THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

XI. The Influence of the Spleen on Iron Metabolism.*

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This investigation was undertaken to determine whether the tendency to anemia in splenectomized dogs and the delayed regeneration of the blood after the administration of hemolytic agents to such dogs[1] might be due in part to some influence of the spleen upon the iron metabolism, as has been claimed by Asher (1).

Our present knowledge concerning iron metabolism may be summarized as follows: Iron is absorbed only to a very limited extent from the gastro-intestinal tract, so that when abundant in the food it passes for the most part unchanged from the intestine in the feces. As much as is absorbed is taken up chiefly from the small intestine and carried by the lymph to be deposited in the liver and to a lesser extent in the spleen, bone marrow, and perhaps elsewhere, and this occurs whether the iron be in intimate organic combination, the so called food iron, incapable of giving the characteristic microchemical reaction, or whether it be in the form of an organic or inorganic salt of iron. Moreover, from the work of Häusermann (2) and of Abderhalden (3), it appears that though iron salts are absorbed, the body is unable, or but very poorly able, to utilize them for the building of hemoglobin, being dependent for this constructive work upon the intimately combined food iron. On the other hand, iron salts are effective stimulants to the blood-

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forming organs and conspicuously increase the utilization by them of the food iron.

The elimination of iron occurs almost wholly through the intestines, especially the colon, the quantity passing out in the urine constituting less than 1 per cent. of the total excretion in man and the dog. In the fasting dog the output found by von Voit (4) was 0.60 of a milligram per kilo of body-weight per day, and on an adequate, but iron-poor diet, Gottlieb's (5) dog excreted 0.34 of a milligram. For man the figures are lower. Cetti and Breithaupt (6), while fasting, eliminated about 0.10 to 0.13 of a milligram per kilo per day; and in various studies on man 0.10 to 0.25 of a milligram per kilo per day have been found to be the intake required to maintain iron equilibrium. However, there is every reason to believe, as is suggested by the work of Schmidt (7), who fed mice for months on a diet extremely poor in iron, but obtained no fall in the hemoglobin, that the organism possesses great power of conserving its iron and of reutilizing it through some form of intermediary metabolism. When, however, Schmidt withdrew iron from the diet for several generations, the younger generations were extremely anemic and this anemia disappeared upon restoring iron to the diet. As the iron-poor diet led to the disappearance of microchemically demonstrable iron from the liver, but affected to a much slighter degree that of the spleen, Schmidt concluded that the liver is the depot for iron from the food, and that the spleen, on the other hand, is the depot for iron from tissue and erythrocyte catabolism and thus an important factor in the intermediary metabolism of iron.

If the spleen plays this part in iron metabolism, its absence might well interfere with the reutilization of iron by the organism and lead to an increased iron elimination, and this Asher and his coworkers, Grossenbacher and Zimmermann, claim to have demonstrated in dogs. They studied the iron elimination of four puppies from two litters; one from each litter was splenectomized and one from each was kept as a control. The iron estimations were made at intervals of a few weeks, two months, and ten months after splenectomy, and in all their experiments they found an output much higher, often double, in the splenectomized animals as compared with the controls.

Bayer (8) has made some studies on man of the iron elimination following splenectomy for rupture of the spleen or for Banti's disease, and has compared the output for a certain number of days on known diets with that of control cases on the same diets. His results are summarized in the following table.

TABLE I.

Disease.	Age in yrs.	Time after splenectomy.	Output of iron in mg. per kilo per dy.	
			Splenectomized.	Control.
Spleen rupture.....	16	2 wks.	0.22	0.16
			0.15	0.18[2]
Spleen rupture.....	16	3 wks.	0.30	0.22
			0.17	0.08
			0.51	0.50
Banti's disease.....	25	6 mos.	0.19	0.18[2]
Banti's disease.....	19	2 yrs.	0.19	0.18[2]

From these experiments the author concludes that there is an increased output of iron soon after splenectomy, as shown by the second observation in the table, but that later the elimination returns to normal. Bayer's statement that certain of his diets contained 0.24 of a gram of iron per day is probably an error since a diet of the general character that he describes would certainly have a much lower iron content.

METHODS.

In our earlier experiments we studied the iron elimination during four-day periods, but found that these periods led to irregular results. In the work here reported, therefore, we present only observations based on periods of nine or ten days' duration.

The animals were placed in metabolism cages with glass floors and after they had been fed for several days on constant weighed amounts of the diet selected, the rectum was emptied by the use of morphin; iron-free charcoal was added to the next feeding, and the collection of feces was begun from the appearance of the charcoal; at the close of the period the rectum was again emptied with morphin, carmine was added to the next feeding, and the feces were collected until carmine appeared in them. In the earlier experiments

[2] These three figures are merely repetitions of a single control experiment.

the urine also was analyzed, but as only traces of iron, less than 1 per cent. of the total elimination, were found the urine was omitted in our later analyses. To avoid the introduction of extraneous iron, the feces were collected by means of a nickel spatula soon after being passed.

In one group of experiments representing the earlier periods after operation, we have studied the output of iron on the same dogs, both before and after splenectomy, without a change in diet. In another group, representing later periods, we have compared the output of normal control dogs with that of splenectomized dogs of approximately the same weight on corresponding diets.

The analyses were made by the method of Ripper and Schwarzer (9), slightly modified. The feces collected for the entire period are placed in a quartz dish, dried, and ashed dry. The ash is extracted with boiling concentrated hydrochloric acid and filtered, and the residue washed with 20 per cent. hydrochloric acid. The residue and filter paper are re-ashed and the extraction is repeated. This ashing and extraction is continued until the extract ceases to give a positive test with potassium sulphocyanide.

The total filtrate is made up to a known volume and two duplicate portions, containing presumably two to five milligrams of iron, are taken. To each is added one cubic centimeter of hydrogen peroxide (Merck's Blue label), and the solution evaporated to dryness on a water bath. The residue is then redissolved in one cubic centimeter of 20 per cent. hydrochloric acid and twenty cubic centimeters of boiling water are used in four small portions, and then this washing with acid and water is repeated. In the course of the manipulation the entire solution is brought into a 200 cubic centimeter Erlenmeyer flask.

All the specimens to be analyzed at one time having been brought to this stage, a standard is prepared by placing into each of two 200 cubic centimeter Erlenmeyer flasks forty cubic centimeters of a quantitative ferric chloride solution containing about 0.002 of a gram of iron. To each of the flasks, those containing the specimens and the two containing the standard, there are added in rapid succession four grams of potassium iodide; the flasks are then immediately stoppered and placed in a water bath at 60° C. for ten

minutes. At the end of this time the flasks are removed, and to each 100 cubic centimeters of cold water are immediately added and the flask is restoppered.

To each flask in turn is added starch solution, and the contents are titrated with sodium thiosulphate solution, approximately N/250, until disappearance of the blue color, and then they are immediately titrated with weak iodin solution back to the first reappearance of the blue color. In each analysis the thiosulphate solution is freshly prepared and standardized against the two flasks of known ferric chloride solution, and the iodin solution also is freshly prepared and standardized against the thiosulphate solution. The precision of the titration method is found to be greatly enhanced by the titration back with iodin to the first reappearance of the blue color and calculation accordingly of the thiosulphate end point.

In control experiments performed by adding known amounts of iron to one of identical pairs of samples of ash of feces, an error of about 2 per cent. was observed.

The food used in these experiments consisted of casein, cracker meal, lard, and fresh beef heart in proportions designed to give the desired amount of iron. The iron content of the food was determined by analyzing many large portions (each 50 to 400 grams) of the beef heart, cracker, and casein and obtaining average figures for use in calculating the iron content of the diets employed.

RESULTS.

In the accompanying tables are given in detail the final figures obtained in our studies. The experiments are divided into two groups. First, five animals were studied both before and for two weeks after splenectomy, on a constant diet throughout; these are arranged in table II according to the iron content of the diet. Second, a group of six animals (table III), three normal controls and three splenectomized animals, were studied at longer periods after splenectomy; these were of about the same weight and were on diets of the same general character, but varying in the content of iron.

Inspection of table II shows that the iron output of dogs 88 and

35 is unchanged by splenectomy, but that dogs 30, 44, and 79 show some increase. On the other hand, in table III, it will be seen that all three splenectomized dogs exhibit an output of iron as compared with the intake closely comparable with that of the controls. From our studies it would appear therefore that during the first two weeks after splenectomy, some, but not all dogs show a slight increase in the output of iron, but that at 1 month, 9 months, and 20 months after splenectomy we find no indication of an increased iron output. The occasional evanescent and inconstant increase in elimination of iron does not justify the conclusion that the spleen exerts an important influence on iron metabolism. Our

TABLE II.

Dog No.	Average weight.	Duration of periods.	Intake.[3]	Output.[3]		Time after splenectomy.
				Before splenectomy.	After splenectomy.	
88	7,000	10 dys.	0.27	0.67	0.70	4–14 dys.
30	5,340	9 dys.	0.30	0.36	0.55	1–10 dys.
35	7,720	9 dys.	0.64	0.87	0.81	1–10 dys.
44	9,000	9 dys.	1.57	1.89	2.10	1–10 dys.
79	9,000	9 dys.	1.71	1.88	2.21	6–15 dys.

TABLE III.

Dog No.	Controls.				Time after splenectomy.
	Average weight.	Duration of period.	Intake.[4]	Output.[4]	
79	9,000	9 dys.	1.00	1.42	
44	9,000	9 dys.	1.57	1.89	
79	9,000	9 dys.	1.71	1.88	
		Splenectomized.			
83	8,400	10 dys.	1.42	1.39	27–37 dys.
9	8,800	9 dys.	1.35	1.56	9 mos.
51	10,000	9 dys.	1.32	1.42	20 mos.

results are obviously different from those of Asher and his associates, and as a possible explanation of this we would call attention to the extreme shortness of the periods—one to three days—employed

[3] Figures expressing intake and output indicate milligrams of iron per kilo per day.

[4] Figures expressing intake and output indicate milligrams of iron per kilo per day.

by Asher and Grossenbacher, and to their failure to mark in any way the stools. In the studies of output ten months after splenectomy, as given by Asher and Zimmermann, the splenectomized dog in most of the experiments was much larger than the control, so that if the iron output of their dogs be calculated per kilo of body-weight it will be found that the output of the splenectomized animals approaches very closely that of the normal controls, and is in some instances identical. It seems possible that in these studies ten months after splenectomy the increased iron output of the splenectomized animals was due rather to the size of the animal than to the splenectomy, and it is doubtful, therefore, whether the conclusions of Asher and Zimmermann, based on these experiments, are justified.

CONCLUSIONS.

Our studies give evidence of increase in the iron elimination in three of five dogs during a period of two weeks following splenectomy, but not in two other dogs. The occasional increased output of iron may have some relation to the anemia which occurs in the early weeks after splenectomy and which varies in degree in different animals.

No evidence was secured of an increase in the iron output at 1, 9, and 20 months after splenectomy.

From our own studies and from examination of the literature of the subject, we conclude that the spleen does not exercise a constant and important influence upon the iron metabolism of the body.

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