THE ELIMINATION OF IRON AND ITS DISTRIBUTION IN THE LIVER AND SPLEEN IN EXPERIMENTAL ANEMIA.

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It has been shown in previous reports (1) from this laboratory that, in the normal dog, when the spleen is removed little or no change in the elimination of iron occurs. On the other hand, in man, splenectomy, in the course of congenital hemolytic jaundice (2) and pernicious anemia (3), is followed by a decrease in the elimination of iron equal to about 40 per cent. Moreover, in hemolytic jaundice (2) the amount of iron eliminated before splenectomy is twice as great as that taken in the food. These observations have been interpreted as indicating that in diseases of the spleen, accompanied by anemia, splenectomy removes or inhibits some factor responsible for blood destruction. This view is supported by the observation that after splenectomy there occurs also a decrease in the elimination of urobilin. On the other hand, if one compares the figures for iron elimination in anemia with those of normal individuals (4), it is found that the former fall, as a rule, within the range of the latter. This naturally brings up the question of the factors determining the storage and elimination of iron in conditions of blood destruction. Very few data concerning this subject are at hand (Table I). The only detailed observations aside from those made in this laboratory are the studies of McKelvy and Rosenbloom (5) in congenital hemolytic jaundice, and of Bayer (6), and of Roth (7) after splenectomy for Banti's disease and hemolytic anemia, respectively.

It is obviously difficult to carry out in man prolonged studies, in the different stages of anemia, of the distribution of iron in the various organs. Only end-results, as in the study of bronzed diabetes of Muir and Dunn (8), are possible. We have, therefore, in the present work attempted, by experiments on animals, to throw some light not only upon the distribution and storage of iron but also upon its elimination in various types of blood destruction due to a single or transient injury. This work we consider to be necessary as a

TABLE I.

Elimination of Iron in Healthy and Anemic Individuals.

Observer.	Sex.	Age.	Iron.		Remarks.
			Intake per day.	Output per day.	
		yrs.	*mg.*	*mg.*	
Lehmann, Mueller, Munk, Senator, and Zuntz (4).	Male.	26	Fasting.	7.3*	Professional fasters; 10 and 6 day periods respectively.
	"	21	"	7.7	
Stockman and Greig (4).	Male.	20	6.2†	6.32‡	Healthy individuals.
			5.6	11.46	
	"	35	6.2	8.33	
	Female.	23	3.5	3.73	
Von Wendt (4).	Male (1st period).		11.0†	9.0‡	Nine periods of observation on two healthy individuals.
	(2nd ").		6.0	11.0	
	(3rd ").		10.0	14.0	
	(4th ").		8.0	9.0	
	(5th ").		17.0	42.0	
	(6th ").		7.0	15.0	
	(7th ").		19.0	24.0	
	(8th ").		28.0	34.0	
	(9th ").		27.0	32.0	
Sherman (4).	Male.		5.7§	5.5‡	Three healthy individuals.
	"		6.5	8.7	
	"		7.1	12.6	
McKelvy and Rosenbloom (5).	Female.	11	8.8†	32.51‡	Congenital hemolytic jaundice; 5 day period.
Roth (7).	Male.	26	90.0§	6.25‡	Hemolytic anemia; splenectomized 3 yrs. previously.
			150.0	4.32	
	"	37	90.0	12.18	Splenectomized 1 mo. previously for trauma of spleen.
			200.0	33.07	
Bayer (6).	Male.	16	240.0†	19.38*	2 wks. after splenectomy for traumatic spleen rupture.
			140.0	7.41	
			130.0	14.54	3 mos. later.
			80.0	5.92	
			300.0	26.73	

* Feces only.

† Iron intake determined by actual analysis.

‡ Urine and feces.

§ Iron intake estimated from tables.

TABLE I—*Concluded.*

Observer.	Sex.	Age.	Iron.		Remarks.
			Intake per day.	Output per day.	
		yrs.	*mg.*	*mg.*	
Bayer (6).	Male.	16	240.0	8.40	Control; fracture of tibia.
			140.0	7.29	
	"	16	130.0	8.57	Control; osteomyelitis operation 14 days previous.
			80.0	3.57	
			300.0	23.49	
	Female.	19	130.0	13.86	Banti's disease; 2½ yrs. after splenectomy.
	"	25	130.0	10.20	Banti's disease; ½ yr. after splenectomy.
	"	27	60.0	21.46	Basedow's disease; before thymectomy.
			60.0	32.70	3 wks. after.
			60.0	12.83	6 " "
			60.0	19.00	10 " "
	Male.	22	130.0	3.59	Banti's disease; before splenectomy.
Goldschmidt, Pepper, and Pearce (2).	Male.	5	3.77§	8.29*	Congenital hemolytic jaundice. Before splenectomy (10 day period).
			4.56	4.11	After splenectomy (10 day period).
Pepper and Austin (3).	Male.	40	16.5§	17.0*	Pernicious anemia. Before splenectomy (5 day period).
			16.5	10.0	2 wks. after splenectomy (4 day period).

preliminary to further experimental studies of the spleen in its relation to blood destruction, as also of the mechanism by which splenectomy in man, in the course of anemia, causes a decrease in the elimination of iron.

Our procedure, in brief, has been to determine in normal dogs and in dogs rendered anemic by the administration of various hemolytic agents (sodium oleate, toluylenediamine, and hemolytic immune serum), (*a*) the elimination of iron in the urine and feces, and (*b*) the iron content of the liver and spleen.

Observations of this nature are few in number. Corper's (9) figures for iron in the normal spleen of the dog, calculated on the basis of dry weight, are 0.26 to 0.98 per cent. Samuely (10), working likewise with the dog, gives figures for the spleen and liver, in the anemia produced by pyrodine (acetylphenylhydrazine) as 0.3921 gm. and 0.2298 gm. respectively per 100 parts of dry weight of blood- and fat-free organ and 0.4819 gm. and 0.3299 gm. per 100 parts of dry weight of fat-free but blood-containing organ. In two other animals after recovery from anemia the comparable figures were 0.1892 and 0.0721 and 0.3892 and 0.3307 gm. Tartakowky (11), after feeding iron to dogs with experimental anemia, found that the iron content of the liver rose from 0.1048 to 2.068 gm. and that of the spleen from 0.09 to 0.172 gm. Boycott and Price-Jones (12), in a study of experimental trypanosome anemia in rabbits, found the iron of the liver to be double that of normal animals, while the iron of the spleen was increased twenty times. As to the elimination of iron in normal animals, von Voit's (13) fasting dog put out 0.6 mg. per day per kilo of body weight, while Gottlieb's (14) dog on an iron-poor diet eliminated 0.34 mg. Hamburger's (15) figures with animals on a meat diet show that the output of iron in the urine is increased but slightly when large amounts of iron sulfate are fed. No other figures for the dog appear to be available.

An interesting series of experiments on rabbits, analogous to ours on the dog, has been made by Muir and Dunn (16). They produced rapid, severe anemia by the use of an hemolytic immune serum, and when the anemia was sufficiently severe, as shown by daily blood counts, the liver, spleen, kidney, stomach, and intestine were removed, after the circulating blood had been washed out, and the iron content was determined and compared with that of normal organs similarly treated. At the same time the blood volume was estimated by bleeding and a colorimetric determination of the hemoglobin content of the washings. By comparing, therefore, the iron content of normal and anemic organs, the excess in the latter was easily estimated, and by comparing hemoglobin figures during life with total blood volume at death, the amount of iron lost from the blood could be determined. Their conclusions are as follows: (1) With destruction of more than half the blood within 3 days, nearly all the iron from injured cells is deposited in the liver, spleen, and kidneys; a certain amount escapes in the urine when hemoglobinuria is present and the amount deposited in the kidneys is roughly proportional to the hemoglobinuria. (2) A third of the total iron of the blood may be deposited in the liver, spleen, and kidneys in 24 hours. Their figures show that in anemia the spleen contained three times, and the liver five times the amount of iron found in normal animals.

In a second series (17) the organs were examined when blood counts showed that the anemia had been completely repaired. The iron content of the organs was found to be only slightly above that of normal organs, indicating that the iron deposited as the result of hemolysis had been nearly all absorbed during the process of blood regeneration—presumably utilized, according to Muir and

Dunn, in the formation of new blood cells. In neither of the investigations was the elimination of iron in urine and feces determined.

Methods.

As our principal object was to determine the relation between the amount of iron lost and that stored in certain organs, we limited our study to the estimation of iron in the urine and feces on the one hand, and in the liver and spleen on the other. We have used blood counts to obtain an approximate idea of the degree of blood destruction, but have not attempted to determine the actual total blood destruction. In the earlier experiments, to provide against error dependent upon a possible variation in the iron content of the organs of normal dogs, a portion of the liver and of the spleen was removed at a preliminary operation,[1] and the iron content determined. When later the same animal was used for the study of anemia, exact control figures for the same animal were thus at hand.

The intake of iron in food was not determined in these experiments. Previous studies (1) of iron metabolism in this laboratory have shown that with the diet used, the iron intake is relatively constant. As the diet was the same in both the control and experimental periods we have felt warranted, therefore, in ascribing changes in elimination and storage of iron as due to the experimental lesion produced.

We have also considered it unnecessary to wash out the liver and spleen in order to remove contained blood. The content of blood in each organ is relatively constant, and as it was impossible to remove the blood from the pieces of organs taken out at the preliminary control operations, we have felt that our figures would be more nearly comparable if this was not done at autopsy.

The amount of iron in the urine was determined by Wolter's (18) modification of Neumann's method, and in the feces, liver, and spleen according to Neumann's method (19), with certain modifications. A weighed amount of the substance, calculated to contain from 3 to 5 mg. of iron,[2] is transferred quantitatively to a 750 cc. Kjeldahl

[1] All operations were performed under ether anesthesia.

[2] For feces, weigh out 3 gm.; for spleen, 1.5 gm.; for liver, 2.5 gm.

flask; 10 gm. of potassium sulfate and 30 cc. of concentrated sulfuric acid are then added; the contents of the flask are heated over a free flame and the heating is continued for 15 minutes after the liquid assumes a straw color. After cooling[3] for about 5 minutes, 75 cc. of cold distilled water are slowly added, and after transfer[4] to a 500 cc. Erlenmeyer flask, the mixture is diluted up to about 150 cc. The rest of the analysis is carried out as described by Neumann.[5]

As far as accuracy is concerned, there is little to choose between this modified method of ashing and that of Neumann, for corresponding results are obtained with each. However, considering the factors of time and convenience of manipulation, a marked difference is noted in favor of our modification. With the use of sulfuric acid and potassium sulfate, the ashing is complete in from 1 to $1\frac{1}{2}$ hours with practically no further attention after setting up. On the other hand, Neumann's method, involving as it does the use of nitric acid in addition to sulfuric acid, requires almost constant attention for a long period of time. Another advantage of the modification is that it does away with objectionable nitric acid fumes during ashing, and obviates the necessity of subsequent boiling to remove all traces of nitric acid.

The dogs, kept in metabolism cages, were fed on a standard diet containing 0.4 gm. of nitrogen per kilo and 70 calories per kilo of body weight. The diet consisted of beef heart, lard, bread crumbs, sugar, a little salt, and sufficient bone ash to ensure well formed feces. The food was mixed with about 300 cc. of water, the intake of the latter being constant for each day, except in such animals as received toluylenediamine *per os*. The animals were not catheterized, the urine being collected daily and preserved by cold storage; the feces were marked by carmine.

[3] Too much cooling should not be allowed, otherwise the addition of water will cause solidification, which is not desirable.

[4] In the analysis of feces, it is necessary at this point to filter off the calcium sulfate, care being taken to wash the filter thoroughly with hot water.

[5] Where Neumann suggests the bubbling of air through the solution to prevent overboiling, we have found it practicable to use glass beads. The water which evaporates during the boiling is replaced in order to prevent the potassium sulfate from crystallizing out during the cooling attending the filtration.

The dogs were placed on the standard diet a few days before the beginning of an experiment. After a period of 1 week, during which the normal elimination of iron in the urine and feces was determined, the animals were operated upon[6] and pieces of the liver and spleen were removed for the determination of the normal iron content.[7] Upon recovery, the animals were again placed under observation and the urine and feces analyzed to determine whether there had been any change in the iron output. Following this, an hemolytic agent was administered, and after studying the output of iron for varying periods of time (3 to 10 days), the animals were chloroformed, and the liver and spleen taken for analysis. In all cases these organs, together with the contained blood, were dried on the water bath, ground up into a fine powder, dried again at 100°C., and analyzed for iron. The feces were analyzed in a similar manner. Throughout the work all precautions were taken to prevent the introduction of extraneous iron.

Anemia was produced in one of three ways: (*a*) sodium oleate (Merck) given intravenously, (*b*) toluylenediamine (Merck) administered *per os*, and (*c*) hemolytic immune serum[8] injected intravenously.

Hemoglobin estimations and red blood cell counts were made from time to time to determine the course and severity of the anemia produced.

Influence of Sodium Oleate.

Sodium oleate was selected as an hemolytic agent which produces a single injury to the red cells without continued action. The hemoglobin is set free in the circulating blood and is rapidly removed in large part by the kidney without the retention in the organs of agglutinated and injured red cells gradually undergoing disintegration.

[6] For the operative work we are indebted to Dr. J. E. Sweet of the Department of Surgical Research.

[7] As the results obtained for normal animals were so uniform, this procedure was abandoned in some of the later experiments.

[8] The hemolytic immune serum was prepared by injecting a rabbit repeatedly with 5 cc. of dog blood at intervals of about 5 days. 1 week after the fifth injection, the rabbit was bled and the serum collected.

The results of two of our experiments with this substance are shown in Tables II and III.

In a third experiment similar results were obtained. There was an increase of iron in the urine, corresponding to the period of hemoglobinuria, with a return to normal figures when the urine cleared. No change in the percentage of iron in the feces or liver and spleen was evident. These results indicate that in hemolysis caused by a

TABLE II.

Dog 1. Effect of Sodium Oleate.

Period.	Iron* in.					Remarks.
	Urine per day.	Feces per day.		Spleen.	Liver.	
	mg.	*mg.*	*per cent*	*per cent*	*per cent*	
Oct. 1–7. Control period (7 days).	1.2	19.4	0.138			
Oct. 8. Control operation.				0.187	0.069	Removal of small portions of organs.
Oct. 11–17. Control period (7 days).	1.9	20.7	0.100			
Oct. 23. Sodium oleate given.						100 cc. of 1 per cent solution in vein.
Oct. 24.	8.0					Hemoglobinuria.
" 25, 26.	2.0					No hemoglobinuria.
" 26. Sodium oleate given.		14.6 (Oct. 23–29)	0.100			200 cc. of 1 per cent solution in vein.
Oct. 27, 28.	9.5					Hemoglobinuria.
" 29, 30.	2.0					No hemoglobinuria.
Nov. 1.				0.173	0.069	Animal chloroformed.

* In this, as in all the other tables, the percentage of iron is calculated in terms of dry weight.

simple hemolytic agent the free hemoglobin is rapidly removed by the kidney and the increase of iron in the urine represents the iron of this hemoglobin. If hemoglobin does not appear in the urine the iron of the urine is not increased in amount. Analyses of the organs indicate that if hemoglobin is retained, the amount is insufficient

to change the iron content of the spleen or liver. No change is seen in the figures for the feces.

TABLE III.

Dog 2. Effect of Sodium Oleate.

Period.	Iron in.					Remarks.
	Urine per day.	Feces per day.		Spleen.	Liver.	
	mg.	*mg.*	*per cent*	*per cent*	*per cent*	
Oct. 9–15. Control period (7 days).	1.3	20.1	0.117			
Oct. 19. Control operation.				0.175	0.112	Removal of small portions of organs.
Oct. 27. Sodium oleate given.						Hemoglobin 90 per cent; red blood cells, 5,260,000. 200 cc. of 1 per cent solution in vein.
Oct. 27–29.	1.2	22.3 (Oct. 27–31)	0.114			Oct. 28. Hemoglobin 85 per cent; red blood cells 5,180,000. No hemoglobinuria.
Oct. 30. Sodium oleate given.	1.4					300 cc. of 1 per cent solution in vein. No hemoglobinuria.
Oct. 31.	3.9			0.181	0.108	Hemoglobin 72 per cent; red blood cells 4,950,000. Slight hemoglobinuria. Animal chloroformed.

Influence of Toluylenediamine.

In the second series of experiments toluylenediamine was selected because it produces a destruction of blood characterized, when moderate doses are given, by choluria rather than by hemoglobinuria, thus making it possible to determine the elimination of iron in a type of anemia in which, if iron is excreted by the kidney, the excretion is not complicated by the presence of free hemoglobin. Moreover, toluylenediamine, unlike sodium oleate, which produces its

effect in a short space of time, has a more prolonged cumulative effect and destroys red cells, according to Joannovics and Pick (20) and Maidorn (21), in part at least, through an hemolytic agent which is intimately connected with the products of fatty degeneration. The mechanism of blood destruction is, therefore, of a more complicated type than that due to sodium oleate. An experiment of this type, with intoxication for 1 week only, is shown in Table IV.

TABLE IV.

Dog 3. Effect of Toluylenediamine.

Period.	Iron in.					Remarks.
	Urine per day.	Feces per day.		Spleen.	Liver.	
	mg.	*mg.*	*per cent*	*per cent*	*per cent*	
Nov. 25–Dec. 1. Control period (7 days).	1.2	19.7	0.101			
Dec. 8. Control operation.				0.187	0.104	Removal of small portions of organs.
Dec. 11–17. Toluylenediamine for 7 days.	1.6	21.6	0.108			0.2 gm. of toluylenediamine *per os* daily. Choluria daily; no hemoglobinuria.
Dec. 18.				0.283	0.146	Animal chloroformed.

In this animal, despite the fact that a moderately severe anemia was present, as shown by 3,300,000 red cells and 58 per cent of hemoglobin on December 17, practically no change in the elimination of iron occurred. On the other hand, the figures show a considerable increase in the percentage of iron in the liver and spleen.

In a second experiment in every way similar, except that toluylenediamine was administered for 9 days instead of 7, analogous results were obtained. The iron in the urine in the period of anemia was 1.6 mg. daily as compared with 1.4 mg. for the control period, and the percentage of iron in the feces, 0.110 and 0.103 for the respective periods. The iron in the spleen and liver, respectively, in the anemia period amounted to 0.299 and 0.154 per cent, as compared with 0.190 and 0.104, respectively, for the normal. The urine

of this animal was constantly bile-stained and the hemoglobin content of the blood fell from 86 to 58 per cent.

In another pair of experiments, toluylenediamine was given for 1 week and discontinued for the same period. The object of these experiments was to determine whether a delayed elimination of iron occurred simultaneously with a resorption of iron from the liver and spleen.

In these and some of the later experiments the preliminary control operation, for the purpose of obtaining tissue for iron analysis, was not done. As may be seen in Table V, the figures for iron in tissues obtained by this preliminary procedure are fairly uniform, averaging 0.104 per cent for the liver and 0.185 for the spleen. In view of this uniformity, the control operation was, therefore, not always done as a necessary part of each experiment, and it was assumed that the normal iron content of the liver is never more than 0.112 per cent, and that of the spleen never more than 0.190 per cent. Reference to Table V will show that these figures are secured by excluding only one extremely low figure, that for the liver of Dog 6.

TABLE V.

Per Cent of Iron in the Liver and Spleen of the Normal Dog.

Dog No.	Liver.	Spleen.
4	0.104	0.187
5	0.104	0.190
6	0.069 (?)	0.187
7	0.098	0.180
8	0.108	0.187
9	0.100	0.188
10	0.112	0.175
Average.....	0.104	0.185

The results of an experiment in which a recovery period of 1 week was allowed after the administration of toluylenediamine are shown in Table VI.

In a second experiment carried out on exactly the same lines, the urine figures in mg. for the three periods were 1.4, 1.6, and 1.5; those for the feces were 18.6, 20.7, and 19.3 with percentages of 0.107, 0.110, and 0.100. The percentage of iron in the liver was 0.132 and in the spleen 0.233. The urine was bile-stained throughout the

period of intoxication. The blood examination gave 101 per cent of hemoglobin in the first period, 70 at the end of the second, and 98 at the end of the third.

These experiments show no evidence of immediate or delayed iron elimination by the kidney or intestine and the figures for iron in the liver and spleen, definitely higher than those for normal organs, would appear to indicate that the increased amount retained, as the result of blood destruction, is not readily given up by these organs.

TABLE VI.

Dog 11. Toluylenediamine with an Interval for Recovery.

Period.	Iron in.					Remarks.
	Urine per day.	Feces per day.		Spleen.	Liver.	
	mg.	*mg.*	*per cent*	*per cent*	*per cent*	
Jan. 19–25. Control period (7 days).	1.5	16.6	0.106			Jan. 26. Hemoglobin 102 per cent; red blood cells 5,240,000.
Jan. 26–Feb. 1. Toluylenediamine for 7 days.	1.4	18.2	0.100			0.2 gm. of toluylenediamine daily. Urine constantly bile-stained. Feb. 1. Hemoglobin 70 per cent; red blood cells 3,580,000.
Feb. 2–8. Recovery period.	1.7	17.2	0.103			
Feb. 9.				0.253	0.140	Hemoglobin 95 per cent; red blood cells 4,990,000. Animal chloroformed.

Influence of Hemolytic Immune Serum.

Hemolytic serum was used in part on account of its definite hemolytic effect, but chiefly because of its agglutinative effect on red cells and their consequent accumulation in the liver, spleen, and lymph nodes. The red cells responsible for the spodogenous tumor of the spleen and enclosed in the endothelial cells of the liver and lymph nodes represent an accumulation of hemoglobin gradually disintegrating and therefore altering profoundly the iron content of these organs. All injections of serum[8] were made intravenously.

Two experiments (Table VII) support the histological evidence that agglutinated and altered red cells are held in the organs immediately after the administration of an agglutinative and hemolytic

TABLE VII.

Effect of Hemolytic Serum.

Dog No.	Period.	Iron in.		Remarks.
		Liver.	Spleen.	
		per cent	*per cent*	
12	Nov. 3. Control operation.	0.098	0.180	
	" 9. Serum given.	0.177	0.287	0.5 cc. of serum per kilo. Died in 3 hrs.
13	Nov. 9. Control operation.	0.100	0.188	
	" 11. Serum given.	0.176	0.301	0.4 cc. of serum per kilo. Died in 3 hrs.

TABLE VIII.

Dog 14. Effect of Hemolytic Serum.

Period.	Iron in.					Remarks.
	Urine per day.	Feces per day.		Liver.	Spleen.	
	mg.	*mg.*	*per cent*	*per cent*	*per cent*	
Dec. 6–12. Control period (7 days).	1.7	11.0	0.110			
Dec. 15. Control operation.				0.108	0.187	Hemoglobin 105 per cent; red blood cells 9,220,000.
Dec. 17–23. Period of anemia* with 4 days of hemoglobinuria.	6.1	31.2	0.120			Dec. 22. Hemoglobin 55 per cent; red blood cells 5,630,000.
Dec. 24.				0.204	0.447	Animal chloroformed.

* On Dec. 17, the animal received 0.06 cc. of serum per kilo and on Dec. 18, 0.09 cc. per kilo. These injections were followed by the appearance of bile pigment in the urine. On Dec. 19, 0.18 cc. of serum per kilo was administered; this was followed by hemoglobinuria persisting for 4 days.

serum. The dogs in question died within 3 hours after receiving the serum, and the increase in iron content cannot therefore be ex-

plained by a deposition of free iron but as due to the masses of erythrocytes held in the endothelial cells and small vessels of the organs. It should be noted that in these two experiments we returned to the practice of performing a preliminary control operation in order to determine the exact amount of iron in the normal organs. The estimation of iron in the urine and feces was impossible on account of the short time the animals survived the injection.

TABLE IX.

Dog 15. Effect of Hemolytic Serum.

Period.	Iron in.					Remarks.
	Urine per day.	Feces per day.		Liver.	Spleen.	
	mg.	*mg.*	*per cent*	*per cent*	*per cent*	
Control period (7 days).	1.8	21.1	0.098			Mar. 28. Hemoglobin 98 per cent; red blood cells 5,120,000.
Mar. 29–Apr. 4. Period of anemia* with 3 days of hemoglobinuria.	11.1	30.8	0.105			Mar. 30. Hemoglobin 50 per cent; red blood cells 3,980,000.
Apr. 5–11. Choluria but no hemoglobinuria.	1.5	23.6	0.103			Apr. 10. Hemoglobin 40 per cent.
Apr. 17.				0.245	0.490	Hemoglobin 30 per cent; red blood cells 2,780,000. Animal chloroformed.

*On Mar. 29, the injection of 0.17 cc. of serum per kilo caused the appearance of bile pigment in the urine; 0.23 cc. on Mar. 31 caused hemoglobinuria lasting 3 days, and the persistence of bile pigment until the end of the experiment.

In two other experiments of the same type, with smaller doses of serum (0.25 cc. per kilo) and without preliminary operation, the animals were killed by chloroform at the end of 18 and 24 hours respectively. The same increase of iron in the liver and spleen was found—0.176 and 0.178 per cent for the respective livers and 0.318 and 0.307 per cent for the spleens.

In a fifth experiment (Table VIII), with preliminary operation,

the analysis of the feces shows that although the total iron in the feces is increased, the percentage remains practically unchanged. The increase of iron in the urine is due to the presence of hemoglobin. As usual, the iron content of the liver and spleen is increased.

In the final experiment (Table IX) of the series, the observations were continued for 2 weeks through a period of prolonged severe anemia. This experiment shows clearly that the iron of the urine is increased only in the presence of hemoglobinuria. During the period of April 5 to 11, blood disintegration continued, as shown by the continued fall of hemoglobin and red cell count and by the presence of bile in the urine, but the iron content of the urine was lower than in the control period. The total elimination of iron in the feces was appreciably higher in the first period of anemia, but the percentage was altered but little. These variations are to be explained, as also those given in Table VIII, by differences in the bulk of feces. The figures for iron in the liver and spleen showed the usual increase.

SUMMARY.

Blood destruction due to a single injury, as by sodium oleate, or acting through a short period of time, as by toluylenediamine or hemolytic immune serum, is not characterized, in the absence of hemoglobinuria, by an increased elimination of iron in the urine. This holds, not only for the evanescent injury caused by sodium oleate, but also for the severe type caused by hemolytic immune serum, in which a progressive destruction of the blood may persist for 2 weeks or more with constant evidence of the disintegration of erythrocytes as shown by bile pigment in the urine. This finding is in accord with previous investigations of anemia in both man and animals.

Likewise, no striking increase is evident, under such circumstances, in the percentage of iron excreted in the feces. The total amount of iron in the feces has been notably increased in two experiments with hemolytic serum, but as the percentage was not appreciably altered, the difference depends presumably on variations in the bulk of feces rather than upon increased elimination.

This evidence of the power of the body to conserve the iron re-

sulting from erythrocytic disintegration is further emphasized by the increased storage of iron in the liver and spleen. This storage is not evident in the slight transient injury caused by sodium oleate, but in the more prolonged anemia due to toluylenediamine and hemolytic serum, the iron content of the spleen and liver is greatly increased, and in the case of anemia due to hemolytic serum is more than doubled.

In view of this definite evidence of the power of the animal body to conserve iron, it is obvious that in the hemolytic anemias in man characterized by excessive elimination of iron in the feces, some other factor than mere blood destruction is operative. Theoretically, it may be assumed that it is a disturbance of the mechanism concerned in the retention or conservation of iron. This phase of the problem we are now studying in experimental types of chronic progressive anemia due to a persisting and ever active cause; and we trust that the studies may throw some light on the iron exchange in anemia and in particular upon the class of hemolytic anemias so markedly benefited by splenectomy.

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