STUDIES ON BARTONELLA MURIS ANEMIA

VIII. CHANGES IN THE CHEMICAL CONSTITUENTS OF THE BLOOD DURING THE ANEMIA

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The purpose of this investigation was to determine the effect, if any, of *Bartonella muris* infection, the resulting anemia, and of the removal of the spleen itself on the protein, fat and mineral metabolism of the rat. Changes in the constituents of the blood of normal adult rats and of adult splenectomized rats at various stages of *Bartonella muris* anemia were studied. In instances in which significant chemical changes in the constituents of the blood were observed in the course of *Bartonella muris* anemia, similar determinations were made as controls on splenectomized rats of Wistar Institute non-carrier stock 1 week after the operation.

Methods and Results

Estimations were made of the total serum proteins, serum albumin, serum globulin, non-protein nitrogen, uric acid, total fat, lecithin, total cholesterol, fatty acids, calcium, phosphorus, carbon dioxidecombining capacity, chlorides, blood sugar, and the glycogen content of the liver at the height of the *Bartonella muris* anemia.

The blood was withdrawn from the heart with the animal under ether anesthesia. All rats studied were 3 to 4 months of age and raised under constant environmental and dietary conditions.

Total Serum Proteins, Serum Albumin, Serum Globulin, Non-Protein Nitrogen and Uric Acid.—Total proteins, albumin and non-protein nitrogen were determined according to the method of Van Slyke (1). Globulin was calculated from the difference between total proteins and albumin. Normal rats, splenectomized anemic rats with a hemoglobin content of 30 per cent and splenectomized rats with a hemoglobin content of 10 per cent were studied. The total proteins of the normal rats vary from 5.5 per cent to 6.4 per cent. Only a slight drop is noted in rats with a low hemoglobin content (4.8 to 5.3 per cent). The albumin of the normal group varies from 3 to 3.4 per cent; in rats with a hemoglobin content of 30 per cent and 10 per cent the albumin drops slightly (see Table I). Total proteins, albumin and globulin drop slightly during the height of the anemia.

TABLE	1
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Total Serum Proteins, Serum Albumin and Serum Globulin in the Blood of Splenectomized Adult Rats with Bartonella muris Anemia

Rat No.	Operation	Hemoglobin determination	Total proteins	Albumin	Globulin
			per cent	per cent	per cent
1	None	Normal	6.1		
2	"	"	5.9	3.4	2.5
3	"	"	6.4	3.4	3.0
4	**	"	6.0	3.0	3.0
5	"	"	5.5	3.3	2.3
6	"	"	5.7	3.0	2.7
		per cent			
7	Splenectomy	20-30	5.1	2.6	2.5
8	"	20-30	4.8	2.6	2.2
9/10*	"	20-30	5.1	2.6	2.5
11/12/13/14/15*	66	2030	4.9	2.6	2.3
16	"	10	5.3	2.8	2.5
17	"	10	5.3	2.8	2.5
18	"	10	5.1	3.1	2.0
19/20/21*	"	10	4.9	2.7	2.2

* Pooled samples.

Non-protein nitrogen in our normal rats averages 40 mg. in 100 cc. of blood. In rats with a hemoglobin content of 20 to 30 per cent the non-protein nitrogen averages 44 mg. in 100 cc. of blood. In rats with a hemoglobin content of 10 per cent the non-protein nitrogen averages 62 mg. in 100 cc. of blood. The non-protein nitrogen definitely rises with the increase in severity of the anemia (see Table II). The uric acid of the blood as determined according to the method of Benedict (2) remains unchanged (see Table II). In human beings, anemia appears to have no characteristic effect on nitrogen metabolism. In pernicious anemia normal non-protein nitrogen values have been reported by Kahn and Barsky (3), though Gettler and Lindeman (4) found the non-protein nitrogen of the

TABLE :

Non-Protein Nitrogen and Uric Acid in the Blood of Splenectomized Adult Rats with Bartonella muris Anemia

Rat No.	Operation	Hemo- globin determi- nation	Non- protein nitrogen	Rat No.	Operation	Hemo- globin determi- nation	Uric acid
			mg. in 100 cc.				mg. in 100 cc.
22	None	Normal	40	43	None	Normal	2.3
23	"	"	38	44	"	"	3.0
24	"	"	40	45	"	"	2.4
25	"	"	41	46	"	"	3.0
		per cent					
26	Splenectomy	20-30	49	47	"	"	2.7
8	"	20-30	46	48	"	"	2.5
27/28*	"	20-30	38	49	"	"	2.5
29/30*	"	20-30	45	50	"	"	2.7
31/32*	"	20-30	47	51	"	"	2.7
						per cent	
9/10*		20-30	36	8	Splenectomy	20-30	2.8
33	"	20-30	45	52	"	20-30	2.3
34	"	10	52	29/30*	"	20-30	3.3
35	"	10	46	31/32*	"	20-30	2.5
36	"	10	60	9	"	20-30	3.3
37	"	10	59	33	"	20-30	2.5
38	"	10	73	53	"	10	2.2
39	"	10	78	18	"	10	2.7
40	"	10	67	35	"	10	1.9
41	"	10	62	34	"	10	2.5
42	"	10	48				
Wistar	"	Normal	38				
"	**	"	39				

* Pooled samples.

blood slightly elevated. Some impairment of the ability to concentrate nitrogen in the urine in patients with severe primary or secondary anemia was observed by Mosenthal (5).

The definite rise in the non-protein nitrogen of splenectomized rats at the height of Bartonella muris anemia is probably attributable to the severity of the Bartonella infection and not to the anemia. During the febrile stage of most acute infectious diseases the nitrogen metabolism as well as the total metabolism is increased (6). This is due not only to increased energy requirements but also to an active destruction of tissues resulting in increased protein catabolism. In severe infectious diseases an increase in the blood non-protein nitrogen may be observed (7-12). In view of the fact that the rise in non-protein nitrogen in severe infections is usually unassociated with the presence of albumin or casts or with an increase in urinary nitrogen (13), it is unnecessary to assume anatomical injury of the kidneys to explain the high non-protein nitrogen in the blood. In Bartonella anemia anatomic evidence of a severe parenchymatous and tubular degeneration (14) is found in many instances and may contribute to the production of a high non-protein nitrogen, but it is unlikely that this plays an important part. In Bartonella muris anemia the high nonprotein nitrogen is probably due to the toxic destruction of protein and a relative oliguria, which is accentuated by the renal injury. Linton (15) observed in experimental studies on T. equiperdum infection of rats a rise in non-protein nitrogen and uric acid of the blood.

Chlorides.—The chloride content of the blood and of the serum were determined by Wilson's modification of the method of Van Slyke (16). Normal blood chlorides vary from 489 to 511 mg. of NaCl in 100 cc. of blood, serum chlorides from 604 to 640 mg. of NaCl in 100 cc. of serum. The chloride content of the blood rises with the anemia to an average of 564 to 609 mg. of NaCl in 100 cc. The serum chloride remains practically unchanged. The difference in the content of chlorides between the blood and the serum follows the drop in the hemoglobin content of the blood. The chloride concentration in the cells is only half of that in the plasma. The high concentration of chlorides in the whole blood of anemic rats is due to the decreased proportion of red blood cells.

Calcium and Phosphorus.—The calcium of the serum was determined according to the Clark-Collip (17) modification of the method of Kramer and Tisdall; the phosphorus according to the method of Fiske and Subbarow (18). The serum of normal rats was found to contain

TABLE III

Chlorides in Blood and Serum of Splenectomized Adult Rats with Bartonella muris Anemia

Rat No.	Operation	Hemoglobin	NaCl				
	Operation	determination	Blood	Serum			
			mg. in 100 cc.	mg. in 100 cc.			
54	None	Normal	498	604			
55	"	"	511	640			
56	"	"	489	634			
		per cent					
52	Splenectomy	2030	602	646			
29/30*	"	20-30	583	592			
31/32*	"	20-30	580	611			
9	"	20-30	598	646			
33	"	20-30	609	665			
53	"	10	592	621			
57/58/59/60*	"	10	564	620			

* Pooled sample.

TABLE IV

Serum Ca and P in Blood of Splenectomized Adult Rats with Bartonella muris Anemia

Rat No.	Operation	Hemoglobin determination	Ca	Р
			mg. in 100 cc.	mg. in 100 cc.
115	None	Normal	9.9	7.7
61	"	"	10.0	7.8
62	"	"	10.0	7.7
63	"	"	10.0	7.8
		per cent		
8	Splenectomy	20-30	9.4	7.3
31/32*	"	2030	9.0	7.8
9/10*	"	20-30	9.2	7.4
29/30*	"	20-30	8.7	—
64/65/66*	"	10	8.5	7.1
67/68*	"	10	8.2	7.6
19/20/21*	"	10	8.2	7.7
116/117/118*	Laparotomy	Normal	8.5	7.2

* Pooled sample.

10 mg. of calcium and 7.7 to 7.8 mg. of phosphorus, in 100 cc. With the development of the severe anemia the calcium decreases slightly,

while the phosphorus remains within the normal range. In anemias in human beings the inorganic phosphorus is not significantly altered. Though the total phosphorus of the whole blood is often reduced the concentration in a unit volume of cells is normal (19, 20).

Carbon Dioxide-Combining Capacity.—The normal carbon dioxidecombining capacity in volume per cent was found to be 52 to 61. With a severe drop in the hemoglobin content, the carbon dioxidecombining capacity of the blood dropped to 26 to 47 volumes per cent.

Rat No.	Operation	Hemoglobin determination	CO ₂
			vol. per cent
69	None	Normal	61
70	"	"	52
71	"	"	57
		per cent	
72	Splenectomy	20-30	48
73	"	20-30	55
74	"	20-30	59
75	"	20-30	48
76	"	20-30	55
77	"	20-30	52
78	"	20-30	62
79	"	10	37
80	"	10	47
81	"	10	26
82	"	10	37
83	"	10	42
84	**	10	26
85	"	10	44

TABLE V

Carbon Dioxide-Combining Capacity of Blood of Splenectomized Adult Rats with Bartonella muris Anemia

The data reported in anemias of human beings indicate that the anemia of itself has little influence on the acid-base balance of the blood or the organism in general (21-24). The carbon dioxide content of the whole blood may be high because the ratio of plasma to cells is high. The carbon dioxide content of plasma is about 1.6 times that of the cells and the carbon dioxide content of whole blood will, therefore, increase with the proportion of plasma which it contains. Severe infections, however, result in reduction of the carbon dioxide content of the plasma, usually attended by an elevation of pH(25-28).

Linton (15), in a study of the blood chemistry of rats infected with T. equiperdum, found a well defined lowering of the carbon dioxide capacity in all the infected rats. An acidosis is present in this disease from an early stage but is not progressive with the severity of the infection. In *Bartonella muris* anemia the acidosis is moderately severe at the height of the infection (as manifested by the height of the anemia) and is apparently attributable to the infection itself and not to the anemia.

Blood Sugar and Liver Glycogen.—Blood sugar determinations were made according to the method of Hagedorn and Jensen (29). The blood sugar of normal adult rats was found to be fairly constant (123 to 147 mg. per 100 cc.). The blood sugar of the splenectomized anemic rats determined under the same conditions—though showing considerable variation, is very low in many instances when the hemoglobin has fallen to 10 per cent.

In pernicious anemia in human beings the blood sugar is found to be high with considerable frequency (30-34) and the degree of hyperglycemia is proportional to the severity of the anemia (31). In most severe infections the decrease in carbohydrate tolerance that develops in the course of the disease may be serious. The metabolic disturbance depends less on the severity than on the type of infection and the association of pyodermic infections with hyperglycemia is well known (35, 36).¹

Linton (15), however, found a terminal drop in blood sugar in pathogenic trypanosome infections in rats. In parasitic infections of this type, Schern (37), von Fenyvessy (38), Scheff (39) and Regendanz (40) likewise observed a drop in blood sugar concentration at the height of the disease.

Liver glycogen was determined according to the method of Slosse (41). In the splenectomized rats glycogen rapidly disappears from the liver with the progress of the *Bartonella muris* anemia. The normal

¹ Hyperglycemia can be produced by injection of killed suspensions of *B. coli*, *B. proteus*, *B. paratyphosus* and *B. enteritidis* (36). Injections of other organisms, such as *Strept. hemolyticus*, *Strept. viridans*, *Strept. aureus*, *B. pyocyaneus* and *B. typhosus*, do not affect the blood sugar.

rats gave readings of 42 to 69 mg. of glucose per 1 gm. of liver. In rats with a hemoglobin content of 30 per cent the liver glycogen dropped to 2 to 16 mg., and it dropped to 0.5 to 0.6 mg. in rats with a hemoglobin content of 10 per cent. Depletion of liver glycogen was found to be complete in rats with T. equiperdum infection by Linton (15).

The liver glycogen is readily depleted in many conditions; for example, starvation, cold, exercise, diabetes, phlorizin poisoning. Starvation may be a factor in the depletion of glycogen in *Bartonella muris* anemia, as the anorexia becomes increasingly severe with the onset of the anemia.²

Lipoid Phosphorus and Lecithin.—Lipoid phosphorus was determined according to the method of Krasnow and Rosen (42). As shown in Table VII, there is a decrease in the lipoid phosphorus of the blood and in lecithin in the anemic rats. The lipoid phosphorus in normal rats is 8.9 to 10 mg. in 100 cc. of blood. In rats with a hemoglobin content of 10 per cent the lipoid phosphorus drops to 5.4 to 7.5 mg. in 100 cc. of blood. Lecithin decreases correspondingly. The decrease varies from approximately 30 to 37 per cent of the values found in the normal animals.

Cholesterol.—Total cholesterol in whole blood was determined according to Sackett's modification of Bloor's method (43). With an increase in the *Bartonella muris* anemia in splenectomized rats there is a decrease in the total cholesterol of the blood. In order to determine the possible influence of operative trauma on the cholesterol content of the blood, cholesterol determinations were made on a series of normal rats before and after laparotomy with the spleen intact. The cholesterol determinations were made 1 week after the operation and showed a drop of about 15 per cent. The decrease in blood cholesterol noted in rats with a hemoglobin content of the blood of 10 per cent varies from 30 to 50 per cent. The cholesterol content of the blood of splenectomized rats uninfected with *Bartonella muris* (Wistar Institute stock) is in the same range as that of the normal laparotomized rats.

Total Fats and Fatty Acids.—Total fats were determined gravimetrically. Fatty acids were calculated from the difference between total fats and the sum of lecithin and cholesterol. Table VII shows

² The liver cells in rats with *Bartonella muris* anemia contain a striking amount of lipoid material.

	Glycogen as mg. glucose in 1 gm. of liver	mg. in I gm.	45	69	42	47		6		4	13	3	2	16	9	2	0.5	0.6	0.5	2		
nemia	Hemoglobin deter- mination		Normal	εί	3	ÿ	per cent	20-30		20-30	20-30	20-30	20-30	20-30	20-30	10	10	10	10	10		
biood Jugar and Liver Glycogen as Glucose in Splenectomized Adult Rats with Bartonella muris Anemia	Operation		None	3	3	3		Splenec-	tomy		3	ų	"	33	3	"	3	3	33	3		
artone	Rat No.		102	103	104	105		75		6	10	72	73	74	17	35	80	81	82	85		
with B	boold ni seosulo	<i>mg. in</i> 100 cc.	66		147	132	72	69	106	106	134	40	58	<u> </u>	132							
Kats	Hemoglobin deter- mination	per	10		10	10	10	10	10	10	10	10	10	10	10							
sed Adult	Operation		Splenec-	tomy	`	33	ï	¥	"	3	"	z	ÿ	z	ÿ							
sctomi	Kat No.		40		41	34	35	18	83	100	85	37	84	39	101							
Splen	Glucose in blood	m g. in 100 cc.	137		108		117	150	129			143	144									
se m	Hemoglobin deter- mination	per cent	20-30		20-30	20-30	20-30	20-30	20-30	20-30	20-30	20-30	20-30									
as Giuco	Operation		Splenec-	tomy	"	"	ÿ	23	3	"	3	ų	ÿ									
rycogen	Rat No.		∞		52	27/28*	29/30*	31/32*	33	72	73	74	75									
1aar	boold ni szozułd	mg. in 100 cc.	130	127	133	133	140	122	147	124	133	131	125	137	123	131	137		123	130	115	
ar ana 1	Hemoglobin deter- mination		Normal	3	3	3	33	3	3	3	¥	3	y,	3	3	3	z		3	3	3	
Suc 20010	Operation		None	¥	¥	ÿ	z	¥	ÿ	z	3	3	y,	3	ť	ų	Splenec-	tomy	3	÷	3	* Pooled sample.
	Rat No.		86	87	88	89	8	91	92	93	94	95	8	67	<u>8</u>	66	Wistar		3	ÿ	3	* Pool

TABLE VI

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that there is a slight rise in total fats and fatty acids with the increase in severity of *Bartonella muris* anemia. The rise in blood fats averages approximately 20 per cent in the rats with a hemoglobin content

	Splenectomized						
Rat No.	Operation	Hemoglobin determina- tion	Total fat	Lipoid P	Lecithin	Total choles- terol	Fatty acids
			mg. in 100 cc.	mg. in 100 cc.	mg. in 100 cc.	mg. in 100 cc.	mg. in 100 cc.
106	None	Normal	1425	8.9	223	114	1088
107	"	"	1275	10.0	249	114	913
108	"	"	1294	9.5	237	116	941
109	"	"		9.1	227	118	
110	"	"				116	
		per cent					
111	Splenectomy	20-30	1462	6.4	161	61	1240
52	· "	20-30	1650	5.2	129	78	1443
33	"	20-30	1587	5.5	138	70	1379
26	"	20-30				91	
112/113*	"	10	1594	5.5	138	91	1365
114	"	10	1525	6.1	152	70	1303
16	"	10	1618	6.5	163	52	1403
17	"	10	1625	6.3	158	66	1401
42	"	10	1700	5.4	134	51	1515
Wistar†	"	Normal	1332	11.4	286	86	960
"	"	"	1550	11.7	291	88	1171
"	"	"	1288	10.8	269	72	947
"	"	"	1332	9.3	232	76	1033
116	None	"				112	
116	Laparotomy	"				90	
117	None	"				105	
117	Laparotomy	"				89	
118	None	"		l		102	
118	Laparotomy	"	1			91	

TABLE VII

Total Fat, Lipoid P, Lecithin, Total Cholesterol and Fatty Acids in the Blood of Normal Rats, of Splenectomized Adult Rats with Bartonella muris Anemia and of Splenectomized Adult Rats Uninfected with Bartonella muris

* Pooled sample.

† Estimations were done 1 week after splenectomy.

of 10 per cent. The rise in fatty acids averages approximately 30 per cent.

In the case of T. equiperdum, Linton (15) found no appreciable

change in the blood cholesterol. In human beings, blood cholesterol appears to be regularly lowered in febrile infectious diseases. The drop follows the severity of the infection. The change, however, in cholesterol and lecithin in severe anemias is well known and is an expression of a general reaction to the loss of red blood cells from the circulating blood (44). This reaction consists in the appearance of an excess of fat and fatty acids in the blood, with a simultaneous decrease of phosphatides and cholesterol.³

The increase in total fats, and the reduction in total cholesterol and lecithin appear only after the red cell count falls below 50 per cent of the normal (45) in human anemias but they bear no direct relation to the degree of anemia.

DISCUSSION

The chemical changes in the blood of splenectomized rats suffering with *Bartonella muris* anemia consist of an increase in 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 in the rats with a hemoglobin content of the blood of 10 per cent. The changes in the lipoids and the rise in blood chloride concentration are attributable to the anemia and are found only when the anemia is severe. The moderate acidosis is an expression of the severity of the systemic infection. The hypoglycemia and the depletion of liver glycogen are terminal phenomena. The hypoglycemia and the low liver glycogen are probably an expression of relative starvation due to the severe anorexia that develops in rats suffering with *Bartonella muris* anemia.⁴

It is plain that none of the chemical changes in the blood can be attributed to a direct disturbance of splenic function. Blood chemistry studies on splenectomized rats (Wistar Institute) in which no infection

³ The one exception seems to be in the chronic anemia of rabbits produced by repeated bleeding. In this condition numerous investigators have demonstrated high blood phosphatides and cholesterol.

• The chemical changes observed by Linton in T. equiperdum infections of rats may in part be due to the *Bartonella muris* anemia which spontaneously occurs as a complication of trypanosome infections (Mayer (46), Marmorston-Gottesman and Perla (47)). occurs and studies on splenectomized rats of carrier stock recovered from the severe anemia reveal no significant deviation from the normal. Splenectomy in the rat has no effect on metabolism that is demonstrable in the usual chemical analyses of the blood.

SUMMARY

1. The blood chemistry of normal adult rats and of splenectomized adult rats suffering with *Bartonella muris* anemia has been studied.

2. Non-protein nitrogen rises with the increase in severity of the anemia. Total serum proteins, serum albumin and serum globulin decrease slightly. Uric acid remains unchanged.

3. Serum chloride concentration remains unchanged, but the concentration of chlorides in the blood increases.

4. An acidosis as estimated by the carbon dioxide-combining capacity of the blood is observed at the height of the infection.

5. The blood sugar decreases and the liver glycogen is depleted in rats with a hemoglobin content of the blood of 10 per cent.

6. Total fats and fatty acids are increased at the height of the anemia. Total cholesterol, the lipoid phosphorus and lecithin are diminished in amount.

7. These changes result from the infection or the anemia. Splenectomy itself plays no part in the changes observed in the chemical constituents of the blood in *Bartonella muris* anemia of adult albino rats.

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