FORMATION OF A FACTOR INHIBITING ERYTHROPOIESIS IN MOUNTAINEERS AFTER DESCENDING FROM HIGH ALTITUDES

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A single ascent to an altitude of 3900 m leads to an increase in the hemoglobin concentration and reticulocyte count in the blood and to an increase in the erythropoietic activity of the plasma. However, 24 h after descent, a factor appears in the plasma which inhibits mitosis in cells of the erythroblastic series. This factor passes through the kidneys and is found in the urine.

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An increase in the formation of erythropoiesis inhibitors has been found after experimental division of various nerves [1-3, 10], in polycythemia produced in sheep by repeated transfusions of homogeneous erythrocytes [12], in rabbits with the same polycythemia [15], in diffuse kidney lesions in man and animals [5, 11, 14], in radiation sickness, pernicious anemia, etc. [4]. Carmena and co-workers [9] described the development of aplasia of medullary erythropoiesis in mountain dwellers 230 h after coming down to sea level. It has also been shown that the plasma of mountain dwellers obtained 24 h after descent to sea level, if injected into rats, inhibits incorporation of Fe^{59} into erythrocytes [13].

The fact that inhibitors of erythropoiesis are formed in man in response to removal of hypoxic influences is interesting from several points of view. Its analysis may prove valuable as an aid to understanding the mechanisms of adaptation to changing oxygen conditions, and in connection with the study of the role of erythropoiesis inhibitors in the system of control of erythropoietic function. We therefore decided to study erythropoiesis inhibitors in mountaineers after climbs of different durations and difficulties.

In the present investigation we studied the effect of plasma and urine of mountaineers who had completed one climb to a relatively low altitude on a bone marrow culture.

EXPERIMENTAL METHOD

Observations were made on 17 healthy trained mountaineers (14 men and 3 women) aged from 19 to 26 years. The mountaineers were based at the "Uzon-Kol" Camp situated in the Western Caucasus at an altitude of 2000 m above sea level. They made their first climb to an altitude of 3900 m a few days after their arrival. The climb and the stay at the high altitude took about 3.5 days, but they came down quickly, within 1.5-2 h. Blood and urine were taken for investigation the day before the first climb, on the day of descent, and on the next two days. The hemoglobin concentration was studied by Sahli's method, reticulocytes were counted (after staining with brilliant cresyl blue), and the effect of the plasma and urine on mitotic activity of cells of the erythroid series was studied in a bone marrow culture. Plasma in a volume of 3-5 ml and urine in a volume of 10 ml were treated with ion-exchange resins of the IR-120 type. We used this type of resin previously to study inhibitors of erythropoiesis and erythropoietin [6, 7]. The resulting eluates were sterilized through a Seitz bacterial filter and added to a suspension of rabbit bone marrow. Incubation took place for 20 h at 37° in an incubation medium containing colchicine in a concentration of 1:500,000. At the end of incubation, films were prepared from the bone marrow residue and stained by Pappenheim's method. In the stained films 200 cells of the erythroblastic series capable of division were counted and the percentage of cells in metaphase calculated. The results were compared with those obtained

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during control cultivation, when Hanks's isotonic salt solution was added to the bone marrow suspension instead of the test sample. The method of cultivation of the bone marrow was fully described elsewhere [7, 8].

EXPERIMENTAL RESULTS

The hemoglobin concentration in the blood of the mountaineers increased during the period of observation. Before climbing its mean value was 14.2 ± 0.2 g%, on the day of descending from the high altitude it was 14.6 ± 0.17 g%, and on the next two days it was 15.2 ± 0.18 ($P < 0.002$) and 15.5 ± 0.16 g% ($P < 0.001$).

The reticulocyte count increased considerably. Before the climb its mean value was 0.8 ± 0.07%, but on the day of descent and on the following day its value was 4.8 ± 0.8 and 8.8 ± 2.7%, respectively. By the third day the reticulocyte count began to fall (to 4.6 ± 1.3%), but it was still much higher than originally. The increase in the reticulocyte count was highly significant ($P < 0.001$). Plasma obtained from the mountaineers before starting their climb, when added to bone marrow cultures caused only a slight decrease in the percentage of cells of the erythroid series in metaphase (by 1.2 ± 0.9), and the decrease was not statistically significant. Plasma obtained on the day of descent from the high altitude caused a slight, but statistically significant increase in the percentage of cells in metaphase compared with the control (by 4.2 ± 1.0; $P < 0.001$). Plasma obtained on the following day, on the other hand, caused a significant decrease in the percentage of erythroblasts in metaphase (by 3.2 ± 1, $P < 0.01$). This decrease was more marked on the third day (5.5 ± 1%; $P < 0.001$). Urine obtained from the mountaineers before the climb, when added to a culture of bone marrow, caused a slight but significant decrease in the percentage of erythroid cells in metaphase (by 2.7 ± 1.03; $P < 0.02$). Urine obtained on the day of descent had lost its ability to inhibit mitotic division and it caused only a slight increase, which was not significant, in the percentage of dividing cells (by 0.7 ± 1.12). Urine collected on the second day after descent caused a decrease in the percentage of erythroid cells in metaphase by 6.9 ± 1.55 ($P < 0.05$). Urine obtained on the third day after descent caused only a slight decrease, which was not significant, in the percentage of cells in metaphase (by 1.6 ± 0.78).

Several investigations have now shown that under the influence of any type of hypoxia, erythropoiesis in man and animals is stimulated. As a result of this stimulation the indices of the erythrocyte composition of the blood are increased. Activation of the erythropoietic system in response to hypoxia takes place with the participation of erythropoietin, formation of which is intensified in hypoxic states. Some of the results obtained in the present investigation are fully compatible with this point of view. An increase in hemoglobin concentration and in the reticulocyte count in the blood was observed in all the mountaineers even though they had made only a relatively short climb – from 2000 m above sea level to 3900 m, and plasma taken from them immediately after descent caused an increase in the percentage of erythroblastic cells undergoing mitotic division, demonstrating erythropoietic activity of the plasma.

A different picture was seen when plasma taken on the second and third days after descent was investigated. When added to the bone marrow culture not only did it not increase the number of mitoses in the erythroblastic series, but decreased it significantly by comparison both with the control and with results obtained during investigation of plasma taken before the climb. These results suggest that 24 h after the end of hypoxia, substances inhibiting erythropoiesis may appear in human blood. The erythropoiesis-inhibiting action of the plasma reached its maximum (48 h after descent) at the same time as the reticulocyte count began to fall.

It could be assumed that the erythropoiesis-inhibiting action of plasma is relative and is associated with termination of increased erythropoietin formation after descending from high altitudes. However, this assumption is contradicted by the fact that the inhibition observed after descent was greater than that before the climb. It is evident that the normalization of the blood erythrocyte composition which takes place after the end of exposure to hypoxia is not a passive process but an active one in which the formation of erythropoiesis inhibitors participates.

When studying the action of the mountaineers' urine on the bone marrow culture we obtained results repeating in principle those for the effect of plasma, but quantitatively less marked. However, it is difficult in such cases to make quantitative comparisons because the amounts tested were hardly comparable: 5 ml plasma and 10 ml urine. At the same time, the discovery that urine taken from mountaineers on the second day after the descent possesses an inhibitory action on erythropoiesis is evidence that this inhibitor passes through the renal filter and is lost to some extent in the urine.

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