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THE WHITE CELL PICTURE IN HOOKWORM DISEASE OF DOGS.[1]

BY

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In a previous report (Foster and Landsberg, 1934) in which several lines of evidence were presented in support of the hypothesis that hookworm anemia is essentially the result of blood loss, we discussed the changes in the red cell picture of dogs harboring experimental infestations with the dog hookworm, *Ancylostoma caninum*. It is desired to present at this time certain of the observations upon the white cell changes in these same animals. The data were obtained concurrently with the red cell data previously discussed.

Standard hematological procedures were used in these studies which have been described previously in detail (loc. cit., pp. 262–263). It is sufficient to note here that the blood sample, either 3 or 5 cc., was obtained by cardiac puncture and coagulation prevented by the use of dry potassium oxalate (2 mg. per cc. of blood). From this sample were taken dilutions for the enumeration of the total white cells, with Turck's solution as a diluent. The dilutions were made with a Thoma pipette and counted in a Levy counting chamber, both of which had been certified by the U. S. Bureau of Standards. The blood smears for the differential counts were made directly from the needle tip immediately following cardiac puncture, by use of Ehrlich's cover slip method and Wright's Romanowsky stain. One hundred white cells in consecutive fields were counted.

Routine helminthological methods used previously in this laboratory and reviewed elsewhere (Herrick, 1928; Scott, 1928) were followed.

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Experimental results.

A comparison of the leukocytic pictures from infection by skin and by mouth (D812, D813, D814).

This study includes the data on three litter mate dogs which were 128 days old at the beginning of the experiment. They had harbored light experimental hookworm infestations previously, but were treated to negative about 3 weeks before the present observations were begun. After two complete determinations, D812 was infected by skin and D813 by mouth. The experimental procedure in these animals was identical except for the mode of infection. The third animal, D814, was retained as an uninfected control.

The data on these animals have been summarized in table 1. It is shown in this table that both of the infected animals had a slight increase in the total number of leukocytes which was greatest during the 49 to 80-day period. This is supported by a few high determinations, which were masked by averaging, in both animals earlier in the infestation. Thus the animal infected by skin (D812) showed 17,300 leukocytes per cmm. on the thirty-eighth day and the one by mouth 18,400 per cmm. on the twenty-seventh day. As shown in table 1 there was a slight, but constant increase in the percentage of juvenile cells in both infested animals. This increase, however, cannot be correlated with the method of infection or the infestation as the uninfected control (D814) showed a similar increase. The polymorphonuclear neutrophils presented a picture just as difficult to interpret. The animal infected by skin (D812) showed a general increase, whereas D813 showed only a slight rise at the end of the experiment; but these percentages in both cases were scarcely above the level, which tended to remain constant, of the uninfected control (D814). It is interesting, however, that D812 had the highest individual polymorphonuclear neutrophil count which was 84 per cent on the sixtieth day.

The interpretation of the eosinophil picture was difficult due to the marked variability. There was apparently a marked rise which appeared soon after infection by mouth (D813) (table 1) but it was not present following skin infection (D812). The animal infected by skin (D812) showed a constant eosinophil count of about 10 per cent until the forty-ninth to eightieth day period when the count actually dropped. The uninfected control showed a high eosinophil percentage at the beginning of the experiment which gradually decreased (table 1). There are several individual determinations, higher than

shown in the table, which warrant mention. After the first infection, D812 showed 19 per cent eosinophils on the thirteenth day; following the second infection, this animal showed 19 per cent 3 days later. D813 showed 22 per cent eosinophils 10 days after the first infection

TABLE 1.

The total and differential leukocyte count for each of three litter mate dogs, two of which were infected with Ancylostoma caninum. *The figures are averages for the days indicated.*

Days	Larvae given	Total white blood cells	Myelocytes	Juveniles	Polymorph.	Eosino.	Baso.	Lymphocytes	Monocytes
		Infected by skin (D812)							
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–3		10,300	0	1.5	49.5	10.5	0	37.0	1.5
5	4,000								
11–18		9,925	0	4.0	44.7	10.0	0	39.2	1.2
19	3,500								
20–38		12,025	1.5	4.0	60.0	11.5	1.0	22.8	0.8
42	15,000								
49–80		14,900	1.5	3.7	69.7	8.0	0.4	18.7	0.2
		Infected by mouth (D813)							
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–3		9,400	0	2.0	60.0	2.5	0	33.5	2.0
5	4,000								
11–18		10,562	0	2.5	54.0	16.2	0	25.7	0.5
19	3,500								
20–38		12,467	0.1	3.7	64.3	11.1	0.1	18.7	1.7
42	15,000								
49–80		13,208	0.4	5.8	65.0	7.4	0.4	19.4	0.8
		Control (D814)							
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0–3		12,000	0	0	63.0	13.0	0	21.0	3.0
11–18		10,247	0	2.5	64.0	3.7	0	29.0	1.0
20–38		11,131	0.1	4.5	64.4	9.5	0	20.9	0.7
49–80		10,105	0	5.0	63.2	5.0	0	26.2	0.5

and 16 per cent on the twenty-ninth day after the second infection. The uninfected control (D814) had several counts of 13 and 14 per cent during the period. It is also worthy of note that both infected animals showed a gradual but constant decrease in lymphocytes (table 1).

The leukocytic picture of dogs with infestation of A. caninum *during iron, copper, and cobalt therapy (D801, D803, D804, D805, D806).*

This group includes five litter mate dogs, each of which was given 1,000 larvae *per os* at the beginning of the experiment. They were then 165 days old. Three of these animals (D801, D803, D804) were given daily administrations of iron, copper, and cobalt [3] therapy, throughout the entire course of the experiment. The two others (D805, D806) received no therapy at all.

The determinations for the three dogs receiving iron, copper, and cobalt therapy have been averaged and those for the two dogs receiving no therapy were treated similarly (table 2). The individual de-

TABLE 2.

The total and differential leukocyte count on five litter mate dogs infected with 1,000 larvae each by mouth immediately after the first blood determination.

Days infected	Average findings for three dogs (D801, D803, D804) receiving Fe-Co-Cu * therapy							
	Total white blood cells	Myelocytes	Juveniles	Polymorph.	Eosino.	Baso.	Lymphocytes	Monocytes
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0	12,450		5.6	63.3	2.3		28.6	
6	12,950		3.0	67.3	11.0		17.3	1.3
13	12,183		7.6	61.0	7.0		19.0	2.0
17	17,433		3.0	70.3	6.6		20.0	
20	16,483		4.6	67.6	3.3		21.0	1.3
24	14,900		4.0	61.6	6.6		26.3	1.3
27	12,823		3.0	63.3	11.6		21.3	0.6
31	14,143	1.0	3.6	61.0	8.6		22.0	1.6
34	15,606	0.3	6.6	63.3	3.3		24.3	
45	12,333	0.3	3.6	60.0	3.3		28.0	0.6
55	13,466		1.3	64.3	4.0	1.0	29.0	0.3
	Average findings for two dogs (D805, D806) receiving no therapy							
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0	12,350		7.0	64.5	2.0		24.0	2.5
6	13,350		7.0	64.0	1.5		24.5	1.0
13	16,000		2.5	70.0	5.5		21.5	1.0
20	10,650		3.5	58.0	11.0		27.0	1.0
27	11,335		3.5	64.5	6.0		26.5	1.0
45	12,350		3.0	63.0	10.0		24.0	
55	10,800	3.0	8.0	63.5	2.5		24.5	

* Given 2.28 gm. per day of a 100 : 1 : 5 mixture of iron citrate, copper sulphate and cobalt chloride.

[3] Given 2.28 gm. per day of a 100: 1: 5 mixture of iron citrate, copper sulphate, and cobalt chloride.

terminations for the animals in these groups were not materially different from the averages given in the table. The group receiving iron, copper, and cobalt therapy showed an increase in the total leukocyte count from the seventeenth to the twenty-fourth day after infection and apparently another rise 2 weeks later. The group not receiving iron therapy showed an increase in leukocytes occurring only on the thirteenth day. In both groups, however, the increase seems to have been accompanied by a slight increase in the percentage of neutrophils. In each group there was an increase in the eosinophil count on two different days but the days did not coincide. It is of interest to note that unlike the previous experiment there was no evidence of a constant increase in percentage of juvenile cells. There was not a constant decrease in the lymphocytes although the group on iron therapy showed low percentages on the sixth and thirteenth days (table 2).

Comparison of the leukocytic picture of an infected, a bled, and a normal animal (D807, D808, D809).

For this experiment three litter mate dogs, 31 days old when consigned to these studies, were used. After 32 days of observation D807 was cutaneously infected with 100 larvae. It was given a second infection of 372 larvae, by the same route, 18 days later. A third infection of 2,000 larvae was given after 17 days and this followed 22 days later by a fourth injection of 1,000 larvae. Eleven days later a final infection of 1,000 larvae was given. After 83 days of observation, during which time it served as a control for D807, D809 was given a single oral infection of 2,000 larvae. Seventeen days later it was given another 1,000 larvae. The third animal (D808) was artificially bled by cardiac puncture. The bleeding period was started on the seventy-first day of the experiment (807 up to this time had received 2,472 larvae) and was continued for 62 days with an average blood loss of 41 cc. per day.

It will be seen (table 3) that in this experiment again there was some increase in the total leukocyte count after infection (D807 and D809), but that there was actually a greater increase in the worm-free dog (D808) which had been bled. This is further supported by a consideration of the individual peak determinations in these animals. After the fourth infection (1,000 larvae) the leukocyte count of D807 was found to be 20,300 cells per cmm. However, during the period in which D809 served as a control it showed several high counts, the highest being 17,600; but after infection this same animal never had

a leukocyte count above 17,500. In this experiment, the highest leukocyte determinations were found during the bleeding period of D808 when counts of 20,600 and 22,400 were observed.

The animal cutaneously infected (D807) showed an increase in

TABLE 3.

The total and differential leukocyte count for each of three litter mate dogs, two of which were infected with Ancylostoma caninum *and one bled by cardiac puncture. The figures are averages for the days indicated.*

Days infected	Larvae given	Total white blood cells	Myelocytes	Juveniles	Polymorph.	Eosino.	Baso.	Lymphocytes	Monocytes
			Infected (D807)						
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1–28		13,110	0.6	5.1	70.1	1.7		25.0	1.4
32	100								
32–50		12,844	0.6	7.0	66.6	1.3		23.7	0.6
50	372								
53–65		12,625	0.8	8.3	70.4	4.5		19.5	0.3
67	2,000								
69–88		11,138	0.5	3.2	60.0	13.3		22.6	0.5
89	1,000								
90–99		12,840	0	3.0	64.8	7.6		24.6	1.2
102	1,000								
102–116		14,249	0.1	2.8	69.8	4.2	0.7	22.0	0.4
118–147		11,175		4.2	62.5	7.0	0.2	25.2	0.7
			Infected (D809)						
			per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1–81		12,352	0.5	3.7	57.7	1.5		31.4	1.0
83	2,000								
83–99		11,243		0.8	56.8	4.5		36.5	1.6
102	1,000								
102–116		13,825		2.3	63.3	6.6		27.5	0.1
118–147		12,037	0.2	3.2	59.7	10.2	0.2	22.7	0.5
			Bled (D808)						
	cc. bled *		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1–71		12,414	0.3	4.6	58.3	1.4		29.3	1.2
72–106	92	12,152	0.2	2.1	58.2	1.4		30.6	1.0
109–116	150	15,462		3.7	64.2	1.5		29.2	1.2
118–133	75	12,756		1.6	67.6			30.0	0.3

* Average blood loss per day during specified interval.

eosinophils following the third infection, averaging 13.3 per cent for the 69 to 88-day period, during which time one peak of 30 per cent was reached. This was the highest peak recorded in our studies. The control animal (D809) when later infected also showed an in-

crease in eosinophils. Moreover there were observed in this dog two high individual determinations of 13 and 17 per cent, occurring after the first and second infections respectively and before the general increase at the end of the experiment. A temporary increase in juvenile cells was observed in one animal (D807) and there was no evidence of a decrease in lymphocytes.

Discussion.

A comparison of the animals infected by skin (D807, D809, D812) and by mouth (D801, D803, D804, D805, D806) revealed that the route of infection had little, if any, influence upon the total white cell count. The differences between the total leukocyte counts of these two groups were so small that they may be considered negligible. Not all the animals infected, by either route, showed a definite increase in total white cells following each separate infection, although most of them showed increased white cell counts at some time during infestation. In addition, the animals (D801, D803, D804) receiving iron, copper, and cobalt therapy exhibited a slightly higher total leukocyte count (table 2) than did the litter mates with no therapy. The total white cell count, in the artificially bled animal (D808), during the period of bleeding showed average counts (table 3) approximating (actually being higher during one period than) those found in the infested animals. This finding agrees with Sarles' (1929) observation that in young dogs a leukocytosis was not a constant occurrence following infection. Also it was noted that the dogs with the heaviest infestations did not necessarily show the highest total leukocyte counts.

In all the infested animals the myelocytes and juveniles presented about the same picture. The percentage of these cells was low and they were found at scattered intervals. Their number and occurrence were not affected by the mode of infection or by the infestation itself. In the individual animals, before and after infection, there was little variation from the uninfected control. This is demonstrated in table 1.

In all the infected animals in which the total white cell counts were increased, the percentages of polymorphonuclear neutrophils were usually increased also. The absolute number of polymorphonuclear neutrophils when plotted with the total white cell count on arithlog paper produced parallel curves. It seems possible that the total increase in white cells was created by an increase in these mature

cells (polymorphonuclear neutrophils) and that a definite "shift to the left," in which the number of immature granulocytic cells is increased (myelocytes and juveniles), did not occur.

An increase in eosinophilic cells was not the usual occurrence after each infection, irrespective of whether the infection was given orally or cutaneously. Although a skin infected dog (D807) showed the highest eosinophil count (30 per cent), the number of eosinophils did not appear to be related to the method of infection. Nor was the number of eosinophils influenced by the administration of iron. We agree with Sarles (loc. cit.) that in young dogs, infection whether cutaneous or oral apparently does not produce a marked eosinophilia. On the other hand, our observations do not support Sarles' finding that the increase in eosinophils coincided with the time at which the hookworms reached maturity (14 to 17 days after infection). Our findings also differ from those of Yamaguchi (1928) who found that after oral infection the eosinophils reached their highest peaks on the seventh day and following cutaneous infection in from 6 to 9 days. In our studies the increase in eosinophils did not occur at any uniform interval after infection. Likewise we did not find, as did Yamaguchi, that oral infection produced a greater increase in eosinophils than did cutaneous infections. Ashford, Payne and Payne (1933) in acute human cases found that "infestation, when sufficiently massive, produces leukocytosis, and this leukocytosis is eosinophilic." This was not observed in our experimental animals, yet it must be emphasized that the two situations are not quite parallel as our animals were not suffering from acute infestations.

In some cases it appeared that the percentage of lymphocytes was decreased when there was an increase in the polymorphonuclear neutrophils or the eosinophils. The method of infection apparently played no rôle in the reduction of lymphocytes. These findings agree partially with the observations of Kawanishi (1932) and Oba (1928) in man, who found that lymphocytopenia appeared simultaneously with eosinophilia.

The basophils and monocytes remained unchanged in the infested animals. Kawanishi (loc. cit.) found no change in these cells in human cases of hookworm infestations with *Necator americanus*.

It should be added that the important white cell changes did not appear to coincide with the greatest red cell changes as published earlier. Finally we have been impressed during these studies with the necessity of making frequent white cell determinations if the results are to have significance.

Summary.

Some limited data on the white cell picture of dogs experimentally infected with larvae of *Ancylostoma caninum* are presented. A marked leukocytosis did not follow every infection whether cutaneously or orally given. The route of infection did not seem to influence the degree of leukocytosis. An increase in the total white cell counts when it occurred, was due apparently to an increase in the mature neutrophilic cells, the polymorphonuclear neutrophil. An increase in the eosinophils did not occur after every infection, but when it did occur the maximum increases came at varying intervals after infection. There was little difference in the magnitude of the eosinophilic responses following oral and cutaneous administration of larvae. Lymphocytopenia occurred in some cases but was not constant and there was no appreciable change in the basophils or monocytes.

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