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Response of the Polycythemic Mouse and Polycythemic Guinea Pig To Human Erythropoietin.* (29409)

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The inconsistent erythropoietic response of guinea pigs to injections of erythropoietin extracts from other species has been described. Gordon(1) found an erythropoietic response in normal guinea pigs following injections of extracts of urine from a child with Thalassemia, but the guinea pigs did not respond to extracts from other sources which had been shown to stimulate erythropoiesis in rats. Guinea pigs have failed to respond to plasma from anemic ponies(2), erythropoietically active human urine extract(3), and erythropoietically active rabbit plasma filtrate(4). It has been reported, however, that erythroblasts in hanging drops of bone marrow cultures from guinea pigs or rats increase in number when serum or filtrates of boiled plasma from anemic patients or guinea pigs are added to the cultures(5). Recently, Schooley and Garcia(6) reported that an extract of human urine which produced an erythropoietic response in rats also stimulated erythropoiesis in hypertransfused guinea pigs.

Because of the paucity of information on the response of the guinea pig to erythropoietin from other species, the activity of an extract of urine from a patient with hypoplastic anemia was compared in polycythemic mice and polycythemic guinea pigs.

Materials and methods. 1. *Extraction of erythropoietically active human urine.* The human urine extract used in these experiments was obtained from a patient with hypoplastic anemia induced by chloramphenicol whose hemoglobin level was 5 g %. Urine extraction was performed by a modified method of Gordon(7). His procedure was followed up to the final step where, instead of lyophilization, 5 volumes of acetone at 0°C were added to each volume of the clear supernatant, and the mixture was allowed to stand overnight at 5°C. The precipitate which appeared was collected and washed with ether, evaporated to dryness *in vacuo* in the cold, and stored in a desiccator at 5°C until used. The final extract was light tan in color.

2. *Measurement of incorporation of Fe59 into erythrocytes of hypertransfused guinea pigs.* Inbred guinea pigs receiving a standard guinea pig laboratory diet with supplementary carrots and weighing 225 to 275 g were transfused intraperitoneally on 2 consecutive days with 5 ml of 80% suspension of washed homologous red cells in normal saline. Endogenous erythropoietin production was suppressed in these animals by the fifth day following the last transfusion. At this time, 50 mg of urine extract dissolved in 1 ml of normal saline were injected subcutaneously

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TABLE I. Effect of Injection of 50 mg Urine Extract from a Patient with Hypoplastic Anemia upon Fe59 Incorporation into Erythrocytes of Hypertransfused Guinea Pigs.*

Interval between inj of urine extract and of Fe59 C13 (hr)	Injected with extract†			Controls			P
	No. animals	Hct. %	% Fe59 incorp.	No. animals	Hct. %	% Fe59 incorp.	
24	3	52.7 ± 1.45	8.05 ± .41	5	53.2 ± 1.07	2.68 ± .28	<.01‡
48	5	53.2 ± 1.16	11.56 ±1.81				<.01‡
72	5	53.8 ± 2.21	8.82 ±1.51	3	53.2 ± 1.2	6.72 ±1.22	>.1

* All values given are mean ± standard error of mean (S.E.).
† Avg dose = 18 mg urine extract/100 g body wt.
‡ Values for control animals at 24 and 48 hr were combined since there was no significant difference.

into the hypertransfused guinea pigs (average dose 18 mg of extract per 100 g body weight) and another group of these hypertransfused guinea pigs receiving 1 ml of normal saline subcutaneously served as controls.

The test animals were divided into 3 identical groups for administration of Fe59 C13. Two μc of Fe59 C13 in 0.1 ml of normal saline were administered by cardiac puncture 24 hours after injection of the urine extract to the first group, after 48 hours to the second group and after 72 hours to the third group. Saline injected controls were similarly injected with Fe59 C13 with each experimental group. Forty-eight hours after injection of Fe59 C13, the animals were sacrificed by exsanguination and a one milliliter aliquot of blood was counted in a well-type scintillation counter. Duplicate microhematocrits were obtained in heparinized capillary tubes. At autopsy animals with evidence of excessive pericardial or mediastinal bleeding were discarded. The Fe59 red cell utilization was calculated by:

$$\frac{\text{CPM/ml sample} \times .07 \times \text{body wt (g)} \times 100}{\text{CPM† injected}}.$$

Calculations were based on the assumption that total blood volume equals 7.0% of body weight. Ancill(8) reported that the blood volume of the normal guinea pig is equal to 7.2% of the body weight. Since our guinea pigs were hypertransfused this assumed total blood volume is probably lower than the true blood volume and the results obtained in our experiments can represent only an estimate of Fe59 RBC incorporation.

† CPM—counts per minute above background.

3. *Measurement of Fe59 incorporation into erythrocytes of polycythemic mice.* Male Swiss white mice from an inbred strain were made polycythemic as previously described (9). On the fifth day following removal from the tank, when erythropoiesis was suppressed, 25 mg of urine extract in 1 ml of normal saline was injected subcutaneously (119 mg/100 g body weight). A group of polycythemic control mice received 1 ml of normal saline subcutaneously. Sixty hours later, all mice were injected with 0.5 μc Fe59 C13 in 0.1 ml normal saline into the tail vein. Blood was obtained by cardiac puncture from half of the mice at 48 hours and from the remainder 72 hours after the Fe59 C13 injection. Blood samples of 0.5 ml were counted in a scintillation counter. Duplicate microhematocrits were obtained from each animal. The calculations of Fe59 red cell utilization were based on previous observations(10) that total blood volume is equivalent to 7.5% of body weight.

Results. 1. *Effect of human urine extract on erythropoiesis in the hypertransfused guinea pig.* As shown in Table I and Fig. 1, increased Fe59 incorporation into red cells was found following injection of human urine extract into hypertransfused guinea pigs. Twenty-four hours after one injection of the extract, Fe59 incorporation of 8.05% was observed in the erythrocytes, which was 300% of the incorporation into the red cells of the uninjected control animals ($P<0.01$).

TABLE II. Effect of Injection of 25 mg of Urine Extract from a Patient with Hypoplastic Anemia upon Fe59 Incorporation into Erythrocytes of Polycythemic Mice.*

Interval between Fe59 C13 inj and sacrifice (hr)	Injected with extract†			Controls			
	No. animals	Hct. %	% Fe59 incorp.	No. animals	Hct. %	% Fe59 incorp.	P
48	5	59 ± 2.42	21.43 ± 5.36	3	61 ± 2.34	.52 ±.17	<.05
72	5	57 ± 3.3	53.5 ±11.2	2	57.7 ± 2.84	.43 ±.11	<.01

* All values given are mean ± standard error of mean (S.E.).
† Avg dose = 119 mg/100 g body wt.

A maximum incorporation of 11.56% was obtained when the Fe59 was administered 48 hours after injection of the urine extract. This was 431% of the observed value for the uninjected control group at that time (P<.01). When Fe59 was injected 72 hours after the extract was administered, the Fe59 incorporation into erythrocytes was not significantly greater than the observed control value at this time (P>.1). Fig. 2 shows the per cent of injected Fe59 appearing in the peripheral blood at various time levels after urine extract administrations. It can be seen that the saline injected controls at 72 hours following initiation of the experiment showed the beginning of escape of polycythemic suppression of erythropoiesis. Thus, differences in per cent of Fe59 incorporation into erythrocytes at this time were not significant.

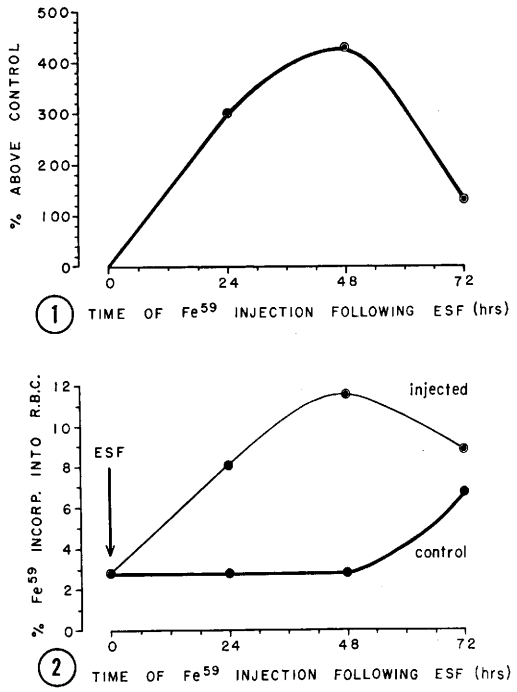

FIG. 1. Incorporation of Fe59 into erythrocytes of hypertransfused guinea pigs following injection of 18 mg/100 g body weight of human urine extract. Points ⊙ represent % of control for the mean value of each group of guinea pigs receiving Fe59 C13 at 24, 48 and 72 hours after administration of urine extract. Blood was taken for assay 48 hr after injection of the Fe59 C13.

FIG. 2. Comparison of incorporation of Fe59 into erythrocytes of hypertransfused guinea pigs receiving 18 mg/100 g body weight of human urine extract and normal saline. Note beginning of escape of polycythemic suppression of erythropoiesis in controls at 72 hr.

2. *Effect of human urine extract on erythropoiesis in the polycythemic mouse.* As shown in Table II polycythemic mice receiving Fe59 C13 60 hours after injection of the human urine extract showed 21.43% Fe59 incorporation into erythrocytes 48 hours later (P<.05) and 53.5% incorporation 72 hours after injection of Fe59 (P<.01). These results were significantly different from the saline injected controls. In this experiment the dose of human urine extract was considerably greater per gram of body weight than that which was used in the guinea pigs; this may account for the greater Fe59 incorporation into erythrocytes observed in the mouse. It is noteworthy that the maximal erythropoietic effect was attained 72 hours after injection of Fe59 C13.

Discussion. The data presented indicated that human urine extract obtained from a patient with hypoplastic anemia which shows marked erythropoietic activity in the polycythemic mouse also stimulated Fe59 incorporation into the erythrocytes of polycythemic guinea pigs. The injections of Fe59 C13 into the guinea pigs were made at inter-

vals of 24, 48 and 72 hours after injection of the urine extract to determine when maximum uptake of Fe59 by erythroid precursors occurs. This was found to be at 48 hours after initiation of erythropoietic stimulation. It would appear that some escape from the polycythemic suppression of erythropoiesis in the hypertransfused guinea pigs occurs when the assay is prolonged to 72 hours as noted by the increased incorporation of Fe59 into erythrocytes of the 72-hour controls.

The previously reported failure of human erythropoietin to stimulate erythropoiesis in the normal guinea pig could have been due to formation of antibodies to the heterologous erythropoietin, as suggested by Garcia and Schooley(6). The short duration (of some) of these experiments, however, make this explanation unlikely. Another possibility is that the assay animals used were not sensitive enough under the experimental conditions to record an erythropoietic response. However, it might have been postulated that human erythropoietin has a different molecular structure from guinea pig erythropoietin and consequently has a limited ability to activate erythropoiesis in the guinea pig and possibly other species(9). The possible species differences of molecular structure of erythropoietin has not been sufficiently explored because of the difficulties of erythropoietin purification. However, as has been shown in this experiment the hypertransfused guinea pig is able to respond to human erythropoietin. A dose response curve with a standardized erythropoietin preparation must be determined in order to compare the guinea pig's response to that of other species.

Summary. Fe59 RBC incorporation was increased in the hypertransfused guinea pig following administration of an extract of urine from a patient with chloramphenicol-induced hypoplastic anemia. This extract also had erythropoietic activity in the polycythemic mouse. Maximum Fe59 incorporation into erythrocytes of guinea pigs occurred when the Fe59 C13 was given 48 hours after administration of the erythropoietically active human urine extract.

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Dextran-Glucose and the Cultivation of Leucocytes. (29410)

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Arakaki and Sparkes(1) have described a micromethod for cultivation of leucocytes of whole blood which makes possible short term cultures with a small inoculum. However, it requires the addition of serum or plasma enrichment, and thus the medium contains an ingredient which may vary in its content. It would therefore be of advantage if, instead, a more nearly inactive standardized substance could be substituted for the enrichment ingredient. Such a substitute would be particularly desirable if it were inactive either as a mitotic stimulator or inhibitor. A commercial 6% dextran solution containing 5.0% glucose (Abbott) has been found to be at least a partially effective substitute for the