toxic when given orally were lethal to guinea pigs at a level of 0.5 mg/kg intravenously. Pretreatment of the animals with atropine, epinephrine or Neo-Antergan failed to prevent or modify the effect of a lethal dose of the polypeptide thus excluding liberation of histamine or acetylcholine as the principal cause of toxicity.

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Humoral Regulation of Erythropoiesis V. Relationship of Plasma Erythropoietine Level to Bone Marrow Activity. (24516)

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Erythropoietine has been demonstrated repeatedly in plasma of experimental animals following exposure to acute hypoxia and also in certain clinical disorders with severe chronic hypoxia(1). Rate of release of erythropoietine, its disappearance following discontinuance of the stimulus, and maintenance of plasma levels have not been extensively studied. Prentice and Mirand(2) noted a decrease in plasma level of erythropoietine following prolonged exposure to low O_2 concentrations at ambient pressure. Erslev(3) was unable to confirm these observations in anemic rabbits or those exposed to low O_2 concentrations. Since the conditions which influence plasma level of erythropoietine are important in the role of erythropoietine in clinical anemias, a further evaluation of those factors is here reported.

Methods and materials. Erythropoietine was assayed in fasted Sprague-Dawley rats as suggested by Fried *et al.*(4) and described elsewhere(5). Release of erythropoietine was stimulated by bleeding rats 2% of body weight or by exposing them to simulated altitude of approximately 23,000 feet (310 mm Hg) in decompression chamber. In studies on effect of chronic anemia in dogs, plasma was acidified (pH 5.5), heated 10 minutes at 90°C (internal temperature). The material was lyophilized and final preparation concentrated 5-fold to compensate for loss of activity due to heating(6) and dialyzed against isotonic phosphate buffer at pH 7.0. The effect of bone marrow function on plasma erythropoietine levels was studied in rats in which erythroid hypoplasia was produced by ionizing radiations. A dose of 400 r was delivered using a 2.5 MEV Van de Graaff generator at average dose rate of 50 r/minute measured with Bendix ionization chamber known to be air equivalent at that energy.

Results. It was possible to demonstrate erythropoietine in plasma of rats exposed for 2-3 hours at a simulated altitude of 23,000 feet. Plasma concentration of erythropoietine

TABLE I. Relationship of Plasma Erythropoietine Level to Duration of Exposure to Hypoxia.

	Altitude plasma*						
	Duration of exposure at altitude (hr)						
Normal plasma	3	6	12	18	24	36	48
5 ± .4†	11 ± 1.4	13 ± .5	17 ± 1.0		20 ± 2.0		
6 ± .9			22 ± 1.2		23 ± 1.5	16 ± 2	10 ± 1.3
6 ± 2.0				29 ± .9			12 ± 1.2

* Plasma obtained from donors following exposure at simulated altitude for varying periods.
† These values represent % of total inj. Fe^{59} present in circulating red cells 16 hr after intrav. inj. of Fe^{59}. Each figure represents mean value for group of 6 animals ± a single stand. error.

continued to rise until peak levels were observed after 12 to 24 hours of exposure (Table I). When length of exposure was prolonged to 48 hours, a substantial fall in plasma level of erythropoietine was noted (Table I). In experiments where a single bleeding of 2% of body weight was used as stimulus for erythropoietine release, a similar curve was noted, although peak values of erythropoietine were substantially less than those observed in altitude-exposed animals (Table II). Due to low peak values the decrease with time appeared relatively small, although in all 3 instances the values at 48 hours were less than those at 24 hours.

Chronic anemia with hematocrits of 20-35 was produced by multiple phlebotomies in dogs whose iron stores were maintained by parenteral administration of Imferon®. In those instances where the hematocrit was in the range of 20-28, significant levels of erythropoietine were demonstrable (Fig. 1). In dogs with higher hematocrits, however, erythropoietine could no longer be demonstrated.

The disappearance rate of erythropoietine was determined by obtaining samples of plasma from rats at various intervals after discontinuance of 16 hour exposure at simulated altitude of 23,000 feet. Results are presented in Fig. 2. The disappearance was curvilinear. An approximation of the half disappearance time was made by comparing the disappearance curve with values obtained using 25% and 50% dilutions of plasma obtained immediately after discontinuance of exposure. The half disappearance time estimated in this fashion was 3-5 hours.

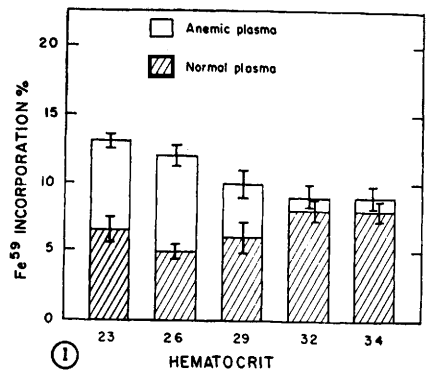
TABLE II. Relationship of Plasma Erythropoietine Level to Duration of Anemia.

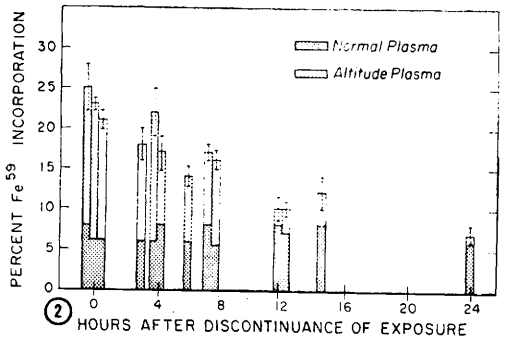
	Anemic plasma*			
Normal	Duration of anemia (hr)			
plasma	12	18	24	48
7 ± 1.7†		13 ±1	12 ± .6	10 ± .9
6 ± 1		10 ± .9	13 ± 1	9 ±1.9
10 ± 1.4	14 ± 1.7		14 ± 1.7	10 ± .9

* Plasma obtained from rats at various intervals after removal of blood equivalent to 2% of body wt.
† These values represent % of total inj. Fe^{59} present in circulating red cells 16 hr after intrav. inj. of Fe^{59}. Each figure represents mean value for group of 6 animals ± a single stand. error.

Since plasma level of erythropoietine decreased in presence of continued hypoxia, where the only apparent change was increase in amount of erythroid tissue, the relationship of bone marrow function to plasma erythropoietine was studied. Bone marrow hypoplasia was induced in rats by exposure to 400 r of ionizing radiations. At an interval of 8 hours after irradiation, the animals were exposed to simulated altitude and erythropoietine levels compared to those of simultaneously exposed non-irradiated animals. The results are given in Table III. Plasma collected from irradiated animals exposed for 48 hours increased Fe^{59} incorporation of assay animals to a level of 18-22% in contrast to the 8-12% values seen in non-irradiated altitude exposed animals.

Rate of disappearance of erythropoietine from plasma of irradiated rats when exposed to altitude was compared with that of non-irradiated altitude exposed animals. The data from these experiments are given in Fig. 3. In 2 experiments altitude-exposed animals received 400 r and in one 700 r. Clearance of erythropoietine from the plasma is somewhat slower in those animals with radiation-induced hypoplasia than in those with normal mar-





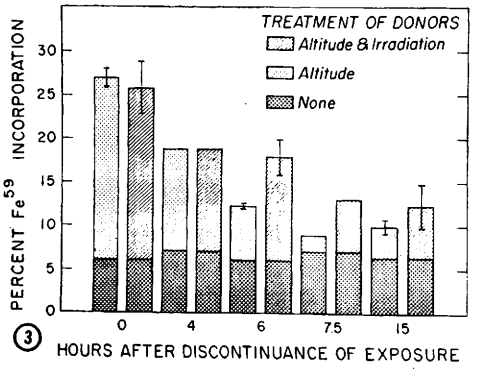


FIG. 1. Relationship of severity of anemia to plasma erythropoietine levels in the dog. Each bar represents mean 16 hr Fe^{59} incorporation of 6 animals. Small cross-bars represent a single stand. error.

FIG. 2. Disappearance of erythropoietine from plasma after discontinuance of hypoxia. Plasma was obtained from donor rats at time intervals indicated on horizontal axis, after 16 hr exposure at simulated altitude of 23,000 ft. Each bar represents mean Fe^{59} incorporation of 6 animals. Small lines or cross-bars represent a single stand. error.

FIG. 3. Disappearance of erythropoietine from plasma of irradiated and non-irradiated animals, at various time intervals following exposure to altitude. Each bar represents 1-3 experiments of 6 animals each. At those instances where more than one experiment was done the small cross-bar indicates range of mean Fe^{59} incorporations.

TABLE III. Comparison of Plasma Erythropoietine Levels in Altitude Exposed Animals with and without Radiation Induced Marrow Hypoplasia.

	Altitude plasma*			
	Irradiated		Non-irradiated	
Normal plasma	18 hr	48 hr	18 hr	48 hr
9 ± 1.2†	29 ± 1.5 29 ± 1.2 31 ± 2.7	17 ± 1.3 21 ± 1.5	30 ± 1.6	12 ± 1.1
7 ± .6	29 ± 2.3	17 ± 1.5	28 ± 1.9	8 ± 1.6
9 ± 1.2	25 ± 1.2	18 ± 2.6	28 ± 2	11 ± 1.4

* Plasma obtained from donors following exposure at simulated altitude for varying periods.
† These values represent % of total inj. Fe^{59} present in circulating red cells 16 hr after intrav. inj. of Fe^{59}. Each figure represents mean value for group of 6 animals ± a single stand. error.

rows. In all experiments average erythropoietine levels observed in irradiated animals 6-15 hours after discontinuance of exposure was greater than that of normal animals exposed to altitude.

Discussion. Data presented demonstrate that following acute exposure to hypoxia there is prompt release of erythropoietine into the plasma, peak values being achieved within 12-24 hours. In animals exposed over a longer period a fall in erythropoietine titer occurred. The data presented herein as well as that previously reported indicate that magnitude of initial response is dependent upon severity of the hypoxia.

The level at which plasma erythropoietine is maintained in chronic hypoxic states appears determined by state of bone marrow function as well as degree of hypoxia. Thus in irradiation-induced marrow hypoplasia, higher erythropoietine levels were present than in non-irradiated animals exposed to a similar degree of hypoxia. Moreover rate of removal of erythropoietine from plasma was somewhat faster in those animals with normal or hyperplastic marrows than in those with marrow hypoplasia.

The implication of these observations in considering the role of erythropoietine in clinical disorders is important. Thus one might anticipate a low plasma level of erythropoietine in mild to moderate anemias asso-

ciated with marrow hyperplasia. Conversely, high levels of erythropoietine would be expected in anemias with similar hemoglobin levels but where marrow is hypoplastic or aplastic. Such a relationship has been noted by us as well as others(1,5,7). We observed the highest erythropoietine levels in aplastic and refractory anemias, where Fe^{59} incorporation values as high as 50% have been produced in the starved assay animal. In patients with sickle cell anemia and hemoglobin levels of 7-8% with marked erythroid hyperplasia, values for Fe^{59} in assay animals were 10%.

The factor of marrow utilization could also account for the observation that erythropoietine is difficult to demonstrate in animals with phenylhydrazine induced anemia unless the hematocrit is in the range of 10-20. In Erslev's studies where erythropoietine was found in animals 48 hours after induction of anemia, hemoglobin values were less than 7(3). Similarly in our experience with chronic blood loss in dogs it was necessary to produce a substantial anemia before erythropoietine could be demonstrated. It is not readily possible to gauge degree of hypoxia produced by a given degree of anemia. However, the supposition that the graded erythropoietine response to severe degrees of anemia is mediated though production of hypoxia is compatible with previously demonstrated correlation between degrees of hypoxia and level of erythropoietine (8).

We concluded that plasma level of erythropoietine in chronic hypoxia is dependent upon severity of hypoxia together with the state of bone marrow function. Urinary excretion of erythropoietine has also been demonstrated (9,10) but the threshold for excretion remains to be determined as does the possible role of liver inactivation(2,11).

Summary. Data have been presented on rate of appearance and disappearance of erythropoietine. Plasma level of erythropoietine rises promptly in response to hypoxia but on continued exposure declines. In rats with radiation-induced hypoplasia, this fall in erythropoietine titer was modified. Accordingly, it is suggested that the marrow utilizes erythropoietine and the clinical implications are discussed.

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