TABLE IV. Effect of Barium Hydroxide and Barium Acetate on Growth, Mortality, and Feed Efficiency of Chickens between 1 Day and 4 Weeks of Age: Exp. 2.

	Barium hydroxide series			Barium acetate series		
Level of added barium, ppm	Gain in live wt, g	Mortality	Gain / Feed	Gain in live wt, g	Mortality	Gain / Feed
0	483	1	.554	435	2	.541
250	No chicks at this level			436	2	.566
500	450	0	.538	410	0	.538
1000	428	0	.552	448	0	.535
2000	406	0	.518	428	1	.540
4000	410	1	.537	357	1	.518
8000	231	14	.510	218	12	.485
16000		20			20	
32000		20		No chicks at this level		

the same basal diet in the barium acetate series. Chickens in lots fed diets containing barium hydroxide gained an average of 401 g, while those in lots fed diets containing barium acetate gained 390 g. Thus it appears that the 2 compounds had similar effects on growth of chickens. If the 2 series of lots in Exp. 2 are considered as duplicates, average gain in weight, for same level of barium, indicates that 1,000 ppm of barium was tolerated and that 2,000 ppm produced slight growth depression.

Addition of barium to the diet of chickens had little or no effect on efficiency of feed utilization, at least at levels to 4,000 ppm and possibly to level of 8,000 ppm.

Summary. The LD_{50} dose of barium for young growing chickens was 623 ± 156 mg/kg of live weight. When barium was fed continuously in the feed, either as barium hydroxide or barium acetate, chickens tolerated levels to 1,000 ppm without apparent ill effects. A slight depression in growth occurred when 2,000 ppm of barium was added to the diet. 8,000 ppm of barium caused death of more than one-half of the chicks in 4 week feeding experiment; and the higher levels (16,000 and 32,000 ppm) caused death of all chicks.

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Relation Between Plasma Iron Turnover and Plasma Iron Concentration at Different Levels of Erythropoiesis. (25867)

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When tracer doses of siderophilin-bound Fe^{59} are injected intravenously in rabbits, plasma radioactivity declines as a simple exponential function of time ($Fe^{59} = 1.e^{Kt}$) (1). The value of time constant K is an estimate of fraction of plasma iron turned over per unit time. Plasma iron turnover is calculated as the product of K, plasma iron concentration and plasma volume. From results of

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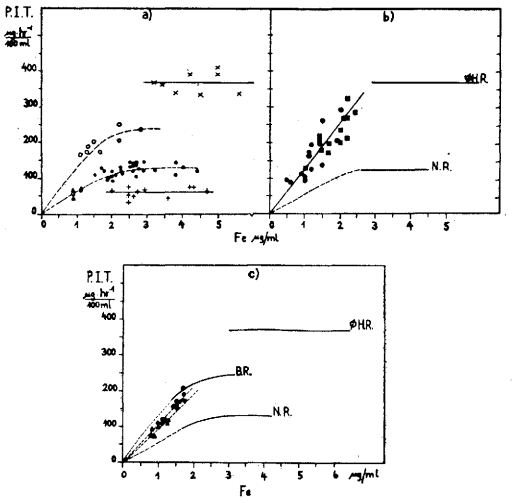

FIG. 1. Relation between plasma iron turnover (P.I.T.) expressed as $\frac{\mu g/hr}{100\ ml\ plasma}$, and plasma iron concentration, [Fe].

1 a.) ● Normal rabbit (N.R.). × Rabbit after 5 inj. of phenylhydrazine (ϕH.R.). ○ Rabbit (B.R.) 72 hr after 20 ml/kg hemorrhage. △ Rabbit inj. with acetone extract of plasma from bled rabbits. + Fasted rabbits inj. with 0.9% NaCl.

1 b.) ● Rabbit inj. with plasma from phenylhydrazine treated donors. ■ Rabbit inj. with boiled plasma of phenylhydrazine treated donors. N.R., normal rabbits; ϕ H.R., phenylhydrazine treated rabbits; B.R., bled rabbits.

1 c.) ▲ Rabbit inj. with plasma of bled donors. ● Rabbit inj. with dialyzed plasma of bled donors.

studies of plasma iron turnover in humans (2), it has been postulated that plasma iron turnover is independent of plasma iron concentration [Fe], and thus that the value of K varies reciprocally with [Fe], and $T\frac{1}{2}\ Fe^{59} = \frac{0.693}{K}$ increases as a linear function of [Fe]. This type of relationship has also been assumed to apply in rabbits(3). This communication presents studies of the relation between plasma iron turnover and plasma iron concentration in rabbits in which level of erythropoiesis was varied by fasting, bleeding, phenylhydrazine injections or treatment with plasma or extracts of plasma from animals rendered anemic by bleeding or phenylhydrazine.

Methods. Plasma iron turnover and Fe^{59} incorporation into erythrocytes were studied in rabbits of both sexes weighing 2-2.5 kg using procedures described previously(1).

Results. Plasma iron turnover and plasma iron concentration. In animals with normal erythropoiesis, which includes untreated controls as well as rabbits which received 0.9% NaCl solution or normal rabbit plasma, P.I.T. is independent of [Fe] over a certain range, but then drops in relation to [Fe] (Fig. 1a). In animals treated with acetone extracts of plasma of bled rabbits, plasma iron concentration is low and so is P.I.T. The experimental points for these animals are close to a line extrapolating the trend in normal animals. These animals had 70% of Fe^{59} in red cells at 48 hours. In fasted rabbits with low P.I.T. and high iron levels, and in phenylhydrazine treated animals with high turnover and high plasma [Fe], P.I.T. is independent of plasma iron concentration. However, in rabbits which had been bled 72 hours previously, P.I.T. increases as function of plasma [Fe]. Both bled and phenylhydrazine treated animals had 90 to 100% Fe^{59} in erythrocytes 48 hours after tracer injection.

P.I.T. and plasma [Fe] in animals treated with plasma or plasma extracts obtained from bled or phenylhydrazinized animals. Fig. 1b shows the relation between P.I.T. and plasma [Fe] in animals receiving plasma or extracts of boiled plasma obtained from phenylhydrazine-treated rabbits. The dependence of P.I.T. on plasma iron is clear. All animals shown had 80-90% Fe^{59} in red cells at 48 hours. In normal rabbits which had received native and dialyzed plasma from severely bled donors (Fig. 1c), P.I.T. is a linear function of plasma iron concentration, and in animals injected with dialyzed plasma from bled donors, P.I.T. and [Fe] are higher than in those animals receiving native plasma.

Discussion. In rabbits with increased erythropoiesis, the ratio between iron going to the marrow and P.I.T. is approximately one. Experimentally the values determined range between 80-100% of Fe^{59} in red cells at 48 hours. Under these conditions plasma iron turnover can be assumed to reflect closely iron uptake by the marrow. Plasma iron turnover is a linear function of plasma iron concentration below about 2 μg/ml (Fig. 1).

These results could be explained by assuming that the limiting factor at low plasma iron concentration is the iron supplied to the marrow by the blood. Uptake of iron (Q_{Fe_M}) by the marrow can be calculated as the product of blood flow to marrow (Q_{b_M}) and the arterial-venous difference in iron concentration ($Q_{Fe_M} = Q_{b_M}[Fe_a - Fe_v]$). When arterial plasma iron level drops below a certain concentration, (Fe_v) might drop to zero; under these conditions ($Q_{Fe_M} = Q_{b_M}[Fe_a]$) marrow iron consumption would be a linear function of Q_{b_M} and plasma iron clearance would be a measure of the blood flow to marrow. The observed clearance values in our experiments suggest that it is highly improbable that blood flow is the limiting factor for, if this were the case, it would mean that only about 2%† of blood volume per minute goes to marrow, a very low figure. Therefore, an explanation for the findings in Fig. 1 may be sought by examining the relation between iron uptake by erythrocyte precursors and iron concentration. It can be postulated that P.I.T. at low plasma [Fe] concentration is a function of plasma iron concentration and number of erythrocyte precursors, including circulating reticulocytes, forming Hb; while at high [Fe] level, P.I.T. is a function only of number of precursors. These postulates are analogous to those used for describing the relation of substrate concentration to rate of an enzymatically catalyzed reaction. In the case of P.I.T., the number of rbc precursors would represent total enzyme. Much experimental evidence points to the existence of an enzymic mechanism incorporating iron into heme(4,5). Recent experiments carried out with reticulocytes(6) and rbc(7) precursors show that iron uptake in reticulocytes is independent of Fe concentration above, and dependent below certain levels(6). The same applies to marrow cultures where at high plasma iron concentration, Fe uptake depends only on number of rbc precursors present while below a certain level it depends linearly on [Fe] for a given number of precursors(7). The hypothesis describing the relation between P.I.T. and plasma [Fe], enables one to infer the erythropoietic activity of marrow under conditions of low or optimal concentration of plasma iron. When plasma iron is optimal, P.I.T. is proportional to number of red cell precursors (P.I.T. $= k_1 \times Np$); when plasma iron is low, P.I.T. is proportional to the product of [Fe] and number of red cell precursors, and plasma iron clearance is a function of the red cell precursors $\left(\frac{\text{P.I.T.}}{[Fe]} = k_2 \times NP\right)$.

The finding described previously(1) that plasma of severely bled animals does not significantly increase average P.I.T. in normal animals but that a significant increase is seen after injecting dialyzed plasma can be explained, in the light of the present findings, as a consequence of the existence in plasma of severely bled rabbits of a factor, probably dialyzable and extractable in acetone which lowers plasma iron concentration. The fact that plasma Fe^{59} clearance is high in these animals can be taken as evidence of increased number of red cell precursors in the marrow, and thus of enhanced erythropoiesis. The finding of increased reticulocytes in the blood of animals treated with bled rabbit's plasma supports this view(8,9).

Analysis of the different aspects of iron metabolism in bled, and phenylhydrazinized rabbits and in animals injected with plasma extracts with erythropoietic activity, suggests an explanation for the findings that plasma iron is usually low in animals injected with extracts and in bled animals, whereas in animals with hemolytic anemia, it remains normal or high. Plasma iron level is a function of the balance between input and output of iron. The most important input of Fe comes from destruction of red cells, while the most important output is that of iron going to the bone marrow. In bled animals and in those injected with erythropoietically active extracts, iron output increases with no increase of input from destroyed red cells, and plasma iron initially will drop, as output outbalances input, until a level is reached when input from depots and intestinal tract again equals out-

† This value is obtained by taking a value of K of 120%/hr or 2% per minute.

put. In animals with abnormal hemolysis, on the other hand, input from destroyed cells is high and initially above output; plasma iron thus rises to a certain level when, mainly as a consequence of increased red cell production, output again equals input.

Summary. 1) The relation between plasma iron turnover and plasma iron concentration is studied in normal and fasted rabbits, in bled and phenylhydrazine treated rabbits, and in rabbits treated with extracts of plasma of bled and phenylhydrazine-treated donors. 2) In normal, phenylhydrazine-treated and starved rabbits, plasma iron turnover is independent of iron concentration over the range of 2-5 μg Fe/ml; below this range plasma iron turnover in normal rabbits tends to drop. Bled rabbits and rabbits receiving extracts with erythropoietic activity show lower plasma iron concentrations than normal, and in these animals plasma iron turnover depends on iron concentration.

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Effects of Urinary Hemopoietine on Fe^{59} Distribution in Rats Studied While Plasma Fe^{59} is High. (25868)

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In the majority of studies in which Fe^{59} has been used in rats to assay extracts with erythropoietic activity, the criterion employed has been the fraction of injected Fe^{59} (% Fe^{59}) appearing in erythrocytes from 16-24 hours after tracer injection(1,2,3). Although extracts rich in erythropoietic activity increase erythrocyte Fe^{59} uptake, this method does not permit quantification of increase in erythropoiesis. Determination of plasma iron turnover and uptake of iron by marrow would give data more directly related to magnitude of erythropoiesis(4,5). Since, when large numbers of rats are employed, it is not practical to carry out accurate plasma iron turnover measurements requiring serial blood samples, we devised a technic in which plasma iron turnover and mean plasma Fe^{59} specific activity (between 0 - t hours) can be estimated from one plasma sample taken at a time (t hours), when plasma Fe^{59} is still high in controls. From mean plasma specific activity and % uptake by different organs, quantity of iron going to these organs can be calculated. We here describe the technic and its application to assay of urinary erythropoietic activity. The results of this method are compared with 24 hour Fe^{59} assay.

Methods. Extracts with erythropoietic activity were obtained from urine of phenylhydrazinize-treated rabbits and patients with aplastic anemia by methods described elsewhere(6). The kaolin adsorption procedure used was similar to that employed by Winkert *et al.*(7). Grey rats, both sexes of a x c strain obtained from Inst. de Biol. Juan Noé,

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