majority of these neoplasms probably were the result of the escape of the cells from the diffusion chambers. This conclusion is based upon the localization of tumor growth to the region near the chamber, and the short temporal relationship of tumor development to chamber implant. It is the author's opinion that migration of both normal and malignant lymphocytic cells through Millipore membranes of the mean pore size employed here remains a possibility which must be considered in all experiments of this type. Liberation of a subcellular tumorigenic agent by the viable lymphoma cells within the chambers could possibly explain the development of some of the malignancies observed.

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Iron Metabolism in Acute Starvation. (29493)

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One consequence of starvation, the cause of a number of metabolic abberations(1,2), is a diminution of erythropoiesis(3). This is manifested by parallel decreases in marrow erythroblasts, reticulocytes in the peripheral blood and presumably hemoglobin synthesis since there is decreased red blood cell radioiron incorporation(3,4). These changes are partially reversible by erythropoietic stimulation and starvation has been utilized to diminish erythropoiesis in animals prepared for bioassay of erythropoietic activity(4,5). Coincidental alterations in iron metabolism which occur with starvation would be reflected in the results of these assays and leave interpretations uncertain(6,7).

This report details preliminary observations on iron absorption, plasma iron, plasma iron clearances, iron turnover, blood volume and iron partition in normal and acutely starved rats. Acute starvation did not alter blood volume nor gastrointestinal uptake of radioiron. Starvation produced a prolongation in plasma iron clearance and a diminution in plasma iron turnover. Starved rats had decreased radioiron in spleen and bone marrow. These alterations were partially reversed by erythropoietic stimulation.

Materials and methods. Female Sprague-Dawley rats were utilized in all experiments. Iron absorption studies were undertaken in 200-300 g rats, whereas 180-200 g animals were used in the other experiments. Fed animals were provided a regular Purina Rat Chow* diet. Starved rats previously fed a diet of Purina Rat Chow* were deprived of all food for 96 hours prior to study. The fed animals used in the iron absorption experiment were deprived of food for 2 hours prior to study in order to clear the upper gastrointestinal tract. Water was allowed all animals *ad libitum*.

Blood volumes were measured in fed and in starved rats by a method previously described(8). Iron absorption, plasma iron(9)

* Iron content 275 p.p.m.

and plasma iron clearance rates(12) were determined in fed rats, starved rats, and in both fed and starved rats following erythropoietic stimulation. Plasma transferrin levels were not measured. Iron partitions were determined in fed rats, starved rats and in starved rats following erythropoietic stimulation.

Erythropoiesis was stimulated by the intraperitoneal injection of 5 units sheep erythropoietin† 24 and 48 hours prior to measurement of iron absorption. In all other experiments erythropoiesis was stimulated by intraperitoneal injection of 0.5 ml of a urine concentrate from anemic patients(4) at 24 and 48 hours prior to study. Packed cell volumes were determined on each animal by the micro technique(10).

I. *Iron absorption.* Rats were lightly anesthetized with ether and given 1 μc Fe^{59}/20 μg Fe^{56} citrate in 1 ml 0.9% saline by stomach tube. Total radioactivity in each animal was determined by means of a small animal body counter.‡ After 2 hours, the rats were killed, the gastrointestinal tract removed intact, opened and repeatedly washed in tap water until the radioactivity remained constant. Dissection was carried out on absorbent paper and the unopened gastrointestinal tract was blotted free of blood to minimize discrepancies in total radioactivity due to blood loss. The gastrointestinal tract was then replaced in the carcass and residual radioactivity measured. The percent iron absorption was calculated by comparing the initial radioactivity of each intact animal with that of the carcass including the absorbent paper.

II. *Iron partition.* Two hours following intravenous injection of 2 μc Fe^{59}/2 μg Fe^{56} citrate the rats were killed and the liver, kidneys, spleen, 1 ml blood, and the femurs removed. The radioactivity of these tissues was then determined in a scintillation counter and expressed as percent of total radioactivity injected. Total blood radioactivity was calculated on the basis of a 5% blood volume by weight. This value, previously determined in this laboratory by direct measurement of the red cell mass and coincident hematocrit(4) was verified in the present experiments as noted below. Correction for total marrow was made by considering the marrow in both femurs to be equal to 16% of the total(11).

III. *Plasma iron clearance.* All animals were weighed, and 2 μc Fe^{59}/2 μg Fe^{56} citrate was injected *via* the tail vein. Groups of 5 rats each were exsanguinated by closed chest cardiac puncture at 15, 30, 60, 90 and 120 minutes following the initial injection. Following centrifugation, 1 ml of plasma was withdrawn and the radioactivity of this sample and the standard was determined in a well type scintillation counter. Plasma iron clearances were determined and T 1/2 clearance time calculated(12).

Results. Starvation did not alter the gastrointestinal absorption of radioiron; however, erythropoietic stimulation increased gastrointestinal iron absorption in both normal and starved animals. This augmentation was of greater magnitude in the starved erythropoietin treated rat (Table I). Blood volume was not altered appreciably in the starved animal (19 starved rats: 5.0 $\pm$ 0.7§ ml/100 g, 21 normal rats: 4.8 $\pm$ 0.7§ ml/100 g), whereas venous hematocrits were elevated following 96 hours of starvation, (starved rats: 52.9 $\pm$ 1.9%, normal rats: 44.3 $\pm$ 1.7%).

Although prolongation of the T 1/2 plasma iron clearance and diminution of the plasma iron turnover followed starvation, there was no alteration in plasma iron (Table II). The T 1/2 plasma iron clearance time was shortened (Fig. 1) and plasma iron turnover in-

TABLE I. Gastrointestinal Absorption of Radioiron.

	No. rats	% GI absorption ± S.D.
Normal rats	19	8.2 ± 3.6
Starved rats	16	8.9 ± 3.1
Normal + erythropoietin	14	13.3 ± 4.1
Starved + erythropoietin	8	26.4 ±10.5

† Prepared by Armour and Co. Research Division under USPHS Grant No. H5393 to University of Chicago and obtained through the Hematology Study Section, Nat. Inst. Health.

‡ Packard Armac Liquid Scintillation Counter.

§ Standard deviation of mean.

TABLE II. Iron Turnover Studies.

	Normal rats		Starved rats		Normal + erythropoietin		Starved + erythropoietin	
	No. rats	Result ± S.D.	No. rats	Result ± S.D.	No. rats	Result ± S.D.	No. rats	Result ± S.D.
Plasma iron (μg/ml)	28	2.88 ±.91	40	2.72 ±.75	30	2.36 ±.52	26	1.13 ±.26
T 1/2 plasma Fe clearance (min)	*	74	*	106	*	52	*	36
Plasma iron turnover (μg Fe/ml plasma/day)	t	38.9	t	25.9	t	45.4	t	31.4

* Mean T 1/2 determined by mean plasma iron clearance curve (Fig. 1)(12).
t Determined by using mean of plasma iron and mean T 1/2 plasma iron clearance(12).

creased by approximately equal increments in both normal and starved animals following erythropoietic stimulation (Table II).

Iron partition studies (Table III) demonstrated a diminution in radioiron in bone marrow and spleen of starved animals. Erythropoietic stimulation with exogenous erythropoietin in the starved rat increased splenic radioactivity to normal and increased bone marrow radioactivity. Whole blood radioactivity at 2 hours represents primarily plasma radioactivity and reflects the plasma iron clearance (Fig. 1).

Comment. Altered erythrocyte radioiron incorporation following starvation has been commonly used to estimate erythropoiesis in the bioassay of erythropoietic activity(4,5). Although changes in erythrocyte radioiron incorporation tend to parallel other measures of erythropoiesis such as percent reticulocytes(4), the use of starvation to diminish erythropoiesis in preparation for bioassay has been criticized because ferrokinetic alterations coincident with starvation complicate interpretations of assay results(6,7). Since most assays presently in use are based on the comparison of relative changes in erythrocyte radioiron incorporation, these objections do not seem particularly pertinent. Nevertheless, changes in the total iron pool, plasma iron or iron turnover rates must be considered if any quantitative interpretations of the assay results are to be made. Such quantitative determinations are essential prerequisites for standardization and comparison of assay results from various sources and may provide guidelines for developing new and more suitable assay methods. The studies

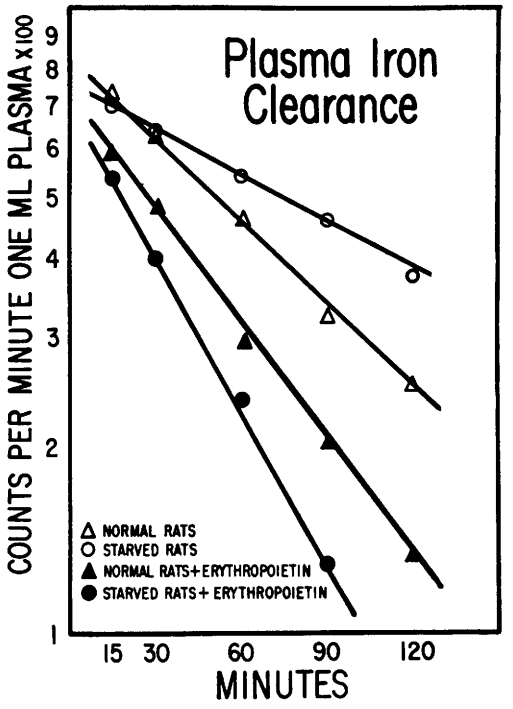

FIG. 1. Radioiron plasma clearance. Each point on the curve represents the mean of 10 determinations at time interval indicated.

herein reported were undertaken to delineate the effect of starvation on ferrokinetics in the starved and the starved erythropoietically stimulated animal in an attempt to illuminate certain aspects of these problems.

Total food deprivation for 96 hours did not appreciably alter the rats' gastrointestinal absorption of radioiron nor the plasma iron. Prolongation of the T 1/2 plasma iron clearance and diminution of the plasma iron turnover are consistent with decreased erythropoiesis and iron utilization. Further support

TABLE III. Iron Partition Studies.

	Normal rats		Starved rats		Starved + erythropoietin	
	No. rats	Result ± S.D.	No. rats	Result ± S.D.	No. rats	Result ± S.D.
Iron partition (%)						
Liver	10	12.0 ± 1.8	11	12.8 ± .9	9	10.3 ± 1.6
Spleen	10	1.5 ± 1.0	11	.6 ± .1	9	1.3 ± .3
Kidneys	10	1.6 ± .2	11	1.8 ± .3	9	1.2 ± .2
Bone marrow	10	19.4 ± 2.6	11	13.1 ± 2.4	9	26.6 ± 4.8
Total blood	10	27.8 ± 1.9	11	27.0 ± 1.8	9	22.8 ± 5.4

of this is provided by the decreased radioactivity found in the bone marrow. These findings complement prior reports of decreased red blood cell radioiron incorporation in starvation(3-5) and indicate that the changes in iron metabolism coincident with starvation are in major part explained by diminished erythropoiesis.

Stimulation of erythropoiesis by exogenous erythropoietin increased gastrointestinal absorption of radioiron in both the normal and starved animal, with greater increase in the starved rat. Accelerated erythropoiesis apparently accounted for this increase. The reason for a disproportionally greater increase in absorption in the starved erythropoietin treated rat is not obvious, but may be related to diminished gastrointestinal mucosal sequestration of iron as recently suggested by Conrad *et al*(13).

A 2-fold increase in the bone marrow radioiron content was produced by erythropoietin administration in the starved animal and approximately equal increases in plasma iron turnover were produced in both fed and starved rats by administration of equivalent amounts of erythropoietin. These alterations are undoubtedly related to accelerated red blood cell production. Enhancement of iron turnover of approximately equal degrees in both starved and fed animals injected with equal amounts of erythropoietin suggests a quantitative significance which is now being investigated.

Summary. Iron metabolism was studied in acutely starved and erythropoietically stimulated rats. Acute starvation did not alter blood volume or gastrointestinal absorption of radioiron. However, prolongation of the plasma iron clearance with concomitant diminution in plasma iron turnover and radioiron in the bone marrow was demonstrated in starved animals. These results can be explained by diminished erythropoiesis. Erythropoietin administration reversed these changes, augmenting plasma iron turnover, gastrointestinal absorption of radioiron and bone marrow radioiron content. Approximately equal increases in plasma iron turnover were produced in both the fed and starved animal by injection of equal amounts of erythropoietin.

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