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HIGH ALTITUDE METAHEMOGLOBINEMIA

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SUMMARY.— Atl high altitudes the human erythrocyte, subject to a permanent hypoxy, is the site of metabolic changes among which metahemoglobin has drawn our attention. The present work presents an argument in favor of the hypothesis that metahemoglobin correspons to a easily mobilized, reserve hemoglobin, in case of emergency. At the time of a maximum physical effort, the metahemoglobin level decreases quite significantly and follows a corresponding decrease in reduced glutation. Moreover, a very significant increase of hematological constants (hematocrit, hemoglobin, red cell count) is seen during exertion to return to normal after a few minutes of recuperation.

INTRODUCTION

The erythrocyte of populations living at high altitudes and exposed to a permanent hypobaric hypoxia, suffers many metabolic changes so as to fulfill its function of respiratory gas transportation. The displacement of the oxyhemoglobin curve (ODC) toward the right, on one hand, and the deviation of arterial and venous PO_2 toward the left, allow oxygen extraction through the tissues, the same as in low altitudes. Nevertheless, at the blood level, the saturation percentages of arterial and venous hemglobin are inferior to those found normally at sea level[8, 16, 17, 18, 21]. This is why the red cell, which assures an adequate oxygenation of the tissues, is in permahent hypoxia.

We have already indicated the metabolic consequences of this erythrocytary hypoxia and especially of the metahemoglobin state (MetHb), which is very

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high[1, 14]. This paradoxically, allowed us to formulate a hypothesis from the work of Darling and Toughton[9] and of Gourdin[14] on the role played by MetHb.

1. Regulation of the respiratory function of the erythrocyte through a control of the ODC position. Metahemoglobin has an antagonic effect to that of phosphoreted molecules, such as A.T.P. adn 2-3 D.P.G. This means that it would produce an OCD deviation to the left[9].

2. Hemoglobin reserve in a non-functional state can be mobilized at any moment in case of emergency[14].

In this paper we present the defense of this second part of the hypothesis. For this purpose we have followed the rates of MetHb variation and of reduced glutation (G.S.H.), in the case of physical exertion of athletes at 3,600 m. altitude.

MATERIAL AND METHODS

For this experiment we used Bolivian athletes, preselected for the Bolivian Games, which took place in La Paz, Bolivia (October 1977). A study was made of 23 male athletes with an average age of 21, 6 $\pm$ 4,5 years. Eighteen of the athletes were natives of the Bolivian plateau (La Paz, 3,600 m.) and represented group A. Five others, Group B, came from altitudes under 1,500 m. and were residents of La Paz for only a few months.

After these athletes were submitted to a series of medical controls and after they had become familiar with the laboratory environment, they were asked to participate in an experiment carried out on a Monarck bicycle. Cardiac frequency was determined and the first sample of elbow venous blood was taken for the determination of hematological constants (hematocrit, hemoglobin, red cell count), of the MetHb and the G.S.H. (Time I).

There was a 10 minute warming-up period during which athletes did moderate excercise, without exceeding a cardiac frequency of 120 beats/min. At the end of this periods a new blood sample was taken. (Time II).

Immediately thereafter, with the athletes still pedaling, the 50% maximum oxygen consumption was determined considering the cardiac frequency[3]. After this pysical level was established, a new blood sample was drawn (Time III).

Sub-maximum and maximum effort tests were started at once, increasing progresively the work potential from the medium potential of 3,5 Kgp. Every minute and a half the load was increased (Balke-method) until sought levels were reached and blood samples were taken (Time IV and V).

Finally, a final sample was collected 25 minutes after exertion had terminated and after athletes had rested in a reclining position (Time VI).

The total blood volume obtained represented approximately 15 ml. and the following evaluations were made on each heparinized blood sample:

Hematocrit (Ht), expressed in % (microhematocrit).

Hemoglobin (Hb), expressed in g/100 ml. of total blood (II).

Red cell count (No. G.R.) expressed in 10^6 G.R./m^3 (In Thoma cell, with dilution in B.D. Unopettes).

Metahemoglobin (MetHb), expressed in % of total hemoglobin and grams of MetHb in each 100 ml of blood[13]

Reduced glutation (G.S.H.), following Kaplan's technique[15] expressed in mg/100 ml. of red cells.

RESULTS

Chart N° 1 shows the hematological and biometrical values of the groups. The statistical study effected with the help of the Student t test allows us to observe that the two groups are not significatively different ($p > 20$) except for weight ($p < 5\%$). Only the MetHb values show a significative difference between both groups ($p < 1\%$). The metahemoglobin values expressed in MetHb Grams/100 ml. of the total blood, are in parenthesis.

Chart II follows the evolution of all the hematological data related to intensity of exertion. The statistical study of this chart is the result of comparing Times II and VI with reference Time I (repose), and results show the following differences:

—Not significant between two successive times ($p > 5\%$) except MetHb which will be studied in detail;

—Not significant between Times I and VI, on one hand, and between Times I and III on the other (except for MetHb).

—Significant ($p<1\%$) between Times I and IV, and less for MetHb and G.S.H. (very significant $p < 0.1\%$).

—Very significant ($p < 0.1\%$) between Times I and V.

We have summarized these data on a graph (Fig. 1) where we can follow the evolution of several parameters in relation to exertion time.

DISCUSSION

In this study we discovered many phenomena which we will discuss separately altough they represent a chain of physiological mechanisms during exertion:

1. Evolution of hematological constants;
2. Evolution of MetHb and G.S.H. rates and
3. Different erythrocytary responses between the natives of higher and lower altitudes.

A. HEMOGRAM AND EXERTION.

The three hematological data studied have a very similar evolution (Fig. 1). There is a clear increase of globular mass in the face of plasmatic volume. This may be due to two mechanisms:

1. A massive liberation of reservoir of erythrocytes.

2. A hemoconcentration by elimination of plasmatic water and better interstitial liquid distribution in different organs[19, 23].

The latter seems to be the most probable.

1. On one hand because of the very significant difference observed between Times I and V ($p < 0.1\%$). The erythrocyte reservers would not permit such a difference[12].

2. On the other hand, after recuperation difference can be seen between times I and VI ($p > 5\%$). That would suppose a physical mechanism.

Nevertheless, it is difficult to affirm that the second mechanism is the origin of our observation in absence of a more complete study on blood and plasmatic volume variations during excercise.

B. EVOLUTION OF THE MetHb AND G.S.H. LEVELS.

The exertion made by these athletes was sufficient to mobilize all the media on which the erythrocyte depends to insure its respiratory gas distribution, mainly oxygen, to the tissues. A grater oxygen consumption provokes and increased demand. In the face of this new situation, the erythrocyte puts into motion all its media for the required adaptation.

We can therefore observe a very significant decrease in the rate of MetHb, from 3.769% to 0.944%; that is, from 0.60 to 0.15 g/10 ml. of total blood (Chart II). In this way every 100 ml. of blood receives 0.44 g. of supplementary functional hemoglobin.

The theoretical oxyphoric potential (P.O.x) of 1,39 cm^3 of oxygen for each gram of Hb[6] decreased to 1.34 as a result of the high percentage of MetHb seen at high altitudes (3,600 m). The needs produced by exertion and variation in the MetHb consecutive rate change the P.O x to 1.38. This theoretical calculation only takes into account the variation due to the changes in MetHb quantity. Actually there are other intervening factors which are not studied in this paper.

Nevertheless, this exertion had as a consequence an increased oxygen content (0.02) in and oxygen carrying capacity of the blood.

MetHb mobilization is renewed. So, after twenty-five minutes of recuperation, levels return to normal for the altitude[2, 14] G.S.H. varied in a similar way during exertion. Starting from an elevated value, characteristic of high altitudes, this molecule decreased at the end of excercise to a value which is comparable to that observed at sea level[10, 2]. Like the Methb, the G.S.H. levels return to normal after twenty-five minutes of recuperation. (Fig. 1, Chart II).

A relation between the G.S.H. and MetHb rates appears reminding us of the work of Scott[20] who showed a direct chemical reducing action of the MetHb by the G.S.H. (12% of the total erythrocyte reducing activity).

We have noted that diminished MetHb levels increase oxygen carrying capacity of the erythrocyte, due to the liberation of hemoglobin reserves. Oxygen liberation and transport to the tissues can also be improved by the MetHb rate activity on the oxyhemoglobin dissociation curve (O.D.C.)

Benesh[5] and Chanutin[7] have shown that A.T.P. and 2-3 DPG displace the ODC to the right (dicreased affinity).

Darling[9] have pointed out the role played by MetHb on the O.D., an antagonic role for the phosphoric derivatives since MetHb favors deviation to the left (increased affinity).

At high altitude strong concentrations of ATP and 2-3 DPG on one hand, and MetHb on the other, establish an equilibrium slightly towards the right of the position seen in those living at sea level[2].

At the point of maximum exertion, the fall of the MetHb level must, therefore, accentuate the deviation phenomena of O.D.C. to the right. This is due to a very slight decrease in oxygen fixation, but specially to the great liberation increase of this gas to the tissues.

Influences in the face afinity between hemoglobin and oxygen result in the displacement of the O.D.C. as result of complex mechanisms.

Exposition to high altitude and physical effort modify all equilibriums allowing us to observe regulatory mechanisms.

C. ADAPTATION AND ACCLIMATION

Both groups studied:

18 natives of high altitudes, and

5 natives of lower lands

do not present any significant difference with regard to hematological and biometrical data, and to GSH levels (Chart 1). On the contrary, the MetHb contained in their erythrocytes is, even in repose, very different. Native of lower lands show a more significant amount of MetHb.

We have studied in this situation the evolution of this non-functional hemoglobin through exertion.

Since both groups have an identical erythrocyte and hemoglobin content, we can represent MetHb evolution schematically, expressed in percentage of Hb in Fig. 2. This way, starting from time of repose (I) with significant different values, we reach maximum exertion (V) with a lower indentical value. After the twnty five minute recuperation (VI), both groups separate again.

The hemoglobin reserve, liberated during exertion by the erythrocytes of both groups is different (Chart III).

Group A gains:

0.57 — 0.15 = 0.42 g. Hb/100 ML. S.T.

Group B gains:

0.71 — 0.16 = 0.55 g. Hb/100 ML. S.T.

It seems that we have a good example here of the difference between adaptation and acclimation at high altitudes.

High altitude athletes show more favorable morphological and physiological characteristics than those from lower altitudes[22].

Acclimation of biochemical mechanisms to altitude does not need to be so intense in these as in athletes of group B.

The whole of acclimation mechanism to high altitude hypoxia seems to reach a state of equilibrium after a few weeks of permanence at the altitude. We have set as a hypothesis that MetHb intervenes as a regulating molecule. This difference between the two groups makes us suppose a mechanism of equilibrium at the level of ATP and 2-3 DPG regulation, and antagonism to very slow MetHb. This corresponds to a point that we have just proven in our laboratories (COUDERT, Unpublished work).

CONCLUSSIONS

The discovery of an elevated MetHb level at high altitude, an apparently paradoxical characteristic, awakened in us a special interest for a better knowledge of said molecule.

We have proposed the hypothesis that MetHb plays an important part in the regulatory phenomena in the erythrocyte adaptation to hypoxia conditions (of altitude and maximum exertion).

This work has allowed us to bring forth new arguments in favor of this hypothesis:

1. MetHb, a hemoglobin reserve, in case of need;
2. MetHb, regulator of oxygen liberation phenomena to the tissues;
3. MetHb, regulator of all processes of erythrocyte acclimation to high altitude hypoxia.

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