ance indicating that this drug has no effect on non-immune clearance of foreign proteins.

It is evident that foreign gamma globulins or albumins are cleared more rapidly from the blood during the exponential phase than are homologous gamma globulins or albumins. The reasons for this phenomenon are not clear; it can be stated rather definitely that this second or catabolic phase of antigen disappearance is non-immunologic in the sense that antibodies are not found at this time. At least 2 possibilities explain these findings: (1) the same enzyme systems responsible for breakdown of homologous protein also catabolize heterologous proteins, the different rates being due to substrate differences. (2) Different enzyme systems acting at different rates catabolize homologous and heterologous proteins, even though they are of the same molecular species. These possibilities are now under study.

Summary. Rates of elimination of iodinated heterologous and homologous albumins and globulins were studied in normal and 6-mercaptopurine treated animals. The half-life of exponential phase of antigen elimination was the same in normal and in 6-mercaptopurine treated animals. These results were similar to those obtained by others in X-irradiated and tolerant rabbits and support the view that the exponential phase of antigen elimination is not due to antibody formation.

1. Talmage, D. W., Dixon, F. J., Bukantz, S. C., Dammin, G. J., *J. Immunol.*, 1951, v67, 243.

2. Benacerraff, B., Sebestyen, M., Cooper, N. S., *ibid.*, 1959, v82, 131.

3. Cinader, B., Pearce, J. H., *Brit. J. Exp. Path.*, 1958, v39, 8.

4. Schwartz, R., Eisner, A., Dameshek, W., *J. Clin. Invest.*, 1959, v38, 1394.

5. Weigle, W. O., Dixon, F. J., *J. Immunol.*, 1959, v82, 516.

6. Mascuredis, S. P., Editor, *Ann. N. Y. Acad. Sci.*, 1957, v70, 1.

7. Weigle, W. O., Dixon, F. J., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 213.

Received February 23, 1960. P.S.E.B.M., 1960, v104.

Erythropoietin and Known Erythropoietic Stimuli.* (25789)

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It has been adequately demonstrated that hemorrhagic anemia, hypoxia, or administration of cobalt will stimulate erythropoiesis(1). Carnot(2) originally suggested the presence of an erythropoietic stimulating agent, erythropoietin, in plasma of anemic rabbits, and subsequent work has confirmed its existence (3). It was of interest to determine the effect of this erythropoietic factor derived from the plasma of anemic rabbits on erythropoietic response induced by hemorrhage, hypoxia, or chronic cobalt administration.

Materials and methods. Male and female New Zealand rabbits were made anemic by repeated injections of phenylhydrazine hydrochloride(4). Erythropoietically active plasma obtained from the anemic rabbits was boiled, filtered as described(5) and frozen until used. Male rats weighing 140 to 160 g were bled 2% of body weight by cardiac puncture under light ether anesthesia. Immediately following hemorrhage, the rats were injected intraperitoneally with an equivalent volume of erythropoietically active boiled anemic rabbit plasma or isotonic saline. Each day thereafter, 1 ml of the respective materials was administered by subcutaneous injection. Blood samples were obtained from the tail at 1, 2, 3, 4, 5, 7, and 11 days after hemorrhage for various determinations. Rats weighing 140 to 160 g at start of experiment were exposed to reduced barometric pressure (310 mm Hg) for approximately 8 hours a day, 5

* Presented at Fed. Meeting, April 1957.

TABLE I. Effect of Injections of Boiled Anemic Rabbit Plasma Filtrate or Saline on Blood Values in Hemorrhaged Rats.

Injection	Control Ret	Control Hct	Day-1 Ret	Day-1 Hct	Day-2 Ret	Day-2 Hct	Day-3 Ret	Day-3 Hct	Day-4 Ret	Day-4 Hct	Day-5 Ret	Day-5 Hct	Day-7 Hct, %	Day-11 Hct, %
	%													
Saline	2.1	44.3	11.5	32.8	7.8	31.9	12.7	32.8	16.4	34.6	16.8	35.5	40.6	44.3
Boiled filtrate	2.4	44.7	13.1	31.8	8.7	32.6	10.9	33.9	15.7	35.5	13.6	35.4	40.4	45.6

Each figure represents avg of 6-8 rats.

to 6 days a week for 4 weeks. When not in the low pressure chamber, animals were maintained at room conditions which approximated sea level pressure. One group of hypoxic rats was given 1 ml boiled rabbit plasma daily by subcutaneous injection during the 4 weeks in the low pressure chamber and for 3 weeks after removal from the chamber. The other group of hypoxic rats received 1 ml isotonic saline. Two groups of control rats for the hypoxic groups were similarly injected, but were maintained at room pressures at all times. All groups were injected for 7 weeks. Blood samples were obtained at weekly intervals. Rats were fed a diet of ground rat pellets with 0.04% $CoCl_2 \cdot 6H_2O$ added to the diet. One group received 1 ml boiled anemic rabbit plasma daily by the subcutaneous route. The other group received 1 ml isotonic saline. Two groups of rats without cobalt in the diet were similarly injected with boiled anemic rabbit plasma or saline. Blood samples were obtained at weekly intervals for reticulocyte and hematocrit percentage determinations.

Results. Standard hemorrhage. One day following hemorrhage, the hematocrit per cent of both groups decreased to minimum values (Table I). Increased erythropoiesis occurred during the first day as indicated by the increase in circulating reticulocytes, although no change in hematocrit per cent was evident until the third to fourth day after hemorrhage. A rapid increase in hematocrit per cent took place after the fifth day. By the eleventh day after hemorrhage, normal values were obtained. There appeared to be no difference in rate of recovery from a standard hemorrhage whether the animals received injection of boiled anemic rabbit plasma or isotonic saline.

Hypoxia. Exposure of rats to decreased oxygen tensions induced a marked increase in hematocrit percentages and a moderate increase in circulating reticulocytes (Table II). A plateau effect was encountered after a 2 week exposure and continued until the hypoxic stimulus was removed. During the period of increasing hematocrit percentage, there was no difference in response between the boiled anemic rabbit plasma and saline injected groups. However, animals maintained at room pressure and injected with boiled anemic rabbit plasma showed a statistically significant increase in hematocrit over those injected with saline. After the hypoxic stimu-

TABLE II. Effect of Injections of Boiled Anemic Rabbit Plasma Filtrate or Saline on Blood Values in Rats Rendered Discontinuously Hypoxic in a Low Pressure Chamber (LPC).

Treatment	Room pressure Control Ret	Room pressure Control Hct	310 mm Hg 1 wk Ret	310 mm Hg 1 wk Hct	310 mm Hg 2 wk Ret	310 mm Hg 2 wk Hct	310 mm Hg 3 wk Ret	310 mm Hg 3 wk Hct	310 mm Hg 4 wk Ret	310 mm Hg 4 wk Hct	Room pressure 5 wk Hct	Room pressure 6 wk Hct	Room pressure 7 wk Hct
	%												
Saline LPC	1.6	44.5	3.4	51.6*	1.6	61.0*	4.8	60.3*	4.8	61.6*	51.6*	46.0	41.8
Boiled filtrate LPC	1.3	45.5	4.5	53.4*	2.6	60.5*	5.9	61.0*	6.0	59.2*	50.8*	51.2*	52.1*
Saline	1.6	44.0	2.4	44.0	4.0	44.0	3.0	41.0	2.5	44.0	43.9	45.2	44.1
Boiled filtrate	1.6	43.6	2.2	47.6*	2.4	47.3*	2.0	48.3*	2.9	50.0*	49.4*	47.2†	50.3*

Each figure represents avg of 5-6 rats.

* Significant difference from saline controls, 1% level.

† *Idem*, 5% ".

TABLE III. Effect of Injections of Boiled Anemic Rabbit Plasma Filtrate or Saline on Blood Values in Rats Fed Cobalt.

Treatment	Initial	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk
	Hct %							
Cobalt and saline	44.4	44.8	45.7	50.8*	53.6*	53.5*	55.3*	56.0*
Cobalt and boiled filtrate	44.8	48.0*‡	50.5*‡	53.5*	52.3*	59.4*	57.0*	62.2*‡
Saline	44.0	44.0	44.0	41.0	44.0	43.9	45.2	44.1
Boiled filtrate	43.6	47.6*	47.3*	48.3*	50.0*	49.4*	47.2†	50.3†

Each figure represents avg of 5-7 rats.
* Significant difference from saline control at 1% level.
† *Idem* 5% " .
‡ " cobalt and saline group at 1% level.

lus was removed, hematocrit values of the saline injected group returned to normal. On the other hand, after return to sea level pressure the hematocrit percentages of rats which received boiled anemic plasma remained above those of the saline injected controls.

Cobalt feeding. Feeding 0.01% cobalt produced a polycythemic response in normal rats as indicated by an increase in hematocrit value (Table III). When boiled anemic rabbit plasma was administered in conjunction with the cobalt feeding an additive effect was observed.

Discussion. Boiled anemic rabbit plasma filtrate containing erythropoietin as well as anemia, hypoxia and cobalt feeding stimulate erythropoiesis. However, when prolonged erythropoietin administration is combined with either anemia or hypoxia, no additional effect is noted. Possibly the endogenous erythropoietin produced by hemorrhage or hypoxia exceeds and masks the effect of the relatively small quantity of exogenous and possibly partially denatured material injected.

The mode of action of cobalt in producing polycythemia is unknown. Unlike its effect in anemia or hypoxia, erythropoietin in boiled filtrate has an additive action in cobalt polycythemia which is evident in the early stages of combined stimulation. This result may be explained by assuming that the cobalt stimulus is suboptimal or that a different mechanism is activated by cobalt.

Summary. 1. Repeated administration of erythropoietin fails to increase the erythropoietic response to hemorrhage or hypoxia, measured by hematocrit or reticulocyte values. 2. Polycythemia is greater after simultaneous administration of cobalt and erythropoietin than after cobalt feeding alone.

1. Grant, W. C., Root, W. S., *Physiol. Rev.*, 1952, v32, 449.
2. Carnot, P., DeFlandre, C., *Compt. Rend. Acad. Sci.*, 1906, v143, 384.
3. Gordon, A. S., *Physiol. Rev.*, 1959, v39, 1.
4. Linkenheimer, W. H., Grant, W. C., Berger, H., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 225.
5. Borsook, H., Graybiel, G., Keighley, G., Windsor, E., *Blood*, 1954, v9, 734.

Received February 26, 1960. P.S.E.B.M., 1960, v104.

Localization of Fluorescent Antibodies to Human Growth Hormone in Human Anterior Pituitary Glands.* (25790)

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The antigenicity of human growth hormone (H.G.H.) has now been clearly established (1,2,3,4). With the advent of more highly purified preparations of H.G.H. the specificity of the corresponding antisera has been supported by studies using hemagglutination-inhibition(1,2,4) and agar gel-diffusion(3) and biological inhibition technics(3). Using