

that in supernatant fluid 4 must be above 7.6. The original precipitin gave a maximum precipitation at pH 7.6 as previously reported.

These results show that ammonium sulphate can separate the precipitin into fractions with different isoelectric points. It will be recalled that the isoelectric point of the precipitin of Type I Pneumococcus prepared by Heidelberger and Kendall's³ method of dissociation with a concentrated NaCl solution is 4.8, whereas that prepared by alkali treatment is 7.6. This difference may indicate that different fractions of the antibody are isolated by these two different methods.

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Hyperlipemia in Experimental Trypanosomiasis of Rabbits.

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Although changes in the lipid content of the blood have been described in a variety of physiological and pathological conditions, they have not been sufficiently studied in animals with protozoal infections. Nakanishi¹ observed some increase in the serum cholesterol, fatty acids and phosphatides in the early stage of syphilis in rabbits but all these elements decreased when generalized infection was established. Santos² and Borel, *et al.*,³ found that in patients suffering from malaria the serum cholesterol was subnormal. On the other hand, a slight increase in the blood cholesterol in filariasis was described by Boyd.⁴ Linton⁵ demonstrated some increase of plasma lipid phosphorus in rats acutely infected with *Trypanosoma equiperdum*. In a study of anemia produced by the same organism Dubin⁶ found in one dog a slight increase in total lipid of whole blood and plasma accompanied by a decrease of cholesterol. Lannoy

³ Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1936, **64**, 161; Heidelberger, M., Pedersen, K. O., and Tiselius, A., *Nature*, 1936, **165**, 138.

¹ Nakanishi, M., *Jap. J. Derm. and Urol.*, 1936, **40**, 215.

² Santos, De Paula, *Trop. Dis. Bull.*, 1927, **9**, 377.

³ Borel, Pons, Advier, and Guillermin, *Ann. Inst. Pasteur*, 1926, **40**, 152.

⁴ Boyd, T. C., *Indian J. Med. Research*, 1930, **17**, 949.

⁵ Linton, R. W., *J. Exp. Med.*, 1930, **52**, 103.

⁶ Dubin, H., *J. Biol. Chem.*, 1913, **33**, 377.

and Lagodsk⁷ noted some elevation in the serum cholesterol of rabbits infected with *Trypanosoma annamense* without mentioning any variation in the total lipid content. The changes so far recorded have not been constant and the variation from normal limits has been rather slight. In view of the meagre information available regarding the abnormalities of blood lipoids further investigation of the changes of these substances in the course of an experimental protozoal infection seems desirable. The present study was, therefore, undertaken to determine whether or not there is any change in the plasma lipoids of rabbits infected with *Trypanosoma brucei*, a strain which hitherto has not been used for the study of blood chemistry.

Two series of rabbits were used, one consisting of 5 test animals, and the other, 4 controls. These animals were kept in separate cages and fed exclusively on 50 gm. of black beans per kilo weight plus a little cabbage. On account of the fact that during the later course of the infection the rabbits became sick and did not take as much food as the controls, the food of the latter was reduced by one-third in order to make it more or less comparable to the amount taken by the test animals. Body weight, temperature and blood count were determined once before inoculation and repeated at each bleeding. About 100,000,000 *Trypanosoma brucei* were injected intraperitoneally into the test animals on the day when the first bleeding for chemical determination was done. The establishment of the trypanosome infection in the test animals was proved by direct finding the organisms in peripheral blood in one case, and in 4 when they could not be so demonstrated by infecting white mice intraperitoneally with 0.5 cc. of whole blood. This suggests that invasion of the blood stream was usually not severe. Concurrent bacterial infection was ruled out by culturing the blood at short intervals. About 15 cc. fasting blood was obtained from ear-vein each time for chemical analysis. The total plasma lipid, cholesterol, protein, non-protein nitrogen and sugar were determined. Bloor's titration method⁸ was used for the estimation of total lipid calculated in terms of oleic acid, Bloor's colorimetric method⁹ for cholesterol and Howe's and Van Slyke's gasometric methods^{10, 11} for protein. All chemical determinations were done in duplicate, and repeated when significant variations were encountered.

⁷ Lannoy, P. L., and Lagodsk, H., *Bull. Soc. Path. Exot.*, 1937, **30**, 57.

⁸ Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

⁹ Bloor, W. R., Pelkan, F. K., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

¹⁰ Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 109.

¹¹ Van Slyke, D. D., *J. Biol. Chem.*, 1927, **71**, 235.

TABLE I.

Time of Examination	Plasma lipoid		Blood picture	
	Total fat, mg./100 cc.	Total cholesterol, mg./100 cc.	Hgb. grams	RBC mill.
Test animals				
Inoculation	152	50	10.6	5.79
2 weeks later	261	64	9.8	4.99
3 " "	702	163.5	7.7	4.55
*4 " "	5050	476	7.0	3.40
Inoculation	245	67	10.0	6.32
2 weeks later	309	54	8.0	5.02
3 " "	376	75.5	6.5	3.96
*4 " "	4500	370	7.0	4.01
Inoculation	324	72	10.0	7.35
2 weeks later	547	118	7.0	3.80
3 " "	670	185	6.4	4.64
4 " "	1188	182	7.5	4.09
†5 " "	1510	247	7.0	4.05
Inoculation	422	125	10.5	4.91
2 weeks later	551	154	9.0	4.37
3 " "	679	200	6.7	3.81
4 " "	1300	266	7.0	3.61
*5 " "	566	154	7.0	Not done
Inoculation	402	102	10.0	5.78
2 weeks later	435	88	9.0	4.37
*3 " "	688	133	6.3	3.81
Control animals				
Chemical analysis and	149	40	11.2	
blood count were done	196	57	11.0	
at the same times as	187	51	10.0	
those on the test ani-	225	40	10.0	
mals.	168	32	11.0	
	227	57	11.1	
	182	34	9.6	
	152	47	10.0	
	249.5	61	11.5	
	331	93	10.2	
	161	36	9.8	
	152	34	10.5	
	166	44	10.0	
	144	42	11.5	
	177	42	10.8	
	200	32	8.5	
	191	38	10.5	
	122	20	10.0	
	105	19	9.8	
	200	38	9.0	

*Rabbits died few days after the last bleeding.

†Rabbit still living.

It is seen from Table I that spontaneous variation in the total lipid and cholesterol content occurs in the blood plasma of rabbits of the control series. However, the change in fatty constituents in normal rabbits during 6 weeks' observation has never been significant. On the other hand, in the animals of the test series, there is a considerable increase both in the total lipid and cholesterol content in 4 out of 5 animals. The maximal increases of total lipid are 33, 18, 5 and 3 times the control figures respectively. Increases in the total cholesterol are also observed, namely 10, 6, 3 and 2 times respectively. It is interesting that normally the ratio of total lipid to total cholesterol was 4 to 1, but after the infection was well established, in 3 animals, the ratio was changed to 12, 11 and 6 to 1. This disproportion of the total lipid to the cholesterol clearly indicates that fatty constituents of the blood plasma, other than cholesterol, were also markedly increased. In one of the test animals which did not survive long enough to permit further investigation, the increase of the total lipid was nevertheless significant. It may be mentioned that the plasma protein and sugar of the infected animals remained more or less within normal limits although the lipid content was greatly increased. The explanation of these metabolic disturbances, however, is unknown at present.

Increased blood destruction induced by venesection and phenylhydrazine poisoning caused an increase of blood lipoids in rabbits.¹² However, the increase was not constant and was not significant unless the red blood cells and hemoglobin had declined below 50% of normal. In the present cases the anemia of the test animals produced by infection in addition to frequent bleeding undoubtedly played some contributory rôle toward the production of hyperlipemia and hypercholesterolemia, yet the increase in total lipid and cholesterol was extremely marked in spite of the fact that the anemia was not severe. Moreover, the lipid content was rapidly and continuously increasing while the anemia remained stationary.

Besides the data presented, it may be briefly stated that guinea pigs subjected to an overwhelming trypanosome infection with the same strain and surviving for 3 to 4 weeks, did not show any appreciable variation in their plasma lipoids.

¹² Bloor, W. R., *J. Biol. Chem.*, 1920, **45**, 171.