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NEW CONCEPTS ON CHRONIC MOUNTAIN SICKNESS

ZUBIETA-CASTILLO, G. AND ZUBIETA-CALLEJA, G.

High Altitude Pathology Institute (IPPA). La Paz, Bolivia

ABSTRACT

The adaptation capacity of human beings to atmospheric pressure changes is remarkable. In high altitude adaptation (HAA) we should consider: that of normal man and that of the diseased. The acute HAA can be more dramatic and problematic than chronic HAA. The diseases are the same as those at sea level and have hypoxic physiognomies. The term Chronic Mountain Sickness (CMS), has created confusion, because it includes pulmonary diseases that cause Excessive Erythrocytosis (EE). EE, is a mechanism of adaptation that increases the oxygen carrying capacity of the blood by increasing the number of red blood cells. The term "dysadaptation to high altitude", for CMS patients that present with EE, seems inappropriate and does not provide a pathogenesis. Above 3000 m, in the Bolivian Andes, respiratory disease with EE affects thousand of persons. With the availability of pulmonary function tests and blood gas techniques, it is increasingly evident that EE is due to some ventilatory or respiratory alteration. Patients with EE show aberrations of one or more of the following: FVC; FEV.1/FVC;FEF 25-75%, Alveolar ventilation; $PaCO_2$; pulmonary shunts; uneven ventilation; TLC; CC/TLC; CV/VC; blood pressure; or chest x-ray. All of our patients had a $PaCO_2$ below 56 mmHg. In patients with EE there is a tendency for the hematocrit to increase with age ($r = 0.35$) with a plateau at around 60 years of age. We found an inverse relationship between FVC ($r = 0.45$) and RV/TLC ($r = 0.10$) with the hematocrit. In conclusion, EE (appearing as CMS) is an adaptation to hypoxia caused by disease at high altitude. **(Acta Andina 1996, 5:3-8)**

RESUMEN

La capacidad de adaptación de los seres humanos a los cambios de la presión atmosférica es notable. En la adaptación a la altura (HAA) debemos considerar: la del sano y la del enfermo. La adaptación aguda puede ser mas dramática y peligrosa que la adaptación crónica. Las enfermedades son las mismas que las del nivel del mar pero con una fisonomía hipóxica. El termino Mal de Montaña Crónico (CMS) ha creado confusión, debido a que incluye enfermedades pulmonares que ocasionan la eritrocitosis excesiva (EE). Esta última es un mecanismo de adaptación que incrementa la capacidad de transporte de oxígeno de la sangre, incrementando los eritrocitos. El termino "desadaptación a la altura", para los pacientes con CMS que tienen EE, parece inadecuado y no explica la patogenesis. En los Andes Bolivianos por encima de los 3000 m., miles de pacientes son portadores de enfermedades respiratorias con EE. Con el acceso a técnicas de estudio de la función pulmonar y de los gases en sangre más eficientes, se hace mas evidente que la EE se deba a alguna alteración ventilatoria o respiratoria. Los pacientes con EE muestran alteraciones en uno o varios de los siguientes: FVC; FEV.1/FVC; FEF 25-75%, ventilación alveolar; $PaCO_2$; shunts pulmonares; ventilación no uniforme; TLC; CC/TLC; CV/VC; presión arterial ó radiografía de tórax. Todos nuestros pacientes tenían un $PaCO_2$ por debajo de 56 mmHg. En estos pacientes hay una tendencia a que el hematocrito aumente con la edad ($r = 0.35$) estabilizándose alrededor de los 60 años de edad. También se encontró una relación inversa entre el FVC ($r = 0.10$) con el hematocrito. En conclusión, el mal de montaña crónico es una adapatación a la hipoxia, debido a enfermedad en la altura. **(Acta Andina 1996, 5:3-8)**

When the first French medical books translated into Spanish, arrived in America, physicians studied medicine at high altitudes and compared the signs and symptoms of permanent residents with similar sea level illnesses. Treatments followed sea level guidelines. Clinicians making careful observations noted that at high altitude the normal inhabitants and their pathology had differences worthy of clarification. In 1928, Monge described an illness that he called high altitude erythremia, defined by markedly increased red blood cell levels (RBC). Initially he linkened it to Vaquez-Osler's disease [10]. He later modified this concept, attributing the disease to a loss of adaptation to high altitude, suffered by some subjects, who surprisingly returned RBC levels to normal upon descent to normal sea level [9,8]. This important discovery of illness at high altitude, is comparable to the observation of physiologic polycythemia in normals at high altitude by Viault [12]. Further reports, improved by quantity and quality gave more details of the characteristics of the disease, given the name of chronic mountain sickness (CMS), Monge's disease and more recently, excessive erythrocytosis (EE) [11,6]. At sea level, patients with cor pulmonale and chronic tissue hypoxia, also have been found to have erythrocytosis.

With time, patients at variable altitudes were studied, and it was concluded that adaptation is more difficult with higher altitudes and faster ascents [4,13,5]. It follows that one must consider the adaptation of normal man and that of the diseased man, since acute adaptation is much more dramatic and dangerous than chronic adaptation.

In the Bolivian Andes, where large populations live above 3000 m, pulmonary disease with EE is present in thousands of patients. With the arrival of improved pulmonary function and

Corresponding author:
Gustávo Zubieta-Castillo IPPA La Clinica del Instituto de Patologia en la Altura Av. Saavedra 2302 Casilla 2852, La Paz, Bolivia
Tel:591-2-368734
Fax:591-2-357159
E-mail: zubieta@oxygen.bo

blood gas equipment and with improved clinical experience, it seems to the authors more evident that EE results from some respiratory or ventilatory alterations. Our report substantiates this theory.

Materials and Methods

Patients with EE who consulted us for treatment of cardio-pulmonary disease or because EE itself turned them cyanotic , were studied from 308 cases diagnosed as having secondary EE, 10 were chosen randomly and cardio-respiratory function studies were performed (Table 1) at High Altitude Pathology Institute (IPPA) in the city of La Paz, at 3600 m (PB x = 495 mmHg). The 10 patients were all male, born in and residents of this area. Control studies of patients' EE, age and pulmonary function studies for larger groups are also described.

Hematocrits were determined using the microhematocrit technique. Forced vital capacity (FVC), forced expiratory volume in 1 second (FEV.1) and forced expiratory flow (FEF) were obtained with the use of a Purittan Bennett Remac adapted through an analog-digital conversed to a PC, previously calibrated with a 3 liter syringe. Expired ventilation (VE) was obtained in a Tissot during 5 minutes and expired samples were analyzed along with blood gases in a Radiometer model MK2Phm Acid-Base analyzer.

Carbon dioxide production (VCO_2) and oxygen consumption (VO_2) were calculated using standard equations described elsewhere [2]. Alveolar ventilation was calculated from VCO_2 and alveolar CO_2 tension ($PACO_2$), that was sampled by the end-tidal method and analyzed in the same blood gas machine. Hyperoxic testing using the same method was performed where arterial oxygen tension (PaO_2) was measured from a blood sample taken from the radial artery at rest. Inability to achieve a PaO_2 above 200 mmHg was considered as an intra-pulmonary shunt. Nitrogen washout curves using the same Remac, were analyzed for uneven ventilation, where the slope of the alveolar plateau greater than 0.2 was considered abnormal. Blood pressure taken at rest in the supine position was also recorded in order to associate possible reno-vascular disorders. The chest x-ray was studied in each case.

Results

In table 1, shadowed results identify abnormals in 10 patients with pulmonary volumes below 90% of predicted. Alveolar ventilation below 3000 ml/min/m² and arterial oxygen tension (PaO_2) below 56 mmHg were considered abnormal for this altitude.

NM	Ht %	FVC % PR	FEV.1 % PR	FEF % PR	VA ml/min/m2	PaO2 mmHg	PaCO2 mmHg	SHUNT PaO2 w/O2	UNV Slope	B.P. mmHg	X-Ray
RC	71	132	120	75	2448	46	27	75	0	109/80	Abnor
FG	67	79	77	59	2115	39	39	104	0.1	120/90	Abnor
ET	72	94	101	85	3535	48	33	114	0.1	130/95	Abnor
CV	78	90	93	80	2360	44	38	115	0.1	110/80	Abnor
DB	77	60	66	101	3969	50	26	135	0.1	160/105	Abnor
RL	69	97	103	118	1564	43	40	190	0.07	120/90	Abnor
IC	72	100	103	96	2456	49	31	200	0.1	139/84	Abnor
LP	83	49	43	23	2210	42	35	210	0.5	120/90	Abnor
JV	61	89	71	33	2728	43	38	230	0.27	140/80	Abnor
HR	65	93	95	87	3252	55	42	250	0.1	180/90	Abnor

Table 1. Excessive Erythrocytosis in 10 randomly chosen from 308 cases reveal pulmonary or blood pressure anomaly (shaded areas). NM = Name, Ht = hematocrit, FVC = forced vital capacity, FEV.1 = forced expired volume in 1 second, FEF 25-75% = forced expiratory Flow, VA = alveolar ventilation, PaO2 = arterial oxygen tension, PaCO2 = arterial carbon dioxide tension, SHUNT = PaO2 reached during breathing 100 % oxygen, UNV = uneven ventilation slope from nitrogen washout curve, B.P. = Blood Pressure, X-ray = chest x-ray, % Pr = % of Predicted

The existence of confluent nodules, scars, opacities, interstitial fibrosis, and patchy shadows on chest x-ray were considered abnormal. Age distribution in a large group of miners with EE compared to normals are shown in fig. 1.

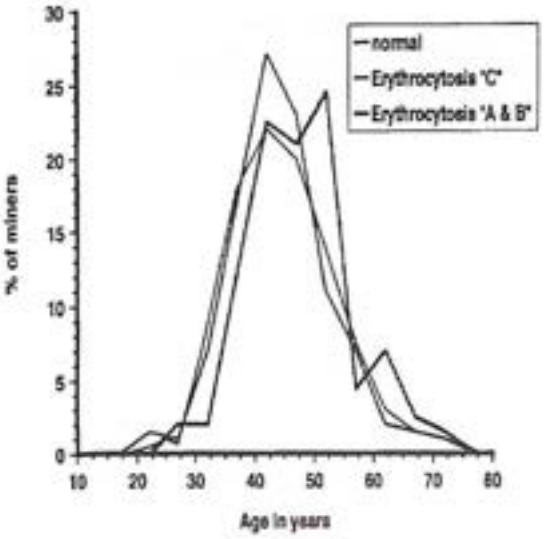

Fig. 1. Percentage distribution of ages for 129 miners with erythrocytosis types A & B, 395 miners with erythrocytosis type C and 1739 miners with no erythrocytosis at 3600 m. (From Zubieta et. al. Revista de la Academia Nacional de Ciencias de Bolivia 1985) [15].

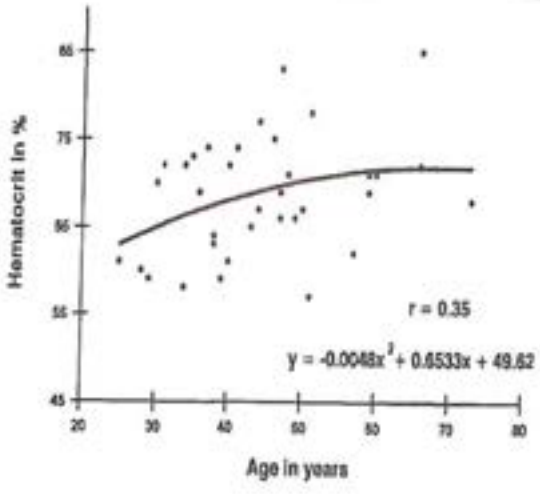

Fig. 2. Correlation between hematocrit and age in 35 patients with EE.

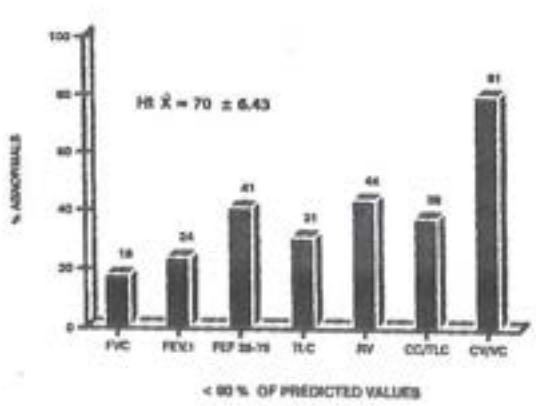

Fig. 3. Percentage of abnormals in pulmonary function testing in 17 patients with EE.

The tendency to increase EE with age in 35 patients with EE is shown in fig. 2. In fig. 3 abnormal pulmonary function tests are shown in 17 patients. FVC and RV/TLC in relation to hematocrit in 17 and 15 patients respectively are depicted in fig. 4 and 5. The correlation between PaO_2 breathing ambient air during hyperoxic tests in EE patients with pulmonary shunt is shown in fig 6.

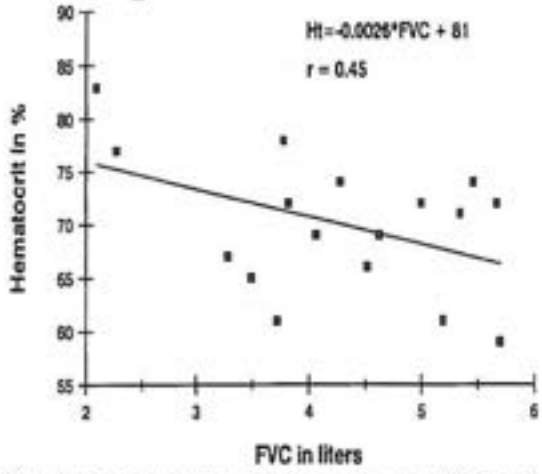

Fig. 4. Relation between hematocrit and FVC in 17 patients with EE.

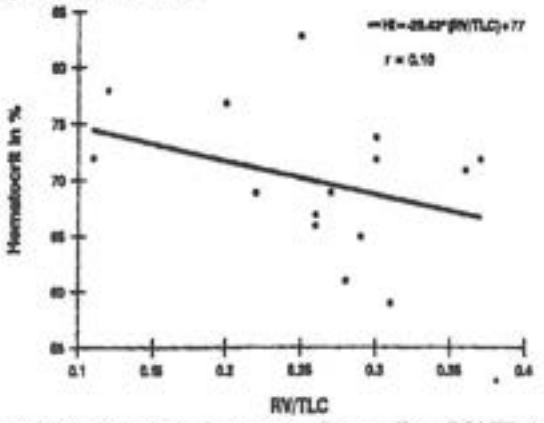

Fig. 5. Relation between the radio RV/TLC obtained from the nitrogen washout curve with the hematocrit in 15 patients with EE.

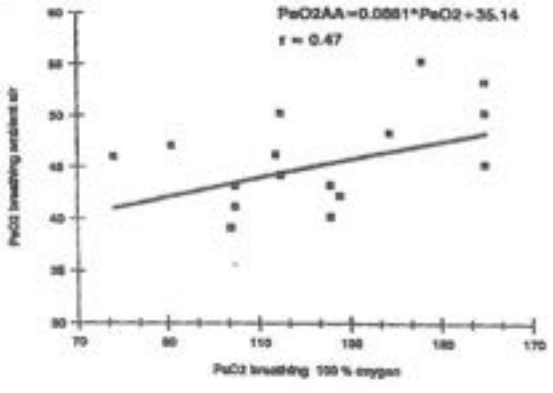

Fig. 6. Relation between the pulmonary shunt determined by breathing 100 % oxygen at 3600 PB = 495mmHg, and the PaO2 breathing ambient.

Discussion

Previous studies [15,16,14] in 2263 patients with silico-tuberculosis, with evident pulmonary lesions, whose diagnosis was based solely on the physical exam, chest radiology and increase of RBCs; showed 5.7% with severe erythrocytosis (types "A" & "B"): $> 7.5 \times 10^6$ GR and 17.45% with moderate erythrocytosis (type "C") $> 6.5 \times 10^6$ and $< 7.5 \times 10^6$ GR.

They all had the same characteristics typical of "chronic mountain sickness", that is, over 40 years old and overweight (fig. 1) [14,15].

With respect to the relation between hematocrit and age in 35 patients with EE there is a tendency for the hematocrit to increase with age ($r = 0.35$) with a plateau at around 60 years of age (fig. 2), showing the adaptation to disease without reaching extremely high levels of the hematocrit. Multiple observations were made by other authors [7].

In another group of 17 subjects with a mean hematocrit of $70 \pm 6.43\%$, the results of pulmonary function tests below 90% of predicted are shown in fig. 3. The greater percentage of abnormals corresponds to the relation closing volume/vital capacity (81%), which merits further observations and analysis.

Fig. 4, confirms the observations of other authors of lower FVCs and larger hematocrits [6,7]. A further observation is that there is an inverse relation between RV/TLC and hematocrit in patients with EE (fig.5).

A direct relation ($r = 0.45$) was obtained between pulmonary shunt determined by hyperoxic tests, expressed as the PaO_2 reached while breathing 100% oxygen and the PaO_2 while breathing room air (fig.6).

From all this data it can be appreciated that the EE is due to some form of pulmonary function abnormality. Adequate technology and experience in chest x-ray interpretation is required. All 10 subjects have a low PaO_2, with or without CO_2 retention.

It is evident that pulmonary lesions of any origin, give rise to an accentuated erythrocytosis, depending on the severity of the lesions, the altitude, and time of residence at altitude, with an awareness that some subjects are more susceptible than others to this accentuation. On the other hand, obvious lesions although not extensive on the chest x-ray, may create pulmonary shunts evident during pulmonary function testing, and cause a severe EE, attributed to ventilation-perfusion mismatch in lobes or segments of the lungs. Analogously, lesions that preserve pulmonary vessels, create shunts that are more erythrogenic than the total destruction of the bronco-alveolar and vascular structure. This leads us to propose the theory that a lobectomy of a compromised lobe, may eliminate the hypoxic stimulus to the kidney and therefore overcome secondary erythrocytosis.

Chronic diseases at high altitude, are the same as those at sea level but with hypoxic physiognomies. When the term "chronic mountain sickness" was originally coined, it created confusion because it included diverse pulmonary diseases that have an increased hematocrit, which is a mechanism of adaptation, that increases the oxygen transportation capacity of blood. In the patients suffering from "chronic mountain sickness" with EE, seems inappropriate and does not explain the pathogenesis of this chronic disease. In our experience, it is always due to some form of pulmonary, cardiac or renal disease, improperly diagnosed at high altitude. Accordingly, the terms secondary erythrocytosis,excessive erythrocytosis and increased polycythemia [1] have been created for diseases whose etiologies are clearly identified. The Cudkowicz scientific mission to La Paz in 1971, which we witnessed, studied 20 patients with CMS. In our opinion, all of them had pulmonary disease [3].

Therefore, it is important not to loose perspective, that adaptation of the human organism to changes in atmospheric pressure and hypoxia are remarkable, both for normal subjects as well as for the diseased.

We believe diseased persons have a slower and more progressive adaptation than residents of high altitude, and we should not consider this a "loss of adaptation". On the contrary they are well adapted to withstand even advanced degress of their disease and its limitations in the hypoxic environment of high altitude

Acknowledgement

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HORMONAL CHANGES IN HIGH ALTITUDE RESIDENTS

ANA MARIA ANTEZANA[1], JEAN PAUL RICHALET[1], R.CARREON[3] AND GERARDO ANTEZANA [2]

[1]Association pour la Recherche en Physiologie de l'Environnement (A.R.P.E.). Bobigny France, [2]Instituto Boliviano de Biologia de Altura (I.B.B.A) and [3]Departamento de Cardiologia-Hospital Obrero, La Paz Bolivia.

ABSTRACT

The response of renin and aldosterone is attenuated under acute and chronic exposure to high altitude (HA); the release of atrial natriuretic peptide (ANP) in sea level (SL) natives is also modified. No data are available in HA residents in whom increased total blood volume and the pulmonary arterial hypertension may influence the hormonal release. Twenty four men residing at 3600 ± 200m, La Paz, were included either in a normocythemic group "HAN" (hematocrit, Ht <57%, n=13) or in a polycythemic group "HAP" (Ht>57%, n=11). A control group of SL residents was studied in normoxia "SLN" and at 4,350m "SLH". Plasma active renin, aldosterone, ANP, potassium and norepinephrine concentrations were measured at rest and after a maximal exercise. Pulmonary artery pressure was assessed by Doppler at rest.
In SLH, renin and aldosterone were blunted, and ANP was unchanged.
In highlanders, the renin response was normal in HAN but decreased in HAP. The exercise-induced increase in aldosterone was lower in HA natives than in SL natives. The correlation between renin and aldosterone observed in SLN and HAN was not found in the group HAP. A protective mechanism against water and salt retention in polycythemic subjects may be evoked . ANP in highlanders was lower than in SL natives. Lower ANP in HA residents in spite of higher pulmonary pressure could be an adaptation to chronic distension of atrial stretch receptors. **(Acta Andina 1996, 5:9-13)**

Keywords: renin, aldosterone, atrial natriuretic peptide, exercise, pulmonary arterial pressure.

RESUMEN

La respuesta de renina y aldosterona está atenuada en la exposición aguda y crónica a las grandes alturas; la liberación del péptico atrial natriurético (PAN) en nativos de nivel del mar también se encuestra modificada. No existen datos disponibles respecto a estas hormonas en nativos y residentes de grandes alturas, en quienes determina eritrocitosis, aumento de la masa sanguínea e hipertensión arterial pulmonar, son factores que potencialmente pueden modificar la s liberación hormonal.Se estudiaron 24 residentes de La Paz, Bolivia, distribuídos en 2 grupos según el hematocrito (Ht):"HAN" normocitémicos Ht<57%, y "HAP" policitémicos Ht>57%.Un grupo de residentes del nivel del mar constituyó el grupo control estudiado en normoxia "SLN", y en hipoxia a 4,350 m "SLH". Fueron medidas en plasma la renina, aldosterona, peptido atrial natriurético, noradrenalina, dopamina y electrolitos, en condiciones de reposo y despúes del ejercicio. La presión arterial pulmonar fué medida por Ecocardiografía - Doppler. En los grupos HAN y HAP hubo bloqueo de la renina y aldosterona y el ANS no se modificó. En los sujetos de altura, la respuesta de renina fué normal en el grupo HAN pero disminuyó en el grupo. El incremento de aldosterona inducido por ejercicio fue menor en los nativos de altura que en los de nivel del mar. La correlación entre renina y aldosterona observada en los grupos SLN y HAN no se encontrón en el grupo HAP. Puede evocarse un mecanismo protector contra la retención de salu y agua en sujetos policitémicos. La ANS en sujetos de altura fue menor que en nativos de nivel del mar. **(Acta Andina 1996, 5: 9-13).**

Palabras claves: renina, aldosterona, peptido atrial natriurético, ejercicio, hipertensión arterial pulmonar.

High altitude hypoxia blunts the renin and aldosterone response during acute and chronic exposure in sea level subjects exposed to high altitude (5,7,9,10,12). Acute mountain sickness is associated to fluid retention with increased antidiuretic hormone (16), aldosterone (2) and atrial natriuretic peptide concentration (3), particularly in pulmonary oedema (11). No study is available in healthy natives residing above 3,000m.Chronic hypoxia might interfere with these hormones since tissular hypoxia, greater volume load secondary to polycythemia, and pulmonary arterial pressure are factors that may modify the hormonal release (10). In moderate altitude natives, 2,200m, the aldosterone response has been shown to be decreased when subjects were exposed to a more severe simulated hypoxia (9,13).

The purpose of this work was to study two hypotheses: (a) residents at 3,600 m show a persistent blunting of the renin aldosterone system, and this could be an adaptive response to chronichypoxia, or (b) the renin aldosterone system is over-stimulated and impairs water and sodium excretion, suggesting a lack qf adaptation.

Address for correspondence:
Dr. Ana Maria Antezana
A.R.P.E 74 rue Marcel Cachin 93012 BOBIGNY CEDEX FRANCE
Phone: 33 1 Fax: 33 1 48387777

Methods

The normocythemic group "HAN" (n=13,33 ± 7yr), had an hematocrit <57%, and the polycythemic group "HAP" (n=11,35 ± 10yr) had an hematocrit >57%. Subjects were born between 3,200m and 4,000m, and were residing at La Paz most of the time (3,600m, P_B=500mmHg). Polycythemic subjects were not receiving any treatment. Arterial blood gases, ventilatory mechanics and spirometric measurements were performed in HAN and HAP groups before inclusion to eliminate a respiratory disease at the I.B.B.A. A control group included 8 subjects from sea level (35±6 yr, 66±3kg studied in normoxia "SLN", and

after 4 days at 4,350m "SLH", at the Vallot's Observatory, France (4,350m, P_B=450mmHg).

Blood samples were obtained at rest and at the end of exercise. The samples were centrifuged and stored in liquid nitrogen for ulterior examination in France. Plasma active renin was measured as active renin immunologically recognized by specific monoclonal antibodies according to the IRMA technique (14); sensitivity was 10 pg/l and the intra-and interassay coefficients of variability were 4 and 6%, respectively. Plasma aldosterone concentration was measured with a radioimmunoassay (Aldosterone II RIA kit, Abbot Laboratories). Aniline-8-naphtalene-1-sulfate was used for extraction. Recovery was 106.5%. Sensitivity was 61 pmol/l and the intra-and interassay coefficients of variability were 3.7 and 4.4 %, respectively. Plasma immunoreactive-atrial natriuretic peptide was measured by a direct radioimmunological technique (7); sensitivity was 3.6 pg/ml with rabbit antiserum, and the intra-and interassay coefficients of variability were 6 and 9%, respectively. Catecholamines were measured by a high-pressure liquid chromatography method (15).

Maximal exercise, performed in a cycloergometer was limited by exhaustion. Oxygen comsumption (vO_2) was measured at the end of each stage through collection of expired gas in a Douglas bag. Expired volume was measured by a Tissot Spirometer, and expired O_2 and CO_2 concentrations were measured by Servomex 570 A and Capnograph Gould, respectively. Heart rate, systolic and diastolic arterial blood pressure and oxygen saturation were continuously monitored. Mean arterial pressure was obtained as 2/3xDBP+1/3xSBP.

Pulmonary artery pressure was assessed at rest by means of a 2.5 mHz probe (4).

Statistical analysis was performed by analysis of variance and covariance. Data are presented as means ± SD.

Results

Resting heart rate and maximal heart rate were not different between highlanders and SLN (Table 1). In SLH resting heart rate was significantly higher than in SLN whereas maximal heart rate was lower. Blood pressure did not show significant differences between the HA groups and SLN in normoxia; MBP was lower in highlanders at rest and during exercise.

Maximal oxygen consumption (VO_2) was lower in highlanders: 32±6 and 31±5 ml.kg-1.min-1 for HAN and HAP respectively, than in lowlanders, 44±4 ml.kg-1.min-1 in normoxia.

Table 1. Hematological characteristics, heart rate and blood pressure.

		SLN	SLH	HAN	HAP
SaO_2 (%)	r	97±1	92±4 **	95±2	94±4
	e	97±1	89±2 **	91±4 **	90±4 **
CaO_2 (ml.100ml $^{-1}$)	r	18.8±0.8	18.4±1.0	21.2±1.0	24.7±1.2
Ht (%)	r	44±3	44±3	52±3**	62±5**
Hb(g.dl)	r	14.5±0.8	15±1.3	16.7±1.0**	19.6±1.5**
HR (bpm)	r	66±7	91±8 **	57±1	69±7
	e	182±8	164±10 **	181±12	173±12
MBP (mmHg)	r	95±6	97±5	91±5	91±8
	e	139±8	127±15	124±12	122±14

Values are mean ± SD : * $p<0.05$, ** $p<0.001$ different from SLN.
r = rest, e = exercise. SLN: sea level natives in normoxia. SLH: sea level natives in hypoxia . HAN: high altitude normocythemic natives. HAP: high altitude polycythemic natives.
Hb: hemoglobin concentration; Ht: hematocrit, SaO2: oxygen saturation; arterial oxygen content; HR: heart rate; MBP: mean systemic blood pressure.

Plasma norepinephrine concentration increased during exercise in all groups (Table 2). Highlanders showed higher levels of norepineprine than SLN, but lower than SL($p<0.01$).

Plasma active renin (PAR) decreased in SLH at rest and after exercise.Resting PAR was higher in highlanders than in SLN; the exercise-induced increase in PAR was lower in HAP (-43%) than in the other groups (Table 2); the response of aldosterone to renin was reduced in HAP as it is shown in Fig.1, where no correlation was found, conversely to the three other groups.

Table 2. Hormonal concentrations at rest and after maximal exercise.maximal exercise.

		SLN	SLH	HAN	HAP
PAR	r	21±7	12±8 *	33±17 *	41±36
pg.ml^{-1}	e	69±44	30±18 *	63±41	58±50
PAC	r	364±139	193±107 *	332±141 *	277±79 *
pmol^{-1}	e	903±268	363±129 **	456±157 *	444±150 *
ANP	r	56±25	38±28	16±7 *	34±40 * +
pg.ml^{-1}	e	91±32	92±41	40±5 *	66±56
NE	r	280±117	734±204 **	550±204 **	632±417 *
pg.ml^{-1}	e	1439±794	3724±1566**	2409±1264*	2486±1690*
DA	r	56±51	37±14	51±27	31±11
pg.ml^{-1}	e	31±18	157±78	143±75 *	76±60 +

Values are means ± SD r = rest, e = exercice.
*$p<0.05$, **$p<0.01$ different from SL, +$p<0.05$ HAP different from HAN.
PAR:plasma active renin. PAC:plasma aldosterone concentration. ANP:plasma atrail natriuretic peptide concentration. NE, DA: plasma norepinephrine and dopamine concentrations.

Resting and exercise plasma potassium concentration was not different between groups (SLN: 4.1, SLH:3.8, HAN: 4.4, and HAP: 4.6 mmol.l-1). ANP was lower in highlanders than in SL natives; it was higher in HAP than in HAN. In HA natives low ANP concentrations were associated with higher pulmonary pressure, when compared to SL natives (Fig. 2). Systolic pulmonary arterial pressure at rest in SLN was 21±5 mmHg, in HAN 26±3 and in HAP 30±5 mmHg. There was a loose correlation between ANP and pulmonary pressure in HAP (r=0.664).

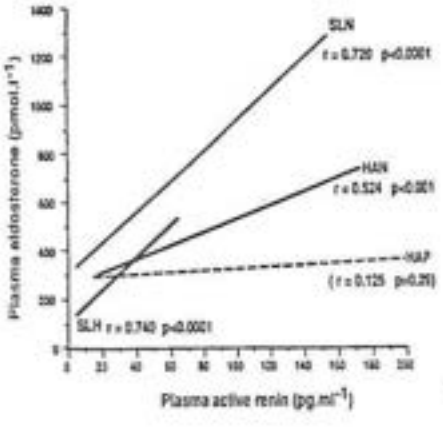

Figure 1.-Correlation between active renin and aldosterone. The aldosterone response to renin is significantly attenuated in the polycythemic group.

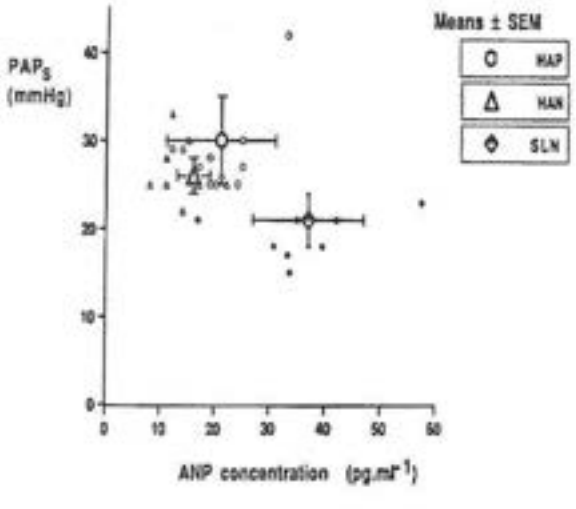

Figure 2.-Relationship between plasma atrial natriuretic peptide (ANP) and systolic pulmonary arterial pressure. Low values of ANP are observed in HA residents despite higher pulmonary pressure.

Discussion

The purpose of the present study was to determine the response of the renin-aldosterone system and the natriuretic peptide under chronic hypoxia. An attenuated response would account for an adaptive phenomenon; an excessive release of these hormones would account for a lack of adaptation as occurs in acute mountain disease. Comparisons of our results should be interpreted with caution, since groups were exposed to a different hypoxic stimulus: 4,350m for SL natives vs 3,600m for bolivians.

Higher resting renin concentrations were observed in four normocythemic subjects, and in one polycythemic subject; aldosterone levels in these subjects were normal. Higher renin secretion may occur when renal perfusion pressure decreases, but mean blood pressure was not lower in these subjects. Also hypohydration and low sodium intake may be responsible for increased renin and aldosterone secretion (13,16) but this was unlikely in our groups. A higher norepinephrine secretion may explain a higher resting renin in those subjects. The higher norepinephrine levels in highlanders were within normal physiological ranges. A blunting in the renin-aldosterone system was observed in the SL subjects exposed to 4,350 m; this agrees with previous studies (16,17). A downregulation of peripheral beta-adrenoceptors, renal beta-receptors, secondary to the sympathetic hyperactivity has been evoked to explain this feature. Those residents from group HAN who showed high levels of NE did not show any attenuation of the renin system; the correlation between NE and PAR was only slightly decreased in HAN.

A positive relationship between renin and aldosterone was observed in SL groups and in HAN, but not in HAP. Higher resting renin concentrations were observed in four normocythemic subjects, and in one polycythemic subject; aldosterone levels in these subjects were normal. Higher renin secretion may occur when renal perfusion pressure decreases, but mean blood pressure was not lower in these subjects. Also hypohydration and low sodium intake may be responsible for increased renin and aldosterone secretion (13,16) but this was unlikely in our groups. A higher norepinephrine secretion may explain a higher resting renin in those subjects. The higher norepinephrine levels in highlanders were within normal physiological ranges, suggesting a blunted response of aldosterone to the renin stimulation in this polycythemic group. Besides a lower exercise induced increase of aldosterone was observed in HAP. Factors which may inhibit the aldosterone secretion are hypokalemia, simultaneous and great ANP release, increase dopamine release and low tissular PO_2 in the adrenal cortex (9,16). None

of these factors could explain the aldosterone attenuation in HAP. Nevertheless, a lower synthesis secondary to a lower tissue PO_2 in the adrenal cortex particular during exercise could happen, since an inhibition of 18-hydroxylase under hypoxia has been described (16).

Resting plasma ANP concentration was lower in highlanders than in SL natives. ANP release is stimulated by atrial distension, increased central blood volume, increased heart rate, hypoxia and cold (19). Heart work, assessed by the double product HR x BP, and work load were equivalent in both SL and HA groups, so the intensity of the exercise was the same. A rise in pulmonary arterial pressure triggers ANP release during exercise (1,3,11); for technical reasons pulmonary pressure was assessed only at rest. Moderate polycythemia could also account for an increased blood volume and for a greater ANP release in HAP when compared to HAN.

In conclusion, renin was not blunted in HA natives. The higher concentration of active renin could result from a higher sympathetic tone in some subjects. A lesser exercise induced increase in aldosterone observed particularly in polycythemic subjects, could be interpreted as a protective adaptive mechanism from water and salt retention and central overload; nevertheless, a deeper adrenal hypoxia is not excluded. These results are in agreement with those observed in moderate-altitude residents (6), in which water and salt retention are probably also avoided by an aldosterone blunting. The low values of ANP in the HA groups in spite of higher pulmonary arterial pressures could be due to an adaptive phenomenon of overstimulated arterial stretch receptors. A progressive distension of these receptors at later stages could be associated to higher ANP concentrations, desensitization of peripheral receptors to ANP, and development of pulmonary hypertension, with no more pulmonary vasodilation and with impairement of renal function. Studies including renal function, hemodynamics and biological markers are needed to better understand the processus of decompensation in some high altitude residents.

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TRIPLE HYPOXIA SYNDROME

ZUBIETA - CASTILLO, G. AND ZUBIETA - CALLEJA, G.

High Altitude Pathology Institute (IPPA). La Paz, Bolivia.

ABSTRACT

Many patients with **excessive erythrocytosis (EE)**, with hematocrit greater than 55%, but lower than 70%, apparently are normal. They work, play soccer, develop intellectual activities and frequently perform better than sedentary normal people (3500 m). They request medical attention, only when they present symptoms similar to those of **acute mountain sickness (AMS)**, such as: headache, dyspnea, nausea, lassitude and indigestion. Without going higher they have been said to experience "sorojchi (AMS) in bed". They show extreme hypoxia with an oxygen arterial tension (PaO_2) near 20 mmHg, with or without hypercapnia and a normal or acidotic pH. We have previously named this complication of **EE, as the triple hypoxia syndrome (THS)**. It is due to: [1] Normal high altitude adaptation to hypoxia, [2] EE hypoxia (CMS) and [3] acute hypoxia that can be reversed by oxygen. The THS is similar to "surviving" on the summit of Mount Everest. It may be caused by viral infections (flu) or some other acute respiratory disease, with malaise that lasts several days without treatment and typically is reversed by 24 hours of oxygen to PaO_2 baseline values of their chronic condition with EE. The diagnosis is important, since the THS is an acute transitory condition, that when not recognized and treated with oxygen can possibly lead to cardiac, pulmonary or cerebral complications. **(Acta Andina 1996, 5:15-18)**

RESUMEN

Muchos pacientes con eritrocitosis excesiva (EE), con hematocritos mayores a 55%, pero menores de 70% aparentemente se desarrollan normalmente. Trabajan, juegan fútbol, y desarrollan actividades intelectuales incluso en mejores condiciones que las personas normales sedentarias de la misma altura (3500m). Ellos solicitan atención médica, solamente cuando presentan síntomas similares a los del sorojchi o mal de altura, tales como: cefaleas, disnea, nausea, lasitud e indigestión. Sin ascender a la altura se dice que sufren "sorojchi en cama". Sus gases en sangre muestran extrema hipoxemia con tensiones parciales de oxígeno (PaO_2), alcanzando los 20 mmHg, con o sin hipercapnia, y pH normal o acidosis. Hemos denominado previamente a esta complicación de la EE, como **síndrome de triple hipoxia (THS)**, el cual se debe a la suma de las tres siguientes hipoxias: [1] hipoxia normal de altura, [2] hipoxia de la EE [3] hipoxia aguda reversible después de la administración temporal de oxígeno . La THS se asemeja a "sobrevivir" por encima de la cima del monte Everest. Puede deberse a infecciones virales (gripe) o alguna otra forma de enfermedad respiratoria aguda, con malestar que dura varios días sin tratamiento y típicamente se revierte con 24 horas de oxigeno a un PaO_2 basal de la EE. Su diagnóstico es importante, ya que el THS es un transtorno agudo, que si no es reconocido y tratado con oxígeno puede llevar a complicaciones cardiacas, pulmonares o cerebrales.**(Acta Andina 1996, 5:15-18)**

More than 10% of the adult population in the Bolivian Andes, above 3000m, have increased numbers of red blood cells (RBC), commonly described as increased polycythemia, secondary erythrocytosis or recently Excessive Erythrocytosis (EE). This pathological entity is secondary to pulmonary disease of various etiopathogenesis associated with a low inspired partial pressure of oxygen in hypoxic environments [4,13,14,15]. It is well known as chronic mountain sickness (CMS) or Monge's disease [7,8,9,10] characteristically present in patients of 40 years or older, overweight, with hypoventilation, low arterial oxygen tension, low oxyhemoglobin saturation and cyanosis with or without CO_2 retention. There are different grades of this disease, in relation to the extent of the pulmonary lesions and the individual susceptibility. It affects natives and all races. They work, play soccer, develop intellectual activities and frequently perform better than sedentary normal people.

Periodically, these patients have aggravations of their bothersome symptoms, which force them to consult a physician. The symptoms include intense headaches, loss of appetite, nausea and sometimes bleading from gastro-intestinal ulcers or the nose. They look more cyanotic and have to interrupt their daily activities. In these circumstances many physicians perform phlebotomies, as the only therapeutic alternative. Although controversial, it seems that the phlebotomy temporarily alleviates the symptoms, without an adequate explanation.

Previously, we have described the first case and named it **Triple hipoxia Syndrome** (THS) a complication of EE [2,12,13]. Here, we present further observations in three cases at 3600m, with an average barometric pressure (PB) of 495 mmHg.

Case 1

Patient NPC, male 49 years old, born and living in La Paz, (3600 m), weight 88.6 Kg, height 162 cms who three days before consultation caught a cold, with malaise and headaches that interrupted his work as a truck driver. Five years ago he had similar symptoms but not as intense. Two years previously, polycythemia was found in a routine check-up, and phlebotomy was performed, with no further examination or treatment.

Corresponding author:
Gustavo zubieta-Castillo IPPA La Clinica del Instituto de Patologia en la Altura. Casilla 2852, La Paz, Bolivia.
Tel. 591-2-368734
Fax. 591-2-357159
E-mail:zubieta@oxygen.bo

On physical examination, he was slightly overweight, cyanosis of the hands, face, mucous membranes and palpebral conjunctives was present and he was able to walk around without severe impairment. The face was swollen, the skin dropsy and he was oliguric. His blood pressure was 160/100 mmHg. The hematocrit was 78%, hemoglobin 25.4 gm% the RBC count 8.736.000 per cubic mm. The white blood cell count was 5500 with 69% neutrophils, 29% lymphocytes, 1% eosinophiles and 1% monocytes. The erythrocyte sedimentation rate was 0 mm, the first hour. Uric acid was 4 mg%. Serum protein electrophoresis
with albumin, beta and gamma fractions within normal limits, but alpha 1 and alpha 2 were slightly diminished. The urine analysis was normal.

The electrocardiogram showed a small increase in P-wave amplitude and a left posterior hemiblock, typical of chronic cor pulmonale. The chest x-ray revealed a discrete increased hearth volume (+), pulmonary arch slightly prominent and an increase of the bronco-vascular shadows. Vital capacity in BTPS was 5024 ml (normal expected), FEV.1/FVC was 81%. The ventilatory and blood gas values are given in Table 1. PaO_2 before treatment was 20 mmHg.

		PIO2 mmHg	Hb gm%	VE(BTPS) ml/min	RF /min	VT ml	PaO2 mmHg	PaCO2 mmHg	pH art	CaO2 vol%
A	NORMAL	94	16.0	8577	17	486	57	28	7.40	20.57
B	Typical CMS	94	21.0	8013	17	477	46	31	7.38	29.29
C	Case 1	94	25.4	8505	19	447	20	30	7.42	11.29
D	Case 1 w/O2	430	25.4	9739	5	1947	54	32	7.40	30.36
E	Case 2	94	25.0	9856	23	428	19	35	7.31	10.07
F	Case 2 w/O2	430	25.0	13935	29	480	82	26	7.47	33.36
G	Case 3	94	25.4	6838	24	284	47	28	7.40	28.95
H	Case 3 w/O2	430	25.4	7612	16	475	185	27	7.37	34.95

Table 1.A)Mean values found in normal subjects, B)Chronic mountain sickness C,E & G)Cases with THS in the acute phase. D,F & H Cases during the administration of 100% oxygen while the shunt test is performed; PIO2 = Partial inspired oxygen tension; VE = ventilation per minute; RF = respiratory frequency; PaO2 = Partial arterial oxygen tension; PaCO2 = Partial arterial carbon dioxide tension; pH = arterial pH; CaO2 = arterial oxygen content. PB = 495 mmHg at 3600 m.

Ventilation was measured during 15 minutes collecting expired samples in a Tissot along with radial blood samples for their analysis in a PHmk2 Radiometer acid-base analyzer. Prior calibration followed manufacture's recommendations. Ventilation was repeated having the subjects inspiring 100% oxygen from a Douglas bag, during 5 minutes. Twenty four hours following oxygen treatment through nasal prongs at 2 liters/minute, the PaO_2 breathing ambient air returned to baseline values of 44 mmHg. Chest x-rays did not show any changes after the oxygen treatment.

Case 2

Patient CGG, male, 63 years old, born and living in La Paz, weight 92.4 Kg, height 168cm was diagnosed as having increased polycythemia several years earlier. On consultation he had fever, vomiting, dorsalgia and pain in both legs. One year before, he had a similar problem, and had to be hospitalized. On two occasions, he had had phlebotomies, the last, one month before consultation. Four years before he had

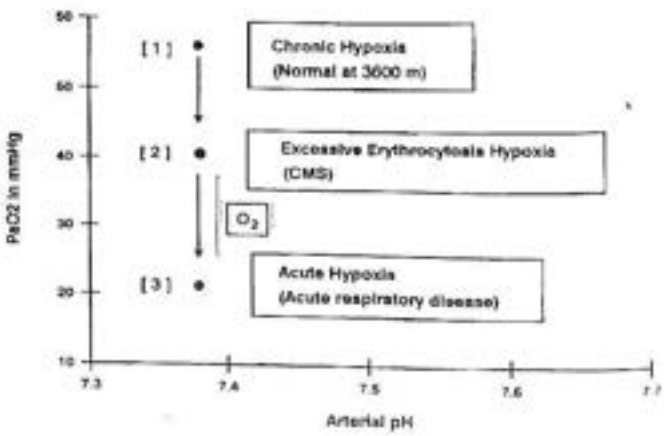

Fig 1. Triple Hypoxia Syndrome (THS). PaO2 showing the three levels of hypoxia. Twenty four hours of oxygen by mask reverse the THS to level 2.

had an episode of gastro-duodenal bleeding. He had never smoked. On physical examination, his tongue was white and he had tachycardia and cyanosis. His blood pressure was 174/103 mmHg. His hematocrit was 75% with 8,400,000 RBC`s and hemoglobin 25 gm%, white blood cell count was 9800, with 83% neutrophils, 15% lymphocytes, 1 %bands eosinophils. Urine analysis showed shown in Table 1. protein 500 mg/dl. His Vital Capacity in BTPS was 4330ml, normal expected, with a FEV.1/FVC of 97%. His blood gases are shown in table 1 along with ventilation studies. He had PaO_2 of 19 mmHg, $PaCO_2$ of 35 mmHg, pH of 7.31 and O_2 saturation of 29%. His ventilation studies following the same technique described in case one breathing ambient air and with 100% oxygen are shown in Table 1. The electrocardiogram with 125 beats/min showed elevated P-waves, negative T waves and left posterior hemiblock. He received oxygen at 3 liters/min. The next day his PaO2 without oxygen was 35 mmHg and the day after 41 mmHg, when he was discharged . No phlebotomy was performed, and his chest x-rays remained unchanged.

Case 3

Patient GAQ, was a male, 37 years old, 51 Kgs weight and 157 cm tall. He felt dyspneic, and had headaches. He had been diagnosed as having pulmonary tuberculosis 16 years before, and received adequate treatment. On consultation he was dyspneic and cyanotic. His tongue was white and his blood pressure was 105/80 mm Hg. His chest x-ray had many nodular callificcations and remained unchanged after treatment.

His hematocrit was 81% with 9.072.000 red blood cells per mm. and 27 gm% hemoglobin. His white blood cell count was 5250 with 64% neutrophils, 2% band, 1% eosinophils, 32% lymphocytes and 1% monocytes. Urine analysis showed protein 350 mg/dl. The electrocardiogram showed an elevated P-wave, with left ventricular hypertrophy. His vital capacity was 3709 ml (normal expected) with a FEV.1/FVC of 74%. His blood gases were: pH = 7.35, $PaCO_2$ = 32 mmHg and PaO_2 = 47 mmHg with a calculated O_2 Saturation (SaO_2) of 82%. Following 24 hr oxygen therapy, his pH returned to 7.38 and PaO_2 increased to 52 mmHg (SaO_2 = 87%), at room air. Ventilation results are

Discussion

These patients have symptoms similar to those present in acute mountain sickness (AMS), which are headaches, loss of appetite, nausea and malaise [5,6] with the absence of respiratory alkalosis instead hypoventilation and a tendency towards acidosis. The syndrome resembles the ascent of a normal subject to altitudes above the summit of Mount Everest, where the PaO_2 is near 28 mmHg [11]. Hence the name "sorojchi (AMS) in bed". The low levels of PaO_2 near 20 mmHg reached by some, can only be explained by the increase of oxygen content in the blood of these patients with hematocrits above 80%. At sea level, such intense hypoxemia would be classified as acute respiratory failure [1] and would be treated in an intensive care unit [3]. Acute respiratory failure is not evident in these ambulatory patients at high altitude. The normal pH of 7.4 in one of these cases shows the remarkable capacity to compensate acid-base equilibrium in chronic hypoxia. Although the triple hypoxia syndrome (THS) was initially described as having a hematocrit of 80% or more, normal pH, $PaCO_2$ of around 30 mmHg and a PaO_2 of 20 to 30 mmHg [12], further observations show that different patterns exist. The acute lowering of the PaO_2 appears to be the most significant finding. In some cases, the pH has been observed to be below 7.34 and the $PaCO_2$ above 32 mmHg, as in case 3.

The symptoms seem to disappear following the administration of 24 hours of 2 liters/min of oxygen by mask. The PaO_2 returns to baseline values of their chronic condition with EE.

Further study is required to analyze about the possibility that the hematocrit increases acutely during THS, which may be probably atributed to hemoconcentration. It later decreases by approximately 4%, following 24 hours of oxygen therapy.

We believe that this acute transitory condition, ocurring with seasonal or climatological changes, coincides with influenza epidemics, or some other acute respiratory illnesses usually of viral origin, evident in all three cases. The Triple Hypoxia Syndrome is due to the addition of the 3 hypoxias: (1) Normal high altitude chronic hypoxia, (2) EE

hypoxia (CMS) and (3) acute disease hypoxia (Fig. 1). The third hypoxia is an essential cause of complaint in patients with EE and reversible at the same altitude after 24 hour oxygen administration, avoiding unnecessary phlebotomies frequently used.

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PHYSIOLOGICAL CHANGES RELATED TO RAPID ALTITUDE SHIFTS IN LA PAZ, BOLIVIA

TRIPLETT, JC., ZUBIETA-CALLEJA, L., ZUBIETA-CASTILLO, G., AND ZUBIETA-CALLEJA, GR.

High Altitude Pathology Institute (IPPA). La Paz, Bolivia

ABSTRACT

Due to the bowl shaped topography of La Paz, Bolivia ,the residents of the city are constantly changing altitude from 3100 meters, in a common residential area to 4100 m at El Alto, an upper industrial city and airport area. In order to access cardio-respiratory alterations occurring with rapid altitude changes, 15 soldiers [average age = 19 years, Weight = 59.07 Kg., Height = 165 cm], residents of El Alto (4000 m), were examined in a mobil unit equipped with a computerized cardio-ventilatory apparatus. Following calibration of the ventilatory apparatus, and after 15 minutes of rest, over the next 6 hours, subjects were connected via mouthpiece to a one way flow valve for 15 minutes. Expired gas was collected in Douglas bags. The electrocardiogram was monitored with 3 standard chest leads. Finger oximetry was recorded. Blood was drawn for arterial gases analysis. These were analyzed at 3600m with a Radiometer Blood Gas MK2 pH. The same procedure was repeated the next day on descent to the forest of Aranjuez (3100 m). Saturation rose from 87.8 ± 2.83% to 91.47 ± 1.40% ($p < .001$). Heart rate changed from 72.5 ± 10.2 to 68.3 ± 7.9 ($p < .05$) beats per minute. Alveolar ventilation (BTPS) diminished from 7,117 ± 1,232 ml to 6,197 ± 844 ml ($p < .05$). Oxygen consumption greatly increased from 172 ± 30 to 300 ± 42 ml/min ($p < .001$). Carbon dioxide production did not change significantly 266 ± 43.23 to 245 ± 32 ml/min. The respiratory Quotient diminished from 1.5 to 0.8 Arterial partial oxygen tension (PaO_2) increased from 54 ± 2.79 to 64 ± 6.33 mmHg ($p < .001$). $PaCO_2$ changed from 33 ± 2.2 to 34 ± 1.57 ($p < .05$). The pH decreased from 7.434 ± 0.026 to 7.381 ±0.335 ($p < .001$). This research demonstrates that when patients are analyzed in a cardio-pulmonary laboratory after they descend or ascend to it, the change in altitude must be considered in interpretation of results. Rapid altitude shifts in La Paz, force residents to constantly adapt, producing respiaratory acidosis on descent within the city. **(Acta Andina 1996, 5:19-22)**

RESUMEN

Debido a la topografía montañosa de la ciudad de La Paz, Bolivia, sus residentes están constantemente cambiando de altura trasladándose entre los 3100 m de la zona residencial a los 4100 m en el Alto y el aeropuerto. Para valorar las alteraciones cardio-respiratorias que ocurren debido a estos cambios, 15 soldados (edad promedio = 19 años, peso = 59.07 Kg., Talla = 165 cm), residents de El Alto (4000 m), se examinaron en una unidad móvil equipada con aparatos computarizados. Después de la calibración del equipo respiratorio, los sujetos previo reposo de 15 minutos fueron conectados mediante una válvula bucal unidireccional durante 15 minutos, cada uno. El gas expirado fue recolectado en bolsas de Douglas y el electrocardiograma fue monitorizado con 3 derivaciones precordiales standard. Fue registrada la oximetría de dedo y se tomaron muestras de gases en sangre arterial, éstas fueron analizadas en nuestro laboratorio a 3600 m con un Radiometer Blood MK2 pH. El mismo procedimiento fue repetido al día siguiente inmediatamente después del descenso al bosque de Aranjuez (3100 m). La saturación de oxígeno se elevó de 87.8 ± 2.83 a 91.47 ± 1.40 ($p < .001$). La frecuencia cardiaca cambió de 72.5 ± 10.2 a 68.3 ± 7.9 ($p < .05$). La ventilación alveolar (BTPS) disminuyó de 7,117 ± 1,232 ml a 6,197 ± 844 ml ($p < .5$). El consumo de oxígeno se incrementó significativamente de 172 ±30 a 300 ± 42 ml/min ($p < .001$). La producción de anhidrido carbónico no cambió significativamente de 266 ± 43.23 a 245 ± 32 ml/min. El cociente respiratorio disminuyó de 1.5 a 0.8. La presión parcial arterial de oxígeno (PaO_2) aumentó de 54 ± 2.79 a 64 ± 6.33 mm Hg ($p < .001$). La $PaCO_2$ cambió de 33 ± 2.2 a 34 ± 1.57 ($p < .05$). El pH disminuyó de 7.434 ± 0.026 a 7.381 ± 0.335 ($p < .001$). Esta investigación demuestra que cuando los pacientes son analizados en un laboratorio cardio-pulmonar después de descender o ascender a la misma altura, el cambio de altura debe tomarse en cuenta en la interpretación de los resultados. Los cambios de altura bruscos y constantes en la ciudad de La paz, obliga a sus residentes a adaptarse constantemente, produciendo acidosis respiratoria al descender. **(Acta Andina 1996, 5:19-22)**

There are related to acute exposure to hypoxia of high altitude many study published in the literature [6,5,2]. Studies at fixed points of altitude of permanent high altitude residents have also been performed [3,4]. However, high altitude shifts of normal highland residents have not been studied. Nearly to 1 million inhabitants of high altitude in the city of La Paz (3600 m), have adapted to the altitude and varying topography constructing their residences and places of work at altitudes varying from 3100 to 4100 m. Some live in the residential areas in the lower part of the city and work in the higher part of the city then, they experience up to 1000m of altitude shifts daily and sometimes four times a day since the habit of going home for lunch persists. In order to observe the physiological changes that constant altitude shifts at high altitude impose on healthy humans, cardio-respiartory studies were performed at both altitudes.

Corresponding author:
John C. Triplett IPPA La Clinica del Instituto de Patologia en la Altura Av. Saavedra 2302 Casilla 2852 La Paz, Bolivia
Tel: 591-2-430251
Fax: 591-2-357159
E-mail: zubieta@oxygen.bo

Methods and Materials

A mobil camper-truck was equipped with computerized cardio-respiratory equipment. A Puritan-Bennett model REMAC adapted through an analog digital converter to a PC was used to measure ventilation during 15 minutes, through a mouthpiece one-way flow valve constructed in our laboratory with a noseclip. After 1/2 hour warm-up, calibration was made with a 3 liter syringe. Barometric pressure was taken from the military airport located at the First Brigade of the Bolivian Air Force in the Alto at 4005 m. with a

barometric pressure (PB) of 478 mmHg. Fifteen soldiers with age (19 years ± 1.55 S.D), height of 165 cm ± 6.46 and weight 59.07 Kg. ± 7.42, were observed following 15 minutes of rest. Expired gas was collected during 15 minutes of ventilation in Douglas bags and later after being appropriately mixed by flushing a disposable 60 cc plastic syringe, a sample was collected in this syringe. We had previously checked that these syringes were able to adequately store expired samples for days. Simultaneously; ECG monitoring was completed with 3 standard chest leads hooked-up to the same computer. Earlobe oximetry was performed with a portable NONIN oximeter Model 8500. Arterial blood samples were taken from either the radial or humeral artery while the subjects were lying down. The observations were done during a 6 hour span. Blood gases and expired gases were analyzed in our laboratory at 3500 m in the PhmK2 Acid-Base analyzer of Radiometer, properly calibrated according to manufacturer`s recommendations. The following day the same procedure was repeated in the same group of soldiers on descent to the bosque of Aranjuez (3100 m).

Statistical analyses was performed calculating averages and standard deviations in a PC with the software statpro and probability was calculated using paired and non-paired student's test.

Results

Table 1 shows the average and standard deviation of heart rate, blood pressure and ventilation values at both altitudes.

Alveolar ventilation (VA) was calculated from $VA = VCO_2/PACO_2 * 0.863$, where VCO_2 = Carbon dioxide production. Tidal Volume (Vt) from VE/RR where VE = Expired ventilation/min and RR = respiratory rate. Dead space volume (VDS) was derived from Bohr's equation. Fifteen soldiers were observed and any observation with systematicerrors were left out and hence the number of observations (n) varies.

		HR /min	BP Syst mmHg	BP Diast mmHg	RR /min	VE ml/min	VA ml/min	Vt ml	VDS ml
EL ALTO	n =	15	15	15	15	15	14	15	14
4005 m	X =	72.47	106.0	66.4	18.9	12944.8	7117.6	689.6	309.8
Pb = 471 mmHg	SD =	10.18	13.55	9.64	3.73	2607.08	1323.53	97.03	58.77
ARANJUEZ	n =	15	15	15	15	15	11	11	11
3140 m	X =	68.33	103.5	66.8	17.2	13031.7	6192.8	771.6	416.8
Pb = 524 mmHg	SD =	7.90	9.87	6.87	3.49	2116.72	885.33	110.12	56.17
	p	<.05	NS	NS	<.05	NS	<.1	<.01	NS

Table 1. Heart rate, blood pressure and ventilation changes in both altitudes. HR = heart rate, BP Syst = systolic blood pressure, BP Diast = diastolic blood pressure, RR=Respiratory rate, VE=Expired ventilation (BTPS), VA=Alveolar ventilation (BTPS), Vt=Tidal volume, VDS=Deadspace.

								STPD		
		SAT %	pH	PaCO2 mmHg	PaO2 mmHg	PEO2 mmHg	PECO2 mmHg	VO2 ml/min	VCO2 ml/min	RQ
EL ALTO	n =	15	14	14	14	15	15	14	14	14
4005 m	X =	87.8	7.434	32.5	54	77	17.9	172.5	266.2	1.56
Pb = 471 mmHg	SD =	2.83	0.026	2.20	2.79	2.25	1.95	30.59	43.23	0.16
ARANJUEZ	n =	15	11	11	11	14	14	11	11	11
3140 m	X =	91.5	7.382	34.2	64	79	16.4	300.7	245.4	0.82
Pb = 524 mmHg	SD =	1.41	0.034	1.57	6.33	2.94	1.76	42.22	32.52	0.06
	p	<.001	<.001	<.05	<.001	NS	NS	<.001	NS	<.001

Table 2. Ventilatory and blood gases changes in both altitudes. SAT = Oxygen saturation, PaCO2 = Arterial carbon dioxide partial pressure, PaO2 Arterial oxygen partial pressure, PEO2 = Expired gas oxygen partial pressure, PECO2 = Expired gas carbon dioxide partial pressure, VO2 = Oxygen consumption, VCO2 = Carbon dioxide production, RQ=respiratory quotient.

Table 2 shows the average and standard deviation of the respiratory parameters and blood gases. Carbon dioxide production (VCO_2) was calculated from VE * $FECO_2$, where $FECO_2$ = Fractional expired carbon dioxide. Oxygen consumtion on (VO_2) was derived from the classic oxygenconsumption equation.

Discussion

Previous studies of normal values of residents in La Paz, have been made considering the city of LaPaz as one point and relating its values to sea level or mountain regions near La Paz [1]. To our knowledge, no studies have been made in normal residents within the same city acutely exposed to altitude shifts. La Paz residents are constantly adapting and shifting to a relative environment of hyperoxia or hypoxia. The city's paved roads permit the ascent of close to 1000 m in 1/2 hour. The airport is located at the higher part of the city at 4050 m.

We found that the heart rate change from 72 to 68 was significant at the .05 level. VE,VA and VDS did not change, however changes in Vt and RR were significant at the .01 and .05 level, respectively. Arterial oxygen tension (PaO_2) and carbon dioxide tension ($PaCO_2$) and Oxygen saturation (%SAT) increased significantly, as expected. The decrease in ventilation upon going lower was confirmed. However the most important observation in our study dealt with VO_2. It is striking to observe a low

Air Force base (4005 m) altitude for these soldiers, and correspondingly the respiratory quotient was quite high (1.56). It seems that at this permanent altitude people consume less oxygen. Their physical capabilities, were not measured in this report, but are known to be excellent because as a military group they are constantly involved in conditioning exercise. On descent, their VO_2 increased significantly ($p < .001$) to 300 ml/min, although their VA diminished, and they maintained a similar VCO_2 and therefore lowered the RQ to 0.82. Finally the pH diminished significantly from 7.434 to 7.382 ($p < .001$). This is the opposite reaction for man on rapid ascent, where respiratory alkalosis develops because respiratory adaptationis inmediate but metabolic adaptation takes several days.

The same phenomenon is observed in this study, although inverse i.e. respiratory acidosis is produced by going down 1000 meters. This implies that the inhabitants of La Paz are permanently changing from respiratory alkalosis to respiratory acidosis, and viceversa. It is surprising to many visitors, that such a large population could have settled at this altitude. This observation should be taken into consideration when we draw blood for gas analysis in patients if they happen to come from the higher or lower parts of the city. Although they should adapt to the PO_2 of the altitude of the lab, the pH value may tend towards a respiratory acidosis or alkalosis.

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THE LIMITS OF PERFORMANCE AT HIGH ALTITUDE

BENGT KAYSER

Département de Physiologie, Centre Médical Universitaire, Genevé, Switzerland.

ABSTRACT

The intensity of maximum exercise involving a large muscle mass that an acclimatized lowlander can sustain at altitude is reduced compared to sea level. In fact, exercise is stopped before the appearance of biochemical signs of peripheral fatigue. One possible hypothesis to explain the reduction of classical biochemical signs of fatigue in the muscle after exhaustive exercise at high altitude could be that central fatigue, induced by a maximally stressed respiratory system, would possibly limit such exercise with large muscle groups before their full potential is elicited. It appears that in controlled laboratory conditions at 5050m the muscular apparatus by itself remains in good condition and in principle capable for work .This seems to be the case only when a small muscle mass is activated. Exhaustion time of dynamic fore arm work at the identical absolute (maximum) load is the same at sea level and altitude, and similar signs of peripheral muscle fatigue develop before exhaustion is reached. By contrast, for similar exhaustion time, the absolute maximum cycling load maintained at 5050m is ~20% lower than at sea level. Furthermore, while exhaustion during leg exercise at sea level is accompanied both by biochemical and electromyographical signs of peripheral fatigue, this is not the case at high altitude. Thus, at altitude, central rather than peripheral fatigue limits exhaustive exercise carried out with large muscle groups. Such mechanism could , at least partially, explain the decreased accumulation of lactic acid in blood in acclimatized subjects during exhaustive exercise at high altitude (''lactate paradox') and may represent a possible strategy for preserving vital respiratory functions from failure at altitude. Indeed at high altitude, the diaphragmatic contribution to ventilation during exercise at the same relative load decreases with time. This seems to be due to diaphragmatic fatigue, which hypothetically may contribute to exercise limitation at high altitude, although other mechanisms, like decreasing oxygen availability at the level of the central nervous system, may also play a role in limiting the duration of exhaustive exercise in conditions of chronic hypobaric hypoxia. **(Acta Andina 1996, 5:23-30)**

Keywords: exercise, lactic acid, muscle, fatigue, altitude, diaphragm

RESUMEN

La intensidad del ejercicio máximo, comprometiendo una masa muscular grande, que un sujeto puede sostener en la altura está reducida. En realidad, el ejercicio se interrumpe antes que aparezcan los signos bioquímicos de fatiga periférica. Una posible hipótesis para explicar la reducción de los clásicos signos bioquímicos de fatiga en el músculo después del ejercicio exhaustivo en la altura podría ser que la fatiga central, inducida por un sistema respiratorio máximamente estresado, podría posiblemente limitar tal ejercicio con largos grupos musculares, antes que se evidencie su pleno potencial . Parece ser que bajo condiciones de laboratorio controladas a 5050m el aparato muscular en sí, permanece en buenas condiciones y en principio apto para trabajar. Este parece ser el caso solo cuando una pequeña masa muscular es activada. El tiempo de agotamiento de trabajo dinámico del antebrazo a idéntica carga absoluta (máxima) es el mismo a nivel del mar que en la altura, y los signos de fatiga muscular periférica se presentan antes que se llegue al agotamiento. En contraste, para tiempos similares de agotamiento, la carga máxima absoluta en la bicicleta ergométrica mantenida a 5050m es ~20% mas baja que a nivel del mar. Más aún, mientras el agotamiento durante el ejercicio de piernas a nivel del mar es acompañado por signos bioquímicos y electromiográficos de fatiga periférica, este no es el caso en la Altura. Por lo tanto, en la altura, el agotamiento central en vez de periférico limita el ejercicio exhaustivo llevado a cabo en grandes grupos musculares. Tal mecanismo podría, por lo menos parcialmente, explicar la disminuida acumulación de ácido láctico en la sangre de los sujetos aclimatizados durante el ejercicio exhaustivo en la altura (lactate paradox') y puede representar la posible estrategia para evitar la falla de las funciones respiratorias vitales en la altura. En la altura la contribución diafragmática a la ventilación durante el ejercicio a la misma carga relativa disminuye con el tiempo. Esto parece ser debádo a fatiga diafragmática, que hipotéticamente puede contribuir a la limitación del ejercicio en la altura, a pesar de que otros mecanismos, como el disminuir la disponibilidad de oxígeno a nivel nervioso central, puede jugar un rol en limitar la duración del ejercicio exhaustivo en condiciones de hipoxia hipobarica crónica. **(Acta Andina 1996, 5:23-30)**

Palabras claves: ejercicio,ácido láctico, músculo, fatiga, altura, diafrágma.

The aim of this article is to briefly discuss the so-called "lactate paradox", i.e. the drastic reduction of the maximum accumulation of lactate in blood ([La] max) during intense exercise observed in acclimatized lowlanders at altitude when compared to sea level.

Before October 1996:
Bengt Kayser MD PhD Meakins Christie Labs University of McGill 3626 St Urbain Street Montreal H2X 2P2 Canada
tel: +1-514-398 4455 ext 3004
fax: +1-514-398 7483

After October 1996 :
Bengt Kayser M D PhD Dépt de Physiologie C entre M édicale Universitaire 1211 G enevé 4C Switzerland
tel: +41-22-702 5360
fax: +41-22- 702 5402
e mail: Kayser @ cmu.unige.ch

Lactate at altitude : the early observations

The first observations on blood lactate ([La]) during exercise at altitude were made by Dill et al. (1931). At altitude at a given work load [La] initially rose higher than at sea level, but after acclimatization reached sea level values again. In subjects acutely exposed to altitude Edwards (1936) also reported higher [La] levels at sub-maximum power outputs but similar [La]max. After acclimatization [La] levels at sub-maximum power outputs had returned close to sea level values whereas [La]max had decreased. Edwards thought that the decrease in [La]max during acclimatization to altitude might be explained by the reduced alkali reserve, by an altered enzyme activity or by diaphragmatic fatigue (Edwards, 1936). Cerretelli (1967) showed

that the decreased alkali reserve at altitude indeed shifts the slope of the line relating [H+] to [La] in blood after exercise and he argued that a lower intracellular pH for a given [La] could possibly impair the activity of glycolytic enzymes like phospho-fructo-kinase.

The paradox

In 1986 West measured a $PaCO_2$ on Everest of 7.5 Torr and calculated a resting PaO_2 of 28 Torr implying an even lower value during exercise. Despite this extreme hypoxemia no [La] accumulation would take place when extrapolating West's graph (adapted from Cerretelli, 1980) (Figure 1): *'If this extrapolation held good, a well acclimatized climber who reached the summit of Mount Everest without suplementary oxygen would have no blood lactate. This is a paradox indeed, because such a climber is apparently more hypoxic during maximal exercise than in any other known situation'* (West, 1986). In contrast with this prediction, during Operation Everest II (OE-II, a simulated climb of Mt. Everest) it was observed that exhausting exercise at a pressure equivalent to the summit of Mt. Everest resulted in a small but significant increase in blood [La] (Sutton et al., 1988; Young et al., 1992).

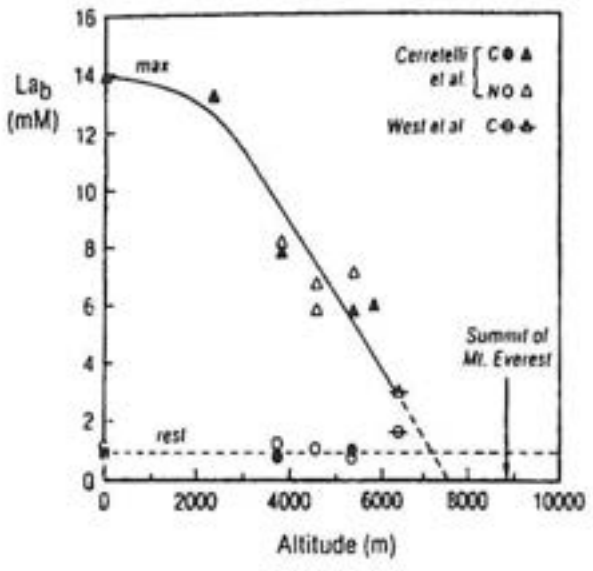

Figure 1:Maximal blood lactate (Lab) as a function of altitude. Most of the data are redrawn from Cerretelli et al (1982). The filled circles and triangles show data for acclimatized Caucasians (C); the open circle and triangles are for high-altitude natives (N). The data for 6300m are from acclimatized lowlanders. Extrapolation of the lineline to the horizontal axis suggests that a climber who reaches the summit of Mount Everest without supplementary oxygen will have no blood lactate (from West,1996).

The term *"lactate paradox"* was introduced by Hochachka(1989). He defined it as *"during incremental aerobic exercise test to fatigue* under hypoxic conditions they **(the Quechuas, South-American high altitude natives)** ***form less lactate and accumulate it to lower levels than normoxic lowlanders'*** **(Hochachka, 1989)**. After 6 weeks of deacclimatization at sea level these subjects were still unable to accumulate as much [La] as lowlanders during fatiguing cycling exercise and he concluded that it was therefore the expression of a fixed metabolic feature (*'the perpetual lactate paradox'*). Recently in a review by Reeves et al. (1992) of data obtained on lowlanders during OE-II and in work carried out on Pike's Peak, the lactate paradox was defined at the situation *'in which blood lactate accumulation during exercise is increased on arrival at high altitude but falls with acclimatization'*

Recent observations

The buffer capacity hypothesis was tested at 5050m during exercise at V O_2max, with or without prior oral sodium-bicarbonate ingestion (Kayser et al., 1993b). Despite normalized buffer stores after bicarbonate ingestion at altitude [La]max levels did not increase and exercise was interrumpted with both muscle and blood pH higher than at sea level. The hypothesis was therefore rejected.

The muscle resting glycogen level seems unaffected by chronic hypoxia (Green et al., 1989; Young et al., 1982). Upon arrival at altitude a standardized exercise causes a glycogen depletion greater than at sea level. After acclimatization the depletion is the same again as at sea level (Green et al., 1992). This muscle glycogen sparing effect upon acclimatization was atributed to a lesser B-adrenergic stimulation of glycogenolysis (Brooks et al. 1991c) and to a lesser dependance on intra-muscular glucose sources (Green et al., 1992; Brooks et al., 1992). In any case, a lack of muscle glycogen stores does not seem to be at the basis of the "lactate paradox".

The activity of several glycolytic enzymes in the vastus lateralis of climbers after a 6-8 week exposure to 5000-8600m was unchanged compared to pre-exposure (Howald et al. 1990)

which confirmed earlier results, including data on total glycogen phosphorylase (Green et al. 1989; Young et al. 1984). A slight decrease in phospho-fructo-kinase (PFK) activity at 4300m was found, but that was explained as a consequence of a changed glycolytic control rather than a cause (Green et al. 1992). By and large, it seems that the main regulatory enzymes of the glycolytic pathway are not affected by chronic pypoxia.

But, since enzyme activity is measured on homogenized muscle tissue in vitro it can only partially describe regulatory metabolic mechanisms in vivo and the hypothesis that a modulation of enzymatic metabolic control of glycogenolysis or glycolysis may be at the basis of the "lactate paradox" can not be ruled out. Indeed, even if total muscle glycogen phosphorylase is not altered at altitude (Green et al., 1992;Young et al., 1984) it remain possible that an increased activation of phosphorylase "b" to its active form a, for example during the initial phase at altitude when adrenaline levels are high, may lead to increased glycogenolysis.

After 18 days acclimatization at 4300m a decrease in the net lactate release from the exercising legs was observed (Bender et al. 1989). This decrease could be to a reduced production or to an increased removal of lactate. Recently the net rates of appearance and disappearance of lactate and glucose at rest and during exercise at sea level and at 4300m were determined (Brooks et al., 1991b; Brooks et al.;1991c; Brooks et al.,1992; Green et al.,1992; Mazzeo et al.,1994). Infusing isotope labeled lactate and glucose and sampling effluent blood from the limbs these authors were able, among others, to measure the net release and uptake of lactate and glucose by the limbs. Acute exposure to hypoxia would increase adrenaline release, which would stimulate glycogenolysis and glycolysis, leading to increased lactate release (Brooks et al.,1992). With acclimatization adrenergic drive would subside and the stimulation of glycolysis would decrease. Indeed, B-blockade decreased the amplitude of the effect of acute altitude exposure on glycolysis, and blood [La] during exercise was lower than in the control condition, although still higher than at sea level. However, it did not prevent the progressive decrease in exercise blood [La] that accompanies acclimatization to high altitude, although the amplitude of the decrease was considerably less (Mazzeo et al.,1994). Thus, the "lactate paradox" cannot be fully explained by an alteration of adrenergic control of glycolysis alone (Mazzeo et al., 1994).

During OE-II at low barometric pressures bicycle exercise induced exhaustion with less biochemical signs of muscle fatigue than at sea level (Green et al., 1989). On the other hand, since nerve conduction velocity, muscle end-plate transmission and muscle excitation-contraction coupling appear not to be altered by prolonged altitude exposure, the function of the neuromuscular system does not seem to be affected (Garner et al.,1991; Kayser et al., 1993a, Kayser et al., 1993d). Bigland-Ritchie and Vollestad (1988) hypothesized that at high altitude central (neural) rather that peripheral (metabolic) fatigue limits dynamic exhaustive exercise with large locomotor muscle groups. A maximally stressed respiratory system would, via the central nervous system, limit the central drive to large muscle groups before their full potential is reached. Indeed, acclimatized subjects at 5050m could sustain exercise with a small muscle group at the same absolute load, for the same time and with similar signs of peripheral fatigue as at sea level, whereas exhaustion of maximum whole body exercise, although performed at a lower absolute load, was reached after similar time but with no signs of peripheral fatigue (Kayser et al. 1994). At altitude the diaphragm fatigues during cycle exercise at 75% of V O_2max (Cibella et al., 1992;Kayser et al., 1993b) and could provide an input to the central nervous system leading to a decrease in central drive to the active locomotor muscles. A recent experiment performed at 5200m does not seem to support this hypothesis.

Acclimatized subjects performed repeated maximal isometric voluntary contractions before and during exhausting bicycle exercise with or without 4% inspiratory CO_2. With added CO_2 ventilation was higher, whereas the maximum voluntary contraction force of the forearm flexors at exhaustion was unchanged. The authors concluded that no decrease in central drive to the respiratory muscles or locomotor muscles had ocurred (Savard and Saltin, 1991). Thus, the origin of the signals leading to the cessation of the central drive at exhaustion from leg exercise at altitude remains unclear. The respiratory and/or other higher nervous centers, with or without contribution of the fatigued respiratory muscles and/or of decreased arterial O_2 saturation, are possible candidates. Nevertheless, the proposed mechanism of central limitation is compatible with the absence of signs of peripheral fatigue at the end of

exhausting maximum exercise with large muscle groups at altitude and with the lower levels of [La]max.

A working hypothesis: Acute hypoxia

Lowlanders exposed to acute hypoxia react to this stressful event with increased circulating adrenaline levels (Young et al., 1989; Young et al., 1991; Mazzeo et al., 1991; Mazzeo et al., 1994). This would stimulate, through muscle ß-receptors, the transformation of phosphorylase "b" to its active form "a", thus increasing the rate of splitting of glycogen to glucose monomers.

Since for a given level of sub-maximal power output oxidative phosphorylation (i.e. VO_2) would remain constant, through a mass-action effect, disproportionate high levels of pyruvate would result in a spill-over into lactate formation leading to higher muscle and blood [La] (Brooks et al., 1991c; Green et al., 1989; Green et al., 1992). During prolonged sub-maximal exercise a new steady state would be attained with stable but higher [La] levels than in normoxia but the same $V O_2$ (Brooks et al., 1992). Since maximum power output is decreased, the maximum rate of oxidative phosphorylation (i.e $V O_{2max}$) is also diminished and $[La]_{max}$ is similar to that at sea level, whereas for any given absolute sub-maximum work rates, [La] is higher. As a result, glycolysis appears to be slightly uncoupled from oxidative phosphorylation (Green et al., 1992). (also see figure 2).

Chronic hypoxia

At altitude the maximum power output that can be sustained during whole-body exercise reduced, possibly by a mechanism of central fatigue. Since the energy cost of muscular contraction *per se* is not altered by hypoxia, the maximal rate of TCA cycling is decreased in proportion. Thus, glycolytic flow into the TCA cycle is also decreased, simply by mass-action effect; and, if the coupling of glycolysis to oxidative phosphorylation would remain constant, [La] accumulation would depend only on exercise intensity and duration, and on the blood lactate removal.

It is conceivable that, in analogy to what happens on lymphocytes for ß2-receptors as well as in the heart for ß1-receptors (Richalet et al., 1990), chronic hypoxia may induce a down regulation of ß-receptors also in skeletal muscle, thereby diminishing the glycogenolytic effect of adrenaline. Whatever the mechanism, in the course of acclimatization, [La] values attained at sub-maximal exercise intensities decrease as compared to acute hypoxia. Since the maximum power output that can be sustained and consequently the maximal rate of oxidative phosphorylation (VO_{2max}) are decreased, $[La]_{max}$ would also decrease and the "lactate paradox" would be established. As a result, glycolysis would appear to be more tightly coupled to oxidative phosphorylation in chronic hypoxia than in acute hypoxia.

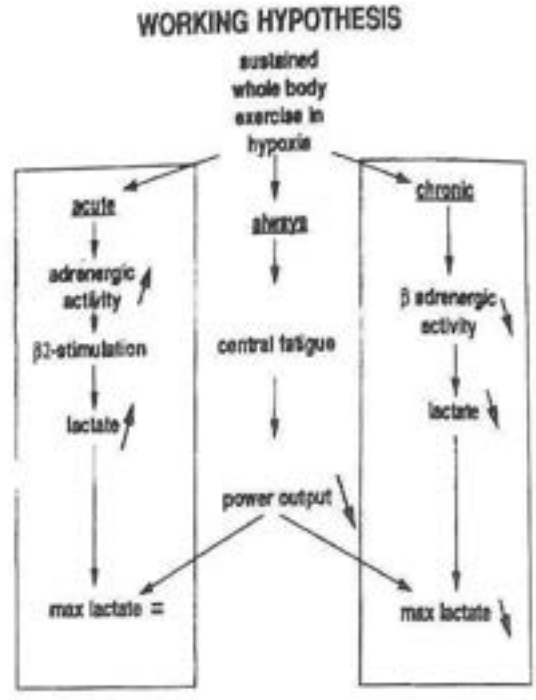

Figure 2.-A diagramatic representation of a hupothetical explanation of the "lactate paradox" in lowlanders going to high altitude. The middle panel shows that maximum vhole-body exercise capacity is decreased through a mechanism of central limitation of the sustainable maximum power output of dynamic exercise. The left panel explains why through the increased adrenergic drive in acute hypoxia increased glycolysis may still lead to high maximum lactate levels, be it is shown how in the acclimatized state a cedreased glycolytic activity in chronic huposia, coupled to the decreased maximum power output, would lead to near normal sub-maximal, but lower maximum blood lactate levels, the latter being the "lactate paradox".

Since, in acclimatized subjects exercising in acute normoxia, the maximum power output and the maximum oxygen uptake are increased as compared to hypoxia, one also would expect $[La]_{max}$ to increase. Grassi *et al.*(pers.comm.) recently observed that $[La]_{max}$ of acclimatized lowlanders at 5050m does not attain sea level values while breathing an oxygen enriched gas mixture, confirming previous observations (Cerretelli,

1980; Edwards, 1936). These results suggest that the "lactate paradox" is not only dependent on the rate of oxidative phosphorylation and that other unknown regulatory mechanisms of metabolism also play a role.

Lactate accumulation in altitude natives

Also Sherpas, high altitude natives of Tibetan origin living in the Nepalese Himalaya, as well as high altitude South-American Indians are characterized by low $[La]_{max}$ values (about 6 mM) [Cerretelli, 1980; Cerretelli et al. 1982; Cerretelli and diPrampero, 1985]. Most research corcerning adaptation to high altitude in altitude native populations has been conducted by taking scientific equipment into the field and by studying the experimental subjects in their habitat. Few observations were performed on natives brought down to sea level (Cerretelli, 1980; Cerretelli et al. 1982; Cerretelli and diPrampero, 1985; Hurtado, 1932; Paz Zamora et al., 1982). Hochachka and colleagues changed the research strategy by taking six Quechua Indians, born and living essentially all of their lives at altitudes ranging from 3600 to 4500m, to sea level (Hochachka, 1989; Hochachka et al., 1991; Matheson et al., 1991). Among other results they reported that the Andean natives also display the "lactate paradox" (Hochachka, 1989). In lowlanders acclimatizing to high altitude the onset of the "lactate paradox" has a half time of about ~5 days (Grassi et al., 1992). Upon return to sea level normal $[La]_{max}$ values are attained after a few weeks, also with a half time of ~5 days (Grassi et al., 1992). By contrast, even after 6 weeks of deacclimatization the Quechuas were still unable to accumulate as much [La] as lowlanders during exhausting bicycle exercise (Hochachka et al., 1991). Based on results obtained by ^{31}P-NMR spectroscopy they tentatively explained their findings by a tighter coupling of glycolysis to oxidative phosphorylation in the working muscle of the Quechuas. Skeletal muscle of these subjects would possess characteristics similar to those found in heart muscle (Katz et al., 1989). Such a shift towards the "cardiac spectrum" would be presumably mediated through a different enzymatic control of aerobic glycolysis (Hochachka, 1992).

Tibetans born at low altitude (1300m, Kathmandu, Nepal) from parents that came originally from the Tibetan plateau (3500-5000m) have the same maximum aerobic power, $[La]_{max}$ mechanical efficiency during cycling and t½ VO_2-on at ~50% of $VO_{2\,max}$ when compared to a local lowland control group, indicating that the metabolic response to an exercise stress, in particular lactate accumulation in blood, are similar in the investigated groups (Kayser et al., 1994). Thus, the "lactate paradox" is not present in subjects that genetically are highlanders but that are born and living at low altitude. The presence of the "lactate paradox" in highlanders born and living at high altitude must therefore be an acquired characteristic as it is for acclimatized Caucasians. The fact that in the Quechuas it does not disappear within six weeks of sojourning at sea level is intriguing, but does not nesessarily imply that it is a permanent feature. It cannot be excluded that deacclimatization over a longer period than 6 weeks would, also in Quechuas, increased [La] max.

Conclusions

Since, at altitude, at any given whole-body exercise intensity, mass oxygen transport to the contracting locomotor muscles is not altered by the process of acclimatization, the gradual reduction in [La] max in lowlanders exposed to chronic hypoxia (i.e. the "lactate paradox") is not due to changes in oxygen availability at the tissue level. At present it appears that the "lactate paradox" is the result of a least two mechanisms: a) a decrease in maximum substrate flux through oxidative phosphorylation due to central limitation of sustained maximum power output in hypoxia, and b) alterations in the metabolic control of glycogenolysis and glycolysis at the cellular level. With regard to the differences in lactic anaerobic metabolism observed between lowlanders and highlanders, both groups being acclimatized or not, these do not seem merely to reflect points along the same continuum of phenotypic adaptation of which the location depends on the time spent at altitude. In any case, the exact mechanisms at the base of the "lactate paradox", both in lowlanders and highlanders, are still matter for further investigation.

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CAN HAPE BE DIAGNOSED THROUGH THE TONGUE?

ZUBIETA-CALLEJA, G.R., ZUBIETA-CASTILLO, G AND ZUBIETA-CALLEJA L.

High Altitude Pathology Institute (IPPA). La Paz

ABSTRACT

High Altitude Pulmonary Edema (HAPE) is an acute illness with dramatic manifestations in the lung that also affects all systems of the body. Hence, very careful observations are required to discover all of these manifestations.

It is our observation that patients with HAPE frequently have ulcers on the tongue. The tongue appears white with one or more reddish colored ulcers that have rough irregular borders. It is non painful and without bleeding. The alterations of the mucosa of the tongue appear similar to geographic tongue. It seems to be related to the severity of the HAPE, and disappears rapidly with oxygen treatment of the pulmonary edema.

Over the past two years, we have seen 6 cases of HAPE of whom 4 presented with tongue ulcers. The other two cases' tongues were pale white and could have formed ulcers had they not received oxygen therapy. The origin of the ulcers is unknown, although it has been hypothesized to be manifestation of viral infection, associated to peripheral vasoconstriction, and/or dehydration.**(Acta Andina 1996, 5:31-34)**

RESUMEN

El Edema Agudo Pulmonar de Altura (HAPE) es una enfermedad con dramáticas manifestaciones en el pulmón, que también afecta a otros sistemas. Por lo tanto, se requieren observaciones cuidadosas para descubrir estas manifestaciones. Hemos observado que los pacientes con HAPE frecuentemente tienen ulceras en la lengua. La lengua se presenta blanca con una o más ulceras rosadas de bordes irregulares. No existe dolor ni sangrado. Estas alteraciones de la mucosa de la lengua son semejantes a la lengua geográfica. Parecen estar relacionados a la gravedad del HAPE, y desaparecen rápidamente con la oxígeno-terapia del edema pulmonar.

En los últimos 2 años, se ha observado 6 casos con HAPE, de los cuales 4 presentaban ulceras en la lengua. Los otros 2 casos, tenian lenguas blancas y es posible que si no hubieran recibido tratamiento, las úlceras se hubieran manifestado. El origen de estas úlceras es desconocido, a pesar que se ha hipotetizado que es una manifestación de infección viral asociada a vasoconstricción periferica y/o deshidratación. **(Acta Andina 1996, 5:31-34)**

High Altitude Pulmonary Edema (HAPE) is a well known illness that can occur on rapid ascent to high altitude [1,3,6,7]. The pulmonary manifestations are evident during auscultation with the presence of rhonchi, rales accompanied by pink sputum and a history of progressive dyspnea over a period of one to four days after rapid ascent to 2500-3000 m [1]. The hemodynamic characteristics have been amply studied [11,4] and responses of the autonomic nervous system in HAPE have been reviewed [9].

To our knowledge, examination of the tongue in this condition has not been described elsewhere. We present two cases with photographs to illustrate this observation.

Cases

In the course of routine treatment of HAPE in a 12 year old boy who had arrived to La Paz, Bolivia (3500 m) the previous day after spending 15 days of vacation at sea level, we observed the previously described symptoms of HAPE. The diagnosis was confirmed by chest x-rays, with typical opacities of a patchy distribution of edema in both lungs. On physical examination the tongue appeared white with two ulcers on the left side. (Fig. 1). One was about 8 mm in diameter, medially located with rough borders, a pink base, non hemorrhagic and non painful to the touch. The second ulcer was 3 mm in diameter laterally located with the same appearance as described above.

Figure 1.-Photograph of the tongue showing two ulcers in a patient with HAPE.

The second case was that of a 43 year old man, native to sea level who came to the city of La Paz (3500 m), and presented with radiographically diagnosed HAPE that was treated with oxygen. He presented with several ulcers on the tongue

Corresponding author:
Gustavo Zubieta-Calleja IPPA La Clinica del Instituto de Patologia en la Altura Av.Saavedra 2302 Casilla 2852, La Paz, Bolivia.
tel: 591-2-368734
Fax 591-2-357159
E. mail: zubieta@oxygen.bo

as shown in Fig 2. This case was more severe clinically.

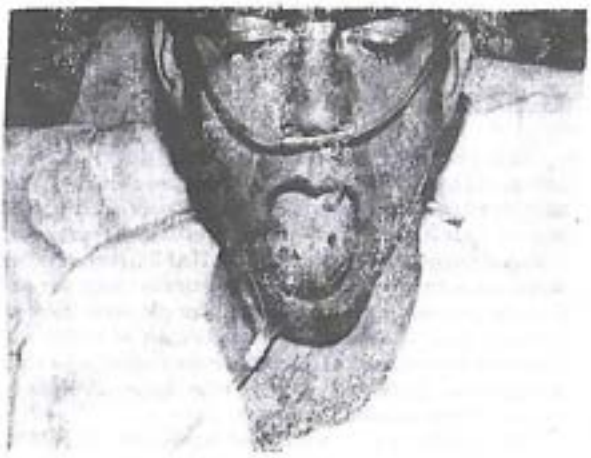

Figure 2.- Photograph of the tongue showing multiple ulcers in another patient with HAPE.

Discussion

The symptoms of HAPE include: headache, nausea, anorexia, insomnia, cough, dyspnea and production of pink frothy sputum. The physical findings included diffusely located rales that may acquire an asymetrical pattern, tachycardia, tachypnea, mild fever and cyanosis. Laboratory analysis finds a mild leukocytosis in some cases. Hematocrit and hemoglobin may be normal or increased. Chest x-rays show opacities of a patchy distribution, occasionally appearing in only one lung [6]. The electrocardiogram with inverted T waves in the right precordial leads and a left shift in the mean T wave vector representing right ventricular strain due to acute pulmonary hypertension has been observed [10]. The inverted T waves have also been attributed to myocardial ischaemia secondary to hypoxia and were reversible on descent to lower altitudes or by the administration of oxygen [8].

Hemodynamic studies [2,4,10], show pulmonary hypertension (PH), increased pulmonary vascular resistance (PVR), normal or decreased pulmonary capillary pressure (PCP); and a markedly decreased arterial oxygen saturation (O_2 Sat), not initially corrected with 100% oxygen. Pathologically, fibrin deposits have been found at autopsy within the alveoli and small capillaries, along with focal interstitial myocarditis [5]. There also may be focal inflammatory in the myocardium.

Since our original description of tongue ulcers with [12], we have been surprised to find these ulcers in HAPE frequently.

Treatment of the pulmonary edema is accompanied by the disappearance of the tongue ulcers in one or two days. These lesions should be looked for when observing a patient under suspicion of having HAPE. If present they may aid in the diagnosis, particularly for non-medical mountain climbers.

It has been hypothesized that due to their clinical similarity to herpes lesions in the mouth, the origin of these ulcers may be due to a viral infection.

As an aside, we have observed that if oxygen treatment is interrupted while capillary blood samples are being drawn from the earlobe of patients with HAPE, the bleeding stops, but is easily resumed if oxygen treatment is reinstated. This may be explained by peripheral vasoconstriction in the earlobe during hypoxia, however further investigation is required.

We speculate if vasoconstriction can partially affect areas of the mucosa of the tongue, producing the ulcers. The highly vascularized mucosa of the tongue is also very sensitive to fever, diet irregularities and dehydration among other things. Further studies are necessary to define the pathogenesis of ulcers on the tongue associated with HAPE.

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ERYTHROPOIESIS IN HUMANS EXPOSED TO SEVERE ALTITUDE HYPOXIA.

ANA MARIA ANTEZANA, JEAN CLAUDE SOUBERBIELLE, JEAN LOUIS LE TRONG AND JEAN PAUL RICHALET

Association pour la Recherche en Physiologie de l'Environnement, Faculté de Médecine, 93012 Bobigny; Laboratoire de Physiologie, Faculté de Médecine Necker, 75014 Paris, France.

ABSTRACT

Some of the factors involved in the control of altitude polycythemia were studied in ten subjects (4 women, 6 men) exposed for 3 weeks to extreme altitude (6542 m). Blood was withdrawn in normoxia (N), after one (H1), two (H2) and three (H3) weeks at 6542 m, for the measurement of serum erythropoietin (EPO, mU/ml), blood hemoglobin (Hb, g/dl), hematocrit (Ht, %), intra-erythrocyte folate (FOL, ug/l) and plasma ferritin (FER, ug/l) concentrations. Renal absolute proximal reabsorption rate (APR) were measured by the lithium clearance method, in N and H2 conditions. After an initial sharp increase in EPO (N: 8 ± 2, H1: 302 ± 282, mean ± S.D.), EPO decreased at H2 (161 ± 151) and H3 (174 ± 212). Ht and Hb increased from N (43 ± 3; 13.8 ± 0.7) to H1 (51 ± 6, 15.7 ± 2.3) and H2 (53 ± 7, 16.1 ± 2.3) and then decreased from H2 to H3 (49 ± 7, 15.0 ± 2.5). Increase in EPO at H1 varied from 3 to 134-fold among individuals. Two women showed a large increase in EPO without increase in Hb. FER showed a marked decrease in H1 (8.1 ± 4.9) and H3 (9.2 ± 3.8) as compared to N (28.0 ± 24.5). Hb was positively related to FER in hypoxia. Iron intake in food was markedly decreased during the 2 weeks of ascent, before arriving at 6542 m. EPO was inversely related to CaO_2 and positively related to APR. The increase in Hb at H1 may have restored the oxygen availability in the kidneys and reduced the formation of EPO. The decrease in Hb from H2 to H3, in spite of a high EPO, may be due to a chronically reduced substrate (iron) availability, as suggested by the decrease in FER favoured by a low iron in take. We conclude that there is a great interindividual variability in erythropoiesis response to EPO in hypoxia. Factors involved in the modulation of this response include nutritional and sex differences, iron stores and tubular function that determines O_2 supply to renal sensors responsible for EPO secretion. **(Acta Andina 1996, 5:35-40)**

Key words: hypoxia, erythropoietin, hemoglobin, folate, ferritin, renal blood flow, renal tubular function.

RESUMEN

La hipoxia de altura estimula la eritropoyesis. Se estudiaron algunos de los factores que intervienen en el control de la poliglobulia de altura en diez sujetos (4 mujeres y 6 varones) expuestos a 6.542m durante tres semanas. Se obtuvieron muestras sanguíneas en condiciones de normoxia (N), después de una (H1), dos (H2) y tres (H3) semanas a 6,542m para dosificación de hemoglobina (Hb), hematocrito (Ht), eritropoyetina sérica (EPO), ferritina plasmática (Fer). Se midió la tasa de reabsorción proximal absoluta (APR) mediante clearance de litio en las condiciones N y H2. EPO aumentó inicialmente y disminuyó en H2 y H3; este incremento varió de 3 a 134 veces según los individuos. La Hb y el Ht aumentaron de N hasta H2, y curiosamente disminuyeron de H2 a H3. La Fer disminuyó en H1 y H3. El aporte en hierro disminuyó substancialmente durante la segunda semana del ascenso. En las mujeres se observó: el mayor aumento de EPO, y el menor incremento de Hb y Ht. Se encontró una relación inversa entre EPO y CaO_2, así como una relación positiva entre EPO y APR. El aumento de la Hb en H1 pudo haber restituído la disponibilidad del oxígeno a nivel renal, disminuyendo la síntesis de EPO. La disminución de la Hb de H2 a H3, a pesar de niveles altos de EPO, puede explicarse por la reducción crónica del substrato (hierro), como lo sugiere la disminución de la ferritina. Existe una gran variabilidad interindividual de la respuesta hematopoyética a la EPO en hipoxia. Los factores implicados en esta modulación incluyen diferencias según el sexo, el estado nutricional y la reserva en hierro, así como la función tubular renal, que determina el aporte de oxígeno a nivel de las células renales productoras de EPO. **(Acta Andina 1996, 5:35-40)**

Palabras claves: hipoxia, eritropyetina, hemoglobina, folato, ferritina, flujo sanguíneo renal, función tubular renal.

The altitude-induced increase in the number of erythrocytes is considered as the main feature of human long term acclimatization to high altitude [12;15;16]. However, the optimal range for hemoglobin concentration at high altitude has been questionned, at least in high altitude natives [16;17]. The role of erythropoietin (EPO) in the polycythemia induced by high altitude has been studied in humans by various authors [1,2,5,8,14]. EPO concentration in serum increases as soon as 90 minutes after induction of hypoxia [2], rises progressively during the first 48 hours [1], then declines to a lower level, but still above basal normoxic values [3]. To our knowledge, no attempt has yet been made to better understand the various factors involved in the human erythropoietic response to EPO in high altitude conditions.

Address for correspondence:
Pr. Jean-Paul Richalet A.R.P.E. 74 rue Marcel Cachin 93012 BOBIGNY CEDEX FRANCE
Phone : 33 1 48 38 77 57
Fax : 33 1 48 38 77 77

Iron or vitamin stores were proved as essential for a normal response to EPO stimulation in other pathologic conditions associated with anaemia. Renal hemodynamics or proximal tubular function may also interfere with EPO secretion [4]. The sympathetic nervous system, activated by altitude hypoxia, interacts with EPO production [6]. Most studies have been performed in male subjects and no data are available on hematological response of women at high altitude. The purpose of the present study was to evaluate the respective role of the above factors (nutrition, kidney, sex), liable to control EPO secretion and erythrocyte production in severe altitude hypoxia. Results were published in details elsewhere [13].

Subjects and Methods

Ten healthy subjects (4 female and 6 male, aged 35 ± 6, 27-44 yrs) participated in the study during a scientific expedition to Mount Sajama (Bolivia) organized by the "Association

pour la recherche en physiologie de l'environnement". Nine subjects were sea-level natives, one subject (woman) was native from La Paz (3600 m) and was resident at low altitude (Paris, France) for 6 years before the expedition. All subjects were moderately trained and had a previous experience of high altitude. They were nonsmoking or occasionally smoking subjects. Two subjects had vegetarian habits at sea level (#2 and #3). Subjects were flown from France to La Paz (3600 m) where they stayed 5 days , then reached by car the altitude of 4200m within 3 days. From there, they walked and climbed for 10 days to reach the summit at 6542 m, where they installed the laboratory and stayed for 3 weeks, without intense physical activity (Fig. 1).

Effectiveness of acclimatization was evaluated daily by a clinical score of acute mountain sickness (AMS) [13]. Blood was withdrawn from an antecubital vein after a 30-min rest in normoxia (N) and after one (H1), two (H2) and three (H3) weeks at 6542 m for the measurement of serum immunoreactive EPO (125 I-EPO COATRIA, BioMérieux, France). Hematocrit (Ht) and hemoglobin concentration (Hb) were measured by means of a microcentrifuge and a microphotometer respectively. Serum ferritin concentration was measured in H1 and H3 conditions, using an immunoradiometric assay (RIA-gnost Ferritin, Behring, Germany). Food intake was evaluated by means of a personal diary where subjects wrote down all their food and liquid intake during three consecutive days in four conditions : normoxia (N), during the ascent period between 4200 and 6542 m (HA), during the first (H1) and the third (H3) week at 6542 m; detailed results are presented elsewhere [13]. Renal function was explored by means of para-amino-hippurate, inulin and lithium clearances measured in N and H2 conditions. Methodology of clearance measurements is presented elsewhere; absolute proximal reabsorption rate (APR) is the difference between glomerular filtration rate and lithium clearance [13]. Arterial O_2 saturation (SaO_2) was evaluated by ear oximetry, allowing the calculation of arterial O_2 content (CaO_2). Values are means $\pm$ S.D. Statistical differences between normoxic and hypoxic conditions were assessed by an analysis of variance with repeated measures. Linear regressions between increase in EPO and various parameters were calculated. A p value of less than 0.05 was considered to be statistically significant.

Results

AMS was slightly felt by the subjects when arriving at La Paz and rapidly disappeared with acclimatization (Fig. 1).

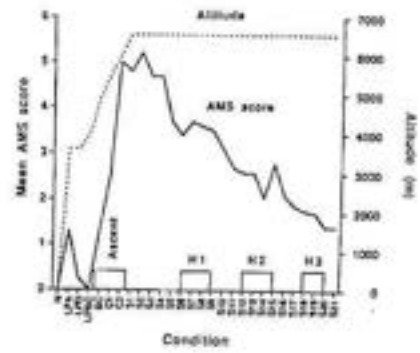

Fig. 1. Ascent profile (Altitude, broken line) and mean score of acute mountain sickness (AMS score, solid line). N: normoxia, LPA, LPD: La Paz, arrival and departure; LPBC: La Paz to base camp; BC: base camp; C1: camp 1 (5500m); C2: camp 2, (6000m); S1 to S21: days at the summit lab (6542m). Ascent period from 4200 to 6542m; H1, H2 and H3 : periods of experiments at 6542m [Modified from ref. 13].

Maximal scores were observed during the ascent, above 6000 m, and during the first 4 days at 6542 m. Scores progressively declined during the stay in the summit. Severe hypoxemia was evidenced by low values of SaO_2 in altitude conditions. After a sharp increase at H1, EPO decreased in most subjects from H1 to H2 and remained stable between H2 and H3. There was a great variability in individual EPO response to the hypoxia. In H1, EPO was 3 to 134-fold greater than in N condition depending of the subject. As expected, Ht and Hb increased from N to H1, then plateaued from H1 to H2 and unexpectedly decreased in all subjects from H2 to H3. Sex differences were evidenced in the hematological response to high altitude [13]. When comparing N and H2, women showed a smaller increase than men in Ht (+14% vs 32%) and Hb (+4% vs 24%). EPO decreased between H1 and H2 or H3 in male but remained high in female subjects during the whole stay at high altitude. Individual response to EPO is illustrated in Fig. 2, showing for each subject the increase in hemoglobin concentration at H1, as the physiologic response of bone marrow to EPO.

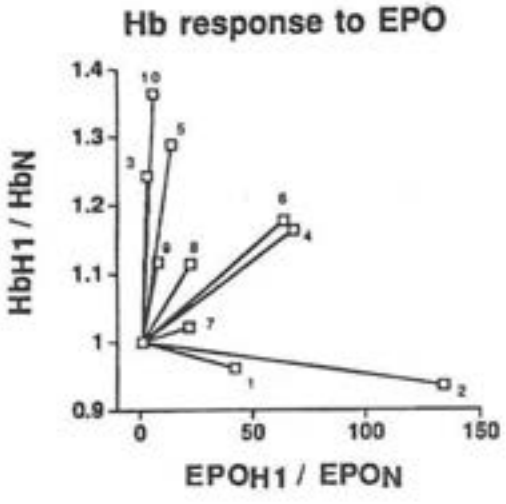

Fig. 2. Ratio of Hb concentration in H1 condition over Hb in normoxia, as a function of the same ratio for erythropoietin. The variation from N to H1, for each of the 10 subjects, is presented : female subjects are # 1, 2, 8 and 9. [Modified from ref. 13].

The slope of each line, illustrating the sensitivity of the erythropoietic response to EPO, is variable among subjects: subjects #3, 5, 10 and 9 are "brisk responders", subjects #8, 6, 4 and 7 are "moderate responders" and subjects #1 and 2 are "low responders". The four female subjects are #1, 2, 7 and 8. Serum ferritin concentrations decreased from normoxia to H1 and H3 by 71% and 67% respectively, reaching values far lower than normal range (Table 1).

Table 1. Hematological parameters and related hormones and substrates.

	Normoxia	H1	H2	H3
SaO_2	97.2 ± 1.2***	70.1 ± 6.1***	74.4 ± 6.8***,*	77.7 ± 8.5***
Ht	42.5 ± 2.9	50.9 ± 6.3***	53.2 ± 6.7***	49.1 ± 7.5***,*
Hb	13.8 ± 0.7	15.7 ± 2.3**	16.0 ± 2.3***	15.9 ± 2.5*
EPO	8 ± 2	302 ± 282**	161 ± 151**,*	174 ± 212**
NEp	333 ± 129	1404 ± 525***	/	1165 ± 653**
Ferritin	28.0 ± 24.5	8.1 ± 4.3**	/	9.2 ± 3.8**
Proteins	74 ± 5	75 ± 5	/	74 ± 4

SaO_2: arterial oxygen saturation (%), Ht: hematocrit (%), Hb: hemoglobin concentration (g/dl), EPO: serum erythropoietin concentration (mU/ml), NEp (pg/ml): norepinephrine, ferritin (µg/l), plasma protein concentration (g/l). Mean ± SD, n=10, H1, H2 or H3 vs normoxia : *: p<0.05, **: p<0.01, ***: p<0.001. H2 vs H1 : *: p<0.05, H3 vs H2 : *: p< 0.001.

Iron intake in food was markedly decreased during the ascent period (HA), in parallel to the overall food intake [13]. There was a significant relationship between increase in EPO at high altitude and decrease in CaO_2; increase in EPO was related to the increase in APR (Fig. 3).

Discussion

The altitude of 6542m was the most severe hypoxic stress ever studied in humans, with respect to serum EPO concentrations. Very high values of EPO were observed, due to severe hypoxia evidenced by very low values of SaO_2. They were higher, in most subjects, than values obtained in alpinists after 2 to 4 weeks at 6300m on Mount Everest [8]. As shown by the decline in EPO during the stay at 6542m, the oxygen sensor adapts to the hypoxic stimulus by an unknown mechanism. Different hypotheses can be evoked to explain this decline in EPO: 1) a negative feedback involving an effect of the hormone itself on its production; 2) the existence of an inhibitory factor of erythropoiesis [9]; 3) modulation by factors progressively released in hypoxia (adenosine, prostaglandins, norepinephrine, cyclic AMP, free O_2 radicals). Stimulation of the adrenergic system seems to enhance hypoxia-induced EPO production by the kidney [6]. As expected, norepinephrine concentrations were elevated in hypoxic conditions, probably enhancing hypoxia-induced EPO production [6]. Thus, a decrease in adrenergic activation cannot be put forward to explain the decrease in EPO from H1 to H3. A down regulation of adenosine and adrenergic receptors on the membrane of renal peritubular cells could be responsible for a decrease in EPO production with prolonged hypoxia, as previously shown in rat heart [7;10]. The inverse relationship between the increase in serum EPO and CaO_2 is an evidence of hypoxemia as a main determinant of EPO production (Fig. 3).

The improvement in CaO_2 due to acclimatization could account by itself for a lowered hypoxic signal to the O_2 sensitive cells producing EPO. High and low responders can be described. Subjects with the lowest Hb response to EPO were two female (#1 and #2). Altitude-induced modifications in plasma volume depend on the accomplishment of acclimatization processes: water retention at high altitude may interfere with our results and partly account from low Hb values in subjects

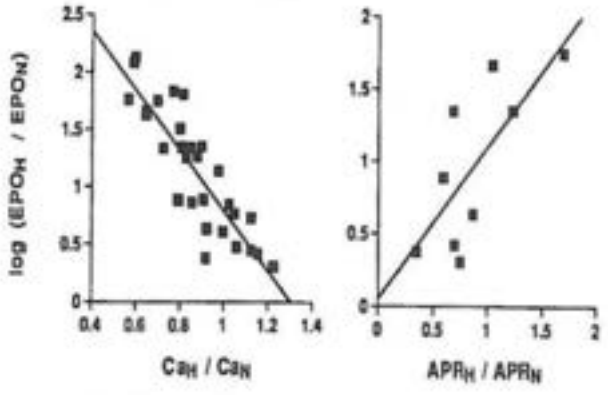

Fig. 3. Increase in EPO from normoxic value as a function of increase in arterial oxygen content (Ca, left: y = 3.40-2.61.x, r =-0.86, p=0.0001, n=30), absolute proximal reabsorption rate (APR, right: y = 0.051 + 1.05.x, r =0.74, p =0.024, n=9) (Ca=CaO2 for a simplified presentation). [Modified from ref. 13].

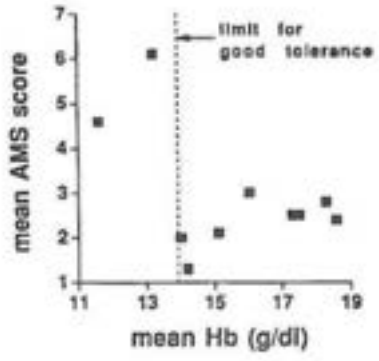

Fig. 4. Mean AMS score plotted versus mean value of hemoglobin concentration in H1, H2 and H3 conditions. [Modified from ref. 13].

suffering from AMS [11]. Dehydration is not likely to account for hemoconcentration since plasma protein concentration was not modified [13]. Decrease in Hb and Ht from H2 to H3 can be due to a decrease in bone marrow response to EPO: this resistance to EPO can be related to a decrease in substrate availability as shown by the strongly lowered ferritin content. The iron deficit in a period of high demand is not compensated by an increased iron intake in food and is parallel to the well-known decrease in overall food intake in hypoxia. The question of an optimal value for hemoglobin concentration is raised. Whereas anaemia is considered as a contra-indication to a stay at high altitude, excessive polycythemia (Hb > 23 g/dl) in high altitude dwellers is detrimental [16;17]. The optimum Hb at altitudes above 5000 m would be in the region of 18 g/dl [16]. However no systematic study is available whichcorrelates a wide range of Hb values with AMS or physical performance. In the present study, the two subjects with the lowest mean Hb values (#1 and #2) had the highest mean AMS score (Fig. 4).

In the range from 14 to 18.6 g/dl, no relation seems to exist between Hb and AMS. It can be concluded that a minimum value for Hb is necessary for a good acclimatization (> 14 g/dl) and that no beneficial or detrimental effect of Hb increase can be observed when Hb is lower than around 19 g/dl. Pre-expedition serum ferritin levels might be determinant for a normal erythropoiesis during altitude exposure. A control of this parameter should be done in subjects, especially women, preparing for a high

altitude expedition and an iron suppletive treatment proposed if necessary. A minimal prior serum ferritin value of 50 ug/dl could be proposed as a safety limit [13]. Gender differences here observed may be related to lower iron stores, classically observed in women. None of the four women suffered from excessive menstrual bleeding before or during the expedition. Two out of the 6 men showed low ferritin concentrations before the expedition (8 ug/l); one of them is a vegetarian. Whatever the sense of variation of APR with altitude exposure, this parameter is well related to EPO production (Fig. 3). This close relationship is in favour of the presence, inside or in the neighbourhood of the proximal tubule, of O_2 sensitive cells the stimulation of which determines EPO production [4]. Kidney function, which has been scarcely studied in altitude hypoxia, may thus play an important role in modulating one of the major components of acclimatization of humans to hypoxia. Extrarenal O_2 sensitive mechanisms involving nervous or humoral factors could also account for the good correlation between CaO_2 and with sub-chronic AMS, EPO would remain

elevated, as an index of chronic tissue hypoxia. We propose that persistent high EPO can be considered as a good marker of unaccomplished acclimatization to high altitude.

Acknowledgments

The 1991 Sajama expedition was possible thanks to all its members, who participated as scientists and volunteers for all the protocols. Logistics have been organized by the "Association pour la Recherche en Physiologie de l'Environnement" (ARPE, Bobigny, France), with the important contribution of the "Instituto Boliviano de Biologia de Altura" (La Paz). This experiment is part of the parthership between ARPE and Sandoz France Laboratories.

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OXYGEN SIGNALLING IN METABOLIC REGULATION : SHORT, INTERMEDIATE, AND LONG TERM ROLES

P.W.HOCHACHKA

Dept. of Zoology, Univ. of British Columbia
Vancouver, B.C., Canada V6T 1Z4

ABSTRACT

We are led at least tentatively to conclude from this analysis that O2 sensing and signal transduction pathways are critically involved.

(i)in the regulation of acute changes in ATP turnover rates in response to increasing or decreasing oxygen availability (or to change in work and perfusion rates),

(ii)in the regulation of intermediate term responses to hypoxia (such as EPO biosynthesis, *cfos* and *cjun* activation, or suppression of gluconeogenic pathway enzymes).

(iii)in the regulation of longer term responses to moderate hypoxia (for example, up regulation of tissue mitochondrial volume densities, of oxygen carrying capacitites, or of oxygen efficient metabolic pathways), and

(iv)in the regulation of long term responses to extreme hypoxia(for example, down regulation of tissue mitochondrial volume densities, adjustments in anaerobic/aerobic metabolic capacities, or adjustments in oxygen efficiency of metabolic pathways).

Perhaps even more evident than the above is the conclusion that oxygen sensing, signal transduction, and expression pathways in metabolic adjustments to hypoxia are still largely unexplored, especially in the comparative field. Although current models for coupling O2 sensing to regulation of ATP turnover are still a long way from being complete or confirmed, they are, in principle, useful and attractive for several reasons: (i)they can account for the often observed relationship between VO2 and perfusion, (ii)they develop a mechanistic basis for O2 conformity (VO2 varying with [O2] or with O2 delivery) over broad concentrations ranges usually very much higher than the Km for O2 for mitochondrial metabolism, (iii)they supply a mechanism for coordinate and near-instantaneous regulation, upwards, of all components in ATPase and ATP synthesis pathways with change in O2 availability, (iv)they supply a mechanistic explanation for up or down regulation of ATP turnover rate with minimal change in concentrations of intermediates in pathways of ATP utilization or of ATP production, and (v)they display interesting potential for strategic transfer to clinical situations, because they seem to be generally applicable to most tissues. Whereas for such obvious reasons these models may be useful, it is not yet clear whether or not they will turn out to be correct. For this further research is clearly required.**(Acta Andina 1996, 5:41-56)**

RESUMEN

Podemos tentativamente concluir a partir de este análisis que las vías sensoras y las de transducción de las señales de oxígeno están críticamente involucradas en las siguientes funciones:

(i) en la regulación de los cambios agudos en las tasas de recambio de ATP en respuesta al aumento o disminución de la disponibilidad de oxígeno (o cambio en las tasas de trabajo y perfusión).

(ii) en la regulación de las respuestas intermediarias a la hipoxia (como la biosíntesis de EPO, la activación de *cfos* y *cjun*, o la supresión de las enzimas de la vía gluconeogénica)

(iii) en la regulación de las respuestas prolongadas a la hipoxia moderada (por ejemplo, el "up regulation" de las densidades volumétricas mitocondriales, ajustes en las capacidades metabólicas anaeróbico/aeróbicas, o ajustes en la eficiencia de las vías metabólicas).

Quizás, aún algo más evidente es la conclusión que el sensor de oxígeno, la transducción de la señal, y las vías de expresión en los ajustes metabólicos a la hipoxia todavía no han sido explorados a profundidad, especialmente en el campo comparativo. Aunque los modelos corrientes para el acoplamiento del sensor de oxígeno a la regulación del recambio de ATP aún no han sido confirmados, ellos son, en principio, útiles y atractivos por diversas razones: (i) pueden dar explicaciones a la relación frecuentemente observada entre VO2 y perfusión, (ii) pueden desarrollar las bases mecánicas para la conformación de 02 (variación de VO2 con la "O2" o con la entrega del 02) sobre rangos amplios de concentraciones usualmente mucho más altos que el Km para O2 para el metabolismo mitocondrial, (iii) proporcionan un mecanismo para la regulación coordinada y estrecha de todos los componentes en las vías sintéticas de la ATPasa y ATP con los cambios en la disponibilidad de O2, (iv) ellos proporcionan una explicación para el "down" y "up regulation" de la tasa de recambio de ATP con cambios mínimos en las concentraciones de los intermediarios en las vías de utilización de ATP o de la producción de ATP, y (v) muestran interesante potencial para la transferencia estratégica a situaciones clínicas, porque parecen ser generalmente aplicables a muchos tejidos. Si bien para tales razones obvias.estos modelos pueden ser útiles, aún no está claro si ellos serán correctos. Para ello se requiere de ulteriores investigaciones..**(Acta Andina 1996, 5:41-56)**

Introduction

A short time ago, two Canadian adventurers in Nepal were asked to assist in a rescue operation; a climbing team caught in a storm and on its way down from the peak of Mt. Everest was in dire straits and its location was uncertain. Along with three Sherpas, the Canadians were assigned the task of trying to locate the climbing team's position from a small plane. When the plane reached about 3000 m, one of the Canadians (unacclimated to altitude) put on his mask and began breathing supplementary oxygen. At about 4000 m, the second Canadian (who had been at altitude in Nepal for some weeks and was thus acclimated) did the same. The plane continued to climb, then finally began circling the peak of Mt. Everest at altitudes over 9000 m; they circled at these altitudes for approximately an hour, but at no point in the reconnaissance did the Sherpas bother to put on their masks and breath supplementary oxygen, so well tuned were they to life under chronic hypobaric hypoxia.

This anecdote, in a nut shell, describes all three aspects of human hypobaric hypoxia problem which form the basis of this paper. In biology, it is almost axiomatic that the response of organisms to varying [O_2] depends upon the time available for the adaptation. Most studies in this area concentrate on the way bioenergetic processes are organized in response to O_2 availability and, traditionally, the literature recognizes three categories (Hochachka and Somero, 1984): (i) short or near-instantaneous response, (ii) intermediate term or acclimation response, and (iii) long term or adaptational response. The boundaries between these three time courses are not rigidly defined and may vary between cells, tissues, and organisms. For example, very soon after an acute response to an hypoxic stress is stabilized (with appropriate adjustments in O_2 fluxes to aerobic pathways of metabolism), the cell may activate or silence batteries of genes in order to begin orchestrating longer term backup defenses against extended O_2 limitation. Although *in vivo* it may take days to weeks to reach a new steady state, the entire process is included in the term acclimation the important point to bear in mind is that in response to intermediate time courses of exposure (acclimation times of hours to days to weeks), cells and tissues are able not only to adjust overall ATP turnover rates, but they can also reorganize pathways of ATP demand and supply (by selective activation or repression of specific genes) so as to improve function (or improve chances of organismal survival) under hypoxic conditions. Populations exposed to hypoxia over phylogenetic time (generations) have even greater cell-level adaptation options in terms of maximizing survival chances under chronic hypoxia.

Although the empirical effects of short, intermediate, and long term exposure to hypoxia are extensively described, in none of this literature are the roles of O_2 sensing and O_2 signal transduction in metabolic adaptation and regulation emphasized; indeed, they usually are overlooked. In this paper, we shall mainly focus on recent developments on the roles of these two processes in acute and acclimatory responses to hypoxia; however, we will briefly consider their roles in long term metabolic adaptation as well.

Our recent analyses of short term (essentially instantaneous) responses to varying availability of O_2 arose almost by chance in the context of evaluating metabolic regulation concepts (Hochachka et al, 1991; Hochachka and Matheson, 1992; Hochachka , 1994). Our evaluation led to several conclusions:

(i)For over 30 years metabolic biochemists have been searching, without success, for metabolite signals which might account for large scale changes in ATP turnover rates in tissues. Most current metabolic regulation theories are based on feedback and feedforward control loops where substrates and products serve to activate or inhibit key enzymes in pathways of ATP u tilization or ATP production; ADP and Pi are often central to these models (Balaban, 1990; Connett, 1988; Connett and Honig, 1989; From et al, 1990; Funk et al, 1990; Kushmerick et al, 1992; Nioka et al, 1991; Rumsey et al, 1990).

(ii)A major problem, not adequately addressed by the above regulation models, is that for many enzymes in pathways of ATP utilization and of ATP synthesis, there is a notable absence of large changes in substrate or product concentrations during up or down regulation of ATP turnover rate. We argue that this leaves effective enzyme concentration as the regulatable parameter. In this view, the traditional roles of metabolite concentrations changes are modified and are assumed to play fine tuning regulatory functions ather than major on-off switching functions (Hochachka and Matheson, 1992).

Hypometabolism means masking of catalytic potentials.

(iii)At this time, only one metabolite 'signal' -oxygen delivery - correlates directly with (upwards or downwards) change in ATP turnover rate and since its regulatory effects clearly occur at concentrations well above the apparent Km for mitochondrial metabolism, it is postulated (Hochachka, 1994; Thurman et al, 1993) that an O_2 sensing system must be involved in transduction of the O_2 signal and in regulation of ATP turnover

.

(iv)O_2 sensing and transduction mechanistic details are not known; however, two key conditions (near instantaneous transmission to all parts of the cell and near simultaneous activation of multipe enzymes in pathways of ATP turnover) must be satisfied for this kind of control to be workable. Finally,

(v)A parsimonious model satisfying these two critical constraints assumes that O_2 signal transduction is coupled (presumably through a secondary signal or signals such as Ca++ ions) to changes in the physical state of the milieu which changes accessibility of enzymes to their substrates, in this way unmasking otherwise 'latent' catalytic power (Hochachka, 1994). Our appreciation of these special functions for O_2 arose from recent studies in two different lines of research - both pointing to the possibility of such roles for O_2 and O_2 sensing systems in regulating aerobic metabolism.

Metabolic Regulation Requires [Oxygen] Sensing

The first of these two study lines initially was concerned mainly with hypoxia acclimation (not acute responses), specifically with hypoxic stimulation of erythropoietin (EPO) synthesis, and this work simultaneously may have exposed a universal O_2sensing system. Earlier studies had already confirmed the ocurrence of an O_2 sensor located primarily in special kidney cells, but also to some extent in liver cells; the experimental evidence was consistent with a heme protein as the O_2 sensor, which in its deoxy form triggers an activationpathway culminating in increased transoription of the EPO gene. Later studies suggested that (i) an O_2 sensing system and (ii) an enhancer sequence regulating gene transcription are much more widespread than had been previously believed (Tsuchiya et al, 1993; Eckardt et al, 1993; Maxwell et al, 1993; Wang and Semenza, 1993). These studies, based on the development of a molecular reporter system allowing rapid scanning of many cell types *for enhancer-specific hypoxia sensitivity, showed that O_2 sensitive gene expression is nearly universal* (Maxwell et al, 1993). When O_2 is limiting (Figure 1), the signal (reduced O_2) mediates the production of an hypoxia-inducible factor (HIF-1) which binds to a specific 'hypoxia induction site' on DNA and accelerates transcription of hypoxia regulated genes (including the EPO gene and possibly genes for at least some glycolytic enzymes and the GLUT1 isoform of the glucose transporter (Bashan et al, 1992)). Even if most studies of this complex O_2 sensing system are directed mainly towards hypoxic regulation of EPO production (an intermediate time-course response), by serendipidy they may have revealed a universal molecular pathway for sensing declining O_2 availability at the cell level.

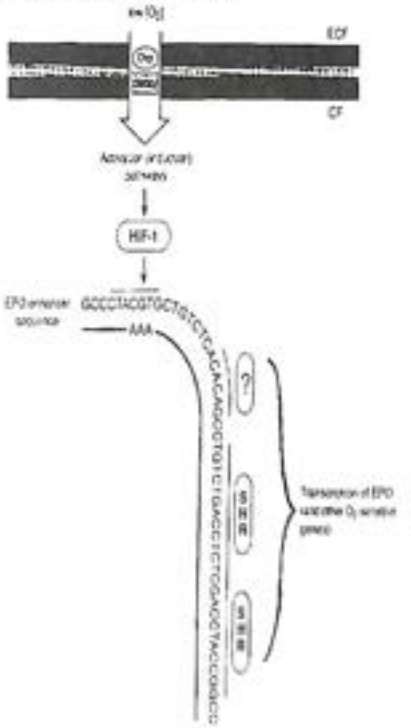

Figure 1. Diagrammatic structure of the hypoxia-inducible enhancer of the EPO gene. Under hypoxic conditions, the percent deoxygenation of the putative O2 sensor increasesand leads to the induction of HIF-1 (hypoxia inducible factor-1). Only a part of the 50 nucleotide sequence of the EPO enhancer fronts the HIF-1 responsive (binding) element. Transcription of reporter genes (e g. the gene for chloramphenicol acetyltransferase) containing the EPO gene enhancer sequence is induced by hypoxia in many (and possibly all) cell types. Since mutations which eliminate HIF-1 binding eliminate hypoxia induction, this factor is considered pivotal in this pathway of O2 sensitivity. Modified from Wang and Semenza (1993)

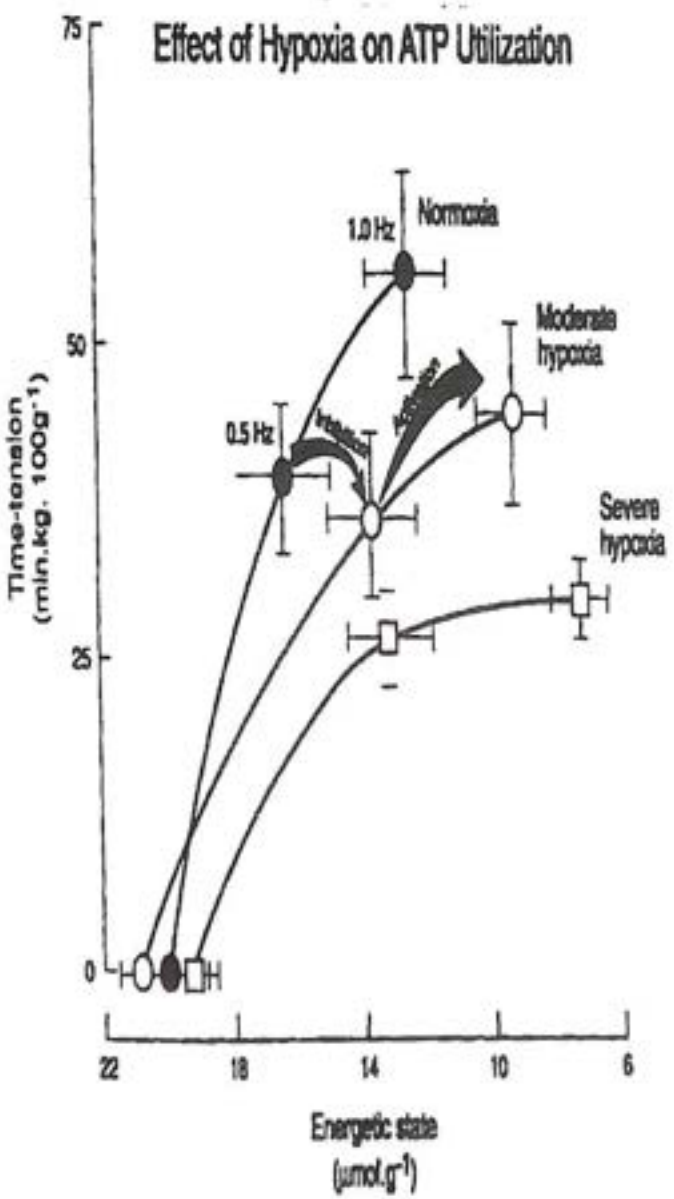

Figure 2. Effect of hypoxia on muscle ATP utilization (proportional to time-tension integral) as a function of the utilizable (high energy) phosphate (defined as the energetic state). Data in each case shown for resting conditions (high energetic state), for 0.5 Hz stimulation (intermediate energetic state) and for 1 Hz stimulation (lowest energetic state). Note that under normoxia, increasing stimulation frequency (decreasing energetic state) increases the work output. At any given conditions, hypoxia decreases contraction-linked ATPase activity (shown by arrow moving from normoxia to moderate hypoxia). This is a regulated response because increasing stimulation from 0.5 to 1.0 Hz increases ATPase to near normoxic levels (shown for moderate hypoxic conditions by arrow moving from intermediate to low energetic state). These kinds of data are consistent with ATP demand being set by stimulation frequency but being modulated by O2 availability. Data from Arthur et al (1992) and Hogan et al (1992).

sensing system powerfully influenced subsequent interpretations in the second of the above two lines of research, which was designed to tease out the roles played by O_2 availability in control of ATP turnover during muscle work under normoxia, moderate hypoxia, and extreme hypoxia. A dog muscle preparation was used in order to bring the system as much under the experimenters' control as possible (Arthur et al, 1992; Hogan et al, 1992). These studies confirmed that none of the usual putative regulatory metabolites (such as ADP or Pi) can account for the changes in ATP turnover rates observed-*except possibly for oxygen itself*. The roles of O_2 are complex and are best explained by looking at ATP demand (proportional to ATPase) first, then at the metabolic response.

Under normoxia (Figure 2), increasing stimulation frequency from 0.5 to 1 Hz leads to increasing ATPase activity (increasing work), which, of course, is expected. What is somewhat surprising is that moderate hypoxia at 0.5 Hz brings about a *regulated* decline in ATPase activity. We Know this is an hypoxia-regulated rate because if the muscle is stimulated harder (at 1 Hz), its ATPase activity reaches that characteristic of normoxic conditions at 0.5 Hz. A similar if more extreme situation occurs at extreme hypoxia. In strictly empirical terms, these data show that the ATP demand during mechanical work ***is set by the ATPases but regulated at least in part by oxygen availability***, and the question arises of how the ATP supply side of the system responds.

ATP supply mechanisms (Figure 3) respond to increasing stimulation wtih an 18 fold increase in O_2 consumption at 1 Hz under normoxic conditions. Again, at 0.5 Hz, moderate hypoxia brings about a *regulated* decline in ATP synthesis rate. As before, we know this is a regulated rate because at double the stimulation frequency the metabolic rate in hypoxia increases to normoxic 0.5 Hz values. In empirical terms, these results show that the ATP supply side of the system is also regulated by O_2 availability. What is more, the regulation is coordinated, for the % of change in ATP demand is almost perfectly balanced by % change in ATP supply; that is why a nearly 20-fold change in O_2 consumption rate occurs with but a 2-3 fold change in free ADP concentrations; although ADP is often considered as a major regulatory metabolite, the above discordance is widespread (Hochachka and Matheson, 1992). In contrast, and because of this coordination, ATP turnover rate in dog muscle is a linear function of O_2 delivery. In this preparation, as in many other systems studied

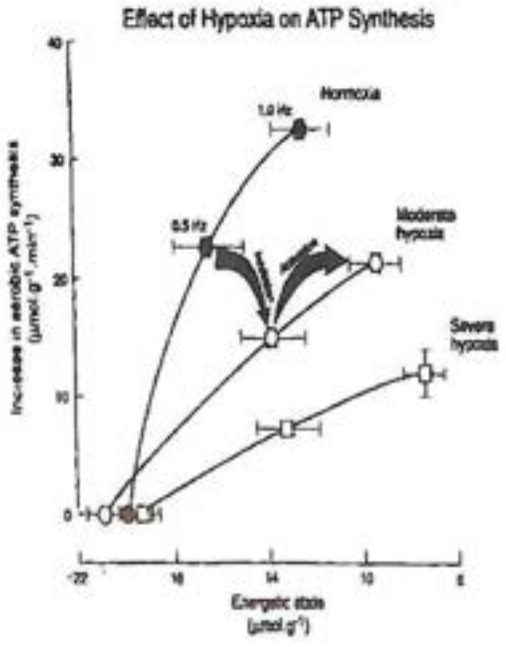

Figure 3. Effect of hypoxia on ATP synthesis in dog muscle gastrocnemius at various work levels. Data are shown for resting conditions (highest energetic state), for 0.5 Hz stimulation (intermediate energetic state, and for 1 Hz stimulation (lowest energetic state). Under normoxia, increasing stimulation (decreasing energetic state) correlates with increasing ATP synthesis rates. On imposition of hypoxia at 0.5 Hz, ATP synthesis rates decline (shown by arrow going from normoxia to moderate hypoxia). That this too is a regulated response is indicated by the fact that at higher stimulation frequency (1Hz), ATP synthesis rates increase to normoxic range , despite the sustained hypoxia. These data are consistent with ATP synthesis rates being set by the ATP demand but being modulated by O2 availability. Data replotted from Arthur et al (1992) and Hogan et al (1992).

by other workers, *the only currently known 'signal' which is large enough and which is directly proportional to the observed change in ATP turnover is in fact O_2 availability.*

An important point to emphasize is that in all these Kinds of systems, O_2 regulation is not mediated simply by O_2 as a substrate for mitochondrial metabolism. Because of (i) a very high O_2 affinity of mitochondrial cytchrome oxidase and (ii) very shallow gradients between cytosol and mitochondria (Gayeski and Honig, 1986; Connett and Honig, 1989), cytochrome oxidase could not possibly act as an O_2 sensor over physiological O_2 tensions. In dog muscle (Figure 4), as in other tissues where similar effects have been observed (Hochachka and Guppy , 1987; Thurman et al, 1993), O_2 regulation cuts in at extracellular concentrations that are in the range of 20-30 torr; under these conditions, intracellular O_2 is estimated to be about 5 torr which would be fully saturating for mitochondria (Gayeski and Honig, 1986). This means that mitochondrial metabolic pathways (as well as other enzymes in ATP turnover) are responding to O_2, but not only to O_2 as a substrate. Put another way, O_2 is affecting ATP turnover rates at concentrations well above the Km for mitochondrial metabolism per se. To accomodate such striking and often-observed dependence of ATP turnover on [O_2], we and others (see Hochachka, 1994; Thurman et al, 1993) postulate ***an O_2 sensor which displays a much lower apparent O_2 affinity than typical of mitochondria and which (directly or indirectly)modulares both ATP demand and ATP supply pathways in a simultaneous coordinated way*** (Figure 4).

Rather than linked to HIF-1 induction, the O_2 sensor in this case would have to be linked to an intracellular, long-range, multitarget activation pathway. In outline, such a model can be summarized as follows: ischemia or hypoxia -> declining O_2delivery to cells --> increasing [deoxy form]/[oxy form] of the sensor --> --> masking of many catalytic potentials --> down regulation of ATPases and of ATP producing pathways. The reverse is postulated for improved O_2delivery, with increasing oxygenation state of the putative O_2 sensor and with enzyme unmasking causing ATP turnover rates to accelerate.

From our present understanding, there are two minimal requirements which this kind of model must satisfy: (i) it needs to account for the simultaneous activation of both ATP demand and ATP supply pathways during activation of cell work and O_2 delivery, and (ii) it needs to account for intracellular long range activation; i,e., for a mechanism to 'spread' the signal to

Current Concepts in O_2 Sensing and Signalling Pathways

The process of O_2 sensing of course has already been examined by physiologists and biochemists, mainly in the context of carotid body chemoreception. At the 1993 International Union of Physiological Sciences conference in Glasgow, for example, three O_2 sensing models were reviewed in detail: a high Km mitochondrial model, a nitric oxide (NO) based model, and an O_2-derived free radical model. Other evidence has pointed in the direction of an unique outwardly

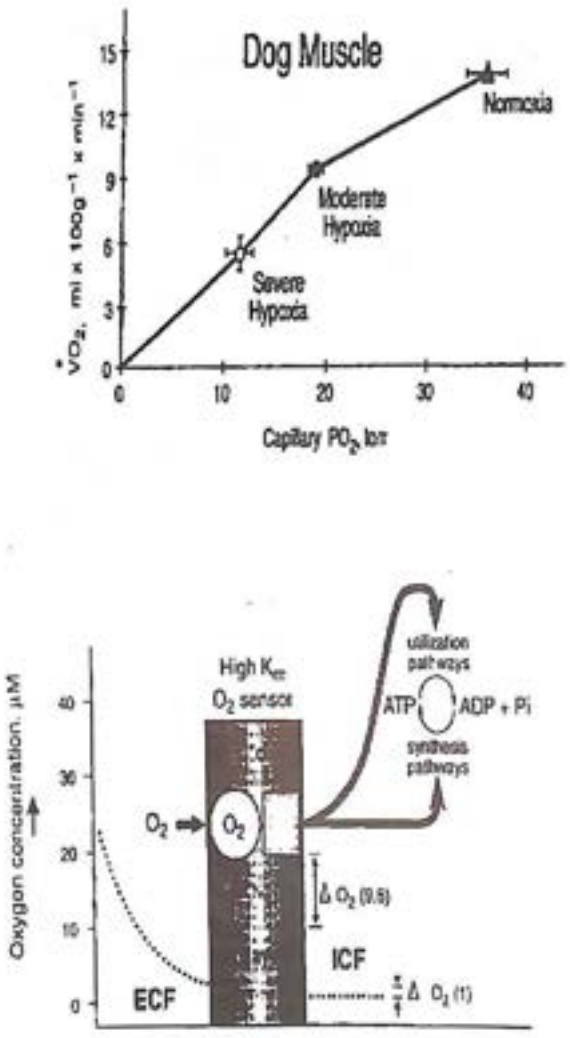

Figure 4. Potential roles for an O2 signalling mechanism in the regulation of metabolism at varying O2 availability. A. **Upper panel.** Changing O2 consumption rates (VO2 in ml O2 100g-1 min-1) as a function of capillary O2 concentrations in working dog gastrocnemius (Hogan et al, 1992). It is important to emphasize that the relationship is between VO2 and extracellular PO2; under these conditions, intracellular PO2 is thought to be fully saturating to mitochondrial metabolism (Gayeski and Honig, 1986). A diagrammatic interpretation of how an O2 sensor might be involved in regulating ATP turnover rates is shown in B, **Lower Panel.** To relate this model to experimental data such as those in the upper panel, the oxygen profile from the ECP to the ICF refers to oxygen concentration (uM units), not to oxygen partial pressure (recall that PO2 = [O2]/solubility coefficients). The problem of sensing O2 for a working cell is complex since the higher solubility of O2 in the lipid bilayer of membranes creates higher O2 concentrations and gradients. Under the conditions described, the difference in concentration across the lipid bilayer is about 9.6 times the difference between the two aqueous compartments; [O2] in aqueous solution at 1.23 x 10-6 M torr-1 corresponds to [O2] in the lipid bilayer at about 1.18 x 10-5 M torr-1. What part of this complex situation is sensed by cells is unclear at this time; in this diagram the sensor is positioned in the membrane (where the external to internal gradient is maximized) to emphasize the correlation between extracellular O2 and VO2 (shown in upper panel). [O2] values given in lower panel are arbitrary, chosen only to quantitatively illustrate the situation O2 signal transduction is linked to regulation of both ATP demand and ATP supply pathways to account for the oft-observed relationship between O2 and ATP turnover rates.

the scope of our analysis here. In the present context, it is sufficient to point out that to this point research into the possible role of O_2 sensing in regulation of ATP turnover can be categorized into direct or indirect models. The first assumes an O_2 sensing system analogous or homologous to that involved in EPO regulation. An alternate framework for O_2 sensing in regulation of ATP turnover considers an indirect pathway which is based on studies showing that nitric oxide inhibits both ATP production, indirectly measured as O_2 uptake rates, and ATP utilization, measured as mechanical work (King et al, 1993). Based on these kinds of observations, an indirect O_2 'sensing' pathway might be summarized as follows: ischemia and /or hypoxia--> reduced O_2 delivery--> increasing intracellular Ca++/calmodulin concentrations --> nitric oxide synthase (NOS) activation--> increasing NO flux --> several effects including simultaneous inhibition of ATPases and ATP synthesis pathways. Increased NO availability also could act via guanylate cyclase activation and cGMP to down regulate energy demand and energy supply pathways, as postulated for vascular smooth muscle (Knowles and Moncada, 1993; Toda and Okamura, 1992).

The flipside of this model postulates a reverse set of processes to regulate rate transitions during improved perfusion and oxygenation characterizing muscle activation. Preliminary evidence favoring such an indirect O_2 response pathway comes from administration of agonists or antagonists designed to specifically block or enhance NO production; perfusion, O_2 consumption and work output are proportionately up or down regulated (King et al, 1993; Sun and Reis, 1992). There are serious difficulties with this indirect model of the role of O_2 in metabolic regulation (NO has a notably finite life time and is destroyed on encounter with hemoglobin); however, it has the distinctly favorable features of being predictive and testable Unfortunately, neither of the above two approaches confronts the issue of a long range intracellular 'signalling' system to instantaneously 'spread' the activation signal to all parts of the cell. A quick search of popular texts and reviews will indicate that approaches to intracellular signalling are currently dominated by target-specific second messenger concepts. Indeed, we have to keep open the possibility that some potent second messenger systems are in fact pivotal in turning on cell ATP .turnover processes; Ca++ has clearly been identifed as playing such a pivotal role in regulation

of actomyosin function. A fundamental limitation of all such second messenger frameworks is the requirement for numerous highly specific intracellular targets. The complex biological reality in most (and probably all) tissues is that every protein in every step in ATP demand and ATP supply pathways must be almost simultaneously activated, and it is hard to visualize a second messenger hitting so many targets so rapidly. In some cases, the time course for metabolic change is awesome. For example, there is more than a 2000 fold activation of Na+ K+ ATPase within less than 300 ms during electric organ discharge (Blum et al, 1990; 1991); there is a nearly 1000 fold, essentially square wave activation of mitochondrial metabolism in insect flight muscle (Sacktor, 1976; Wegener et al, 1991), and a similar (Dobson and Hochachka, 1987; Dobson et al , 1988; Hochachka et al, 1991; Moyes et al, 1992; Suarez et al, 1990)) more than 100 fold activation of ATP turnover during maximum work of fast twitch muscles in vertebrates! The number of targets, their speed of activation, and their degree of activation would seem to stretch second messenger frameworks to the breaking point. Thus it appears that something is missing in target specific second messenger views of cell activation, especially in tissues like muscle where response time in evolutionary terms may influence survival of the organism. We are considering the hypothesis that the missing element in current models of control of ATP turnover may be the intracellular milieu itself.

Intracellular Fluids Are Not Simple Aqueous Solutions

The insight that the physical state of the cytosol may play a role in regulating metabolism arose when we turned to barnacle muscle cells to try get estimates of true resting concentrations of metabolites such as ADP (Hochachka, P.W., unpubl. observations). The giant barnacle is well known to have giant muscle cells (1-3 mm in diameter and 10-30 mm in length!) and our initial goal was to obtain small (picoliter or nanoliter quantities) of cytosol from the resting cell. To our surprise, the cytosol could not be micropipetted; nor, when the muscle fibers were cut, did it leak out; instead, the cytosol behaved more like a gel than a sol. (This has been well known for over 30 years by neurophysiologists studying the giant squid axon; they routinely roll out axoplasm gel toothpaste out of a tube, but the metabolic and regulatory significance of this unexpected physical state of the ground substance in these cells has been pretty well ignored.) Many years ago, researchers such as Heilbrunn (1956) knew of this behaviour, but methods for studying the properties of the cytoplasm were not advanced enough to evaluate its physical state in vivo. Today the situation is different. Using a variety of new and penetrating approaches, we now know that cytoplasm is remarkably viscous, in the range of 10-15 cP, not the 1 cP we normally assume which is typical of water (Dix and Verkman, 1990).

Under in vivo conditions, diffusion rates may be very different from those expected in simple solutions. Using a reference phase technique, workers in this area find (i) that many proteins are diffusive, many are not, and (ii) that even small solutes (disaccharides are often used as metabolic models) may show varying diffusion rates (Mastro et al, 1984). Concentrations of small molecules and metabolites may be similar in different regions of the cell or may vary (Horowitz and Miller, 1984). Even more relevant to our analysis is the observation that all of the above, especially microviscosities in different regions of the cell, are not static physical states; instead, they change with the activity or metabolic status of the cell (Paine, 1984), and the same situation is even more exaggerated in the mitochondrial matrix. The viscosity of the inner compartment of mitochondria (i) varies with the metabolic state of the mitochondria (*more* viscous in state 3 than in state 4!) and (ii) can be up to *40 times* higher than the viscosity of dilute buffers (Scalettar, et al, 1991). These observations raise the possibility that transitions in the physical state of intracellular fluids could alter substrate accessibility to enzymes (the catalytic power of enzymes with no access to substrate is 'latent'; providing substrate accessibility is potentially a simple but effective way of 'unmasking' enzyme catalysis) and this information could be instantaneously transmitted throughout the cytosol to all reaches of the cell during large scale upwards or downwards shifts in ATP turnover rates in tissues like muscles. If this were linked to O_2 supply (for example, through Ca+ +) it would account for two of the chief experimental observations that frequently arise in *this field: the direct relationship between O_2 availability and the ATP turnover rate and the near-instantaneous spread of the activation or deactivation 'signal' to essentially all protein*

components involved in integrated cell and tissue function at once.

In that the above model makes clear cut for further research. However, from this discussion it will be evident that the roles of acute O_2 sensing and transduction mechanisms in mediating the metabolic regulatory effects of this metabolite, although recognized, are poorly understood and are barely described. In contrast, oxygen signalling pathways over intermediate time courses are better worked out.

Intermediate Term (Acclimatory) Responses to Hypoxia

Based on early studies on hypoxia sensitive systems, it was at first believed that, when under O_2 limiting conditions, cells and tissues would make up energy deficits by activating anaerobic pathways. However, in its simplest form, this strategy is to down regulate ATP turnover rates. In early acclimation stages, anaerobic metabolic pathway fluxes may remain unchanged (which by virtue of the inefficiency of anaerobic glycolysis leads to a decline in ATP turnover rates) or may gradually decline, especially after acclimation time is extended. Because of sustained O_2 limitation, however, a large fraction of overall ATP turnover necessarily depends upon anaerobic glycolysis, which is why in such cells and tissues, the ratio of anaerobic/aerobic metabolic pathway capacities may well be regulated upwards (further discussion of strategies of hypoxia acclimation is available in Hochachka and Randall, 1978; Hochachka and Somero, 1984; Hochachka and Guppy, 1987).

Similar but less exaggerated processes appear to be found during hypoxia acclimation in mammals. In rats, acclimation to hypobaric hypoxia increases capillarity and the glucose transporter capacity of the brain (Harick et al, 1991); presumably these adjustments are coordinated with simultaneous up regulation of overall tissue glycolytic capacities to allow for sustained ATP synthesis rates despite chronic O_2 limitation (LaManna et al, 1992; Harick et al, 1991; 1994). Earlier studies of other tissues in animals undergoing high altitude acclimation suggested increases in capillarity and in oxidative capacities; these results are consistent with between-species studies of high vs low altitude animals. However, later studies found decreases in mitochondrial [enzymes] with little or no change in capillarity (see Hoppeler and Desplanches, 1992). Part of the explanation of the confusion in this research may be methodological. For example, exposure to hypoxia leads to reduced exercise capacity and often to caloric imbalance and gradual loss of body (especially muscle) mass. Hence, changes observed in muscle aerobic metabolic capacities might be due to changes in activity (or training) state, to caloric imbalance, or to hypoxia *per se* - or all of the above, since all three factors in theory could influence cell metabolic organization. Takahashi et al (1993) recently reexamined the issue trying to correct for activity; they found that hypoxia acclimation in rats led to a measurable decrease in total malate dehydrogenase (MDH) activity per g of muscle, but to no change in hydroxyacyl CoA dehydrogenase (HOAD). MDH is a poor marker of mitochondrial metabolism since most of total MDH reflects cytosolic activity; thus the lack of effect on HOAD implies little or no effect on muscle mitochondrial oxidative capacity. Even more convincing is a recent study of three months of 3800 m acclimation on heart enzymes in non-pregnant sheep, in near-term mothers, and in the sheep fetus. Citrate synthase, as a marker of mitochondrial oxidative capacity, increased about 50% in the adults and nearlycoupled with training programs (Terrados et al, 1990; Terrados, 1990). However, this effect is not seen when elite endurance -trained athletes are acclimated for 2 weeks at modest altitude (Swedish cross country skiers were used, known to have one of the highest maximum aerobic metabolic rates thus far measured in humans! (Mizuno et al, 1990)). In contrast, similar exposure of humans to extreme altitudes (over 5000 m and up to 8800 m) consistently leads to a down regulation of oxidative capacities (and to an increase in the ratio of anaerobic/aerobic metabolic capacities) at least of skeletal muscles (Cerretelli et al, 1990; Hoppeler and Desplanches, 1992). Unfortunately, the basis for the striking difference between extreme and moderate hypoxic challenge in humans is not yet understood.

However, even if the phenomenological description of acclimatory responses of human muscles to hypoxia are incomplete, it seems fair to conclude that at least during acclimation to relatively extreme hypoxia, one notable effect involves an up regulation of the ratio of anaerobic/aerobic pathway capacities. Similar adjustments of anaerobic/aerobic pathways are to be expected for other tissues, such as kidney (Halasz et al, 1974) and liver (Longmuir, 1992; Tran-Thi et

al, 1994; Yoshino et al (1991), although it is beyond our mandate to review these effects in detail here. Suffice to emphasize that, at the cellular level, O_2 sensing and transduction processes probably form an integral part of the regulation of these complex acclimaticion processes to extreme hypoxia. For illustration, we will discuss their nature and roles (i) in anoxia acclimatory responses in turtle hepatocytes, (ii) in the hypoxia acclimatory response of gluconeogenesis and glycolysis to hypoxia in rat hepatocytes and (iii) in the *cfos* and *cjun* hypoxia acclimation responses in rat cardiac myocytes. All of these *in vitro* studies used extreme O_2 limitation and thus would be most comparable to *in vivo studies* of humans acclimating to extreme (not moderate) hypoxia.

O2 Sensing, Signal Transduction, and Hypoxia Acclimation

One of the experimentally more useful outcomes of the EPO studies to the hypoxia field in general is the development of protocols useful in identifying the occurrence of O_2 sensing systems. Three such protocols are particular widely used (for literature in this area, see Land and Hochachka, 1995): (i) the O_2 sensing response is independent of the ocurrence of oxidative metabolism (for example, a cyanide block is insufficient signal for the induction of hypoxia-associated genes), (ii) nickel and cobalt, by locking heme proteins in deoxy conformation, should mimic the effects of anoxia on heme protein based O_2 sensing pathways, (iii) carbon monoxide, by locking heme proteins in the oxy conformation, should reverse anoxia effects, and (iv) inhibitors of heme protein synthesis pathways should inhibit or abolish heme protein based O_2 sensing. We used these probes to identify the ocurrence of a heme protein based O_2 sensing and signal transduction system in turtle hepatocytes (Land and Hochachka, 1995). In these cells, the short term (acclimatory) response to anoxia involves sensing of O_2 lack by a heme protein based pathway and the pathway and the subsequent example, a cyanide block is insufficient signal for the induction of hypoxia-associated genes), (ii) nickel and cobalt, by locking heme proteins in deoxy conformation, should mimic the effects of anoxia on heme protein based O_2 sensing pathways, (iii) carbon monoxide, by locking heme proteins in the oxy conformation, should reverse anoxia effects, and (iv) inhibitors of heme protein synthesis pathways should inhibit or abolish heme protein based O_2 sensing. We used these probes to identifi the ocurrence of heme protein based O_2 sensing. We used these probes to identify the ocurrence of a heme protein based O_2 sensing and signal transduction system in turtle hepatocytes (Land and Hochachka, 1995). In these cells, the short term (acclimatory) response to anoxia involves sensing of O_2 lack by a heme protein based pathway and the pathway an the subsequent preferential pression of genes for glycolytic enzymes while the expression of genes for gluconeogenic enzymes is decreased (Figure 6) In turtle hepatocytes (Buck et al, 1993; Land and Hochachka, 1995), these gene-expression responses to hypoxia are thought to defense adaptations that are well known charateristic of this: (i) down regulation of ATP utilization pathways (e.g. Na+ K+ATPase, protein turnover, gluconeogenesis, ureagenesis, etc.) coordinately with the down regulation of O_2 dependent ATP synthesis pathway, (ii) the decreased conductivity of cell membranes (referred to as 'channel arrest' in the literature), and (iii) the maintenance of conditions which protect the cells against recovery and reperfusion damage (this latter hypoxia defense strategy is largely assumed to be self-evident since these species clearly survive any ppossible 'reperfusion' damage after extreme hypoxia and ischemia; it is, however, a poorly understood and largely unexplored area in research on hypoxia tolerant species (for metabolic aspects of this problem, see Schulte et al, 1992). In other cells, hypoxia-mediated induction of detoxification pathways such as catalyzed by heme oxigenases (Murphy et al, 1991) are thought to protect cells and tissues from free radical damage during recovery. However, it is not known if similar intracellular messenger system whose primary defense strategies are utilized in hypoxia tolerant species.

Similar, acclimatory (or intermediate time course) responses to low O_2 tensions in rat cardiac myocytes have been explored by Webster and his colleagues (1994). Their observations also indicate the ocurrence of an O_2 sensing and signal transduction system that is involved in regulating the expression of two oncogenes, *cfoc* and *cjun*. In this case, the signal transduction pathway seems to involve protein kinase C and the phosphorylation of target proteins which regulate the expression of *cfos* and *cjun*; the products of the two latter genes are separately

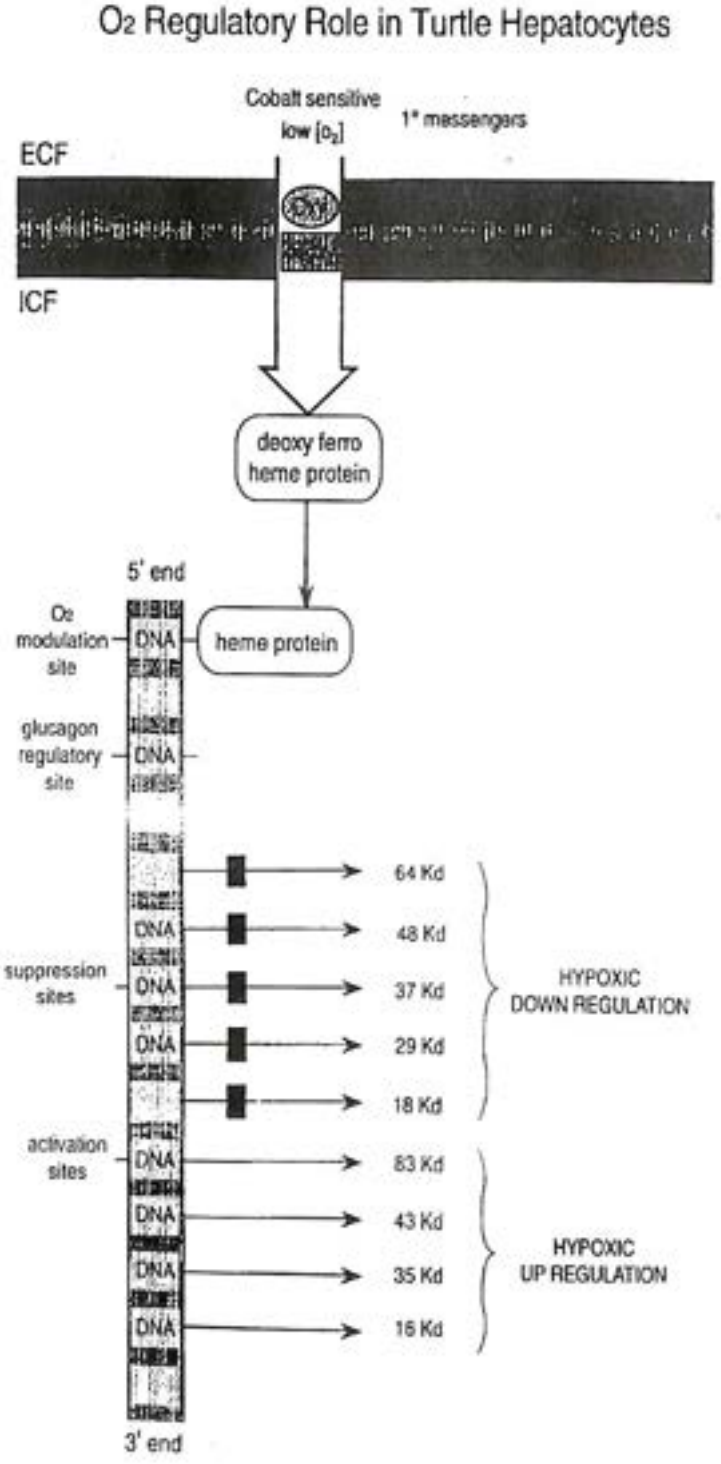

Figure 5. A diagrammatic summary of the postulated role in an hipoxia tolerant cell (turtle hepatocyte) of a heme protein based 02 sensing system that through a currently unknown signal transduction pathway leads to the accelerated expression of several genes (some of which, on the basis of comparable studies with other cells, may be glycocytic enzymes) with the simultaneous suppression of several other genes. At this stage, the regulated proteins are only identified according to size. Summary is based upon Land and Hochachka (1995).

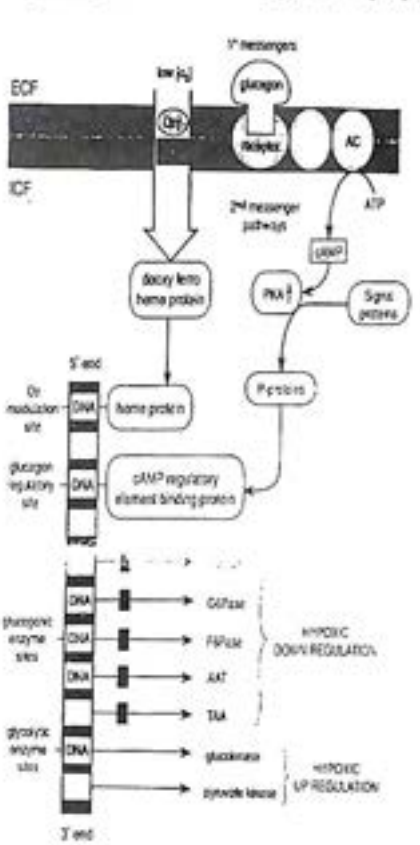

Figure 6. A diagrammatic summary of a heme protein based O2 sensing system whose primary role appears to be the modulation of glucagon (and insulin?) regulation of genes specifying enzymes in glucose homeostasis. Summary is based on studies of rat liver hepatocyte cultures by Keitzmann et al (1992; 1993).

inactive, but self-assemble into heterodimers that serve as a Kind of 'tertiary' function is to regulate the expression (through the AP1 site) of several additional genes (Figure 7). Although the physiological functions of the latter gene products are not fully worked out, it is probable that they too are involved in orchestrating the hypoxia defense adaptations that these cells undergo during hours-todays exposure to reduced atmospheric oxygen: overall reduction in ATP turnover (suppressed ATP utilization pathways with simultaneous adjustments in the membrane structure-function adjustments mentioned above, although not directly demonstrated in hypoxia acclimated rat heart myocytes, may be orchestrated by *cfos* and *cjun* regulated proteins.

Metabolic Adaptations to Long Term Exposure to Hypoxia

Just as phenomenological short term responses to O2 availability are well described, so too are long term adaptational responses. Thus, if one

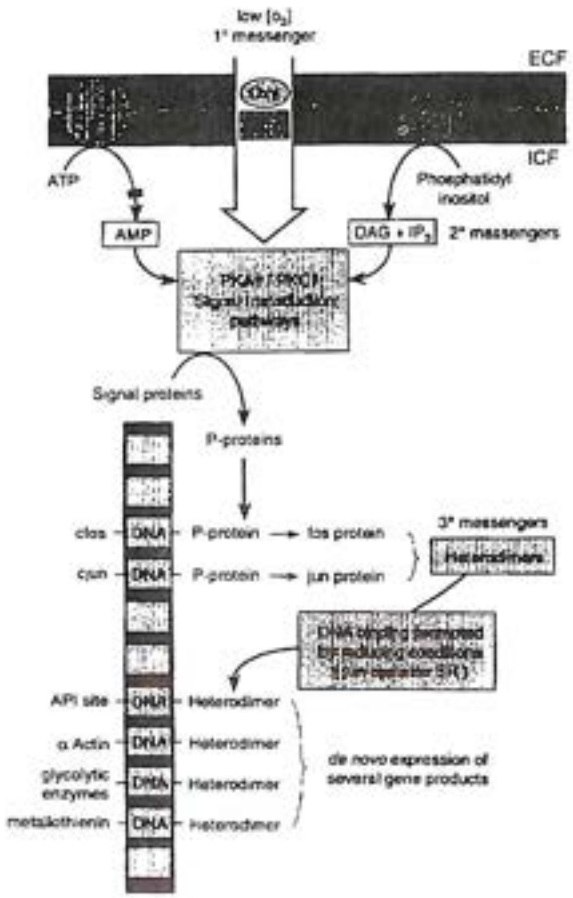

Figure 7. A diagrammatic summary of an O2 sensing and signal transduction system in rat cardiac myocytes. The transduction pathway in these cells appears to depend largely upon protein kinase C phosphorylation of currently unidentified 'signal' proteins involved in regulation of two oncogenes, *cfos* and *cjun*. Alone, the products of these two genes are inactive; however, they self assemble into heterodimers which serve as tertiary messengers: by binding to the AP1 site they are crucially involved in regulating the expression of several additionalgenes. The protein products of these latter genes in turn appear to be involved in orchestrating hypoxia defense mechanisms of cardiac myocytes exposedc for hours to days to extreme hypoxia. Summary based on Webster et al (1994).

compares a given homologous tissue among the vertebrates, say skeletal muscle, one observes about a 500 fold range over which the oxidative capacities vary among species. With citrate synthase (CS) as a mitochondrial marker enzyme, muscle catalytic capacities range from less than 1 in white muscles of some Amazon fishes (see Hochachka and Randall, 1978) to nearly 500 umol per g per min in hummingbird flight muscles (Suarez et al, 1990)- a capacity range presumably arising at least in part as a function of the chronic availability of molecular O_2 at the cellular level. In mammals, the effect of hypoxia on cell oxidative capacities seems to vary with the intensity of the stress. In moderate hypoxia, tissues seem to adapt by increasing capillarity, O_2 carrying capacity in myoglobin containing tissues, and concentrations of enzymes in aerobic pathways of

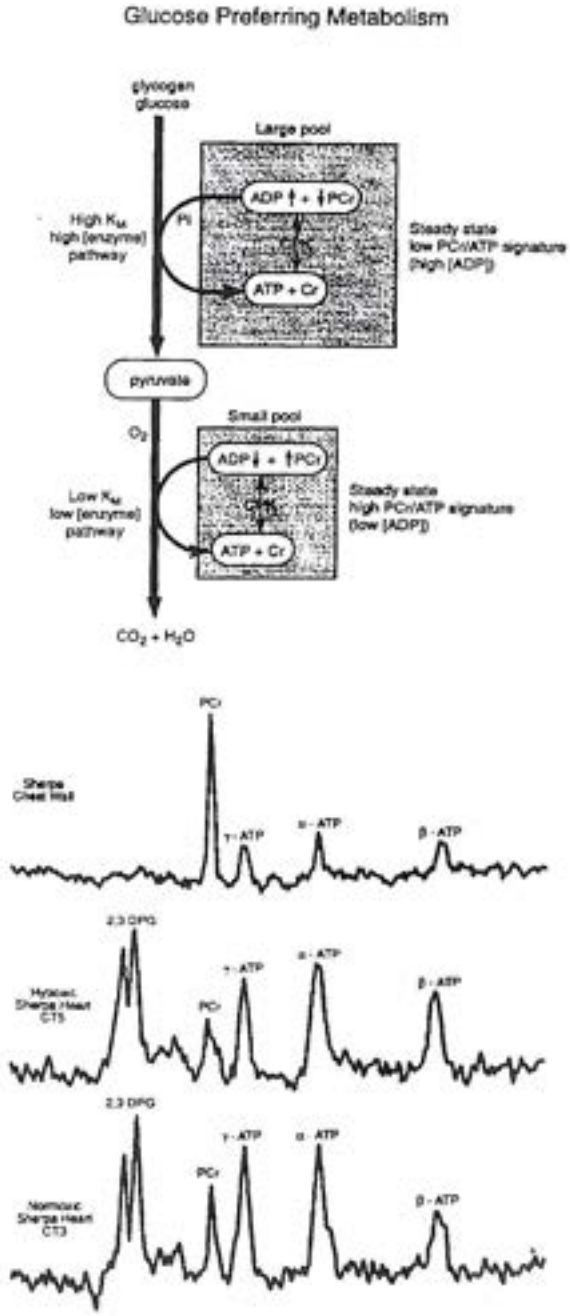

Figure 8.**Upper Panel**: The participation of an active aerobic glycolysis forces heart creatine phosphokinase to equilibrium at reduced [PCr], stable [ATP], elevated [Cr] and elevated [ADP]. This situation is the reason why a low [PCr]/[ATP] is taken as a signature of an O2 efficient, carbihydrate based fuel preference in heart metabolism. **Lower Panel.** ^{31}P M agnetic Resonance Spectra from the heartof an individual Sherpa at 4 tesa during normoxia (CT3) and hypoxia (CT5), compared to the chest wall (phosphate peaks mainly from chest skeletal muscle). The 2,3 diphosphoglycerate (2,3 DPG) peaks arise from red blood cells. The phosphocreatine (PCr) and ATP peaks arise from the left ventricle and the septal wall. Using the gamma phosphate of ATP for reference, the saturation correct heart PCr/ATP ratio in this subject was 0.8 under normoxia and 1.0 under hypoxia, substantially less than the 1.8 average found in hearts of lowlanders and from the value of 6.0 found for the chest wall muscles. The chest wall PCr/ATP ratios in Sherpas are similar to those found in lowlanders. Modified from Hochachka et al (1995).

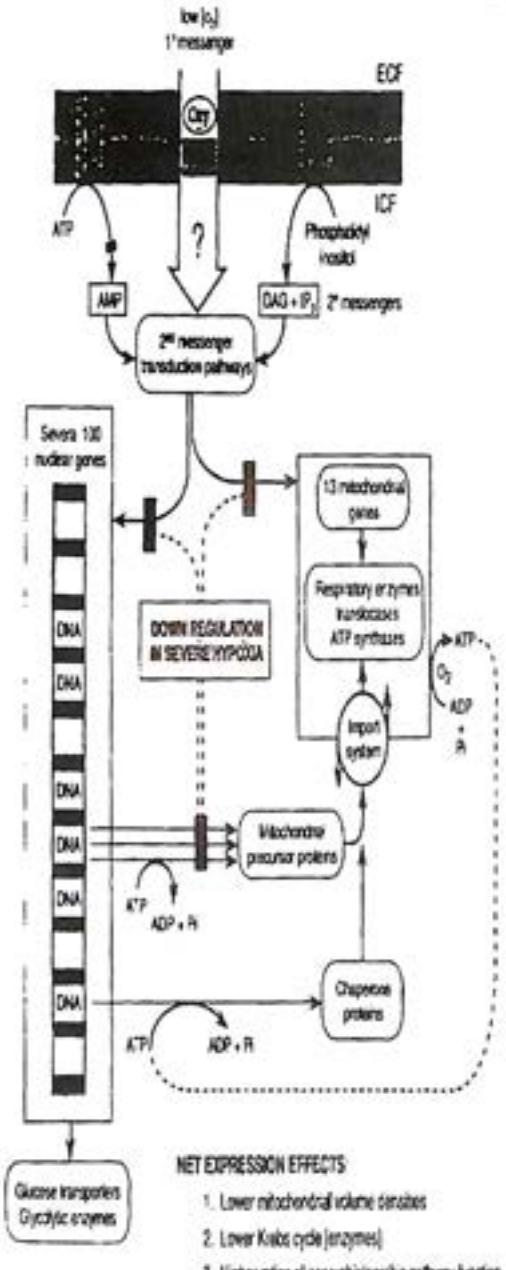

Figure 9. A diagrammatic summary of the kinds of regulatory processes which are presumed to occur during long term adaptation to severe hypoxia in vertebrate animals, including humans. Some of the major steps involved in mitochondrial biogenesis are based on recent reviews (Ceurzva et al, 1993); Nagley, 1991). Net expression effects are reported frequently in studies of adaptation to chronic hypoxia (see Cerretelli et al (1990), Hochachka and Randall, 1978; Hochachka and Somero, 1984; and Hochachka (1985)). Although no direct information is available on O2 sensing and transduction pathways, some possibilities are included based on hypoxia acclimation studies. The purpose of this kind of sketch is to outline as efficiently as possible the areas in greatest need of further research.

metabolism (Hochachka, 1985). In more extreme hypoxia exposure, in humans under hypobaric hypoxia for generations, the oxidative capacities at least of skeletal muscles seem to be down regulated (Kayser et al, 1991; Hochachka et al, 1992). Even lactate dehydrogenase (LDH) capacity, often used on an interspecies basis as an approximate indication of absolute anaerobic glycolytic capacity, is on the lower end of the spectrum for humans (Hochachka et al, 1992). However, the ratio of anaerobic/aerobic metabolic pathway capacity is up regulated in muscle of altitude natives such as Sherpas from the Himalayas or Quechuas from the Andes; for example, the ratio of muscle LDH/CS differs by a factor of 2-3 compared to lowland athletes. It is possible that the difference in response direction in moderate vs extreme chronic hypoxia is due to the inordinate hypoxia sensitivity of protein biosynthesis (Buc-Calderon et al, 1993; Land and Hochachka, 1993; Land and Hochachka, 1995)), although this concept remains to be more firmly established.

In addition to the above metabolic adjustments to chronic hypoxia, the regulation of fuel selection may be adjusted to maximize the amount of ATP conserved per O_2 utilized, an adaptation which would be particularly advantageous under chronic hypoxia.

This stoichiometric efficiency adjustment (achieved by preferential carbohydrate rather than free fatty acid - FFA - utilization and thus by avoidance of the 'FFA oxygen wasting effect') is evident in animals and in humans exposed to extreme chronic hypoxia over generational time (Hochachka, 1993). A consequence of this metabolic organization is that the glycolytic path influences the concentrations of the adenylates (the ADP-dependent pyruvate and phosphoglycerate) kinases of the glycolytic at are high activity, high Km(ADP) enzymes. As a result, the creatine phosphokinase near-equilibrium reaction shifts to lower phosphocreatine concentrations (Figure 8). These adjustments are clearly visible in whole body 4T magnetic resonance spectroscopy of the Sherpa heart *as* lower than expected PCr/ATP ratios - telling signatures of a heart biochemically organized for preferential aerobic carbohydrate metabolism (see Hochachka et al, 1995, for further literature and discussion in this area).

Although these kinds of empirical observations on adaptation to chronic hypoxia are widespread in the literature, the mechanism sunderlying them are not well understood. Diagrammatic summaries of the kinds of processes that may be operative under long term exposure to severe hypoxia are given in Figures 8). These kinds of summaries are based upon current conceptsol mitochondrial biogenesis (Cuezva et al, 1993, Nagley, 1991) and upon phenomenological observations onphylogenetic adaptations to

phylogenetic adaptations to relatively severe chronic hypoxia; to our knowledge, there is no information whatever on what the O_2 sensing mechanismsand signal transduction pathways here may actually be. The rough sketches given in Figures 9 are thus to be considered speculative and are prompted by studies of O_2 signalling systems in intermediate time courses of exposure. This clearly is an area in dire need of much more research.

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ERYTHROCYTE SHAPES AND PEROXIDATION DURING HIGH ALTITUDE ADAPTATION ON TIEN-SHAN-PAMIR REGION

I. B. HODJAMBERDIEV MD,[1] A. A. KUCHERBAEV MD,[2]

Biochemistry Department, Tuberculosis Research Institute and Central Research Laboratory, Medical Institute, and Hematology Department, Pediatrics, Obstetrics & Gynecology Research Institute.[2] [1]

ABSTRACT

Immigrants (adults) and aborigens (adults, new-borns, children) of several Tien-Shan-Pamir zones have been studied. Stomatocytes and degenerative forms are incresed 2-3-fold in immigrants of the Internal Tien-Shan zone, with a simultaneous peroxide stimulation in erythrocyte membranes.**(Acta Andina 1996, 5:57-60)**

Key words: Pamir-Shan mountains, immigrants, erythrocyte membranes, adaptation.

RESUMEN

Fueron examinados los imigrantes (adultos) y los aborígenes (adultos, niños y recién nacidos) en unas zonas de la región de Tian-Shan y Pamir. Fue registrado un crecimiento considerable (en 2-3 veces) de los estomatocitos y de las formas degenerativas en las células de los inmigrantes que viven en la región de Tian-Shan Interno, con la estimulación paralela de los procesos de peróxido en las membranas de los eritrocitos. **(Acta Andina 1996. 5:57-60)**

Palabras claves: Pamir-Shan, inmigrantes, membrana eritrocitaria, adaptación.

Introduction

There is little knowledge of the cellular membrane mechanisms of adaptation in alpine zones. Erythrocytes suitable object for this research. The complex geographical-ecological ranking made previously (Khodjhamberdiev et al., 1994) is used in this work.

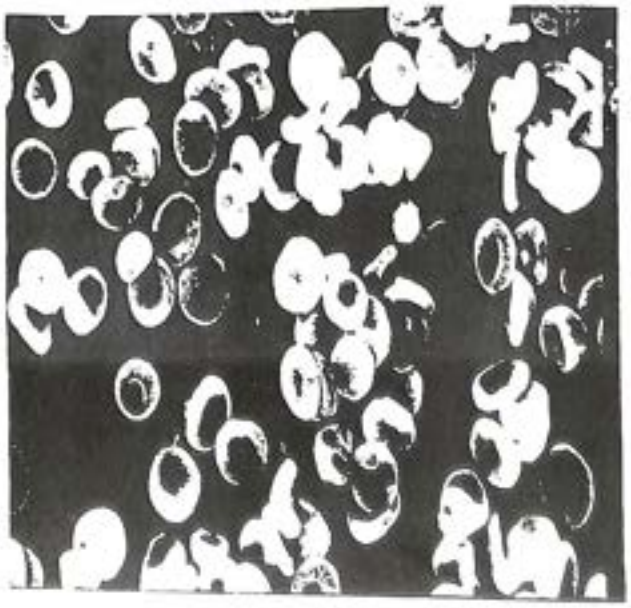
Material and Methods

Aborigen-mountaineers (adults, mewborns, children) and immigrants (adults) of several Tien-Shan-Pamir zones have been studied. One group of anaemic child-mountanineers has been added. Erythrocytes have been examined: 1) for shape - by scanning electron microscopy, SEM, (glutaraldehyde-fixed and dehydrated with "critical point" apparatus) at 20 kv in JEOL (Dewar, 1982) and sometimes by phase-contrastwet drop microscopy, 2) for membrane characteristic - by transmission electron microscopy (glutaraldehyde-OsO4-fixation, epon-araldyt for hardening, ultramicortomy) in JEM-100, 3) for membrane elasticity - by the microcapillary technique (parallel using 3uk and 7uk), 4) for lipid structure of membrane by thrinlayer chromatography of lipid fraction, previously purified membranes 5) for lipoperoxide processes by luminoldependent hemiluminescence, by malonyldialdehyde estimation., 6) for antiperoxide antiperoxide (antiradical) processes membranes by superoxydismutase activity (adrenochrome method), 7) for peroxide substrate by free fatty acids estimation (with Rhodamine 6G), 8) for energetic of erythrocyte by ATP content in erythrocyte cytoplasm (Boehringer Chemicals, with HAD/HAD H).

Igor B. Hodjamberdiev, MD.
Biochemistry Department, Tuberculosis Research Institute and Central Research Laboratory, Medical Institute, P.O Box 1451, Bishkek, 720040 Kyrgyz Republic

Results

We calculated percent of different forms per 1 thounsand cells (by Bessis classification)in SEM pictures. In samples from immigrants there were many spherocytes and planocytes (photo 1).



We found some "lumbar" forms of cells, in immigrants and in children-mountaineers with iron deficient anaemia, especially. There are wider fluctuations of microcytes-macrocytes variation in newborn mountaineers. Sometimes we found cytoplasmic ponticuli among erythrocytes forming adhesion groups in immigrants and newborn, child mountaineers. Membrane loosening and exfoliation were present in immigrants.

The prevailing tendency in the erythrocyte peroxide system of mountaineers and immigrants is of higher levels (in both peroxid and antiradical systems in membranes). But if altitude factors are complicated with iron deficient anaemia in children, peroxide levels rise moderately.

The most visible damaging effect on the erythrocyte shape and chemistry was observed (table 1) in samples from the hazard climate part of Tien-Shan-Pamir (if geographical region are compared), and in immigrants in the initial stage of adaptation (if human groups are compared).

Discussion

We suppose that there are two stages in the imigrants high altitude adaptation. First stage, "energency", with two variants, with a physiological one percent of abnormal erytrhocytes and filterability sharply increases, due to membrane peroxide rise. With a pathological one - hemolysis occurs. The second stage, stabilization, (after the first stage by the physiological variant) is characterized by moderate increase of echinocyte levels and partly reversed filterability due to inert membrane lipid concentration rise ("biomechanic balance system" autonomic mechanism (khodzhamberdiev, 1994).

Table 1. Erythrocyte shapes and chemistry in adult persons after the first week of altitude adaptation

Physico geographic region	Middle asian desert plain country	Country of middle-asian mountains		
		Province of North and Central Tien-Shan	Mountain province of Tien-Shan sirts	Mountain province of Pamir sirts
Ecology rank	I	II	IV	IV
Observation point, m. above sea level	740	2,200	3.100	3,300
Echinocytes I, %	9.1	9.4	10.0	12.6*
Echinocytes II, %	3.3	2.3	13.9	8.1*
Stomatocytes and degenerated forms, %	1.67	3.1*	7.18*	6.37*
ATP, nmol/ml	640	628	467*	434*
FFA, nmol/mg protein	16.2	25.3*	31.0*	46.1*
Cholesterol/phospholipid	0.86	0.96	0.62*	0.72*
MDA, nmol/g Hb	26.8	22.0	31.2*	33.1*

FFA: Free fatty acids; MDA - malonildialdehyde; (*): a statisctically significant difference from a control plain group.

Our results suggest the use of the morphological criteria as the most constant and adequate indicator for diagnosis of adaptation-cost, specially if it is complicated by geochemical anemia.

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EL PROGRAMA DE REPRODUCCION HUMANA DEL INSTITUTO DE INVESTIGACIONES DE LA ALTURA DE LA UNIVERSIDAD PERUANA CAYETANO HEREDIA. CENTRO COLABORADOR DEL PROGRAMA DE REPRODUCCION HUMANA DE LA ORGANIZACION MUNDIAL DE LA SALUD. 1965-1995

Gustavo F. Gonzales
Instituto de Investigaciones de la Altura. Universidad Peruana Cayetano Heredia
Apartado 1843. Lima, Perú

HISTORIA

El Instituto de Investigaciones de la Altura inicia el Programa de Reproducción Humana allá por el año 1965 cuando del 26 al 29 de abril se desarrolla un Symposium sobre Problemas de Población y Altitud, que se plasma en la publicación del libro **POBLACION Y ALTITUD**, editado por los Dres Luis A. Sobrevilla, José Donayre, Federico Moncloa y Roger Guerra-García. En este simposio participan 17 expertos tanto en población como en altura entre los que destacan los Dres Carlos Monge Medrano, Alberto Hurtado, Javier Arias-Stella, Leonel Alvarez, Luis Barua Castañeda entre otros. El Dr Leopoldo Chiappo desarrolla los comentarios finales del symposium concluyendo que se observan como características especiales del problema de población los siguientes:

a) Problema de migración interna, obra del proceso del excesivo desplazamiento rural-urbano.

b) Problema de composición poblacional manifiesto en el alto índice de desempleo y subempleo, con el consiguiente desperdicio de aprovechamiento de recursos humanos potencialmente utilizables dado el alto porcentaje de población en edad de trabajar.

c) Problema de elevación e incremento de las tasas de reproducción y crecimiento demográfico.

Tomando como base las conclusiones de este symposium, el Instituto de Investigaciones de la Altura desarrolla una intensa actividad de investigación que se desarrolla principalmente en el Laboratorio de Endocrinología.

El Laboratorio de Endocrinología en sus primeros veinte años, con la sucesiva dirección de Federico Moncloa y Roger Guerra-García ha realizado numerosas e importantes investigaciones en los nativos de Cerro de Pasco (4340 m).

José Donayre y Marco García-Hjarles han demostrado que el espermatograma y la bioquímica seminal son normales.

La fisiología y la bioquímica de la gestación y del recién nacido fueron estudiados a inicios de los 70s, y los resultados han sido publicados en un volumen de la revista peruana GINECOLOGIA Y OBSTETRICIA, editado por Roger Guerra-García.

La fecundidad de la población de Cerro de Pasco está incrementada; así lo demuestran dos encuestas realizadas en un grupo representativo de esa población, que fueron publicados por el Instituto de Investigaciones de la Altura a inicios de los 70s. Entre ellos se destaca el libro "Cambios de la Fecundidad en Cerro de Pasco" publicado por Delma del Valle y Luis A. Sobrevilla.

En los 80s, Gustavo F. Gonzales demuestra que el efecto inhibitorio sobre la fertilidad masculina de la exposición aguda a la altura es mediada por la serotonina, y que el tratamiento previo con antiserotoninérgicos a animales de laboratorio previene este efecto de la altura.

Es de destacar que en sus primeros 25 años de vida entre 1961 y 1986 el Instituto de Investigaciones de la Altura ha mantenido una continuidad en sus actividades, con participación importante de profesionales jóvenes, lo que ha permitido la necesaria renovación con el tiempo.

En la primera década (1961-1970) destacan en el campo de la reproducción las figuras de Roger Guerra-García, Federico Moncloa, Luis Sobrevilla y José Donayre.

En la segunda década (1971-1980) destacan las incorporaciones importantes de Luis A. Llerena, Juan Coyotupa y Marco García-Hjarles.

En la tercera década (1981-1990) se incorporan Gustavo F. Gonzales, y Carlos Carrillo

En el último quinquenio (1991-1995) ocurre una participación masiva de profesionales de distintas disciplinas interesados en reproducción humana.

EL CENTRO COLABORADOR DEL PROGRAMA DE REPRODUCCION HUMANA DE LA ORGANIZACION MUNDIAL DE LA SALUD.

En 1986, el Instituto de Investigaciones de la Altura fue invitado a participar de una competencia para elegir centros de América Latina con capacidades de desarrollo en el campo de la Reproducción Humana, cuyo término en el futuro se cambió a Salud Reproductiva.

El Instituto de Investigaciones de la Altura incorporó a la propuesta al reciente formado Instituto de Estudios de Población y al Departamento de Ginecología y Obstetricia.

A partir del 1 de diciembre de 1986, la Universidad Peruana Cayetano Heredia recibió la aprobación de un apoyo por cinco años con revisión anual. El Instituto principal era el Instituto de Investigaciones de la Altura y su Investigador Principal, el Dr Roger Guerra-García. Intervenían como coordinadores de cada unidad, el Dr Gustavo F. Gonzales, Jefe del Laboratorio de Endocrinología del Instituto de Investigaciones de la Altura; el Dr Luis Sobrevilla, Director del Instituto de Estudios de Población, y el Dr Manuel Gonzalez del Riego, Jefe del Departamento de Ginecología y Obstetricia.

El Programa permitía entre otros:

1.- el entrenamiento de profesionales de las diferentes unidades con la finalidad de desarrollar una masa crítica importante para el desarrollo del PROGRAMA DE REPRODUCCION HUMANA.

2.- el equipamiento de los laboratorios del Instituto de Investigaciones de la Altura, y en lo pertinente de las otras unidades participantes.

3.- el apoyo para el desarrollo de investigaciones enmarcadas en el PROGRAMA DE REPRODUCCION HUMANA.

4.- la asistencia de consultores expertos en reproducción humana de diferentes centros en el mundo.

5.- el apoyo para programas de docencia y entrenamiento en investigación.

EL PROGRAMA DE REPRODUCCION HUMANA

El Instituto de Investigaciones de la Altura desarrolla el Programa de Reproducción Humana como Centro Colaborador del Programa Especial de Investigación, Desarrollo y Entrenamiento en Investigación en Reproducción Humana de la Organización Mundial de la Salud, desde Diciembre de 1986. Este Programa incluye la participación de miembros del Departamento de Ginecología y Obstetricia y del Instituto de Estudios de Población.

Las actividades de Investigación se desarrollan en base a la determinación de prioridades de investigación en Salud Reproductiva elaborado con la consultoría de expertos de la Organización Mundial de la Salud, el Dr Gregorio Pérez-Palacios de México, el Dr Ramiro Molina de Chile, el Dr Juan Díaz, de Brasil y el Dr Luis Bahamondes, de Brasil.

El análisis de los problemas en Salud Reproductiva identificados por la Universidad Peruana Cayetano Heredia se enmarcan en los siguientes programas:

1.- Salud Reproductiva de los Adolescentes.
2.- Andrología
3.- Infecciones del tracto reproductivo
4.- Planificación Familiar
5.- Reproducción en las grandes alturas

El desarrollo de estos programas de investigación apuntan a identificar las causas que determinan una alta tasa de morbilidad y mortalidad asociados a los fenómenos reproductivos, y a plantear estrategias para disminuir dichas tasas.

RECURSOS HUMANOS

Para el cumplimiento de los objetivos planteados en la identificación de prioridades en investigación en Salud Reproductiva, el IIA ha conformado una importante masa crítica de caracter multidisciplinario, quienes en su mayoría han recibido adiestramiento en el extranjero, principalmente con el apoyo financiero de la Organización Mundial de la Salud, PLACIRH, y la Fundación Rockefeller.

Los investigadores del Instituto de Investigaciones de la Altura que actualmente participan como investigadores activos del Programa de Reproducción Humana pertenecen a las siguientes Unidades del Instituto:

1.- Unidad de Reproducción

Dr Gustavo F. Gonzales, Coordinador del Programa
Dr Carlos Carrillo, jefe de la Unidad
Dr Juan Coyotupa
Dr Arturo Villena
Dr Francisco Escudero
Dra Carmen Góñez
MSc Jorge Franco
MSc Diana Garayar
Biol. Mary Takara
Biol. Leyda Cabrera
Enf. Doris de la Cruz
Obst. Dicina Torres
Obst. Rosa Zela
Obst. Laura Delgadillo

2.- Unidad de NeuroCiencias

MSc Ida Alarcón, jefe de la Unidad
Psic. Olga Salaverry
Psic. Nancy Silvestre

3.- Unidad de Morfología y Genética.

Dr Máximo Salazar, jefe de la Unidad

INFRAESTRUCTURA

El Instituto de Investigaciones de la Altura cuenta con un edificio en Lima adjunto al Hospital Cayetano Heredia, y otro en la ciudad de Cerro de Pasco a 4340 m de altura adjunto al Hospital Daniel Carrión.

EQUIPOS DE LABORATORIO

La mayoría de equipos de laboratorio han sido donados por la Organización Mundial de la Salud. Algunas de las Unidades se han adquirido con recursos autogenerados por la Institución. Las facilidades de laboratorio con que cuenta el Instituto son las siguientes:

1.- Equipo de Cromatografía liquida de alta presión (HPLC)
2.- Equipo de Electroforesis de foco isoeléctrico
3.- Contador de radiación gama
4.- Lector de ELISA
5.- Espectrofluorómetro
6.- 1 centrifuga refrigerada
7.- 3 centrifugas de mesa
8.- 3 microscopios compuestos
9.- 2 microscopios invertidos
10.- 1 microscopio estereoscópico
11.- 3 incubadoras de CO_2
12.- 2 Gabinetes de Flujo laminar
13.- 1 equipo de agua millipore
14.- 2 espectrofotómetros.
15.- 1 cuarto frio
16.- 1 congeladora
17.- 3 baños María

EQUIPOS DE INFORMATICA

Nuestro instituto tiene la característica de que todos sus integrantes conocen y aplican, los procesadores de texto, los programas de base de datos, y algunos conocen y usan los programas estadísticos (STATA, SPSS).

El Instituto posee los siguientes equipos de informática:

1.- 6 computadoras IBM compatibles AT-286
2.- 4 computadoras IBM compatibles AT-386
3.- 5 computadoras IBM compatibles AT-486
4.- 1 computadora Laptop AT-486.
5.- 3 impresoras EPSON LX-810
6.- 1 impresora HP-III
7.- 1 impresora HP-IV Mplus
8.- 1 impresora deskjet 550
9.- 1 scanner
10.- 1 computadora AT-486, 100 MHz con Multimedia.

EQUIPO DE ENSEÑANZA AUDIOVISUAL Y DE IMPRESION

Siendo la docencia una de las actividades más importantes del Instituto, se ha logrado adquirir una serie de facilidades para la misma. Entre ellas tenemos:

1.- 1 proyector de diapositivas
2.- 1 proyector de transparencias
3.- 1 placa de data display portatil
4.- 1 equipo de data display incorporado al proyector.
5.- 1 videograbadora Panasonic
6.- 1 cámara fotográfica CANON EOS 1000
7.- 1 VHS Samsung
8.- 1 televisor SONY 25"
9.- 1 fotocopiadora CANON.

ACTIVIDADES

Las actividades del Programa de Reproducción Humana del Instituto de Investigaciones de la Altura son las siguientes:

1.- Investigación
2.- Docencia
3.- Publicaciones
4.- Servicios.

Investigación

Los investigadores del IIA han realizado una serie de investigaciones que se enmarcan dentro de las prioridades de Investigación en Salud Reproductiva identificadas por nuestro Centro.

1.- Salud Reproductiva de los Adolescentes.

- Comportamiento sexual en adolescentes de las ciudades de Lima, Cusco e Iquitos.
- Factores psicosociales asociados al embarazo en adolescentes de Lima, Arequipa, Cusco, y Pucallpa.
- Efecto del embarazo en la adolescencia sobre el desarrollo visomotor de los hijos en edad escolar.
- Las Creencias, los estilos de afrontamiento al estrés y la autoestima en adolescente y su relación con la actividad sexual en la adolescencia.
- Factores sociales y biológicos asociados a la menarquia en el Perú.
- Aspectos clínicos del embarazo en adolescentes

2.- Andrología.

- Mecanismos de la regulación de la producción de inhibina
- Factores de los túbulos seminíferos que

influencian la secreción de las células de Sertoli y la espermatogénesis.
- Desarrollo de nuevos métodos diagnósticos en la infertilidad masculina.
- Desarrollo de nuevos métodos en el tratamiento de la infertilidad masculina

3.- Infecciones del tracto reproductivo

- Prevalencia de leucocitospermia en parejas infértiles
- Efecto de la leucocitospermia en la calidad del liquido seminal.
- Inmunologia de la reproducción masculina.

4.- Planificación Familiar

- La lactancia materna como método contraceptivo en la altura
- Desarrollo de un contraceptivo masculino: Estudios en ratas
- Factores socioculturales asociados a la práctica y actitudes de los varones hacia la planificación familiar.
- Conocimiento y práctica de la planificación familiar por los adolescentes de ambos sexos.

5.- Reproducción en las grandes alturas

- El ciclo menstrual en la altura: Correlato ultrasonográfico y hormonal.
- Factores biomédicos asociados a la alta tasa de fertilidad en la altura.
- Prevalencia de partos pre-términos en la altura.
- Adrenarquia en la altura
- Menarquia en la altura
- Menopausia en la altura
- Edad gestacional al parto en diferentes altitudes del Perú.

Docencia

La actividad de docencia ha sido bastante activa y ha tenido tres componentes:

a.- Entrenamiento en investigación
b.- Cursos cortos
c.- Maestria en ciencias con mención en Fisiologia de la Reproducción.

Entrenamiento en investigación

Profesionales y estudiantes de la Universidad Peruana Cayetano Heredia como de otras instituciones, particularmente del Instituto Peruano de Seguridad Social han recibido entrenamiento en investigación en Salud Reproductiva por periodos de 2 a 24 meses.

Cursos cortos

El IIA ha desarrollado una gran actividad tratando de difundir los resultados de sus investigaciones, asi como tratando de que profesionales extranjeros de primera linea contribuyan con su experiencia en una serie de cursos cortos que se han realizado en diferentes provincias del pais.

Esta actividad ha permitido mantener una estrecha interrelación entre nuestro Instituto y centros de diferentes partes del pais, asi como identificar potenciales profesionales que pueden conformar una masa critica de investigadores en su región tratando de identificar sus problemas de salud reproductiva y desarrollar sus programas de investigación orientados a dichas prioridades.

Maestria

Con el Departamento de Ciencias Fisiológicas se ha venido desarrollando una actividad conjunta para la implementación de la Maestria en Ciencias con mención en Fisiologia Reproductiva. Esta maestria se lleva desde la década de los setentas.

El Programa incluye la aprobación de 64 horas créditos que se cubre en promedio en 4 semestres. A mitad de dicho periodo el alumno debe rendir un examen de candidatura que consiste en la preparación de un proyecto de investigación y la sustentación ante un jurado calificado designado por la Escuela de Post-Grado de la Universidad Peruana Cayetano Heredia.

Los cursos son de tipo tutorial y se distribuyen en Tópicos selectos en Fisiologia, y Cursos de Investigación en Fisiologia. Para el cumplimiento del Programa se requiere la aprobación de 32 créditos en Investigación en Fisiologia.

Al término de los cursos el estudiante prepara y desarrolla una tesis que debe ser sustentada ante un jurado designado por la Escuela de Post-Grado.

A la fecha han culminado satisfactoriamente esta maestria los siguientes profesionales:

1.- Marco Garcia-Hjarles, 1973
Asesor: Dr Roger Guerra-Garcia

2.- Gustavo F. Gonzales, 1978
Asesor: Dr Roger Guerra-Garcia

3.- Sadot Villarreal Vargas, 1984
Asesor: Dr Gustavo Gonzales

4.- Gustavo Velásquez, 1984
Asesor: Dr Gustavo Gonzales

5.- Ricardo Zorrilla, 1984
Asesor: Dr Gustavo F. Gonzales

6.- Jorge Franco, 1989
Asesor: Dr Roger Guerra-Garcia

7.- Diana Garayar, 1989
Asesor: Dr Roger Guerra-Garcia

8.- Santiago Pastor
Asesor: Dr Enrique Fernández

Están llevando estudios de maestria, los siguientes profesionales:

1.- Rosa Zela
Asesor: Dr Gustavo F. Gonzales

2.- Javier Garcia
Asesor: Dr Gustavo F. Gonzales

Publicaciones

El Programa de Reproducción Humana del Instituto de Investigaciones de la Altura ha desarrollado una intensa actividad en el rubro de publicaciones tanto en revistas nacionales como internacionales, asi como en la preparación y edición de libros y contribuyendo con capitulos de libros.

La participación de los investigadores del IIA ha sido impresionante lo que se evidencia en el número de resúmenes presentados a congresos nacionales e internacionales.

Otras Actividades.

El Programa de Reproducción Humana ha permitido mantener una excelente interrelación y trabajo en conjunto con profesionales de otros centros tanto nacionales como extranjeros (Chile, Argentina, Brasil, México, Australia, USA).

Esta interrelación ha permitido la venida de expertos extranjeros al Perú, o que profesionales de otros centros del pais se entrenen en el extranjero. Se ha logrado igualmente realizar investigaciones conjuntas que han permitido tener varias publicaciones en revistas internacionales.

Miembros de nuestro Instituto participan en las siguientes redes Latinoamericanas:

1.- Red de Investigación y Entrenamiento en Inmunología de la Reproducción. Esta red es coordinada por la Dra Deborah Anderson de los Estados Unidos y participan profesionales de Lima, Buenos Aires, y la Habana. Han solicitado su incorporación profesionales de Panamá y México.

2.- Red del Programa Regional sobre Aspectos Sociales de la Reproducción Humana. Esta red es coordinada por la Dra Edith Pantelides de Argentina y participan profesionales de 11 paises, incluyendo nuestro pais. Por el IIA participa la psic. Olga Salaverry, quien recibió entrenamiento en el centro de la Dra Pantelides.

Los siguientes miembros del IIA forman parte de la Junta Directiva de la Asociación LatinoAmericana de Investigadores en Reproducción Humana (ALIRH)

De 1993 a 1995

Presidente: Dr Roger Guerra-Garcia
Vice Presidente: Dr Gustavo F. Gonzales
Tesorero: Dr Carlos Carrillo
Secretario: Dr Marco Garcia-Hjarles

De 1995 a 1997

Presidente: Dr Gustavo F. Gonzales
Vice Presidente: Dr Carlos Carrillo
Tesorero: Dr Arturo Villena
Secretario: Dr Marco Garcia-Hjarles

Las actividades de investigación de los profesionales del IIA han sido recompensadas por una serie de premios nacionales entre los que destacan el Premio Hipólito Unanue y el Premio Roussell, los más importantes del pais.

PUBLICACIONES

A. Número de publicaciones durante el periodo de participación como Centro Colaborador de la Organización Mundial de la Salud.

	Nacional	Regional	Internacional	Total
Artículos originales (Revistas)	12		52	64
Artículos de Revisión (Revistas)	2		6	8
Capítulos de libros	1		4	5
Libros	6			6
Resúmenes de Congresos	70		76	146
Reportes Oficiales				
TOTAL	91		138	229

PUBLICACIONES HRP-OMS (1987-1995)

LIBROS

1.- **Gonzales GF** (1987) Instituto de Investigaciones de la Altura 1961-1986. Ed. R. Guerra-García, F. Sime y GF Gonzales. Lima-Perú. pp. 103-229. (Español)

2.- **Gonzales GF.** (1991) Diagnóstico y Manejo de la Pareja Infértil. Instituto de Investigaciones de la Altura. Lima-Perú. ed. GF Gonzales. 208 pp.

3.- **Gonzales GF.** (1992) Andrology. Fertility and Infertility. Instituto de Investigaciones de la Altura. Lima-Perú. ed. GF Gonzales. 318 pp. (Español).

4.- **Gonzales GF.** (1993) Reproducción Humana en la Altura. Instituto de Investigaciones de la Altura. Lima-Perú. ed. GF Gonzales. 208 pp.

5.- **Gonzales GF** (1994) La Adolescencia en el Perú, Instituto de Investigaciones de la Altura. Lima-Perú. ed. GF Gonzales. 328 pp.

6.- **Gonzales GF., Villar J., Lavin P** (1995) Metodología de la Investigación en Reproducción Humana. Lima-Perú. ed. GF Gonzales. 350 pp

CAPITULO DE LIBROS

1.- **Gonzales GF** (1986) Influencia del status socioeconómico y la altitud de residencia en la antropometría de los estudiantes nativos de diferentes altitudes del Perú. En: Perinatalidad, Crecimiento y Desarrollo en el Perú. Ed. AMIDEP, Lima-Perú pp. 93-133. (Español)

2.- de Kretser,DM., Robertson,DM., Risbridger,GP., **Gonzales,GF.**, Drummond,A., Burger,HG (1989) Inhibin and related peptides. In Molecular and Cellular Endocrinology of the testis. Serono Symposia Publications. Raven Press 50: 11-20. (Inglés)

3.- de Kretser DM., Sun YT., Drummond AE., **Gonzales GF.**, Robertson DM., Risbridger GP (1990). Physiological mechanisms controlling spermatogenesis. In: Gamete Physiology. (Eds). RH Asch, JP Balmaceda y Johnston I. Serono Symposia USA. pp 19-29 (Inglés)

4.- de Kretser DM., Risbridger GP., Drummond AE., **Gonzales GF.**, Sun YT (1991) Paracrine mechanisms in the regulation of testicular function. In: Growth Factors & Fertility Regulation. (eds) Haseltine & Findlay JK. pp 143-155. (Inglés)

5.- **Gonzales GF.**, Guerra-García R (1993) Algunas características del embarazo y del recién nacido en la altura. En: Hipoxia. Investigaciones básicas y clínicas. F. León-

Velarde and A. Arregui. 374 pp. (Español).

REVISTAS

Revistas Nacionales

1.- Ramirez T., Cajahuaman T., **Gonzales GF.**, **Carrillo C** (1987) El peso de la placenta en las grandes alturas. **Diagnóstico** (Lima-Perú) 19: 74-76.

2.- **Gonzales GF.**, Ramirez T., Cajahuamán S (1987) Estudios en recién nacidos de gestantes añosas de Cerro de Pasco (4340 m). **Diagnóstico** (Lima, Perú) 19: 146-149.

3.- **Coyotupa J.**, Koremblit N., Maradiegue E., **Carrillo C.**, Noriega L., **Gonzales GF.** (1987) Sindrome del Foliculo Luteinizado no roto. **Ginecología y Obstetricia** (Lima, Perú) 31: 53-55.

4.- **Gonzales GF.**, **Coyotupa J.**, Noriega L., **Carrillo C.**, Gutierrez R., Quintana L (1988) Hiperprolactinemia en el sindrome de ovario poliquistico: ¿Causa o Consecuencia? **Diagnóstico** (Lima, Perú) 21: 106-111.

5.- **Gonzales GF.**, **García M.**, Velasquez G., **Coyotupa J** (1988) Prolactina e Infertilidad Masculina. **Diagnóstico** (Lima, Perú) 21: 47-57.

6.- **Gonzales GF.**, Bacini J., Noriega L., Prazak L., Tremolada J., Zapana M (1989) Nuevas técnicas para selección de espermatozoides mótiles para un programa de fertilización asistida. **Revista Médica Peruana** 61: 15-22.

7.- Quintana L., **Coyotupa J.**, **Carrillo C.**, Ramirez T., **Gonzales GF.** (1991) Prolactinemia en mujeres a nivel del mar y en la altura. **Ginecología y Obstetricia** (Lima, Perú) 36: 88-93.

8.- **Coyotupa J.**, Gonzalez S., Zorrilla R., Ramirez T., **Gonzales GF.**, **Guerra-García R** (1991) Menarquia y menopausia en la altura. **Ginecología y Obstetricia** (Lima, Perú) 37: 43-50.

9.- Whilhem J., López G., Gil K., Muro L., Donayre M., Arevalo J., Ramirez C., **Carrillo C.**, **Gonzales GF.** (1991) La edad materna como factor de riesgo en el embarazo en la selva del Perú. **Diagnóstico** (Lima, Perú) 28: 80-84.

10.- Román V., **Carrillo C.**, Román C (1992) Características maternas y complicaciones neonatales de la macrosomia fetal. **Diagnóstico** 29: 77-82.

11.- Angeles M., **Carrillo C** (1994) Depresión en el puerperio inmediato. **Diagnóstico** (Lima, Perú). (en prensa).

12.- **Torres D.**, **Gonzales GF** (1995) El factor masculino en un servicio de infertilidad de Lima. **Diagnóstico** (Lima, Perú). 34: 17-22.

INTERNACIONAL

1. **Gonzales,GF.**, Risbridger,GP., de Kretser,DM (1988) In vitro synthesis and release of inhibin in response to FSH stimulation by isolated segments of seminiferous tubules from normal adult male rats. **Molecular and Cellular Endocrinology**. 59:179-185

2. **Gonzales,GF.**, **Garcia-Hjarles,MA.**, Napuri,R (1988) Corrected seminal fructose levels:Index of secretory activity of seminal vesicles. **Archives of Andrology** 21:135-142

3.- **Garcia MA** (1989) Spermatogram and seminal biochemistry of high altitude natives at sea level and patients with chronic mountain sickness. **Arch. Biol. Medic. Expe. (Chile)**. 22: 61-68.

4. **Gonzales,GF** (1989) Functional structure and ultrastructure of seminal vesicles. **Archives of Andrology**. 22:1-14

5. **Gonzales,GF.**, **Garcia-Hjarles,MA.**, Napuri,R., **Coyotupa,J** (1989) Blood serotonin levels and male infertility **Archives of Andrology**. 22:85-90

6. **Gonzales,GF.**, Risbridger,GP., de Kretser,DM (1989) The effect of insulin on inhibin production by isolated seminiferous tubule segments from adult rats cultured in vitro. **Molecular and Cellular Endocrinology**. 61:209-216

7. **Gonzales,GF.**, **Garcia-Hjarles,MA.**, Velazquez,G., **Coyotupa,J** (1989) Seminal Prolactin and its relationship to sperm motility in men. **Fertility and Sterility** 51:498-503

8. **Gonzales,GF.**, Risbridger,GP.,de Kretser,DM (1989) Epidermal growth factor increases inhibin synthesis by isolated segments of rat seminiferous tubules. J. Endocrinology 123:213-219

9. **Gonzales,GF.**, Risbridger,GP.,de Kretser,DM (1989) In vivo and in vitro production of inhibin by cryptorchid testes from adult rats. **Endocrinology**. 124: 1661-1668

10. **Gonzales,GF.**, **Garcia-Hjarles,MA.**, Gutierrez,R., **Guerra-Garcia,R** (1989) The secretory activity of seminal vesicles and its relationship with sperm motility: Effects of infections in the male reproductive tract. **International Journal of Andrology** 12: 286-294.

11. **Guerra-Garcia R.**, Franco J ., **Gonzales GF** (1989) Serum inhibin is inversely correlated with serum FSH levels in adult men. **Archives of Andrology** 22: 35-40.

12. **Gonzales GF.**, Risbridger GP., Pollanen P., de Kretser DM (1989) Stage-specific inhibin secretion by rat seminiferous tubules. **Reprod. Fert. Dev** 1:275-279.

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