THE BLOOD CHEMISTRY OF AN ACUTE TRYPANOSOME INFECTION

By RICHARD W. LINTON, PH.D.

(From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York)

(Received for publication, April 14, 1930)

The recognition of the fact that the blood chemistry is altered has given a new direction to the study of experimental trypanosome infections.

Most of this work has dealt with the effect of the disease on the blood sugar, owing in part to the relative simplicity with which this factor may be studied, and in part to the claim of Schern, one of the first students of these changes, that the trypanosomiases are diseases in which the host's sugar is utilized by the parasites in a manner entirely analogous with that of bacteria growing in a test tube. The literature dealing with this point has been reviewed by Regendanz (1). Schern (2), von Fenyvessy (3), and Scheff (4), have published figures which show that the blood sugar concentration falls as the number of parasites increases, while other workers, amongst whom Regendanz (1) has published the most complete data, believe that the hypoglycaemia is only a terminal phenomenon. It has become evident that the true state of affairs in these infections is more complicated than Schern supposed, and in the field of carbohydrate metabolism difficulties of interpretation have increased rather than diminished as more investigators have reported their results.

Other blood changes have been much less thoroughly explored. Scheff (2) has published a report dealing with various aspects of the problem, beside the carbohydrate metabolism. He emphasizes the amount of oxygen consumed by the trypanosomes *in vivo*, a phenomenon which gives rise to an oxygen lack in the host, and leads eventually to death by "inner asphyxia." The upset in oxygen metabolism, the exhaustion of the free and reserve carbohydrates, and the probable presence of a toxin, determine the death of the host, in Scheff's opinion.

Scheff's data on the oxygen content of the blood could not be confirmed by Kligler, Geiger, and Comaroff (5). They found but little difference in this respect between normal and infected rats. Nor were the experiments which they devised to show a toxin successful. They incline to the view that the rapid multiplication of the parasites leads to the production of lactic acid in quantities which cannot be

successfully cared for by the host. The oxidative metabolism is thus upset, the alkali reserve depleted, and death ensues.

Discordant results have also been reported in regard to the liver glycogen. Scheff's data point to an exhaustion of this substance as the infection progresses, while Regendanz believes that the liver glycogen is always present in sufficient amount to keep the blood sugar at a normal level, and that the terminal lowering of the glucose is due to the action of a trypanosome toxin on the glycogen-glucose mechanism.

The strain of *T. equiperdum* was obtained from the Hygienic Laboratory in Washington. In rats it produces an acute septicaemic infection which terminates fatally in from 72 to 96 hours, after an incubation period which varies with the infecting dose. The majority of animals will not survive a concentration of trypanosomes much greater than one million per cubic millimeter of blood, although occasionally a concentration of nearly two million is observed. The rat does not show any resistance to the invader, so far as can be discovered. On the other hand, individual animals show marked variations in the number of parasites which they can tolerate before death ensues. Furthermore, the speed of reproduction as shown by the length of time in which the number of parasites doubles, shows considerable variation from rat to rat. Whether these are the results of individual resistance cannot be stated. It is clear, however, from the total counts, that the infection is by no means such a clear-cut, mathematically reproducible one as other workers have described for other strains.

Our present report deals with the foregoing matters and with certain other factors which have not yet been emphasized in trypanosome infections. These are points of attack by the parasites on the host's metabolism, the understanding of which is necessary for a complete picture of the disease. We have been interested in this infection also because of the information it may yield for the study of acute septicaemias in general. Infection with our strain of *Trypanosoma equiperdum* in the rat presents a relatively uncomplicated septicaemia: the infectious agents are confined nearly exclusively to the blood stream, while other tissues are involved to only a slight and irregular degree, and then only when the disease has almost run its course. Antibody production and leucocytic reactions are absent or evident to only a very slight degree; and the reticulo-endothelial system, the fundamental importance of which is well recognized in a large number of infections, is not involved (Dwijkoff (6)).

Methods

The CO_2 combining capacity, non-protein nitrogen, uric acid, and chloride determinations were made by the usual laboratory methods.* Micromethods were used for the other determinations, as follows: Liver glycogen—method of Slosse (7); blood sugar—method of Folin (8); lipoid phosphorus—method of Krasnow and Rosen (9); cholesterol—method of Ling (10). Blood was obtained by heart puncture under light anaesthesia with iso-amyl-ethyl barbituric acid (amytal), approximately 7 mg. being given per 100 gm. of rat. In a few instances where the animals were *in extremis* anaesthesia was not used.

The data on the animals are arranged in the tables according to increasing numbers of trypanosomes per cubic millimeter of blood. It must be emphasized, however, that this arrangement shows only roughly the increasing severity of the disease. With this strain of trypanosomes, and with the stock of rats used, the lethal concentration varies greatly from one animal to another. For example, Rat 9, in Table II, with 925,000 organisms per cubic millimeter was moribund when bled, while Rat 16 in Table I, showing twice as many trypanosomes, still had several hours of life, to judge from its general appearance, breathing, activity, etc. This example is the most extreme one that occurs in our data, but it serves well to illustrate that the arrangement of figures shows a relative rather than an absolute severity of infection.

Carbon Dioxide Combining Capacity

In Table I are presented the data for the CO_2 combining capacities of 14 infected and 2 normal rats. Beginning with the fourth animal in the list (137,000 trypanosomes per cubic millimeter) all the infected rats show a well defined lowering of the CO_2 capacity. An acidosis is thus present from an early stage of the disease, varying in intensity from moderate to severe. The acidosis does not vary with the progress of the infection, as is shown by the figures for Rats 15 and 16, but drops while the number of trypanosomes is still small to varying levels below normal, and remains subnormal throughout.

Non-Protein Nitrogen, Uric Acid, Chlorides

The data on these constituents are presented in Table II, which gives the figures for 4 normal and 10 infected animals. Up to and including Rat 7 the figures fall within the normal ranges, but beginning with

* I wish to thank Mr. S. Graff, of the Department of Biochemistry, for these determinations, and Mr. R. Rebold, of the same department, for the sugar determinations on the hydrolized glycogen.

a higher concentration of trypanosomes (760,000 per cubic millimeter) there is a definite rise in the non-protein nitrogen and in the uric acid to figures which indicate pathological changes in the kidneys. These changes appear as preterminal rises, occurring at a time when 4 to 6 hours represents the estimated length of life.

Histologically the kidneys are normal until a late period in the disease. The changes which they show are those of an early nephrosis. The blood vessels are greatly swollen and in some areas hemor-

TABLE I

Carbon Dioxide Capacity of Serum in Rats Infected with Trypanosoma equiperdum

Rat No.	Trypanosomes per cu. mm.	CO_2 capacity. Vol. per cent
1	Normal	59.5
2	Normal	62.0
3	75,000	65.0
4	137,500	37.0
5	200,000	13.6
6	225,000	45.0
7	275,000	35.0
8	525,000	25.0
9	820,000	47.8
10	825,000	36.0
11	830,000	31.9
12	850,000	20.2
13	1,000,000	31.5
14	1,125,000	14.5
15	1,175,000	46.0
16	1,850,000	40.4

rhages have occurred; the glomeruli are enlarged and fill the capsules; sections stained with Giemsa show them to be swarming with trypanosomes. They seem, however, to be intact, and undamaged, and the same is true of the collecting tubules. The secreting tubules, however, have undergone marked changes; they are swollen, and in some places the cells are broken down; fat has appeared in them, in the form of droplets, in large amounts, while it is absent in the glomeruli and in the collecting tubules. In some cases albumin is present in the urine.

The chlorides do not show any significant variations. In the three

TABLE II

Non-Protein Nitrogen, Uric Acid, and Chlorides in the Blood of Rats Infected with Trypanosoma equiperdum

Rat No.	Trypanosomes per cu. mm.	Non-protein nitrogen. Mg. per 100 cc.	Uric acid. Mg. per 100 cc.	Chlorides. Mg. per 100 cc.	
				Blood	Serum
1	Normal	39.6	2.8	482	590
2	Normal	33.0	3.0	461	592
3	Normal	29.0	1.9	453	—
4	Normal	32.0	2.6	468	—
5	475,000	37.0	2.9	470	—
6	525,000	36.0	3.1	432	573
7	600,000	36.4	2.7	465	575
8	760,000	42.0	4.8	472	—
9	925,000	138.0	11.3	381	—
10	1,050,000	73.0	7.8	420	566
11	1,100,000	47.0	5.6	459	572
12	1,275,000	57.0	5.6	453	—
13	1,450,000	56.0	6.1	470	—
14	1,800,000	40.0	3.9	—	—

TABLE III

Lipoid Phosphorus and Lecithin in Whole Blood of Rats Infected with Trypanosoma equiperdum

Rat No.	Before infection		After infection		Percentage increase	Trypanosomes per cu. mm.
	Lipoid P. Mg. per 100 cc.	Lecithin. Mg. per 100 cc.	Lipoid P. Mg. per 100 cc.	Lecithin. Mg. per 100 cc.		
1	8.05	201	10.35	258	28	25,000
2	7.05	176	9.22	230	30	292,000
3	9.25	231	10.00	250	8	300,000
4	8.9	222	10.85	271	21	575,000
5	8.1	202	11.75	293	42	575,000
6	8.9	222	10.8	270	21	600,000
7	9.6	240	12.5	312	30	625,000
8	7.8	195	10.55	253	35	740,000
9	8.8	220	13.3	332	51	850,000
10	9.05	226	11.6	290	28	1,200,000
11	8.8	220	17.5	437	98	1,800,000

instances in which they differ at all markedly (Rats 6, 9, and 10) they are lower than the normal range.

Lipoid Phosphorus and Lecithin

Table III contains the data for lipoid phosphorus, and the calculated lecithin content in eleven animals before and after infection. Without exception the animals show increases in these constituents. The increase begins very early in the course of the infection, when the parasites have been in the blood but a few hours (Rat 1), and has become nearly twice normal when the disease ends. With two exceptions (Rats 3 and 11) the increase varies between approximately 20 per cent and 50 per cent of the figure obtained from the normal animal.

TABLE IV

Cholesterol in Whole Blood in Rats Infected with Trypanosoma equiperdum

Rat No.	Cholesterol before infection. Mg. per 100 cc.	Cholesterol after infection. Mg. per 100 cc.	Trypanosomes per cu. mm.
1	76		
2	81		
3	68		
4	71		
5	87		
6	80		
7	86		
8	82		
9	80	74	717,000
10	74	76	750,000
11	67	67	820,000
12	76	85	830,000
13	73	69	850,000
14	76	78	900,000
15	74	84	1,250,000
16	76	83	1,275,000
17	66	69	1,850,000

Cholesterol

Cholesterol determinations are given in Table IV. The figures show that no changes in this blood constituent occur as the result of

the trypanosome infection. The amounts of cholesterol during the severe stages of the infection are all within the normal range, and vary but a few milligrams from the determinations before infection.

TABLE V

Liver Glycogen, as Glucose, and Blood Sugar, in Rats Infected with Trypanosoma equiperdum

Rat No.	Trypanosomes per cu. mm.	Grams of liver taken	Glucose. Mg. per gram of liver
1	Normal	1.162	20.1
2	Normal	1.117	16.3
3	87,500	1.043	0
4	100,000	1.269	0
5	112,500	2.085	3.7
6	137,000	1.064	6.0
7	225,000	1.108	3.4
8	300,000	1.198	7.0
9	500,000	0.688	0
10	660,000	2.426	0
11	825,000	1.172	0
12	1,000,000	1.081	0
13	1,075,000	2.098	0

Blood Sugar

Rat No.	Trypanosomes per cu. mm.	Blood sugar. Mg. per 100 cc.
1	112,500	67
2	292,000	112
3	300,000	141
4	575,000	79
5	660,000	88
6	740,000	111
7	1,200,000	75

Liver Glycogen and Blood Sugar

Determinations of liver glycogen and blood sugar are given in Table V. The two rats in the earliest stages of the infection were without glycogen demonstrable by the method used. The other rats which were positive had low glycogen values, compared with the controls. The last five animals, with counts of 500,000 trypanosomes per cubic

millimeter and over, did not show any glycogen, even when, as in two of the cases, over 2 gm. of liver were taken. Histologically, the livers of these animals show an enormous accumulation of fat.

DISCUSSION AND SUMMARY

Our results show that animals infected with *T. equiperdum* suffer from an acidosis that is present continuously from an early stage in the disease, and that this condition is complicated subterminally by the breaking down of the kidney structure. It is difficult to estimate how much influence the renal condition may have on the animal's death, but it is probably an important contributory cause.

The increase in lecithin may be of significance in relation to the disappearance of the liver glycogen and the maintenance of a normal blood sugar level. It is known that fat circulates in the blood as a lecithin-like compound (Bloor), and the production of sugar from fat, and of dextrose from glycerol (12), in the animal body, appears to be possible. For trypanosome infections, however, such statements can only be considered as suggestive possibilities, particularly in view of the fact that a hypoglycaemia develops while large amounts of fat are still present in the liver.

The absence of any change in the blood cholesterol in *T. equiperdum* infections is in contrast with the changes found for this constituent in various diseases such as typhoid fever, scarlet fever, and erysipelas; and in chronic infections such as malaria and syphilis. Our results also differ from those of Dubin (13) on one dog infected with this same organism. In this case, the infection was presumably a chronic one, and the dog had a cholesterol and lecithin blood content lower than normal. Our results for normal rats, using Ling's technique, are about 10 per cent lower than those reported by Randles and Knusson (14), who used Bloor's method.

SUMMARY

The CO_2 capacity of the serum is markedly lowered early in infection with *Trypanosoma equiperdum*.

The non-protein nitrogen and uric acid constituents of the blood are increased in the terminal stages. The kidneys also show terminal degenerative changes.

The cholesterol remains unchanged throughout.

Lecithin is markedly increased, most of the observations showing a 20 per cent to 50 per cent rise in this substance.

Liver glycogen is lower than normal in the early stages and could not be demonstrated in the later stages of the infection.

The blood sugar remains normal until a very late period in the disease.

BIBLIOGRAPHY

1. Regendanz, P., *Arch. f. Schiffs- u. Trop. Hyg.*, 1929, **33,** 242.
2. Schern, K., *Biochem. Ztschr.*, 1928, **193,** 264.
3. von Fenyvessy, B., *ibid.*, 1926, **173,** 309.
4. Scheff, G., *ibid.*, 1928, **200,** 309.
5. Kligler, I. J., Geiger, A., and Comaroff, R., *Ann. Trop. Med. and Parasitol.*, 1929, **23,** 325.
6. Dwijkoff, P., *Folia haematol.*, 1926, **33,** 1.
7. Slosse, A., *C. R. Soc. Biol.*, 1927, **97,** 1810.
8. Folin, O., *J. Biol. Chem.*, 1928, **77,** 421.
9. Krasnow, F., and Rosen, A. S., *Proc. Soc. Exper. Biol. and Med.*, 1928, **26,** 67.
10. Ling, S. M., *J. Biol. Chem.*, 1928, **76,** 361.
11. Linton, R. W., *Ann. Trop. Med. and Parasitol.*, 1929, **23** 307.
12. Ferber, J., and Rabinowitsch, S., *Am. J. Med. Sci.*, 1929, **177,** 827.
13. Dubin, H., *J. Biol. Chem.*, 1918, **33,** 377.
14. Randles, F. S., and Knudson, A., *ibid.*, 1929, **82,** 57.