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Influence of Plasma Filtrate Containing Erythropoietic Factor on Intestinal Iron Absorption in Rats.* (27361)

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It has been shown that acute exposure to altitude increases intestinal iron absorption in men(1,2). These findings suggested that the increased intestinal absorption of iron was associated with increased red cell production rather than lowered oxygen saturation of the blood or oxygen tension at the intestinal level. The close relationship between the level of red cell production and iron absorption suggests that the regulating mechanisms of iron absorption during altitude changes must take place in intimate relation to or through a mechanism similar to that controlling erythropoiesis. To test this hypothesis the effect of plasma filtrate obtained from rabbits submitted to simulated high altitude on intestinal absorption of iron in the rat was studied.

Material and methods. Thirty-three white, adult, male rats, weighing 150 to 200 g, were divided into 3 groups: 1) 11 rats were given plasma filtrate obtained from "altitude" rabbits; 2) 11 rats received plasma filtrate from "sea level" rabbits; 3) 11 rats were injected with saline.

The plasma filtrate was obtained from "sea level" rabbits and from rabbits exposed to a simulated altitude of 22,000 feet for 24 hours in a low presure chamber. Following Borsook's method(3), the plasma was acidified to pH 5.5 and heated in boiling water for 5 minutes; the suspension was filtered and frozen until ready for assay.

The test solutions were administered by the intraperitoneal route daily for 6 days, in a dose of 4 cc/day, 2 cc in the morning and 2 cc in the afternoon. On the fifth day 0.5 cc of a solution of ferrous sulfate (about 0.5 mg of metallic iron per kg of body weight) labelled with Fe^{59} was given by mouth under light pentothal anesthesia by means of a tuberculin syringe fitted with a curved No. 16 needle. The tip of the needle was blunted and smoothed to prevent damage to the esophagus. All the recipient animals were starved for 2 days prior to injection and remained without food for 3 days after injection. The day before receiving iron and 2 days after, the rats were fed with milk to restore normal intestinal function after the starvation; this food was chosen because of its low content of iron. From this time until the end of experiment they received a normal rat diet.

Blood samples were taken by heart puncture 7 days after the rats were given the iron solution. Feces were carefully collected for 5 days to determine the unabsorbed portion of iron. The total amount of feces from each rat was ground and treated with sulfuric acid. These solutions were kept in containers of uniform geometry for 3 to 4 days, then water was added to achieve a standard 500 cc volume. Radioactivity of Fe^{59} was measured in 2 cc samples of blood and in the whole samples of the stools in a gamma scintillation counter. In determining the total amount of absorbed iron appearing in red blood cells a blood volume of 7% of body weight was estimated according to previous determinations carried out in our colony of rats.

Results. Rats that received plasma from rabbits subjected to a simulated altitude of 22,000 feet showed a higher percentage of radioiron in their blood than other rats which were injected with either plasma from "sea level" rabbits or saline (Fig. 1). There was a corresponding decrease in unabsorbed iron

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TABLE I. Influence of Plasma Filtrate from "Altitude" and "Sea Level" Rabbits on Iron Absorption in Rats.

	No. of rats	Fe^{59} appearing in blood (%)	Unabsorbed Fe^{59} in feces (%)	Reticulocytes (%)*	Hemoglobin (g %)†
Plasma filtrate of "altitude" rabbits	11	19.9 ± 2.6‡	71.1 ± 2.3‡	4.4 ± .73‡	15.1 ± .49‡
Plasma filtrate of "sea level" rabbits	11	7.1 ± 1.4	86.2 ± 1.7	1.2 ± .42	15.2 ± .16
Saline	11	5.8 ± 1.0	87.0 ± 1.8	.8 ± .11	15.3 ± .26

* Reticulocytes: Observed the day that iron was given.
† Hemoglobin: Taken at beginning of experiment.
‡ Mean ± S.E.

in feces of the "altitude" rats compared to the other 2 groups. No significant differences were found between the 2 groups of rats which received "sea level" plasma and saline.

From 1 to 14% of the administered iron did not appear in either blood or feces. This portion was partially used for other needs or went to storage organs, or may have been excreted in the urine.

The reticulocytes were measured the day that iron sulfate was given, *i.e.*, 5 days after injections. They were increased in rats given "altitude" plasma filtrate and within normal limits in rats receiving "sea level" plasma filtrate or saline (Table I).

Discussion. These data show that plasma filtrate obtained from rabbits exposed to high altitude hypoxia has a definite influence on iron absorption. However, it cannot be determined from these experiments whether the action of plasma filtrate containing erythropoietic factor acts directly on the intestine to stimulate iron absorption or whether its role is simply to increase red cell production, which in turn might control iron absorption. Observations in polycythemic mice have failed to demonstrate increased iron absorption by exogenous plasma erythropoietine, which has been interpreted as showing no direct action on iron absorption(4). Further experiments are necessary to explain how the plasma filtrate exerts its action. The possible existence of another factor different from erythropoietine cannot be excluded.

Summary. A plasma filtrate from rabbits exposed to a simulated altitude of 22,000 feet increased intestinal absorption of ferrous iron in rats compared with that in rats receiving plasma filtrate of "sea level" rabbits and rats receiving saline.

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Myocardial Oxygen Consumption as Influenced by Isotonic Contractions.* (27362)

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Although available evidence indicates that myocardial oxygen consumption may be greatly influenced by many factors(1-4), the role of isotonic contractions as a determinant of the myocardial oxygen consumption is undetermined. Sarnoff(4) and Wollenberger (5) have provided evidence from studies on

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