6 subjects before and after simulation in an altitude chamber of a 40-day ascent to 8848 m. They reported a persistent decline in visual long-term memory A higher ventilatory response to hypoxia correlated with a reduction in verbal learrling ($r = -0.88$, $P < 0.05$) and with poor long-term verbal memory ($r = 0.99$, $P < 0.01$). An increase in the number of aphasic errors on the aphasia screening test also correlated with a higher ventilatory response to hypoxia in both the simulated-ascent group and a subgroup of 11 mountaineers. Because the functional decrements were greater in those who were believed to be less hypoxic, they suggested the possibility that the injury in climbers with strong HVR might have resulted from hypocapnic cerebral vasoconstnction, combined with the Bohr effecton capillary PO_2 ofthe extreme arterial alikalosis pH was predicted to be about 7 75 from the measured end tidal PCO_2 on Everest (60) but was measured at 7.57 and BE was -10 mM in the subjects in the Everest 11 chamber expenment (61), inducing a Bohr effect left shift of about 20%.

Song et al (62) reported cerebral thrornbi in several climbers who had gone higher than 5,000 m for longer than 3 weeks. They speculated that the cause was hemoconcentration resulting from secondary polycythemia and dehydration at altitude. In the fetus and infant, hypoxemia whether from high altitude or other causes, is associated with increased cerebrovascular morbidity. Longo et al (63) compared cerebral arteries obtained from normoxic and chronically hypoxic sheep adults and fetuses. Long-term hypoxemia was associated with generalized increase in base-soluble protein (5-5%), a depression of the maximum potassium-induced tensions (16-49%), and a depression of the relaxation responses to S-nitroso-N-acetylpenicillamine (1-11%), which releases nitric oxide into solution upon hydration. They concluded that chronic hypoxemia depresses cerebral vascular smooth muscle and endothelial hypoxic response to a greater- extent in the fetus than in adults

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INCREASE IN SUCCINATE DEHYDROGENASE ACTIVITY IN MICE BRAIN DURING CHRONIC HYPOXIA

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RESUMEN . Se midió la actividad de la succinato deshidrogenasa (SDH) en siete regiones cerebrales de ratones expuestos a tres semanas de hipoxia hipobárica (450 torr, 4380 metros por encima del nivel del mar), y se comparó con los controles normóxicos de nivel del mar. En el grupo hipóxico se encontró un aumento del 40% en la actividad de la SDH en la corteza y el hipocampo. Éste podría ser un mecanismo compensatorio frente a la disminución de la actividad de otros componentes de la cadena respiratoria en condiciones de hipoxia hipobárica, como una estrategia para mantener la producción normal de ATP.

Palabras claves: Metabolismo cerebral; Succinato deshidrogenasa; Hipoxia; Ratones

RÉSUMÉ: Accroissement de l'activité de la succinate déshydrogénase dans le cerveau des souris au cours de l'hypoxie chronique.

On a mesuré l'activité de la succinate déshydrogénase (SDH) dans sept régions cérébrales de souris exposées à trois semaines d'hypoxie hypobare (450 torr, 4 380 m d'altitude) et on a comparé avec les contrôles normoxiques du niveau de mer. Dans le groupe hypoxique on a constaté une augmentation de 40 % de l'activité de la SDH dans le cortex et dans l'hippocampe. Cette augmentation pourrait être un mécanisme compensateur face à la diminution de l'activité d'autres composants de la chaîne respiratoire dans des conditions d'hypoxie hypobare, comme une stratégie pour maintenir la production normale d'ATP.

Mots-clés : Métabolisme cérébral, Succinate déshydrogénase, Hypoxie, Souris.

SUMMARY: Succinate dehydrogenase (SDH) activity was measured in seven brain regions of mice exposed to three weeks of hypobaric hypoxia (450 torr, 4380 meters above sea level) and compared to to normoxic sea level controls. In the hypoxic group we found an increased activity of SDH in cortex an hippocampus of 40%. These results can be explained as a compensatory mechanism for the decreased activity of other components of the respiratory chain in hypobaric hypoxia, as an strategy to mantain the normal ATP production.

Key Words: Brain metabolism; Succinate dehydrogenase; Hypoxia; Mice

INTRODUCTION

Human brain has one of the highest metabolic rates in the body, while representing just 2% of total body weight. It accounts for 20% of the total body weight oxygen consumption and 95% of its metabolic demands is aerobically accomplished (1,2), so the normal function of the brain is almost entirely dependent on an adequate supply of oxygen for the synthesis of ATP via oxidative energy metabolism..

At high altitudes, where the oxygen arterial PO_2 is reduced almost to half sea level pressure, it would be expected that brain function is affected in some degree. An extreme case is the Chronic Mountain Sickness, which is characterized in part by neurologic and psychiatric symptoms such as headache, paresthesias, depression, physic and mental fatigue (3). Also the risk of migraine and strokes is considerably increased (4).

Changes induced by prolonged exposure to hypoxia in cerebral energy metabolism have been reported (5), such as increase in glucose transport across the blood brain barrier (6) and increased brain glucose consumption (7), reduction in the respiratory activity of mitochondria, including citochrome C oxidase (complex IV) and NADHCo Q reductase activities (Complex I) (8,9). Both enzymes are located in the inner mitochondrial membrane. The succinate dehydrogenase is a component of the Krebs cycle and is also a member of the mitochondrial respiratory chain (complex II) (10).

In order to determine if other enzymes of the mitochondrial respiratory chain are also altered under this metabolic stress, we evaluated the activity of Complex II in mice brains exposed to prolonged hypoxia.

MATERIALS AND METHODS

Litter mates of male Balb/c mice 3 were obtained after weaning at 21 days of age. One group (n=7) was transported to Cerro de Pasco (4380 meters above sea level. A second group remained at sea

level under similar conditions as the control group (n=7).

After three weeks of exposure to hypoxia mice were decapitated and brains quickly removed and stored at -70 oC until use. The following brain regions were dissected: frontal cortex, remainig cortex, cerebellum, hippocampus, hypotalamus, talamus, and corpus striatum. All the procedures were carried out at 4 oC. In brief, tissue samples were homogenized in phosphate buffer 0,3M ph 7,4 (10% w/v), and the SDH activity was assayed spectrophotometrically on 600 nm using Phenazin Methosulfate (PMS) and 2,6 Dichlorofenol indophenol (DCFIF) as final electron acceptor as previously described by Singer (11). Concentrations of proteins were measured by the method of Lowry using bovine serum albumine as the standard (12). The results were expressed as the mean ± SD values and comparison between controls and hypoxic animals were evaluated by Student´s t test (table 1).

Table 1. Succinate dehydrogenase activity (mmol/min/mg protein) in brain regions of mice exposed to three weeks of hypobaric hypoxia.

	Control	**Hypoxic**
Frontal cortex * (n=7)	61,9 ± 12,8	100,7 ± 37,6
Rest of cortex * (n=7)	43,2 ± 10,7	60,7 ± 14.6
Cerebellum (n=7)	68,8 ± 12,8	76.0 ± 22,9
Hippocampus * (n=5)	16,4 ± 2.6	21,2 ± 3,9
Talamus (n=5)	17,5 ± 4.9	20,3 ± 3,9
Hypotalamus (n=7)	19,6 ± 9,3	17,9 ± 9,3
Striatum (n=7)	15,1 ± 6,1	12,7 ± 2,1

Values are means ± SD; n = number of mice.
Significantly different from normoxic controls by Student´s t-test, p<0,05.

RESULTS

After three weeks of exposure to hypoxia mice showed reduced body weight and had higher hematocrits when compared to controls. The mean body weight (g ± SD) was 24,9 ± 1,1 for controls and 19,6 ± 2,1 for hypoxic mice (p<0,005). The hematocrit (% ± SD) was 47,7 ± 3,2 in controls and 60 ± 1,8 in hypoxic mice (p<0,001).

Succinate dehydrogenase activity was increased 40% in the cortex and hippocampus of hypoxic animals when compared with sea level group. The other areas evaluated did not show a significant change (table 1).

DISCUSSION

Prolonged exposure to chronic hypoxia induces changes in brain energetic metabolism as described previously, including a reduction of complex I and complex IV activities (8,9). These changes in the respiratory chain may affect the synthesis of ATP. However Harik et al. (7) have demostrated that ATP levels in brains of rats exposed three weeks to hypobaric hypoxia remain unchanged. This normal ATP levels may be achieved at least in part by an increment in SDH activity as a compensatory mechanism for the maintenance of normal electron flux through the respiratory chain. Previous studies have also demonstrated changes in the SDH activity induced by hypoxia in some brain regions and other tissues (13-16).

As Robin stated, there could be alternative pathways as adaptations to chronic hypoxia in mammals (17). One of the possible involved mechanisms is the increase of succinate thiokinase within the Krebs cycle, produced by the anaerobic metabolism of glutamate as occurs in heart (18,19). The proper knowledge of this pathway in chronic hypoxia warrants further investigation.

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ADAPTATION OF THE BRAIN'S MICROCIRCULATION TO PROLONGED HYPOBARIC HYPOXIA

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RESUMEN: Adaptación de la Microcirculación Cerebral a la Hipoxia Hipobárica Prolongada

Se revisa algunos de los efectos de la hipoxia hipobáricamoderada (0.5 atmósferas) sobre la microcirculación cerebral en ratas adultas. La vascularización cerebral aumenta luego de hipoxia prolongada, pero el curso en el tiempo de la respuesta adaptativa y su reversibilidad no se conocen. No hemos encontrado un aumento significativo en la vascularización cerebral luego de 4 días de hipoxia, pero sí un incremento significativo de de aproximadamente de 33% luego de 1 semana.La vascularización cerebral continuó aumentando, alcanzando un máximo de aproximadamente 70% sobre los valores controles luego de 2 semanas de hipoxia, pero sin aumento significativo adicional entre las 2 y 3 semanas de hipoxia. Tres semanas de normoxia luego de 3 semanas de hipoxia revirtieron la hipervascularización inducida por hipoxia. Esta hipervascularización se asoció con una densidad incrementada de la proteína transportadora de glucosa (GLUT-1) en microvasos cerebrales aislados. esta expresión incrementada de GLUT-1 fue evidente luego de 1 semana de hipoxia, permaneció constante luego de 3 semanas y no revirtió luego de 3 semanas de recuperación normóxica. Así, a diferencia de la vascularización cerebral incrementada inducida por hipoxia, la densidad de GLUT-1 incrementada inducida por hipoxia en microvasos cerebrales aislados no es rápidamente reversible. La combinación de vascularización cerebral incrementada y la expresión incrementada de GLUT-1 en los microvasos cerebrales de ratas sometidas a hipoxia hipobárica se asocian con un transporte incrementado de glucosa de la sangre al cerebro y una tasa metabólica cerebral regional de glucosa incrementada. Estos hallazgos documentan varias respuestas adaptativas a la hipoxia prolongada y sugieren una glicólisis incrementada en el cerebro de ratas adultas luego de hipoxia moderada prolongada.

Palabras claves: Hipoxia, Microcirculación, Cerebro, Glicólisis.

RÉSUMÉ: Adaptation de la microcirculation cérébrale à l'hypoxie hypobare prolongée.

Quelques effets de l'hypoxie hypobare modérée (0,5 atm) sur la microcirculation cérébrale de rats adultes ont été revus. La vascularisation cérébrale augmente à la suite d'une hypoxie prolongée, mais le déroulement dans le temps de la réponse adaptative et sa réversibilité ne sont pas connus. Au bout de 4 jours d'hypoxie aucune augmentation significative de la vascularisation cérébrale n'a été observée, mais au bout d'une semaine on a pu noter une augmentation d'environ 33 %. Cette vascularisation a continué à se développer jusqu'à un maximum d'environ 70 % par rapport aux valeurs de référence, après 2 semaines d'hypoxie, mais sans accroissement supplémentaire significatif entre la 2e et la 3e semaine. Au bout de 3 semaines de normoxie faisant suite à 3 semaines d'hypoxie, la vascularisation cérébrale est redevenue normale. Cette hypervascularisation induite par hypoxie a été associée à une densité accrue de la protéine transportant le glucose (GLUT-1) dans des vaisseaux capillaires cérébraux isolés. Cet accroissement de densité de GLUT-1 dans ces capillaires a été notoire après 1 semaine d'hypoxie, se maintenant à ce niveau après 3 semaines d'hypoxie et 3 semaines de récupération normoxique. Ainsi, à la différence de la vascularisation cérébrale induite par hypoxie, l'accroissement de la densité de GLUT-1 induit par hypoxie dans les vaisseaux capillaires cérébraux isolés n'est pas rapidement réversible. La combinaison de la vascularisation cérébrale et de l'expression accrue de GLUT-1 dans les vaisseaux cérébraux de rats soumis à l'hypoxie hypobare est associée à un transport accru du glucose du sang au cerveau, accompagné d'une élévation des concentrations cérébrales de glucose et de lactate et d'un taux métabolique cérébral régional accru du glucose. Ces découvertes expliquent plusieurs réponses adaptatives à l'hypoxie prolongée et suggèrent une glycolyse accrue dans le cerveau de rats adultes après une hypoxie modérée et prolongée.

Mots-clés : Hypoxie, Adaptation, Microcirculation, Cerveau, Glycolyse.

SUMMARY: We review some of the effects of moderate hypobaric hypoxia (0.5 atmosphere) on the brain microcirculation of adult rats. Brain vascularity increases after prolonged hypoxia, but the time course of the adaptive response and its reversibility, were not known. We found no significant increase in brain vascularity after 4 days of hypoxia but noted a significant increase of about 33% after 1 week. Cerebral vascularity continued to increase, attaining a maximum of about 70%, over control values after 2 weeks of hypoxia, but without significant further increase between 2 and 3 weeks of hypoxia. Three weeks of normoxia after 3 weeks of hypoxia reversed the hypoxia-induced cerebral hypervascularity. The cerebral hypervascularity induced by hypoxia was associated with an increased density of the glucose transported protein (GLUT-1) in isolated cerebral microvessels. This increased expression of GLUT-1 in cerebral microvessels was evident at 1 week of hypoxia, remained constant after 3 weeks of hypoxia, and was not reversed after 3 weeks of normoxic recovery. Thus, unlike the hypoxia-induced increased brain vascularity, the hypoxia-induced increased density of GLUT-1 in isolated cerebral microvessels is not readily reversible. The combination of increased brain vascularity and increased GLUT-1 expression in cerebral microlvessels of rats subjected to hypobaric hypoxia is associated with increased blood-to-brain glucose transport, increased brain concentrations of glucose and lactate, and increased regional cerebral metabolic rate for glucose. These findings document several adaptive responses to prolonged hypoxia and suggest increased glycolysis in the adult rat brain after moderate and prolonged hypoxia.

Key Words : Hypoxia, Adaptation, Microcirculation, Brain, Glicolysis

INTRODUCTION

The mammalian brain requires uninterrupted perfusion for its normal functions. Blood flow to the brain delivers primarily oxygen and glucose; and every clinician knows the dire consequences of severe brain hypoxia. When hypoxia is brief and moderate, regional brain oxygen tension may be maintained by increasing cerebral blood flow, which is associated with shorter capillary mean transit time, increased red cell velocity, and possible capillary recruitment. However, in prolonged moderate hypoxia, other adaptive (or maladaptive) mechanisms become manifest (1-3). These differ by species, but the rat's response is similar to man's and for that reason, it is believed that studies of chronic moderate hypoxia in the rat are of clinical relevance (2). Chief among the adaptive mechanisms to prolonged moderate hypoxia are increased red cell mass and changes in ventilation and arterial blood gases, both of which increase the oxygen carrying capacity of the blood. In fact, LaManna *et* al. (4) examined rats exposed to hypobaric hypoxia at 0.5 atmosphere (ATM) for 3 weeks and found that although cerebral blood flow in hypoxic rats was not significantly increased, the marked increase in arterial hematocrit allowed similar oxygen delivery to the brains of hypoxic rats as that to the brains of normoxic controls. However, if the increased hematocrit and hyperventilation were the only adaptive responses to hypoxia, brain oxygen tension in hypoxic rats would have remained low because of the lower capillary PO2 which is the driving force for oxygen diffusion into tissues. Thus, increased hematocrit and hyperventilation are not sufficient for adequate brain oxygenation; other adaptive responses are needed.

Over the last few decades, a considerable body of experimental evidence led to the observations that the brain's vascularity increases in prolonged hypoxia, presumably yielding smaller intercapillary distances which would improve oxygenation (4-9). The main aim here is to summarize pertinent results from our laboratory concerning the alterations in the cerebral microcirculation that take place in adult rats subjected to hypobaric hypoxia at 0.5 ATM for periods of up to 3 weeks. Because of the substantial enrichment of brain capillaries with the glucose transporter protein, GLUT-1 (10), we will also review the effects of hypoxia on GLUT-1 in cerebral microvessels, and therefore on blood-to-brain glucose transport and brain glucose metabolism.

In all experiments, adult male Wistar rats aged 3-6 months were kept in hypobaric chambers maintained at 0.5 ATM (380 torr) for the indicated times except for 1 hour per day when the pressure was returned over 10 minutes to atmospheric for cage cleaning and for water and food replenishment (4). In some experiments, the reversibility of these changes were studied in rats that were subjected to hypoxia for 3 weeks but then were allowed to recover at normal atmospheric pressure for another 3 weeks. In all experiments, hypoxic rats were compared to normoxic littermates that were kept outside the hypobaric chamber, but which were treated in an otherwise identical manner.

CHANGES IN CEREBRAL VASCULARITY

LaManna *et* al. previously reported increased brain vascularity after 3 weeks of hypoxia in the same experimental hypoxia model that was described above (4). The increased vascularity ranged from 40 to 70% above control regional values that were measured in coronal brain sections that were stained for alkaline phosphatase as a marker of cerebral capillaries. However, the time course of the development of increased brain vascularity and the reversibility of this response remained unknown.

Harik *et al.* measured cerebral vascularity in rats that were subjected to hypoxia for periods of 1, 2, or 3 weeks and in rats subjected to 3 weeks of hypoxia but then allowed to recover for another 3 weeks (11). For measurement of cerebral vascularity, the rats were anesthetized, perfused-fixed *in situ*, and coronal brain sections were immunostained using GLUT-1 antibody and the peroxidase-antiperoxidase method as described previously (12). Two regions representing the frontal motor cortex were studied where microvessel profiles were counted and exoressed as microvessel profiles/ mm^2 of cerebral cortex as described before (4).

Hypoxic rats developed polycythemia within days of hypoxic exposure (Fig. 1). Significant differences in the hematocrit between hypoxic and normoxic rats was evident at 1, 2, and 3 weeks of hypoxia (11). Three weeks after normoxic recovery, the hematocrit returned to control values indicating full reversibility of the hypoxia-induced polycythemia (Fig. 1).

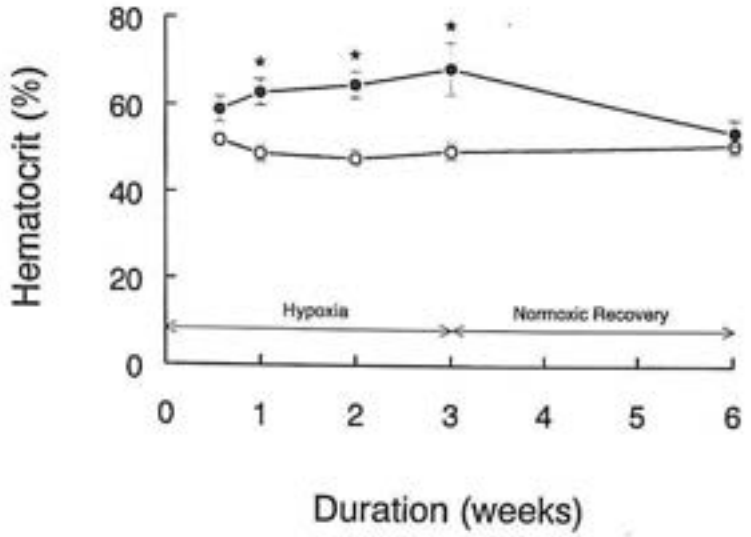

Figure 1. Effects of hypoxia and recovery from hypoxia on hematocrit.
The venous hematocrit in normoxic (o) and hypoxic (·) rats is plotted as a function of experimental duration. The data represent means ±SD of 3 rats in each group at 4 days, 20 rats in each group at 1 week, 3 rats in each group at 2 weeks, 20 rats in each group at 3 weeks and 6 rats in each group at 6 weeks. The hematocrit was significantly increased in the hypoxic group at 1 week, 2 weeks and 3 weeks, but there was no significant difference in the hematocrit values at 6 weeks. Taken from ref. 11.

On the other hand, cerebral vascularity was not altered significantly after 4 days of hypoxia (Fig. 2). After one week of hypoxia, there was a 33 % increase in cerebral vascularity which continued to increase attaining a maximum of about 70% above control values after 2 weeks of hypoxia (Fig. 2). The increased cerebral microvessel density like polycythemia, was reversible in rats that were allowed to recover for 3 weeks (11).

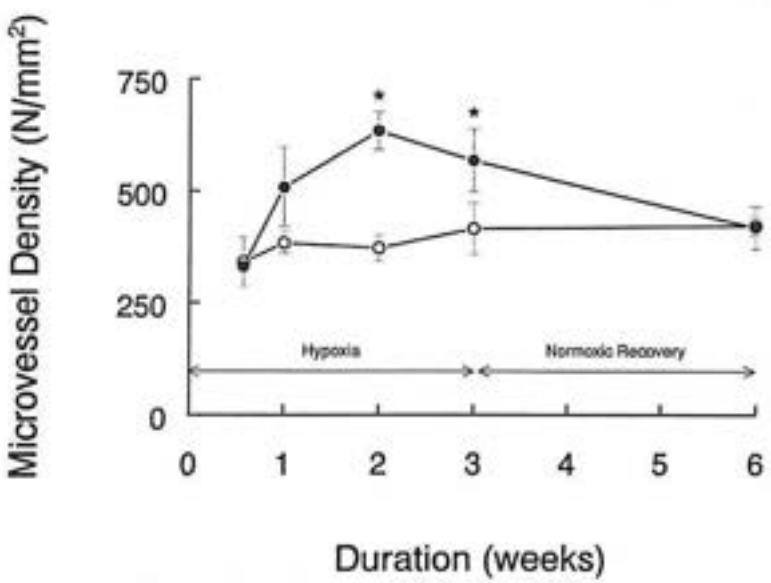

Figure 2. Effects of hypoxia and recovery from hypoxia on cerebral vascularity.
The cerebral microvessel density in normoxic (o) and hypoxic (·) rats as a function of the experimental duration. The data represent ±SD of 3 rats in each group at each time point. Analysis of variance showed an effect of hypoxia on the cortical microvessel density at 2 weeks and 3 weeks of hypoxia but not at the other experimental durations. There was no effect of duration on the cortical microvessel density in control rats. There was an effect of time on cerebral microvessel density in hypoxic rats in that the cerebral vascular density increased significantly between 4 days and 7 days of hypoxia ($P<0.001$) and decreased significantly during the 3 week recovery period ($P<0.04$). Taken from ref. 11.

These results from two dimensional studies of the effects of moderate prolonged hypoxia on brain vascularity are compatible with the results of investigations using three dimensional geometry of the microvascular network in the cerebral cortex of rats exposed to the same hypoxic insult as reported by Mironov et al. (13). In that study, the vascular pattern in hypoxic rats was similar to that of normoxic rats except that there was increased vascular density, tortuosity and loop-like formation, which were most evident in the deeper part of the cerebral cortex. Quantitative assessment of the mean capillary segment length showed a significant increase in the deep parietal cortex of hypoxic rats. The increased capillary segment length in the deep parietal cortex of hypoxic rats was also evident in comparisons of the frequency distribution of capillary segment lengths (13). In hypoxia, the peak frequency was significantly increased from 37.1 ± 1.7 pm to 52.3 ± 1.9 pm (means ± SD). If sprouting was the only mechanism of the increased vascularity of the hypoxic cerebral cortex, then a smaller capillary segment length would be predicted. The findings to the contrary indicate elements of capillary elongation and hypertrophy which are induced by hypoxia.

CHANGES IN GLUCOSE TRANSPORT AT THE BLOOD-BRAIN BARRIER

Brain capillaries are known to have a high density of the glucose transporter protein (GLUT-1) in their plasma membranes (9-12). This is understandable since brain capillaries, which constitute less than 0.5 % of the wet weight of the brain, have to transport glucose for the overwhelming mass of surrounding neurons and glia. Because oxygen, but not glucose, is limited in hypobaric hypoxia, one would suspect that newly formed brain capillaries would have a lower GLUT-1 density in their plasma membranes to avoid a mismatch between oxygen and glucose delivery.

Harik et al. quantitated GLUT-1 in isolated cerebral microvessels obtained from rats subjected to hypobaric hypoxia for 1 or 3 weeks, and in their littermate controls (9). The isolated cerebral microvessels were assayed for their D-glucose-displaceable cytochalasin-B binding. The results showed that the maximal number of cytochalasin-B binding sites was significantly increased by about 25 % at 1 and at 3 weeks of hypoxia while cytochalasin-B binding to particulate fractions of the cerebral cortex was not affected by hypoxia.

Because cytochalasin-B binds to numerous glucose transporters, and is not specific to GLUT-1, and because the normoxic reversibility of this phenomenon was not studied, Harik et al. recently measured GLUT-1 in isolated cerebral microvessels from rats subjected to various periods of hypoxia with and without recovery, using quantitative immunological determination of GLUT-1 by Western blots (11). The effect of hypoxia on the protein expression of GLUT1 in isolated cerebral microvessels was calculated as the ratio of the optical density of the autoradiograms of the GLUT-1 band in Western blots of hypoxic and normoxic samples of microvessels from littermate rat groups that were assayed simultaneously (11). The expression of GLUT-1 in isolated cerebral microvessels was significantly increased after 1 and 3 weeks of hypobaric hypoxia (Fig. 3). However, the increased expression of GLUT-1 in cerebral microvessels isolated from hypoxic rats remained significantly higher than control levels after 3 weeks of normoxic recovery (Fig. 3). Thus, it appears that the increased density of GLUT-1 in isolated cerebral microvessels is not easily reversible (11).

The results obtained by the Western blot immunochemical method for quantitating the density of GLUT-1 are consistent with those measured in the earlier study (9) where cytochalasin-B binding was used to quantitate the glucose transporter in isolated cerebral microvessels. Both studies showed about 50% increase in the density of the glucose transporter in the cerebral microvessels of rats subjected to hypoxia. This was not unexpected given the fact that GLUT-1 is the major, if not the only, glucose transporter in cerebral microvessels. These results are consistent with the increased blood-to-brain glucose transport in vivo in rats that were exposed to hypobaric hypoxia (9). In contradistinction to the decline in the cerebral vascular density in hypoxic rats that were allowed 3 weeks of normoxic recovery, the GLUT-1 density in isolated cerebral microvessels of recovering rats did not decline (11). This suggests that either the half life of GLUT-1 in cerebral microvessels is prolonged so that GLUT-1 persists for extended time periods, or that the stimulus underlying GLUT-1 synthesis does not shut off easily.

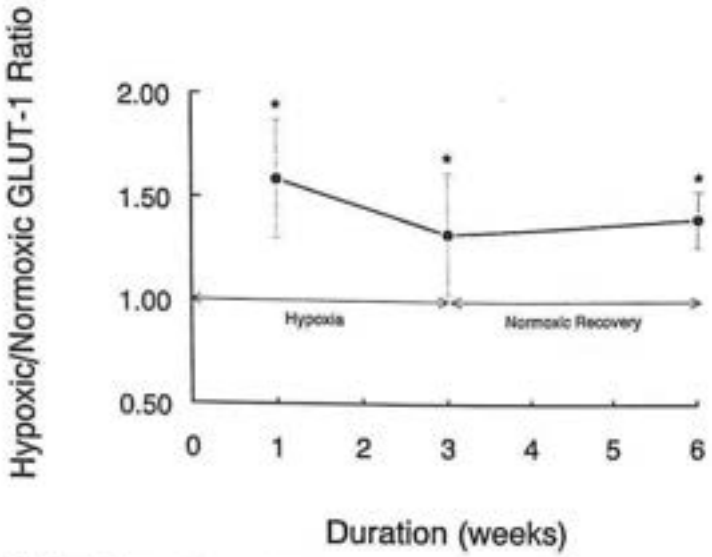

Figure 3. Effect of hypoxia on GLUT-1 density in isolated cerebral microvessels.
GLUT-1 density ratio of hypoxic:normoxic preparations as a function of the experimental duration. The data represent means ± SD of 3 microvessel preparations after 1 week, 5 preparations after 3 weeks, and 4 preparations after 6 weeks. The Student t-test showed a significant effect of hypoxia at all 3 time periods that were studied ($P < 0.05$), but no effect of duration of hypoxia or recovery by analysis of variance. Taken from ref. 11.

The combination of the increased brain vascularity and the increased density of GLUT-1 in cerebral microvessels of rats subjected to hypobaric hypoxia was found to be associated with increased blood-to-brain glucose transport in vivo in rats subjected to 3 weeks of the same hypobaric condition described above (9). Regional blood-to-brain D-glucose transport and blood flow were determined by the double-label, single-pass indicator-fractionation atrial bolus injection method. Cerebral blood flow was slightly higher in hypoxic rats but the difference did not reach significance in any of the brain regions. Also, there were no significant alterations in regional brain L-glucose space in hypoxic rats. The extraction fraction values of D-glucose were increased by about three-fold in hypoxic rats and the unidirectional blood-to brain influx was doubled in those rats. That the increased brain glucose influx in hypoxia was not commensurate with the increased extraction fraction despite the similar regional blood flow and the similar L-glucose space in both groups of rats is probably a reflection of the markedly decreased blood plasma fraction which is a consequence of the increased hematocrit and the decreased blood plasma fraction in hypoxic rats. The calculated maximal transport capacity was significantly increased in hypoxic rats (9).

Thus, these findings indicate that in addition to the increased vascularity of the brain in hypobaric hypoxia, there is an increased density of the glucose transporter moiety at the blood brain barrier which contributes significantly to the increased unidirectional transport of glucose into the brain. The physiological concomitants of these observations have been followed up in other studies that were performed using the same model of hypobaric hypoxia. Harik et al. measured regional brain concentrations of glucose, glycogen, lactate, adenosine triphosphate, phosphocreatine, regional intracellular pH and regional cerebral metabolic rate for glucose in rats subjected to 3 weeks of hypobaric hypoxia (14). The results obtained from that work indicated a significant increase in brain glucose and lactate levels and a significant decrease in brain glycogen levels in hypoxic rats but without a significant change in brain ATP, phosphocreatine, and pH values. Thus, the increased density of the glucose transporter at the blood-brain barrier, and the increased blood-to-brain glucose transport result in higher brain glucose concentrations. The lack of differences between hypoxic and normoxic brains in their high energy phosphate content further indicates that at this level of hypoxia, there is adequate compensation for oxygen lack. The similar regional brain intracellular pH in hypoxia and normoxia is another indication of the compensation which prevents tissue acidosis in the face of higher lactate concentrations in the brains of hypoxic rats. Harik et al. speculated that the increased brain lactate levels in hypoxia are useful for maintaining brain pH in the presence

of low brain tissue CO_2 tension, thereby avoiding the necessity to export brain bicarbonate (14).

The concomitant findings of decreased brain glycogen and increased brain lactate suggest increased anaerobic glycolysis in the hypobaric brain. The other important finding was the increased cerebral metabolic rate for glucose in hypoxic rats (14). The authors suspected that the increased cerebral metabolic rate for glucose in hypoxia, presumably for anaerobic glycolysis, combined with the low brain plasma flow in hypoxic rats and the fact that rats erythrocytes do not carry glucose, are logical explanations for the increased glucose transport at the bloodbrain barrier. The exact mechanism of how the higher cerebral metabolic rate for glucose in prolonged moderate hypoxia eventually causes up-regulation of glucose transport at the blood-brain barrier remains unknown.

The increased reliance of the brain on possible anaerobic glucose metabolism is probably one of several compensatory mechanisms that underlie adaptation to prolonged hypoxia which was previously suspected in the heart (15). Perhaps this is the reason why a carbohydrate rich diet is believed to be beneficial for subjects exposed to high altitude (16).

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DEVELOPMENT AT HIGH ALTITUDE: INTRAUTERINE AND EXTRAUTERINE

HIGH ALTITUDE, HYPOXEMIC-INDUCED RESPONSES IN ADULT AND FETAL CEREBRAL BLOOD VESSELS

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RESUMEN: Respuestas de Vasos Sanguíneos Cerebrales Fetales y Adultos Inducidas por Hipoxemia de Altura

En respuesta a la hipoxemia de altura prolongada, las arterias cerebrales de fetos de ovejas y de ovejas adultas muestran respuestas contráctiles disminuidas a la noradrenalina y a otros agonistas. En esta revisión examinamos los mecanismos contráctiles pre-sinápticos y post-sinápticos que podrían explicar estos cambios. Además, examinamos la relajación de los vasos cerebrales y el rol del embarazo en la alteración de estas respuestas. Los resultados revelan la profundas diferencias de respuesta de las arterias carótidas común e intracraneal, así como las diferencias significativas existentes en las respuestas del feto y del adulto. Dichos resultados también enfatizan el rol de la hipoxemia de altura prolongada en la modulación del acoplamiento farmaco-mecánico a nivel de la microcirculación cerebral.

Palabras claves : Aclimatación; Hipoxia; Sistema nervioso simpático; Noradrenalina; Desarrollo

RÉSUMÉ: Réponses des vaisseaux sanguins cérébraux foetaux et adultes, induites par hypoxémie d'altitude.

En réaction à l'hypoxémie prolongée d'altitude, les artères cérébrales de foetus de brebis et de brebis adultes présentent des réponses contractiles amoindries à la noradrénaline et à d'autres agonistes. Dans cette étude de révision nous examinons les mécanismes contractiles pré-synaptiques et post-synaptiques qui pourraient expliquer ces changements. Nous étudions également la relaxation des vaisseaux cérébraux et le rôle de la gestation dans l'altération de ces réponses. Les résultats révèlent de profondes différences entre les réponses des artères carotide commune et intracrânienne, ainsi que des différences significatives entre les réponses du foetus et celles de l'adulte. Ces résultats mettent aussi en relief le rôle de l'hypoxémie prolongée d'altitude dans la modulation de l'accouplement pharmaco-mécanique au niveau de la microcirculation cérébrale.

Mots-clés : Acclimatation, Hypoxie, Système nerveux sympathique, Noradrénaline, Développement.

SUMMARY: In response to high altitude long-term hypoxemia, the cerebral arteries of adult and fetal sheep show decreased contractile responses to norepinephrine and other agonists. In this review we examine the pre-synaptic and post-synaptic contractile mechanisms which might account for these changes. In addition, we examine cerebral vessel relaxation and the role of pregnancy in altering these responses. The results highlight the profound differences in response of common carotid and intracranial arteries, as well as the significant differences in responses of fetus and adult. They also emphasize the role of high altitude, long-term hypoxemia in modulating pharmaco-mechanical coupling in the cerebrovasculature .

Key Words: Acclimatization, Hypoxia, Sympathetic nervous system, Norepinephrine, Development

INTRODUCTION

In the late 1920s and the 1930s several investigators determined that in man and other mammals, fetal umbilical blood had low oxyhemoglobin saturation and high O_2 capacity, as compared to adults. This led to the concept of

the fetus, as a mountaineer at high altitude, being exposed to a low O_2 tension, and thus being at "Mt. Everest in Utero" (1, 2). The question arose, if the fetus carried by a mother at sea level is in effect living at simulated high altitude, how does a fetus survive which is borne by a mother at high altitude in whose arterial blood the O_2 tension is much lower than that at sea level? What are the mechanisms, both maternal and fetal, which enable man and many mammals to reproduce at altitudes up to 4,500 to 4,880 m (15,000 to 16,000 ft)? In addition to women at high-altitude, fetuses of a large number of other pregnant women may experience prolonged hypoxemic stress. Thus, the problem of long-term hypoxemia, and the mechanisms whereby mother and fetus attempt to maintain tissue and cellular oxygenation, is of great physiologic importance and clinical relevance.

The cerebral vasculature is richly innervated by sympathetic nerves (3, 4), and the neurotransmitter norepinephrine acts post-synaptically on smooth muscle adrenergic receptors as a principle determinant of vascular contractility (5, 6, 7). The reactivity of vascular smooth muscle to adrenergic agonists has been shown to be modulated by a number of factors including hypoxia (8, 9, 10). In the adult, high altitude, long-term hypoxia is associated with a number of cerebrovascular, cardiovascular, metabolic, and other adaptations (11), the cellular bases of which are poorly understood. For the fetus, which at sea level thrives at an arterial PO_2 value comparable to an adult at high altitude, almost nothing is known of its cerebrovascular acclimatization responses when its mother is at high altitude.

Here we review what is known of cerebrovascular acclimatization responses to prolonged hypoxemic stress in the adult and the developing fetus. Most of the data is derived from studies in sheep. Because it is outside the scope of this presentation, we will not attempt to review pulmonary, cardiovascular, hematologic, and other responses to high-altitude (>3000 m), long-term hypoxemia in the adult or fetus.

Several definitions are in order. Generally speaking "hypoxia" refers to a relative lack of oxygen (e.g., low O_2 content or tension) in the ambient air and/or tissues. Strictly speaking, "hypoxia" or "dysoxia" is O_2 limited energy flux and electron transfer in the mitochondrial cytochrome oxidase system rather than a low O_2 content or tension, *per se* (12). "Hypoxemia" is a state of less than normal O_2 in the arterial blood. "Acclimatization" to high altitude is the process of becoming accustomed to an environment with a relatively low O_2 tension, as ambient PO_2 is inversely related to altitude. The process of acclimatization occurs over a period of days to weeks, but is believed to be essentially complete within five to six weeks (13). This is to be distinguished from "adaptation" which occurs over a period of decades or generations.

High Altitude, Long-Term Hypoxemia "Model"

During the past decade, we have explored a number of facets of the process whereby the fetus, pregnant mother, and nonpregnant adult acclimatize successfully to the stress of high-altitude, long-term hypoxemia. The high-altitude "model" which we have used is as follows. Western grade Suffolk ewes (both 30 days pregnant and nonpregnant) are transported to the White Mountain Research Station (WMRS), White Mountain, California, where they are maintained, at 3,820 m (12,470 ft). After three to four months of acclimatization, the near-term (~140 of 147 days gestation), and nonpregnant ewes are transported to our laboratories for study. In addition, some pregnant ewes are returned to our laboratories at ~115 to 120 days gestation for chronic catheterization and in vivo studies. After arrival at LLU, the maternal arterial O_2 tension of all long-term hypoxemic ewes is maintained at that level which they experienced at high-altitude, e.g., ~60 Torr.

Table 1. Maternal and Fetal Physiologic Responses to High Altitude, Long-Term Hypoxemia

Physiologic Response	Normoxia	High Altitude	% Change
Mother			
PO_2 (Torr)	102±2	64+2*	-37.1
PCO_2 (Torr)	35±1	29_1*	-17.9
pH	7.44±0.01	7.46_0.01	0.3
[Hb] (g/dl)	8.7±0.3	10.5_0.4*	20.7
BP (mm Hg)	81±3	88_4	8.6
Fetus			
Weight (g) (at 140 days gestation)	4640±180	862±300	-
PO_2 (Torr)	23±1	19±1*	-17.2
[HbO_2] (%)	59±3	50±3♦	-15.9
[Hb] (g/dl)	10.1±0.7	12.6±_0.6*	24.7
O_2 content (mUdl)	7 7±0.5	7.8±0.5	1.0
PCO_2 (Torr)	48±1	4±1 *	-18 4
pH	7.36±0.01	7.37±0.01	0.1
Lactate (mg/dl)	13 1±07	14 4±1	9.9
Heart Rate (beats/min)	168±5	165±5	-1.6
Arterial Pressure (mmHg)	44±1	52±1*	17.1
Right Ventricular Output(ml min^{-1} kg^{-1})	276±10	183±10*	-33.6
Left Ventricular Output(ml min^{-1} kg^{-1})	166±16	142±16	-14.5
Right Stroke Volume (ml/kg)	1.66±0.05	1.11±0.05*	-33.1
Left Stroke Volume (ml/kg)	0.97±0.09	0.84±0.08	-13.4
Combined Ventricular Output (ml min^{-1} kg^{-1})	441±23	335±28♦	-24.1
Breathing Incidence (min/h)	25	25	-
Norepinephrine (pg/ml)	553±55	635±65	14.8
Epinephrine (pg/ml)	81±19	113±12	39.5
ACTH (pg/ml)	66±8	60±9	-10
Cortisol (ng/ml)	47±3	50±1	6.4
Erythropoietin (mU/ml)	23±2	31±17	35

Data from References 19 and 20. Values are means ± SE; ** $p<0.01$; ♦ $p<0.05$ from control

General Fetal Responses/Adaptations to Long-Term Hypoxemia

In humans, newborn birth weight and perinatal mortality rate serve as fairly universal measures of the effects of high altitude on fetal growth and development. Over the past five decades, a number of studies on several continents and many ethnic groups have demonstrated lower mean birthweight, elevated placental to fetal weight ratios, and higher neonatal mortality and morbidity including intraventricular hemorrhage (14) in such infants (15, 16, 17, 18). Some specific physiologic responses to high altitude, long-term hypoxemia in chronically catheterized fetuses and adults are given in Table 1, with comparisons to normoxic control animals. The values are based on results in 10 to 40 adult or singleton fetuses in each group (19, 20).

Cerebrovascular Changes

General. In chronically catheterized near-term fetal sheep, cerebral blood flow (as measured with radioactive labeled microspheres) was not significantly different in the high altitude, acclimatized fetus (110±19 mUmin/100 g) as compared with sea level controls (139±12 mUmin/100 g). This, despite the fact that total cardiac output was 27% lower (351±55 mUmin/Kg) that in normoxic controls (483±12 ml/min/Kg) (20).

From a structural standpoint, the common carotid arteries of fetal and adult sheep showed ~50% increase in base soluble protein (percent of dry weight) as compared with normoxic controls (Fig. 1, upper panel). However, these vessels showed no significant differences in vessel resting inside diameter, wall thickness, or percent water (9). Fetal, but not adult, middle cerebral arteries

showed a less dramatic, 22% increase in base soluble protein (Fig. 1, lower panel). The reason for these large increases in protein is not clear.

Contractile Responses to Potassium and to Amines. In both common carotid and the cerebral arteries of adult and fetus, chronic hypoxemia depressed maximum contractile tension to depolarization by $1.2x10^{-1}$ M potassium. However, when these contractile responses to K^+ were normalized relative to vessel cross-sectional area, the responses of fetal and adult vessels differed. In the fetal arteries, the hypoxic-induced depression of response persisted. In contrast, adult arteries showed no significant changes in K^+-induced stress. When normalized to cross-sectional area, the potassium responses reveal not just the contractile capacity of the tissue, but also the relative proportion of contractile to noncontractile elements in the wall. That potassium-induced stresses decreases in the fetal common carotid suggests that either contractile protein synthesis decreased or that non-contractile elements increased, in response to hypoxia. That no other changes were noted suggests that hypoxia exerted few effects at the level of the contractile apparatus. As noted above, hypoxia generally increased protein content. If this occurred without a change in stress, then hypoxia must have exerted a parallel effect on both contractile and non-contractile elements in the adult common carotid, but may have preferentially stimulated noncontractile protein synthesis in the fetal common carotid.

Some may argue correctly that $1.2x10^{-1}$ M potassium is an unphysiologic stimulus. Thus, we repeated this comparison by the use of exogenous amines serotonin (10^{-5} M) and histamine ($2x10^{-5}$ M). In fetal common carotid and intracranial arteries, chronic hypoxemia depressed the maximal contractile response to a mixture of serotonin and histamine 37% and 26%, respectively. In contrast, the adult common carotid and cerebral arteries showed no significantly different responses. Of interest, when these maximum responses to serotonin and histamine were normalized relative to the maximum response to potassium, the changes in the fetal arteries became not significant, while the adult intracranial arteries showed significant 50 to 87% increases in the ratios (9). The amine-potassium ratio serves to normalize out any effects of changes in the basic contractile apparatus. Thus, changes in protein, thickness, and potassium-responses are all excluded from interpretation of these data. Instead, the data indicate the extent to which receptor effector coupling is changing as a consequence of hypoxia.

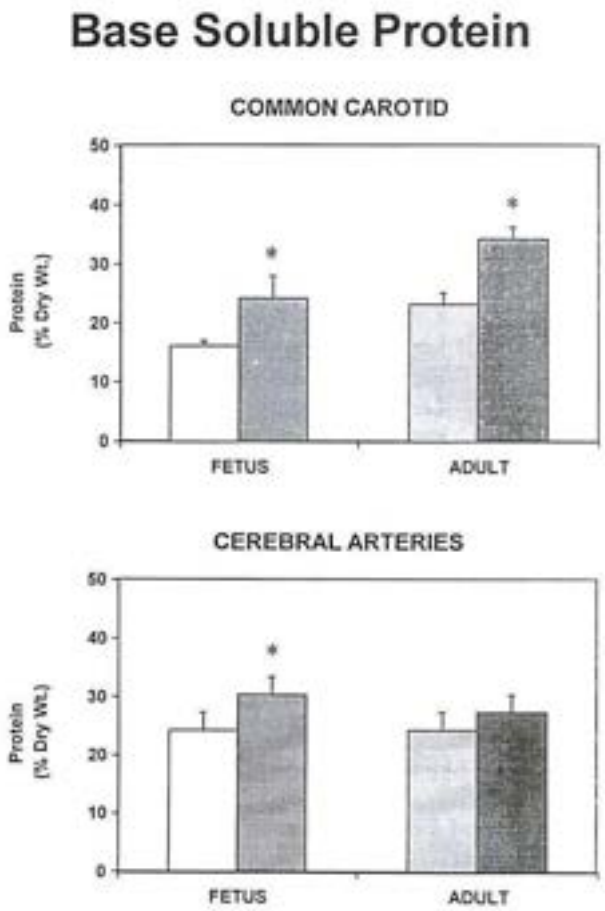

Figure 1. Upper Panel. Effects of long-term hypoxia on base-soluble cellular protein content, expressed as percent dry weight, in common carotid of fetal and non-pregnant adult sheep maintained at either sea level (open or lightly shaded bar) or at 3280 m (heavily shaded bar) for 110 days. Values are mean ± SEM; *p<0.05.

Lower Panel. Shown here are mean ± SEM, expressed as percent dry weight, measured in middle cerebral arteries of fetal and non-pregnant adult sheep maintained at either sea level or at 3280 m for 110 days (the last 110 days of gestation for the fetuses). Note that hypoxia was associated with significantly elevated protein levels in arteries of both age groups. *p<0.05.

Contractile Responses to Endogenous Norepinephrine. To examine adrenergic-mediated contractile mechanisms, the arterial nerves were directly and selectively stimulated at physiological frequencies resulting in norepinephrine (NE) release with contractile effect (21, 22). For all arteries, the contractile response of normoxic arteries were significantly greater in adult than in fetus (21). In the adult common carotid and intracranial arteries, long-term hypoxemia was associated with significant depression of contractile response. In contrast, in fetal common carotid and intracranial arteries, long-term hypoxemia was associated with a markedly greater contractile response (21). We emphasize that these responses were to endogenous norepinephrine, and that all normal uptake systems were intact and not blocked. Thus, the data reflect the true functioning of the adrenergic receptors in these arteries. If the blood brain barrier is intact, circulating norepinephrine would never reach the α-receptors on cerebral arteries and only NE from sympathetic neurons would activate these receptors. As the adrenergic neuroeffector pathway is not completely developed in the fetus, hypoxia appears to accelerate development of this system in the fetus, but retard it in the adult.

Pre-Synaptic Noradrenergic Function. In a series of studies, we have attempted to dissect the hypoxic-mediated NE-induced responses in terms of pre-synaptic (e.g., neuronal neurotransmitter release) versus post-synaptic (e.g., factors which determine contractile response) components. One measure of pre-synaptic noradrenergic functional capacity is NE content. In fetal intracranial arteries, but not common carotid, long-term hypoxemia was associated with a slight decrease in NE content. In contrast, both common carotid and cerebral arteries of the adult showed significant increase in NE content (21). These changes may appear paradoxical in light of the depression of neurogenic vasoconstriction in adult arteries, and its augmentation in fetal arteries. However, NE content is the product of both nerve number and the mean NE content per nerve fiber. In addition, NE content may be less in those nerves which fire often, as compared with those that are more quiescent. Thus, these results suggest that in comparison with normoxic, control fetal arteries, hypoxic vessels have fewer adrenergic nerve terminals or less NE per nerve. The opposite would be expected in the adult arteries, e.g., increased adrenergic nerve density or greater NE content per nerve. These results emphasize the contrasting effects of long-term hypoxia on fetal and adult common carotid and cerebral arteries.

Another index of pre-synaptic noradrenergic function is nerve density. This can be determined indirectly by quantifying cocaine-sensitive NE uptake, a selective measure of NE containing nerve terminals. As shown in Figure 2, upper panel, in adult and fetal common carotid arteries exposed to 10^{-5} M cocaine, long-term hypoxia was associated with elevated NE uptake of 86% and 132%, respectively (i.e., greater adrenergic nerve density). Other intracranial arteries except the middle cerebral also showed a similar increase (21). Importantly, long-term hypoxia had a similar effect in both fetus and adult. Thus, nerve density changes alone cannot explain the age related differences in NE content (hypoxemic-induced decrease in fetus and increase in adult). Thus, it would appear that long-term hypoxemia is associated with decreased NE content per nerve, possibly resulting from increased nerve recruitment in fetal arteries. Adult arteries show the opposite response, e.g., increased NE content per nerve, due possibly from decreased nerve recruitment (21).

An additional factor that may account, in part, for the hypoxic-induced alterations in contractile response to nerve stimulation, is the co-release with NE from adrenergic nerve terminals of neuropeptide Y (NPY). Following NE depletion from adrenergic nerves by pretreatment with guanethidine (10^{-6} M), the remaining tetrodotoxin-sensitive neurogenic responses can be attributed to NPY release. In response to long-term hypoxemia, both adult common carotid and middle cerebral artery showed significant decreases in maximum response to stimulation at 8 Hz. In contrast, fetal arteries showed enhanced responses. That is, long-term hypoxemia decreased the NPY component in adult common carotid and intracranial arteries, while enhancing it in the fetal vessels (21). Thus, the pre-synaptic effects of long-term hypoxemia were similar for NE and NPY, but are opposite for adult and fetus. In the adult, long-term hypoxemia appears to increase neurotransmitter content per nerve, possibly due to increased adrenergic nerve activity. In contrast, long-term hypoxia decreases neurotransmitter content per nerve in the fetus, possibly as a result of increased neuronal activity

(21).

Post-Synaptic Noradrenergic Function. Post-synaptic mechanisms in adrenergic-mediated vascular contractility include a multitude of factors, some of which are: vascular smooth muscle plasma membrane α_1-,α_2-, and ,ß-adrenergic receptors (AR), their density and binding affinity, the second messengers (inositol 1,4,5-trisphosphate ans(1,4,5)P_3) for α_1-AR, and cyclic adenosine monophosphate (cAMP) for α_2- and ß-AR), the Ins(1,4,5)P_3 receptor (Ins(1,4,5)P_3-R) on endoplasmic reticulum, intracellular calcium, several enzymes and their state of activation, and so forth.

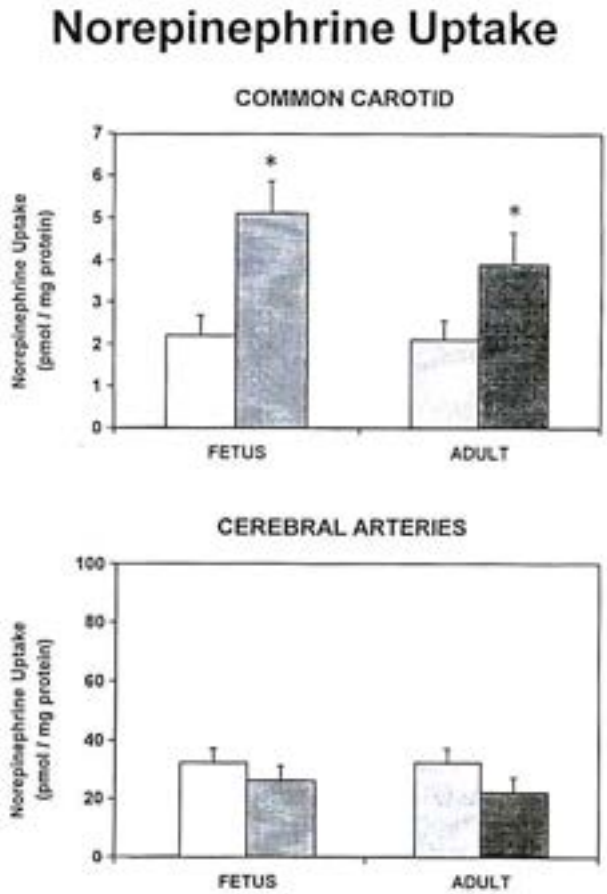

Figure 2. Upper Panel. Effects of long-term hypoxia on norepinephrine uptake in fetal and adult common carotid artery. Paired arteries were exposed to [^{3}H]-norepinephrine with and without the presence of 10^{-7} M cocaine. The differences between control and cocainetreated arteries are shown. Note that in the common carotid hypoxia appears to increase nerve density in both fetal and adult arteries. Values are mean ± SEM; *p<0.05 Lower Panel. Effects of long-term hypoxia on norepinephrine uptake in fetal and adult middle cerebral arteries. Paired arteries were exposed to [^{3}H]-norepinephrine with and without the presence of 10^{-7} M cocaine. *p<0.05.

To examine the role of α_1-adrenergic receptors and their alteration with long-term hypoxia, we quantified these receptors in common carotid and anterior, middle, and posterior (AMP) cerebral arteries of fetuses and adult sheep acclimatized to high altitude. Figure 3, upper panel, shows the α_1-adrenergic receptor density values (B_{max},in fmol/mg protein), as measured with saturation binding of the α_1-AR antagonist [^{3}H]prazosin, for normoxic control and high altitude, hypoxemic fetal and adult common carotid artery. In the fetus and adult, in response to long-term hypoxemia, α_1-AR density decreased 75% and 66% from normoxic control values, respectively. For both the hypoxemic fetus and adult, these receptor density values were significantly different from normoxic controls (p<0.01). Figure 3, lower panel, shows •$_1$-AR density values in fetal and adult combined anterior, middle, and posterior cerebral arteries. High altitude, long-term hypoxemia was associated with decreases of 76% and 61% from control values, respectively (p<0.01). [3H]prazosin binding affinity values (K_D) of both common carotid and AMP cerebral arteries averaged 0.13±0.07 nM, and did not vary significantly as a function of hypoxemia, developmental age, or vessel type (23).

To examine the effect of long-term hypoxemia on the NE dose--Ins(1,4,5)P_3 response relationship for cerebral arteries, we quantified Ins(1,4,5)P_3 (maximal response at 45 sec) as NE varied from 10^{-7} to 104 M in both adult and fetal AMP cerebral arteries. For normoxic fetal AMP cerebral arteries, 10^{-3} M NE-induced Ins(1,4,5)P_3 increased 212% from the basal value. In hypoxic arteries the basal value was similar to normoxic control, increasing only 96% with 10^{-3} M NE. The EC_{50} values for hypoxic adult and fetal AMP cerebral vessels did not differ significantly from normoxic control. Figure 4, upper panel, shows these responses, expressed as percent of basal value, for the common carotid artery of the fetus and adult. The normoxic fetal common carotid showed essentially no response to NE, and this was not altered by hypoxemia. In contrast, in adult common carotid, long-term hypoxemia was associated with a decrease of NE-induced Ins(1,4,5)P_3 response of 51% from control. In fetal and adult AMP cerebral arteries alFig. 4, lower panel), long-term hypoxemia was associated with decreases in Ins(1,4,5)P_3 response

of 35% and 44%, respectively, from normoxic NE-induced values (23).

To examine inositol 1,4,5 trisphosphate receptor binding in hypoxic vessels, we quantified the Ins(1,4,5)P_3-R in the hypoxemic fetal and adult common carotid and AMP cerebral arteries, and compared these values with normoxic controls. In the common carotid artery of the fetus and adult, Ins(1,4,5)P_3-R density fell 32% and 70% from normoxic control values, respectively. Ins(1,4,5)P_3-R density decreased 80% and 47% from control values for fetal and adult AMP cerebral arteries, respectively. In the hypoxic vessels Ins(1,4,5)P_3-R affinity did not change significantly from control (23).

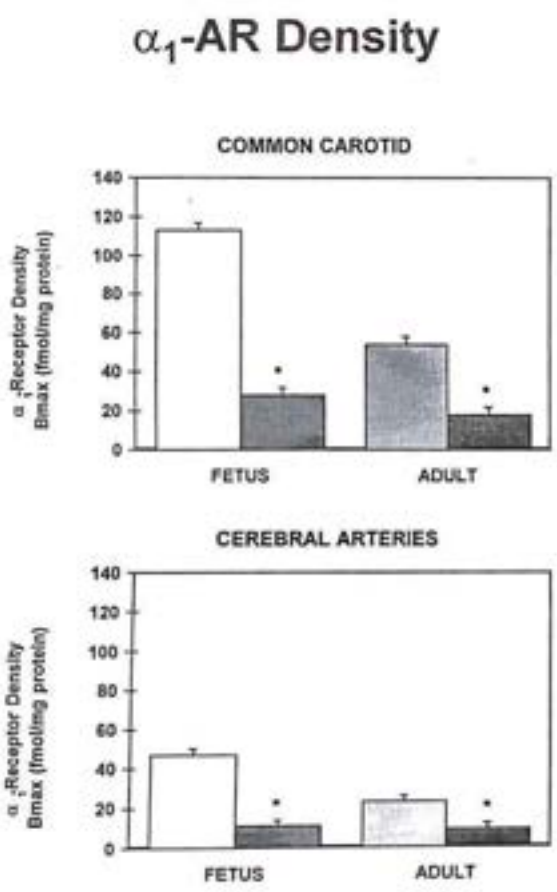

Figure 3. Upper Panel. α_1-adrenergic receptor density (B_{max}) values (fmol/mg protein) as determined with ^{3}H-prazosin, in the common carotid artery of normoxic control (open or lightly shaded bar) and high altitude, hypoxemic (heavily shaded bar) fetal and adult sheep. Data are mean values ± SEM. For both fetal (n=4) and adult (n=5) COM the receptor density values for hypoxemic vessels were significantly different from normoxic controls. *p <0.01

Lower Panel. α_1-adrenergic receptor density of combined anterior, middle, and posterior cerebral arteries for the normoxic control and hypoxemic fetus and adult. Data are mean values ± SEM. The receptor density values of both fetus (n=4) and adult (n=4)were significantly different from the values for the normoxic controls. *p<0.01.

Relaxation Responses. In response to the endothelium-dependent, receptor-independent, vasodilator (by release of endothelium-derived relaxation factor) A23187, chronically hypoxemic fetal common carotid and middle cerebral arteries showed less relaxation than normoxic controls. In contrast, adult common carotid (and to a less extent other cerebral arteries) showed enhanced responses to A23187. By comparison, both hypoxemic fetus and adult showed decreased relaxation responses to the endothelium-independent relaxant nitrosothiol SNAP (s-nitroso-N-acetylpenicillamine). Thus, the ratios of A23187 to SNAP were lower in hypoxemic fetal arteries, while they were higher in the adult vessels (9).

Pregnancy-Associated Effects on Contraction and Relaxation Responses. In the normoxic adult, pregnancy is associated with profound alterations of vascular structure and function, including the cerebrovasculature (24, 25, 26). These changes are believed to be mediated in part by the marked increases in circulating estrogen, progesterone, and other hormones (27, 28). To examine the combined challenge of hypoxia and pregnancy, we examined contractile and relaxation responses in pregnant and nonpregnant adult sheep acclimatized to high altitude hypoxemia. Although both pregnancy and high altitude produced significant alterations in cerebrovascular function, these were not simply the sum of responses in the two groups. Both hypoxic pregnant and hypoxic nonpregnant common carotid and middle cerebral arteries showed increased contractility to potassium depolarization, compared with normoxic pregnant or nonpregnant controls (29). For common carotid (but not middle cerebral) these changes were similar when corrected for vessel thickness (e.g., stress). We observed no significant difference in α_1-AR density or affinity, NE-induced Ins(1,4,5)P_3 response, or Ins(1,4,5)P_3-R density or affinity between pregnant and nonpregnant common carotid or AMP cerebral arteries. For

common carotid from pregnant animals, endothelial function, as measured by relaxation response to A23187, was decreased. However, this was markedly enhanced by hypoxia. Such changes were not notable in middle cerebral or other intercranial arteries (29). Clearly, cerebral vascular responses and dynamics of hypoxemia and pregnancy affect vessels in an artery specific manner.

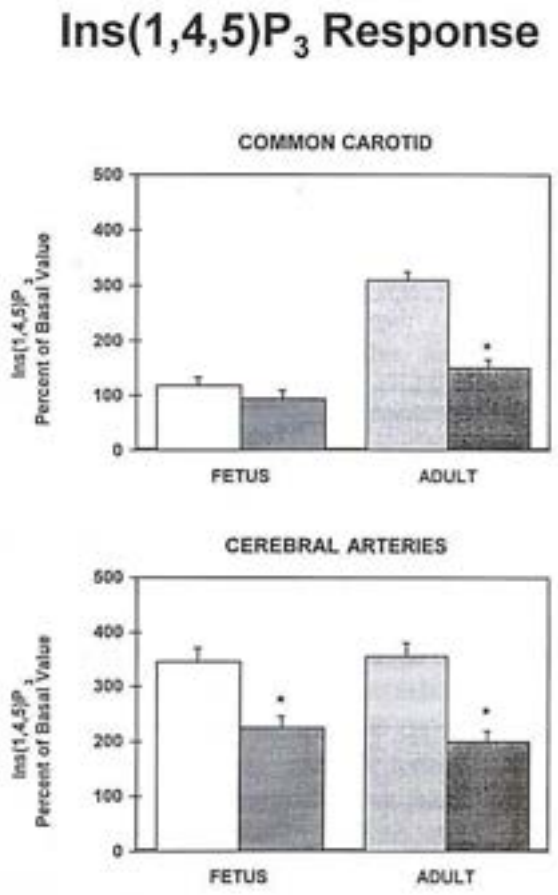

Figure 4. Upper Panel. Norepinephrine-stimulated (10^{-4} M) Ins(1,4,5)P_3 values (expressed as percent of basal value) in normoxic and hypoxemic term fetal and adult ovine common carotid artery. Values are expressed as mean ± SEM. The value for hypoxic adult COM (n=5) was significantly different from that of the normoxic control. **p <0.01.

Lower Panel. NE-induced Ins(1,4,5)P_3 values for AMP cerebral arteries of the normoxic (open bar) and hypoxemic (shaded bar) fetus and adult. The values for hypoxemic fetus (n=4) and adult (n=5) were both significantly different from normoxic controls, *p<0.01

COMMENT

For the adult, high altitude has served as a useful model of how the body's physiological mechanisms acclimatize over a period of days or weeks, or genetically adapt over the course of generations. For the fetus, because of its relatively low arterial PO_2values simulating "Mt. Everest *in utero*" under normoxic, control conditions, the question of how it can grow and develop in a mother at high altitude has posed a dilemma. Because of its obvious relevance to neuronal function, the regulation of cerebral blood flow is important.

Hopefully, the present studies will help to illuminate a rather obscure corner of regulatory physiology. That different elements of the adrenergic-mediated pre-synaptic and post-synaptic signal transduction cascade are independently regulated, both in the adult and in the fetus, should come as no surprise. Because in our studies of the fetus under conditions of long-term hypoxemia the circulating concentrations of epinephrine and norepinephrine were high normal to elevated (30), this chronic vasoconstrictor influence may down-regulate the several portions of the signal transduction cascade. Such a modified regulatory response may help insure that vessel contractility is decreased, so that cerebral oxygenation is not compromised. In addition, many of the hypoxic-induced changes observed in the fetal vessels are similar to those associated with developmental maturation. Nonetheless, the manner in which these changes serve to regulate cerebrovascular tone under these circumstances in vivo must await further studies. In addition, the present studies may provide useful leads to examine the critical issue of hypoxic-mediated gene transcription in modulating the adrenergic signal transduction pathway.

CONCLUSIONS

In adult common carotid and AMP cerebral arteries, acclimatization to high altitude, long-term hypoxemia was associated with significant alterations in pre-synaptic and post-synaptic adrenergicmediated mechanisms; however, these were not necessarily the same in the two vessel groups. Fetal common carotid and AMP cerebral arteries also showed considerable alterations in these mechanisms in response to long-term

hypoxemia. Nonetheless, there were significant differences in these responses between the two vessel groups in the fetus, and between adult and fetus. These findings illustrate the complexity of acclimatization responses to high altitude, and probably account, in part, for the significant hypoxic-induced differences in NE-induced contractility in adult and fetal cerebral vessels. Of course, other elements in the adrenergic-mediated signal transduction pathway may also differ under these conditions. Alterations in these or other signal transduction mechanisms may also play a key role in dysregulation of cerebral blood flow in the adult subjected to high altitude, long-term hypoxemia, as well as in the fetus or newborn subjected to prolonged hypoxemia.

ACKNOWLEDGMENT

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CARDIOPULMONARY TRANSITION IN THE NEONATE AND INFANT AT HIGH ALTITUDE

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RESUMEN: Transición Cardiopulmonar en la Altura: el Neonato y el Lactante

Objetivo: En la altura , ocurren alteraciones relacionadas con el nivel de altitud en la saturacidn arterial de oxígeno (S_aO_2), tanto en el neonato como en el lactante.

Diseño: La evidencia tomada de investigación original y de la literatura publicada permite la comparación de varios grupos poblacionales residentes en altura, tanto en América del Norte y del Sur como en Asia.

Material y Métodos: Datos de oximetría de pulso, pletismografía respiratoria, ecocardiografía, cateterización cardíaca y examen histológico de neonatos y lactantes ilustran alteraciones en la función, ejemplos de adaptación exitosa, y ejemplos de morbilidad y mortalidad relacionados con el nacimiento o la residencia en altura.

Resultados: La S_aO_2 cae al aumentar la altura; sin embargo, este efecto no tiene relación lineal con la altura o la presión barométrica. La S_aO_2 varia marcadamente con el estado conductual. En contraste con los patrones observados a nivel del mar, la S_aO_2 disminuye luego de una semana de vida en ciertas poblaciones de altura. La prevalencia y la duracidn de la respiración periódica de los lactantes aumenta en la altura en comparacidn con los del nivel del mar. La respiración periódica ocurre más comúnmente durante el sueño activo y tranquilo y se asocia con un patrón cíclico de saturación de oxígeno. La presión de la arteria pulmonar cae lentamente luego del nacimiento en altura extrema. En altura moderada, las presiones de la arteria pulmonar pueden normalizarse, pero el lecho vascular pulmonar sigue siendo susceptible al desarrollo de hiperntensión pulmonar sintomática durante un periodo prolongado de transición. El sindrome de mal de montaña subagudo infantil y la persistencia de cortocircuitos de derecha a izquierda por el foramen oval y el conducto arterioso reflejan una presión de arteria pulmonar y una resistencia vascular elevadas en la infancia.

Conclusión: El recién nacido de la altura experimenta una transición más lenta de patrones fetales a patrones maduros de función cardiopulmonar. Ocurren effectos diferenciales al aumentar la altura y se observan diferencias en respuesta entre varios grupos poblacionales a alturas similares.

Palabras claves: Adaptación fisiológica; Altura; Hipoxia; Oximetria de pulso; Hipertensión pulmonar; Mal de montaña subagudo infantil; Perú; Tibet; China.

RÉSUMÉ: Transition cardiorespiratoire à grande altitude : le nouveau-né et le nourrisson.

Objectif : Des altérations de la saturation artérielle d'oxygène (S_aO_2) liées au niveau d'altitude se produisent aussi bien chez le nouveau-né que chez le nourrisson.

Plan : L'évidence obtenue de l'investigation originale et de la littérature publiée permet la comparaison entre plusieurs groupes humains habitant des régions de grande altitude en Amérique du Nord, Amérique du Sud et Asie.

Matériel et méthodes : Des données d'oxymétrie de pouls, de pléthysmographie respiratoire, d'échocardiographie, de cathétérisation cardiaque ainsi que l'examen histologique de nouveau-nés et de nourrissons illustrent les altérations de la fonction, les exemples d'adaptation réussie et les exemples de morbidité et de mortalité liées à la naissance ou à la résidence en altitude.

Résultats : Bien que la S_aO_2 diminue quand augmente l'altitude, cet effet n'est pas lié linéairement à l'altitude ou à la pression barométrique. La S_aO_2 varie très nettement en fonction de l'état comportemental. Contrairement aux modèles observés au niveau de la mer, chez certaines populations vivant à grande altitude la S_aO_2 diminue au bout d'une semaine de vie et la prévalence et la durée de la respiration périodique du nourrisson sont en augmentation. La respiration périodique se produit le plus souvent pendant le sommeil actif et paisible et elle est associée à un *pattern* cyclique de saturation d'oxygène. A très grande altitude la pression de l'artère pulmonaire diminue lentement après la naissance. A une altitude modérée, les pressions de l'artère pulmonaire peuvent se normaliser mais le lit vasculaire pulmonaire reste susceptible au développement d'une hypertension pulmonaire symptomatique pendant une période prolongée de transition. Le syndrome du mal des montagnes subaigu infantile et la persistance de courts-circuits de droite à gauche par l'orifice ovale et le conduit artériel reflètent une pression de l'artère pulmonaire et une résistance vasculaire élevées pendant l'enfance.

Conclusion : Chez l'enfant nouveau-né de grande altitude, la transition des *patterns* foetaux aux *patterns* matures de la fonction respiratoire se fait plus lentement. Des effets différentiels se produisent quand l'altitude augmente et l'on observe des différences dans les réponses de plusieurs groupes de population vivant à des altitudes similaires.

Mots-clés : Adaptation physiologique, Altitude, Hypoxie, Oxymétrie de pouls, Hypertension pulmonaire, Mal des montagnes subaigu infantile, Pérou, Tibet, Chine.

SUMMARY:

Objective: Altitude-related alterations in arterial oxygen saturation (S_aO_2), ventilation, and the pulmonary circulation occur during cardiopulmonary transition in the neonate and infant at high altitude. Design: Evidence gathered from original research and the published literature allows comparison of various population groups resident at high altitude in North and South America and Asia.

Material and Methods: Data from pulse oximetry, respiratory plethysmography, echocardiography, cardiac catheterization and histologic examination of neonates and infants illustrate alterations in function, instances of successful adaptation, and examples of morbidity and mortality related to birth or residence at high altitude.

Results: S_aO_2 falls with increasing altitude; however, this effect is not linearly related to altitude or barometric pressure. S_aO_2 varies markedly with behavioral state. In contrast to patterns observed at sea level, S_aO_2 decreases after 1 week of life in certain populations at high altitude. Periodic breathing in infancy increases in prevalence and duration at high altitude as compared to sea level. Periodic breathing occurs most commonly in active and quiet sleep and is associated with a cyclic pattern of oxygen saturation. Pulmonary artery pressure falls slowly after birth at extreme high altitude. At moderate high altitude, pulmonary artery pressures may normalize, but the pulmonary vascular bed remains susceptible to development of symptomatic pulmonary hypertension during a prolonged transition period. The syndrome of subacute infantile mountain sickness and persistence of right-to-left shunts at the foramen ovale and ductus arteriosus reflect elevated pulmonary artery pressure and pulmonary vascular resistance in infancy.

Conclusion: The newborn infant at high altitude experiences a slower transition from fetal to mature patterns of cardiopulmonary function. Differential effects occur with increasing altitude and differences in response are observed among various population groups at similar altitudes.

Key Words: Adaptation, Physiologic; Altitude, Hypoxia, Pulse oximetry, Pulmonary hypertension, Subacute infantile mountain sickness, Peru, Tibet, China

Background

Altitude-related alterations in arterial oxygen saturation (S_aO_2), ventilation, and the pulmonary circulation occur during the cardiopulmonary transition after birth and during infancy at high altitude. A comparative approach using data from various population groups resident at high altitude in North America, South America, and Asia illustrates the effect of increasing altitude and the differences observed among certain population groups at similar altitudes. Among these examples exist instances of successful adaptation, altitude-related alterations in function, and increased morbidity and mortality due to birth or residence at high altitude.

A vignette from the history of Spanish settlement of the Andes in in the mid- 16th century points out the hazards of birth at high altitude. Antonio de la Calancha wrote that in the first years of the settlement of Potosi, a silver-mining community at an altitude of 4000 m, no Spanish infants born there survived the neonatal period (1). Women of European descent chose to descend to nearby valleys, where their infants remained during infancy. Only after more than half a century is there record of a Spanish infant surviving after delivery in Potosi.

Arterial Oxygen Saturation

Oxygenation is the critical function assumed by the lungs at the moment of birth. The transition from fluid-filled to air-filled lungs not only effects oxygen transfer across the alveolar-capillary membrane, but the physical inflation of the lungs and the increased alveolar oxygen serve to dilate the pulmonary vascular bed and facilitate increased pulmonary blood flow, the other key component in raising the P_aO_2 after birth. At sea level, S_aO_2 rises from 47 to 61% immediately after birth to more than 80% by 7 minutes of life (2). In the first week, S_aO_2 remains in the low-to-mid 90% range and reaches adult levels of 94 to 98% by the end of the first month of life (3).

At 1610 m in Denver, Colorado, mean S_aO_2 during infancy ranged from 92 to 94% (4). After the first month of life, S_aO_2 varied with infant activity; S_aO_2 was higher while awake and feeding as compared to active and quiet sleep (Figure 1). At 2800 m in Summit County, Colorado the mean S_aO_2 for healthy awake infants was 92% (5).

At 3100 m in Leadville, Colorado, mean S_aO_2 ranged from 81 to 91% (Figure 1) in the first 4 months of infancy (6). Arterial oxygen saturations were initially at the upper limits of this range; S_aO_2 fell by one week after birth to a mean of 81% in quiet sleep, at the lower end of the range. Infant activity had notable effect on saturations at and after one week of life, when values were higher during wakefulness than during sleep. S_aO_2 during feeding was intermediate between saturations while awake and asleep. Values in quiet sleep rose to 86% by 2 and 4 months; the highest saturations were achieved in the awake state at 4 months. Although all infants in the study cohort remained healthy, the drop in SaO2 at one week of age coincided with the reported onset of symptoms in other babies who developed clinical signs of hypoxemia (e.g. cyanosis, irritability, poor feeding, failure to thrive).

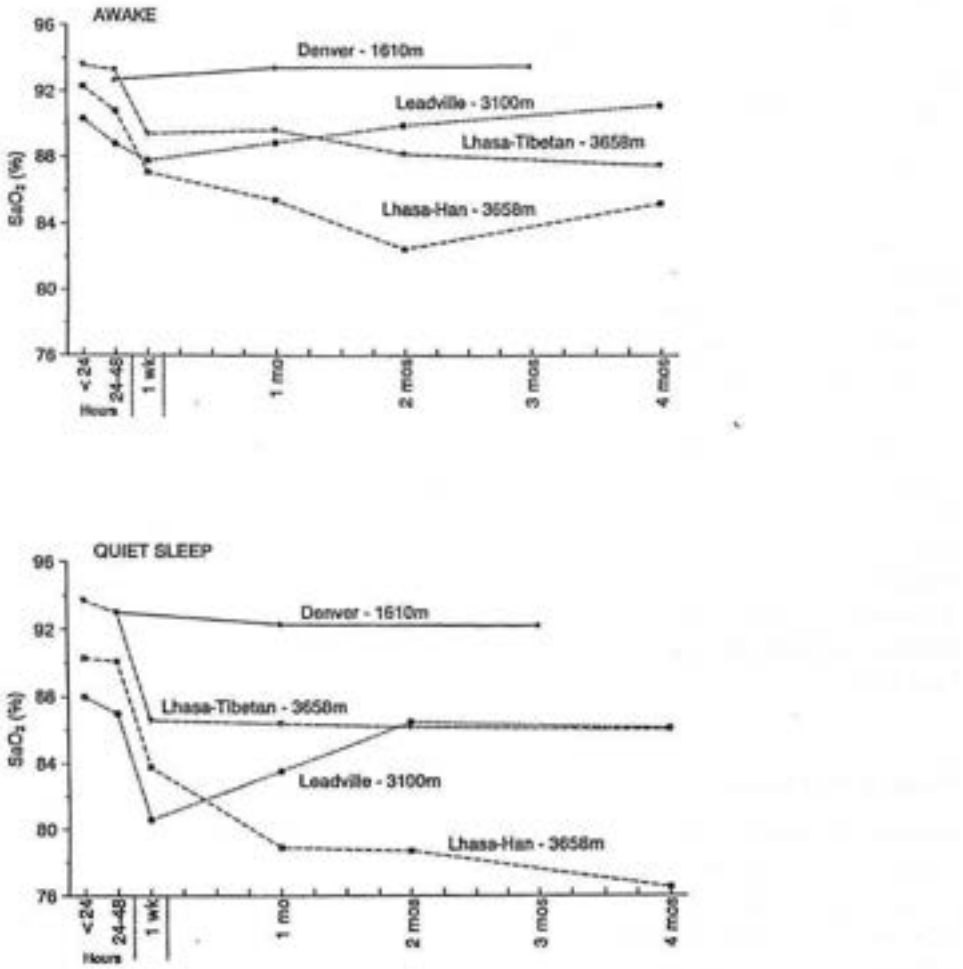

Figure 1. Arterial oxygen saturation in awake (upper panel) and quietly sleeping (lower panel) infants decreased with increasing altitude. At altitudes above 3000 m, S_aO_2 fell by one week of life from initial values at the upper end of the observed ranges. In Tibetans at 3658 m and residents of Leadville, CO at 3100 m, arterial oxygen saturations then stabilized through 4 months of age. Han infants in Lhasa, Tibet at 3658 m showed a progressive decline in saturations, most marked in quiet sleep, through 4 months.

At 3658 m in Lhasa, Tibet mean S_aO_2 for Tibetan infants ranged between 86 and 94% (7). The highest S_aO_2 occurred in the first two days after birth while awake. A fall in saturations occurred by one week of life. Thereafter, saturations stabilized in the Tibetans, with S_aO_2 only slightly greater while awake than in active or quiet sleep. Han infants born in Lhasa to mothers of low-altitude ancestry showed lower S_aO_2 than the Tibetans from birth through 4 months of age (Figure 1). Again, the highest saturations occurred in the first two days after birth (91 to 92%); a fall in S_aO_2 occurred by one week to 87%. However, mean S_aO_2 declined progressively in the Han to 76% by 4 months, while the Tibetans' values remained stable during this period.

At an altitude of 3750 m in La Oroya, Peru the mean S_aO_2 value was 88% in native Quechua infants from 2- to 5-months old (8). At extreme high altitude in Morococha, Peru (4540 m), directly measured arterial saturations ranged from 57% to 75% in newborns from 1/2 hour to 72 hours of age (9). Arterial saturations remined in the range of 74 to 80% throughout infancy (10).

The decrement in S_aO_2 observed with increasing altitude is not simply linear. This is due, at least in part, to varying degrees of hyperventilation, resulting in smaller drops in alveolar oxygen. Hyperventilation also increases pH and thereby shifts the oxyhemoglobin dissociation curve to the left, at least temporarily; metabolic compensation for respiratory alkalosis and intermittent

hypoventilation may decrease pH, resulting in a lower saturation for a given P_aO_2 (11). The prominent differences in SaO2 between sleep and wakefulness and the fall in S_aO_2 at one week likely relate to respiratory pattern. However, the dramatic differences between saturations in the Tibetan and Han infants cannot be explained on the basis of respiratory rate and pattern alone. Tibetans are unique in their length of settlement at high altitude, the lack of admixture with other populations, and the absence of migratory patterns to low-altitude regions. While the native populations in the Andes are analogous to the Tibetans with respect to long ancestry at high altitude, there has been much greater opportunity for admixture with lowland populations and migration to low altitude. North Americans and the Han are both newcomers to high altitude in the genetic sense. The Tibetan-Han differences which exist at the same altitude illustrate the probable role of genetic adaptation to high altitude.

Respiratory Patterns

Periodic breathing is a recognized feature of infancy at sea level, where it occurs in up to 78% of full-term neonates in the first two weeks of life (12). Periodicity in the respiratory pattern declines with postnatal age, from one month through 5-6 months (13).

Early studies in Denver (14) suggested that periodic breathing occurred more frequently and for a greater duration at 1610 m than previously reported from sea level. At 3100 m (Leadville, CO) the reported incidence of periodic breathing was 100% in neonates (15). The pattern of periodicity exhibited was a cycle of 4 to 6 breaths over 6-7 seconds with a subsequent pause of 6-7 seconds.

Periodic breathing likely represents an exaggeration of normal oscillations in respiratory frequency and tidal volume; as such it may be a normal phase in the development of respiratory control (16, 17). Peripheral chemoreceptor reflexes and the interaction of central with peripheral chemoreceptors may be important in the genesis of periodic breathing in newborns (13). The functional inactivity ofthe carotid chemoreceptor in the first 48 hours of life may account for the virtual absence of periodic breathing during this period (18, 19, 20).

Pulmonary Circulation

In normal postnatal transition, the fall in pulmonary vascular resistance and pulmonary artery pressure (P_{pa}) is the cornerstone to achieving higher P_aO_2 and effecting functional, then anatomic closure of the fetal atrial and ductal shunts. At sea level, pulmonary artery pressure falls to adult levels within the first 3 days of life, with the steepest decline in the first 24 hours (21).

At 3100 m in Leadville, CO the the ratio of right ventricular pressure to left ventricular pressure (RVP/LVP) fell within the normal to moderately elevated range during the first week of life. All 2 and 4 -month infants had values in the normal range, using the echocardiographic technique of LVSCI (left ventricular systolic circular index) (6). However, all infants born in Leadville routinely received supplemental oxygen at delivery and during postnatal transition. From the era before routine oxygen supplementation, 5 infants and 6 older children from Leadville were reported with a syndrome of pulmonary hypertension. Cardiac catheterizaton in three infants confirmed the clinical diagnosis. One infant who died exhibited medial hypertrophy and intimal thickening of pulmonary arterioles and disruption of the internal elastic lamina on autopsy (22).

In Lhasa at 3658 m, fifteen infants and children aged 3 to 16 months at death defined the syndrome of subacute infantile mountain sickness, characterized by pulmonary hypertension and right heart failure (23). All children were Han with the exception of one Tibetan boy; 13 of 15 were born at low altitude and brought to Lhasa an average of two months prior to onset of illness. Clinical signs which characterized the syndrome included: dyspnea, cough, cyanosis, sleeplessness and irritability, facial edema, hepatomegaly, and oliguria. Histology was characterized by medial hypertrophy of small pulmonary arteries, muscularization of pulmonary arterioles, and severe right ventricular hypertrophy and dilation.

The most striking data in support of a prolonged postnatal fall in pulmonary artery pressure come from extreme high altitude. Newborns at 4540 m in Peru had nearsystemic pulmonary artery pressures for several days following birth (9). Administration of 100% oxygen to 3 infants at 72 hours age resulted in normalization of P_{pa} values to levels near those of infants at sea level.

Other evidence supporting delayed fall in P_{pa} at

extreme high altitude comes from cardiac catheterization studies and pulmonary histology. Right heart catheterization of children under 5 years living at 4330 m and 4540 m in Peru confirmed instances of elevated PPA and increased pulmonary vascular resistance (10). In healthy children who died of non-pulmonary causes, there was evidence of slow regression of pulmonary arteriolar muscularization and thickening of the muscular layer of small pulmonary arteries. Delay in regression of the fetal pulmonary vascular pattern was so pronounced in some cases that a fully adult pattern was never achieved (24).

Delayed functional closure of fetal shunts gives further clinical evidence of the prolonged decrease in pulmonary vascular resistance and pulmonary artery pressure. The incidence of patent ductus arteriosus in Peruvian children at 4330 m (Cerro de Pasco) was 0.74%, in contrast to 0.05% at sea level (25). In Qinghai Province, PRC, the prevalence of atrial septal defect and patient ductus arteriosus increased from zero at sea level to > 5% at 4500 m (26). Hypoxemia is presurned to be the stimulus contributing to the increased prevalence of patent ductus arteriosus at high altitude (25-27).

CONCLUSION

The newborn at high altitude experiences a slower transition from fetal to mature patterns of cardiopulmonary function. Arterial oxygen saturation remains lower than corresponding adult values through early infancy. S_aO_2 declines in the first week and remains lower in sleep than the awake state. Extreme high altitude results in persistently low saturations, and certain populations show an exaggerated and prolonged fall in S_aO_2 associated with clinical signs of hypoxemia or right heart failure in infancy. Periodic breathing patterns are common to all neonates at high altitude. Periodic breathing in sleep is associated with cyclic changes in S_aO_2. Pulmonary artery pressure falls very slowly with persistence of fetal pulmonary vascular patterns and a higher prevalence of persistent right-to-left shunts. Symptomatic pulmonary hypertension may develop in susceptible infants at high altitude.

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WOMEN, ENDOCRINE AND REPRODUCTIVE PHYSIOLOGY

EFECTO DE LA MENOPAUSIA EN LA RELACIÓN ENTRE LAS PRESIONES ALVEOLARES DE O_2 Y CO_2 Y EL MAL DE MONTAÑA CRÓNICO.

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RESUMEN: Este estudio se llevó a cabo con el fin de determinar el rol que tiene la llegada de la menopausia en la relación entre las presiones alveolares de O_2 Y CO_2 y el Mal de Montaña Crónico (MMC) o Enfermedad de Monge. El estudio se realizó en una muestra poblacional femenina residente de Cerro de Pasco (4,300 m) entre los 26-62 años de edad (n=41). Se midieron, entre otras, las variables fisiológicas involucradas en la secuencia fisiopatológica del MMC: presiones tidales de O_2 Y CO_2 ($P0_2T$ y $PC0_2T$, torr), saturación de oxígeno (SaO_2,%), y concentración de hemoglobina (Hb, g/dl). Los resultados mostraron que, en las mujeres de altura después de la menopausia, se observa una disminución de la $P0_2T$ (48.4±3.3 vs 52.7±3.1 torr; p<O.OOOl) y de la SaO_2 (81.9±4.12 vs 84.7±3.14%; p<O.OOl), así como un aumento de la PC02T (34.5+2.2 vs 30.4±3.3 torr; p<O.OOOl) y de la Hb (18.6±1.7 vs 15.9±2.3g/dl; p<O.OOl). En las poblaciones masculinas de altura se observa un aumento de la prevalencia de eritrocitosis excesiva en forma continua con la edad, en el caso de las mujeres de altura, postulamos, en base a nuestros resultados, que un factor contribuyente adicional para la aparición abrupta (y no continua) del MMC sería la disminución de la ventilación, como lo indica el aumento de la $PC0_2T$. Esta, al disminuir, traería como consecuencia hipoxemia y eritrocitosis excesiva, el signo preponderante del MMC.

Palabras claves: Menopausia, Mal de montaña crónico, Presión tidal de O_2, Presión tidal de CO_2, Policitemia, Saturación de oxígeno.

RÉSUMÉ: Effet de la ménopause sur la relation entre les pressions alvéolaires de 0_2 et CO_2 et le Mal des Montagnes Chronique.

Le but de cette étude était de déterminer le rôle de la survenue de la ménopause dans la relation entre les pressions alvéolaires de O_2 et CO_2 et le Mal des Montagnes Chronique (MMC) ou Maladie de Monge. L'étude a été réalisée à partir d'un échantillon de population féminine résidant à Cerro de Pasco (4 300 m d'altitude), dont l'âge variait entre 26 et 62 ans (n=41). Ont été mesurées, entre autres, les variables physiologiques présentes dans la séquence physiopathologique du MMC : pressions courantes de O_2 et CO_2 (PO_2T y PCO_2T, torr), saturation en oxygène (SaO_2, %) et concentration d'hémoglobine (Hb, g/dl). Les résultats ont montré que chez les femmes ménopausées vivant en altitude il y a une diminution de la PO_2T (48.4±3.3 vs 52.7±3.1 torr; p<0.0001) et de la SaO_2 (81.9±4.12 vs 84.7±3.14 %); p<0.001), ainsi qu'une augmentation de la PCO_2T (34.5±2.2 vs 30.4±3.3 torr; p<0.0001) et de la Hb (18.6±1.7 vs 15.9±2.3g/dl; p<0.001).

Parmi les populations masculines d'altitude on observe une augmentation de la prévalence d'une polyglobulie excessive et permanente avec l'âge. Dans le cas des femmes vivant en altitude, nous formulons l'hypothèse, sur la base de nos résultats, qu'un facteur supplémentaire contribuant à l'apparition brusque (et non continue) du MMC serait la diminution de la ventilation respiratoire, comme l'indique l'augmentation de la PCO_2T. Celle-ci, en diminuant, aurait pour conséquence l'apparition d'une hypoxémie et d'une polyglobulie excessive, signe prédominant du MMC.

Mots clés : Ménopause, Mal des montagnes chronique, Pression courante de O_2, Pression courante de CO_2, Polyglobulie, Saturation en oxygène.

SUMMARY The objective of this study was to investigate the role of menopause in the relation among the end tidal O_2 Y CO_2 pressures and chronic mountain sickness (CMS) in a female high altitude population. The females studied were 26-62 years age (n=41) and have permanent residence in Cerro de Pasco (Pasco, Perú; 4,300 m.). The sample was divided in post-menopausal (n=21) and pre-menopausal groups (n=20) for comparison. End tidal pressures (PO_2T y PCO_2T, Torr), blood oxygen saturation (SaO_2) and hemoglobine concentration (Hb, g/dl) were measured, among others, as the main variables involved in the physiopathological sequence of CMS. Postmenopausal women had lower PO_2T (48.4±3.3 vs 52.7±3.1 torr; p<0.0001) and SaO2 values (81.9±4.12 vs 84.7±3.14%; p<0.001), and higher

PCO_2T (34.5±2.2 vs 30.4±3.3 torr; p<0.0001) and Hb values (18.6±1.7 vs 15.9±2.3g/dl; p<0.001). than premenopausal women.

Men's hemoblobin levels, at high altitude, rise continuously with age. In contrast, in high altitude women, Hb rises only after the cessation of menstruation. In the case of men, the suggestion has been made that the increase in Hb is the result of the simultaneous drop of ventilatory and/or pulmonary function as a function of age. In the case of women, one can postulate that an additional contributing factor for the higher Hb values, is the greater hypoventilation (higher PCO_2T values) which appears with menopause.

Key words: Menopause, Chronic mountain sickness, end tidal O_2 pressure, End tidal CO_2 pressure, Polycythemia, Oygen saturation.

INTRODUCCION

En 1928, Monge Medrano describió con el nombre de Enfermedad de los Andes a la pérdida de aclimatación a la altura. Esta puede ocurrir por la incapacidad de algunos individuos de aclimatarse en forma integral a la residencia en alturas considerables. Esta enfermedad, llamada ahora enfermedad de Monge o mal de montaña crónico (MMC), se caracteriza principalmente por sus síntomas neuropsíquicos, como cefaleas, mareos, somnolencia, insomnio, fatiga, dificultades del movimiento, falta de concentración mental y alteraciones de la memoria. Una elevada cifra de glóbulos rojos, de hemoglobina y de hematocrito acompañan al cuadro clínico. Estas cifras se encuentran por encima de aquellas esperadas para la altura de residencia (eritrocitosis excesiva - EE).

Nuestros estudios muestran que la prevalencia de síntomas asociados al MMC aumenta con la edad y que la EE en hombres cercanos a la quinta década sobrepasa el 20% (Arregui et al., 1990). Asímismo, la ventilación, la capacidad vital, la saturación de oxígeno y el flujo espiratorio máximo decaen con la edad (Sime et al., 1975; Monge C. et al., 1992; León-Velarde et al., 1993; León-Velarde y Arregui, 1994), generando la secuencia de eventos: disminución de la función ventilatoria, caída de la capacidad vital, disminución de la saturación de oxígeno, hipoxia arterial, EE.

Los problemas de salud de los hombres nativos y residentes de las punas, como el MMC o enfermedad de Monge, han sido obieto de numerosos estudios nacionales e internacionales (Monge M. et al., 1928; Hurtado et al., 1942; 1955; 1956; Monge M. y Monge C. 1966; Ergueta et al., 1971; Heath y Williams, 1981; Wu et al., 1987; Winslow y Monge C. 1987; Pei et al., 1989; Arregui et al., 1991; Vargas y Villena, 1994), sin embargo, la fisiopatología de la mujer andina ha sido menos estudiada que la del hombre, y son aún menos conocidas las características y/o frecuencia del MMC en esta población. Este hecho se ha debido en parte a la consideración que las mujeres estarían protegidas de la enfermedad, i.e. de la EE, por la menstruación, hecho fisiológico que mantendría normales los volúmenes sanguíneos, aún en la altura. Las mujeres de la tercera edad (postmenopausia), no sólo no estarían protegidas por la pérdida del exceso de sangre, sino que además, su lecho vascular no se encontraría preparado para un mayor volumen de sangre, desencadenándose la sintomatología propia del MMC con mayor intensidad.

Recientemente, León-Velarde et al., (en prensa) han encontrado, en base a un estudio de tipo epidemiológico, que las mujeres de altura después de la menopausia tienen mayores hematocritos y signos y síntomas asociados al MMC y menores flujos espiratorios máximos y saturaciones sanguíneas de oxígeno. También mostraron valores de hematocritos más altos para un mismo valor de saturación de oxígeno. Así mismo, estos autores determinaron que el valor de eritrocitosis considerada como excesiva para mujeres de Cerro de Pasco (4,300 m) es de 56% y que, basados en esta cifra, la prevalencia de mujeres en riesgo de mal de montaña sería de 8.8%. La menosausia aparece más tempranamente en la altura que a nivel del mar (Zhang et al., 1991; Gonzales, 1993; Villena et al., 1993), lo que conllevaria a la aparición del MNC en mujeres a una edad anterior a la que corresponderia como tercera edad para nivel del mar. Si la etapa premenopáusica protege a las mujeres andinas de la eritrocitosis patológica de la altura, y con ello de adquirir el MMC, aquellas, al llegar a la menopausia, tendrian una mayor predisposición para desarrollar la enfermedad. Esta predisposición se deberia a una disminución abrupta de la ventilación, la que se veria reflejada por el aumento de la presión tidal de anhidrido carbónico (PCO_2T, torr) Con respecto a mujeres en etapa pre-menopáusica.

El objetivo de este estudio fue determinar si Con la llegada de la menopausia, disminuye la ventilación en las mujeres de altura constituyendo un factor de riesgo para la aparición del mal de montaña crónico.

MATERIAL Y METODOS

La población blanco del estudio estuvo constituida por una muestra de mujeres residentes por más de 10 años en la ciudad andina de Cerro de Pasco (4,300 m). La muestra estuvo conformada por dos grupos: 1. **Pre-menopausia**: mujeres que no habian llegado a la menopausia, i.e. que se encontraban menstruando de manera regular (n=l9). 2. **Post-menopausia**: mujeres que habian dejado de menstruar por lo menos Un año antes del comienzo del estudio (n=l9).

Se midió la hemoglobina (Hb, g/dl) en sangre venosa mediante espectrofotometría por medio del Hemocue. Las presiones tidales de O_2 Y CO_2 (PO_2T y PCO_2T, torr) se determinaron utilizando el Normocap directamente del aire espirado. La medida de saturación arterial (SaO_2, %) se realizó por triplicado mediante un oxímetro de pulso Nellcor en posición sentada, tuviendo cuidado en que las manos se encontraran a una temperatura constante. Esto se logró solicitando a la voluntaria que sumergiera la mano izquierda en un baño temperado durante un minuto. También se evaluaron el pulso (FP, pulsaciones/minuto) y las presiones arteriales sistólica (PAS) y diastólica (PAD).

La estimación de estos parámetros permitió discernir en qué medida el aumento de la PCO_2T aumenta la Hb, trayendo como consecuencia la EE, y por ende la aparición del mal de montaña crónico

Análisis de datos.

El procesamiento de la información cuantitativa se realizó con el programa SPSS-PC+. En los casos de una variable discreta (menopausia) y varias continuas (PO_2T y PCO_2T, SaO_2 y Hb), se utilizó el ANOVA de una vía, y en el caso de una variable discreta y dos contínuas el ANCOVA. Las variables presentaron varianzas homogéneas con 95% de confiabilidad. Los resultados fueron considerados como significativos para una $p<0.05$.

RESULTADOS.

La tabla 1 muestra las diferencias en edad, presiones arteriales sistólicas, diastólicas y frecuencia de pulso entre las mujeres pre-menopáusicas y post-menopáusicas. Puede verse una diferencia en las PAS ($p<0.03$) y en las PAD ($p<0.05$), siendo ambas mayores para las mujeres post-menopáusicas. Esto probablemente se explique por las diferencias de edad ($p<O . 0001$) .

Tabla 1: Edad (años), peso (kg), presión arterial sistólica (PAS, mm Hg) y diastólica (PAD, mm Hg) y frecuencia de pulso (FP, pulsaciones/min) en mujeres pre-menopáusicas y post-menopáusicas.

N	EDAD	PESO	PS	PAD	FP
Pre-menopáusicas					
X	34	55.6	92.6	53.2	73
DS	6.96	8.78	13.31	10.29	7.72
Post-menopáusicas					
X	53	57.5	103.7	59.5	77.4
DS	4.36	8.25	17.54	8.87	10.70
p	< 0.0001	N.S.	< 0.03	< 0.05	N.S.

En la tabla 2 se muestra las diferencias en PO_2T y PCO_2T, SaO_2 y Hb entre los dos grupos de mujeres. Es aquí donde se observa como en las mujeres post-menopáusicas se cumple la secuencia fisiopatológica que lleva al MMC. En las mujeres post-menopáusicas se observa una menor PO_2T ($p<0.05$); una mayor PCO_2T ($p<0.0006$), indicación de hipoventilación y

una menor SaO_2 ($p<0.05$), indicación de hipoxemia. Esta llevaría a la mujer menopáusica a una elevación de la concentración de Hb ($p<0.005$), principal signo diagnóstico del MMC. Para eliminar el efecto de la edad, esta se especificó como covariable en el análisis de covarianza, las relaciones arriba mencionadas siguieron guardando su significancia. Cuando se realizó el análisis de regresión con la PCO_2T como variable independiente se encontró una p significativa en función de la Hb ($p<0.0006$).

Tabla 2. Presión tidal de O_2 (PO_2T, torr) y de CO_2 (PCO_2T, torr), saturación de oxígeno (SaO_2, %) y concentración de hemoglobina (Hb, g/dl) en mujeres pre-menopáusicas y post-menopáusicas.

	PO_2T	PCO_2T	SaO_2	Hb
Pre-menopáusicas				
	52.7	30.4	87.4	15.9
	3.07	3.26	2.32	2.31
Post-menopáusicas				
	48.3	34.5	82.3	18.6
	3.34	2.23	3.19	1.70
p	< 0.05	< 0.0006.	< 0.01	< 0.005.

La figura 1 muestra como la SaO_2 se modifica en función de la ventilación. Los valores más altos PCO_2T corresponden a una mayor hipoxemia y estos se presentan en el grupo postmenopausia. Esta hipoventilación generaría el aumento de Hb que se observa en la Tabla 2.

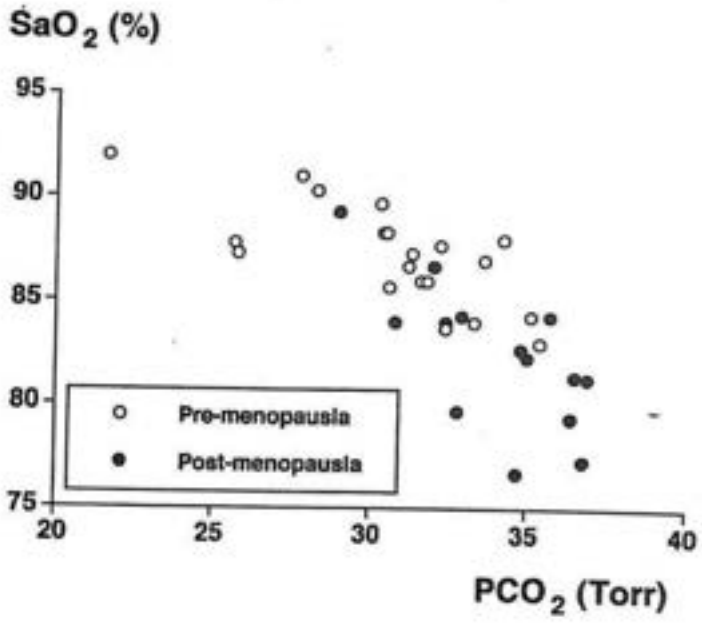

Figura 1. Relación entre la saturación arterial de oxigeno y la ventilación en mujeres premenopáusicas

DISCUSION

Los resultados de esta investigación demuestran que los indicadores fisiopatológicos que llevan al MMC están significativamente más presentes en las mujeres postmenopáusicas. La asociación entre la disminución de la ventilación (aumento de la $PC0_2T$), aumento de la hipoxemia (baja $Sa0_2$), con el consecuente aumento de la Hb, sustentan la hipótesis que la menopausia sería un factor contribuyente a la aparición del MMC en mujeres residentes de la altura.

Cabe resaltar, que a diferencia de las poblaciones masculinas de altura en las que se observa un aumento de la prevalencia de EE en forma contínua con la edad (Whittembury y Monge, 1972; Monge C. et al., 1989; León-Velarde et al., 1993), en el caso de las mujeres de altura, un factor contribuyente adicional para la aparición abrupta (y no contínua) del MMC con la edad sería la disminución de las hormonas femeninas en la etapa post-menopausia. La progesterona aumenta la ventilación (Regensteiner et al., 1989; Tatsumi et al., 1995), tanto durante el embarazo como en la fase luteal del ciclo menstrual (Goodland et al., 1954; Takano et al., 1981). En ratas sometidas a hipoxia crónica, se ha descrito adicionalmente, que la ausencia de hormonas sexuales femeninas aumentan las respuestas eritrémicas y cardiopulmonares propias del MMC (0u et al., 1994). Por otro lado, se ha demostrado que los estrógenos reducen la producción de eritropoyetina (Mirand and Gordon, 1966). En la etapa de la menopausia, en la altura, la disminución de estas hormonas disminuiría la ventilación y aumentaría la producción de eritropoyetina, esto incrementaría la hipoxemia de altura y la Hb, conllevando a la aparición de la sintomatología propia del MMC en las mujeres de altura.

Santolaya et al. (1982), han estudiado un grupo de 162 mujeres que viven a 2,800 m., y han encontrado que también la presión arterial de CO_2 aumenta muy por encima de los valores para hombres a partir de la década de los 40; no obstante, estos autores no relaciona este hallazgo con la menopausia. Moreno-Black et al. (1984) no encuentran un aumento de la Hb en mujeres estudiadas a 3,700 m, pero debe aclararse que sólo estudiaron mujeres hasta los 45 años de edad. Sin embargo, Ruiz (1973), sí encuentra un aumento del hematocrito en mujeres de más de 45 años que viven a 4,100 y 4,260 m. Este autor no incluye a la menopausia en su análisis, no obstante sus gráficos muestran claramente el incremento de las pendientes de aumento del hematocrito a partir de la edad de la menopausia para esa altura.

Se ha sugerido que la menstruación podría ser considerada como una auto-sangría que protegería a la mujer en la altura del MMC. También se ha mencionado que la menstruación produciría deficiencia de hierro causando una ligera anemia. Hannon et al. (1969) han mostrado que las mujeres tienen una menor tasa de incremento del hematocrito que los hombres cuando su dieta no es suplementada con fierro. Desafortunadamente, en la literatura relacionada a hipoxia o altura, no se encuentran datos suficientes ni para apoyar estas hipótesis, ni para refutarlas.

El hecho que en la altura se preserven las diferencias en las concentraciones hematológicas entre hombres y mujeres que se encuentran a nivel del mar (Berendsohn and Muro, 1957) ha apoyado la idea que los bajos valores de Hb y hematocrito encontrados en las mujeres de altura las protegeria contra el desarrollo del MMC. En este estudio demostramos que éstas, no sólo presentarían MMC, sino que además podrían desarrollarlo a un menor nivel de Hb y hematocrito, y a una edad más temprana. Este hallazgo, no ha sido señalado antes en la literatura nacional o internacional; creemos que es una información que es necesario difundir dada su importancia en las decisiones médicas relacionadas a la menopausia en la altura.

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EFFECT OF MENSTRUAL CYCLE ON INCIDENCE OF ACUTE MOUNTAIN SICKNESS IN WOMEN: PRELIMINARY RESULTS FOR TWO STUDIES

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RESUMEN: Efecto del Ciclo Menstrual en la Incidencia de Mal de Montaña Agudo en Mujeres: Resultados Preliminares de Dos Estudios

La mayor parte de estudios de mal de montaña en mujeres premenopáusicas que viajan a ambientes de altura no han tenido en cuenta la fase del ciclo menstrual en la que se encontraban las mujeres. Consecuentemente existe poca información acerca de los posibles efectos que tienen las fluctuaciones cíclicas que ocurren en las hormonas esteroideas en la ocurrencia de mal de montaña agudo (AMS). Nosotros hemos determinado la incidencia de AMS en 12 mujeres voluntarias durante una exposición de 36 h a 446 torr (aproximadamente 4300 m) en una cámara hipobárica durante la fase folicular temprana (EF) y la fase luteal (L) de su ciclo menstruall a través de un diseño de estudio transversal. Adicionalmente, hemos determinado la incidencia de AMS en otras 16 mujeres voluntarias durante una residencia de 12 d a 4300 m en la cima del Pikes Peak, Colorado, en la fase EF (8 sujetos) o en la fase L (8 sujetos), usando un diseño de estudio comparativo. Los resultados preliminares de estos estudios sugieren que la incidencia de AMS no difiere mucho entre las fases del ciclo menstrual.

Palabras claves: Mal de montaña agudo, Mujeres, Fases del ciclo menstrual

RÉSUMÉ: Effet du cycle menstruel sur l'apparition du mal aigu des montagnes chez les femmes : résultats préliminaires de deux études.

La plupart des études sur le mal des montagnes chez les femmes pré-ménopausiques se rendant dans des régions de haute montagne n'ont pas pris en compte la phase du cycle menstruel dans lequel elles se trouvaient. Il existe donc peu d'informations relatives aux effets possibles des fluctuations cycliques des hormones stéroïdes sur l'apparition du mal des montagnes aigu (AMS). Nous avons déterminé l'incidence de l'AMS chez 12 femmes volontaires soumises à une exposition de 36 heures à 446 torr (environ 4 300 m) dans une chambre hypobare, pendant la phase folliculaire précoce (EF) et la phase lutéale (L) de leurs cycles menstruels, en utilisant un plan d'étude transversale. Nous avons en outre déterminé l'incidence de l'AMS chez 16 autres volontaires au cours d'un séjour de 12 jours à 4 300 m, au sommet du Pikes Peak, Colorado, pendant la phase EF (8 sujets) ou la phase L (8 sujets), en utilisant un plan d'étude comparative. Les résultats préliminaires suggèrent que l'incidence de l'AMS ne diffère guère, quelle que soit la phase du cycle menstruel.

Mots-clés : Mal des montagnes aigus, Femmes, Phases du cycle menstruel.

SUMMARY: Most studies of altitude illness in premenopausal women traveling to high mountain environments have not controlled for phase of the menstrual cycle. Consequently, little information exists about the possible effects of cyclic fluctuations in ovarian steroid hormones on the occurrence of acute mountain sickness (AMS). We determined the incidence of AMS in 12 women volunteers during a 36 h exposure to 446 torr (~4300 m.) in a hypobaric chamber during the early follicular (EF) and luteal (L) phases of their menstrual cycle using a cross-over study design. Additionally, we determined the incidence of AMS in 16 additional women volunteers during a 12 d residence at 4300 m on the summit of Pikes Peak, Colorado in EF (8 individuals) or L (8 individuals) using a group comparison study design. Preliminary results of these studies suggest that the incidence of AMS in women does not differ greatly between menstrual cycle phases.

Key words: Acute mountain sickness, Women, Menstrual-cycle phase

Acute mountain sickness (AMS) is a syndrome which occurs in unacclimatized individuals from low altitude who travel to altitudes above approximately 2438 m and remain there for more than a few hours. The most common symptoms of AMS include headache, nausea, vomiting and lassitude. Altitude-associated sleep disturbances are often also considered to be a manifestation of AMS (1), although disrupted sleep can occur in well acclimatized individuals who lack other symptoms. The fundamental cause of AMS is prolonged hypobaric hypoxia. The pathophysiology is thought to involve hypoxia-induced subclinical cerebral edema that resolves with altitude acclimatization (1). Consequently, alterations in respiratory and fluid/volume responses to hypoxia could affect the occurrence of AMS.

The menstrual cycle of women during their reproductive years is characterized by regular fluctuations of ovarian steroid hormones (estrogens and progesterone) controlled by the hypothalamic-pituitary-ovarian axis. These fluctuations and their physiologic effects function to assure recurrent

physiologic opportunities for reproduction. Ovarian steroid hormones have discernable and well documented effects on respiratory function and body fluid/volume relationships. The different concentrations of ovarian hormones associated with different phases of the menstrual cycle could affect the degree of hypoxia and extent of fluid shifts experienced by women during high altitude exposure, which might alter the occurrence of AMS symptoms.

Although AMS has been investigated frequently in males, it has not been studied adequately in women to discern whether there is an effect of ovarian steroid-hormone fluctuations. Two types of studies exist within the English literature that report AMS in women. First, there are studies in which an explicit or implicit assumption was made that no difference in response to altitude exists between men and women. Those studies examined both genders together without differentiating between them (2-6). The second type of study reported is that in which women were compared directly to men within the same investigation (7-10) or were compared to male historical controls (11,12). No consistent conclusions can be drawn from the studies. Grollman (7) reported "mild" AMS symptoms in his wife, but not in himself, during the first 36 h on the summit of Pikes Peak in Colorado, USA. Harris et al. (11) found that the pattern of AMS symptoms in women was different than that in men. Hannon (13) suggested that women acclimatized to high altitude "more readily" than men based upon several studies his group performed on the summit of Pikes Peak. The other studies did not demonstrate a discernable difference between men and women in AMS symptoms (8- 10, 12), but none of the reported studies controlled for menstrual cycle phase.

We hypothesized that the different levels of estrogens and progesterone present during different phases of the menstrual cycle would alter the physiologic response to altitude exposure and cause differences in the occurrence of AMS symptoms. We tested that hypothesis by assessing AMS symptoms in women volunteers during a 36 h exposure to 446 torr in a hypobaric chamber and in other women volunteers during a 12 day exposure to 4300 m on a mountain during two different phases of their menstrual cycle. The preliminary results from these recently completed studies are presented.

Hypobaric Chamber Exposure

The purpose of the hypobaric chamber study was to determine if there were differences in occurrence of AMS symptoms between the early follicular (EF) and luteal (L) phases of the menstrual cycle during early altitude acclimatization. The chamber was used to facilitate precise control of ambient environmental conditions (barometric pressure, temperature, humidity).

The subjects for this study were 12 women volunteers with normal menstrual cycles. They had a mean (±S.E.M.) age of 26.3±1.2 years, a height of 60.1±2.4 cm and a weight of 55.6±7.9 kg. All were low-altitude residents and had not been exposed to altitudes greater than 1500 m for at least six months prior to their participation in the study. All were nonsmokers.

A within-subjects factorial design was used in which each volunteer was evaluated at sea level and at simulated high altitude (446 torr; ~4300 m) during both EF and L of their menstrual cycle. The follicular phase was defined as beginning with the first day of menses and lasting until detection of"ovulation" using a commercial assay for LH in the urine (First Response, Tambrands Inc.). The luteal phase was defined as beginning the day of detection of "ovulation" until the onset of menses.

During each menstrual cycle phase, the volunteer was first evaluated in the hypobaric chamber for 24 hours at sea level. The chamber was then decompressed at a rate of 15 torr/minute to a pressure of 446 torr, and the volunteer remained at that pressure 32 hours. The ambient temperature and relative humidity were maintained at 23±1 °C and 55±5% throughout all exposures. Volunteers had unrestricted access to a balanced diet and fluid for consumption throughout the study, but were not allowed to ingest caffeine.

Symptoms were assessed in the volunteers at sea level and at 4 and, 24 hours after decompression ("ascent") using the Environmental Symptoms Questionnaire (ESQ). The ESQ is a self-administered 68-question inventory of symptoms that occur in stressful environments (14). It is well validated and has been often used for detection of AMS. Weighted averages of cerebral symptoms designated "AMS-C" and respiratory symptoms designated "AMS-R" were calculated (14) for each subject at each assessment.

The symptom score data was evaluated for statistical significance using a two-way ANOVA (altitude/time, menstrual cycle phase) with repeated measures in both factors. Significant differences were localized by *post hoc* analysis using Student-Newman-Keuls method. All tests were two-tailed and the level of significance was designated as $p=0.05$.

The overall pattern of symptoms in the volunteers conformed to the pattern seen in previous chamber studies with males at similar altitudes, i.e., there was onset of symptoms by 3-_ hours after ascent with maximal symptom intensity during the first 24 hours followed by a progressive decrease in symptoms. Mean AMS-C scores at 4 and 24 hours of altitude exposure and mean AMS-R scores and 24 hours of altitude exposure were significantly increased over the mean scores at sea level.

There were no statistically significant differences in ESQ scores between menstrual cycle phases.

Pikes Peak Study

The second study was designed to determine whether menstrual cycle phase affected AMS symptoms by altering the normal course of altitude acclimatization. The study was performed in the research facility on the summit of Pikes Peak in Colorado, USA (4301 m) to allow prolonged altitude exposure without restricting volunteers to the confined space of the hypobaric chamber. Ambient barometric pressure was uncontrolled in this setting and fluctuated from 458 to 464 torr during the study.

Sixteen women volunteers with normal menstrual cycles performed as subjects for this study. They had a mean age of 21.7±0.5 years, height of 167.4±1.1 cm and weight of 62.2±1.0 kg. All were low altitude residents, and all but one had not been exposed to altitudes greater than 1500 m for at least six months prior to their participation in the study. All were nonsmokers.

The study used a mixed factorial design in which one group of volunteers (n=8) was assessed for AMS symptoms at sea level and high altitude during the early follicular phase of their menstrual cycle and were compared to another group (n=8) assessed under the same conditions during the luteal phase of their cycle. The menstrual cycle phases were defined as in the previous chamber study (see above).

Assessment of symptoms at sea level was accomplished on multiple occasions in each phase of the menstrual cycle during a five month period prior to beginning the altitude exposure. Following sea-level exposure, the volunteers were assigned to either EF or L groups. They were then transported by commercial airplane and automobile to the laboratory facility on the summit of Pikes Peak over a period of approximately six hours on the second day of the cycle phase corresponding to their assigned group. The volunteers remained on the summit for 10-12 days. During the entire study period they consumed a controlled, vegetarian diet which was designed to minimize weight change. They were restricted from consuming caffeine.

As in the previous chamber study, symptoms were assessed at 4 and 24 hours after ascent using the ESQ. Weighted symptom averages (AMS-C and AMS-R, see above) were calculated for each volunteer from the ESQ data.

Symptom score data were evaluated for statistical significance using a two-way ANOVA (altitude/time, menstrual cycle phase) with repeated measures within the altitude/time factor. Significant differences were localized by post hoc analysis using the Student-Newman-Keuls method. All tests were two-tailed and the level of significance was designated as p=0.05.

The overall pattern of symptoms in these women volunteers generally conformed to the pattern seen previously in men and women at the Pikes Peak facility. Mean AMS-C and AMS-R scores were significantly increased over sea-level scores 24 hours after ascent. There were no statistically significant differences between menstrual cycle phases in ESQ or LLS scores.

Figure 1 presents the mean ESQ scores during follicular and luteal menstrual cycle phases from both studies combined. Each cycle phase group represents a total of 20 women.

DISCUSSION

Although we postulated that fluctuations in ovarian steroid levels associated with different phases of the menstrual cycle might alter the occurrence of AMS symptoms by altering the physiologic response to high altitude exposure, the preliminary analysis of the data from the two studies suggests that menstrual cycle phases have little effect. The explanation for these results is not clear from the limited information available at this time. At least three possible explanations should be considered: l) altitude exposure suppressed ovarian steroid hormone levels, 2) the magnitude of ovarian steroid hormone effects is insufficient to affect AMS, or 3) the inherent variability of hormone levels between and within individuals is sufficiently great to preclude detection of statistical significance with the sample sizes used for these studies. Some observations from previous studies (11, 12) suggest that altitude exposure might alter the menstrual cycle, but we are not aware of any definitive data in the English literature concerning this subject. Likewise, we are not aware of previous literature concerning the magnitude of physiologic effects of ovarian steroids relative to

altitude acclimatization and AMS symptoms. The occurrence of individual variability in ovarian steroid levels is well known, but the extent of individual variability in these two studies has not been evaluated yet. Hopefully, planned analysis of additional data from these studies will help to clarify the relationship of menstrual cycle phase to AMS in women exposed to high altitude;

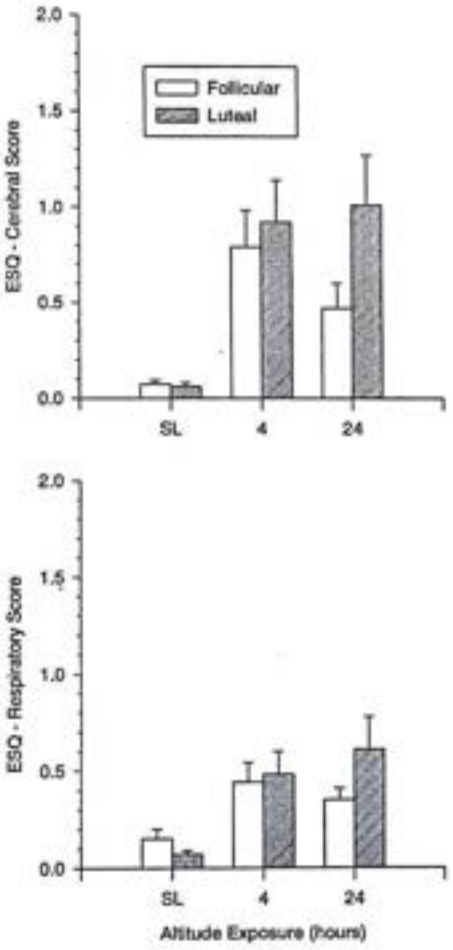

Figure 1. Environmental Symptom Questionnaire (ESQ) scores at sea level and at 4 and 24 hours decompression to 446 torr in a hypobaric chamber or ascent to 4300 m at Pikes Peak. Volunteers were exposed in the follicular (n=20) and luteal (n=20) phases of their menstrual cycle. Bars indicate standard error of the mean (SEM).

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PHYSIOLOGY OF INTERMITTENT EXPOSURE TO HIGH ALTITUDE

ERYTROPOIETIN AND CENTRAL VENOUS PRESSURE IN HIGH ALTITUDE SHIFT WORKERS

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RESUMEN: Eritropoyetina y Presión Venosa Central en Trabajadores de Altura Por Turnos

Se planteó la hipótesis que en trabajadores por turnos expuestos a hipoxia intermitente (10 días de trabajo a más de 3,600 m, seguidos de 4 días de descanso a nivel del mar) por más de 5 años, las respuestas de eritropoyetina (EPO) y los cambios en la presión venosa central (CVP) son diferentes de las que se observan en caucásicos que viven a nivel del mar. dado que el compartimiento intersticial pertenece al sistema de baja presión, un objetivo adicional del estudio fue cuantificar los cambios de distribución de líquido en los tejidos superficiales a lo largo del eje corporal durante tal hipoxia intermitente. Se usó un ultrasonógrafo para medir los cambios de espesor tisular de la cabeza (frente) y la tibia en trabajadores de turnos y en caucásicos de nivel del mar. Además se analizaron muestras sanguíneas para medir presión coloido-osmótica (COP) y concentraciones de albúmina (ALB) con la finalidad de determinar si el pasaje de líquido del compartimiento intravascular al extravascular se debe a variaciones de la COP o de la concentración de ALB. Se estudió a personal de cocina (N=11) de la mina de oro La Coipa (3600 m) y a un grupo de caucásicos de nivel del mar (N=5). Se tomó muestras de sangre y se realizó varias mediciones de CVP y densidad tisular (TT) antes, durante y luego de un turno típico. En las muestras basales (BDC) antes del cambio, los trabajadores por turnos presentaron concentraciones de EPO de 5.2±2.4 $mU.ml^{-1}$, que aumentaron en altura ($P<0.01$) y retornaron a valores BDC durante la recuperación (día 16). Los caucásicos mostraron la misma evolución en el tiempo. Los valores CVP en los trabajadores por turnos fueron generalmente superiores a los valores de los caucásicos. La densidad tisular de la región frontal en los primeros aumentó de manera definitiva durante la exposición a la altura ($P<0.05$) y se mantuvo a un nivel elevado durante el período de recuperación ($P<0.05$). la densidad tisular en la tibia no mostró cambios significativos; cambios similares se observaron en los caucásicos de nivel del mar. En conclusión, refutamos la hipótesis según la cual la respuesta inicial de EPO al estímulo hipóxico está alterada en los trabajadores en cuestión. Las más altas concentraciones de hemoglobina y/o de CVP halladas en los trabajadores por turnos podrían explicar las concentraciones más bien bajas de EPO observadas en la BDC. Además, los trabajadores por turnos y los caucásicos de nivel del mar mostraron acumulaciones medibles de líquidos en el tejido superficial de la parte superior del cuerpo, luego del cambio de nivel del mar a la altura.

Palabras claves: Salud, Mineros, Hipoxia intermitente, Presión venosa central, Densidad tisular, Ultrasonido.

RÉSUMÉ: Erythropoïétine et pression veineuse centrale chez les personnes travaillant en altitude et par roulement.

Une hypothèse a été émise selon laquelle, chez les personnes travaillant par roulement et qui ont été exposées de façon intermittente à l'altitude (10 jours de travail à plus de 3 600 m, suivis de 4 jours de repos au niveau de la mer) pendant plus de 5 ans, les réponses de l'érythropoïétine (EPO) et les variations de la pression veineuse centrale (CVP) sont différentes de celles des caucasiens vivant au niveau de la mer. Etant donné que le compartiment interstitiel appartient au système de basse pression, un objectif supplémentaire de l'étude a été de quantifier les changements de distribution de fluide dans les tissus superficiels tout au long de l'axe corporel, au cours du stress hypoxique intermittent. A l'aide d'un appareil à ultrasons on a donc mesuré les changements de densité tissulaire dans la tête (front) et le tibia des travailleurs par roulement et des caucasiens du niveau de la mer. Des prélèvements de sang ont en outre permis de mesurer la pression colloïdo-osmotique (COP) et la concentration d'albumine (ALB), dans le but de déterminer si le passage des fluides du compartiment intravasculaire au compartiment extravasculaire est dû à des variations de la COP et des concentrations d'ALB. L'étude a été faite sur le personnel des cuisines (N=11) de la mine d'or de La Coipa (3 600 m) et sur un groupe de caucasiens du niveau de la mer (N=5). On a effectué des prélèvements de sang et on a mesuré à plusieurs reprises la CVP et la densité tissulaire (TT), avant, pendant et après un roulement normal d'équipes. Dans la collection de base de données (BDC), avant la transition, les travailleurs par équipes présentaient des concentrations d'EPO de 5.2±2.4 $mU . ml^{-1}$ qui augmentèrent en altitude ($P<0.01$) et redescendirent à des valeurs BDC pendant la récupération (le 16e jour). Les caucasiens montrèrent la même évolution dans le

temps. Les valeurs CVP chez les travailleurs des équipes de roulement furent généralement supérieures à celles des caucasiens. La densité tissulaire de la région frontale chez les premiers augmenta de façon significative pendant l'exposition à l'altitude ($p<0.05$) et se maintint à un niveau élevé pendant la période de récupération ($p<0.05$). La densité tissulaire dans les tibias n'a pas montré de changements significatifs; des changements similaires ont pu être observés chez les caucasiens du niveau de la mer. En conclusion, nous réfutons l'hypothèse selon laquelle la réponse initiale d'EPO au stimulus hypoxique est altérée chez les travailleurs en question. Les plus hautes concentrations d'hémoglobine et/ou de CVP trouvées chez les travailleurs des équipes de roulement pourraient être responsables des concentrations plutôt basses d'EPO observées dans la BDC. En outre, les travailleurs des équipes de roulement et les caucasiens du bord de mer montrèrent des accumulations mesurables de fluides dans le tissu superficiel de la partie supérieure du corps, après passage du niveau de la mer à l'altitude.

Mots-clés : Santé des travailleurs, Hypoxie intermittente, Pression veineuse centrale, Densité tissulaire, Ultrasons.

SUMMARY: It was the hypothesis that in shift workers with a history of intermittent hypoxic stress (working 10 days at >3,600 m, then 4 days rest at sea-level) for >5 years the initial erythropoietin (EPO) response, and the changes in central venous pressure (CVP) are different from Caucasian low-landers. Because the interstitial compartment belongs to the low pressure system, it was an additional aim of the study to quantify fluid distribution changes in the superficial tissues along the body axis during such an intermittent hypoxic stress. Therefore, an ultrasound device was used to measure the tissue thickness changes at the head (front) and tibia in shift workers and Caucasian low-landers. In addition, blood samples were analysed for colloid osmotic pressure (COP) and albumin (ALB) concentrations to evaluate whether fluid shifts from the intravascular to the extravascular compartment are probably due to changes in COP and ALB concentrations. We studied the kitchen personnel (N=11) of the goldmine La Coipa (3,600 m) and a group of Caucasian low-landers (N=5). Blood samples were taken and CVP and tissue thickness TT determined several times before, during, and after a typical shift. At baseline data collection (BDC) prior to transition the shift workers had EPO concentrations of 5.2 + 2.4 mU-ml-1, which increased at altitude ($P<0.01$) and returned to BDC values on the recovery (day 16). The Caucasians showed the same time course. CVP values in the shift workers were generally higher than in the Caucasians. The tissue thickness at the front in shift workers increased significantly at altitude exposure ($P<0.05$) and remained elevated in the recovery period altitude ($P<0.05$). The tissue thickness at the tibia showed no significant changes. Similar tissue thickness changes could be observed in the Caucasian low-landers. In conclusion, the hypothesis has to be refuted that the initial EPO response to a hypoxic stimulus is altered in these shift workers. Higher hemoglobin concentrations and/or CVP values found in shift workers might be responsible for the rather low EPO concentrations observed in shift workers at BDC. Furthermore, shift workers and Caucasian low-landers showed measurable fluid accumulations in the superficial tissue of the upper part of the body after transition from sea-level to high altitude.

Key words: Occupational Health, Intermittent Hypoxic Stress, Central Venous Pressure, Tissue Thickness, Ultrasound Method

INTRODUCTION

Generally, the mines in the South American Andes are among the highest in the world. Today there is increasing mining activity at altitudes between 3,000 and 6,000 m. In previous times, usually long-adapted local people were recruited for mining at altitudes above 3,000 m. Growing economic interest especially in Peru, Bolivia, and Chile for copper, gold, and silver led to plans to expand exploration and exploitation of these natural resources in the Andes (Monge et al. 1990). It is easy to conceive that the increasing number and the growing size of these mines such as Minera Doña Inés de Collahuasi (Chile) with a population of about 20,000 cannot be run by local personnel only and that people had to be recruited from all over the country. Thus, people from low altitudes (sea-level) were recruited. It is apparent that new physiological, medical, and/or psychological problems related to the establishment of these mines could be predicted, but only scanty information is available on how workers tolerate the high altitude exposure (Jalil 1995; Jimenez 1995; Ward et al. 1995). La Coipa (3,600 - 4,000 m) in the southern Atacama desert of Chile is one of these new mines. Nowadays about 600 people are working permanently in what is one of the richest goldmines in the world. Personnel are recruited from all parts of the country especially from the coastal areas of Chile.

For our study we have chosen the kitchen personnel of the surface-mine La Coipa (location of the study, see figure 1). The reason for choosing this group was to avoid factors which might influence the erythropoietic system by environmental pollution, as is known from Andean underground-mines (Frisancho 1988). The workers usually had continued for more than 5 years a shift of 10 working-days at 3,600 - 4,000 m and a four day rest period at sea level. The mining management determined this schedule, which was most likely tolerated by most of the miners (personal communication). A scientific evaluation on the occupationai health problem still needs to be performed.

It was the hypothesis that in these shift workers the initial erythropoietin (EPO) response and the central venous pressures (CVP) are different from a control group of Caucasian low-landers, who prior to the study had not been exposed to altitudes >3,000 m during the previous six months before the expedition. CVP measurements were included in this study, since previous experimental data in dogs have shown that changes in CVP modulate.

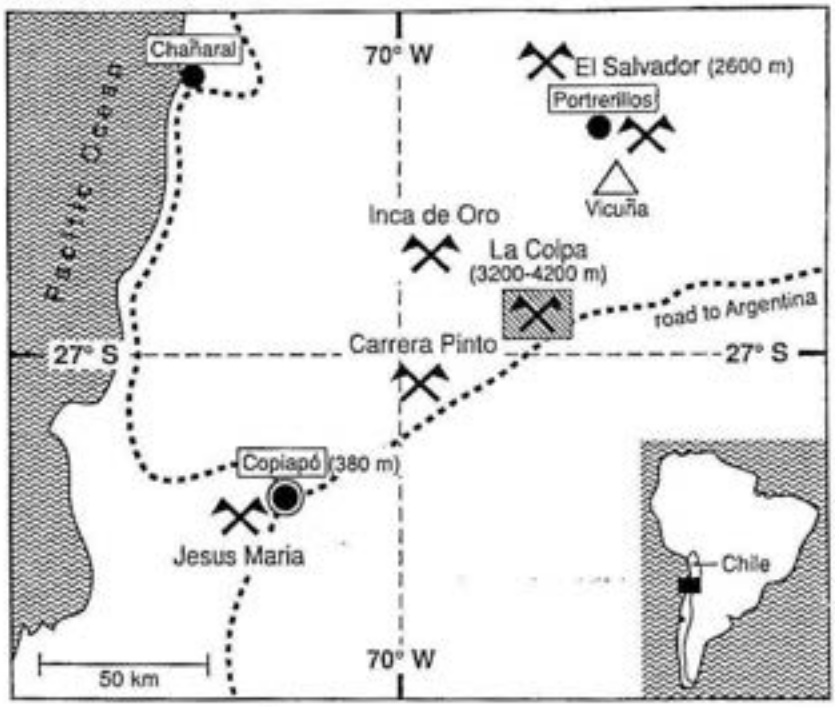

Figure 1. Location of the goldmine La Coipa (3200-4200 m) in Chile. (Map modified after Sylvester and Palacios 1991).

EPO production and release from the kidneys (Ehmke et al. 1995; Just et al. 1993), and similar considerations have been made for man (Gunga et al. 1994). Furthermore, it was assumed that in both group tissue thickness changes at the front (head) and tibia might occur at high altitude, which are known to be present already at moderate altitude exposure (Gunga et al. 1995).

MATERIAL AND METHODS

Location of the study. The study was performed in the southern Atacama desert of the Chilean Andes during April 1994. The baseline data collection (BDC) was taken in Copiap= (380 m, Chile) prior to transition to La Coipa, a new gold mine at an altitude of 3,600 - 4,000 m. The mine is a four-hour drive by car from Copiapó.

Subjects and Protocol. The shift workers (N=11, male, age 34 + 10.3 years, height 1.67 + 0.04 m, body mass 67.0 + 10.3 kg) were working as kitchen personnel in La Coipa. One Chilean shift worker was withdrawn from the study during the first BDC due to several vasovagal syncopies during the blood withdrawal procedure.

The shift workers usually had a shift of 10 days at high altitude in La Coipa and a four day rest period at sea-level. During daytime they were working in the mine between 3600 and 4000 m and slept overnight in a camp at a lower level (3000 m). EPO, reticulocytes, hematocrit, hemoglobin, plasma volume and tissue thickness changes (Δ%) were determined in the shift workers during and after transition to high altitude. The percentag changes in plasma volume (Δ% plasma volume) were calculated from hemoglobin concentrations and hematocrit using standard methods (Strauss et al. 1951). Blood samples from the shift workers were taken on the 1st (=BDC), 6th, 11th, and 16th day during the expedition.

Parallel to the Chilean shift workers, a control group of Caucasian low-landers (N=5, male, age 40.8 + 5.5 years, height 1.84 + 0.09 m, body mass 82.5 + 5.8 kg) was studied. During daytime they were working between 3600 and 4000 m and slept overnight in the same camp of the shift workers at a lower altitude level (3000 m). Blood samples from the Caucasian control group were taken on the 2nd (=BDC), 5th, 7th, 11th, 13th, and 15th day of the study. In addition, EPO values from the shift workers were compared with data obtained by us from a larger group of male Caucasiens (N=49, age 20-50 years).

CVP and ultrasound equipment. The equipment to measure CVP consisted of a small conventional strain gauge connected to a 19-gauge needle, a preamplifier, a small oscilloscope, and a tape recorder to store the signals. This equipment was used during earlier space flights for measuring

CVP under micro-gravity conditions (Kirsch et al. 1984). Pulse-coded modulation was used for data acquisition. CVP was measured by the arm-down method according to Gauer and Sieker (Gauer and Sieker 1956) in 6 out of the 11 shift workers and in all 5 Caucasian control subjects. The CVP measurements were taken at several locations (Caucasians: 3th, 5th, 8th, 11th, and 13th day; shift workers: 4th, =th, 11th, and 16th day of the expedition). Every measurement was made in the morning between 10 a.m. and 12 noon, after the subjects had rested in a supine position for 15 min.

For the tissue thickness measurements an ultrasonic pulse echo equipment was used (CL3DL Kräutkramer and Co, Cologne, FRG). The CL3DL operated on 10 Mhz (Kirsch et al. 1980a; Kirsch et al. 1980b), an equipment which was used sucessfully during space flights (Kirsch et al. 1993), clinical (Gunga et al. 1994) and other field studies (Gunga et al. 1995). The probe (0 = 1.0 cm) transmitts a brief burst of ultrasonic energy that propagates through different materials and is received by the same probe (A-mode). The probes were connected with the instrument by means of a flexible cable. The probe was fitted into a teflon ring which stabilized the system. The weight of the probe and the teflon ring together was 6.5 g. The coupling of the equipment with the tissues induced a deformation from an undisturbed level by less than 2 % assuming a tissue thickness between 2-8 mm. This was experimentally tested. The data could be read directly from a display. Since the values depend on many physiological variables an in situ analysis of the error is mandatory. In order to determine the error of the method in 4 subjects 10 measurements in one location were done within one hour. This gave an standard deviation of 0.04 mm (1 %) for the mean values. The resolution of the ultrasound sensor is 0.5 % of the values obtained in subjects assuming an average tissue thickness of 4.0 mm.

Analytical methods. The blood was centrifuged, and serum was stored immediately at -30°C until tested. EPO was measured by an ELISA distributed by IBL (Hamburg, Germany) with an intra-assay coefficient of variation of 4.8 %. All samples were assayed together in duplicate. The COP was measured with the BMT-921-onkometert® (Thomae, FRG) and the CV in one run was 0.44 %. The hemoglobin (HB) concentrations were measured with a Reflotron® by Boehringer (Mannheim, FRG) (CV 2.0 %), the packed cell volume (PCV) with a micro-packed cell volume centrifuge by Compur®-Electronic (München, FRG) (CV 0.8 %). The percentage changes in PV (delta % PV) were calculated from control and post ascent HB and PCV measurements according to Strauss et al. (Strauss et al. 1951).

Statistics. The results are expressed as arithmetic means + SD. For statistical analysis we used the ANOVA (Microcal ORIGIN 3.5 software). The null hypothesis was rejected when $P<0.05$.

RESULTS

The results are summarized in figures 2 - 9.

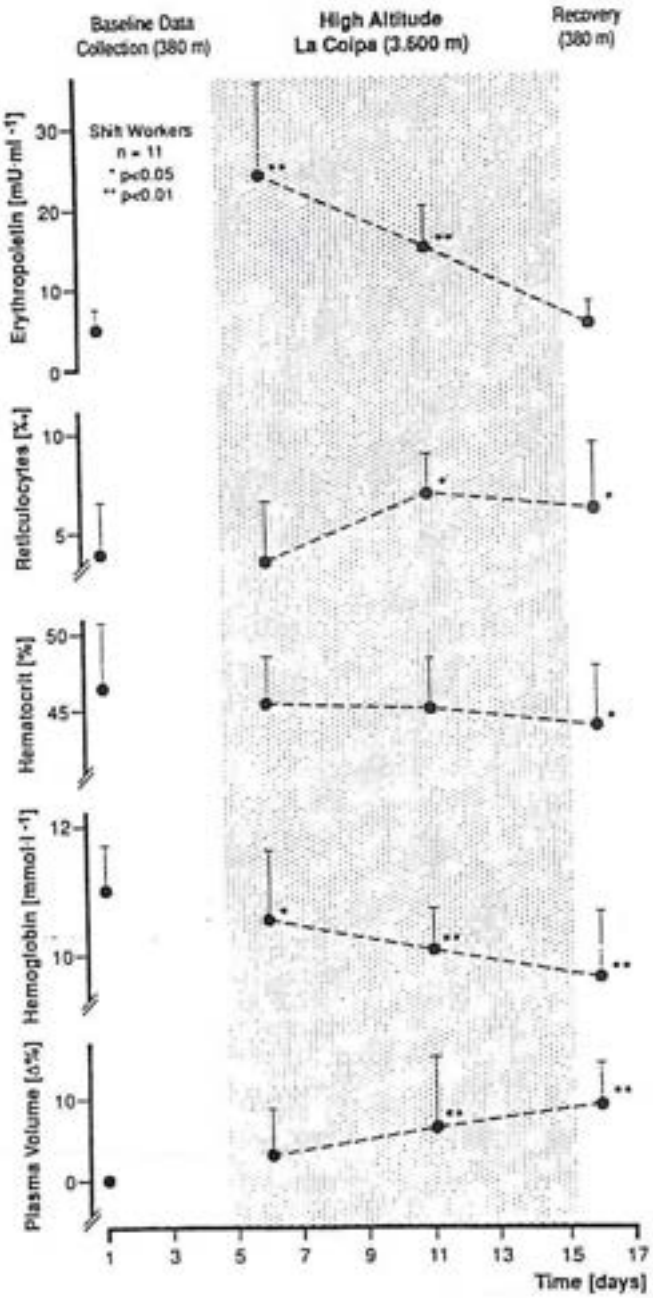

Figure 2. Erythropoietin, reticulocytes, hematocrit, hemoglobin and A% plasma volume changes in Chilean high altitude shift workers before, during, and after 10 working-days at 3,600 m. The time of

exposure to high altitude is shaded (Pc0.05; P<0.01). (After Guno,a et al.1996).

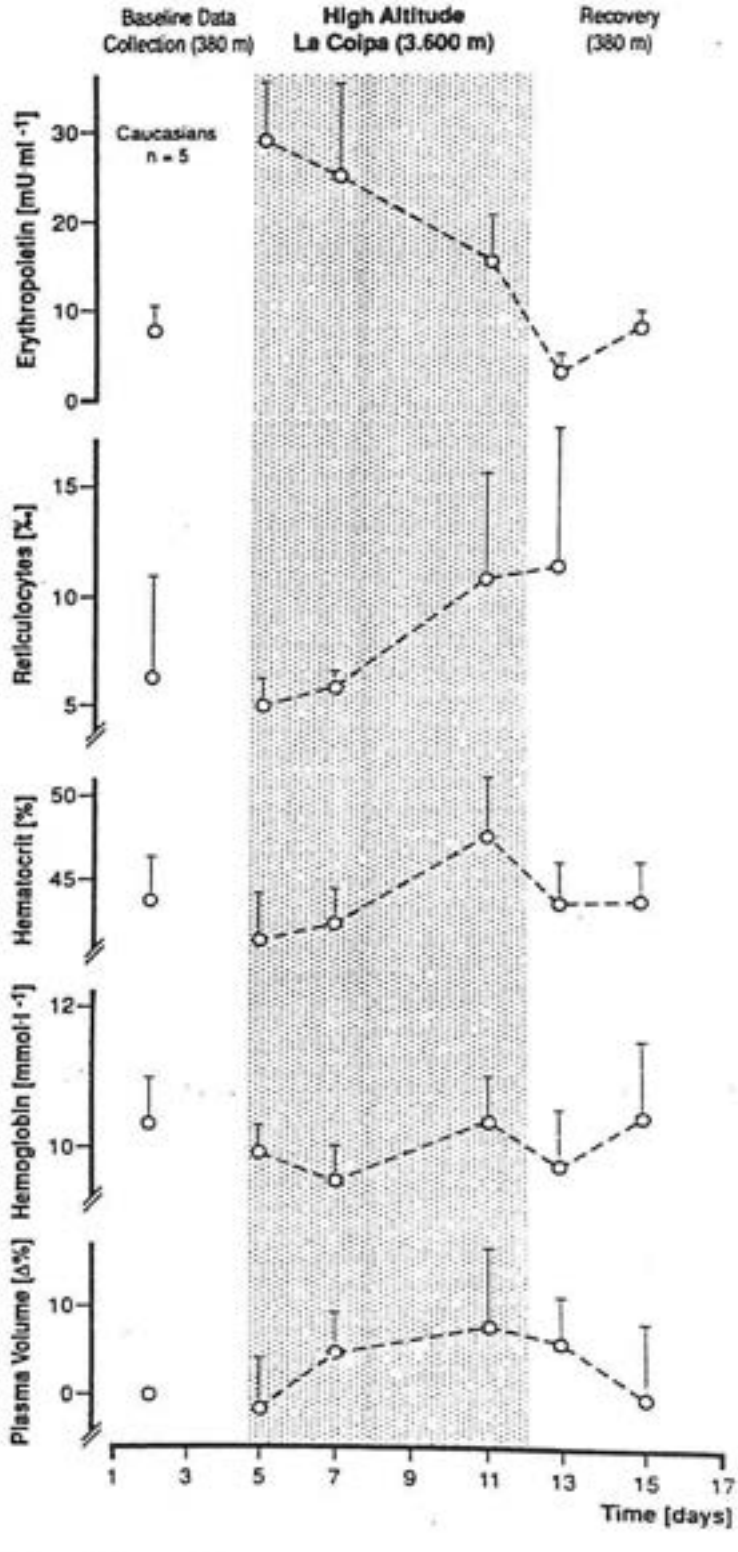

Figure 3. Erythropoietin, reticulocytes, hematocrit, hemoglobin and Δ% plasma volume changes in Caucasian low-landers before, during, and after exposure to 3,600 m. The time of exposure to high altitude is shaded. (After Gunoa et al.1996).

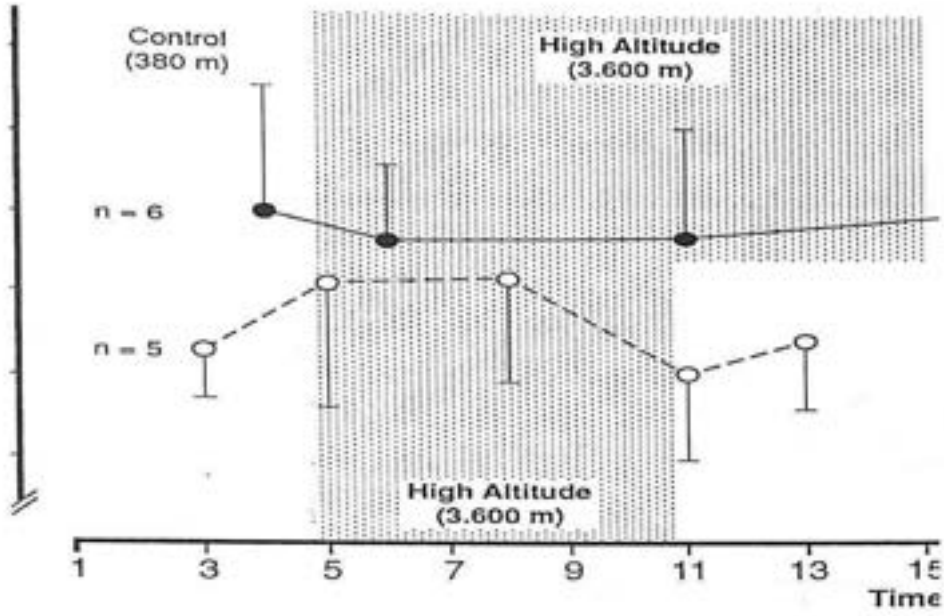

Figure 4. Central venous pressures (CVP) before, during, and after transition from sea-level (380 m) to high altitude (3,600 m) in Chilean high altitude shift workers (•) and Caucasian low-landers (o). (After Gunga et al. 1996)

DISCUSSION

Populations living permanently at high altitude (Quechua Indians, Sherpas) and newcomers (mainly Caucasian mountaineers) have been studied frequently with respect to their adaptation to different altitudes. These studies usually deal with the typical characteristics of altitude adaptations such as erythropoiesis, and cardiorespiratory control at rest and during exercise. Knowledge on high altitude adaptations in these two groups have been reported, but no systematic studies are available in the literature on the effect of professional long-term, high altitude shift working in humans as described in the present paper.

Therefore, the present study focusses on the characteristics of high altitude adaptation in humans induced by weekly altitude shifts (intermittent hypoxic stress) over of period of years rather than days or weeks (expeditions) or generations (Quechua, Sherpas) regarding the erythropoietic response as seen in EPO production and release.

The most prominent findings in this field study are 1) the low EPO concentrations at the baseline data collection in Chilean shift workers, 2) the pronounced initial EPO increase after transition from 380 m to 3,600 m, 3) the decline of EPO in both groups during the prolonged high altitude exposure, 4) the higher CVP found in high altitude shift workers compared with the controls, 5) the increase in tissue thickness at the front during high altitude exposure, and 6) the fall in COP and ALB concentrations in shift workers at altitude.

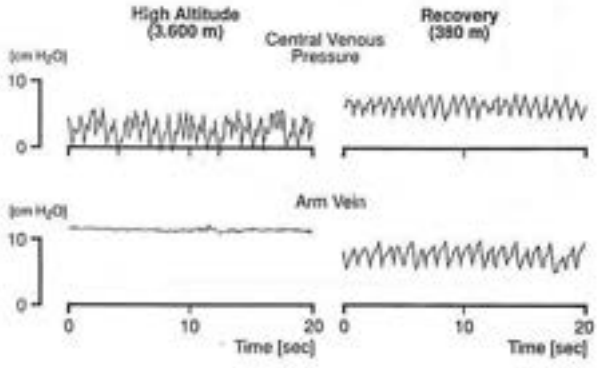

Figure 5. Typical example of central venous and peripheral arm vein pressure recordings in one subject (Chilean shift worker). High altitude left

side, 24 hours after descent right side. (After Gunga et al.1996).

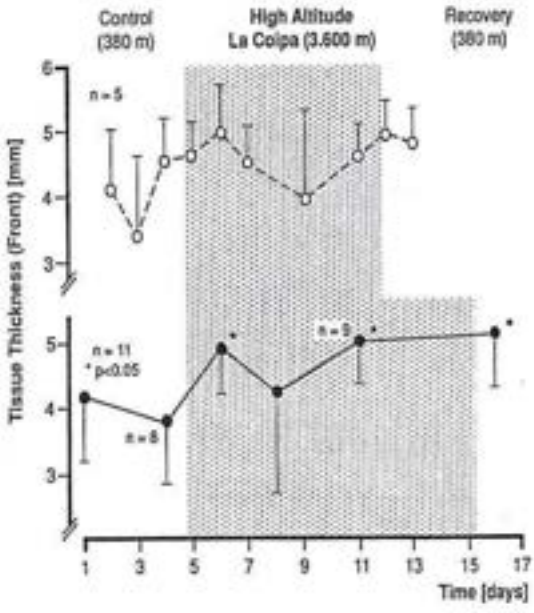

Figure 6. Time course of tissue thickness of the front (head) before, during, and after transition from sea-level (380 m) to high altitude (3,600 m) in Chilean high altitude shift workers (•) and Caucasian low-landers (o). The time of exposure to high altitude is shaded (P<0.05).

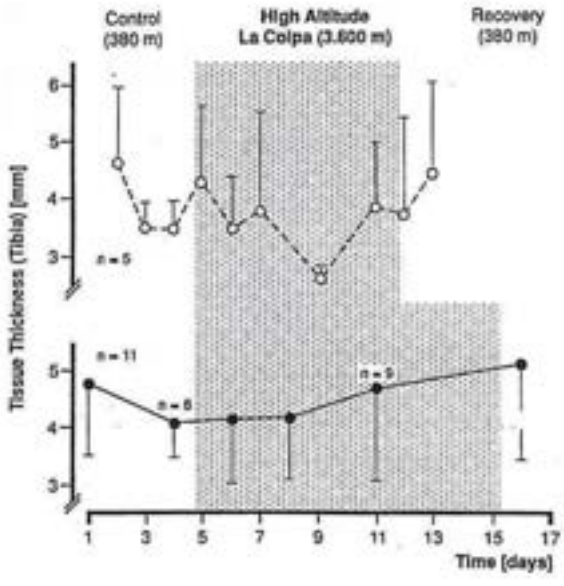

Figure 7. Time course of the tissue thickness at the tibia before, during, and after transition from sea-level (380 m) to high altitude (3,600 m) in Chilean high altitude shift workers (•) and Caucasian low-landers (o). The time of exposure to high altitude is shaded.

It is currently believed that the increase in plasma EPO concentrations is proportional to the severity of the hypoxic stress and that EPO production is regulated by the relative amount of oxygen available to the tissues involved in its production, primarily the renal cortex (Jelkmann 1992). Studies concerning the relationship between hypoxia and EPO production and release are frequent, but they were mainly performed in rodents, often used extreme pathophysiological models and therefore no data are available on EPO levels in shift workers (Jelkmann 1992). So first, a comparison between the basal EPO levels found in shift workers before transition to altitude with a larger group of male Caucasian subjects (N=49) showed that the EPO levels at the baseline data collection were significantly lower in Chilean shift workers than in Caucasian low-landers (Gunga et al. 1996). Furthermore, it appears that between 2,300-4,000m, parallel to decreasing PO2, only a slow progressive serum EPO in crease occurs. This is in accordance with the findings from hypobaric chamber studies in humans (Eckardt et al. 1989), although a field study at 4559 m showed generally lower EPO levels than observed in the present study (Mairbaurl et al.1986). This might be due to the fact that miners and Caucasian controls worked during daytime between 3,600 and 4,000 m and slept during the night at 3,000 m, so that they had a daily intermittent hypoxic stress. Significantly higher EPO levels were found in mountaineers at altitudes >5,000 m (Milledge and Cotes 1985; Richalet 1994) than in the present study.

In both the shift workers and the Caucasians, a gradual EPO concentration decrease was observed during their stay at 3,600 m, although the hypoxic stress is prevailed. This decline in EPO concentrations during the stay at high altitude is an observation, which is consistent with findings reported from moderate altitude (<2,300 m) (Gunga et al. 1994) and higher altitudes (Abbrecht and Littell 1972; Milledge and Cotes 1985; Richalet et al. 1994). The EPO down-regulation is surprisingly similar in shift workers and Caucasians. It was previously suggested (Winslow and Monge 1987) that at this altitude, the rate of EPO turnover could be increased after initial stimulation, precluding its accumutation in the blood. Nutritional factors, such as a low protein and/or carbohydrate intake (Anagnostou et al. 1977; Bethard et al. 1958; Catchatourian et al. 1980; Rosenberg et al. 1989), as a reason for the decline in serum EPO concentrations during the stay at high altitude, which first was theoretically predicted (Dunn et al. 1980), can be excluded

during this study; the mine management took care that a sufficient food supply was guaranteed so that each subject received approximately 50-60 kcal - cal^{-1} 24 hrs. Therefore, our data support the findings of a single earlier study, which came to the conclusion that the fall of the EPO concentration during continuous hypoxia is not primarily related to reduced food intake (Jelkmann et al.1983).

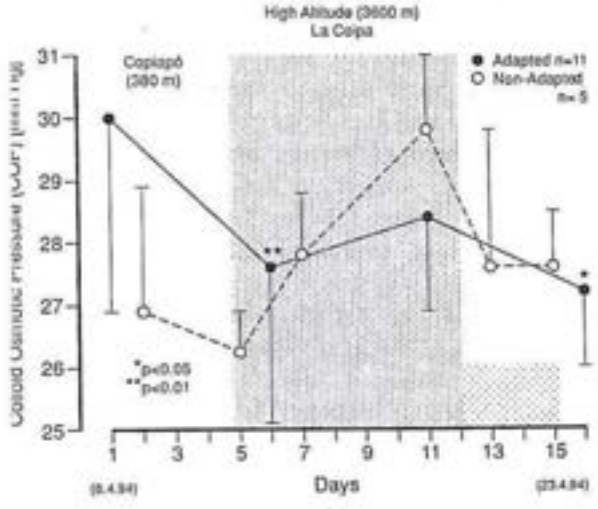

Figure 8. Time course of the colloid osmotic pressure before, during, and after transition from sea-level (380 m) to high altitude (3,600 m) in Chilean high altitude shift workers (•) and Caucasian low-landers (o). The time of exposure to high altitude is shaded (P<0.05; P<0.01).

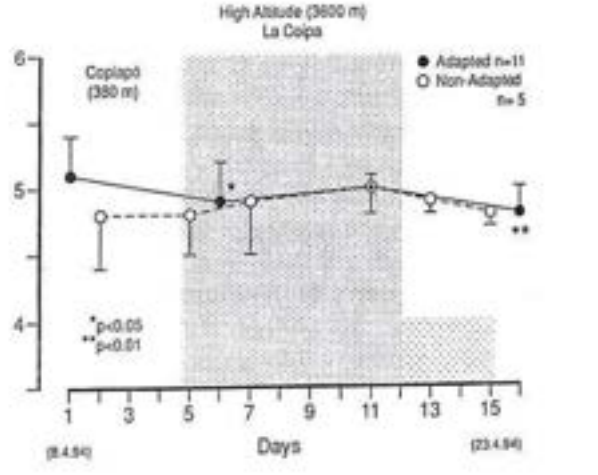

Figure 9. Time course of albumin concentrations before, during, and after transition from sea-level (380 m) to high altitude (3,600 m) in Chilean high altitude shift workers (•) and Caucasian low-landers (o). The time of exposure to high altitude is shaded (P<0.05; P<0.01).

Baseline hematocrit in Chilean shift workers was slightly higher than in the Caucasian control group, but rather low compared to hematocrit values known from rural and urban populations living permanently at altitudes >3,000 m in Bolivia, Peru and Chile (hematocrit >50%) (Monge et al. 1990; Winslow et al. 1989; Winslow and Monge 1987). In contrast, the baseline hemoglobin concentrations of shift workers were comparable to those found in the literature for high altitude populations (Monge et al.1990; Winslow and Monge 1987).

Previous experimental data in dogs have shown that changes in CVP modulate EPO production and release (Ehmke et al. 1995; Just et al. 1993), and similar considerations were made for man (Gunga et al. 1994). According to this hypothesis, changes in CVP should be inversely related to EPO concentrations in blood, i.e. low CVP should lead to an increase in EPO concentrations and vice versa. We tested this hypothesis in the shift workers and the Caucasian control group. Little if any data on the CVP at altitude has been reported in the literature. During Operation Everest II, a simulated ascent to Mount Everest in a hypobaric chamber over 40 days, the surprising observation was made that the mean right atrial pressures tended to be low despite pulmonary hypertension. Compared with sea-level values it was found that high altitude right atrial pressures were decreased for 10 of the 15 resting and for 20 of 23 exercise measurements (Reeves et al. 1987).

The venous recordings in the present study support the idea that the shift workers had a high intrathoracic filling volume, because the CVP values were generally higher than in the low-landers (Figure 4). Furthermore, the wave forms seen in the arm vein after descent indicate that the high filling volume had overcome the collapse of the veins at the point where they enter the thorax (Figure 5). Therefore, the pressure gradient between the intra- and the extrathoracic part of the low pressure system was small, so that both parts of the low pressure system form a unit. This can either occur via high intravascular volume or venous constriction, which moves the blood volume from the extra- towards the intrathoracic vessels or both.

In general, CVP in the shift workers tended to decrease at high altitude. That this was not seen in the Caucasian controls in this study might be due to the fact that the subjects tended to be dehydrated or that they had vasodilation due to the acclimatisation to the Atacama desert climate (Adolph 1969).

In this study at high altitude fluid extravasations into the superficial tissues of the upper part of the

body occured whereas at the lower part of the body (tibia) no significant changes could be detected by the ultrasound method (figure 6 and 7). Simultaneously in shift workers a significant fall of COP and ALB could be observed and it might well be that this fall in intravascular COP is obviously partly due to a protein leakage supporting edema formation in the superficial tissues of the upper part of the body. Those peripheral edemas at high altitude are described frequently (Hackett et al. 1976; Hayashi et al. 1988; Lobenhofer et al. 1982) and data concerning the water turnover, body composition and protein concentrations at high altitude are also available in the literature (Bartsch et al. 1991; Claybaugh et al.1992; Hannon et al.1969; Kryzwicki et al. 1971; Rennie et al. 1972; Surks et al. 1966). In the present study, the ultrasound sensors were attached at points were the underlying tissues consisted predominantely of skin and connective tissues. These tissues are known to be water stores of the body among others (Aukland and Reed 1993). In case the hydration level of the body changes in these tissues the first signs are visible there, provided thermoneutral conditions prevailed so that noteworthy changes of the skin perfusion can be excluded. We could demonstrate this in patients during dialysis treatment and in women during pregnancy and after delivery. In those models within short periods water loading of the tissues and unloading can be followed. With the help of the method these changes could be reliably seen (Kirsch et al. 1993; Gunga et al. 1994; Gunga et al. 1995). Why the edema preventing mechanisms as proposed by Guyton and co-workers (Guyton et al. 1975) got out of control at high altitude remains an open question. The fact remains that the fluid accumulation in the peripheral tissues went hand in hand with a protein leakage which concomitantly led to a decrease in COP. It is tempting to speculate that not only the superficial tissue in the upper part of the body are involved in this fluid accumulation but probably also lung and other tissues of the body. Hackett and Rennie (Hackett and Rennie 1979) described recentiy among other phenomenas the occurrence of peripheral edema in mountaineers. They stated that peripheral edemas are a common problem at altitude and sometimes very dramatic. Out of 200 trekkers they found 23 % having at least in one area of the body peripheral edema. The edemas in their group occurred as well around the eyes and face, the hands, or the ankies and feet. Furthermore, they found that 14 out of 19 who had facial edema had as well signs of acute mountain sickness. Some of the trekkers had eyelid edema which was so extensive that vision was impaired. They came to the conclusion that everyone with peripheral edema must be checked for pulmonary (HAPE) and cerebral edema (HACE) as well. Lobenhofer et al. (1982) who analysed 166 cases of high altitude pulmonary edema found in 10 cases (7 %) local edema. Hayashi et al. (1988) who studied the changes in water balance and in arterial oxygen saturation in 28 trekkers during a mountaineering expedition to Mt.Tharkot (6 100 m) in india found an increased incidence of peripheral edema despite a reduction in total water consumption and the use of Diamox during the ascending phase. In their study peripheral edema occurred from 2 500 m on and the incidence increased with higher altitude. In their study the two subjects who suffered most severly from high altitude exposure showed retinal hemorrhages, coughing and peripheral edema. Carson et al. (1969) who investigated a group at Pike's Pike determined the number and time course of symptoms of the acute mountain sickness (AMS) appearing during a stay at high altitude. They found that the subjects had the most severe symptoms on the 1st and 2nd day at high altitude. It remains to be seen whether the application of this new, non-invasive superficial tissue "edema-detector" can be used as an indicator in case such a fatal development starts.

In conclusion, the hypothesis has to be refuted that the initial EPO response to a hypoxic stimulus equivalent to 3,600 m is altered in Chilean shift workers during a five year period of intermittent hypoxic stress. The EPO concentrations in long-term shift workers are lowered at BDC compared to a large Caucasian control group. The low EPO concentrations found at BDC might be related to higher hemoglobin concentrations or generally higher CVP found in this group compared to the Caucasian controls. Changes in CVP, fluid shifts out of the intravascular compartment and their accumulation in the interstitial space as well as the decrease in COP and ALB concentrations during the altitude exposure deserve further investigations to unterstand the role of the low pressure system in human adaptation to moderate and high altitude.

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SEVERE INTERMITTENT HYPOXIA: HIGH-ALTITUDE MINES AND TELESCOPES AND THE CASE FOR OXYGEN ENRICHMENT

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RESUMEN: Hipoxia Intermitente: Minas de Altura y Telescopios y Argumentos a Favor del Enriquecimiento del Aire con Oxígeno

Se ha discutido previamente los problemas de la hipoxia intermitente en minas nuevas ubicadas entre 4000 y 5000 m de altura. Hay interés creciente en colocar telescopios en altura, especialmente en el norte de Chile, donde las condiciones de observación son excelentes. En una instalación típica propuesta, el telescopio estará a 5000 m y los trabajadores dormirán a 2500 m, dirigiéndose al telescopio cada día. El enriquecimiento del aire ambiental con oxígeno en las minas y los telescopios muestra un gran potencial para mejorar el bienestar y la productividad de los trabajadores.

Palabras claves: Hipoxemia severa, Calidad del sueño, Función psicométrica, Aclimatación.

RÉSUMÉ: Hypoxie intermittente sévère : Mines d'altitude et télescopes; importance d'un enrichissement en oxygène.

Ont été discutés préalablement les problèmes de l'hypoxie intermittente sévère qui se présentent dans de nouvelles mines situées entre 4 000 et 5 000 m. Il existe un intérêt croissant à installer des télescopes dans des zones de grande altitude, spécialement au nord du Chili où les conditions d'observation sont excellentes. Dans une installation type proposée le télescope se trouverait à 5 000 m, les travailleurs dormiraient à 2 500 m et remonteraient chaque jour sur le site du télescope. L'enrichissement en oxygène de l'air ambiant dans les mines d'altitude et sur les sites des télescopes représente un grand potentiel pour l'amélioration du bien-être et de la productivité des forces de travail.

Mots-clés : Hypoxémie sévère, Qualité du sommeil, Fonction psychométrique, Acclimatation.

SUMMARY: The problems of severe intermittent hypoxia in new mines at altitudes of 4000-5000 m have previously been discussed. There is increasing interest in placing telescopes at high alatude, especially in north Chile, where observing conditions are excellent. In a typical proposed installation, the telescope will be 5000 m and the workers will sleep at 2500 m and commute to the telescope each day. Oxygen enrichment of room air at the sites of the mines and telescopes shows great potential for improving the well-being and productivite of the work force.

Key words: Severe Hypoxemia, Sleep Quality, Psychometric Function, Acclimatization

INTRODUCTION

Recently there has been increasing interest in the physiological and medical aspects of severe intermittent hypoxia associated with high-altitude mines and telescopes. These facilities are typically at altitudes between 4000 and 5000 m though the old Aucanquilcha mine in north Chile is at 5950 m. Often workers commute to the facilities from lower altitudes, sometimes sea level. For example, at the new Collahuasi mine at an altitude of 4500-4600 m, most of the workers will live in Iquique at sea level, travel to the mine by bus for 7 days, and then return to their families at sea level for another 7 days with the cycle continuing indefinitely. The medical and physiological problems associated with this severe intermittent hypoxia are new and not well understood.

Proposed Telescopes at High Altitude

Several telescopes are now planned for high altitude, particularly in north Chile. Observing conditions are excellent, partly because the telescopes will be above so much of the interfering atmosphere, and partly because the atmosphere near the Atacama desert is extremely dry and therefore there is a very low concentration of water vapor which normally absorbs the radiation.

One proposed installation is the National Radio Astronomy Organization (NRAO) radiotelescope which will be situated at an altitude of 5000 m in the Andes of north Chile east of San Pedro de Atacama. At that altitude, the barometric pressure is about 415 torr, giving a PO_2 of moist inspired gas of only 77 torr compared with the sea level value of 149 torr. The plan is for the workers to sleep near San Pedro de Atacama at an altitude of about 2500m and commute each day to the telescope. This will be a formidable project with

an investment of $200 million. Other similar installations are being considered by Japan and other countries.

The severe hypoxia of an altitude of 5000 m impairs central nervous system function, reduces the quality of sleep, and limits work capacity. The deleterious effects of hypoxia are reduced somewhat by acclimatization. However workers who are intermittently exposed to severe hypoxia will presumably never acclimatize as well as people who stay permanently at a given altitude. The earlier arrangement of setting up whole towns near facilities at high altitude, such as in Cerro de Pasco and Morococha in Peru, is no longer favored. It is unpleasant for families to live at these high altitudes, children grow more slowly, and in any event it is extremely expensive to set up whole communities together with schools, hospitals, etc. Thus it is likely that the new strategy of commuting with the inevitable exposure to severe intermittent hypoxia is the way of the future.

Potential of Oxygen Enrichment of Room Air

Some of the reasons why oxygen enrichment of room air at high altitude has such potential value have been analyzed previously (1). Briefly, relatively small amounts of oxygen enrichment confer very substantial gains. For example, every 1% rise in oxygen concentration (for example from 21 to 22%) results in a reduction in equivalent altitude of 300 m (equivalent altitude is that which has the same inspired PO_2 value). In addition, improvements in technology allow large amounts of oxygen to be produced relatively cheaply. This can either be done using oxygen concentrators which preferentially adsorb nitrogen and produce an enriched oxygen mixture, or using liquid oxygen itself.

Some of the advantages of oxygen enrichment of room air at high altitude have previously been discussed (1) but some interesting new information is now available. There are now a number of measurements of the arterial PO_2 in lowlanders who have gone to hirh altitude for several days. Typically the arterial PO_2 is lowest during the first day, and it rises by 2-5 mmHg over the next 4 or 5 days. The explanation for the rise is ventilatory acclimatization whereby the alveolar ventilation gradually increases in response to the stimulation from the peripheral chemoreceptors, and the initial inhibiting effects of alkalosis in the blood and cerebrospinal fluid are reduced as bicarbonate is removed from both compartments.

Compilation of the available data show that when lowlanders go to altitudes of 3800 m and above, and stay there for about 7 days, the arterial PO_2 settles out below 55 mmHg. Naturally the PO_2 falls as the altitude increases, and at an altitude of 5000 m, the arterial PO_2 after a few days of acclimatization is typically less than 50 mmHg.

An interesting feature of this degree of hypoxemia is that if it existed in a patient with chronic obstructive pulmonary disease (COPD) it would entitle the patient to continuous oxygen therapy. In other words, the modern management of a patient with severe COPD and an arterial PO_2 of less than 55 includes continuous oxygen therapy by nasal cannulas. Furthermore it has been shown that patients with COPD whose arterial PO_2 is less than 55 mmHg, and who are treated with continuous oxygen therapy, gradually improve their psychometric function (measured during air breathing) over several months (2). Heaton and his colleagues studied both continuous oxygen therapy and nocturnal oxygen therapy in patients with severe COPD and showed that in both instances the "Performance IQ," which is a measure of psychometric function breathing air, improved over the first 6 months, and in the case of continuous oxygen therapy, psychometric performance continued to improve over the subsequent 6 months.

These provocative data show that if workers had their arterial PO_2 reduced below 55 mmHg by COPD rather than living at high altitude, they would be entitled to receive continuous oxygen therapy. Moreover, the therapy would improve their central nervous system function when they were in their hypoxemic state. Given the demanding skills necessary in modern mining with its high degree of mechanization, and also the fact that the frequency of accidents in highaltitude mines is some 3 to 4 times greater than that in mines below 3000 m (Jimenez, personal communication), these data suggest an obligation for oxygen enrichment at high altitude.

Other recent measurements raise very interesting questions about the value of oxygen enrichment in dormitories at high altitude. After initial tests at the Collahuasi mine at 4500 m, an extensive study was carried out at El Tambo mine, altitude 4300 m. The oxygen concentration in the dormitories was raised from a normal value of 21% to about 25%. Sixteen dormitory rooms were used for oxygen enrichment, the oxygen being supplied from a liquid oxygen depot.

Studies of the quality of sleep showed that this improved with oxygen enrichment in that there were fewer apneas, the total time spent in apnea was reduced, there were fewer arousals, and the staging of the electroencephalogram was more like the sea level pattern. These results are hardly surprising because it is common experience that people sleep less well at high altitude than low altitude, and the effect of oxygen enrichment is to reduce the equivalent altitude.

A more provocative finding was that studies of psychometric performance during the day after sleeping in an oxygen-enriched atmosphere showed small but consistent improvements in psychometric performance (Jimenez, personal communication). At first sight this may appear surprising because the oxygen stores of the body are very small and it is difficult to believe that the PO_2 of the brain could be increased some hours later. However, as discussed, there is an improvement in psychometric performance in the COPD patients treated with continuous oxygen therapy (2). In addition, similar findings have been described in patients with disordered breathing during sleep at sea level. For example, Engleman et al. (3) showed that patients with obstructive sleep apnea who were treated with continuous positive airway pressure (CPAP) showed improved daytime psychometric function. Whether this is simply due to less fatigue or whether there is a more subtle explanation is not clear. However in the light of the results found in patients with disordered breathing during sleep at sea level the results at high altitude are not particularly surprlsmg.

The most provocative finding however is that some patients who have slept in an oxygen enriched environment apparently sometimes have a higher arterial PO_2 during the following day (Cantuarias, personal communication). It should be emphasized that this is a very preliminary finding which may or may not stand up to subsequent testing. At first sight it is very difficult to understand how the arterial PO_2 of a worker breathing air during the day could be affected by oxygen enrichment during the previous night. However again a similar finding has been reported in patient with sleep-disordered breathing at sea level. Leech et al. (3) studied 17 patients with obstructive sleep apnea who were treated with CPAP and showed that the daytime arterial PO_2 rose significantly from a mean of 69 mmHg to a mean of 82 mmHg over a period of 3 to 46 months of follow-up. A clue to the mechanism of this was supplied by Berthon-Jones and Sullivan (4) who showed that some patients with obstructive sleep apnea who were treated with CPAP improved their ventilatory response to carbon dioxide. Moreover, the change was seen within 1 or 2 nights of treatment.

It should be emphasized that much more work needs to be done on the physiological responses to oxygen enrichment at high altitude. However the dramatic results in patients with sleep-disordered breathing and consequent arterial hypoxemia at sea level after treatment with continuous positive airway pressure suggest that oxygen enrichment during sleep at high altitude may have a number of beneficial effects.

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X

PUBLIC AND OCCUPATIONAL HEALTH AND MEDICAL EXPERIENCE AT HIGH ALTITUDE

ANTROPOLOGÍA Y MAL DE ALTURA CRONICO: ENFERMEDADES Y MALESTARES DE LOS MINEROS DE HUANCAVELICA

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RESUMEN: El propósito de este trabajo es presentar y analizar la concepción que tienen los mineros de Huancavelica, Perú central, de las enfermedades que les afectan en la mina. Según ellos, se pueden contraer dos tipos de enfermedades: aquellas causadas por la divinidad de la mina (el Muki), las cuales son concebidas como castigos para sancionar faltas cometidas contra el dios minero o contra la colectividad; y aquellas causadas por el contacto con tesoros antiguos, entierros o huacas.

El análisis de los testimonios recogidos, permite sugerir la existencia de una posible coincidencia entre los síntomas del primer tipo de enfermedades y los síntomas del Mal de Altura Crónico.

Este artículo permite así, subrayar el interés de los trabajos antropológicos sobre enfermedades no sólo para los estudios sobre la cosmología y el sistema de representaciones de los mineros y campesinos de los Andes, sino también para investigaciones conjuntas con biólogos y médicos sobre problemas de salud de las poblaciones de altura.

Palabras claves: Antropología, Enfermedad, Mal de montaña crónico, Mineros.

RÉSUMÉ: Anthropologie et Mal des Montagnes Chronique : Maladies et malaises des mineurs de Huancavelica

Le but de ce travail est de présenter et d'analyser la conception que se font les mineurs de Huancavelica, Pérou central, des maladies qui les affectent dans la mine. Selon eux, deux types de maladies peuvent être contractées : celles causées par la divinité de la mine (le Muki) considérées comme des punitions pour des fautes commises contre le dieu de la mine ou contre la collectivité, et celles provoquées par le contact avec des trésors anciens, des sépultures ou des huacas.

L'analyse des témoignages recueillis permet de suggérer l'existence d'une possible coïncidence entre les symptômes du premier type de maladies et ceux du Mal des Montagnes Chronique.

Cet article permet ainsi de souligner l'intérêt des travaux anthropologiques relatifs aux maladies, non seulement pour les études sur la cosmologie et le système de représentations des mineurs et des paysans des Andes, mais aussi pour les recherches conjointes avec des biologistes et des médecins sur les problèmes de santé des populations d'altitude.

Mots-clés : Anthropologie, Maladie, Mal des Montagnes Chronique, Mineurs.

SUMMARY: Anthropology and chronic mountain sickness: Illnesses and ailments of the Huancalican Miners.

The aim of this paper is to present and analyze how miners from Huancavelica, Central Peru, consider ailments contracted in the mines: miners may contract two types of ailments, those brought about by the god of the mines (the Muki) and which are considered to be a punishment for transgressions against the spirit guardian of the mines or the collectivity, and those caused by contact with buried treasures dating from the preChristian past.

The analysis of fieldwork data suggests the existence of a possible relationship between symptoms of the first type of illness and symptoms of chronic mountain sickness.

This paper underlines the interest of the anthropological study of illness not only for investigating the cosmology and the system of representations of Andean miners and peasants but also for carrying out research in cooperation with biologists and doctors on health problems of people living in the highlands.

Key words: Anthropology, Chronic mountain sickness, Illness,Miners.

INTRODUCCIÓN

A inicios de los 80 realizamos un trabajo de campo en un centro minero del departmento de Huancavelica, en el Perú central. En ese entonces, nuestro interés estuvo centrado en reconstruir y analizar desde una perspectiva antropológica, el proceso de transformación de campesinos en mineros. Nuestro trabajo estaba dividido en dos partes, una primera destinada a estudiar las condiciones sociológicas y económicas de la migración y de la instalación en la mina, tanto a nivel de la esfera del trabajo como de la vida cotidiana en el campamento minero. Una segunda, que consagramos a seguir y examinar el proceso de transformación a nivel del sistema de representaciones y prácticas religiosas [1]

Se trataba pues, de un trabajo cuyo centro de interés no era precisamente el estudio de las enfermedades. Sin embargo, muy rápidamente nos dimos cuenta que era imposible sostener una conversación con los mineros sin que el tema de las enfermedades no interviniera. Y es que, como lo hemos señalado en otro trabajo, el relato de las enfermedades constituye una especie de mito personal[2]. Estos relatos, atraviesan toda historia migracional, laboral, o para resumir, toda historia de vida. Porque la etiología siempre está situada en un acto de la vida del individuo, acto que precede la enfermedad y que merecería normalmente el oprobio de la sociedad. De ahí, la relevancia de la historia personal en el estudio de las enfermedades andinas, o más exactamente la importancia de trabajar a partir de testimonios y del relato del enfermo y no solamente limitarse al punto de vista de los especialistas de la terapéutica. Pero el estudio de las enfermedades no puede reducirse tampoco para el antropólogo a un análisis de las creencias religiosas y de la cosmología. Desde el punto de vista del etnólogo no son carentes de interés los síntomas variados y la etiología de esos males, tal como aparecen en los relatos de enfermedades. Esas enfermedades no solamente hacen referencia a una visión del mundo, sino también a una concepción del cuerpo.

Finalmente, antes de pasar a los datos sobre enfermedades quisiéramos abordar brevemente un problema que se nos presentó cuando recogíamos testimonios sobre las enfermedades mineras. Existe una correspondencia entre los malestares y las enfermedades descritas por los mineros y las enfermedades profesionales de estos trabajadores? Como veremos, los síntomas de las enfermedades descritas por los mineros no parecen corresponder a ninguna enfermedad profesional. Hay que señalar, que gran parte de los síntomas de las enfermedades descritas por los mineros son dermatológicos, los que pueden corresponder a enfermedades de la piel que la medicina formal no reconoce como particularmente frecuentes en la mina. Tratamos además de informarnos con los médicos del centro minero, buscando obtener un esclarecimiento del lado de la medicina oficial sobre este tipo de enfermedades. El único resultado fueron respuestas del tipo: "todo esto no son más que pretextos de los mineros para no ir a trabajar". Y debemos admitir que no fue, sino hasta la lectura de una trabajo reciente sobre el Mal de Altura Crónico, que pudimos constatar que muchos de los síntomas de las enfermedades y transtornos relatados por nuestros informantes, podían corresponder a dicho mal[3] De ahí que nos pareciera interesante presentar aquí, cómo un estudio antropológico sobre una población minera de altura, permitía sugerir o insinuar - a través de datos diferentes recogidos por medio de métodos totalmente distintos a los de la medicina y biología de altura una posible coincidencia a nivel de los síntomas con un mal biológicamente existente. Igualmente esto permite subrayar, el interés de los trabajos antropológicos sobre las enfermedades no solamente para los estudios sobre la cosmología o el sistema de representaciones del hombre andino, sino en una colaboración estrecha con biólogos y médicos. Por ello, creemos que sería necesario combinar los esfuerzos de antropólogos y de médicos en investigaciones futuras sobre las enfermedades que aquejan a los mineros y al hombre andino en

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[1] Este trabajo de investigación culminó en una tesis de doctorado titulada: *Pratiques et croyances religeuses des paysans et des mineurs à Huancavelica (Andes Péruviennes)*, Paris, EHESS, 1990.

[2] Salazar-Soler, C., op. cit. En este sentido, nuestro trabajo se sitúa en la perspectiva trazada por C. Bernand para los estudios antropológicos sobre las enfermedades andinas. Ver Bernand-Muñoz, C., *Enfermedad, daño e ideología. Antropología médica de los Renacientes de Pindilij*, Quito, Ed. Abya-Yala, 1986

[3] Arregui, A., León Velarde, F. y Valcárcel, M., Salud y Minería *El riesgo del mal de montaña crónico entre los mineros de Cerro de Pasco*, Lima, ADEC-ATC, 1990.

general, dado que la enfermedad no puede tampoco ser reducida íntegramente a un mal físico. Ella participa también del campo de las emociones y de la cosmología y ocupa un lugar muy importante en la vida de los campesinos y mineros de los Andes

Los datos sobre los cuales se basa el presente trabajo provienen de las investigaciones llevadas a cabo por nosotros en un centro minero del departamento de Huancavelica. Se trata, como ya hemos dicho, de un trabajo de antropología y por lo tanto los casos que hemos recogido representan una muestra pensada en función de una investigación cuyo centro de interés no fue el estudio de las enfermedades. En este sentido, precisemos que no aplicamos ningún cuestionario sobre patologías o enfermedades en la mina. Los datos que presentamos han sido extraídos de entrevistas o de historias de vida que realizamos y recogimos con una muestra de mineros. Hay que señalar que la muestra cubría sobretodo los mineros de socavón, de diferentes oficios, procedencia, antiguedad y edad. Sin embargo, no podemos decir que desde el punto de vista del estudio de las enfermedades en la minería o del estudio del Mal de Altura Crónico, estos casos sean significativos, dado que como lo han mostrado otras investigaciones, para hacer un estudio serio del Mal de Altura Crónico, se necesita trabajar con una muestra que permita medir de una manera sistemática una serie de parámetros con respecto a este mal, tales como el tiempo de trabajo en la mina, la procedencia de los trabajadores y la edad

Nuestro caso de estudio: los mineros de la provincia de Angaraes

El centro minero estudiado se encuentra ubicado en la provincia de Angaraes, en el departamento de Huancavelica, en el Perú central. El campamento principal está situado a 4250 m de altura. La mina produce minerales complejos de plata, plomo, bismuto, zinc, cadmium y tungsteno. La producción de plata es de lejos la más significativa.

En los años 80, cuando estabamos realizando nuestra investigación, el centro minero contaba con 1045 trabajadores: 936 obreros y 110 empleados.

Si existe una división de trabajo compleja entre los trabajadores, la más visible y la más significativa desde el punto de vista de la estructura del grupo es aquella que resulta de la doble oposición entre minero del interior de la mina y el obrero de superficie, de un lado, y entre obrero y empleado, por el otro.

Ya hemos señalado cómo nuestra investigación estuvo centrada sobretodo en el estudio de los mineros de socavón.

La mayor parte de los trabajadores del centro minero estudiado son reclutados en las comunidades campesinas de los alrededores de la mina, en el resto del departamento de Huancavelica y en los departamentos vecinos. En los años 80, 47% provenían de las comunidades campesinas de la provincia de Angaraes; 23% de la provincia vecina de Huancavelica, 6% de las otras provincias del departamento de Huancavelica, 14% del departamento de Junín y solamente 10% provenían de los otros departamentos. Es decir, que se trataba de una población originaria de zonas de altura[4].

El 60% de los trabajadores tenían entre 18 y 33 años; su nivel de escolaridad era muy bajo: existía un 9% de analfabetos y 64% no poseían una instrucción primaria completa. La gran mayoría de ellos eran casados (84%).

Hasta los años 60, se trataba de una mano de obra fluctuante, que venía a trabajar a la mina por periodos cortos de algunos meses, alternando así la actividad minera con las actividades agropecuarias. A partir de 1968 comienza un proceso de estabilización de la mano de obra, que responde tanto a cambios ocurridos en el campo como a una política de la empresa (mecanización, aumento de la producción y necesidad de una mano de obra estable y calificada). Desde esa fecha los trabajadores permanecen en la mina por periodos más largos que corresponden a entre 10 y 20 años.

Otro dato que nos parece significativo de señalar, es la ocupación anterior de esta población minera. En la mayoría de los casos se trataba de campesinos que dejaron sus comunidades para venir a trabajar al centro minero. Muy pocos son los que tenían una experiencia como obreros y en particular como obreros mineros.

Nuestro trabajo de investigación mostró que para esta población rural, instalada en el centro minero, el nuevo universo de trabajo y de vida induce cambios rápidos y profundos en la esfera de l as representaciones sociales des de la concepción del trabajo hasta los hábitos

[4] La mayoría de comunidades de origen de los trabajadores mineros están situadas por encima de los 3000 m.s.n.m. Ver Salazar-Soler, op. cit. (1990).

alimenticios y vestimentarios, incluso si el trabajador del centro minero estudiado no es un proletario en el sentido estricto del término pues sigue conservando lazos muy fuertes con su comunidad campesina de origen; permanece integrado al sistema tradicional de cargos, participa en las labores y las asambleas de su comunidad y continua trabajando e invirtiendo en ella. Por el contrario, el análisis comparativo de creencias y prácticas religiosas de los mineros y de los campesinos de las comunidades campesinas de Angaraes, permitió mostrar que existe una continuidad muy fuerte entre el campo y la mina: misma concepción del tiempo y del espacio, misma percepción del cuerpo y de las enfermedades, las mismas divinidades, las mismas creencias y los mismos ritos. Existe sin embargo en los mineros, una tendencia hacia la individualización del comportamiento ritual, por ejemplo en sus relaciones con el Muki, la divinidad de la mina. Contrariamente a las representaciones sociales, el sistema de creencias religiosas y de representaciones cosmológicas no evoluciona sino en la larga duración y determina en gran medida las modalidades de integración de la población campesina a la modernidad.

Las enfermedades

Tanto los mineros como los campesinos de Angaraes, distinguen principalmente entre: las enfermedades de los Wiracocha o enfermedades microbiosas y las enfermedades de los runa.

Las enfermedades de los Wiracocha (hombre blanco) o microbiosas son aquellas que según ellos aparecieron con la llegada de los españoles. Son enfermedades de los hombres blancos, pero que fueron traídas a los pueblos andinos y por lo tanto pueden atacar a los runa o campesinos, quienes para curarse deben acudir al hospital [5]. A decir de los campesinos y de los mineros de esta zona, estas enfermedades tienen su origen en la ciudad. La ciudad es considerada como un lugar de degradación de las relaciones sociales. Estableciendo así una relación entre enfermedad y comportamiento social. La tuberculosis es considerada como la enfermedad de wiracocha por excelencia. En un trabajo anterior sobre la creencia del Pishtaku (el degollador andino) entre los mineros, analizamos algunas versiones en las que éstos acusaban a un ex-enganchador de mano de obra para las minas de ser además de Pishtaku, incestuoso, borracho, y explotador de los runa, el responsable de haber introducido la tuberculosis en la zona. Enfermedad social y enfermedad física van asociadas. La silicosis es clasificada por los mineros como una enfermedad de Wiracocha. Los mineros de Angaraes dicen que la silicosis fué traída al campo por los hombres blancos cuando estos empezaron a trabajar las minas[6]

Las enfermedades de los runa son aquellas que según nuestros informantes, atacan exclusivamente a los runa pues poseen una constitución diferente a la del hombre blanco. Ellos atribuyen esta diferencia tanto a régimenes alimenticios distintos como a sistemas de pensamiento y de vida social diferentes: los runa creen todavía en las divinidades de la tierra y de la montaña mientras que el hombre blanco ya no cree en nada. Entre las enfermedades de los runa encontramos aquellas concebidas como castigos infligidos por las divinidades o los antepasados para sancionar faltas - transgresión del territorio de las divinidades o transgresión de las leyes que rigen la vida comunal. Son también consideradas como enfermedades de runa, aquellas causadas por el "mal aire"; para los campesinos o los mineros el mal aire son las emanaciones o los vapores que provienen del interior de la tierra y que pueden atacar al hombre andino. Por oposición a las enfermedades de los Wiracocha, las de los runa, son concebidas como enfermedades "antiguas". Ellas son causadas por los gentiles o por los vapores que expelen los vestigios de los gentiles, por los Wamani (dioses de la montaña), los Muki (divinidades de la mina), las divinidades de los pozos, lagunas, o arco iris[7]

[5] Runa es un término quechua que quiere decir la gente o los hombres, por oposición a los animales o a otros seres, o por oposición a la mujer (Mroz, M. *Los runa y los wiraquca: estudios sobre la ideologeia social and¿na a través de la tradición oral quechua*, Varsovia, Tesis de doctorado, Universidad de Varsovia, 1984.). Este es el término utlizado también por los mineros de la provincia de Angaraes para distinguirse por oposición al hombre blanco.

[6] Sobre el Pishtaku en las minas ver el artículo de Salazar-Soler, C., "El Pishtaku entre los campesinos y los mineros de Huancavelica", *Bulletin de l'nstitut Français des Etudes Andines*, 20, n°1, pp. 7-22.

[7] Fuera de estos dos tipos de enfermedades los campesinos y los mineros agrupan las otras enfermedades, que atacan a los runa pero también a los Wiracocha en dos familias: - Aquellas atribuídas a un desequilibrio interno causado por un movimiento brusco de uno de los líquidos del cuerpo tal como la sangre o la

En este trabajo nos limitaremos a las enfermedades de los runa.

Las enfermedades de los runa en la mina

Según los mineros de Angaraes, los trabajadores pueden atrapar dos tipos de enfermedades en la mina: aquellas causadas por el Muki, la divinidad de la mina, y las causadas por los entierros.

En la región de Angaraes, el minero puede sufrir una serie de enfermedades que ellos interpretan como un castigo del Muki por haber olvidado las ofrendas o por haber entrado a la mina sin pedirle su autorización. Estas enfermedades son la mukihuayra y la kutincha. Antes de entrar en el detalle de las enfermedades veamos brevemente quién es el Muki.

Los mineros de la región creen en la existencia de un ser de tinieblas, el Muki, que habita en las entrañas de la tierra. El Muki es frecuentemente descrito como un ser de forma humana , del tamaño de un niño de diez años, que está siempre vestido como un minero salvo que su vestimenta así como sus instrumentos de trabajo son de oro. Según los testimonios de los mineros, el Muki posee unos ojos rojos que brillan en la oscuridad y está dotado de un sentido de la visión muy desarrollado. Posee dos cuernos que sobresalen por encima de su casco y que utiliza para perforar las rocas y extraer el mineral que promete a los hombres. Es descrito también como un hombrecito de piel muy blanca, un gringo, barbudo que lleva frecuentemente un poncho de vicuña. Este ser es concebido por los mineros de Angaraes como el guardián y el dueño del mineral, y en tanto tal es muy celoso y aparece frecuentemente a los trabajadores para solicitarles ofrendas a cambio de dejarlos trabajar en paz. Es un personaje ambivalente, que puede ser generoso con los hombres y brindarles riquezas, pero también es concebido como un ser maligno que castiga a los que entran a la mina sin pedirle su autorización o a los que han olvidado de hacerles ofrendas, causando accidentes o una serie de enfermedades. Los mineros realizan ofrendas y rituales para pedir a la divinidad de la mina protección y fecundidad. Se dice también que algunos trabajadores sellan pactos individuales con el Muki, en los cuales a cambio de ciertas ofrendas y promesas la divinidad de la mina proporciona riquezas y mineral[8]

La mukihuayra o viento del Muki (huayra es un vocablo quechua que quiere decir viento), ataca a las personas que han olvidado las promesas hechas al Muki o que han entrado a la mina por primera vez sin pedirle permiso. La persona que sufre de mukihuayra presenta una irritación en la piel y pequeños granos rojos parecidos a los de la varicela. Estos granos se desarrollan de tal manera que el trabajador, muy disminuído en sus movimientos, termina por quedar completamente paralizado. Esta parálisis afecta primero los miembros, sobre todo los brazos y las manos.

El caso de Tomás, que nos fue relatado por uno de sus compañeros de trabajo, ilustra los síntomas que puede presentar esta enfermedad y que nos interesa particularmente aquí:

> "El Elías maestro que es, ha dicho mañana vamos a empezar tarea 46 así que hay que venir temprano. Yo con el compadre Marcelino, el Ambrosio, el Aucalli, hemos avisado al Tomás para repartirnos para traer su coquita, su cigarro, su traguito para pagar al taytacha Muki no ve que íbamos a abrir nueva tarea diciendo se ha ido el Elías. Pero el Tomás no ha querido diciendo, eso es de chuto, de ignorante diciendo. Así que hemos pagado no más al día siguiente, hemos trabajado tranquilos no más. Así que para que amanezca miércoles sería ha venido la mujer del Tomás ha avisar que se ha puesto mal que no puede venir a la tarea. Así cuando hemos ido a ver todito su cuerpo ha estado con unos chupos grandes, con ojito rojo, grande así, el Tomás llorando de dolor, dice que había amanecido así con unos puntitos colorados en todo el cuerpo que le comía, se ha ido al hospital, ahí el médico le ha dado medicina, nada le ha hecho peor ha crecido chupo grande, por todito el cuerpo así con ojito rojo ha sido, no podía andar siquiera. Llorando ay es que ha avisado al Gregorio diciendo no ha pagado, eso es de chuto dice, ay le avisado tayta Gregorio diciendo eso ha sido de estito, ese chupo con puntito rojo

bilis, y - Aquellas concebidas como el resultado de brujería causada por un pongo a pedido de un vecino o pariente envidioso. Para el detalle de este tipo de enfermedades, ver Salazar-Soler, C., 1990.

[8] Sobre el Muki y en general las prácticas religiosas de los mineros de Huancavelica ver Salazar-Soler, C., op. cit.

estito apostema así ha dicho, el tayta Muki bravo es" (T. minero de socavón).

Para tratar esta enfermedad, y en general todas las enfermedades que presentamos aquí, los mineros acuden por lo general al especialista tradicional o pongo. Quien, en la mayoría de los casos y a fines de poder diagnosticar el mal, hace una serie de preguntas al paciente no sólo sobre los síntomas sino sobre el contexto en el que se presentó la enfermedad. Luego, por lo general, realiza una consulta a la divinidad de la mina para averiguar si se trata de dicha enfermedad. Si la respuesta es positiva, el especialista procede a curarla. El tratamiento es el siguiente: primero el pongo frota el cuerpo de la víctima con tres tipos de piedras: la qocharumi (o piedra del lago), la orqorumi (piedra de la montaña) y la cachirumi (piedra de sal). Luego prepara dos llampus (polvos de maíz), uno con maíz negro y el otro con maíz blanco. Cubre en primera instancia el cuerpo del enfermo con el llampu de maíz negro para "capturar la enfermedad", y luego después de haberlo limpiado, con el llampu de maíz blanco para "purificarlo". Finalmente, frota el cuerpo del enfermo con una mezcla hecha a base de un poco de tierra del lugar en donde el enfermo dice haber visto al Muki, toronjil, claveles de color rojo, llima-llima, orkosunchu (vigudera Pflanzii) y kuya-kuya (senciro vulgaris). Cada una de estas hierbas tiene -según el especialista- una propiedad específica. El pongo dice que los claveles son una ofrenda para el Muki, la lima-llima y el orkosunchu sirven para favorecer la "reproducción del mineral" al interior de la tierra pero también para curar ciertas enfermedades pulmonares; la kuya-kuya, utilizada en otros lugares para la fabricación de filtros de amor, es usada aquí para favorecer la buena voluntad del Muki hacia el enfermo. El especialista frota el cuerpo del paciente con esta mezcla y una pequeña serpiente. Realiza las frotaciones invocando al Muki para pedirle protección, perdón y clemencia.

Como el Muki, el Amaru, la serpiente de dos cabezas que según los mineros vive en las entrañas de la tierra y que sale a la superficie en tanto mensajera de la divinidad de la mina, castiga también a las personas que entran a la mina sin pedir permiso al dios minero. Puede causar una en fermedad muy cercana a la mukihuayra: la víctima de este mal presenta "ápostemas" que cubren todo el cuerpo pero sobre todo los brazos. Esos apostemas "maduran" hasta reventar, dejando escapar un líquido negruzco e inclusive gusanos cuando se trata de casos graves. Este líquido negro que despiden los apostemas puede contaminar el resto de la piel, "comen la piel" si no se los cura a tiempo. Algunos mineros señalaron que ellos tuvieron compañeros de trabajo que fallecieron atacados por esta enfermedad, la cual comió una parte de los órganos externos: las orejas, nariz o las manos por ejemplo. Ellos dicen también que cuando alguien muere víctima de este mal es porque no quiso escuchar los consejos del Muki o del Amaru, "se hizo el sordo". Cuando la enfermedad ataca las manos de una persona es porque ésta excavó en la mina sin perdir permiso al tayta Muki o porque quiso extraer el mineral sin hacerle las debidas ofrendas. A decir de los mineros, en ciertos casos esta enfermedad no tiene manisfestaciones externas, pero carcome interiormente el cuerpo. En este caso los granos se desarrollan al interior del cuerpo , los cuales al reventar dejan escapar un líquido negro que carcome las entrañas.[9]

Esta enfermedad provocada por el Amaru es muy difícil de detectar. Según el pongo, la consulta que se le hace al Muki juega un papel muy importante para el diagnóstico y para la cura. El especialista dice que cuando esta enfermedad ataca a una persona es porque ésta ha tenido un mal comportamiento , o ha cometido faltas graves contra la divinidad de la mina. El tratamiento de esta enfermedad es el mismo que para aquella causada por el Muki. Se reconoce cuando una persona ha fallecido víctima de este mal porque al cabo de algunos minutos después de su muerte el cuerpo comienza a eliminar un líquido negro y apesta.

La kutincha es otra de las enfermedades causadas por el Muki. El enfermo de kutincha empieza teniendo mucho sueño, vértigo y al cabo de algunos días entra en un estado de sonambulismo generador de un debilitamiento progresivo que puede ser fatal. Otro síntoma de esta enfermedad es que la persona comienza a "secarse" comenzando por los miembros superiores.

Según los mineros de la región de Angaraes esta enfermedad es uno de los grandes castigos impuestos por el Muki a aquellos que tratan de traicionarlo, es decir, aquellos que habiendo sellado un pacto con él para obtener mayor cantidad de mineral y más riquezas no respetan sus promesas cuando la divinidad ya cumplió las suyas. Para hacerles recuerdo de ese pacto el Muki puede causar entonces un accidente,

[9] Sobre las creencias en torno al Amaru entre los mineros y los campesinos de Angaraes ver: Salazar oler, op. cit. (1990).

infligirles la kutincha y, en algunos casos, puede causarles directamente la muerte.

Escuchemos el testimonio de un trabajador que sufrió de esta enfermedad:

> "Así yo he sido despedido casi mamay diciendo los inges: indios ociosos, durmiendo no más andas, acaso te pagamos para dormir, para lampear te pagamos!, así me han sacado de la mina. Dice que me han encontrado dormido, como tres veces así no más, dice así sentadito así con la perforadora pero bien dormido dice; me ha vencido el sueño. Saliendo de la mina me ha agarrado dolor de cabeza, todito estito por aquisito todito esto (dice agarrándose la cabeza) por detrasito por mi delante parecía que me iba a reventar. Frío ha de ser me ha dicho la Domitila así que me he tomado un trago, nada peor, las orejas en mi adentro hacían: boom, boom. Así he llegado a mi casa, mal me he puesto, ya ha sido para estar peor. Así he amanecido en la mina dentro, así sentadito dice han encontrado los de turno de la mañana, vuelta he regresado a la casa, vuelta dolor de cabeza vuelta Dicen me han encontrado dice allá detrasito de la plata por ay por el relave juntito por ay, mi mujer ha preguntado, ya no decía nada, así como wawa me he estado, así en mi pantalón no más ensuciaba no ve que ya no andaba, debilidad ha sido, mi mujer me alcanzaba un caldo diciendo para frío de la cabeza, pero no podía comer que será, así me he puesto pues enflaquecido, sequito, huesito no más era. Como upa no más era. Ay casi me he finado, si no fuem por el taytacha Gregorio que ha hecho el uywachi ha dicho sanarás, pero está difícil, el taytacha Muki está bien bravo, kutincha es. Esto más ha sido porque más antes yo he trabajado en una mina por ahí por Castrovirreyna por ahí con otro patroncito, así hemos llamado un día al taytacha Muki diciéndole traerás mineral, y hemos hecho trato el ponía el mineral ahí el patroncito conmigo más ibamos a llevarle una llamita, un carnerito así no más, dice que hasta una pasna ha prometido ese otro patroncito, por eso dice es ahora finado. De haber encontrado mineral hemos encontrado una veta rica, ha sido pero el patroncito ese ha sido abusivo y se ha quedado con harto me ha dado a mí un poco diciendo toma estito y desaparece. Estito he gastado ni sé en nada, cuando se ha acabado he venido acá y entro a la mina y zas! me agarra el taytacha Muki, se pone bravo, porque para serle sincero, ya no hemos pagado, será por eso que me ha agarrado así? como será?" (S.P. minero de socavón).

Otros mineros que declararon tener esta enfermedad dijeron sufrir de constantes dolores de cabeza, dormirse en el trabajo y algunos señalaron además tener problemas para respirar al despertar. Estos últimos, en su mayoría, relacionaban este síntoma con la silicosis; otros con los problemas de cambio de temperatura a los que están expuestos en el trabajo (diferencia de temperatura entre el interior de la mina y la superficie). Incluso uno de ellos, nos contó, muy disgutado, que sintiéndose "ahogado" todas las mañanas, había acudido al hopital para que le revisen los pulmones, pero los médicos no le encontraron nada; y nuestro minero muy enfadado nos dijo "que ni siquiera le habían dado un jarabe", y que sus problemas de ahogo continuaban.

Este mal puede ser curado por el Muki, a través de la mediación de un especialista, solamente una vez que el enfermo cumplió con las promesas hechas en el momento del pacto con la divinidad. El tratamiento de esta enfermedad es el siguiente: el especialista frota el cuerpo del enfermo con un sapo verde, luego hace beber al paciente una bebida hecha a base de hierbas (manzanilla, toronjil, orko wira-wira (Achyroclineable) y retama). Frota enseguida el cuerpo del enfermo con esta mezcla invocando al Muki, pidiéndole que "le devuelva su sonqo". En esta enfermedad se dice que el Muki ha sonqeado a la víctima, la ha "vaceado de su sustancia vital".

Las enfermedades causadas por el Muki como aquellas en el campo causadas por los Wamani, las divinidades de la montaña, son concebidas como castigos por las faltas cometidas por los trabajadores. Esta concepción de la enfermedad recuerda la distinción que según los mineros hace el Muki entre los hombres de "buen corazón" y aquellos de "mal corazón". Son éstos últimos los que son castigados por la divinidad y los que son víctimas de enfermedades como la kutincha o la mukihuayra. Pero que significa poseer un "mal corazón" en el contexto de la mina? Aquél que transgrede las reglas de la reciprocidad andina, aquél que no cumple sus deberes con la divinidad o aquél que rompe el pacto social con sus

compañeros de trabajo o sus paisanos, ése es alguien de "mal corazón". Según uno de los testimonios que recogimos, Hilario, un minero que fue "sonqeado" por el Amaru, no solamente había olvidado de hacer ofrendas al Muki sino que además había ocultado a sus compañeros el mineral que había encontrado para beneficiarse sólo del producto de su venta. Lo que parece resultar del análisis de la concepción de las enfermedades no es más que una ética de trabajo, cuyo garante supremo es la divinidad de la mina .

Como podemos apreciar, muchos de los síntomas de estas enfermedades que según los mineros son causadas por el Muki y que aparecen descritos a lo largo de los testimonios presentados, pueden sugerir una coincidencia con los síntomas del mal de altura crónico: dolor de cabeza, somnolencia, quemazón de las palmas de las manos y/o plantas de los pies, sensación de cansancio físico y/o mental, sensación de tristeza o depresión, falta de aire al despertar, dolores musculares [10]

Puede llamar la atención el hecho de que uno de los principales signos del mal de altura crónico, es decir la cianosis (coloración azul-morada de las manos o labios) no aparezca en los testimonios de nuestros mineros como un signo de algún malestar. Sin embargo, este aparece ya no en los testimonios recogidos sobre las enfermedades, sino en otro tipo de información recolectada. Se trata, de las respuestas que dieron los mineros a las preguntas que les hicimos sobre si podían distinguir a un minero en la calle y si sí, a través de qué rasgos?. 35% de los 490 trabajadores entrevistados afirmaron poder reconocer a un minero. En muchas de estas afirmaciones, los rasgos físicos tenían un importancia significativa. Cabe recalcar que el 13% de los que dijeron poder reconocer un minero en la calle declararon poder hacerlo a causa de su fisionomía, en la cual sobresalía dos rasgos: cara manchada (manchas negras o moradas en los labios pero también en el resto del rostro) y los ojos irritados con una coloración rojiza [11]

Como dicen Arregui, León-Velarde y Valcárcel (op. cit.), con excepción de la cianosis de la cara, labios o manos y la dilatación de las venas de las manos o pies que constituyen signos objetivos del mal de altura crónico, todos los restantes son síntomas subjetivos y sujetos por lo tanto a la objeción que la subjetividad puede tener. Y esta parece en efecto, ser la mayor dificultad que deben enfrentar los mineros cuando van a consultar a un médico. A esto se agrega el hecho de que muchas veces el control o la consulta no se efectúa en el momento ni en las condiciones en que están presentes los síntomas. Y es más, muchos de los síntomas no pueden ser atribuidos solamente al mal de montaña crónico. Por último, como lo señalamos al inicio de esta presentación, los médicos parecían otorgar poca importancia a estos malestares; quizás en parte debida a las dificultades ya mencionadas del diagnóstico.

Según nuestros informantes, el otro tipo enfermedades que atacan a los mineros son causados por los entierros y llevan el nombre de huayra[12]. La Huayra o enfermedad de la antimonia provienen de las emanaciones procedentes de los entierros o huacas en las minas. Para nuestros informantes ciertos lugares en la mina están cargados de una especie de "electricidad" que ellos llaman antimonia. Ellos identifican esos lugares como entierros, es decir los lugares donde los gentiles o los Incas enterraron el oro para protegerlo de la ambición de los Españoles. Esos entierros contienen "oro vivo" y esta es la causa de la enfermedad de la antimonia.

Por lo general, esta enfermedad se atrapa por contacto directo, como en el caso de los mineros que trabajando en la mina, encuentran un entierro o los que trabajan al lado de un tesoro.

Los síntomas de la huayra son los siguientes: el enfermo comienza oliendo mal, como si estuviera en estado de putrefacción, luego se "seca", primero los brazos, las manos y finalmente todo el cuerpo. Una semana después pierde la piel (sobretodo la de los brazos) y finalmente sus miembros se deforman. Esta enfermedad puede ser mortal si no se la cura rápidamente. Para curarla el especialista debe limpiar el cuerpo del enfermo con un sapo verde. Luego prepara una mezcla a base de orina del enfermo, hierbas

[10] Arregui, et al., op. cit.

[11] Sobre los detalles de esta encuesta ver: Salazar-Soler, C., op. cit. (1990).

[12] Huayra es un término quechua que quiere decir viento. El término hace referencia en los Andes al "mal aire" que como dijimos es concebido por los campesinos y los mineros como las emanaciones o vapores que surgen del interior de la tierra y que pueden atacar al hombre andino. El ayahuayra (o viento del muerto) es uno de los huayra que según los campesinos de Angaraes atacan más frecuentemente al runa. Ver para los huayra Bernand-Muñoz, C. op. cit. y Salazar-Soler, op, cit (1990).

(romero, toronjil, retama, y malva) y grasa de cordero o de llama. Enseguida debe frotar varias veces el cuerpo del paciente con esta mezcla. Cuando el estado del enfermo es grave, se le debe dar a beber esta mezcla sin la grasa animal.

Los mineros de la región de Angaraes dicen que esta enfermedad se manifiesta a veces de otra manera, la persona atacada comienza a tener crisis de epilepsia:

> "Un día a la hora del descanso, estábamos tomando la sopa, un minero, un compañero, se acordó que se había olvidado su picota, y regresó a buscarla, pero se demoraba harto y no regresaba. Cuando el timbre ha sonado para el segundo turno lo hemos encontrado en una galería caminando como ciego, con tembladera, botando espuma por la boca, como opa. Nosotros hemos pensado que era la antimonia que lo ha cogido. Ahí entierro dice estaba buscando. La antimonia es muy peligrosa porque podemos estar lampeando tranquilos y vlan! podemos ser castigados por nuestros taytas los Incas y podemos morir pues". (M.S. minero de socavón).

Según los mineros, los casos de epilepsia son debidos al hecho de que el enfermo ha tratado voluntariamente de excavar un entierro. En estos casos, el especialista da de beber al enfermo una bebida hecha a base de orina del enfermo, toronjil, romero y aliso. Luego hace una mezcla con un puñado de tierra del lugar en donde el paciente contrajo la enfermedad y sal y la aplica sobre la frente del enfermo. Según el pongo se deben aplicar sustancias repugnantes y saladas sobre la cabeza, que sean capaces de "absorber" la antimonia. Finalmente el especialista pasa una moneda antigua de oro o de plata sobre todo el cuerpo del paciente para "llamar al oro vivo o la plata viva de los antiguos", y extraerla del cuerpo.

Esta enfermedad es conocida también con el nombre de "agitación", sobretodo cuando se manifiesta con convulsiones o cuando se detecta crisis de epilepsia en los enfermos. Según H. Favre, la agitación y la huayra veta, eran las dos enfermedades más comunes entre los mineros de Huancavelica en los años 60. Este autor, señala además, que el temor de atrapar enfermedades en la mina constituía uno de los factores que pesaban en la decisión de los campesinos de ese decenio de permanecer en la mina solamente por periodos cortos. La agitación era considerada como una enfermedad incurable [13]

La huayra veta es otra de las enfermedades conocida por los mineros de Huancavelica. Ellos dicen que no solamente los vapores que expelen los tesoros o las huacas pueden causar la huayra o antimonia, sino que cualquier veta o filón contiene vapores que salen cuando uno los explota y atacan al minero, produciéndole una serie de transtornos. La huayra veta es una variante de la antimonia y es considerada como menos grave que ésta. Los síntomas que revelan esta enfermedad son similares a los de la antimonia, a excepción de las convulsiones y las crisis de epilepsia. El tratamiento es similar al de la enfermedad anteriormente descrita.

Existe otra variante de la antimonia. Los mineros dicen que los lugares preferidos del Muki son aquellos donde hay agua, que está contaminada por los ácidos de los minerales; agua que tiene una apariencia de suciedad y que es responsable de ciertas enfermedades. Los campesinos dicen que estas "ciénagas" despiden vapores que atacan a las personas que caminan sobre ellas. Esta enfermedad puede atacar a cualquier minero, aunque algunos de ellos dicen que esos vapores atacan sobre todo a la gente que entra a la mina por primera vez sin pedir permiso al Muki.

La persona que sufre de esta enfermedad presenta "apostemas" sobre los brazos y las piernas, que terminan paralizándolo completamente. Para curarla se debe frotar el cuerpo del enfermo con una mezcla hecha a base de un puñado de tierra del lugar, sal y torongil.

Encontramos la utilización del término antimonio o antimonia para describir esos vapores en otras regiones del Perú y del Ecuador [14] En todos los casos, la antimonia está asociada al oro de las huacas y a las emanaciones provenientes de las tumbas. El antimonio es un metal que podemos encontrar en diferentes minas de los Andes, incluso en las de la provincia de Angaraes, bajo la forma de estibina, mineral que tuvo un papel importante en los experimentos de los alquimistas. Si bien como sabemos podemos encontrar antimonio en la fabricación de ciertos medicamentos antiparasitarios, también conocemos que su absorción en altas dosis puede

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[13] Favre, H., "Algunos problernas referentes a la industria minera en Huancavelica", Cuadernos de Antropología, 1965, vol. III, n98, pp. 16-24.

[14] Ver Bernand-Muñoz, C., op. cit.

provocar vómitos y diarreas. Sin embargo, esto no explica la utilización corriente del término antimonia para designar enfermedades causadas por los "vapores".

Por el contrario, a dosis poco elevadas, los vapores y las sales de mercurio, son extremadamente peligrosos y mortales. Ya en la época incaica la explotación del mercurio era considerada como muy peligrosa. B. Cobo, en su Historia del Nuevo Mundo (1653), afirmaba que los Incas no explotaron el metal antes de la llegada de los españoles, y Garcilaso de la Vega en sus Comentarios reales de los Incas (1609), explica que los Incas prohibieron su explotación: "... Los reyes Incas alcanzaron el azogue, y se admiraron de su viveza y movimiento, mas no supieron qué hacer de él ni con él, porque para el servicio de ellos no le hallaron el provecho para cosa alguna; antes sintieron que era dañoso para la vida de los que lo sacan y tratan, porque vieron que les causaba el temblar y perder los sentidos. Por lo cual, como reyes que tanto cuidaban de la salud de sus vasallos, conforme el apellido amador de los pobres, vedaron por ley que no sacasen ni se acordasen de él; y asi lo aborrecieron los indios de tal manera, que aun el nombre borraron de la memoria y de su lenguaje, que no tienen para nombrar el azogue, sino lo han inventado después que los españoles lo descubrieron año de mil quinientos y setenta y siete." (Libro VIII, cap;.XXV).

Los síntomas de la enfermedad del azogue descritos por Garcilaso pueden corresponder a las convulsiones y la pérdida de sentido mencionados por los mineros de la provincia de Angaraes para describir a las personas atacadas por antimonia.

J. de Acosta en su ***Historia natural y moral de Indias*** (1550), describe la utilización del oro para atraer el mercurio que se aspiró: " A los hombres que han echado azogue en los oídos para matarlos secretamente, ha sido el remedio meter por el oído una paletilla de oro, con que llaman el azogue, y la sacan blanca, de lo que se ha pegado al oro.... Y porque el humo del azogue es mortal, me dijeron que se prevenían los of iciales contra ese veneno con tomar un doblón de oro desmenuzado, el cual pasado al estómago llamaba allí cualquier azogue que por los oídos, ojos, narices o boca les entrase de aquél humo mortal y con esto se preservaban del daño del azogue, yéndose todo al oro que estaba en el estómago, y saliendo después todo por la vía natural ..." (Libro IV, cap. XI, pp. 101102). Esta descripción nos recuerda el caso que hemos descrito más arriba, salvo que los mineros actuales no hablan de tragar oro en polvo sino de frotarse con una moneda de oro y plata.

Esta breve explicación sobre la antimonia muestra que esta idea generalizada en los Andes sobre las emanaciones perniciosas no es completamente extranjera a ciertas experiencias concretas. Si bien ciertos síntomas atribuídos a la antimonia tales como las convulsiones y las erupciones sobre la piel están efectivamente asociadas a los vapores metálicos, en la mayoría de los casos, incluso en las minas de la provincia de Angaraes, ningún vapor pernicioso de este tipo es mencionado por la medicina de trabajo.

Algunos datos sobre las enfermedades mineras en la historia

Históricamente cuáles son las enfermedades de los mineros que aparecen descritas en los documentos?

Para el siglo XVI, XVIIy XVIII contamos sobretodo con descripciones de enfermedades en las minas de plata de Potosí y en las de mercurio de Huancavelica. Como sabemos, estas dos minas constituyeron los ejes de la economía minera colonial andina.

En lo que se refiere a las minas de Potosí, se trata -en el caso de los mitayos que trabajaban al interior de la mina (barreteros y apires)- de enfermedades respiratorias ligadas al cambio de temperatura entre el interior de la mina y la superficie, agravadas por la absorción del polvo. Pero la documentación colonial habla también de enfermedades que los trabajadores de los ingenios atrapaban por la absorción del polvo de la molienda mineral. Las fuentes documentales dan cuenta del mal del Chocó, especie de tifus, o tos producida por la absorción del polvo. Así el doctor don Pedro Francisco Arizmendi subdelegado del partido de Chayanta escribe en 1790 a don Francisco de Paula y Sanz, gobernador intendente de Potosí un informe sobre las causas "principales de la mortalidad y enfermedad extraordinarias que se experimentan entre los mitayos de la provincias contribuyentes a la mita potosina". En este documento Arizmendi reduce las causas para la enfermedad del Chocó a: la falta de descanso con que se obliga a trabajar a los mitayos, los precios excesivos de arriendo de los ingenios -lo que obliga a hacer sobretrabajar a los indios-, y los servicios suplementarios con que se recargan a los indios. Sobre el Chocó dice que: "desde que hay mita en Potosí se conoce esta enfermedad y no se ha empeñado diligencia alguna para

cautelarla". "Hallándose las alas de molienda de los ingenios como inundada de polvos metálicos, antimoniales y envenenados, que continuada e inevitablemente respiran los infelices moledores y cernidores, les ulceran los pulmones y les causan la tos incurable que en su idioma llaman choco, a cuyo impulso mueren unos, se estropean otros, y al cabo todos los atacados de ella son víctimas de estos trabajos en la flor de su juventud o de su virilidad" [15]

Otro documento de la misma época nos da cuenta de los estragos causados por esta enfermedad: "Acabo de llegar de dicho pueblo de Moscari, donde se me presentaron 44 enfermos incurables de choco, todos jovenes de modo que ninguno pasaba de 30 años, 10 han muerto de los que vinieron ultimamente de la mitas y los más, según su aspecto y la tos con que se les advierte, no pueden durar mucho. Este contagio no se había experimentado en dicha parcialidad hasta 30 años a esta parte, en que según el dicho de algunos viejos de los de la mita anterior a dicha época fueron trasladados del trabajo del cerro a dicho ingenio o a su morterado, desde cuyo tiempo empezaron a sufrir el choco destructor de los indios, sin duda por la malísima construcción del dicho morterado..." [16]

Las fuentes coloniales no sólo se expanden en la descripción de esta enfermedad, sino que proponen soluciones (que en algunos casos son de una modernidad asombrosa). Tales males podían ser controlados según Arizmendi si tanto los personeros del gobierno como los médicos que deambulan por la villa recurrían al uso de métodos de ventilación y precaución (como por ejemplo mascarillas de vidrio de cristal) utilizadas entonces en Europa: "Porque no pedir a la mayor diligencia un modelo de aquellas mascarillas a las boticas de España o a las extranjeras y enviarlas a Cochabamba donde se fabricaran en el número que se quiera" [17]

En algunos documentos encontramos mención a las enfermedades cuya causa son el cambio de altura y de clima que experimentan los mitayos que vienen a trabajar a las minas de Potosí: "...a estos fundamentos es que tan terriblemente desventura los gravisimos males que sufren los miserables indios en la mita de Potosí debe reflexionarse que si en general causan tantos estragos quales aseran en los de los pueblos Reales en estar por esencia sujetos a dicha presión por la diferencia de temperamento de estos con aquellos, como que los cinco pueblos, de Capinoza, Tapacari, Sipesipe, Porco y Tiquiparra están situados en valles ardientes y la mutación de sacarlos a la rígida puna de Potosí basta para su aniquilación y por lo duro de aquél trabajo en el morterado y molienda de cuyo cernido resulta un sutil polvo de los metales que les lastima el pecho de modo que los enferman de una tísis incurable que de a poco que regresan a sus pueblos mueren los más de ellos" [18]

Los otros males que encontramos descritos en la fuentes del siglo XVI y XVII son las enfermedades producidas por el contacto con el azogue. En efecto, las minas de azogue de Huancavelica fueron las más peligrosas. La roca que rodeaba el mineral era inestable y blanda, siendo propicio a derrumbes. Pero peor aún era la existencia de gases venenosos en las labores, lo que hacía el trabajo azardoso. El proceso de beneficio también conllevaba numerosos peligros, de los cuales dos eran muy severos. "El primero era al momento de la molienda debido a la absorción de polvos minerales. El segundo estaba presente durante todas las etapas del proceso de amalgamación debido a que los

[15] Archivo Nacional de Bolivia (ANB) (Minas, T. 129, n° III).

[16] Recurso ante la Audiencia de La PLata: El doctor Victorian de Villava, fiscal de ella y protector general de indios del distrito, sobre los tratamientos inhumanos, cargas indel idas y defraudaciones de salarios con que don Salvador Fulla, azoguero nuevo y dueno de minas e ingenios en el asentamiento de Huarihuari,términos de Potosí oprime a los mitayos del pueblo de Pocoata partido de Chayanta que trabajan en dichas haciendas, 1797-1799. (ANB, Minas, 130, n°10).

[17] (ANB, Minas t. 129, n°3).

[18] Testimonio de los informes que a instancias del doctor don Victorián de Villava, fiscal de esta Real Audiencia y protector general de naturales, expedieron don Francisco de Viedlna, gobernador Intendente de Cochabamba, al marqués de Casa Hermosa, gobernador intendente de Chucuito, el doctor Felipe Antonio Martínez de Iriarte cura propio de la doctrina de Chaqui, partido de Porco y Vicario pedanero de Potosí y el doctor don José de Osma y Palacios, cura propio que fue de la doctrina de Moscari, partido de Chayanta sobre los prejuicio s que a los pueblos de indios de dichas circunscripciones se siguen de la mita de Potosí (ANB, Minas t.129, n°VIII).

trabajadores estaban expuestos al envenenamiento por mercurio: en la mezcla del mercurio con el mineral que los trabajadores realizaban con los pies, en la destilación de la pella, en el lavado de la misma, etc" [19]

A propósito de los peligros del azogue, ya hemos citado a Acosta y a Garcilaso, escuchémos que nos dice Solórzano y Pereira en su *Política Indiana* (1648): "... de los daños y enfermedades que se contrahen en las de azogue, como yo lo experimenté en las de Guancavelica donde estuve por visitador y gobernador desde 1616 hasta el de 1619, cuyo solo polvillo hace grande estrago a los que cavan, que alli llaman el mal de la mina; y el baho del mismo azogue a los que le cuecen y benefician los penetra en buen tiempo hasta las médulas, y debilitando todos los miembros, causa perpetuo temblor en ellos, de suerte que aunque sean de robusto temperamento, pocos dexan de morir dentro de quatro años, según dicen Matiolo y Bisciola (2), y antes de ellos Plinio, San Isidoro, Dioscósides y otros (3)" .

En el siglo XVIII, las noticias sobre las condiciones de trabajo, las enfermedades y la mortalidad de los mitayos de Huancavelica nos llegan a través de la polémica sobre la abolición de la mita en esta mina. Los denunciadores de la mita señalaban los estragos que este servicio obligatorio causaba en la población mitaya. Por el contrario, los defensores de la mita señalaban en el decenio de los 30 que los antiguos abusos habían sido corregidos y que las mejores condiciones de trabajo en la mina reducían el número de muertes, ya fuera por envenenamiento a causa del mercurio, ya por otras circunstancias [20]

Para ilustrar el tono de la polémica citemos el testimonio de Jerónimo de Sola, quien fue gobernador de Huancavelica durante trece años, y que fue decisivo en la decisión tomada por el Consejo de Indias: "No se habla ya de los miedos que antes tenían de azogarse y perder la vi da o la salud ; no siendo capaz de negar ning ún desapasionamiento era aquí dictamen corriente no haber piquero, por lo general, que aguantase sin arrojar sangre y azogarse de tres a cuatro años en el trabajo; y ahora se les ve entrar y salir tan robustos al fin de este tiempo como el primer día". En esa misma línea Antonio de Ulloa, otro gobernador de Huancavelica, dice que "en tiempo de mas de tres años que llevo corrido de gobierno, sólo han parecido en la mina 4 ó 5 indios". Ninguno de ellos por enfermedad, sino "despeñados por los cerros, o de la embriaguez a la cual son muy propensos" [21] Está demás decir el cuidado con qué debemos tomar estos testimonios vista su procedencia.

Según anota el historiador M. Molina, "el azogamiento no parecía ser ya en esa época un peligro para los trabajadores de la mina. Los efectos mortales del "umpe", "un ayre que mata de improviso si se respira", no preocupaban ya a las autoridades". "En otra época los piqueros eran sus principales víctimas al inhalar el gas venenoso cuando desprendían el mineral con el pico. Al ser sustituido éste por el barreno tal riesgo había desaparecido. Los únicos casos de azogamiento se producían, no en el interior de la mina, sino en los hornos de beneficio. Aquí los más expuestos eran los indios "oyaricos" y "horneros", los encargados de cargarlos"". Al decir de Ulloa estos casos eran también muy raros. Cuando algún indio contraía el mal, el remedio consistía en "irse a algún paraje donde el temperamento sea caliente y usar de la chicha y de otras bebidas que acostumbran los indios, lo cual les mueve a sudar. Así quedan sanos al cabo de 2 ó 3 meses [22]

Como dice M. Molina (op. cit.), Ulloa, como gobernador de Huancavelica tenía especial interés en restar importancia al espinoso tema de los peligros de la mina. Así resulta poco creíble que durante todo su mandato no se hubieran producido ninguna defunción por azogamiento o que las cuatro o cinco muertes se hayan producido a causa de accidente o de la embriaguez . " No cabe duda de que el indice de mortalidad disminuyó respecto de épocas anteriores y que los mineros cuidaban cada vez a una población más escasa y cara. Pero ello no significa negar la evidencia de un tipo de trabajo

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[19] Bakewell, P., "Mining", in Bethell, L., *Colonial Spanish America*, Cambridge, Cambridge University Press, 1984.

[20] Molina, M., Antonio de Ulloa en Huancavelica, Granada, Universidad de Granada, 1995, pp. 86-87.

[21] Relación e informe que hace Don Jerónimo de Sola..., pp. 18-19; Carta de Ulloa al virrey. Huancavelica, 9 de enero de 1762, AGI, Lima 842. Ambos documentos citados en: Molina, M., op. cit. p. 87-88. Antonio de Ulloa ocupó la gobernación y la superintendencia de Huancavelica entre 1758 y 1764.

[22] Molina, M., op. cit., p. 89; Ulloa, A., *Noticias Americanas*, entretenimiento XIV y Carta de de Ulloa al virrey. Huancavelica,9 de enero de 1762. AGI, Lima, 842.

considerado, en sí mismo, "de gran riesgo" y realizado en un lugar que, en la propia expresión de Ulloa, era intransitable y amenazaba constante ruina" [23]

Para el siglo XIX contamos, para el Perú y en particular para las minas de Cerro de Pasco, con las descripciones de Rivero y Ustariz. Este autor menciona, el caso de los azogados ("Se da este nombre a los paralíticos que han respirado vapores mercuriales refogando la pella"), del mal del Chocó ("Enfermedad que acomete a los operarios de ingenios y tragando ellos el polvo del metal que cuando muele se levanta por el recio golpe del almadeneta, les causa una especie de asma de que mueres"), el humpe ("Aire estancado en las labores por falta de ventilación y que corrompiéndose mata a quien lo respire") y el macurque ("Es una incomodidad corporal que siente al día inmediato de haber entrado en una mina profunda y tenido muchas labores, y que causa dolores por cuatro o cinco días, impidiendo aún el andar"). Este autor también habla del soroche, veta o bochorno: "Se llama así la falta de respiración por lo delgado del aire. Los mineros dicen que proviene de los antimonios de las vetas que cruzan la cordillera". Dice también que: "Se observa que las personas que acaban de llegar y las que no están acostumbradas al temperamento y son debiles de pulmón padecen afección al pecho, faltándoles la respiración cuando se agitan; llámase aquí esto veta (en Lampa y Puno soroche) pues el aire de las vetas que cruzan en los paises minerales son las que producen tal efecto, haciéndose estensiva esta falta de respiración o bochorno, que proviene de la poca densidad del aire por la excesiva altura, hasta los animales que caen muertos, cuando los apuran en las subidas de las cuestas, con pesadas cargas. La enfermedad que acomete a los mineros es la parálisis producida por el tránsito repentino de una temperatura elevada a otra fría, y también por el continuo uso que hacen del azogue. Los que padecen de esta enfermedad se llaman azogados. He visto personas atacadas de parálisis que no podían ni aun ponerse los dedos en la boca, pues muchos de ellos habían tenido que sufrir por algunos ratos la respiración de los vapores mercuriales. Pero la enfermedad más común es la pleuresía ó dolor de costado, y la fiebre pétrida o tabardillo. La primera se cura tomando una infusión de mullaca, yierba de muy pequeña talla, que crece en las cercanías, o con las que llaman hueso muerto. La primera planta es de hojas muy menudas y de una frutilla colorada redondita. La segunda crece en los pastos y sus hojas son blancas y cortas".[24]

Igualmente para el siglo pasado, E. Ruck (1890), habla de un mal que afectaba a los mineros bolivianos. Se trataba del casavi, una especie de tisis: "...En el año 1821 murieron casi 300 en la mina de Salomón y en el camino como 90... otros enfermos contraen el casavi que es ponerse como tísicos".[25]

Enfermedades de los runa en el campo

Regresemos a la época actual y a Angaraes. Los males descritos anteriormente no parecen ser exclusivos de los mineros, si no que los campesinos de las comunidades de Angaraes que entrevistamos comparten también una serie de estos transtornos. Lo cual es lógico, si pensamos que la comunidades de Angaraes con la cuales trabajamos están situadas por encima de los 3200 m.s.n.m.. Algunas de estas enfermedades nos fueron explicadas como castigos de las divinidades de la montaña (Wamani) o de la madre-tierra (Pachamama).[26]

Tomemos el ejemplo de la Ccaiccasca, descrita por los campesinos de Angaraes como un castigo infligido por el Wamani, el dios de la montaña. Los enfermos de ccaiccasca comienzan sintiendo dolores de cabeza insoportables y una falta de apetito que conlleva una pérdida importante de peso. En algunos casos las víctimas de esta enfermedad sufren desvanecimientos, pérdidas de conciencia que se prolongan con cada crisis.

Finalmente, según los campesinos, la persona que sufre de ccaiccasca se vuelve opa (tonto). Como en el caso de los mineros, escuchemos el testimonio de un campesino:

> "Así ha sido mi enfermedad, así pues he estado bien grave. Ha sido cuando era jóven todavía, así dice me dolía la cabeza parece que se me iba a partir así avizando he dicho a mi mujer pero

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[23] Molina, M-, op. cit., 90.

[24] Rivero y Ustariz, Mariano de, *Colección de memorias científicas, agrícolas e industriales*, Bruselas, Imprenta de H. Goemare, 1957, 2 tomos: "Memoria sobre el rico Mineral de Pasco" (1828), tomo I, pp. 182-227; p. 187.

[25] Ruck, E., *Diccionario minero hispanoamericano*, Sucre (inédito), 1890. ANB

[26] Sobre las enfermedades de los campesinos de Angaraes ver Salazar-Soler, 1990.

vuelta no más me he ido a trabajar vuelta he regresado, pero así no más he estado, hambre nada tenía, todo botaba, nada se quedaba en mi adentro, enflaqueciendo no más he estado. Cuando ha sabido venir a verme mi hermano de por ahí de Jauja; diciendo como pues en flaqueci do no más estás, oscuro no más, todo s tus terrenitos seco no má s está, como pues irás a verte al hospital. Yo he dicho que voy a estar yendo al hospital he dicho ya se me pasará; pero peor ha sido, peor ha sido. Así pongase yo estaba conversando con mi mujer, paj ! quedaba tieso, ataque me daba, así no más me daba ataques, tieso quedaba, después claro me recordaba. Así no más ha sido, más peor más ataques me han sabido dar hasta quedar no más opa, opa he quedado, tontito, ni hablar he podido miraba no más, parecía al rato que me recordaba, pero no, malo ha sido esta enfermedad. Así no más como opa he quedado, mi mujer sacaba no más tomar el sol, después ni ha sacado tomar el sol porque he sabido ponerme negro, todito mi cuerpo negro. Así un día que mi mujer ha ido a la tienda a comprar fideos ha estado contando así ha encontrado al curioso saliendo de la tienda, él ha dicho traeras para ver, traeras. Mi mujer ha suplicado diciendo, vendras taytacha a ver a mi esposo vendrás porque él ya no puede andar ha dicho. Ha venido pues, me ha visto ha dicho, vamos a ver diciendo curaremos. Así con el pongo, mi mujer, mi compadre Feliciano más me han llevado quipichado a Pajari, ahí hemos subido. Ahí el pongo ha comenzado a llamar al taytacha Pajari. Hemos llevado su coquita, su cigarro, su traguito, sus caramelitos, ha sido ccaicasca ha dicho" (P.S. campesinos).

Una vez más la descripción de esta enfermedad nos pueden recordar algunos de los síntomas de mal de altura crónico. El informante atribuye esta enfermedad al castigo del Wamani. Según él la divinidad de la montaña estaba disgustada con él porque cuando fue reclutado como mano de obra temporal para trabajar en la instalación de la central eléctrica, los ingenieros le hicieron cavar la montaña sin antes realizar ofrendas a la divinidad.

Como en los casos anteriores, antes de emprender el tratamiento propiamente dicho, hay que asegurarse que se trata de una enfermedad causada por el Wamani, a quien el pongo consulta . Si la respuesta es positiva, se procede al tratamiento. El pongo comienza limpiando el cuerpo del paciente con maíz. Luego le hace beber un brebaje preparado a base de ruda, raíces de sutuma (escorzonera, Perezia multiflora), de hojas de toronjil, de saksa kuti (Guazuma, Theobroma ferruginea) molida v romero. Cuando el paciente está grave se aconseja vendarle la cabeza con ranas y claveles o darle de beber una sopa de cabeza de carnero negro. Se les da de beber también una bebida hecha a base de polvo de ñaupa rumi (piedra de los gentiles, aquellas que se encuentran cerca de los vestigios de los gentiles, son de color gris y se disuelven facilmente) mezclada con cañazo. El tratamiento debe durar tres semanas.

Veamos un segundo caso campesino. "El taytacha Canlalay me ha sonqeado", esta fue la frase utilizada por Eusebio para explicarnos la causa de las desgracias que le aquejaban desde hacía cinco años. Un día Eusebio había subido a un camión para ir a visitar a sus parientes, la carretera estaba interrumpida debido a un deslizamiento de terreno y el chofer había pedido ayuda a los pasajeros para reabrirla. Eusebio rehusó y se subió a dormir al cerro que se encontraba al lado de la carretera. Cuando Eusebio fue despertado por los otros pasajeros para continuar viaje, sintió ya en ese momento un intenso dolor de cabeza, y desde ese día cayó enfermo: tenía mucho sueño, sufría de dolores de cabeza cada vez más intensos y "sentía una pesadez de la cabeza", según él sufría de ataques que se fueron prolongando hasta convertirse en convulsiones. Al final se volvió "sonámbulo": "...Dicen que como upa me he andado todos los terrenitos, así comiendo lo que me daban, así andando como upa, grave me he puesto dicen que me daba ataques, botaba espuma por la boca y mi cuerpo todito mi cuerpo temblaba".

El tratamiento de esta enfermedad es a base de frotaciones, con polvo de maíz para captar la enfermedad y limpiar el cuerpo; y con una mezcla hecha a base de un poco de tierra de donde se cogió la enfermedad y hierbas. También se les da a beber una bebida hecha a base de cañazo, y polvo procedente de piedras de la montaña, de polvo de sonqo rumi (una piedra de color amarillo que se encuentra por lo general, según los campesinos, en las riberas de las lagunas o en los lugares que son considerados como los "ojos del Wamani", los orificios de la montaña) y huesos molidos. Si la enfermedad ha

atacado la cabeza hay que vendársela como en el caso de la ccaiccasca con ranas y claveles rojos.

Comentarios finales

Algunos comentarios para concluir esta presentación. El análisis de los testimonios de los mineros de la provincia de Angaraes sobre sus enfermedades , sugiere una posible coincidencia a nivel de los síntomas entre cierto tipo de males que aquejan a los mineros y el mal de altura crónico. Notemos, que son sobretodo las enfermedades concebidas como castigo de las divinidades, tanto en la mina como en el campo, las que presentan estos síntomas coincidentes. Esta es una hipótesis que necesita ser verificada a través del estudio de un número mayor de casos.

En lo que concierne a la parte histórica, las enfermedades de los mineros descritas hacen alusión sobre todo a problemas respiratorios ligados al cambio de temperatura entre el interior de la mina y la superficie y a la absorción del polvo mineral en la molienda. Las fuentes históricas hacen también referencia a la importancia de la intoxicación con azogue.

Creemos haber demostrado la necesidad de realizar trabajos conjuntos entre antropólogos y estudiosos del mal de altura crónico. Si las encuestas médicas o biológicas son indispensables en el estudio de este mal, las entrevistas con los sujetos del estudio realizadas en el campo y con un seguimiento a largo plazo no son carentes de importancia, pues permiten conocer la percepción que tiene los actores mismos del mal que los aqueja.

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Fuentes primarias

(Archivo Nacional de Bolivia = ANB)

- Recurso ante la Audiencia de La Plata: El doctor Victorian de Villava, fiscal de ella y protector general de indios des distrito, sobre los tratamientos inhumanos, cargas indebidas y defraudaciones de salarios con que don Salvador Fulla azoguero nuevo y dueño de minas e ingenios en el asentamien to de Huarihuari , términos de Potosí oprime a los mitayos del pueblo de Pocoata partido de Chayanta que trabajan en dichas haciendas, 1797-1799. (ANB, Minas 130, n° X).
- Testimonio de los informes que a instancias del doctor Victorían de Villava, fiscal de esta Real Audiencia y Protector General de Naturales,

expedieron don Francisco de Viedma, Gobernador Intendente de Cochabamba, al marqués de Casa Hermosa, gobernandor intendente de Chucuito, el doctor Felipe Antonio Martínez de Iriarte cura propio de la doctrina de Chaqui, partido de Porco y Vicario pedanero de Potosí y el doctor don José de Osma y Palacios, cura propio de la doctrina de Moscari, partido de Chayanta, sobre los prejuicios que a los pueblos de indios de dichas circunscripciones se siguen de la mita de Potosi ANB, Minas t. 129, n° VIII).

-Rück, E., Diccionario minero hispanoamericano, (inédito), 1890, ANB.

MOUNTAIN-RESCUE MEDICINE

MOUNTAIN RESCUE MEDICINE IN THE MONT BLANC MASSIF: 3932 ACCIDENTS IN 8 YEARS.

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RESUMEN: Medicina de Rescate en el Maciso del Mont Blanc: 3 932 Accidentes en 8 años

Se efectúan más de 500 salvatajes cada año en el maciso del Mont Blanc y en más del 60% de casos hay un médico presente. La transmisión rápida de alerta gracias a una red radiofónica sofisticada, la extensión relativamente pequeña del maciso y la ubicación ideal del hospital Chamonix hacen que casi todos los rescates se realicen en menos de una hora. El montacargas del helicóptero permite llevar a cabo rescates en las zonas más inaccesibles y en las épocas más peligrosas. Durante la temporada alta (7 meses al año) un médico de guardia se encuentra permanentemente disponible, listo para unirse al equipo de rescate. Para ser un miembro efectivo del equipo los médicos deben estar entrenados en traumatología y medicina de emergencia, así como ser montañistas resistentes y experimentados. El material médico es robusto pero lo suficientemente ligero y compacto como para ser transportado a cuestas en una bolsa. Las víctimas son sobre todo jóvenes extranjeros de sexo masculino. 15% de los pacientes presentan traumatismo grave y 7% están moribundos en el lugar del accidente. El seguimiento a largo plazo de los pacientes con traumatismo grave muestra que 83% se recuperan bien y que su pronóstico es mejor si el tiempo entre el accidente y la atención médica es breve. La patología más común es el traumatismo moderado de los miembros inferiores. Inmobilización, sedación y analgesia son la base del tratamiento en los accidentes de montaña. Más preocupantes son los traumatismos encéfalo-craneanos y de la columna vertebral, así como las cardiopatías isquémicas. La hipotermia es a menudo una consecuencia de otras lesiones o enfermedades. El tratamiento médico en condiciones adversas debe reducirse al mínimo. La experiencia del médico es esencial, pues debe encontrar el mejor equilibrio entre el tratamiento médico en el lugar y la evacuación inmediata para alejar a la víctima del frío, de la hipoxia y del peligro eventual de una avalancha, deslizamiento de terreno, etc. Si el tiempo es favorable o la evacuación inmediata es difícil, pueden aplicarse todas las técnicas habituales de reanimación. Finalmente, el rol de la prevención es siempre esencial.

Palabras claves: Salvataje en montaña, Medicina de urgencia, Traumatología, Hipotermia, Mal de montaña agudo.

RÉSUMÉ: Médecine de sauvetage dans le Massif du Mont-Blanc: 3 932 accidents en 8 ans.

Plus de 500 sauvetages sont effectués chaque année dans le Massif du Mont-Blanc et dans plus de 60 % des cas un médecin est présent. La transmission rapide de l'alerte grâce à un réseau radiophonique sophistiqué, l'extension relativement réduite du Massif et la position idéale de l'hôpital de Chamonix font que presque tous les sauvetages sont menés à terme en moins d'une heure. L'hélitreuillage permet d'effectuer des sauvetages dans les zones les plus inaccessibles et aux périodes les plus dangereuses. En haute-saison (7 mois par an) un médecin de garde se trouve en permanence à l'hélistation, prêt à se joindre à l'équipe de secours quand l'alerte est donnée. Pour faire partie de l'équipe le médecin doit non seulement être compétent en traumatologie et médecine d'urgence, il doit aussi être un alpiniste résistant et expérimenté. Le matériel médical transporté est robuste, mais suffisamment léger et compact pour être transporté dans un sac à dos. Les victimes sont surtout des jeunes de sexe masculin et souvent d'un pays étranger. 15 % des rescapés présentent un traumatisme grave et 7% sont moribonds sur les lieux mêmes de l'accident. Le suivi sur longue période des victimes de traumatismes graves montre que pour 83 % d'entre elles la récupération est satisfaisante et que le pronostic est meilleur si l'intervalle de temps entre l'accident et les soins est court. La pathologie la plus courante est le traumatisme modéré des membres inférieurs. Immobilisation, sédation et analgésie sont la base du traitement des accidents de montagne. Plus préoccupants sont les traumatismes crâniens et de la colonne vertébrale, ainsi que la cardiopathie ischémique. L'hypothermie est souvent une conséquence d'autres lésions ou maladies. Le traitement médical dans des conditions adverses doit être réduit au minimum. L'experience du médecin est ici essentielle, car c'est lui qui doit trouver le meilleur équilibre entre le traitement médical sur place et l'évacuation immédiate qui éloigne ainsi la victime du froid, de l'hypoxie et d'un éventuel

danger tel qu'avalanche, glissement de terrain, etc. Si le temps est doux ou que l'évacuation immédiate s'avère difficile, toutes les techniques courantes de réanimation peuvent être appliquées. Finalement, le rôle de la prévention est toujours primordial.

Mots clés : Sauvetage en montagne, Médecine d'urgence, Traumatologie, Hypothermie, Mal des montagnes aigu.

SUMMARY: More than 500 rescues are made each year from the mountains of the Mont-Blanc Massif and in more than 60% of these rescues a doctor is present at the scene. The rapid transmission of the alert thanks to a sophisticated radio network, the relatively compact size of the Mont-Blanc Massif and the ideal position of the Hopital de Chamonix mean that almost all rescues are completed in less than an hour. The helicopters' winch allows rescues to be carried out in the most inaccessible and perilous places. During high season (seven months of the year) a doctor is permanently on-call at the heli-pad, ready to join the rescue team at a moment's notice. To be an effective member of the team the doctors must not only be competent in traumatology and emergency medicine but also be strong and experienced mountaineers. The medical equipment carried is robust, light and compact enough to be carried in a rucksack. The rescue victims are predominantely young, male and are often foreign. 15% of patients are severely injured and 7% are pronounced dead at the scene. Long-term follow up of the severely injured patients shows that 83% go on to make a good recovery and that their prognosis is better if the time between accident and medical attention is short. The most common pathology seen is a moderately severe, traumatic injury of the lower limb. Immobilisation, sedation and analgesia prove the basis of treatment in the mountains. More worrying are injuries to the head or spine and ischaemic heart disease. Hypothermia is often a consequence of other injuries or illness. Medical treatment in hostile mountain conditions must be kept to a minimum. The experience of the doctor is essential as it is he who must find the best compromise between medical treatment at the scene and immediate evacuation so removing the victim from the effects of cold, hypoxia and objective danger (avalanche, stone-fall etc.) If the weather is mild or if immediate evacuation is difficult, all the usual resuscitation techniques are possible. Finally, the role of prevention remains essential.

KEY WORDS: Mountain rescue, Emergency medicine, Traumatology, Hypothermia, Acute mountain sickness.

INTRODUCTION

Situated at the foot of Mont Blanc, Chamonix is rightly considered one of the world capitals of mountaineering. In summer, climbers come from all over the world to climb such famous faces as the north face of the Grandes Jorasses or the west face of Les Drus but above all they come to climb MontBlanc. Whilst in winter, the descent of the Vallée Blanche remains the reference in high-mountain off-piste skiing. The activity of the rescue service corresponds with the popularity of the massif with currently more than 500 rescues a year (Figure 1). This paper is based on the 3932 rescues that have taken place over the last eight years.

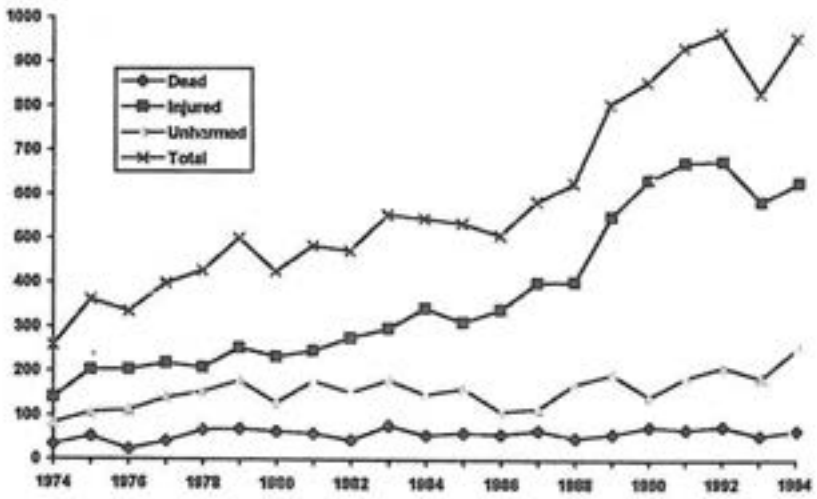

Figure 1 : Activity the last 20 years

THE STRUCTURE OF THE MOUNTAIN RESCUE SERVICE

Initially run voluntarily by mountain guides the rescue service has become more and more complex over the years. Significant developments in the service have often come in response to a major accident, most notable is the story of Vincendon and Henry who in 1956 died of exposure following a badly managed rescue mission, an event which led to the creation of the Peloton de Gendarmerie de Haute-Montagne (PGHM) in 1958 [1].

The present rescue service can be considered in five parts:

1. The helicopter teams of the Gendarmerie and of the Sécurité Civile, who can rescue victims from the most inaccessible of

places, are involved in 95% of all rescues [photos 1&2].

Photo 1

Photo 2

2. The PGHM rescue team members, of whom the majority are guides, deal with the logistics of the rescue including access, rope-work, and evacuation of the patient.

3. The doctors have been regular members of the team since the mid-1980's and are mainly assistants in the Emergency department of Chamonix hospital. In addition to their knowledge of emergency medicine, they are all experienced mountaineers, an essential quality that allows their integration into the team without putting either the team or victim at further risk.

The hospital is ideally situated in the heart of the massif where, at an altitude of 1000m, it is often above the valley fog that prevents the helicopter landing at other lower hospitals. This proximity allows early assessment and treatment to take place. The emergency department with its two resuscitation rooms and heli-pad may seem excessive for a small alpine town but was designed this way to cope with the seasonal influx of patients [photo 3].

Photo 3

5. The ' Société Chamoniarde de Secours en Montagne' (SCSM), is the administrative body behind the mountain rescue service. They supply some medical and technical equipment but above all they finance the radio network, which during the last 10 years has significantly improved both the transmission of the alert and the coordination of the rescue.

Each rescue is coordinated from the PGHM in Chamonix by a member of the rescue team who has an indepth knowledge of massif. The details available to the rescue team as to the state of the victims are often vague and it is often difficult to determine if a doctor is required. This is compensated for by the high percentage (60%) of rescues that have a doctor present, a service that is above the needs of many of the patients.

THE EQUIPMENT

Aside from the mountain rescue equipment used, the medical equipment differs little from that found in a paramedic ambulance.

The stretcher the most frequently used is the 'Perche Piguillem' which is well adapted to winch rescue [photo 1]. The victim is strapped into the stretcher and if necessary can be completly immobilised with neck collar, splints and vacuum stretcher.

A first aid pouch containing syringes, cannulars and IV analgesics is carried around the doctors waist. The doctor's rucksack, developed in conjunction with Lafuma®, is divided into 2 parts, one part for the climbing equipment and one part medical, which further divides into 4 pockets (respiration, circulation, medication and dressing) [photo 4&5].

Photo 4

Photo 5

A standard range of medication is carried with an emphasis towards intra-venous analgesia, sedation, rehydration and coronary artery dilatation.

Vital signs can be monitored at the scene including a cardiac trace by a miniscope the size of a calculator, temperature by an epi-tympanic thermometer and oxygen saturation by a mini pulse oxymeter.

The medical equipment is completed by a resuscitation rucksack containing oxygen, suction, a respirator (Oxylog®) and a defibrilator (Leardal®) all of which are compact and robust

THE GATHERING OF DATA

Each rescue victim has a medical record card on which is recorded their personal details along with the time, place, and circumstances of the accident, the injuries sustained (WHO classification), the state of the patient and the treatment given at the scene (Figure 2).

FICHE MEDICALE SECOURS EN MONTAGNE CHAMONIX

Date Heure Appel Heure Secours

Météo 1.Beau 2.Mauvais Hélico Carav Pc. en charge : 1.Lieu 2.DZ 3.Sans

Alerte : 10.accident 11.accident grave 12.médical 13.médical grave 14.mort 15.égaré 16.recherche 17.évacuation 18.sans précision 19.découverte fortuite

IDENTITE

Nom Prénom Sexe

Date de naissance Nationalité

ACTIVITE

Lieu Altitude

Rando/Prom Ski Alpinisme Parap Delta Vélo Rafting

Cetation Montagne F PD AD D TD ED Ski : F D ED

Circonstances (cocher autant de numéros que nécessaire)

30.Fracher 34.Ski plate 38.Cetation 42.Dévissage 46.Rappel
31.Glace 35.Hors piste 39.Chute Tété 43.Chute pierre 47.Foudre
32.Neige 36.Ski Montag 40.Abertos 44.Chute sérac 48.Pb Techn
33.Mixte 37.Ski Fond 41.Décollage 45.Avalanche 49.Crevasse
50.Autres (préciser) 51.Surf 52.Noyade

Difficulté du secours 1.facile 2.facile et danger 3.difficile 4.difficile et danger

BILAN MEDICAL

	1-4	CONTUS	ENTORS	LUXAT	FRACT	PLAIE	OBLURS	MEDIC
CRANE		10	—	—	13	14	—	16
O.R.L		20	21	22	23	24	25	26
RACHIS		30	31	32	33	34	35	36
THORAX		40	—	—	43	44	—	46
ABDOMEN		50	—	—	—	54	—	56
BASSIN		60	—	—	63	64	—	66
MBRE SUP		70	71	72	73	74	75	76
MBRE INF		80	81	82	83	84	85	86

Gravité initiale : 1.Indemne 2.modéré 3.grave 4.risque vital 5.mort

Polytraumatisé Glasgow Oeil(4-1) + verbal(5-1) + moteur(6-1) =

RESUME CLINIQUE

MISE EN CONDITION

Injection : IM IV SC Abord périphérique Abord central

Intubation Défibrillation Drainage thoracique Immobilisation

Thérapeutiques

EVACUATION

Evacuation 1.coquille 2.barquette 3.Piguillem 4.assis

Treuillage Heure arrivée DZ Hôpital receveur

Evolution 1.stable 2.amélioré 3.aggravé 4.décédé

MEDECIN : Dr

Figure 2 : Medical records card

EPIDEMIOLOGY

The population rescued are young with an average age of 32 years (Sd 13), predominantly male (71%) and often foreign (37%). February, March, July and August are the busiest months, together accounting for 65% of all rescues (Figure 3). The activity of the rescue team is spread evenly throughout the working day with a slightly earlier start in summer.

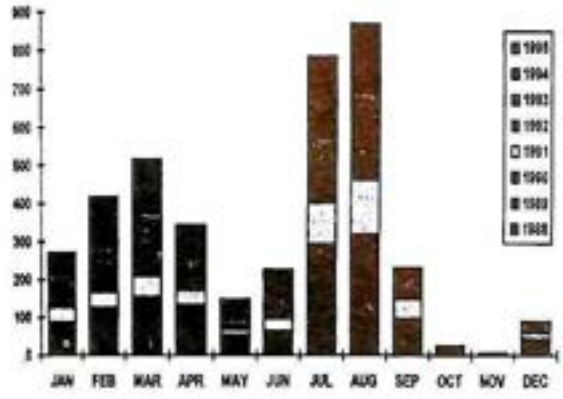

Figure 3 : Monthly distribution (1988 - 1955)

The rescues are generally rapid, with an average time between the alert and the return to the valley of 58mins (Sd 10). The speed of the rescue is aided by the relatively compact size of the massif (25km by 12km), by the central position of the hospital and by the brief and rapid approach to treatment.

The average altitude of rescue is 2406m with 24% taking place above 3500m. The high number of people that ski the Vallée Blanche in winter and climb Mont-Blanc in summer makes these two areas the most common sites for rescues in the massif.

40% of all victims are from ski accidents (half of them on piste) and 44% are due to climbing. However traumatic deaths are four times more common amongst climbers than amongst skiers and are mainly due to falls or stone fall. Hiking in the lower mountains of the massif accounts for 14% of rescues and involves a more standard population. Finally, although paragliding is an increasingly popular sport it accounts for only 2% of all accidents.

SOCIO-CULTURAL FACTORS

Fashion

Mountaineering is and remains a dangerous sport, however recently a worrying trend is emerging. With the increasing popularity of bolted rock climbing, more and more climbers are heading into the mountains with the ability to climb hard rock but with few of the skills necessary to deal with glaciers or mixed ground. With the dangerous result that many climb without a helmet or move on crevassed glaciers unroped. On the other hand, a rope badly used can increase the risk of an accident for example when a climber falls and pulls off the other climber.

The mystique

Each year a number of climbers, inspired by the reputation of Mont-Blanc and encouraged by the media as to the facility of its ascent, choose to ignore the risks and climb it with a minimum of experience. Some succeed, many fail and sadly some have serious accidents (amputations following a winter ascent in light shoes) or even die (diabetic keto-acidosis when a climber was caught out in bad weather with limited supplies).

The other side of the coin

In recent years, the generous nature of a free rescue service has sadly begun to be abused. Occasionally, climbers demand a rescue where in the past they would not have thought to do so. Without doubt they are encouraged by the ease of calling for help with a radio and are used to seeing the helicopter in the massif. Because of the overall increase in the number of rescues, the number of cases of minor trauma or even simple fatigue are increasing. This problem is important as each rescue must be balanced against the risk to the rescue team and the expense of the mission.

PATHOLOGY

The majority of the pathology seen in mountain accidents is traumatic (90%) and can be graded from 1 to 5. Of the 3932 victims rescued, 10% were unharmed (grade 1), 68% had an injury of moderate severity e.g. fracture/dislocation of a long bone (grade 2), 12% were severly injured e.g. fracture of the spine (grade 3), 3% were in a serious state e.g. haemorrhagic shock or profound hypothermia (grade 4), and 7% were dead on rescue (grade 5). It is interesting to note that men account for 83% of all seriously injured patients.

Traumatic injury of the lower limb is common, accounting for 35% of all rescues, although it

rarely leads to serious complications (Figure 4). More worrying are the 22% of victims that present with a head injury, often due to stone or ice fall or to a fall by the climber. 10% of those rescued have a spinal injury, 12% of whom go on to have some residual impairment. 13% of victims have multiple trauma. In many of these neurological signs, hypothermia and haemorrhagic shock are often intricated and it can then prove difficult to distinguish the cause.

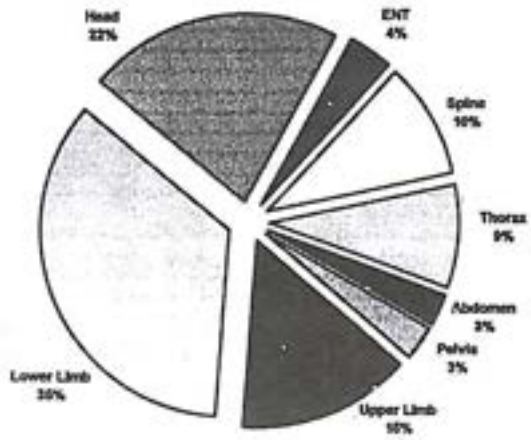

Figure 4 : Traumatic injuries (n = 3932)

At this point it should be stated that hypothermia is frequently secondary to other injuries (78% in a series of 89 cases in 6 years) and less often a pure phenomenon. Finally, although always present in the mind of climbers, lightening strikes are rare with only 2 or 3 cases a year.

Medical problems account for only 10% of rescues amongst which two pathologies are common:

- Coronary artery insufficiency is often exposed in men still in their fifties who are taken quickly to altitude by cablecar and are then very active. This leads to numerous cases of chest pain and around 5 myocardial infarcts a year.
- Acute mountain sickness (25 cases a year) usually begins after a symptom free period of 4-6 hours above 3500m. Although often limited to headaches, shortness of breath and nausea, it can however be serious. This condition is often misjudged by mountaineers who only associate it with expeditions to very high altitude.

TREATMENT

A doctor is present at the scene in 60% of all rescues and in 93% of rescues of seriously injured victims. Some serious cases are still rescued without a doctor either because the details of the accident were vague or because of dangerous flying conditions where a minimum team is carried.

The treatment given in the mountains depends on the state of the patient and on the environment. It is unrealistic to expect to be able to perform advanced resuscitation in the hostile conditions often encountered in the mountains. Therefore after an initial assessment of the patient, the aim is to remove the victim from the effects of cold, hypoxia and objective danger (stone-fall, avalanche etc.). From our experience it is apparent that at 4000m in summer, an adult climber, well equiped but seriously injured (internal bleeding or a head injury) can drop his temperature to 30°C in less than one hour. From this it is clear that only essential treatment should be given in the mountains and that the rest be left to the hospital. The experience of the doctor in this initial assessment is essential, as it is his role to find the compromise between optimal treatment and rapid evacuation.

Treatment in the mountains

Clinical examination of a patient in the mountains is always difficult because of the amount of clothing and equipment worn by mountaineers.

If the air temperature is warm and if immediate evacuation of the patient is not possible, then more advanced resuscitation techniques are possible (central venous line, intra-thoracic drain etc.) without forgetting that the doctor must perform them alone. If the conditions are less favourable one must limit oneself to essential acts such as immobilisation, analgesia, sedation, compressive dressings, intubation and cardiac resuscitation.

The securing of venous access although preferable must not delay the rescue. We rarely put up an infusion because of the difficulty of guarding the line open in extreme conditions and use the catheter mainly for injection of intra-venous drugs. Hypovolaemia can sometimes be dealt with in the field simply by raising the patient's legs. The preferred rehydration fluid is the plasma expander hydroxyethylamidon, as it is more resistant to the cold than the gelatins. In the future we hope to be able to use the new hypertonic 7% saline solutions which have a good relationship between efficacy and weight.

Finally, in the case of an overland evacuation, it is better, if possible, to allow the victim to participate in their own rescue. Patients can often walk with a fractured arm or skull if they have sufficient pain relief.

Rescue and Evacuation

Although very well suited to alpine rescue, the single engined Alouette III faces two technical restrictions in winch rescues (44% of all rescues). Firstly, the stretcher can only be brought on board if it is inclined at an angle of 50°, a manoeuver which is obviously incompatible with a patient who is haemodynamically unstable [photos 1&6]. In such a case the stretcher is winched in a horizontal position to a flat area where it is landed, and from there loaded onto the helicopter. Secondly, as only one person can be winched at a time the doctor is taken up first, followed by the patient.

Photo 6

The cockpit of the Alouette III is cramped and it is our practice to load the stretcher in transversely with the doctor sitting above the patient. Therefore every drug that the doctor may want to use during the flight must be prepared before take off and carried in the hand. In such circumstances the pursuit of a cardio-vascular resuscitation would prove difficult. These inconveniences would disappear with the introduction of a new generation of helicopter which each year is held back for financial reasons.

Finally, in the case of hypothermia, the transport is the period of greatest danger and must be performed gently yet quickly. Also one must be particulaly careful when moving the patient on and off the stretcher to not set off a ventricular fibrilation

The first few hours

The resuscitation and management of a victim of mountain trauma is the same as for any other victim of trauma, however two points specific to the mountains need emphasising.

- Sliding down a snow slope can lead to internal injuries which are not always obvious on initial examination. For these patients abdominal ultra-sound on admission is systematic.
- All multiple trauma cases should be suspected to be hypothermic and the taking of core temperature systematic. Reciprocally, as hypothermia is most often seen secondary to other injuries, a hypothermic patient is presumed to have other injuries until proved otherwise and rehydration remains thats hasis of resuscitation [2].

Transfers

Despite the amount of mountain related medicine that it sees, Chamonix Hospital is still essentially a small hospital. For patients with serious injuries, specifically of the head, spine or thorax, it allows resuscitation and stabilisation before transfer to a specialist centre. For some patients, for example those with internal haemorrhage, this service requires great resources which are proving more difficult to finance each year.

OUTCOME

Long-term follow up of trauma victims shows that of the patients comatosed following head injuries, three quarters go on to make a full recovery. Of those that die from head injuries more do so from haemorrhagic contusions with oedema than from pure haematomas which are usually drained successfully. Spinal injuries can be well immobilised once the patient is on the stretcher, however sometimes irreversible damage is already done. The prognosis for victims of multiple trauma is generally good unless they have a significant head injury.

Follow up of those presenting with medical problems shows that patients with pure hypothermia and a cardiac output, even if the hypothermia is profound, have a good prognosis; that the prognosis of patients with a myocardial infarction in the mountains, as elsewhere, depends largely on the rapidity of treatment; and that all patients suffering from acute mountain sickness recover fully, only occasionally needing a short stay in hospital.

A follow-up study of 145 serious (Grade 3&4) victims of mountain accidents [3] showed that 83% went onto make either a good or complete

recovery, 5% recovered poorly, and that 12% finally died. The total mortality, before and after rescue is 9%, or around 50 per year. They have an average age of 32 years and are predominantly male (87%). This same study showed that the less the delay between accident and rescue and the less the time of the medical intervention, the better the outcome (p=0.006). This result is all the more important as it applies to a population predominantely young and male for whom rapid recuperation has important economic implications.

The presence of a doctor in the rescue team is clearly beneficial and this is supported by the fact that the number of deaths each year has not increased despite the fact that the number of rescues has increased by approximately 9% per year since 1974.

CONCLUSION

Within Chamonix's prestigious and popular massif one finds much mountain related trauma, rescue team members who are guides, doctors who are experienced mountaineers and a highly equipped hospital at the foot of the mountains. Everything is there to maintain and justify an integrated rescue service of the highest standard which is capable, in the majority of cases, of managing the patient from the scene of the accident through hospital to their return home.

Once the alert is received, the rescue almost always goes ahead in a satisfactory manner. Therefore, to reduce mortality in the Mont-Blanc massif one must concentrate on the time before the alert is received and specifically on two areas. Firstly, to encourage the more widespread and more responsible use of radios; and secondly and above all, to promote a higher level of safety in the mountains. For as ever, the prevention of accidents by education and training is essential.

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HIGH ALTITUDE ADAPTATION

PHYSIOLOGICAL ADAPTABILITY, THYROID FUNCTION,BODY COMPOSITION AND GENETIC VARIABILITY IN CENTRAL ASIA HIGH ALTITUDE POPULATIONS.

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RESUMEN: Adaptabilidad Fisiológica, Función Tiroidea, Composición Corporal y Variabilidad Genética en Poblaciones de Altura de Asia Central

Se recogió datos sobre adaptabilidad fisiológica a la altura, función tiroidea, composición corporal y variaciones genéticas, durante el programa CAHAP (Central Asia High Altitude People). Se estudió a más de 400 varones sanos de cuatro poblaciones diferentes: una muestra de la aldea Sary Tash, Kirghiz, 3200 m (HA); una muestra de Talas, Kirghiz, 900 m; una muestra de Kazakh, valle de Keghen, monta_as de Tien Shan, 2100 m (MA); y una población de baja altura (LA), Uighur, 600 m. En este artículo se examinan veinte variables fisiológicas y somatométricas. Los valores de hemoglobina y eritrocitos son significativamente mayores en las muestras de monta_a que en las de baja altura. Las variaciones en los volúmenes pulmonares son controversiales. No se observó diferencias significativas en los indicadores de función tiroidea (T4 libre y hormona estimulante de la tiroides) entre el grupo HA Kirghiz y el grupo LA Kirghiz. Los resultados sugieren la presencia de adaptaciones fisiológicas a la hipoxia hipobárica en el grupo HA Kirghiz así como en el grupo MA Kazakh. La menor adiposidad de los del grupo MA Kazakh comparada con la del grupo La Uighur para relacionarse con el estrés asociado a al ambiente de altura asi como al estilo de vida. El agua corporal total y el agua extracelular, que se predijeron por los métodos de dilución y de impedancia bioeléctrica, mostraron alta correlación, sugiriendo la posibilidad de del uso confiable de las fórmulas predictivas desarrolladas en sujetos caucásicos. Se analizó los sistemas de grupos sanguineos, las isozimas y los polimorfismos de proteinas séricas con métodos multivariantes. Los mapas genéticos obtenidos usando el análisis de componente principal muestran la centralidad de los Uighurs, Kazakhs y Kirghiz dentro de la amplia variabilidad genética de las poblaciones asiáticas, en concordancia con su centralidad geográfica y la historia compleja del poblamiento de la región.

Palabras claves: Altura, Asia Central, Kirghiz, Kazakh, Uighur, Fisiología, Tiroides, Composición corporal, Antropometría, Variabilidad genética.

RÉSUMÉ: Adaptabilité physiologique, fonction de la thyroïde, composition corporelle et variabilité génétique des populations de haute altitude d'Asie Centrale.

Des données sur l'adaptabilité physiologique à la haute altitude, la fonction de la thyroïde, la composition corporelle et la variabilité génétique ont été recueillies au cours du programme de recherche CAHAP (Central Asia High Altitude People). L'étude a porté sur des sujets sains de sexe masculin, appartenant à 4 populations différentes : un échantillon Kirhgiz de haute altitude (HA) du village de Sary Tash, Pamir (3 200 m); un échantillon de référence Kirghiz de Talas (900 m); un échantillon Kazakh de moyenne altitude (MA) de la vallée de Keghen (montagnes du Tien Shan, 2 100 m) et une population Uighur de basse altitude (LA) (600 m).

Dans cette étude ont été examinées vingt variables physiologiques et somatométriques. Dans les échantillons de montagnes les valeurs d'hémoglobine et d'érythrocytes sont nettement supérieures à celles des échantillons de basse altitude. Les variations de volumes pulmonaires sont controversables. On n'a pas observé de différences significatives dans les indicateurs de la fonction thyroïdienne (T4 libre et hormone stimulante de la thyroïde) entre le groupe Kirghiz HA et le groupe Kirghiz LA. Les résultats suggèrent la présence d'adaptations physiologiques à l'hypoxie hypobare dans le groupe Kirghiz HA, ainsi que dans le groupe Kazakh MA.

Il semblerait que l'adiposité moindre des sujets du groupe Kazakh MA en comparaison avec ceux du groupe Uighur LA soit à mettre en relation avec le stress associé à l'environnement montagneux, aussi bien qu'avec le style de vie. L'eau corporelle totale et l'eau extracellulaire, prédites par les méthodes de

dilution et d'impédance biolélectrique ont montré une forte corrélation, suggérant la possibilité de l'emploi confiable des formules prédictives développées chez les sujets caucasiens.

Les systèmes de groupes sanguins, les isozymes et les polymorphismes de protéines sériques ont été analysés par des méthodes multivariantes. Les cartes génétiques obtenues par analyse du composant principal montrent la position centrale des Uighurs, des Kazakh et des Kirghiz au sein de l'ample variabilité génétique des populations asiatiques, en accord avec leur position géographique centrale et l'histoire complexe du peuplement de la région.

Mots-clés : Haute altitude, Asie Centrale, Kirghiz, Kazakh, Uighur, Physiologie, Thyroïde, Composition corporelle, Anthropométrie, Variabilité génétique.

SUMMARY: Data on physiological adaptability to high altitude, thyroid function, body composition and genetic variations were collected during the CAHAP (Central Asia High Altitude People) research program. More than 400 healthy adult males from four different populations were studied: a high altitude (HA) Kirghiz sample of the Sary Tash village in Pamir (3200 m); a reference Kirghiz sample from Talas (900 m); a middle altitude (MA) Kazakh sample from the Keghen valley (Tien Shan mountains, 2100 m) and a lowland (LA) Uighur population (600 m).

Twenty physiological and somatometric characters are examined in the present report. There are significantly higher values of hemoglobin and erythrocytes in the mountain samples than in the lowland ones. The variations in lung volumes are controversial. No significant differences in thyroid function indicators (free T4 hormone and thyroid stimulating hormone) were noted between HA Kirghiz and LA Kirghiz. Results suggest the presence of physiological adaptations to hypobaric hypoxia in HA Kirghiz as well as in MA Kazakhs.

The lower adiposity in MA Kazakhs than in the LA Uighurs seems to be related to stress connected to the mountain environment as well as to the lifestyle. Total body water and extracellular body water, predicted by the dilution and bioelectric impedance methods, are highly correlated, suggesting the possibility of the reliable use of predictive formulae developed on Caucasian subjects.

Blood group systems, isozymes and serum protein polymorphisms were analyzed with multivariate methods. The genetic maps obtained using Principal Component analysis show the centrality of Uighurs, Kazakhs and Kirghiz within the wide genetic variability of Asian populations, in agreement with their geographic centrality and the complex history of the peopling of the region.

Keywords: high altitude; Central Asia; Kirghiz; Kazakh; Uighur; physiology; thyroid; body composition; anthropometry; genetic variability.

INTRODUCTION

The study of human adaptability to high altitude (HA) in Central Asia populations is an interesting field of research. In fact, there is little information on this topic in the international literature in comparison with the data available for other HA populations, such as Andean Aymaras and Quechuas, Ethiopian Ahmara, and Tibetans, Han, Bothia and Sherpas in the Tibeto-Himalayan region.

Although some studies have been promoted by the Kirghiz Institute of Cardiology in Bishkek and by the Institute of Anthropology of the Moscow State University, many aspects of the biology of HA peoples of Tien Shan and Pamir are still unexplored. Moreover, the results of these studies have been published mainly in Soviet or former Soviet national journals. An interesting, but certainly not recent, general review of HA adaptation in the Pamir and Tien Shan mountains was published in "The biology of HA peoples" (Baker ed.) (1978) by Mirrakhimov, who briefly summarized the results of several studies performed by his research group and by many other Soviet investigators. In the same IBP volume, Frisancho (1978) discussed data on the growth of children in the Tien Shan mountains collected by Psyzuk et al. (1967) and by Miklasheveskaya et al. (1972). Miklasheveskaya et al. (1979) published a review of their studies of HA human growth in "Physiological and morphological adaptation and evolution" edited by Stini.

Subsequently, numerous papers have been published but mainly in Russian (Mirrakhimov, 1972, 1978; Mirrakhimov et al., 1981, 1985, 1987, 1988; Mirrakhimov and Ibraimov, 1982; Ibraimov and Mirrakhimov, 1979; Ibraimov et al. 1990; Aitbaevet alP, 1992; Aldashev et al., 1989; Aliev et al., 1993; Daniiarov et al., 1982, 1992; Episkoposyan et al., 1994; Khmelnitskii et al., 1991; Reshetnikova et al., 1991,1994; Tulebekov et al., 1977). In 1983 an interesting new edition of the 1977 "Adaptive reactions in human populations" (edited by Alexeeva) was published, which also contains an overview of HA adaptation studies. Unfortunately, the data reported is not always useful for comparative studies on account of the peculiar topics investigated and the different methods of data collection and analysis.

The CAHAP (Central Asia High Altitude People) research program has been promoted in collaboration with the Laboratory of Anthropology of the Academy of Science of Kazakhstan. The aim of the project is the collection and analysis of new anthropological and genetic data in HA Central Asia populations in order to have results directly comparable with the information already present in the international literature on other HA peoples and in particular with the original information collected in the Peruvian Andes with the same methods and instruments (Tarazona-Santos et al., present issue). The main objectives of the CAHAP Program are

the study of human adaptability to HiA, body composition, nutrition and genetic variability in Kazakhs of the Tien Shan mountains and in Kirghiz from Pamir. Two Italo-Kazakh expeditions were carried out in 1993 in Kazakhstan and 1994 in Kirghizstan in order to collect new information on these topios.

In the present communication we would like to describe our experimental design, to synthesize the obtained results and to give some preliminary indications regarding further analyses currently in progress.

MATERIAL AND METHODS

Kirghiz, Kazakhs and Uighurs are Turkich-speaking (Altaic linguistic family) populations that settled in the Pamir and Tien Shan mountain ranges during the last 4-5 centuries. From an anthropological point of view they have been defined as TurkoMongolic populations (Alexseev and Gochman, 1983).

In the present research, the high altitude (HA) Kirghiz population of the Sary Tash village in Pamir (3200 m) is compared with a lowland (LA) Kirghiz reference sample from Talas (900 m). To detect possible gradients of human adaptability, data were also collected on middle altitude (MA) Kazakhs from the Keghen valley (Tien Shan mountains, 2100 m) and in a lowland (LA) Uighur population from the Pendjim village (600 m), in Kazakhstan.

Sary Tash is a HA village a few kilometers from the border with Tajikistan, in the heart of the Pamir mountains. The local population numbers around 1500 and is largely concentrated in the village or scattered in outlying areas. It is extremely isolated, especially during the long winter months when it is cut off by snow. Hygiene is primitive and food supplies are scarce: there is no sewer system or water mains. The local economy, based on grazing sheep and goats or raising yaks and horses moved to the mountain pastures during the summer, offers bare survival. Only a small amount of food is imported.

The village of Talas is in the most northerly section of Kirghizstan, along the trade routes to Kazakhstan and Uzbekistan, important since ancient times (The Silk Route). The health and hygiene situation is fair and the local economy, based on farming and cattle raising, ensures complete and abundant food supplies. Numerous bazaars and local markets provide good product distribution.

The Keghen valley is on a high plain a few kilometers from the northern slopes of the Tien Shan range. Although isolated, especially during the winter months, it is a rather hospital place for human settlements. The local economy is mainly based on grazing sheep, goats and horses but also on farming.

The village of Pendjim is in the most eastern section of Kazakhstan, only 18 km from the boundary with China. It is inhabited mostly by Uighurs, who emigrated from the Xing-Chang (Chinese Uighur autonomous region) in recent decades. The local economy is based on farming and cattle raising while some bazaars provide products from China and from the rest of Kazakhstan.

After a preliminary medical analysis, more than 400 fully healthy subjects were studied. They were unrelated adult males, native to the study area. In particular, data were collected during the summer of 1993 from 123 Kazakhs from the Keghen valley and 80 Uighurs from the Pendjim village, while in the summer of 1994, 114 Kirghiz from the Sary Tash village and 91 Kirghiz from the Talas plains were examined.

The following physiometric, anthropometric and genetic variables, selected from the wider information collected during the CAHAP expeditions, are investigated in the present report:

1. ***Adaptability to high altitude.*** Forced Expiratory Volume (FEV) and Forced Expiratory Volume in one second (FEV1) were assessed with a Vitalograph Alpha spirograph, while hematological parameters (hemoglobin, erythrocytes and haematocrit) were measured with an Emo-Flash photometer (Menarini, Florence, Italy). The main somatometric characters involved in adaptability to HA were measured according to the Anthropometric Standardization Reference Manual of Lohman et al. (1988). Thyroid function by concentration analysis of free T4 hormone (FT4) and thyroid stimulating hormone (TSH) was studied in relation to hypoxia and to phenylthiocarbamide (PTC) taste sensitivity (by the Harris and Kalmus method, 1950).

2. ***Body composition.*** Eleven anthropometric variables related to body composition and in particular to adiposity (skinfolds) were measured following Lohman et al. (1988). Total body water (TBW) and extracellular water (ECW) were assessed by D2O and NaBr dilution, respectively. Fat patterning was analyzed by multiple frequency

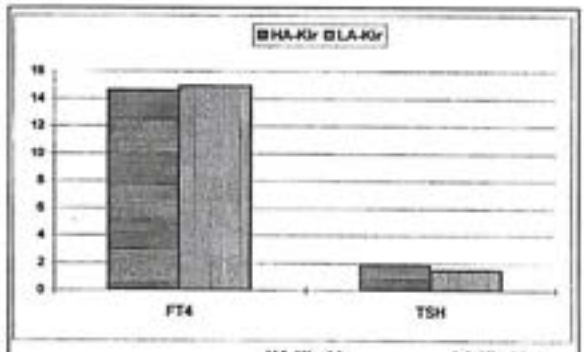
bioelectrical impedance (Human IM Scan tetrapolar impedance plethysmograph, Dietosystem, Milan, Italy).

3. ***Genetic variability***. The distribution of more than 20 "classic" genetic markers and of several mt-DNA (d-loop region) and n-DNA polymorphisms (microsatellites) was examined. In particular, a multivariate analysis of the distribution of blood group systems (ABO, Rh, MNSs, Kell, P, Duffy, Kidd and Diego) is presented in this survey.

RESULTS AND DISCUSSION

1. Adaptability to high altitude

There were no significant differences in thyroid function indicators (FT4 and TSH) between HA Kirghiz and LA Kirghiz (Figure 1). Moreover, no relationships were observed between the PTC Taste Sensitivity distribution and the thyroid function (Facchini et al., 1997).



	HA-Kirghiz			LA-Kirghiz		
	N	Mean	S.D.	N	Mean	S.D.
FT4 (pg/mL)	78	14,6	2,17	70	14,90	2,72
TSH (mIU/mL)	78	1,82	1,11	70	1,49	0,92

Fig.1 Free T4 hormone (FT4) and thyroid stimulating hormone (TSH) values in high (HA) and low (LA) altitude Kirghiz. Differences are not statistically significant.

The discussion of the relations between the hypoxic environment and thyroid function i s interesting because the topicbis still controversial and few is the available data . Many studies have supported the idea that acute, but also chronic (Ramirez et al., 1995), exposure to HA could produce a marked elevation of plasma T3 and T4, while having no influence on (Sawhney et al., 1991) or causing a reduction of (Basu et al., 1995) Thyroid Stimulating Hormone (TSH). More in general, many authors have observed that HA hypoxia could induce an increase in thyroid function (Chakraborty et al. 1987). Nevertheless, studies on animals reported by Frisancho (1993) show that HA exposure leads to reduced thyroid function, perhaps related to a deficient secretion of TSH or a decreased requirement for T4 (and hence a concomitant reduction of hormone synthesis). Also Gambert (1991) supports this last hypothesis and notes that HA populations in the Andes, whose diets are deficient in iodine, have less goiters than people with similar diets living at lower altitude. This should imply that there is an altitude-related increase in T4 sensitivity and thus a decreased requirement for T4.

Finally, during acclimatation to hypoxia, several investigators have reported initial increases in serum T4 and T3 concentrations at HA, followed by decreases toward normal with continued exposure, while TSH levels were normal (Blume, 1984; Ward et al., 1989).

As regards Central Asia, studies carried out in the former USSR (Tien Shan and Pamir Mountains) have shown that healthy local HA populations have a hypofunction of the thyroid gland (Miklashevskaya et al., 1979). Our data are consistent with these observations, suggesting a slightly decreased or normal function of the thyroid gland in HA environments.

In conclusion, studies on humans in acute hypoxia have suggested a temporary increased thyroid function whereas those on humans and animals in chronic hypoxia have indicated decreased or normal function of the thyroid gland. Nevertheless it should be considered that there are two complicating factors in the interpretation of thyroid response to HA: a deficiency of iodine typical of the mountain habitat and the general influence of a cold environment on thyroid function.

As argued by Heat and Williams (1995), both these variables are much easier to control in experimental animals that in humans. In addition, in the study of acute exposure to HA, several experiments have been performed on a limited number of subjects (soldiers or mountain-climbers) but not on large samples (Chakraborty et al. 1987; Blume, 1984; Mordes et al. 1983). There are probablytwo or more different adaptive physiological responses to hypoxia based on the type of exposure (acute or chronic).

Although data analysis of the hematological and pulmonary variations is still in progress, most of the observed values indicate significant physiological adaptive responses to hypoxia both in the MA and HA samples.

In particular, the hematological parameters appear to be altitude-sensitive also at 2100 m. and then they increase at 3200 m. While MA Kazakhs and HA Kirghiz have higher hematological values than

LA Uighurs and LA Kirghiz, no differences were found between MA Kazakhs and HA Kirghiz (Figure 2). The values seem consistent with those reported in the review by Mirrakhimov (1978).

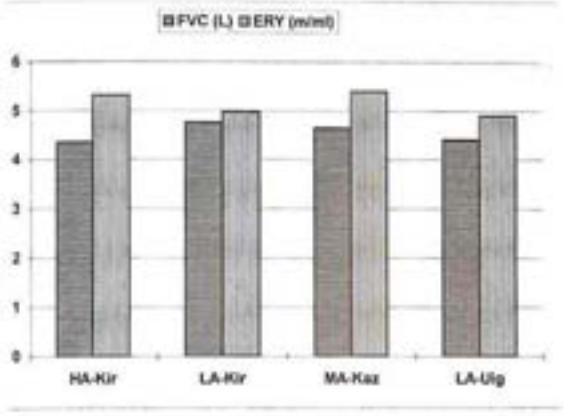


Fig.2 Forced Vital Capacity (FVC) and number of Red Cells (ERY) in the 4 samples: High Altitude Kirghiz (Sary Tash, 3200 m) and Low Altitude Kirghiz (Talas, 900 m), Middle Altitude Kazakhs (Keghen valley, 2100 m) and Low Altitude Uighurs (Pendjim, 600 m). For ERY and FVC the differences are significant ($p<0.001$).

Lung volumes are higher in MA Kazakhs than in LA Uighurs, while the two groups are similar in somatic and chest dimensions. The comparison between HA Kirghiz, LA Kirghiz and MA Kazakhs is complicated by the effects of nutritional stresses in the HA Kirghiz population. HA Kirghiz in fact exhibit lower somatic and chest dimensions than the LA Kirghiz and MA Kazak samples which are characterized by a proper nutrition and a favorable environment (Figure 3). Lung volumes are also lower in HA Kirghiz than in LA Kirghiz and MA Kazakhs (Figure 2).

2. Body composition

The LA Uighurs have higher values than MA Kazakhs for all the characters related to body composition (Table 1). The lower adiposity in MA Kazakhs than in the LA Uighurs seems to be related to stress connected to the environment as well as to the lifestyle (Facchini et al., in press). The data on the HA Kirghiz and LA Kirghiz are still being analyzed, but a clear decrease in adiposity is present in the mountain sample, confirming the effects of the unfavorable HA environment. In fact, the body composition analysis reveals different fat patternings in relation to the different nutritional environments and climatic stresses (hypobaric hypoxia, cold, etc.).

TBW and ECW were assessed in a subsample of 28 MA Kazakhs both by D2O and NaBr dilution, respectively, and by multiple frequency bioelectrical impedance (BI) at 1 and 100 kHz, respectively; formulae developed on a sample of Caucasian subjects with a hydration status similar to that of the study population were applied. TBW and ECW predicted by the two methods are highly correlated and not significantly different. These results suggest that the selected predictive formulae developed on Caucasian subjects may provide a precise and accurate assessment of ECW and TBW in Turko-Mongolic populations (Battistini et al., 1995)

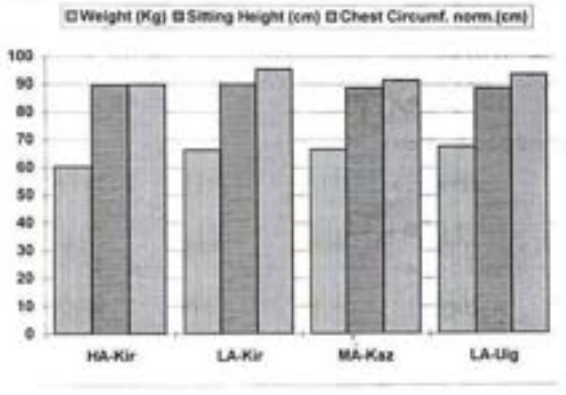


Fig.3 Selected anthropometric variations in the 4 samples: High Altitude Kirghiz (Sary Tash, 3200 m) and Low Altitude Kirghiz (Talas, 900 m), Middle Altitude Kazakhs (Keghen valley, 2100 m) and Low Altitude Uighurs (Pendjim, 600 m). For Sitting Height ($p<0.05$), Chest Circumference ($p<0.05$) and Weight ($p<0.001$) the differences are significant.

3. Genetic variability

The results for the distribution of more than 20 blood group systems, isozymes and serum protein polymorphisms are among the first available for Central Asia. The PTC Taste Sensitivity polymorphism was also tested: in agreement with other data on Kirghiz populations (Ibraimov and Mirrakhimov, 1979; Mirrakhimov and Ibraimov, 1982), a higher PTC Non-Taster frequency was found in HA Kirghiz than in LA Kirghiz (Facchini et al., 1997).

As regards blood group distribution, the samples were typed for the AB O, Rh, MNSs, Kell, P, Duffy, Kidd and Diego systems (Pettener et al., 1996). As an example, Fig. 4 shows the genetic relationships among 31 Asian populations based on the ABO, Rh, Kell and MNSs systems, the most widely studied blood groups.

Tab. 1 Fat patterning variations in Middle Altitude (MA) Kazakhs (2.100 m.) and lowland (LA) Uighurs (600 m.).

	MA-Kazaks (N=122)		LA-Uighurs (N=79)		ANOVA	
	Mean	S.D.	Mean	S.D.	F	p
Age (yr)	32.4	8.7	33.2	12.6		
Height (cm)	169.0	6.4	168.9	5.5	0.02	0.87
Weight (kg)	66.5	8.7	67.6	11.0	0.59	0.45
Body Mass Index (KG/CM2)	23.3	2.6	23.7	3.7	0.94	0.33
Circunferences (cm)						
Upper arm	27.4	2.3	28.4	3.0	7.97	>0.01
Waist	78.5	6.9	81.6	10.3	33.21	>0.01
Hip	90.5	5.6	92.8	6.4	14.46	>0.01
Thigh	46.3	3.2	48.1	3.9	12.92	>0.01
Skinfolds (mm)						
Supraliac	9.1	3.5	12.7	5.9	24.61	>0.01
Subscapular	8.5	2.9	11.5	4.7	31.43	>0.01
Triceps	6.4	2.4	7.6	3.3	6.85	>0.01
Biceps	2.9	0.8	4.2	1.6	66.26	>0.01
Calf	4.6	1.1	6.8	2.4	66.86	>0.01
Arm muscle area (cm2)	50.0	6.9	54.3	9.4	9.25	>0.01
Arm Fat Area (Cm2)	8.5	3.5	10.7	5.3	12.11	>0.01
Ratios (mm;cm)						
Sub. Sk./Tric.sk	1.38	0.29	1.59	0.45	14.5	>0.01
Sup. Sk./Tric.sk	1.46	0.41	1.70	.047	13.6	>0.01
Sub. Sk./Calf sk	1.99	0.64	1.90	0.66	0.7	0.42
Tric. Sk./Calf sk	1.39	0.37	1.14	0.34	18.1	>0.01
Waist/Hip	0.87	0.04	0.88	0.06	2.0	0.16
Waist/Thigh	0.17	0.01	0.17	0.01	0.0	0.92

The first principal component (38.1 % of total variation) describes a regular east to west cline. Caucasoid groups present negative values starting from Turkic, Near East and European averages, while Mongoloids show a tendency to positive values. Along the second component a wide variation is shown by Mongoloid groups in comparison with the narrow range that characterizes Caucasoids. The position of the populations suggests a south to north gradient. On the whole the two components account for 60.3 % of the total variation and give patterns of genetic affinity congruent with the geographic map of Asia. Within this genetic map the four Central Asia samples cluster in the center of the graph at the boundaries between the Caucasoid and Mongoloid groups. The two Kirghiz samples exhibit different trends, the population from the plain being closer to Caucasoids. This is probably related to its more western geographic position and its location along the ancient Silk Route. It is also interesting to note the central position along the first axis of the Himalayan and Tibetan Central Asia mountain populations, which according to geography cluster with negative values for the second axis.

Multivariate analysis shows genetic affinity among Uighurs, Kazakhs and Kirghiz. The new data indicate that the patterns of genetic variability should not affect the reliability of the interpopulation comparisons performed in the present study. Moreover, the four samples present an intermediate position between the major groups of Caucasoids and Mongoloids. The detected genetic centrality of Uighurs, Kazakhs and Kirghiz

is in agreement with their geographic centrality and the complex history of the peopling of the region.

The present results are being confirmed by the assessment of further blood group systems, red cell enzyme polymorphisms and serum proteins. The analysis of several mtDNA (d-loop region) and N-DNA (microsatellites) polymorphisms will allow a complete survey of the genetic composition of the Kazakhs, Kirghiz and Uighurs from Central Asia.

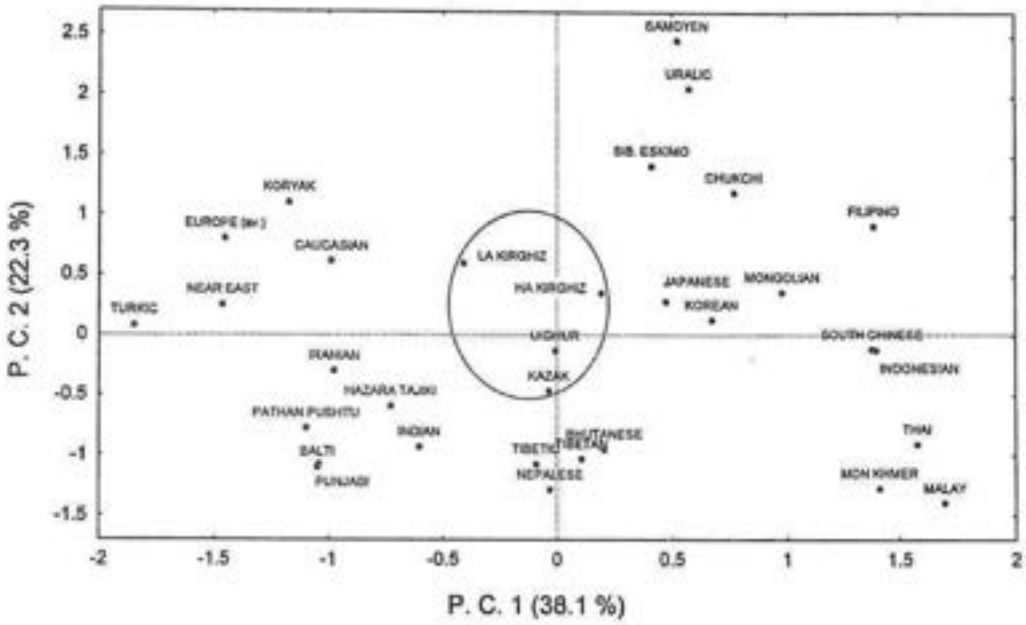


Fig.4 Principal component analysis of genetic variability in selected Asian populations based on blood group systems

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HUMAN ADAPTABILITY IN A QUECHUA POPULATION OF THE PERUVIAN CENTRAL ANDES (HUANCAVELICA, 3680 m). RELATIONSHIPS BETWEEN FORCED VITAL CAPACITY, CHEST DIMENSIONS AND HEMOGLOBIN CONCENTRATION.

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RESUMEN: Adaptabilidad Humana en una Población Quechua de los Andes Peruanos Centrales (Huancavelica, 3680 m). Relaciones Entre Capacidad Vital Forzada, Dimensiones Toráicas y Concentración de Hemoglobina

El estudio intenta brindar datos originales de una población Quechua de un área poco estudiada, los Andes Peruanos Centrales, y busca investigar las relaciones entre variables implicadas en la adaptabilidad humana a la altura. Se examinó una muestra de 77 varones adultos sanos no emparentados, nativos de la provincia de Huancavelica (3680 m). Se investigó las siguientes variables somatométricas y fisiométricas: talla, talla en posición sentada, ancho torácico, espesor torácico, circunferencias torácicas máxima, mínima y normal, capacidad vital forzada (FVC) y concentración de hemoglobina (Hb). Los valores de las variables examinadas son comparables a los de estudios previos de poblaciones Andinas de altura. La Hb y la edad no estuvieron correlacionadas. Para evaluar las relaciones entre las variables, se realizó análisis de regresión múltiple secuencial de la FVC y de las otras variables. Los resultados sugieren que la edad y la mecánica respiratoria, pero no las dimensiones torácicas, están fuertemente asociadas con la funcionalidad pulmonar.

Palabras claves: Adaptabilidad a la altura, Andes, Quechuas, Capacidad vital forzada, Hemoglobina, Antronometría

RÉSUMÉ: Adaptabilité humaine d'une population quechua des Andes centrales péruviennes (Huancavelica, 3 680 m). Relations entre la capacité vitale forcée, les dimensions thoraciques et la concentration d'hémoglobine.

Ce travail tente de fournir des données originales sur une population Quechua d'une région peu étudiée, les Andes centrales péruviennes, et recherche les relations entre les variables impliquées dans l'adaptabilité humaine à la haute altitude. Les 77 sujets d'un échantillon d'adultes masculins, sains et non apparentés, natifs de la province de Huancavelica (3 680 m) ont été examinés. Les variables somatométriques et physiométriques suivantes ont été mesurées : taille, taille en position assise, largeur et épaisseur du thorax, circonférences maximales, minimales et normales du thorax, capacité vitale forcée (FVC) et concentration d'hémoglobine. Les valeurs des variables examinées sont comparables à celles d'études préalables sur des populations andines de haute altitude. Il n'y a pas de corrélation entre l'hémoglobine et l'âge. Afin d'évaluer les relations entre les variables, une analyse de régression multiple séquentielle de la FVC sur les autres variables a été effectuée. Les résultats suggèrent que l'âge et la mécanique respiratoire, mais non les dimensions thoraciques, sont fortement associés à la fonctionnalité pulmonaire.

Mots-clés : Adaptabilité à la haute altitude, Andes, Quechuas, Capacité vitale forcée, Hémoglobine, Anthropométrie.

SUMMARY: The study aims to provide original data on a Quechua population from a less studied area, the Peruvian Central Andes, and to investigate the relationships between variables involved in human adaptability to high altitude. A sample of 77 healthy unrelated adult males, natives of the Province of Huancavelica (3680 m), was examined. The following somatometric and physiometric variables were investigated: height, sitting height, weight, chest breadth, chest depth, maximum, minimum and normal chest circumferences, forced vital capacity (FVC) and hemoglobin concentration (Hb). Values of the examined variables are comparable to those from previous studies of Andean high altitude populations. Hb and age are not correlated. In order to evaluate the relationships between the variables, stepwise multiple regression analysis of FVC on the other variables was performed. Results suggest that age and respiratory mechanics, but not chest dimensions, are strongly associated with lung functionality.

Keywords: high altitude adaptability, Andes, Quechuas, forced vital capacity, hemoglobin, anthropometry

INTRODUCTION

Since Hurtado began the first systematic research on adaptations to high altitude at the population level (1,2), several studies have reported and analysed the differences between high and low altitude populations (3-20). These differences mainly regard anthropometric and physiometric variations concerning the uptake and transport of oxygen. Indeed these functions are the basis of adaptation to the hypoxic environment. The multiple factors that could explain the variability among high and low altitude populations are the subject of an active field of research for anthropologists and a prolific source of theoretical questions in adaptive biology. In the first studies, the differences between high and low altitude populations were attributed to an adaptive genetic component resulting from natural selection (1,18). Starting from the '70s, an alternative hypothesis based on biological plasticity replaced the predominant point of view. This hypothesis gives greater importance to the possibility of modelling

adaptation during growth (6-8). Recent research has tried to quantify the genetic and developmental components of the adaptation to an hypoxic environment and has revealed the importance of both factors (21,22). Other studies have assessed the role of work activity (21,23) and of socio-econornic conditions in the determination of the detected variability among high altitude populations (2426).

Adaptation to high altitude implies the interaction of systems involved in the uptake, transport and delivery of oxygen. Thus it is appropriate to study the interactions between variables that are reliable indicators of the functionality of those systems. This approach is important because Frisancho et al. (5) have shown that particular stresses associated with environments such as high altitude or circumpolar areas could modify the relationships between morphometric and physiometric variables.

This study is part of a joint research program of the University of Bologna and Cayetano Heredia University of Lima, Peru. Besides human adaptability to high altitude, the research aims to study body composition and genetic variability, using surnames, classical markers and DNA polymorphisms, in the populations of the province of Huancavelica (3680 m), in the Peruvian Central Andes. A parallel collaboration with the Academy of Sciences of Kazakhstan will allow comparison of the results obtained in the Andean populations with those obtained in high altitude groups from Central Asia (19, 27-30).

In this paper, we present the first anthropometric and physiometric data obtained in the Quechua population from Huancavelica. The results are compared with those available for other Andean high altitude populations. The study aims to provide original data on a Quechua population from a less studied area, the Peruvian Central Andes. The relationships between lung function measured by forced vital capacity (FVC) and the other variables studied have also been analysed using stepwise multiple linear regression.

MATERIALS AND METHODS

The sample examined

The sample is composed of 77 healthy unrelated adult males, natives of the Province of Huancavelica. All the subjects belong to the Quechua ethnic group and are permanently resident in the area around the city of Huancavelica (3680 m), which is characterised by low rates of admixture with non-indigenous populations. 73% of the subjects are manual labourers (mainly farmers). The age distribution of the sample is shown in figure 1.

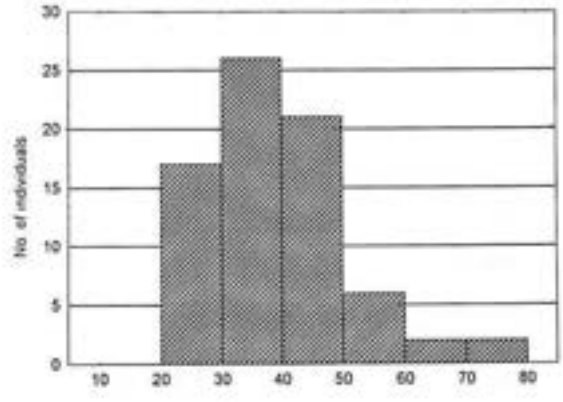

Figure 1 Age distribution ofthe Quechua sample from Huancavelica (3680m).

Variables considered in the study

Each subject was interviewed in order to assess his ethnic origin and his permanent residence in the high altitude environment. The following anthropometric and physiometric variables were measured: height, sitting height, weight, chest breadth, chest depth, maximum, minimum and normal chest circumferences, forced vital capacity (FVC), hemoglobin concentration (Hb). All the somatometric parameters were measured by the same investigator in October 1994 (E. T.S.), according to the recommendations of Weiner and Lourie (31) and Lohman et al. (32). The forced vital capacity (FVC) was measured with a Vitalograph-ALPHA spirometer. Every subject repeated the test twice and the best performance was recorded. The hemoglobin concentration was measured from a capillary blood sample with a portable Hemo-Flash Menarini spectrophotometer. Three readings of hemoglobin concentration were made and the mean value was recorded.

Statistical analysis

The following basic statistics were calculated for each variable: mean, standard deviation, coefficient of variation and Pearson's correlation coefficient with age and its level of significance. Means and standard deviations for subsamples (i.e. ten-year age classes) were also calculated. Stepwise multiple linear regression was used to analyse the relationships between FVC, a reliable indicator of lung functionality, and the anthropometric and physiometric measurements.

RESULTS AND DISCUSSION

Table 1 reports the basic statistical data for the considered variables. Figure 2 shows that the sitting height and weight of the Huancavelica Quechuas are similar to those reported for other Andean populations, while the mean height is lower. Indeed only the mean height of the 20-30 years class is similar to the values of other Andean populations.

	Total sample					20-29 years			30-39 years			40-49 years			>49 years		
	N	Mean	S.D.	r	p	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.
Height (cm)	77	157.90	5.56	-0.30	0.010	17	160.19	6.58	26	157.50	5.07	21	157.44	5.65	10	155.27	3.41
Sitting height (cm)	77	84.52	3.41	-0.30	0.010	17	85.01	4.04	26	84.80	3.38	21	84.52	2.73	10	82.47	3.43
Weight (K)	72	60.00	6.63	0.10	0.400	14	60.90	6.30	25	58.80	5.70	20	62.80	0.66	10	61.40	6.20
Chest breadth (cm)	77	26.07	1.72	0.12	0.300	17	20.15	1.05	26	19.80	1.17	21	21.07	1.11	10	22.24	1.98
Chest depth (cm)	77	20.52	1.53	0.47	<0.001	17	25.66	1.81	26	25.77	1.36	21	27.21	1.63	10	25.36	1.88
Max. chest circ. (cm)	77	96.23	4.15	0.12	0.310	17	96.18	4.39	26	94.66	3.36	21	98.54	3.79	10	96.19	4.92
Min. chest circ. (cm)	77	90.38	4.20	0.25	0.030	17	89.74	4.95	26	88.57	3.38	21	92.84	3.59	10	91.41	4.06
Normal chest. circ. (cm)	77	93.38	4.18	0.17	0.150	17	92.26	4.35	26	90.50	3.48	21	94.88	3.87	10	92.73	4.47
Forced vital capacity (FVC) (l)	77	4.82	0.91	-0.40	<0.001	17	5.12	0.83	26	5.04	0.79	21	4.66	0.87	10	4.02	0.91
Hemoglobin conc (Hb) (g/dl)	77	17.53	1.93	-0.01	0.917	17	18.18	1.53	26	17.23	1.74	21	17.28	1.72	10	17.85	3.23

Table 1. Sample size (N), means and standard deviations (SD) in the total sample and in the four ageclasses. Pearson's correlation coefficients (r) with age and their significance levels (p) are reported for the total Quechua sample from Huancavelica (3680 m).

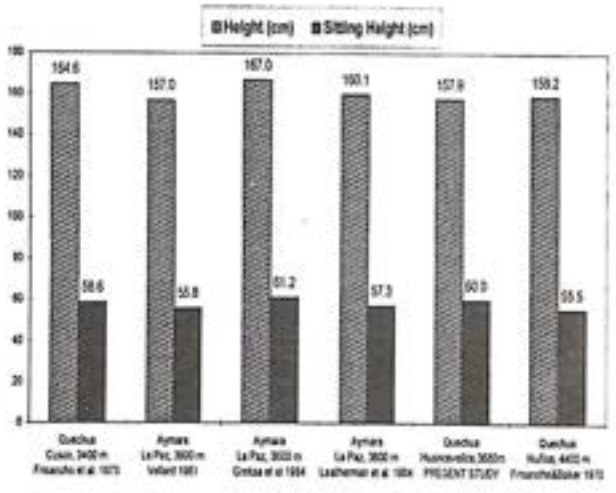

Figure 2 Mean values of height and sitting height in selected Andean populations.

In the Huancavelica sample, there are significant negative correlations with age of height and sitting height (table 1). These correlations could be explained either by the anatomical effects of ageing or by secular trend processes. Gonzales et al. (33) have reported a secular increase in stature in populations of the Peruvian Central Andes between 1937 and 1980, mainly displayed by a relative increase in lower limb length. This differential increase of body segment lengths should imply the diminution of the cormic index with time and a positive correlation between cormic index and age in a transversal sample. However neither feature is present in the Huancavelicans examined (r = -0.06, p= 0.618), which suggests the absence of secular trend in Huancavelica during the last few decades. Thus the negative correlation between age and height (r-0.30) could be a result of ageing processes. The negative correlation between age and sitting height (r=-0.30) strengthens this hypothesis since this variable is affected by two typical aging phenomena: the reduction of intervertebral spaces and the curvature of the vertebral column. The general worsening of socio-economic conditions in this area of Peru in recent decades is in accordance with the hypothesised absence of secular trend. The non-significance of the decrease in mean height with age reported in table 1 (p>0.10) is probably due to the small sample size.

Chest dimensions and FVC values of the Quechua population from Huancavelica are similar to those reported for other Andean native populations (figure 3 and 4). However, comparisons should be performed with caution and taking into account possible bias due to age composition, ethnicity and sample size. As concerns the age composition, our data can be compared with results obtained by Muller et al. (15) in Aymara populations from Northern Chile (fig. 5). FVC for each age class is

higher in the Quechua sample from Huancavelica if compared with Aymara populations from Costa, Sierra (2500-3500 m) and Altiplano (4000-4500 m) (figure 3). The differences between the Peruvian Quechuas and the Chilean Aymaras are probably due to the high level of Caucasian admixture in the Aymara samples, rather than to the different altitude. In fact, in the Altiplano about 30% of the subjects are non Aymara (15) and the percentage of non Aymara individuals increases with decreasing altitude. As recently suggested by Greksa (22), a portion of variance of FVC values in Andean populations is due to a genetic component. This interpretation is consistent with the slight differences in lung volumes between Quechuas and Aymaras obtained by Greksa. (34)..

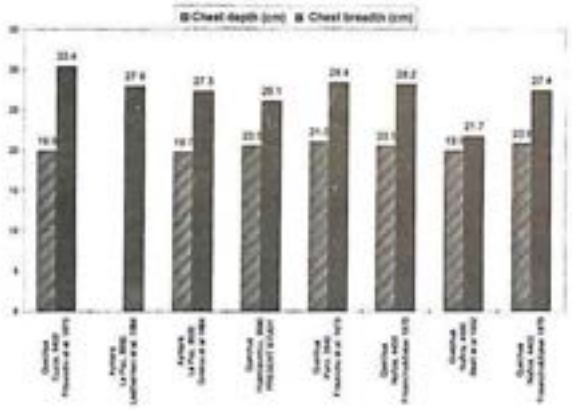

Figure 3 Mean values of chest depth and chest breadth.

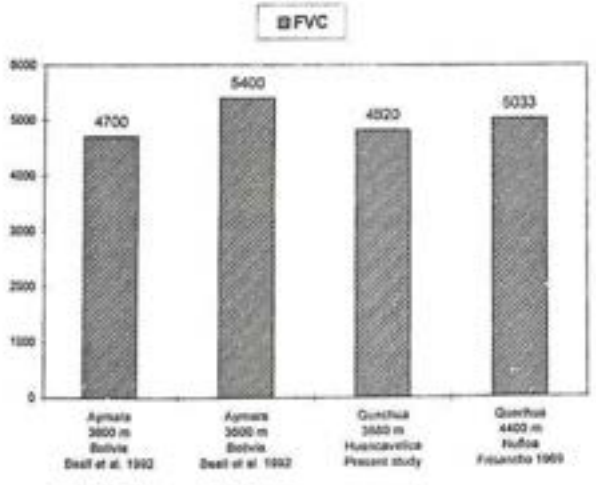

Figure 4 Forced vital capacity (FVC) in selected Andean populations.

The hematological response to high altitude in the Huancavelica sample was measured by hemoglobin concentration. The mean values are close to the values reported for rural Andean populations of comparable altitude (35-39) (Figure 6). There is no correlation between hemoglobin concentration and age, in agreement with results obtained in other rural high altitude populations (37,38,40).

Figure 6 illustrates the results of stepwise multiple linear regression of FVC on the other variables considered in this research. The maximum and minimum chest circumferences and age are the most important explanatory parameters of the lung function variability, as measured by FVC. The high values of the regression coefficient beta associated with maximum and minimum chest circumferences are expected (0.990 i 0.416, p=0.021 and -0.719 i 0.347, p=0.043 respectively). However, not all the chest dimensions are involved in the deterrnination of FVC: normal chest circumference and chest diameters show a low correlation with lung functionality (figure 5). This suggests that, in an adult sample, the parameters of respiratory mechanics, and not chest dimensions, are associated with the forced vital capacity. Frisancho (3) and Mueller et al. (15) have observed that, during growth, chest dimensions and age show the highest correlations with lung functionality, as measured by lung volumes. This relationship has been interpreted as an adaptive mechanism deterrnined by both genetic and developmental components. However, our data suggest that this relationship is not present during adulthood, when the variability of FVC is strongly associated with the ageing process and respiratory mechanics. but not with chest dimensions.

It is interesting to observe that hemoglobin concentration is negatively correlated with FVC. Beall and Goldstein (41) and Pettener et al. (19, 30) have found a similar relationship in Central Asian populations. Since hemoglobin concentration is neither negatively nor positively correlated with age in our sample, it is possible that the negative association with FVC is independent of the are in; process. Multivariate analysis applied to our data corroborates this.

In conclusion, the Quechua sample from Huancavelica is characterised by values for somatometric and physiometric variables that are comparable to those previously reported for Andean high altitude populations. The interactions of physiometric responses implicated in the adaptation to the hypoxic environment have been investigated. A clear negative association between FVC and age has been found, while there was no correlation between hemoglobin concentration and age. Finally, the negative correlation between FVC and hemoglobin concentration, which should be confirmed by further analyses, seems to be independent of age.

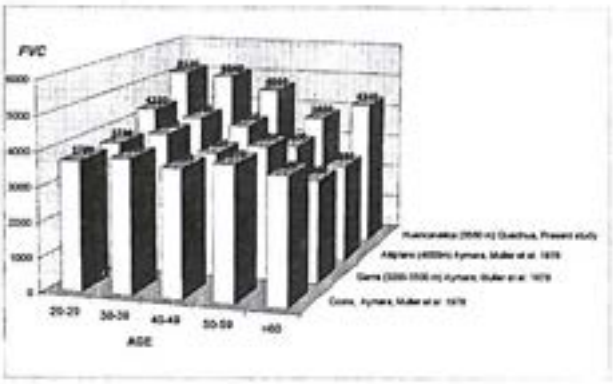

Figure 5 Forced vital capacity (FVC) by age classes in the Quechua sample from Huancavelica and in Aymara populations from Northern Chile.

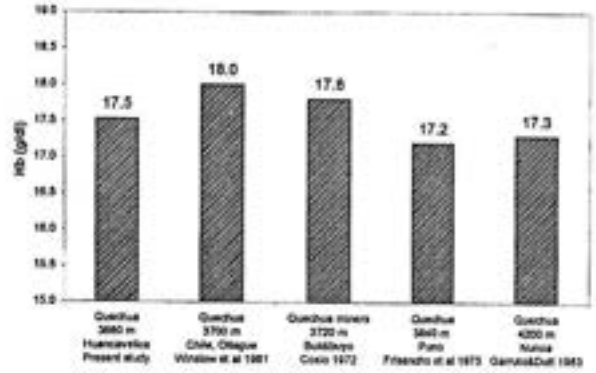

Figure 6 Hemoglobin concentration in selected rural Andean populations.

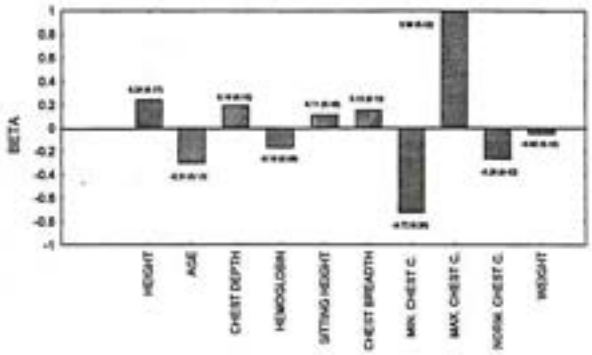

Figure 7 Stepwise multiple linear regression of FVC on the other exam~ined variables: standardised regression coefficients (beta) with standard errors in the Quechua population from Huancavelica.

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WOMEN AT ALTITUDE: BLOOD VESSELS

an evolutionary and integrative review

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RESUMEN: La respuesta fisiológica de las mujeres a la altura ha sido poco estudiada hasta hace un tiempo. Interesa saber si mujeres y varones difieren en su respuesta fisiológica a la altura y si las diferencias se deben a hormonas ováricas o gonadales circulantes. Para tales estudios, es importante recordar que la determinación sexual implica una considerable complejidad evolutiva, genética y de desarrollo. Los estudios de respuesta aguda y crónica a la hipoxia brindan información útil para examinar los mecanismos generales y el significado evolutivo del sexo y de las hormonas sexuales. Los períodos de gestación y desarrollo fetal están sujetos a mayor riesgo de mortalidad que cualquier otro período de la vida. En la altura, la fertilidad se mantiene en niveles de nivel del mar o por encima del nivel del mar, pero el peso al nacer disminuye un promedio de 100 gramos por cada 1000 m de altitud, principalmente como resultado de retardo del crecimiento. También hay una mayor frecuencia de complicaciones maternas durante el embarazo, que pueden contribuir al menor peso al nacer. La gestación, como resultado de acciones combinadas de hormonas ováricas y tasa metabólica incrementada, estimula varios componentes del transporte materno de oxígeno de modo que incrementa la ventilación y la oxigenación arterial. La magnitud del incremento en la ventilación materna y en la oxigenación arterial guardan relación positiva con el peso al nacer. El flujo sanguíneo úteroplacentario también parece estar reducido en la altura, particularmente en mujeres que desarrollan pre-eclampsia. El incremento en la ventilación materna y en el flujo sanguíneo úteroplacentario durante el embarazo en particular y las influencias de las hormonas ováricas sobre los procesos de transporte de oxígeno en general, probablemente han recibido el influjo de la selección natural para lograr la adaptación evolutiva a la altura.

Palabras claves: Estradiol, Progesterona, Testosterona, Transporte de oxígeno, Hipoxia, Cardiovascular, Respiración, Retardo de crecimiento intrauterino, Mortalidad infantil, Pre-eclampsia.

RÉSUMÉ: La réponse physiologique des femmes à l'altitude a été peu étudiée jusqu'à une époque récente. Or, il est intéressant de savoir si leur réponse physiologique à l'altitude diffère de celle des hommes et si les différences sont dues aux hormones ovariennes ou gonadotropes circulantes. Pour mener à bien ces études il ne faut pas perdre de vue que la détermination sexuelle implique une grande complexité évolutive, génétique et de développement. Les études de réponse aiguë et chronique à l'hypoxie fournissent des informations utiles pour examiner les mécanismes généraux et la signification évolutive de l'influence du sexe et des hormones sexuelles. Plus que toute autre période de la vie, les périodes de gestation et de développement foetal sont soumises à de grands risques de mortalité. En altitude, la fertilité se maintient à des niveaux comparables - et même supérieurs - à ceux du niveau de la mer, mais le poids à la naissance diminue de 100 g tous les 1000 m, principalement en raison du retard de croissance. On observe également une plus grande fréquence de complications chez la mère pendant la grossesse, pouvant contribuer au moindre poids à la naissance. La grossesse, résultat d'actions combinées d'hormones ovariennes et d'un taux métabolique en augmentation, stimule plusieurs composants du système maternel de transport d'oxygène, de sorte qu'il y a accroissement de la ventilation respiratoire et de l'oxygénation artérielle. On observe un rapport positif entre l'ampleur de cet accroissement et le poids à la naissance. Le flux sanguin utéro-placentaire semble être également en diminution du fait de l'altitude, particulièrement chez les femmes présentant une pré-éclampsie. L'augmentation de la ventilation respiratoire maternelle et du flux sanguin utéro-placentaire pendant la grossesse en particulier et les influences des hormones ovariennes sur les processus de transport d'oxygène plus généralement, ont probablement reçu l'influx de la sélection naturelle pour parvenir à l'adaptation évolutive à l'altitude.

Mots-clés : Oestradiol, Progestérone, Testostérone, Transport d'oxygène, Hypoxie Cardiovasculaire, respiration, Retard de croissance intra-utérin, Mortalité infantile, Pré-éclampsie.

SUMMARY: The physiological response of women to altitude has received comparatively little study until recently. Of interest is whether women and men differ in their physiological response to high altitude and if so, whether differences are due to circulating ovarian or gonadal hormones or to gender differences unrelated to levels of circulating hormones. It is important to recall that the determination of gender involves considerable evolutionary, genetic and developmental complexity. Studies of acute and chronic response to high altitude provide are useful for examining the general mechanisms and evolutionary significance of the influences of gender and sex hormones on processes of oxygen transport. The period of pregnancy and fetal development is subject to greater mortality risk than any other period during the life cycle. At high altitude, fertility is maintained at or above sea level values but infant birth weight decreases an average of 100 gm per 1000 m altitude gain, principally as a result of fetal growth retardation. An increased frequency of maternal complications of pregnancy also has been reported at high altitude and may, in turn, contribute to the birth weight decline. Pregnancy stimulates several components of maternal O_2 transport, raising ventilation and arterial oxygenation as the result of the combined actions of ovarian hormones and increased metabolic rate. The magnitude of the rise in maternal ventilation and arterial oxygenation relates to infant birth weight at high altitude. Uteroplacental blood flow also appears to be reduced at high altitude, particularly among women developing preeclampsia. Thus the maternal ventilatory and cardiovascular adjustments by which fetal-placental O_2 and other nutrient

delivery is maintained may be crucial in attaining successful adaptation to high altitude.

KEY WORDS: Estradiol, Progesterone, Testosterone, Oxygen Transport, Hypoxia, Cardiovascular, Respiration, Pregnancy, Intrauterine growth retardation, Infant mortality, Preeclampsia

INTRODUCTION

To date there has been little systematic study of women's physiological response to acute or chronic hypoxia. Despite the early, inclusive studies conducted on residents of high altitude by Mabel Purefoy Fitzgerald (1), most of the extensive literature on newcomers or long-term residents of high altitude has not included female subjects. Such an omission is consistent with much of the cardiovascular, respiratory and exercise physiology literature. One suspects that it is due, in part, to a concern that cyclic variation in female hormones may contribute an additional source of variation. Ironically, this very possibility is actively being investigated in contemporary studies of women at altitude.

As described in the preceding papers (2-6), gender comparisons are currently underway on the effects of high altitude on exercise performance and symptoms of acute mountain sickness. Also being studied are the influences of menstrual cycle phase on ventilatory, cardiovascular, hematological, and nutritional acclimatization to high altitude. Progress is being made toward understanding of the factors causing the incidence of the high-altitude disorder of chronic mountain sickness (CMS) to differ between the sexes.

The purpose of this article is to consider the general mechanisms and significance of the influences of gender and sex hormones on hypoxic responses. An evolutionary and integrative approach is used in order to address the importance of gender and sex hormonal influences on human adaptation to high altitude. This approach is developed by considering 1) the evolutionary determinants and influences of gender, 2) the effects of hypoxia on reproductive success, and 3) the influences of reproductive hormones and pregnancy on processes of oxygen transport and their implications for fetal and maternal well-being.

EVOLUTIONARY PERSPECTIVE ON SEX AND GENDER

While sex and gender are often used synonymously, here we use "sex" to refer to the biological attributes of being male or female and "gender" to those attributes generally ascribed to members of a given sex. As such, gender includes socially as well as biologically acquired or assigned attributes and includes the possibility that the attributes ascribed to one gender may vary and at times, include those traits assigned to the other.

For humans and other mammals, sex is determined by having received from one's parents either two X chromosomes (females) or one X and one Y chromosome (males). This chromosomal determination of sex is not universal. Among birds, males are XX and females are XY. Sex determination in most insects is the same as mammals but in two orders, the lepidoptera (moths and butterflies) and trichoptera (caddisflies), is like that of birds (XX males and XY females) (7). In humans, the presence of two X's vs one X and one Y chromosome is usually but not always associated with the presence of female vs. male genitalia and other secondary sexual characteristics. The regulation of secondary sexual characteristics is a function of a gene or genes on the Y chromosome that, together with a brief period of elevation in androgen levels during embryonic life, prompt the formation of testes. If this gene(s) on the Y chromosome is lacking, an XY individual will develop into a female; likewise a prenatal surge in androgens can cause a genetic female to develop testes. Since, in the absence of a stimulus to become male, a female will result, female features appear to be the underlying, primary attribute of mammalian life.

Darwin recognized that sex is subject to natural selection and termed this "sexual selection". For reasons that remain unclear, males in utero are subject to higher mortality than females. Hence the majority of spontaneous abortions are male. Even so, there is an excess of males relative to females at birth; the average sex ratio at birth is 106 males for every 100 females. By adulthood in most societies the sex ratio has evened, again indicating greater male than female mortality. Female mortality remains generally lower than male mortality at every age except during childbearing years. As a result, life expectancy is longer for females than males at nearly every age in most societies. These differences in mortality between males and females indicate that sex and/or gender influences Darwinian (evolutionary) fitness.

One of the mechanisms by which sex and gender influence evolutionary fitness is the presence of sex-specific genetic material. Among mammals, both mother and father contribute an equal, haploid number of chromosomes to form the zygote. In addition, the mother contributes her mitochondrial DNA to the fertilized egg. This mitochondrial DNA reproduces itself with each ensuing cell division. In a somewhat analogous fashion, Ychromosomal DNA is transmitted only by the father. However an important difference is that Y-chromosomal DNA

is acquired only by males whereas both females and males possess the maternally-derived mitochondrial DNA. Thus the developing organism is subject to nuclear genetic influences from both parents but to mitochondrial genetic influences from its mother only.

Imprinting or the disproportionate influence of maternal or paternal genetic material is another mechanism by which maternally and paternally-derived genes influence the developing organism. Recent studies have revealed that, for example, paternal genes exert a greater influence on placentation whereas maternal genes appear to predominate in fetal development (8).

EFFECTS OF HYPOXIA ON REPRODUCTIVE SUCCESS

From an evolutionary perspective, the period of pregnancy and fetal development is subject to greater mortality risk than any period during the life cycle. In this section, we consider whether chronic hypoxia serves to increase selective pressure during this critical period.

Severe, acute hypoxia impairs reproductive function (9) but the effects of more sustained or moderate hypoxia are less clear. Earlier studies suggested a reduction in fertility in South American high- compared with low-altitude populations, as judged by completed family size and an increase in completed fertility in persons who migrated from high to low altitudes, but this has not been supported more recently (10). While menarche is later and menopause earlier, a 1990 survey in Peru demonstrated higher completed fertility in the high than low altitude-departments (11). The high-altitude residents achieved higher fertility by shortening the intervals between menarche and the birth of the first child and between the births of subsequent children and conceiving more often during lactation in comparison with low-altitude residents.

There are a number of factors which might be expected to influence fertility levels in the Andean region. In the setting of high infant mortality (Figure 1), fertility may be elevated, perhaps in an attempt to assure surviving offspring. Alternatively, the reporting of births may be reduced in Peru and Bolivia where most births occur outside of hospitals (12). Cultural considerations pertaining to the contribution and costs of children also influence childbearing patterns. In traditional areas of highland Peru, high completed fertility is desirable in that children from ages 6 - 18 yr generate more resources than they consume (13).

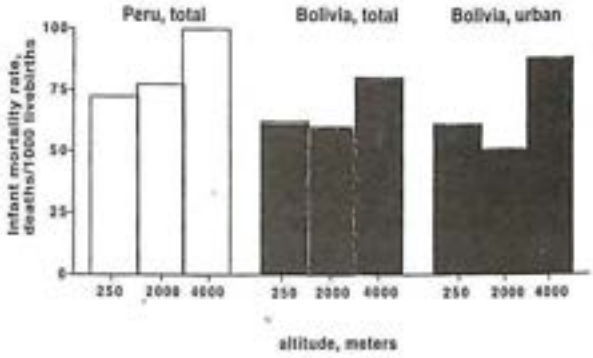

Figure 1: Infant mortality increases with altitude in Peru and Bolivia (12).

An important but unanswered question pertains to the influences of short-term hypoxia in women who are already pregnant. Large numbers of persons travel to Colorado and other mountainous regions (14). Medical advice is inconsistent regarding the safety of travel to high altitude for pregnant women and their babies. The only published data, to our knowledge, is a brief report concerning 13 newcomer residents of La Paz, Bolivia (Table 1) (15). While the numbers are extremely small, it is noteworthy that the women who arrived at high altitude in their first trimester appeared to develop maternal or fetal complications more frequently than those who conceived at high altitude. Needed is an expanded, systematic comparison of maternal and neonatal complications and birth outcome in newcomers exposed to high altitude before vs. during pregnancy. In order to control for the effects of migration independent of altitude, such a study should include an assessment of pregnancy and birth outcomes in women who move from one low altitude to another.

Chronic hypoxia has long been associated with a reduction in infant birth weight, averaging a 100 gm decline per 1000 m altitude gain (9, 16). There is a corresponding increase in the percentage of low-birth-weight babies (< 2500 g) (17). The reduction in birth weight is likely due to high altitude rather than maternal body size or some other population-specific factor since a generally similar reduction in birth weight is seen in most (but not all) human populations and can be observed when comparing birth weights of infants born to the same woman at low vs. high altitude (18). The reduction in birth weight at high altitude has historical significance; the first recognition that fetal growth and length of gestation were separable influences on birth weight was made at high altitude (19).

Table 1. Pregnancy outcome in 13 La Paz newcomers (adapted from 15)

Arrival	No. Complication(s)	Birth
weight. Gm	1 none	2900
Before conception	2 none	2600
	3 none	3000
	4 none	3000
	5 none	3000
	6 none	3100
	7 C-section	2900
	mean ± sem	
	2920 ± 70	
1st trimester	8 eclampsia, preterm, C-section	2100
	9 preterm	2000
	10 preterm	1800
	11 threatened abortion, C-section	2800
	mean ± sem	
	2175 ± 220	
2nd trimester	12 none	3300
	13 preterm, C-section	2500

The primary cause of the reduction in birth weight at high altitude appears to be intrauterine growth retardation (IUGR) (Figure 2). In Colorado, the reduction occurs principally in the third trimester as demonstrated by a progressive decline in fetal weight after 32 weeks at high compared with low altitudes (20 - 21) (Figure 2). Recent data included in Figure 2 from Peru appear consistent with the North American results when the higher altitudes of Peru are taken into account (22 - 23). Growth curves from Peru and Bolivia across the full range of gestational ages at low vs high altitudes are needed but will be difficult to construct given that birth records are not available for most of these countries' population (12). Average gestational ages at high vs. low altitude are similar in North America (16, 19 - 21) but modest reductions, which are generally not sufficient to explain the birth weight reduction observed (24), have been reported in some South American studies (22 - 23). Such altitude-associated reductions in gestational age may be due to differences in medical interventions for preventing preterm births or the extent to which complete samples have been obtained.

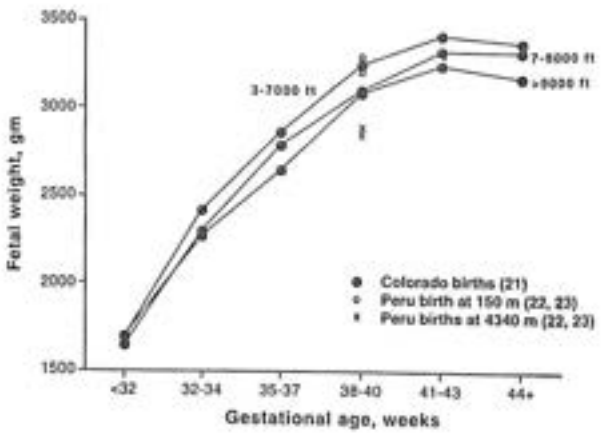

Figure 2: Fetal growth is retarded at high altitude beginning after 32 weeks gestational age (comparison of curves, $p < 0.05$) (21 - 23).

The magnitude of retardation of fetal growth at high altitude appears to vary among populations in relation to their duration of high-altitude exposure. Populations have resided at high altitude for the shortest period in the Rocky Mountains, an intermediate length of time in the Andes, and the longest in the Himalayas (25). Comparing well-matched studies by the same investigator across a 0-3500 m gradient, Zamudio and co-workers found that the reduction in birth weight was greatest in North Americans (-352 grams, p<.001), intermediate in South Americans (-270 grams in Peru, -282 grams in Bolivia, p<.001) and least in Tibetans (-72 grams, p=NS) (24). Of note was that the Tibetans, residing at an altitude equivalent to that present in the South American studies, did not appear to have altitude-associated IUGR (24, 25). This suggests that the Tibetans have adapted to high altitude in a fashion that permits normal fetal growth.

Twenty years ago, infant and neonatal mortality rates were above nationwide levels in Colorado and the other USA mountain states (19). Currently, infant mortality is the same as national values and does not increase with altitude within Colorado (21). The mortality decline in Colorado was associated with a modest increase in birth weight and a fall in the percent preterm births (21) but much of the decline remains unexplained. Being small (reduced birth weight) does not reduce infant mortality at high altitude (26) since low birth weight infants ar an increased mortality risk at every altitude (2 .mproved

medical technol gy and greater utilization of tertiary medical facilities by women from the highest compared with the lowest-altitude areas in Colorado are likely to have contributed to the mortality decline.

Bolivia and Peru currently have the highest infant mortality in South America. The excess mortality compared to other countries has not changed appreciably over the past 20 or more years (12). Within both countries, infant mortality is greatest in the highest altitude regions when all infants or only urban infants are compared (Figure 1). However, the infant mortality data are of poor quality. Payment is required to register a birth or death and only about one-third of the deaths are certified by a physician (12). Such problems are particularly acute in rural regions and other settings where infant mortality is likely to be highest.

The IUGR and greater frequency of preterm deliveries in some studies may be the result of an increased frequency of pregnancy complications, best documented of which is an increased incidence of placental abruptions and preeclampsia. A review of all deliveries in La Oroya, Peru (3750 m) over a 15-year period indicated that placental abruptions were three-fold more common than at sea level and demonstrated an age- and parity-associated relationship, occurring in 6.8% of women over 40 yr and in 3.4% with parity greater than 4 (27). Preeclampsia is the leading cause of maternal and fetal mortality in the industrialized world (28). Preeclampsia is defined as an elevation in blood pressure (>140/90 mmHg, a systolic rise > 30 mm Hg, or a diastolic rise > 15 mmHg) accompanied by proteinuria and/or upper extremity edema in a woman who is normotensive when nonpregnant. - Abnormalities of liver function, coagulation, and the central nervous system are sometimes observed as well. The incidence of preeclampsia increases about three-fold from low to high altitudes in Colorado (29 - 30) but data from South America are equivocal as to whether the incidence of preeclampsia is increased (22, 31).

An increased occurrence of maternal complications during pregnancy is likely to contribute to an elevation in maternal as well as infant mortality. Maternal mortality in Peru and Bolivia is more than twice the South American average (12), rising from 13.2 maternal deaths per 10,000 live births on the coast, to 21.5 in the 2-3000 m region and to 43.1 at elevations above 3000 m in Peru (32).

Impaired placentation and uteroplacental ischemia may be a common pathway whereby pregnancy complications are increased and intrauterine growth retarded at high altitude. Uteroplacental ischemia has been associated with preeclampsia at low altitude (33). An attractive hypothesis is that the uteroplacental ischemia, in turn, is due to impaired trophoblast invasion and remodeling of maternal spiral and other uterine arteries (34).

Historic as well as more recent observations indicate that behavioral adjustments, in addition to alterations in maternal O_2 transport, are important responses to high altitude. Out-migration serves as a mechanism for avoiding the challenges of high altitude. As Antonio de la Calancha observed in 1632 when the Spaniards entered what is now Bolivia, pregnant women of Spanish origin would descend to give birth at lower altitudes and not return until the child was more than a year old (35). A similar practice occurs among Han women living on the Tibetan Plateau who often return to their home districts at lower altitude during pregnancy. After birth, their infants customarily stay with extended family until approximately two years of age, at which time they are brought to high altitude (36).

MATERNAL OXYGEN TRANSPORT RESPONSES TO PREGNANCY AND THEIR IMPLICATIONS FOR MATERNAL AND FETAL WELL-BEING

Because not all women at high altitude deliver growth-retarded babies, we have conducted a series of studies to test the hypothesis that altitude-associated IUGR is due to insufficient maternal O_2 transport to meet fetal-placental demands. The alternate possibilities are that placental diffusing capacity is impaired in such a way as to limit O_2 or other nutrient transfer or that other, fetal-specific factors limit growth. Early reports indicated that the placenta at high altitude was similar in absolute size and larger in relation to fetal size than at low altitude (37). Recent studies indicate that the placenta at high altitude is more vascularized and has a greater diffusing capacity than at low altitude (38, 39). Thus, impaired placental O_2 transfer is unlikely to be the primary cause of altitudeassociated IUGR.

In normal pregnancy, elevated levels of progesterone and estrogen in combination with increased metabolic rate raise peripheral (carotid bódy) and central chemosensory sensitivity and resting ventilation (40 - 42). Investigation of gender differences in ventilation is often framed in terms of the effects of ovarian hormones. It is important, however, to recognize that other factors also contribute to gender differences. Gender may influence ventilation in ways that are unrelated to circulating levels of sex hormones due to, for example, prior hormonal exposure or to other, gender-linked factors. When normalized for differences in body size, neutered as well as intact females have higher alveolar ventilation and HVR than their intact or neutered male counterparts (43, 44). The male hormone testosterone also affects ventilation (45). The rise in resting ventilation after exogenous testosterone administration is due to an increase in metabolic rate but testosterone treatment also raises hypoxic and hypercapnic ventilation responsiveness. Interestingly, the increase in HVR appeared due to a diminution in descending central inhibitory influences on the carotid boy (45).

At low altitude, the increase in ventilation during pregnancy does not appreciably raise arterial O_2 saturation, since it is already nearly maximal. At high altitude, arterial O_2 saturation rises with pregnancy. Our studies in Peru and Colorado have demonstrated that the magnitude of the rise in maternal ventilation, hypoxic ventilatory sensitivity, and arterial O_2 saturation during pregnancy related positively to the birth weight of their infants (18, 46, 47). While suggesting that the factors serving to raise maternal arterial oxygenation help to preserve fetal growth at high altitude, a puzzling aspect of these observations is that arterial O_2 content is similar at high and low altitude as the result of the pregnancyassociated rise in arterial O_2 saturation and higher hemoglobin concentration characteristic of the high-altitude residents (18). Because uteroplacental blood flow increases some 50fold during pregnancy, it is a major influence on uteroplacental O_2 delivery (i.e. the product of uteroplacental blood flow and arterial O_2 content). We therefore asked whether uterine blood flow was altered by residence at high altitude.

Several factors combine to increase uteroplacental blood flow during pregnancy. Of major importance is the remodeling of the uteroplacental circulation. The uterine and radial arteries (the vessels branching from the uterine artery and,entering the uterine wall) at least double in diameter. The downstream, basilar and spiral arteries also enlarge as a result of the trophoblast invasion from the developing placenta. In preeclamptic pregnancies, however, the increase in vessel diameter occurs only in the decidual portion and not in the myometrial region of the uterine vascular wall, resulting in a narrow segment with retained pressor sensitivity that likely raises uterine vascular resistance and diminishes blood flow.

Changes also occur outside the uteroplacental circulation which contribute to the increased uteroplacental blood flow during pregnancy. Blood volume increases approximately 40%, cardiac output rises similarly, and the distribution of blood flow is altered to increase the proportion of flow directed to the uteroplacental circulation as opposed to the lower extremities (48). Blood pressure falls, implying a decrease in systemic vascular resistance, which may in turn be due to altered response to vasoconstrictor, vasodilator, or myogenic stimuli. The increased blood volume implies that venous capacitance is increased, since the major portion of blood volume resides in the venous circulation.

Near term, approximately 1 l/min or 15% of the total cardiac output is directed toward the uteroplacental circuit. In studies conducted at low altitude (1600 m), we demonstrated that the rise in uteroplacental blood flow is accomplished in approximately equal part by a doubling of uterine artery diameter, that was complete by mid-gestation, and a rise in uterine artery flow velocity that continued until term. The overall increase in uteroplacental blood flow during human pregnancy is similar to that observed in other mammals when normalized per kg of fetal weight (48). In studies at

high altitude (3100 m), we found similar external and common iliac artery diameters but smaller uterine artery diameters, resulting in 1/3 lower unilateral uterine artery blood flows in the high-altitude women (49). Pelvic blood flow distribution was also altered near term; 74% of common iliac flow was directed toward the uterine artery in normal pregnant women at low altitude whereas only 47% was directed to the uterine artery at high altitude. Women who developed preeclampsia at high altitude had less redistribution of common iliac flow to favor the uterine artery and no increase in uterine artery flow near term. These differences were present prior to the onset of hypertension, suggesting that lower uterine blood flow may be a cause rather than an effect of preeclampsia (50). Compilation of data from human and experimental animal studies indicates that reductions in uterine or uteroplacental blood flow relate exponentially to declines in birth weight (figure 3), suggesting that an altitude associated decline in uteroplacental blood flow may be a major contributor to the birth weight reduction observed.

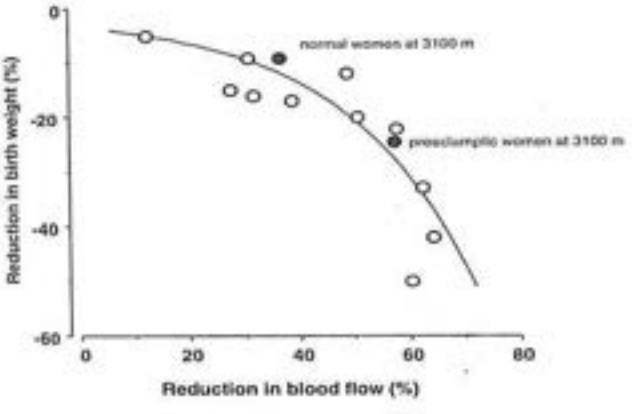

Figure 3: Reduction in birth weight and uterine blood flow in women at high altitude (closed circles) is consistent with that observed in models of IUGR in experimental animals (open circles) (adapted from 51).

SUMMARY AND CONCLUSIONS

The influences of gender and sex hormones on physiological responses to acute and chronic hypoxia are complex. This complexity is due to the many factors determining or, conversely, being determined by sex or gender. While operative under low- as well as high-altitude circumstances, the influences of gender and sex hormones at high altitude may be particularly important given the probable increase in selective pressure demonstrated by the more frequent occurrence of maternal, fetal and neonatal complications.

In general, stimulatory effects of ovarian hormones appear to help defend maternal arterial O_2 saturation and birth weight at high altitude. This has been demonstrated in a series of studies in which we and others have shown that ovarian hormones raise ventilation and are likely involved in the vascular alterations of pregnancy that serve to increase blood flow to the uteroplacental circulation. A lesser rise in uterine artery blood flow, particularly among preeclamptic women, may underlie the reduction in birth weight observed at high altitude and may, in turn, be due to an impaired growth response and/or alterations in responsiveness to vasoconstrictor, vasodilator or myogenic stimuli.

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