EACA without interfering with fibrinogenolytic activity; ovomucoid inhibited fibrinogenolytic activity but did not appreciably inhibit FA. Hopkins, Terner and Pincus(11), by use of an activator prepared from human blood, converted plasminogen to an enzyme possessing ACTH-inactivating activity but without FA. Thus, it seems reasonable to assume that the enzyme activity of activated plasminogen might be determined by the inhibitors and/or activator employed.

EACA above .06 M concentration has been reported competitively to inhibit FA of SK-activated plasminogen, whereas, below .06 M it enhances fibrinolytic activity(12). In our experiments, the failure of EACA effectively to inhibit FA at 0.26 M (50.0 mg, Table I) could be due to the excessive amount of activator employed in the system (2000 U SK).

Summary. Ovine prolactin (0.5 mg) was incubated for 2 hours at 37.5°C with human plasminogen with and without e-aminocaproic acid (EACA). Fibrinolytic activity (FA) and prolactin-inactivating activity (PIA) were increased by streptokinase (SK). EACA at 0.026 M (5.0 mg) and 0.26 M (50.0 mg) increased FA but did not increase PIA. Antifibrinolysin (100 U) completely inhibited both activities.

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Relation Between Erythropoietin Plasma Level and Oxygen Requirements.* (28182)

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It has been postulated that red cell production is regulated by the plasma level of erythropoietin and that production of this hormone is regulated by the balance between tissue oxygen supply and demand(1,2). The titer of erythropoietin in plasma increases when oxygen supply is reduced. It has been shown that in starved(3) and in hypophysectomized(4,5) animals the oxygen requirement is reduced. Very little information is available concerning the erythropoietin production in conditions where a reduced oxygen supply is associated with a reduced oxygen need.

This is a study of the effects of hypoxia, cobalt, or anemia on the titer of erythropoietin in plasma of normal, hypertransfused, starved, or hypophysectomized rats.

Material and methods. Groups of 5 to 12 hypophysectomized, starved, or polycythemic male rats of the Sprague-Dawley strain (weighing between 170 and 220 g) were subjected to 3 known stimuli of erythropoietin production: hypoxia, cobalt, or anemia. In each experiment a group of normal animals

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was subjected at the same time to the same stimulus and their plasma assayed simultaneously for erythropoietin, except for Experiment 3 where all the control animals exposed to a simulated altitude of 24,000 feet died. Therefore the hypophysectomized rats exposed to 24,000 feet were compared to a group of normal rats submitted during the same time to 20,000 feet.

To evaluate the effect of degree of plethora on erythropoietic response, 2 different degrees of polycythemia were achieved. In one experiment rats were made polycythemic by transfusing intraperitoneally 5 ml of packed red cells (hematocrit approximately 70%) and in another experiment 5 ml of packed red cells were given on 2 consecutive days. Polycythemic rats were subjected to various erythropoietic stimuli 24 hours after the last injection. Erythropoietin production was stimulated in the starved groups after 3 days of starvation. Hypophysectomy was performed through the parapharyngeal approach and the erythropoietic stimuli were applied 7 days after surgery. Before pooling the blood samples from a group of hypophysectomized rats, the completeness of the operation was confirmed at autopsy by examination of the pituitary site under a binocular dissecting microscope and samples from incompletely hypophysectomized rats were discarded.

Animals were made hypoxic for 20 hours by an atmospheric pressure equivalent to altitude of 20,000 feet (pO_2 60 mm Hg). In one experiment a 24,000 feet (pO_2 50 mm Hg) simulated altitude was used. The rats were exsanguinated by puncture of the abdominal aorta immediately upon being taken from the low pressure chamber.

According to Goldwasser *et al.*(6) 25 μM of $Co\ Cl_2$ + Co^{60} per 100 g body weight were injected subcutaneously. The rats were bled 12 hours later, and plasma radioactivity was counted to determine the cobalt plasma level.

Anemia was induced by bleeding the rats from tail vein puncture (2% of body weight) 10 minutes after intraperitoneal injection of 100 units of heparin. Five ml of saline were injected intraperitoneally immediately after bleeding. Blood was withdrawn for the erythropoietin assay 24 hours after bleeding. Heparinized plasma samples were kept frozen until assayed.

Plasma level of erythropoietin was determined by the polycythemic mouse assay method recently described by DeGowin *et al.* (7). Six mice were used for each assay. Virgin female Swiss mice (20-25 g) were transfused intraperitoneally with 1 ml packed red cells on 2 consecutive days. Five days after the second transfusion, 1 ml of the plasma to be tested was injected subcutaneously; 2 days later 0.5 μc $Fe^{59}Cl_3$ was injected intravenously *via* the tail vein and 3 days later blood was collected by heart puncture and counted in a scintillation counter. The radioactive iron uptake into red cells was calculated, assuming a blood volume of 7% of the body weight. Hematocrits were determined by a micromethod. The metabolic rate of the starved rats was determined after a starvation period of 3 days with a closed circuit respiration apparatus as described by Kleiber(8).‡

Results are summarized in Tables I and II.

1. *Effects of hypophysectomy on erythropoietin response.* In all experiments the hematocrits of hypophysectomized rats were higher than those of normal rats. This post operative rise previously observed by others (9,10) is probably due to hemoconcentration.

a) Hypoxia. The hypophysectomized rats submitted to a simulated altitude of 20,000 feet had no detectable erythropoietin in their plasma (Exp. 1 and 2). However, after a more severe stimulation (24,000 feet, Exp. 3) a good response was obtained: 8% Fe^{59} uptake into the red cells of recipient mice.

b) Cobalt. As shown in Table I (Exp. 5), the erythropoietic response of the injected mice could not be attributed to the small amount of cobalt present in the injected plasma (0.10 to 0.26 μM/ml). Response to cobalt was not appreciably affected by hypophysectomy as shown in Exp. 1 and 2. Fe^{59} uptake in the recipient animals was 16.5% and 25.1% as compared to the values obtained in plasma of normal rats injected with

‡ Dr. E. S. Evans, Dept. of Anatomy, Univ. of California, Berkeley, kindly performed the metabolic rate determinations and provided hypophysectomized rats.

TABLE I. Effects of Hypophysectomy, Starvation or Polycythemia on Erythropoietin Response To Hypoxia, Cobalt and Anemia.

Exp	Plasma assayed from:	Rats' Het. %	Fe^{59} uptake in recipient mice, %	Cobalt in assayed plasma, μM/ml
1	Normal rats at 20,000 feet	39.8	12.6 ± 2.9*	
	Hypophysectomized rats at 20,000 feet	48.9	1.8 ± 2.4	
	Normal rats inj with Co^{++}	39.7	19.7 ± 5.1	.14
	Hypophysectomized rats inj with Co^{++}	48.5	16.5 ± 4.1	.26
		Saline:	1.8 ± 1.7	
2	Normal rats at 20,000 feet	41.7	28.8 ± 7.2	
	Hypophysectomized rats at 20,000 feet	50.0	1.4 ± .9	
	Normal rats inj with Co^{++}	39.6	30.2 ± 5.8	.11
	Hypophysectomized rats inj with Co^{++}	43.0	25.1 ± 8.9	.20
	Normal rats bled	24.0	14.1 ± 6.4	
	Hypophysectomized rats bled	30.6	4.9 ± 1.6	
		Saline:	0.54 ± .20	
3	Normal rats at 20,000 feet	43.4	18.5 ± 8.5	
	Hypophysectomized rats at 24,000 feet	48.3	8.0 ± 4.3	
		Saline:	0.85 ± .90	
4	Normal rats at 20,000 feet	43.6	24.7 ±12.9	
	Starved rats at 20,000 feet	47.0	8.1 ± 3.6	
	Polycythemic rats at 20,000 feet	51.4	22.8 ± 9.2	
	Normal rats inj with Co^{++}	37.7	23.3 ± 8.0	
	Starved rats inj with Co^{++}	45.0	29.4 ± 3.4	
	Polycythemic rats inj with Co^{++}	50.8	11.3 ± 7.5	
		Saline:	0.35 ± .49	
5	Normal rats at 20,000 feet	36.6	37.2 ± 9.8	
	Starved rats at 20,000 feet	44.6	10.7 ± 4.1	
	Polycythemic rats at 20,000 feet	62.7	2.5 ± 2.0	
	Normal rats inj with Co^{++}	35.8	28.0 ± 9.3	.10
	Starved rats inj with Co^{++}	43.5	22.0 ±12.6	.15
	Polycythemic rats inj with Co^{++}	53.3	18.9 ± 5.5	.10
	Normal rats bled	25.5	10.0 ± 6.3	
	Starved rats bled	28.3	9.9 ± 2.5	
	Normal rat plasma + 30 μM Co/ml	36.0	1.0 ± .9	.30
		Saline:	1.3 ± 2.1	

* Standard deviation.

cobalt, 19.7% and 30.2%.

c) Anemia. Plasma of hypophysectomized anemic rats induced an iron uptake of 4.9% in recipient mice, whereas plasma from normal anemic rats induced an uptake of 10% and 14.1%.

2. *(Exp. 4 and 5). Effects of starvation on erythropoietin response to:* a) Hypoxia. The mean radioactive iron incorporation in RBC of mice injected with plasma of starved hypoxic rats was 8.1% and 10.7%, whereas results after injection of plasma of normal hypoxic rats averaged 24.7% and 37.2%.

b) Cobalt. Response to cobalt was not affected by starvation. In Exp. 4 the uptake was 29.4% after injection of plasma from starved rats and 23.3% after injection of plasma from normal rats. In Exp. 5 values were respectively 22.0% and 28%.

c) Anemia. This stimulus was as effectual in the starved animals as in the fed ones; Fe^{59} uptake was 10.0% with "normal anemic" plasma and 9.9% with "starved anemic" plasma.

3. *(Exp. 4 and 5). Effects of polycythemia*

TABLE II. Comparison of Oxygen Consumption Of Starved and Fasted Young Male Sprague-Dawley Rats.

Group	No. of rats	Oxygen consumption, l/m²/hr	Metabolic rate,† cal/m²/hr
Starved 3 days	7	6.67 ± .56*	31.3 ± 2.0 (-31)
Fasted 4 hr	7	9.39 ± .58	45.1 ± 2.8

* Standard deviation.

† An R. Q. of 0.71 was assumed for starved rats and an R. Q. of 0.80 for fasted rats. Corresponding caloric equivalents are 4.69 and 4.80, respectively.

on erythropoietin response to: a) Hypoxia. A slight degree of polycythemia had no effect on erythropoietin response to hypoxia; plasma of hypoxic rats whose hematocrit was 51% induced an iron uptake of 22.8% into red cells (Exp. 4). A more marked polycythemia (hematocrit 63% against 37% for the control group) inhibited the response to hypoxia, Fe^{59} uptake 2.5% (Exp. 5).

b) Cobalt. The plasma of polycythemic rats injected with cobalt was also less active than that of normal rats. Uptake into red cells of recipient mice averaged 11.3% in Exp. 4 and 19.0% in Exp. 5, while values obtained after injection of "normal cobalt" plasma were respectively 23.3 and 28%.

Discussion. These results show that hypophysectomy and starvation reduce erythropoietin response to hypoxia. This is compatible with the hypothesis that erythropoietin production is related to the proportion of oxygen supply to demand. There seems to be a correlation between the depression of the metabolism and reduction of the response to hypoxia. When the oxygen requirement was mildly reduced by starvation to 70% of the normal (Table II), erythropoietin titer was still increased by hypoxia. When a more pronounced depression of metabolism was induced by hypophysectomy(11), no appreciable response was obtained after using the same degree of hypoxia (20,000 feet). When a more severe hypoxic stimulus was applied to hypophysectomized rats (24,000 feet instead of 20,000) a measurable increase in erythropoietin titer occurred: 8% Fe^{59} uptake into red cells of the recipient mice. These results are not unexpected in view of results reported previously that exposure of hypophysectomized rats to 16,000 feet was not followed by increase of the red cell mass, although such an increase was obtained after exposure to higher altitude(12,13). Jacobson and co-workers(1) have pointed out that rats with reduced metabolism and a normal red cell mass are comparable to polycythemic animals. If this view is correct, polycythemic rats with normal oxygen requirement will respond poorly to hypoxia. In agreement with this hypothesis, a reduced erythropoietin response was found in these conditions, at least when the degree of polycythemia was significant. It has been suggested that an erythropoietin inhibitor appears in the plasma of polycythemic sheep(14). The presence of such an antagonist factor in the plasma of polycythemic rodents is very unlikely since polycythemic rats are as sensitive to erythropoietin as starved rats(15). Moreover it is well established that the polycythemic mouse is the most sensitive preparation used for erythropoietin assay(7).

As Crafts and co-workers(16) and Jacobson and co-workers(1) have shown, an increase in erythropoietin titer can be obtained by bleeding hypophysectomized rats. Starved or hypophysectomized rats responded better to anemia than to hypoxia. Starved rats, made anemic, disclosed the same plasma erythropoietin titer as did well fed anemic rats. After hypophysectomy, anemia induced a higher titer than hypoxia (20,000 feet) despite the fact that normal rats exhibited a lower response to anemia than to hypoxia. In the absence of knowledge concerning the intimate mechanism of erythropoietin production, it is difficult to explain why the hypophysectomized rats are more sensitive to anemia than to hypoxia.

There is experimental evidence that cobalt is a toxic agent that interferes with oxidative enzymes(17,18). It seems that the stimulating action of cobalt, like the action of hypoxia, is related to the proportion of oxygen demand to supply. Erythropoietin response to cobalt was reduced in polycythemic or hypophysectomized rats. Nevertheless, as has been shown by McCarthy *et al.*, erythropoietin response to cobalt was not affected by starvation(19) and only to a small extent by hypophysectomy, but it must be pointed out that the cobalt plasma level was higher in these animals than in the other groups (Table I), probably because the urinary excretion of cobalt was reduced in these conditions. For this reason these rats have been probably submitted to a more potent stimulation by cobalt than rats from other groups. This could explain why the hypophysectomized or starved rats responded better to cobalt than to hypoxia despite their low oxygen requirement.

Summary. The level of erythropoietin has

been measured in the plasma of starved or hypophysectomized rats with lowered oxygen requirement after stimulation by hypoxia, cobalt, or anemia. Also, polycythemic rats have been submitted to hypoxia or cobalt. A reduced erythropoietin response was observed in hypophysectomized and starved rats after stimulation by hypoxia. The diminished response was not so striking after stimulation by cobalt, probably because the concentration of the cobalt in the plasma was higher in these groups. Response to cobalt and hypoxia was also reduced in polycythemic rats.

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Multiple Congenital Abnormalities in the Rat Resulting from Acute Maternal Niacin Deficiency During Pregnancy.* (28183)

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Deficiencies of riboflavin, pantothenic acid, or pteroylglutamic acid, induced by antimetabolites of these vitamins during pregnancy, result in multiple congenital malformations in the offspring(1,2). This study reports the effects of a purified niacin-deficient diet containing the niacinamide antimetabolite, 6-aminonicotinamide (6-AN), on embryonic development. As niacinamide can be synthesized from dietary tryptophan(3,4), deletion of the vitamin from the diet may not affect significantly embryonic development in rats and certain other species(5,6,7).

Methods and materials. Rats (Long-Evans) averaging 100 days of age and 225 g in weight were bred with normal males. All rats were given a stock diet of natural food stuffs‡ and distilled water for drinking; lettuce was furnished twice weekly. Rats were weighed regularly and vaginal smears were examined daily. The morning of finding spermatozoa in the vaginal smear was considered day zero of pregnancy. Rats were transferred to individual cages and given a purified niacin-deficient diet containing 100 mg of 6-AN/kg of diet on days 7-9, 8-10, 9-11 or 10-13 of pregnancy. The animals were then trans-

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‡ Ground whole wheat 67.5%, casein 15%, skim milk powder 7.5%, sodium chloride 0.75%, calcium carbonate 1.5%, hydrogenated vegetable oil 6.75%, 1.0% fish oil (vit. A and D concentrate) and sufficient KI to furnish 0.9 μg iodine/g of diet.