killing fell to below normal levels at 75 and 100°C for the mesophile and thermophile, respectively, possibly due to selection. (2) The relative mutability of the two was also confirmed by the determination of fermentation (mannose) mutants, and by isolation of biochemical mutants using the Davis(12) technic. Such mutants were readily isolated from *B. globigii* cultures, but only a limited number of vitaminless and amino acidless, and no purineless or pyrimidineless mutants could be isolated from the thermophilic cultures. Generally, the thermophile was much less mutabile than the mesophile as compared by streptomycin resistant, fermentation and biochemical mutants.

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Depression Red Cell Iron Turnover by Transfusion. (19257)

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(Introduced by J. H. Lawrence.)
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One of the commonly employed methods for study of the regulation mechanisms of a steady state is to observe the events which follow an increase or decrease in the regulated quantity.

This paper is concerned with the change in erythrocyte production rate, subsequent to an increment in the red cell volume (RCV) of rats, as measured by plasma and red cell iron turnover using Fe^{59}.* The method for increasing the red cell volume was by intraperitoneal transfusions of blood obtained from the same highly inbred strain.

Hess(1) discovered that rabbits which had been rendered polycythemic by repeated transfusions developed marrow aplasia and fibrosis. Boycott and Douglas(2) contended that only excessive red cell destruction, *not* inhibition of production, explained the disappearance of cells from artificially plethoric rabbits. Although some of their own data would now be considered clearly indicative of lessened production, this concept was not accepted until Robertson's(3) work. Later Krumbhaar(4), Boycott and Oakley(5), and others used the reticulocyte percent as an index of marrow erythrocyte production, and they noted a decline in reticulocytes during the early weeks of transfusions. Although most of the early workers used blood which was initially compatible, several noted development of agglutinins. Possible causes for the inconsistent results in early work will be discussed.

Methods. The iron turnover in plasma and red cells was determined using tracer amounts of Fe^{59}. Plasma iron turnover is the quantity

* No attempt is made here to elaborate on the mechanism through which a change is induced in rate of production by the increased RCV.

of iron entering or leaving plasma per unit time. It is calculated as follows: Plasma Fe turnover (μg/day) $= \frac{0.693}{Fe^{59} \text{ half-time (hr)}} \times$ plasma volume (ml) $\times$ concentration of Fe in plasma (μg/ml) $\times$ 24 (hr). The fraction of tracer present in cells at 3 to 5 days after injection may be used as an index of the portion of turnover in plasma going to marrow and thence to red cells. $\frac{Fe^{59} \text{ in all red cells}}{Fe^{59} \text{ injected}} \times$ plasma Fe turnover $=$ iron entering cells (μg/day). The Fe^{59}, of high specific activity, was combined with globulin prepared from fraction IV-7 (Cohn). The resulting solution had a protein concentration from 5% to 7% and contained 0.73 μc per injection of 0.25 ml. The amount of nontracer iron present in the preparation was negligible.

Twelve female Slonaker rats weighing from 180 to 210 g were used. After preliminary red cell counts and reticulocyte counts, 6 of these rats were given 2 ml of blood 7 times during 6 days. The donor blood from lightly etherized animals *of the same sex and highly inbred strain* was obtained from the inferior vena cava in heparinized syringes and pooled before intraperitoneal injection. Red cell counts and reticulocyte counts were done on the control rats 4 times during the first 9 days and on the experimental rats at the times plotted on Fig. 1. Capillary tube hematocrits were obtained on all animals. On the 8th day,

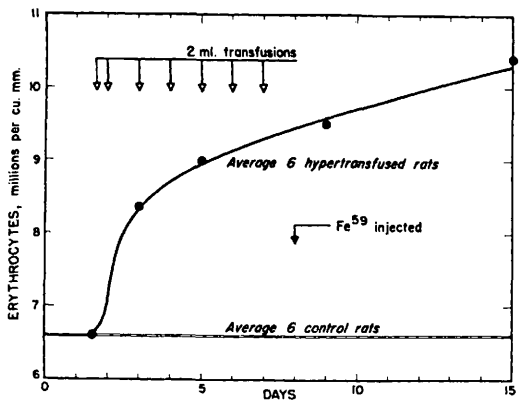

FIG. 1. Erythrocytes, millions/mm^3 in control and transfused rats. Controls S.D. $\pm$ 1.7 million/mm^3.

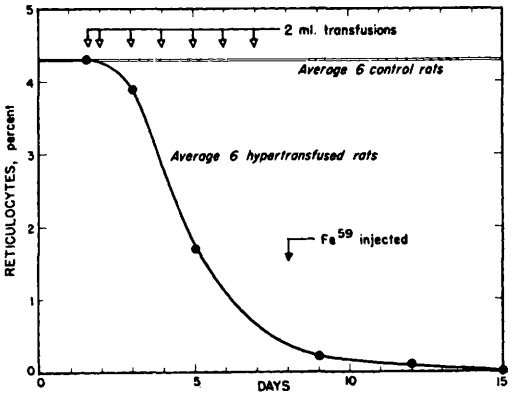

FIG. 2. Reticulocyte decline with increase in red cell volume. Controls S.D. $= \pm 1.6\%$.

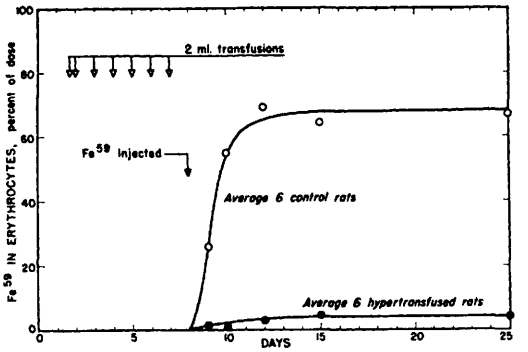

FIG. 3. Percent of injected Fe^{59} in cells. The plateau values were used with the iron turnover in plasma in computation of the rate of iron utilization in erythrogenesis. The red cell iron utilization in the experimental group had diminished to less than 1/10 that of the controls.

one day after the 7th and last transfusion, the Fe^{59} globulin was injected into the tail veins of all 12 animals. Small capillary tube blood samples were taken at 10 minutes, at 3 hours and 50 minutes, and at 10 hours and 48 minutes. After centrifugation, the cell and plasma portions were assayed separately in an efficient gamma fluorescence counter(8). The counting rates per milliliter were computed and plotted as a function of time on semi-logarithmic paper. A line through these points was extrapolated to zero time. The plasma volume was determined by dividing the counts per minute injected by the counts per minute per milliliter at zero time. The total blood volume was computed using the mean hematocrit of the early samples. The red cell volume was found by subtraction. The sam-

TABLE I.

	Plethoric (hyper-transfused)	S.D.	Control animals	S.D.
Avg red cell vol., cc/100 g body wt	5.4		2.53	
Avg hematocrit after transfusion period in %	59.7	± 2.8	40.7	± 3.7
Avg Fe^{59} half-time in plasma (hr)	2.23	± .57	1.58	± .35
% of inj. Fe^{59} in erythrocytes (avg)	4.2	± 3.9	67.6	±12.8
% of inj. Fe^{59} in liver at necropsy	92.9	± 3.5	29	± 3.8
Total plasma iron, μg/ml	5.55*	± .53	3.54*	± .97
" " vol. in ml	5.9	± .82	7.84	± .54
Fe turnover in plasma, μg/day	308		265	
Fe turnover in red cells, μg/day	14.8		177	

* Total iron was determined on plasma removed 17 days after determination of the tracer half-time. Use of this value would be invalid in absolute computation of the turnover but is very useful in comparison between the two groups.

pling was repeated at 24 hours, 53 hours, 4 days, 7 days and again when the animals were killed. Before necropsy the animals were lightly anesthetized with ether; the abdominal cavity was opened; as much as possible of the animal's blood was removed from the inferior vena cava; and samples were taken for Wintrobe tube hematocrits, for red cell radioactivity, and for total plasma iron determinations. The rats, with jugular veins severed, were then perfused with saline until they expired. The livers were excised and total radioactivity of each was determined and expressed as a fraction of the injected radioiron. The red cell volume was used together with the counting rate measurements in calculating the total radioactivity in the red cells.

Results and discussion. Fig. 1 shows the increase in the mean red cell count of the transfused animals. This is in agreement with the previous work(6,7) on transfer of erythrocytes from the peritoneal cavity. The mean of 30 red cell counts in controls during 15 days was 6.6 ± 1.7 (SD) million per cu mm. The rise for several days after stopping transfusions probably results from a readjustment in plasma volume. It is similar to that observed in dogs(4). Fig. 2 indicates the decrease in average reticulocyte percent of transfused rats. The mean reticulocyte percent of the 30 counts on controls was 4.0 ± 1.6 (SD). Fig. 3 expresses the percent of injected iron 59 present in the total red cell volume as a function of time. The differences between the experimental and the control groups are in every detail evidence for a profound decline in production rate in the plethoric animals (Table I). The moderate increase in plasma iron turnover observed in the transfused animals has been associated with red cell-to-plasma transfer increase expected with the normal cell destruction rate of the larger red cell volume.

The normal major pathway, plasma-to-marrow-to-erythrocytes, appears to be greatly diminished with the plethora. The decline in plasma tracer concentration is associated, not with an erythrogenic utilization, but with the transport of nontracer iron from the broken down cells to the iron storage depots. The portion of the plasma iron turnover attributable to red cell production in the plethoric animals was less than 1/10 that of the controls. Radioactivity in the livers of the experimental group was 93% of the Fe^{59} injected whereas only 29% was found in the livers of the control animals. If any liver radioactivity was due to red cells remaining there after the perfusion, it is likely that a greater portion would be present in the control animal livers because of the higher specific activity of their red cells.

The plasma and red cell iron turnover phenomena observed in this experiment are almost identical to those seen in Peruvian natives (14,500 ft.) when they are moved to sea level and studied(9). The shift of tracer from plasma to liver in the hypertransfused animals is similar to that observed by *in vivo* ferrokinetic studies in patients(10,11) who also showed morphologic evidence of marrow hypoplasia.

There are a number of fundamental differences between these experiments and earlier studies. The problem of antibody development is avoided by use of rats whose blood and tissue compatibilities are so complete that parabiotic union can be accomplished and maintained. None of the donors was used more than once.

Experiments at this laboratory in which rats received 4 ml of homozygous plasma per day for 2 weeks, have shown that plasma transfusion does not cause a reduction in red cell production. Others have made the same observation(5).

The inhibition of red cell iron turnover by increasing the red cell volume may be useful in studies of control of erythropoiesis.

Summary. (1) A profound decrease in the red cell iron turnover occurred after experimental increase in the red cell volume of rats. (2) A major portion of the tracer was found in the liver at necropsy.

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Species Specificity of Thromboplastin: Role of the Cothromboplastin Reaction (19258)

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It has been recognized for a long time that species differences may influence the coagulative activity of thromboplastin (tissue extract) on plasma. Thus Quick(1) found that mammalian thromboplastin was relatively inactive with respect to the coagulation of avian plasma and avian thromboplastin was similarly inactive with respect to mammalian plasma. Thromboplastin from rabbit brain was highly active with respect to human plasma but this was not true of thromboplastin from guinea pig brain. Recently Burstein(2) has reported that the activity of mammalian thromboplastin with respect to avian plasma could be greatly increased by the addition of mammalian serum to the reaction mixture: for a maximal effect addition of 0.1 ml of undiluted serum to the 0.3 ml reaction mixture of the prothrombin time test was needed. We have found that normal serum greatly increases the activity of thromboplastin with respect to dicumarol plasma. This effect will occur with highly diluted serum (1:100) provided that the serum and thromboplastin are allowed to react for a brief period before the plasma is added. We have presented evidence (3-5) that this reaction, which we have called the cothromboplastin reaction, is attributable to a specific factor, cothromboplastin, which is present in both plasma and serum and is decreased by dicumarol much more rapidly than is prothrombin. It seemed worth while to determine whether cothromboplastin might be involved in the property which homologous serum has of increasing the activity of thromboplastin with respect to heterologous plasma.