11-1-1933

Observation on the Defense Mechanism in Trypansoma Equiperdum and TypamsomaLewisi Infections in Guinea Pigs and Rats

Hildrus A. Poindexter

OBSERVATIONS ON THE DEFENSE MECHANISM IN TRYPANOSOMA EQUIPERDUM AND TRYPANOSOMA LEWISI INFECTIONS IN GUINEA PIGS AND RATS

BY

HILDRUS A. POINDEXTER

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

REPRINTED FROM
THE AMERICAN JOURNAL OF TROPICAL MEDICINE
VOL. XIII, NO. 6, NOVEMBER, 1933

Reprinted from THE AMERICAN JOURNAL OF TROPICAL MEDICINE
Vol. XIII, No. 6, November, 1933

OBSERVATIONS ON THE DEFENSE MECHANISM IN TRYPANOSOMA EQUIPERDUM AND TRYPANOSOMA LEWISI INFECTIONS IN GUINEA PIGS AND RATS

HILDRUS A. POINDEXTER[1]

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

The defense mechanism in trypanosomiasis has interested students of parasitic infections for many years, and experimental analysis of the problem has been attempted in numerous ways involving studies of the course of the infection under various conditions of the host. In this paper we shall report on modifications in the course of *Trypanosoma equiperdum* and *Trypanosoma lewisi* infections in guinea pigs and rats in which attempts had been made to alter the metabolic balance of the host. We shall also present observations of certain anatomical and blood changes in correlation with different periods of the infection.

The work of Schern (1) (1925) has shown that in samples of blood taken from infected rats during the early part of the infection, the trypanosomes were more active and remained alive for several hours, while the organisms became motionless within about ten minutes in preparations taken only a few hours before death. Furthermore, trypanosomes obtained during the terminal stage of the infection could be reanimated by the addition of dextrose, levulose, or thermostable fermentable constituents of normal serum or liver extracts. Schern (2) and others have published figures which show that the blood sugar concentration falls as the number of parasites increase. Hence the conclusion

[1] Formerly, Fellow, Howard University Medical School, under a grant from the General Education Board. The first two years of the work reported in this paper were performed in the Department of Bacteriology, College of Physicians and Surgeons of Columbia University, New York City. The remainder of the work was done at Howard University Medical School, Washington, D. C.

that trypanosomiasis is a disease in which the host's sugar supply is utilized by the parasite in a manner analogous to that of bacteria growing in a test tube. The hypoglycemia associated with the initial increase of trypanosomes appears to serve as a protective mechanism because as the blood sugar continues to decrease the trypanosomes diminish in number. This, we believe, is due to lack of a sufficient supply of readily absorbable carbohydrates for the parasites. The fall in blood sugar is temporarily compensated by glycogenolytic function of the liver which releases more glycogen. This mobilization of glycogen is probably facilitated by a decreased alkali reserve, but as the infection progresses a central necrosis of the liver develops which interferes with its glycogenolytic function and prevents the release of sufficient glycogen to supply the demand for carbohydrates. The result is a hypoglycemia.

THE EFFECT OF GLUCOSE INJECTION ON THE COURSE OF TRYPANOSOMA EQUIPERDUM INFECTION IN GUINEA PIGS

With the above facts in mind we decided to investigate whether an artificially produced intermittent hyperglycemia would aggrevate the course of infection in guinea pigs inoculated with *Trypanosoma equiperdum*.

Blood sugar determination with the Folin method were made on 21 guinea pigs fed a normal diet of carrots, cabbage, oats, and lettuce. Samples of blood were taken from the heart one to three hours after the morning feedings. The results, as shown in column 3 of table 1, were taken as standards of comparison in this work. One week later, 12 of these guinea pigs were starved for eighteen to twenty hours and the blood sugar was then redetermined. These results are shown in column 4. While there was a slight decrease of blood sugar in all but one of the guinea pigs (no. 202) the change was too slight to be statistically significant.

In our next experiment we determined the immediate changes in blood sugar of animals on a normal diet resulting from the intraperitoneal injection of a small amount of a 10 per cent glucose solution. These results are given in the last five columns of table 1.

It appears from the table that the increase in blood sugar following the injection of glucose is definitely significant and that the time of greatest concentration in the blood is about thirty minutes after the injection, after which it slowly declines.

TABLE 1

Blood sugar changes in non-infected guinea pigs on normal diet, after 18 to 20 hours starvation and at various time intervals after intraperitoneal injections of 10 cc. of an aqueous solution of glucose, per 250-gram guinea pig

GUINEA PIG NUMBER	WEIGHT OF GUINEA PIGS	SUGAR PER 100 CC. OF BLOOD						
		On normal diet	After 18 to 20 hours starvation	After intraperitoneal injection of 10 per cent glucose				
				15 minutes	30 minutes	1 hour	2 hours	3 hours
	grams	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
200	255	77	69	70	79	80	71	68
201	260	83	77	80	94	89	80	80
202	250	54	58	70	75	73	65	60
203	255	74	70	78	83	D		
204	265	74	70	75	91	85	80	73
205	280	87	80	86	99	91	D	
206	270	79	75	80	97	86	80	81
207	260	99	85	90	102	95	89	85
208	250	85	80	90	98	90	85	D
209	245	81	75	82	D			
210	250	67	60	70	80	83	76	70
211	255	83	78	83	95	91	80	82
212	265	74		85	97	87	76	D
213	270	95		105	118	100	98	85
214	250	100		102	134	D		
215	255	65		78	87	90	82	86
216	290	75			111	99	80	76
217	270	77		80	93	90	81	80
218	275	91		110	123	109	D	
219	275	77		87	96	91	83	89
220	225	68		75	90	93	88	D

D represents animals dying from heart injuries due to repeated cardiac punctures.

We then studied the changes in the number of trypanosomes (*T. equiperdum*) in the blood stream of infected guinea pigs one-half hour after the injection of glucose. Observations were made at weekly intervals during the course of the infection.

Three series of guinea pigs were examined: One as a control group without the influence of glucose injection, one on a normal diet and the other after eighteen to twenty hours of fasting. The results are summarized in table 2.

It is evident that an increase in the trypanosome count invariably followed the injection of glucose. The greatest percentage increment occurring during the first to sixth weeks with the count decreasing irregularly in the terminal stages of the infection.

TABLE 2

PERIOD OF INFECTION	AVERAGE PERCENTAGE INCREASE OF ORGANISMS IN CONTROL GROUP WITHOUT GLUCOSE INJECTION	AVERAGE PERCENTAGE INCREASE OF ORGANISMS AS SHOWN BY PARASITE COUNTS, OF GUINEA PIGS INFECTED WITH T. EQUIPERDUM ½ HOUR AFTER GLUCOSE INJECTION	
		In guinea pigs on normal diet	In guinea pigs after 18 to 20 hours fast
	per cent	*per cent*	*per cent*
1st week	0.0800	26.1	8.1
2nd week	0.0970	13.3	11
3rd week	0.0170	41	27.3
4th week	0.0060	14.3	16.3
5th week	0.0003	12	11.8
6th week	−0.0001	9.2	5
7th week	0.0000	4.6	1.6
8th week	0.0015	3	5.7
9th week	0.0001		2.7
10th week	0.0032		6.5
11th week			1
12th week			4.9

The average number of guinea pigs used in the determinations recorded were 8 in the first column, 5 in the second, and 4 in the third.

That this heightening of the trypanosome count was directly related to the amount of glucose in circulation is further supported by the fact that with the rare exceptions the guinea pigs which received glucose after starvation showed a smaller percentage increment than did those on a normal diet. Furthermore, the infection of the former was usually of a chronic type, the animals surviving two to three weeks longer than the other test animals. An additional observation not recorded in the table is that injections of glucose usually reduced the prepatent period from one to three days.

THE RELATION OF THE SPLEEN TO RESISTANCE IN TRYPANOSOME INFECTIONS

One of the common anatomical alterations associated with trypanosomiasis is the enlargement of the spleen. Laveran (3) (1908), studying animals infected with *T. brucei*, observed that the hypertrophy was more marked in acute than in chronic trypanosome infections and also that it was more intensive in such animals as the mouse, guinea pig and dog, which died with relatively larger numbers of parasites in their blood than did rabbits. This concomitant enlargement of the spleen has subsequently been observed in various trypanosome infections. Recently Perla and Marmorston-Gottesman and Vorzimer (4) (1930) have shown that there is a marked hyperplasia of the spleen within a few days after the injection of *T. lewisi* in rats. That the spleen plays an important rôle in the defense against certain trypanosome infections is also shown by the work of Mutermilch (7) (1911), who believed that trypanolysins are formed in the spleen and other haematopoietic organs; of Rosenthal and Spitzer (5) (1924), who showed that in the splenectomized rats infected with *T. lewisi*, no reproduction-inhibiting "reaction product" was formed and of Regendanz and Kikuth (6) (1927), who showed that in rat infections with *T. lewisi* the reproduction-inhibiting "reaction substance" was formed chiefly by the reticulo-endothelium of the spleen. Perla and Marmorston-Gottesman (9) (1930), were able to show the loss of resistance of rats to *Trypanosoma lewisi* infection after splenectomy and its return with splenic autotransplants. Linton (10) (1929), in a review has pointed out the importance of the spleen as an organ of defense in this type of infection.

EXPERIMENTAL

There are reasons to believe that the spleen is the chief reservoir for the trypanosomes during the incubation period and at least during the early part of the infection. This temporary arrest of the trypanosomes in the spleen and other lymphatic organs may be looked upon as an attempt to localize the infection. We decided, therefore, to determine the effect of abnormal contraction of the

spleen on the incubation period and course of *T. equiperdum* infection in the guinea pig. We employed a drug, pilocarpine, which according to Cushny (16) contracts the spleen and as criterion of this action determined the increase in the number of erythrocytes and trypanosomes in the blood stream. This method was used by Linton and Poindexter (8) (1931), in experiments on rats infected with *T. lewisi.*

Upon standardizing the drug for guinea pigs, it was found that 1 mgm. of pilocarpine in aqueous solution when injected subcutaneously into 250-gram guinea pigs would cause an effective increase in the number of erythrocytes in the peripheral blood within twenty-five to fifty minutes. The counts were made one-half hour after injection and it was found that for 12 normal guinea pigs there was an average erythrocyte increase of 241,900 or 4.2 per cent.

Table 3 shows the effect of subcutaneous injections of 1 mgm. of pilocarpine at weekly intervals throughout the course of the infection on the number of red blood corpuscles and parasites in the peripheral circulation (ear vein) of guinea pigs infected with *T. equiperdum.* The table also gives the average size in centimeters and weight in grams of the spleens of animals dying or killed during various periods of the infection.

The analysis of the red blood corpuscles and the trypanosome increase in table 3 shows a significant correlation, except for the eighth week and we feel sure that the two changes are due to the same mechanism, i.e., contraction of some of the inner organs, primarily the spleen. Further evidence that the spleen is the chief reservoir for the parasites is presented by the following observations: Once each day after the infection of guinea pigs with *T. equiperdum,* both wet preparations and stained smears were made from the peripheral blood (ear vein), from splenic punctures, or from the spleens of animals that were killed. Examination of these preparations showed that the trypanosomes were present in the spleen by the third to fifth day after injection, while they did not appear in the peripheral blood until the sixth to eleventh day.

An analysis of the last column in table 3 shows that during the

early part of the infection the increase in size of the spleen was greatest and this fact may be correlated with an endeavor to dispose of the trypanosomes during the prepatent period. As the infection proceeded the size and weight of the organ generally

TABLE 3

The average percentage increases in red blood corpuscles and trypanosomes resulting from subcutaneous injections of 1 mgm. of pilocarpine per 250-gram guinea-pig infected with T. equiperdum are recorded

Also average size and weight of spleen of animals dying of the infection or killed during the different stages thereof.

PERIOD OF INFECTION	AVERAGE PERCENTAGE INCREASE ½ HOUR AFTER PILOCARPINE INJECTION		AVERAGE SIZE AND WEIGHT OF THE SPLEEN
	In number of red blood cells	In number of trypanosomes	
	per cent	*per cent*	
Normal	4.2		2 x 1 x ¼ cm.; 1.75 grams
1st week	9.2	6	3 x 2⅓ x 1 cm.; 3.2 grams
2nd week	4.8	2.1	4¾ x 3¼ x 1¾ cm.; 6.33 grams
3rd week	4.9	11.2	No animals died or were killed during this period of the infection
4th week	5.1	6	3¾ x 2⅓ x 1⅔ cm.; 4.3 grams
5th week	3.1	4.1	3 x 2 x 1⅓ cm.; 3.5 grams
6th week	2.2	1.8	3⅔ x 2⅓ x 1⅓ cm.; 4.2 grams
7th week	2.9	1.5	3½ x 2 x 1¼ cm.; 3.25 grams
8th week	Decrease	3.2	3 x 2½ x 1 cm.; 3.5 grams
9th week	2.3		3 x 1¾ x 1 cm.; 3.5 grams
10th week			3¾ x 2⅓ x 1 cm.; 3.1 grams
11th week			3 x 2 x 1 cm.; 2.9 grams
12th week			4 x 2½ x 1 cm.; 5 grams
13th week			3¾ x 3 x 1½ cm.; 4.5 grams
14th week			3 x 2½ x 1½ cm.; 3.4 grams
15th week			3 x 2 x ¾ cm.; 2 grams
Average number of animals examined each week....	6	6	3

diminished except for 2 of the 3 animals that had a third relapse after the second subpatent period. These gross changes can be correlated with the microscopic findings in which the splenic sinuses were found to be greatly dilated and congested with

trypanosomes and red blood cells during the early part of the infection whereas there was no obvious increase in fibrous tissue or pigmentation. On the other hand, guinea pigs that died or were killed after several weeks of the infection showed an extensive fibrosis and pigmentation, the pathological picture resembling in many respects that of the spleen in chronic malaria. In the spleens of these latter animals, however, few if any trypanosomes could be found.

TABLE 4

The effect of repeated injections of 1 mgm. of pilocarpine at two hourly intervals, on the prepatent period in guinea pigs infected with T. equiperdum is shown by the number of trypanosomes counted in 50 high power fields of stained blood smears

NUMBER OF DAYS AFTER THE INOCULATION OF TRYPANOSOMES							
2nd day		3rd day		4th day		5th day	
Before injection	½ hour after injection	Before injection	½ hour after injection	Before injection	½ hour after injection	Before injection	½ hour after injection
Guinea pig 9		Guinea pig 1441		Guinea pig 11		Guinea pig 1442	
0	0	0	0	0	0	0	39
0	0	0	0	0	30	16	106
0	0	0	7	6	33		
0	0			25	80		
Guinea pig 1433		Guinea pig 1444		Guinea pig 1438		Guinea pig 1443	
0	0	0	0	0	0	0	0
0	0	0	0	0	0	20	176
0	0	0	5	3	7	203	350
0	0	2	16	9	37		

Each injection consisted of 1 mgm. of pilocarpine. The number of milligrams inoculated in each instance is indicated by the number of blood examinations.

It had been noticed in the preceding experiment that the subcutaneous injection of 1 mgm. of pilocarpine per 250-gram guinea pig twice a day during the prepatent period reduced its duration by one-fourth. The organisms appeared and remained in the peripheral blood within four to eight days after the infection whereas in untreated animals they did not do so usually until the ninth day. The temporary appearance of trypanosomes could be demonstrated as early as the third day in some instances. In

an attempt to extend these observations a different pair of inoculated animals was injected with 1 mgm. of pilocarpine at two hourly intervals on the second to fifth days inclusive of the prepatent period. Fifty high power fields of stained blood smears, made before and one-half hour after pilocarpine injection, were examined and the number of trypanosomes counted. The results are presented in table 4.

The table clearly shows that the injections of pilocarpine from the third day onwards was followed in all six animals by an initial appearance of the organisms in the blood stream. One can deduce also that the later in the prepatent period the drug is injected the smaller is the amount necessary to cause the first appearance of parasites in the circulation.

The next possibility that arose was that the increase of organisms in the blood stream was due to a direct stimulation of reproduction by the pilocarpine and not to a contraction of the abdominal organs. Daily parasite counts throughout the course of the infection were made on guinea pigs inoculated with *T. equiperdum* and then treated at various intervals with pilocarpine and on 10 infected but untreated animals.

By statistical analysis of the results it may be concluded that there was a significant increase in the trypanosomes resulting from the injection of pilocarpine although there was no increase in the rate of reproduction. When a week to week comparison was made of the pilocarpine group and the control group, the rate of change was practically the same, the peripheral increase being only temporary.

It was also observed that the incline in the plotted graph representing the trypanosome count following pilocarpine injections varied in different stages of the infection. During the logarithmic phase of reproduction the increase of trypanosomes after pilocarpine injections was greater than during the stationary phase, the stage of decline, or during the period between the relapses. This observation may be correlated with the pathological picture of the spleen as described above; with increased fibrosis there should be less contraction from an equal stimulus.

EFFECTS OF T. EQUIPERDUM INFECTION ON PREGNANT GUINEA PIGS

During three years of study of experimental trypanosomiasis in guinea pigs, we have had the opportunity to observe the effect of pregnancy on the course and termination of the infection and the effect of trypanosomiasis upon the mother and offspring. Four cases have been observed and will be discussed in order. The first guinea pig was found to be pregnant during the third week of the infection. The number of trypanosomes in the peripheral blood did not rise as high as in the average non-pregnant guinea pig infected with the same dose. The guinea pig delivered two macerated fetuses during the fifth week of the infection. There was a long period (four weeks) following the delivery, when no trypanosomes could be found in the peripheral blood, after which the trypanosomes returned causing the death of the animal eighteen weeks after the initial injection.

The second guinea pig was found to be pregnant several weeks after the infection. She was given intraperitoneal injections of arsenoferratose, which caused a disappearance of the trypanosomes. She later delivered three dead fetuses.

The third guinea pig was found to be pregnant soon after infection. She ran a very low grade infection, delivering non-formed embryos and completely recovering so far as the trypanosomes in the peripheral blood were concerned. After several months she became pregnant again and this time delivered one fully formed, but dead fetus. After a long interval the animal became pregnant the third time and gave birth to two living guinea pigs, but this time the mother, having very edematous external genitalia and swollen eyelids, died the day after the youngs were born (eighteen months after the initial infection). The youngs died three days later since we were not able to keep them alive without their mother.

The fourth guinea pig was found to be pregnant a few weeks after the infection. The disease ran a mild course and after delivering two guinea pigs which were found dead on our arrival in the morning (we do not know whether they were born alive and

died later during the night or whether they were born dead), the animal recovered. Several weeks later we attempted to reinfect her with *T. equiperdum* but she was found to be immune. A short time later she became pregnant again and gave birth to a healthy offspring. This mother, which had apparently been immune to the trypanosome infection several weeks before, was again injected with *T. equiperdum*; this time she became infected and had a fairly normal course of a less severe type of disease from which she recovered. The animal died later of some respiratory infection. The period of observation of this fourth guinea pig extended over sixteen months.

It may be pointed out that the effects of trypanosomiasis are similar to the effect of syphilis on pregnancy. It would also appear that the disease is less severe in gravid guinea pigs and that a temporary immunity to super-infection may occur.

TERMINAL DECREASE IN THE NUMBER OF TRYPANOSOMES IN RATS INFECTED WITH T. EQUIPERDUM

It is generally accepted that *T. equiperdum* infection in rats is of the progressive fatal type and the protocols of most observers show that the animal dies while the number of trypanosomes in the blood is still increasing. Our findings in this regard were similar but recently we observed a rat in the agonal stages of the infection which showed marked cyanosis of the head, ear, feet, etc., and extreme dyspnea. Blood for the trypanosome count in stained and wet preparations taken from the tail vein, gave the following findings: The blood instead of being dark as expected was of a pale milky appearance; the number of trypanosomes per cmm. was less than on the previous day; fresh preparations showed the trypanosomes moving at a very slow rate and the stained slide showed the trypanosomes apparently agglutinated and most of the red cells were fragmented.

An experiment using 12 rats infected with equal amounts of trypanosoma equiperdum suspension was carried out to determine the trypanosome changes in the last few hours of the disease. Single daily parasite counts were made until the fourth or fifth days when the animals became visibly ill. We then made hourly

counts until death was imminent whereupon the organisms were counted every half hour. Ten of the animals died on the fifth, one on the fourth and one on the sixth day of infection.

The observations from this experiment showed that the average increase in the number count is fairly regular until the fifth day. However, on this, the last day of the infection, there is a marked decrease, the average number of trypanosomes decreased from about five and one-half millions to almost three millions.

We may conclude, therefore, that in an infection of this type, although the increase in the number of circulating organisms is fairly regular in the presyndromal period, the agonal stages are marked by a disappearance of almost half of the organisms. However, the animals die with a large number of living organisms still in the blood stream.

EFFECT OF TRYPANOSOMIASIS ON THE FRAGILITY OF THE RED CELLS

It is well known that anemia accompanied trypanosomiasis, but its occurrence is generally dismissed from further consideration as being due to the destruction of the red cells by the spleen and other hemophagic organs. Since we had found that there was a coincident destruction of red blood corpuscles and organisms during the terminal stages of certain trypanosome infections we next determined the effect of trypanosome infections on the resistance of the red blood corpuscles to hemolysis by hypotonic solutions. Accordingly, the erythrocytes of 36 rats were examined, 24 of which had been infected with *T. lewisi* and 12 with *T. equiperdum*.

Preliminary experiments had shown that red blood corpuscles of normal rats are generally more resistent to hypotonic saline than are those of human beings. Hemolysis of the corpuscles from many of the animals does not even begin in saline of as low as 0.28 per cent, a concentration which is usually the weakest one used in testing the fragility of human cells. We modified, therefore, the usual test by extending the series of tubes so that the weakest solution contained only 0.20 per cent saline.

The rats infected with *T. lewisi* were examined at four weekly

intervals and the results obtained were compared with tests previously done with normal samples of blood from the same animals. No significant changes in fragility were found. The large variation in the hemolytic zone among normal individuals gives a standard deviation greater than the average change resulting from the infection.

However, with the *T. equiperdum* infected rats the results obtained were of a different nature. These animals were examined

TABLE 5

Fragility tests of the erythrocytes of rats inoculated with T. equiperdum on the third, fourth, and fifth days after infection

RAT NUMBER	ZONE OF HEMOLYSIS			
	Before inoculation	Days of the infection		
		3rd	4th	5th
	per cent	*per cent*	*per cent*	*per cent*
1	0.26–0.22	0.30–0.30		
2	0.22–0.20	0.26–0.24	0.34–0.30	
3	0.28–0.22	0.28–0.26	0.32–0.26	0.34–0.30
4	0.30–0.30	0.32–0.30	0.38–0.32	
5	0.24–0.20	0.28–0.28		
6	0.24–0.22	0.28–0.24	0.20–0.28	
7	0.26–0.20	0.28–0.28		
8	0.22–0.22	0.24–0.22	0.32–0.30	
9	0.28–0.24	0.30–0.26	0.32–0.30	0.40–0.34
10	0.30–0.24	0.32–0.26	0.36–0.36	
11	0.24–0.20	0.30–0.28		
12	0.26–0.24	0.30–0.26	0.32–0.28	

The first number represents the point at which hemolysis begins and the next the point at which it is completed.

daily commencing on the second day and the results are presented in table 5.

Table 5 shows a consistent decrease in the resistance of the red blood corpuscles to hemolysis by hypotonic saline which has a tendency to become more marked as the infection progresses.

The decreased resistance to hemolysis shown by the erythrocytes of rats infected with *T. equiperdum* in contrast to the relative stability of those infected with *T. lewisi* may be due in part to the more marked non-volatile acidosis, which had been shown

by Andrews, Johnson, and Dromal (11) to exist in *T. equiperdum* infected rats and which Linton (12) failed to find in rats infected with *T. lewisi*.

INCREASE IN LARGE MONOCYTES AND PLATELETS IN RATS INFECTED WITH T. LEWISI

The importance of the reticulo-endothelial system in protozoan infections has been well discussed and reviewed by Linton (10). Trypanosome infections, except in rare cases such as in Chagas' disease, involved the lymphatic system and blood stream. It would not be surprising to find, therefore, that the system which is most affected by the trypanosomes shows the effects of this stimulation in the blood stream. Todd (13) has stated that there is an endotheliocyte increase in the blood of trypanosomiasis patients. A similar increase was observed in our trypanosome infected rats and guinea pigs.

The large monocytes, according to Maximow (14) (1927), are derived from the hemocytoblasts, and are known to increase as a result of direct stimulation of the reticulo-endothelial system. As many of the functions of these cells parallel those of the histiocytes and since the endotheliocytes may be confused with the large lymphocytes, we have used in the next experiment the large monocyte count as an indirect index of histiocytic stimulation. Table 6 presents the differential white cell counts of 6 rats infected with *T. lewisi* during various stages of the infection. There are listed also in some instances platelet counts carried out by Fonio's method. In all, 19 animals were examined of which the 6 listed are representative.

In table 6 P. = polymorphonuclear leucocytes, L. = lymphocytes, L. M. = large monocytes, E. = eosinophiles, Pl. = platelets. The most important findings are a consistent increase in the percentage of large monocytes and in the platelets. The increases in both are more pronounced during the early period of the infection; they begin to return to normal as the animal recovers. These findings suggest some stimulation of the reticulo-endothelial system as a result of the infection. A temporary increase in the number of red blood corpuscles appears in the

TABLE 6

Differential white cell count of rats infected with T. lewisi giving the percentage of large monocytes, polymorphonuclear leucocytes, lymphocytes, and eosinophiles; together with the number of trypanosomes per 100 red blood corpuscles, the number of platelets per cubic millimeter of blood; and other rare findings

RAT NUMBER	NORMAL	WEEKS AFTER THE INFECTION			
		1st	2nd	3rd	4th
1	*2% L.M.* 70% P. 28% L. 150,000 Pl.			*21% L.M.* 16% P. 61% L. 1% E. 27 trypanosomes	
2	*4% L. M.* 71% P. 25% L.		*17% L.M.* 61% P. 18% L. 4% E. 430,000 Pl. 1 trypanosome 2 nucleated red cells per 100 white blood cells		
3	*4% L. M.* 50% P. 46% L.	*28% L. M.* 38% P. 28% L. 6% E. 752,000 Pl. 3 trypanosomes		*33% L. M.* 35% P. 22% L. 10% E. 205,000 Pl. 58 trypanosomes	
4	*10% L. M.* 61% P. 29% L. 256,000 Pl.		*35% L. M.* 18% P. 45% L. 2% E 905,000 Pl. 107 trypanosomes	*29% L. M.* 22% P. 48% L. 1% E. 300,000 Pl. 21 trypanosomes	
5	*3% L.M.* 40% P. 56% L. 1% E. 300,000 Pl.				*25% L.M.* 25% P. 48% L. 2% E. 246,000 Pl. 3 trypanosomes

TABLE 6—*Concluded*

RAT NUMBER	NORMAL	WEEKS AFTER THE INFECTION			
		1st	2nd	3rd	4th
6	*8% L.M.* 37% P. 55% L.	*19% L.M.* 27% P. 54% L. 532,000 Pl. 2 trypanosomes 2 nucleated red cells per 100 white blood cells		*28% L.M.* 21% P. 50% L. 400,000 Pl. 50 trypanosomes	*16% L.M.* 33% P. 49% L. 293,000 Pl. 7 trypanosomes

peripheral circulation within the first two to four days after the injection of the trypanosomes. This has been observed in about 57 per cent of a large number of guinea pigs.

SOME OBSERVATIONS ON CROSS-IMMUNITY TO TRYPANOSOME INFECTIONS

It is generally conceded, as was demonstrated by Marmorston-Gottesman, Perla and Vorzimer (4) and others, that rats recovering from an infection with *T. lewisi* acquire an immunity for life. This is not in agreement with our observations in which rats recovering from *T. lewisi* could be reinfected after six to twelve months. These animals however, required larger doses of trypanosomes and the course of the reinfection was of a milder type. Taliaferro (15) (1929), in a general review and discussion, has shown that resistance conferred by a passage strain is specific for that strain but not for the relapse variant of the same species. It is of interest to note in this regard that the relative immunity conferred on these rats was for the homologous passage strain.

Taliaferro (15), has reported that animals previously infected with *T. lewisi* show no cross immunity to *T. equiperdum*. We have also studied the same problem. The results of one experiment in which 12 rats were used are given here. Six rats were infected with *T. lewisi* and the infection was permitted to run its

usual course. Six weeks after infection all trypanosomes had disappeared from the circulation. When the animals were again infected with *T. lewisi* of the same passage strain, each of the six rats was found to be immune. They were then infected along with the other six normal rats with an equal number of *T. equiperdum* organisms. The results were as follows: All of the rats (12 in number) died of the infection. The average length of life in the group immune to *T. lewisi*, however, was 149.3 hours, whereas the corresponding average for the non-immune group was 109 hours. This may be due to some slight over-lapping type immunity but conclusion must be withheld until further experiments are performed.

DISCUSSION

The high positive correlation between the trypanosome increase and the white blood corpuscle increase after the injection of glucose and the lack of such correlation in the red blood corpuscle changes may be explained by pointing out that the mechanism which causes physiological leucocytosis also causes an increase of the trypanosomes. Schern (2) has explained this as due to the trypanosomes utilizing the blood sugar in the same way as bacteria utilize sugar in the test tube. That trypanosomes may utilize the blood sugar as easily absorbable food, resulting in an actual increase in the rate of reproduction, as well as the virulence and activity of the trypanosomes, is shown by the changes in motility on the cover slip, by the more rapid increase of the parasites in the circulation with a higher final count, and by the earlier deaths in the animals that received glucose injections.

The effect on the prepatent period caused by such a drug as pilocarpine (which contracts the spleen without increasing the rate of trypanosome reproduction) probably serves as a reservoir for the trypanosomes, during the early part of the infection. This hypothesis is further supported by observations on smears from splenic punctures, which show the microörganisms to be present in the spleen before they appear in the peripheral circulation. These data, therefore, amplify the current conception which regards the spleen as the most effective organ in the defensive mechanism against certain trypanosome infections.

A temporary increase in the number of red blood corpuscles appears in the peripheral circulation within the first two to four days after injection of the trypanosomes. This we believe is due to an early stimulation of hematopoiesis by the trypanosomes or their products before the subsequent hemolytic activity can manifest itself. There is an increase in both the red blood corpuscle production as demonstrated by the increase in nucleated red blood cells, and in red blood corpuscles destruction as shown by the progressive anemia.

Pregnancy in guinea pigs infected with *T. equiperdum* seems to be favorable to the mother as is suggested by the milder form of the disease, the apparent temporary recovery of the animal and the obvious lengthening of its life. Trypanosomiasis on the other hand has an unfavorable influence on the embryo.

It is not known whether the lysis of trypanosomes and red blood corpuscles are due to the same or different mechanism. The fundamental mechanism of trypanolysis has not yet been experimentally ascertained, but is probably the same as that which causes the crisis and final destruction of the trypanosome in recurrent and non-pathogenic infections.

There is no significant change in the fragility of red blood corpuscles in rats infected with *T. lewisi.* However, there is a significant decrease in the resistance of the red blood corpuscles to hemolysis by hypotonic saline in rats infected with *T. equiperdum.* These findings seem to correlate with the acidosis in the two types of infection, the greater acidosis being associated with the greater fragility of the red blood cells to hemolysis by hypotonic saline.

The increase in the size of the spleen, in the number of trypanosomes after splenic stimulation by pilocarpine, and in the percentage of large monocytes and platelet, are all well correlated with the reticulo-endothelium being the chief system active in trypanosome infections. The early increase and later decrease of the large monocytes and platelets show a suggestive mathematical correlation with the increased size of the spleen in rats infected with *T. lewisi.* The changes in size of the spleens of rats infected with *T. lewisi* almost parallel the changes in size of the spleens of guinea pigs infected with *T. equiperdum* in the first five weeks of their infection.

SUMMARY AND CONCLUSION

Artificial hyperglycemia shortens the prepatent period and increases the rate of reproduction of the trypanosomes in guinea pigs infected with *T. equiperdum*. Death supervenes in such animals from two to three weeks sooner than in control animals with a lower blood sugar. These observations suggest that the trypanosomes in the blood stream require carbohydrates for food and utilize them in a way analogous to the utilization of sugar by bacteria in the test tube.

The intraperitoneal injection of pilocarpine shortens the prepatent period in guinea pigs infected with *T. equiperdum*. It also causes a significant increase in the number of trypanosomes in the peripheral circulation when injected during the logarithmic phase of development. The increase is only temporary and does not represent a stimulation in the rate of reproduction of the trypanosomes. This increase apparently is due to a contraction of some of the abdominal organs, primarily the spleen which serves as a reservoir for the trypanosomes during the early part of the infection.

The trypanosomes or their products cause an early stimulation of the hematopoietic organs as shown by the temporary increase in the number of red blood corpuscles and in the number of nucleated red blood cells in the peripheral circulation.

That the spleen is the most important and most active individual organ of the reticulo-endothelial system, in the defense against trypanosome infections, is further shown by the correlation of the gross and microscopic changes with the response of the animal to a drug which contracts the spleen.

T. equiperdum infection in pregnant guinea pigs appears to run a milder course in the mother but has a deleterious effect on the offspring.

There is a terminal decrease in the number of trypanosomes in rats infected with *T. equiperdum*. This occurs within the last few hours of the disease and is associated with a terminal destruction of the red blood corpuscles. We believe that death is in a large part due to the anoxyemia resulting from this hemolysis which is further aided by the presence of a non-volatile acidosis in the blood.

There is no significant change in the resistance of the red blood corpsucles of rats infected with *T. lewisi*, while in *T. equiperdum* infection a significant decrease in the resistance of the red cells occurs.

The change in the size of the spleen in rats infected with *T. lewisi* almost parallels the change in the size of the spleen of guinea pigs infected with *T. equiperdum* during the first five weeks of the infection.

There is a significant change in the large monocytes and the platelets during the early part of the infection of rats with *T. lewisi*, with a tendency to return to normal as the infection clears up.

The average duration of life of rats infected with *T. equiperdum* was lengthened approximately 40 hours in animals previously infected with *T. lewisi*.

We wish to express our appreciation to Dr. R. W. Linton for his invaluable advice during the early part of these experiments and to the various members of the different departments of the College of Physicians and Surgeons who freely gave their advice.

REFERENCES

(1) Schern, K. 1925 Ueber Trypanosomen. I–VI. Centralbl. f. Bakt., 96.

(2) Schern, K. 1928 Über die Störung des Zuckerstoffwechsels bei Trypanosomiasen und Spirochätosen. Biochem. Ztschr., cxciii, 264.

(3) Laveran, A. 1908 Sur quelques alterations de la rate chez les cobayes infectes de trypanosomes. Bull. Soc. Path. Exot., i, 393.

(4) Marmoston-Gottesman, J., Perla, D., and Vorzimer, J. 1930 Immunological studies in relation to the suprarenal gland. Jour. Exp. Med., lii, 587.

(5) Rosenthal, F., and Spitzer, F. 1924 Weitere Untersuchungen über die trypanoziden substanzen des menschlichen Serums. V. Die Bedeutung des Reticuloendothels fur den Mechanismus der trypanoziden Wirkung des Menschenserums. Ztschr. f. Immunitatsf., xl, 529.

(6) Regendanz, P., and Kikuth, W. 1927 Ueber die Redeutung der Milz fur die Bildung des vermehrungshindernden Reactions produktes (Taliaferro) und dessen Wirkung auf den Infektionsverlauf der Ratten-Trypanosomiasis (*Trypanosoma lewisi*). Versuche der Uebertragung des *Trypanosoma lewisi* auf die weisse Maus. Centralbl. f. Bakt., Orig., ciii, 271.

(7) Mutermilch, S. 1911 Sur L'origine des anticorps chez les cobayes trypanosomies. Ann. de L'Int. Pasteur, xxv, 776.

(8) LINTON, R. W., AND POINDEXTER, H. A. 1931 Artificial acidosis in *Trypanosoma lewisi* infections and its bearing on the pathogenic action of *Trypanosoma equiperdum*. Jour. Exp. Med., liv, 669.

(9) PERLA, D., AND MARMORSTON-GOTTESMAN, J. 1930 Further studies on T. lewisi infection in albino rats. Jour. Exp. Med., lii, 601.

(10) LINTON, R. W. 1929 The reticulo-endothelial system in protozoan infections. Arch. Path., viii, 488.

(11) ANDREWS, J., JOHNSON, C. M., AND DORMAL, V. J. 1930 Lethal factors in experimental infections of *Trypanosoma equiperdum* in rats. Amer. Jour. Hyg., xii, 381.

(12) LINTON, R. W. 1930 The blood chemistry of an acute Trypanosome infection. Jour. Exp. Med., lii, 103.

(13) TODD, J. C. 1924 Clinical Diagnosis by Laboratory Methods. W. B. Saunders Company, Philadelphia, Pa., p. 312 (Text).

(14) MAXIMOW, A. A. 1927 Morphology of the mesenchymal reactions. Arch. Path. and Lab. Med., iv, 557.

(15) TALIAFERRO, W. H. 1929 The Immunology of Parasitic Infections. The Century Company, New York. (Text.)

(16) CUSHNY, A. R. 1915 Text Book of Pharmacology and Therapeutics. Lea and Febiger, Philadelphia and New York.