

A Simple Test for Erythropoietin in Deproteinized Plasma Extract.* (22791)

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The effectiveness of a deproteinized plasma extract from rabbits made anemic with phenylhydrazine in producing erythropoietic stimulation has been questioned by several investigators(1,2). Others(3,4,5,6) have confirmed its erythropoietic stimulating properties by a variety of technics. Most recent publications on the subject have shown little rise in Hb or hematocrit in animals receiving the material but have demonstrated statistically significant rises in total red cell volume, red blood count, and nucleated red cells within the marrow. The above technics are cumbersome in that they require long-term injections of recipient animals (2-6 weeks) and require large amounts of extract. Moreover the results, though statistically significant, are not clear cut.

The following method enables a quick test of plasma from an anemic source, requires only small amounts of plasma and enables cross-species examination of the erythropoietic factor in the plasma. Also, utilizing uptake of Fe^{59} into red cells as the criterion for erythropoietic stimulation, the method requires only 3 or 4 days of subcutaneous injections of the deproteinized plasma extract and produces a clear cut differential in Fe^{59} uptake between control and experimental groups of animals. The relative increase of Fe^{59} uptake into the red cell mass of experimental groups as compared with normal controls was considerably greater in hypophysectomized rats than in normal rats.

Materials and methods. a) Young adult Sprague-Dawley rats, aged 2-3 months and ranging in weight from 250-400 g were employed in the experiments utilizing normal

animals. The hypophysectomized rats[†] were of the same age group and had been hypophysectomized for at least 40 days to insure a stable post-hypophysectomized state. They were maintained on a diet consisting of Purina laboratory chow, and bread and milk. b) White New Zealand rabbits were given 1 cc of 1% phenylhydrazine subcutaneously daily until Hb was less than 5 g. They were then bled and the plasma prepared as described previously(5). Control plasma was obtained from normal non-treated rabbits. c) The deproteinized plasma extract was administered in two ways: 1) In unconcentrated form, the extract being diluted to the original volume of plasma. 2) In concentrated form where the extract was diluted to only $\frac{1}{3}$ the original volume of plasma. d) The normal rats received 5 cc of deproteinized extract (concentrated or unconcentrated, anemic or normal) for 4 successive days subcutaneously. One hour after the last subcutaneous injection of plasma approximately $1 \mu\text{c}$ Fe^{59} in 1 cc saline was given intravenously and 24 hr thereafter the animal was bled from the aorta. The radioactivity in the sample was measured in a Nancy Wood well-type scintillation counter. The radioactivity in an aliquot of the original Fe^{59} solution given each animal was similarly measured. Calculation of Fe^{59} uptake into red cells was carried out as previously described(7). Precaution was taken after each experiment to run hematocrits. Any animal with low hematocrit was not included in our results since animals with low hematocrits had tended to run higher iron uptakes. The hypophysectomized rats received 2 cc of plasma extract daily (concentrated or unconcentrated, anemic or normal) for 3 successive days subcutaneously. One hour after the last injection of plasma, $1 \mu\text{c}$ of Fe^{59} was given intravenously as in the normals and 48 hours thereafter they were bled.

* The authors express their thanks to Andre Bulba, Ruth Deckert, Robert Hamilton, Edward Dywinski for their technical assistance. This investigation was supported in part by research grants from National Cancer Institute, U.S.P.H.S. and the Atomic Energy Commission.

[†] Hormone Assay Laboratories, Inc., Chicago, Ill.

TABLE I. Normal Rats Given Deproteinized Plasma Extract Subcutaneously from Anemic and Normal Rabbits for 4 Consecutive Days.

Weekly groups*	% uptake of Fe ⁵⁹ in RBC, 24 hr after last inj. of plasma			
	#1	#2	#3	#4
<i>Normal plasma</i>				
Concentrated		35.9	54.2	35.6
		28.2	56.2	41.4
		24.3	50.2	56.9
		32.9	56.3	44.8
		27.5	49.7	29.5
		36.1	50.0	47.6
Avg %		30.8	52.7	41.6
Regular	58.1		57.1	51.5
	59.8		56.3	43.5
	39.0		47.7	57.1
	61.9		49.1	61.3
	65.6	—	29.8	51.6
	59.5		53.1	46.7
	52.1			55.6
	54.8			
	61.4			
	50.5			
Avg %	57.7		48.9	51.9
<i>Anemic plasma</i>				
Concentrated		47.8	80.6	69.7
		24.9	91.6	74.1
		40.0	88.7	78.1
	—	47.8	88.2	69.7
		41.8	77.2	64.1
		45.5	70.7	93.1
Avg %		41.3	87.3	73.3
Regular	69.6		84.4	55.8
	83.4		71.8	61.8
	62.5		81.0	40.2
	69.6		88.5	67.7
	67.0	—	92.6	60.4
	74.2		79.6	53.4
	69.6			53.0
	71.1			
	51.4			
	63.4			
Avg %	67.1		83.0	56.6

* Weekly groups #1, 2, 3, 4, 5 represent separate experiments performed on five consecutive weeks. Separately prepared extract was administered on each successive week.

Calculation of Fe⁵⁹ uptake into red cells was carried out in the same way as in the normal rats.

Results. The results are shown in Tables I and II. The normal rats showed considerable fluctuation in uptake values from week to week. However, those receiving anemic

plasma extract were consistently higher than those receiving normal extract. It should be noted that the groups receiving concentrated anemic extract in all instances had higher average uptake values than the groups receiving unconcentrated anemic material. Also, the concentrated anemic extract was more consistently effective than the unconcentrated material in producing a greater relative response in experimental groups as compared with normal controls. We are unable to explain the wide week to week fluctuation in Fe⁵⁹ uptake in these normal animals.

The hypophysectomized animals showed very distinct differences between the groups receiving normal plasma extract and those re-

TABLE II. Hypophysectomized Rats Receiving Regular and Concentrated Deproteinized Plasma Extract from Normal and Anemic (Phenylhydrazine) Rabbits Subcutaneously for 3 Consecutive Days.

Weekly groups*	% uptake of Fe ⁵⁹ in RBC, 48 hr after last inj. of plasma		
	#1	#2	#3
<i>Normal plasma</i>			
Concentrated	.9	1.0	1.5
	1.0	1.2	1.9
	15.4	1.3	9.3
	2.2	4.4	3.0
	7.8	5.2	6.8
Avg %	5.5	2.6	4.5
Regular	3.3	3.6	5.2
	7.8	3.6	1.1
	3.6	6.0	1.3
	1.0	1.7	1.6
Avg %	3.9	4.7	2.3
<i>Anemic plasma</i>			
Concentrated	28.8	26.9	27.6
	45.9	26.8	27.2
	22.0	27.1	27.5
	49.3	21.7	82.0
	26.8	22.9	47.2
		24.6	
Avg %	34.6	25.0	42.3
Regular	18.3	22.7	25.2
	7.5	17.8	21.7
	6.6	14.5	12.6
	12.7	25.8	
	11.7	23.9	
Avg %	11.4	23.5	19.8

* Weekly groups #1, 2, 3 represent separate experiments performed on 3 consecutive weeks. Separately prepared extract was administered on each successive week.

ceiving anemic plasma extract. These differences were considerably more marked than those seen with the normal rats. Groups receiving concentrated anemic plasma had 6 to 9 fold increases in Fe^{59} uptake as compared with those receiving concentrated normal plasma. Likewise, groups receiving unconcentrated plasma had 3 to 8 fold increases as compared with those receiving normal plasma. Again the concentrated material was somewhat more effective than the unconcentrated in stimulating increased Fe^{59} uptake in recipient animals.

Discussion. The above data demonstrate conclusively as judged by Fe^{59} uptake into RBC, the erythropoietic stimulating property of a deproteinized plasma extract from anemic rabbits. Our findings also demonstrate the sensitivity of the hypophysectomized rat as a test preparation for demonstrating the presence of an erythropoietic factor in an extract particularly when incorporation of Fe^{59} into red cells is used as the index of red cell production.

The importance of the deproteinized character of this material is obvious. It enables the testing of erythropoietic activity of plasma from one species into another. More particularly, testing of human plasma from anemia of various sources with regard to its erythropoietic stimulating properties may be possible.

It is of interest that those investigators who have found the deproteinized material inac-

tive have given it intravenously (1,2).[†] Those, on the other hand, who have given it subcutaneously have found it to be effective (3,4,5,6).

The present results with deproteinized plasma extract from rabbits made anemic with phenylhydrazine demonstrated comparable levels of stimulation to those reported by Freid *et al.* (8) using whole plasma from anemic rats given intravenously to hypophysectomized rats. The criterion of stimulation was the same being the uptake of Fe^{59} in red cell mass.

Summary and conclusions. A simple test for erythropoietin in deproteinized plasma extract is described. A total of 3 or 4 days is required for subcutaneous injection of the plasma extract and only one intravenous injection of Fe^{59} is necessary. When concentrated plasma extracts are given to hypophysectomized rats, 6 to 9 fold differences in response between anemic and control groups are achieved. The deproteinized nature of the material enables cross-species examination of the erythropoietic factor.

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[†] Recently, in our laboratory, hypophysectomized rats receiving intravenous injections of deproteinized rabbit anemic plasma extract (regular or concentrated) manifested a definite erythropoietic stimulation of a similar magnitude to those receiving this material subcutaneously.