THE METABOLISM OF COPPER AND IRON IN SPLENECTOMIZED RATS FREE FROM BARTONELLA MURIS INFECTION

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In previous studies it was found that the chemical changes in the blood of splenectomized rats suffering from *Bartonella muris* anemia consist of an increase in the total fats and fatty acids, a drop in lecithin and total cholesterol, a rise in blood chloride concentration, a moderate acidosis, and a variable drop in blood sugar. The liver glycogen is almost depleted. These changes are attributable either to the severity of the anemia or to the infection. Blood chemistry studies on splenectomized rats that were not carriers of *Bartonella muris* and in which no infection or anemia occurred revealed no significant deviation from the normal. Splenectomy in the rat free from *Bartonella muris* infection has no effect on metabolism that is demonstrable in the usual chemical analyses of the blood.

The present studies were undertaken in order to determine the effect of splenectomy on the metabolism of copper and iron in the rat free from latent *Bartonella muris* infection.

A relationship between the function of the spleen and the utilization of copper in the body was suggested by the experiments of Perla and Marmorston-Gottesman, and Perla, who observed the protective effect of an excess of copper in the diet on the natural resistance of albino rats to *Bartonella muris* anemia (1), *Trypanosoma lewisi* (2) and *Trypanosoma equiperdum* (3) infections. These diseases severely injure the spleen. In the case of *Bartonella muris* anemia a specific protective function of the spleen has been established (4). These studies suggested that the utilization of copper in the body may be dependent on splenic function. Further, it was found that in *Trypanosoma equiperdum* infection of albino rats there was a striking decrease in the concentration of copper in the spleen with no change in its con-

centration in the liver. The iron retention in the liver, however, was markedly increased (5).

The extensive literature on the relation of the spleen to the metabolism of iron has been critically reviewed in the monograph of Lauda and Haam (6), and by Wilson and Krumbhaar (7).

There is a considerable difference of opinion to be found in the literature concerning the effect of splenectomy on the concentration of iron in the liver, and on the metabolism of iron. Tedeschi (8) observed that the liver of the rabbit and of the guinea pig contains more iron pigment histologically after splenectomy than the organs of normal animals. Chevallier (9), however, found no increase in iron in the liver of rabbits during a period of 8 weeks following splenectomy. In a series of rabbits, chemical analyses of the iron content of the liver at intervals of 1 to 8 weeks after removal of the spleen did not demonstrate any increase in iron concentration in the liver according to Lauda (6). He observed, however, that in guinea pigs after a postoperative interval of 3 or 4 weeks the iron content of the liver seemed increased. Similar observations were made by Asher, Chevallier, and others. Lauda (6) believed that since the guinea pig was the only laboratory animal in which he found an increase of iron in the liver following splenectomy, some extraneous unknown factor was responsible for the increase, and that this did not reflect a direct disturbance in splenic function. Asher and Tominaga (10) found an increase in iron in the liver and kidneys of white rats following splenectomy, but this undoubtedly was associated with *Bartonella muris* anemia. Lauda observed no increase in the concentration of iron in the liver of splenectomized mice, nor in splenectomized doves.

In studies on rabbits, dogs, guinea pigs, and rats, Asher and his associates (11) found a marked and permanent increase in iron elimination after removal of the spleen. They believe that partial compensation for the loss of the splenic storehouse of iron was demonstrated by an increase in the iron content of the liver after splenectomy. In their opinion the spleen acts to prevent loss of iron from the body and therefore plays a principal rôle in the metabolism of iron.

Pearce and Krumbhaar and their associates (12) were unable to demonstrate a constant increase in the iron output following splenectomy in dogs. In those instances in which there was a significant increase in the elimination of iron in a splenectomized animal, it was associated with a marked secondary anemia. The exact reason for the anemia they were unable to determine. Wilson and Krumbhaar (7) estimated the iron balance in six dogs before and after splenectomy. In two intact dogs and in five dogs before and after control operative procedures other than splenectomy, the iron balance was found to be positive in the intact controls and in three of the operated controls, while a negative balance occurred in two of the operated controls which, however, had developed a mild postoperative anemia. Of the six splenectomized dogs, five showed an increased elimination of iron after splenectomy. This coincided with the period of developing

anemia. The authors suggested that, though the post-splenectomy anemia might have been the cause of the negative balance, splenectomy removed an iron depot and that the loss of iron thus produced was the cause of the anemia.

These investigators failed to stress the presence of latent *Bartonella canis* infection in the dog, a disease which often becomes manifest, following removal of the spleen, and is associated with a prolonged anemia (Kikuth (13)).

From a critical analysis of the literature Lauda concluded that there is insufficient proof that the spleen plays any rôle in the metabolism of iron or in hemoglobin formation.

Methods

In our experiments, twelve male and female albino rats, free from *Bartonella muris* infection, were used. They were all 3 to 4 months of age and of approximately similar weights. The rats were kept in metabolism cages constructed with false bottoms so that the animals had no access to their feces. The cage rested on a funnel, the opening of which was covered with glass wool saturated with toluene and the stem of which fitted tightly into the neck of the collection bottle. Three rats were kept in a single cage. The diet consisted of purina in small checkers, and copper-free water which was supplied through drop bottles fitted with glass nozzles. Copper-free water was used throughout for washing everything that came in contact with the rats, as well as all the glassware used for the chemical determinations.

The urine and feces in each cage were collected twice a week. The urine was analyzed for uric acid (Benedict and Franke (14)), creatine and creatinine (Folin's microchemical modification (15)), total nitrogen (Kjeldahl), and copper (McFarlane (16)). The feces as well as the purina cubes were analyzed for total nitrogen (Kjeldahl), copper (McFarlane), and iron (modified Elvehjem and Hart method (17)). The elimination of iron in the urine is insignificant and was therefore disregarded.

The nitrogen metabolism and the metabolism of copper and iron were studied in the rats during a control period of 8 weeks prior to splenectomy and during 7 weeks after splenectomy. No differences in the results were noted in the males and females.

Nitrogen Metabolism in Splenectomized Rats Free from Bartonella muris Infection

The splenectomized rats remained apparently well and showed no evidence of anemia. The nitrogen metabolism remained unchanged until 3 weeks after splenectomy. Following this there was an increased retention of total nitrogen with a decreased excretion of nitrogen in urine and feces. The creatinine and creatine remained strikingly constant throughout the experimental period. The uric acid remained practically unchanged (see Table I).

Iron Metabolism in Splenectomized Albino Rats Free from Bartonella muris Infection

Following splenectomy there was an increased retention of iron during a period of 4 to 6 weeks after the operation. Then a drop

TABLE I

Nitrogen Metabolism in Normal and Splenectomized Rats Free from Bartonella muris Infection

Date	Daily average per rat											
				Total N								
	Urine	Feces	Purina	Urine	Feces	Total excreted	Intake	Retention	Total creatinine	Preformed creatinine	Creatine	Uric acid
1933	*cc.*	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Nov. 28–Dec. 4	8	1.8	9.6	207	125	332	394	62	9	5	5	1.20
Dec. 5–11	8.6	2	9.6	216	124	340	394	54	10	5	6	1.18
Dec. 12–18	11	1.9	9.6	206	121	327	394	67	11	5	7	1.31
Dec. 19–25	10	1.9	9.8	221	127	348	402	54	10	5	6	1.28
1933–34												
Dec. 26–Jan. 1	10.5	2	9.6	215	125	340	394	54	11	6	6	1.30
1934												
Jan. 2–8	9	2	9.6	223	123	346	394	48	8	4	5	1.21
Jan. 9–15	9.5	2	9.7	203	126	329	398	69	10	6	5	1.34
Jan. 16–22	10.5	2	9.8	221	126	347	402	55	8	5	3	1.31
*Jan. 23–29	9	2	9.7	207	122	329	398	69	10	5	6	1.37
Jan. 30–Feb. 5	8.6	2	9.7	221	126	347	398	51	10	5	6	1.35
Feb. 6–12	11	1.9	9.8	209	124	333	402	69	10	5	6	1.34
Feb. 13–19	8	2	9.9	201	105	306	406	100	9	4	6	1.30
Feb. 20–26	7	1.9	9.8	185	108	293	402	109	11	5	7	1.34
Feb. 27–Mar. 5	8	1.9	9.8	187	105	292	402	110	10	5	6	1.36
Mar. 6–12	7	2	9.9	188	106	294	406	112	10	5	6	1.32

* Jan. 23, splenectomized.

occurred and the iron retention decreased to the level observed before splenectomy. This was unassociated with any evidence of hemoglobin destruction.

Copper Metabolism in Splenectomized Rats Free from Bartonella muris Infection

From Table II it is evident that a disturbance in the copper metabolism occurred, following removal of the spleen. For a period of 2 weeks following splenectomy the copper balance remained positive

TABLE II

Copper and Iron Metabolism in Normal and Splenectomized Rats Free from Bartonella muris Infection

Date	Daily average per rat							
	Cu					Fe		
	Urine	Feces	Total excreted	Intake	Retention	Feces	Intake	Retention
1933	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Nov. 28–Dec. 4	0.0052	0.0980	0.1032	0.1383	0.0351	1.976	1.805	−0.171
Dec. 5–11	0.0062	0.1086	0.1148	0.1383	0.0235	1.661	1.824	+0.163
Dec. 12–18	0.0052	0.1061	0.1113	0.1383	0.0270	1.789	1.843	+0.054
Dec. 19–25	0.0054	0.1055	0.1109	0.1411	0.0302	1.705	1.882	+0.177
1933–34								
Dec. 26–Jan. 1	0.0070	0.1090	0.1160	0.1383	0.0220	1.802	1.843	+0.041
1934								
Jan. 2–8	0.0063	0.1115	0.1178	0.1383	0.0205	1.835	1.800	−0.035
Jan. 9–15	0.0063	0.1099	0.1162	0.1397	0.0235	1.663	1.755	+0.093
Jan. 16–22	0.0067	0.0978	0.1045	0.1411	0.0366	1.674	1.774	+0.100
*Jan. 23–29	0.0066	0.1110	0.1176	0.1397	0.0221	1.437	1.756	+0.319
Jan. 30–Feb. 5	0.0074	0.0965	0.1039	0.1397	0.0358	1.347	1.756	+0.409
Feb. 6–12	0.0057	0.1437	0.1494	0.1411	−0.0083	1.613	1.774	+0.161
Feb. 13–19	0.0065	0.1440	0.1505	0.1425	−0.0080	1.382	1.792	+0.410
Feb. 20–26	0.0058	0.1333	0.1391	0.1411	+0.0020	1.660	1.774	+0.114
Feb. 27–Mar. 5	0.0051	0.1420	0.1471	0.1411	−0.0060	1.610	1.774	+0.164
Mar. 6–12	0.0050	0.1413	0.1463	0.1425	−0.0038	1.734	1.792	+0.058

* Jan. 23, splenectomized.

and the retention was the same as in the normal rats during the preoperative period. Then excretion of copper in the feces increased and a negative copper balance resulted which persisted during the remainder of the experimental period. No change in the elimination of copper in the urine was noted.

DISCUSSION

In previous experiments (18) the changes in the concentration of certain chemical constituents of the blood in splenectomized rats of *Bartonella* carrier stock were found to be associated with *Bartonella muris* infection and anemia and were not due to the removal of the spleen itself. In rats free from this infection no change from the normal concentration in the blood constituents occurred. Removal of the spleen in albino rats free from latent *Bartonella muris* infection and in which no anemia develops, results in a disturbance of the copper metabolism which becomes manifest 2 weeks after operation. The increased elimination of copper in the feces is associated with a transiently increased retention of iron, but the negative copper balance persists. No disturbance in nitrogen metabolism occurs.

The reciprocal relationship in the metabolism of copper and iron is difficult of explanation, but bears some resemblance to the relation of calcium and phosphorus metabolism.

The variability in results of experiments on the metabolism of iron in splenectomized animals, found by previous investigators, was doubtless due in part to the presence of latent infections that became manifest following the removal of the spleen. The complicating anemia that was observed in dogs and rats was due to infection and not to an interference with hemoglobin formation resulting from removal of the spleen. (For a discussion of the spleen and latent infection, see the monograph of Perla and Marmorston.[1]) Many species of dogs are carriers of *Bartonella canis* (Kikuth (13), Perard (19), Regendanz (20)), and under certain conditions an anemia develops following splenectomy due to a flare-up of the *Bartonella canis* infection. Most species of rats are carriers of *Bartonella muris*, and splenectomy is followed by an active infection associated with a fatal anemia. Mice are carriers of eperythrozoon coccoides and *Bartonella muris* infections that become active following splenectomy (Schilling (21), Eliot and Ford (22), and Marmorston (23)). Marmorston observed that a third latent infection in the mouse, *Klossiella muris* of the kidney, was unfavorably influenced by splenectomy. Splenectomy in cattle, horses, and sheep results in activation of latent piro-

[1] Perla, D., and Marmorston, J., Relation of the spleen to resistance, to be published.

plasmidae diseases often associated with severe anemia and death (24). Further studies on the effects of splenectomy on metabolism can be reliable only if the presence of latent infections has been rigidly excluded.

From our experiments, therefore, it seems justifiable to conclude that the disturbance in the copper and iron metabolism noted in the animals following splenectomy is due to the removal of the cells of this organ and not to any known extraneous factor.

No disturbance in copper metabolism was noted for a period of 2 weeks after removal of the spleen. It is possible that some substance elaborated by the spleen and essential for the utilization of copper is stored elsewhere in the body and that this supply is exhausted after a period of 2 weeks.

SUMMARY AND CONCLUSIONS

Removal of the spleen in albino rats free from *Bartonella muris* infection is followed by an increased elimination of copper in the feces, which commences 2 weeks after splenectomy. This is associated with a persistent negative copper balance.

An increased retention of iron occurs during a period of 4 to 6 weeks after splenectomy with a return of the iron metabolism to normal after this period.

No disturbance in creatine or creatinine metabolism occurs. The uric acid amount is unchanged. There is an increase in the retention of nitrogen, which is first noted 3 weeks after splenectomy.

The spleen is essential for the utilization of copper in the body.

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