tion of parental genotypes with respect to the *Dy* and *dy* alleles provides a sound basis for expecting certain ratios of normal:affected offspring. This is particularly useful in the search for early differences between normal and affected individuals.

The method of ovarian transplantation has the added advantage of putting to use the germ cells of affected homozygotes which would otherwise be unavailable for reproductive purposes. Compare the incidence of dystrophic animals in the 1951-55 breeding colony of strain 129 mice with that in the 1956 colony maintained with the help of ovarian transplantation. Between 1951-55, in spite of considerable effort toward selection of matings between heterozygotes, the incidence of dystrophics was 6% (177/2950)(1). In 1956, the combined incidence of dystrophics among offspring of the 3 types of matings listed in Table II was 1048 4439, or 24%. The use of animals with ovarian grafts has greatly facilitated the production of dystrophic mice in quantity and has permitted direct genetic tests which have provided definitive evidence that *dystrophia muscularis* in strain 129 mice is inherited on a unit autosomal recessive basis.

Summary. 1. Critical evidence is given which supports the hypothesis that *dystrophia muscularis* is inherited in strain 129 mice on a unit autosomal recessive basis. 2. Offspring were obtained from ovaries of dystrophic females transplanted to histocompatible normal hosts. The incidence of dystrophy in a large F_2 population descended from these grafted ovaries is 19%, and in a large backcross population 44%. 3. Approximately 70% of the animals bearing ovarian grafts yielded graft offspring. The technic of ovarian transplantation, as modified from that of Robertson is described. 4. Using offspring of known genotype derived from transplanted ovaries, it has been possible to provide a reliable and extensive supply of dystrophic animals and normal littermates for research use.

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Fe^{59} Assessment of Erythropoiesis in Mice Following Injections of Anemic Rabbit Plasma Extracts.* (23154)

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Carnot and DeFlandre(1) postulated the existence of an erythropoietic stimulating factor in plasma of rabbits. Since then much evidence(2-5) has been published favoring a humoral control of red cell production. However, the effectiveness of an erythropoietic factor in deproteinized plasma extract from rabbits made anemic with phenylhydrazine in producing erythropoietic stimulation as judged by Fe^{59} uptake in red cells has not been

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shown to date in mice.

The following report describes specifically the use of the Fe^{59} uptake method to determine effectiveness of deproteinized anemic rabbit plasma extracts in stimulating erythropoiesis in Swiss mice.

Materials and methods. (a) Male Swiss mice (Hauschka ICR Swiss) aged 8-10 weeks and ranging in weight from 18-35 g were employed in all the experiments described below. They were maintained on a diet consisting of Derwood-Morris mouse pellets. (b) White New Zealand rabbits were given 1 cc of 2.5% phenylhydrazine subcutaneously daily until hemoglobin was less than 5 g. They were then bled and the plasma prepared as described previously(2). Control plasma was obtained from normal non-treated rabbits. The deproteinized plasma extract administered was concentrated to ⅓ the original volume of plasma. Swiss mice received subcutaneously 2 cc of rabbit deproteinized concentrated anemic or normal extract for 3 successive days. On the fourth day, 1 μc of Fe^{59} in ½ cc saline was injected into the jugular vein and 24 hours thereafter the mice were bled from the dorsal aorta. The radioactivity in the sample was measured in a Nancy Wood well-type scintillation counter. Radioactivity in an aliquot of the original Fe^{59} solution given each animal was similarly measured. Calculation of the Fe^{59} uptake into the red cells was carried out as previously described(6). Precaution was taken after each experiment to run hematocrits. Any animal with excessively low hematocrit was not included in our results since animals with low hematocrits had tended to run higher iron uptakes. In this study hypertransfusions with mouse blood were used to test radioiron uptakes as a means of reflecting erythropoiesis in the mouse. This will be described separately along with the results for convenience. The Fe^{59} was injected and calculated in the same manner as indicated above.

TABLE I. Percentage Incorporation of a Single Intravenous Injection of Fe^{59}* into Circulating Erythrocytes of Normal Male Swiss Mice.

Hr	No. of mice	Avg uptake, % and range	S.D.	Avg Hct., % and range
24	23	22.8 (7.9-34.7)	± 2.4	41 (28-48)
48	27	30.3 (10.3-53.6)	± 8.7	42.7 (40-48)
72	27	31.3 (12.2-49.6)	± 2.9	42.3 (37-48)

* Fe^{59} uptake at varying intervals after single inj.

TABLE II. Depression of Red Cell Iron Uptake* by Repeated Blood Transfusions in Normal Male Swiss Mice.

Hr	No. of mice	Avg uptake, % and range	S.D.	Avg Hct., % and range
24	11	2.6 (.3- 4.5)	± 1.2	46.8 (43-51)
48	11	4.8 (2.5- 7.7)	± 1.4	45.3 (42-50)
72	12	9.5 (5.3-12.5)	± 2.0	42.5 (40-45)

* Uptake at varying intervals after a single intrav. inj. of Fe^{59}.

Results. Fe^{59} uptake values are shown in Table I along with hematocrits. The percentage incorporation of a single intravenous injection of Fe^{59} into circulating erythrocytes of normal Swiss mice is expressed as a function of time. The radioiron uptake values in a 24, 48 and 72 hour period were respectively 22.8% ± 2.4, 30.3% ± 8.7, and 31.3% ± 2.9.

To test the validity of radioiron in determining red cell production in mice the following experiment was carried out. It is known that when the product of a tissue or cell is supplied exogenously the producing tissues will often atrophy. A group of normal inbred Swiss mice were given intraperitoneally repeated transfusions of ½ cc of whole blood from Swiss mice for 5 days. On the 5th day capillary hematocrits and red cell counts were done on a representative group of transfused and control mice. The mean of red cell counts in controls was 11.2 million per cu mm and in transfused mice 14.7 million per cu mm. The capillary hematocrits in the controls averaged 41.5% and in the transfused mice 47% in one group and 64% in another. On the sixth day following the last transfusion the mice were given a single intravenous injection of Fe^{59} and were bled 24, 48 and 72 hours thereafter (Tables II and III). Tables II and III show the marked failure of the transfused mouse to utilize the radioiron in red cell production. This is particularly observable in the 24-hour values shown in Tables II and III. However, the 72-hour values show a greater percent uptake than in

TABLE III. Depression of Red Cell Iron Uptake* by Repeated Blood Transfusions in Normal Male Swiss Mice.

	No. of mice	Avg wt (g) and range	Avg uptake, % and range	S.D.	Avg Het., % and range
Hypertransfused	7	23.4 (19.5-26.0)	.9 (.4- 2.4)†	± .75	62.6 (47-79)
Non-hypertransfused	7	24.4 (18.0-27.0)	17.9 (9.7-30.3)†	± 8.2	41.6 (37-45)

* At 24 hr. † t = 5.4791 P = 1%.

the 24 and 48-hour values in Table II.

The results obtained with deproteinized anemic and normal rabbit plasma extracts are presented in Tables IV and V. In Table IV the first lot of concentrated anemic plasma gave an averaged Fe^{59} incorporation in 24 hours of 38.3% ± 8.01 and the concentrated normal plasma values were 30.8% ± 8.6. In this experiment the differences in mean Fe^{59} incorporation between mice receiving anemic and normal rabbit plasma were significant at a level between 2 and 5% (t – 2.259). The groups in Table V are similar except an untreated control group was added. The second lot of concentrated anemic gave a mean value of 52.0% ± 9.26, and the untreated control group gave a mean value of 43.5% ± 10.04. The differences in the mean between concentrated anemic and untreated controls have limited significance at a 5-10% level (t – 1.909).

Discussion. The above data demonstrate as judged by Fe^{59} uptake into RBC, the erythropoietic stimulating property of a de-

TABLE IV. Normal Male Swiss Mice Given Deproteinized Plasma Extract (Lot #1) Subcutaneously from Anemic and Normal Rabbits for 3 Consecutive Days.

% uptake of Fe^{59}* in RBC, 48 hr after last inj. of plasma.

	Wt, g	% Hct.	% Fe uptake
Conc. anemic rabbit deproteinized plasma extract (13 mice)	29	46	50.6
	28.5	45	43.7
	28	46	45.3
	27.5	44	43.6
	22	46	42.7
	27	47	36.6
	23	46	38.6
	26	45	45.1
	28	44	32.2
	27	45	46.1
	19	48	30.1
	28	43	45
	23.5	—	23
	Avg	45.4	38.3
	S.D.	± 1.38	± 8.01
Conc. normal rabbit deproteinized plasma extract (12 mice)	32	41	31.4
	29.5	48	36.7
	27.5	43	34.6
	21	45	16.9
	25	45	27.9
	27	43	41.6
	28	45	22.8
	30	46	25.1
	30	42	42.4
	32	41	30.5
	29	46	19.6
	24	44	40.5
	Avg	43.7	30.8
	S.D.	± 1.77	± 8.6

* 1 μc Fe^{59} in ½ cc saline inj. intrav. 24 hr after last plasma inj.
t = 2.259. P = Statistically significant at a 2-5% level.

TABLE V. Normal Male Swiss Mice Given Deproteinized Plasma Extract (Lot #2) Subcutaneously from Anemic Normal Rabbits for 3 Consecutive Days.

% uptake of Fe^{59}* in RBC, 48 hr after last inj. of plasma.

	Wt, g	% Hct.	% Fe uptake
Conc. anemic rabbit deproteinized plasma extract (9 mice)	30	45	54.6
	33.5	42	58.5
	28.5	33	60.6
	25	43	55.3
	22.5	41	43.7
	22.5	42	47.4
	25.5	40	56.2
	25.5	45	32.3
	26	41	59.5
	Avg	41.3	52
	S.D.	± 3.57	± 9.26
Untreated controls (10 mice)	23.5	44	32.2
	22	39	26.1
	31	38	49.7
	24	43	45.6
	30.5	35	48.5
	31	40	60.6
	36.5	43	48.6
	34	38	36.8
	31	39	38.5
	27	40	48
	Avg	39.9	43.5
	S.D.	± 2.77	± 10.04

* 1 μc Fe^{59} in ½ cc saline inj. intrav. 24 hr after last plasma inj.
t = 1.909. P = Statistically significant at a 5-10% level.

proteinized plasma extract (Lot 1) from anemic rabbits. However, plasma extracts from anemic rabbits previously tested in normal and hypophysectomized rats(3) have been found to be much more effective with a smaller dose on a per gram body weight basis inasmuch as a dose 6 times greater to the mouse gave an erythropoietic response of lesser magnitude. It is interesting to point out here that in assaying growth hormone preparations in our laboratory we also repeatedly observe the greater sensitivity of the rat to growth hormone than the mouse. Nevertheless, it seems probable that the erythropoietic principle in anemic rabbit blood is effective generally in experimental animals. Keighley and Borsook(7) have shown the production of increased circulating hemoglobin in Swiss mice with extract from the plasma of rabbits made anemic by phenylhydrazine. Their study using hemoglobin determinations, although on a total of 12 animals, confirms our results using the Fe^{59} method. However, their erythropoietic effect from anemic rabbit plasma was obtained after a longer injection period of 7-20 days.

Fe^{59} studies with normal Swiss mice under biological conditions such as induced plethora which are known to affect the bone marrow's output of red cells in other experimental animals and humans(8,9) lends support to the concept that Fe^{59} uptake in the mouse is a valid representation of RBC production. Much the same responses with the Fe^{59} uptake method are seen in the mouse as are seen in other animals, and human studies(8-11) using the same method. Perhaps the Fe^{59} method might provide a method for studying erythropoietic dynamics and the effectiveness of chemotherapeutic agents on various blood disorders of the mouse. The mouse is an animal widely used in biological research and is readily available and economical to work with. Moreover, strain specificity control is available in the mouse to a much greater extent than rats and other available species of animals.

Summary. The above data demonstrate, as judged by Fe^{59} uptake into RBC, the erythropoietic stimulating property of a deproteinized plasma extract (Lot 1) from anemic rabbits in male Swiss mice. Fe^{59} studies with normal male Swiss mice under the experimental condition of induced plethora lends support to the concept that Fe^{59} uptake in the mouse is a valid representation of RBC production.

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