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ON A PROBABLE RELATIONSHIP BETWEEN ANEMIA AND SUSCEPTIBILITY TO HOOKWORM INFECTION.[1]

BY

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An understanding of resistance to metazoan infections has been encouragingly approached through the isolation of individual factors which affect the balance between resistance and susceptibility in particular host-parasite combinations. In the case of the relationship of the dog to its hookworm parasite, *Ancylostoma caninum*, there has been summarized elsewhere (Foster, 1935) a considerable body of evidence which emphasizes the importance in this respect of such factors as age of host, diet, genetic constitution, degree of infestation, and pathogenicity of parasite. As a working hypothesis it was suggested that factors which militate against a host's well-being are factors which render that host more susceptible to the invasion of hookworms.

In this report it is desired to present briefly the results of experimental studies with two additional factors, namely, periodic blood loss and a milk (iron-deficient) diet, both of which were observed to render dogs and cats more susceptible to parasitism with *A. caninum*. Although these factors were the subjects of separate experimental studies, and are treated as such in the following pages, it is believed that the iron-depletion factor, with its consequent anemia, which was common to both investigations, might presumably have been the factor most responsible for the observed uniform effect upon resistance in both cases.

It should, perhaps, be stated that these investigations were conducted during 1932 and 1933, but were not pursued as extensively as had been originally planned. It has been felt, however, that the re-

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cent experimental activity along precisely the same lines in other host-parasite relationships (Porter and Ackert, 1933; Porter, 1935) has made it desirable to place on record these observations.

The effect of periodic blood loss upon the resistance of dogs to A. caninum.

The literature affords two instances, both involving the relationship of the chicken to its intestinal roundworm, *Ascaridia galli*, wherein the effect of blood loss upon susceptibility to helminthic infection has been observed. Herrick (1925) conducted a preliminary experiment and it seemed clear that in some cases the bled chickens were less resistant. Continuing along the same line, Porter and Ackert (1933) were able to give further support to Herrick's findings. The bled chickens were not uniformly more susceptible, but in 16 out of 20 experimental groups, they yielded at autopsy either more or longer worms than the controls.

In the present study, the procedure consisted in following the courses of hookworm infection and hemoglobin level in resistant mongrel dogs from which large quantities of blood were removed from 1 to 3 times weekly over rather long periods of time. The blood was aspirated by the method of cardiac puncture (Foster and Landsberg, 1934, p. 262).

It was possible to test the effect of bleeding in a preliminary way with an animal (D723) which had already become highly resistant to hookworm infection during the course of earlier experiments. After this animal had reached 8 months of age, it was used for a period of 40 weeks in studies of the effect of a deficient diet upon resistance, and its complete history for this period has been presented elsewhere (Foster and Cort, 1935, p. 304). It may be stated, at this time, however, that during a period of 12 weeks, while on a normal diet immediately preceding the period of bleeding, this dog was given a total of 21,210 larvae in seven doses. In spite of these heavy infections, the daily egg production dropped off to a level of about 150,000 E.P.D.,[3] a reaction which indicated an extremely resistant condition. The hemoglobin level at this time was about 81 per cent and its body weight about 5 kg. It was felt that this dog presented extremely favorable material for the present experiment because of this condition of almost complete resistance to further infection. For the period of bleeding the infective doses were reduced

[3] E.P.D. = eggs per day.

to 500 larvae each biweekly. The animal was bled eleven times during a period of 48 days, taking a total of 1285 cc. of blood. This represented an average blood loss of about 0.583 per cent of body weight per day for the whole period. The hemoglobin fell from 81 per cent to 41 per cent, a drop of about 50 per cent. The resistance of this host was no less affected. For the first 2 weeks of bleeding the daily egg output remained unchanged but by the third week it had increased to more than twice its original level and remained stable at this point (about 300,000 E.P.D.) throughout the bleeding period and for more than a week after bleeding was discontinued. Within 3 weeks of cessation of bleeding, however, the egg production was down to a much lower level than existed before the period of bleeding, indicating that its resistance was regained quickly and effectively when the animal was no longer bled.

Having observed this result in a preliminary test, an experiment was organized to take advantage of five dogs which were available at the time and presented favorable material. Only three of these were carried successfully through the whole experiment; the other two were killed before any effect had been observed, one by excessive bleeding (4.05 per cent body weight in blood taken twice within a period of 5 days), and the other by cardiac embarrassment as a result of hemorrhage into the pericardium. The following discussion will be limited to the results obtained with the three dogs which lived throughout the whole experiment.

Data on D752, D730, D755. These were not litter mates but all were about 10 months old when first infected for use in the present experiment. The data are presented in table 1.

The infection history of D752, shown in graph 1, has seemed to show most clearly the effect of heavy blood loss upon resistance to hookworm infection. An initial infection of 100 larvae was given when the experiment was started, in order to measure the degree of resistance of this dog while still unbled. The resulting infestation, which was followed for 67 days, showed throughout a daily egg production under 50,000 E.P.D. This agrees well with the type of infestation usually encountered in dogs of this age when given small infective doses. The hemoglobin level during this period varied between 72 per cent and 79 per cent, with an average of about 76 per cent. Beginning 48 days after this initial infection, and continuing for a period of 75 days thereafter, this dog was bled, taking an average of 37.8 cc. of blood daily. The average weight of the animal for this period was 8437 gm., making the daily blood loss (37.8

cc. = 39.9 gm.) equivalent to 0.47 per cent of the body weight. In all, a total of 2837 cc. of blood was taken in twenty-four bleedings.

TABLE 1.

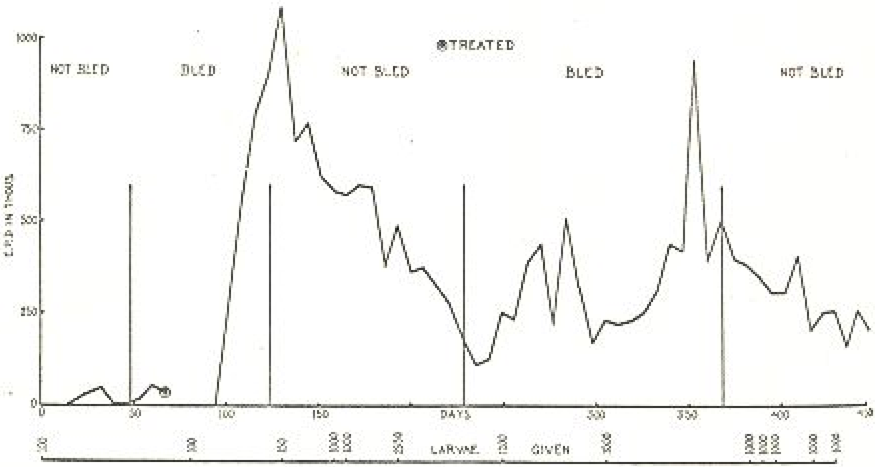
Data on three dogs subjected to periodic bleeding.

Days on exp.	D 730				D 752				D 755			
	Larvae given	Blood drawn cc.	Per cent hemoglobin	E.P.D. (thous.)	Larvae given	Blood drawn cc.	Per cent hemoglobin	E.P.D. (thous.)	Larvae given	Blood drawn cc.	Per cent hemoglobin	E.P.D. (thous.)
0	100		70		100		72		100		70	
25				+			72	34				64
32				94			73	46			68	636
39			76	119			76	2			66	459
46			85	66			79	2			65	526
48			79		Bleeding begun							
49	Bleeding begun											
53		150		84		120	71	13				440
60		300	58	83		340	49	50				463
67		200	44	131		150	47	31			56	427
67	All treated to negative											
81	100	400	53		100	407	45		100		63	
88		550	39			605	44				62	
95		150	42	–		180	37	+				+
102		405	33	–		475	32	274				89
109		350	28	–		250	27	555			70	91
116	1060			–		200	39	802			75	99
123		240	35	–		110	43	999				167
123					Bleeding discontinued							
130		200	33	–	150		38	1165	150			185
130									Bleeding begun			
137				+				738		320		85
144		200	36	+			70	831		200		134
151		560		+				651		510		241
158	1000	200	47	42	1000		64	587	1000	250	38	200
165	1000	275		96	1000			576	1000	300		286
172		255	42	108			67	603		280	37	251
179								599				310
186		610		27				377		710	41	343
193			37	53	2550			496	2550			330
200		535		67			72	369		550	47	452
207				84				375				367
214				137				328				177
221		75		122			77	286		325	52	337
228		500	52	33				172		650	34	402

TABLE 1.—*Continued.*

Days on exp.	D 730				D 752				D 755			
	Larvae given	Blood drawn cc.	Per cent hemoglobin	E.P.D. (thous.)	Larvae given	Blood drawn cc.	Per cent hemoglobin	E.P.D. (thous.)	Larvae given	Blood drawn cc.	Per cent hemoglobin	E.P.D. (thous.)
228					Bleeding resumed							
235		180		37		300	56	110				194
242		280		48		310		125		300		238
249	1200	250		25	1200	390	31	252	1200	405	35	441
256		250	32	94		320		237		400		123
263		250		96		280		339		350		288
270		310		50		310	40	462		310		207
277				23		340		217		360		288
284				86				511				309
291		250		14				320				268
298			38	53		240		169		350		675
305	1000	250		29	1000		51	231	100		36	404
312		300		123		320		222		350		325
319		555		118		210	52	282		300	51	305
326			41	258		375		253		410	25	136
333		610		186				312				142
340				82		620	35	437		590	25	407
347		300	24	197				424				465
354				218				944				237
361		250		289		320		393		300		233
368		770	28	299		680	32	506		680	38	175
368	All bleeding discontinued											
375				172				400				270
382	1000		38	379	1000		54	386	1000		33	155
389	1000		42	200	1000		58	353	1000		39	134
396	1000		51	112	1000		61	308	1000		41	49
403			59	365			67	306			45	93
410			65	494				409				55
417	1000			1379	1000		68	205	1000		56	88
424			60	3149				253				97
431	1000			2950	1000			257	1000			104
438			69	1603			79	159			67	127
445				389				258				255
452			78	396			87	204			75	89

On the sixty-seventh day, 19 days after bleeding was begun, this dog was treated to negative in order that it might be tested again with the same dose of larvae. Accordingly, a second infection of 100 larvae was given on the eighty-first day, the animal having been negative to Lane examination for more than a week. A good infestation developed from this reinfection, the eggs first appearing after a normal prepatent period of 14 days, with gradually increasing egg production throughout the period of bleeding. After the egg count had reached about 1,000,000 E.P.D., 123 days on experiment



GRAPH 1. The curve of daily egg production of dog 752, showing the effect of periodic bleeding.

and 75 days after bleeding was begun, the bleeding was discontinued. The hemoglobin level had been reduced to about 40 per cent, which represents a drop to approximately one-half of the original level. After the cessation of bleeding the egg count fell off rapidly in spite of four additional heavy infections, totaling 4700 larvae. Meanwhile the hemoglobin index returned to normal. Bleeding was resumed after 105 days and continued for a period of 20 weeks. The animal was bled 16 times, taking a total of 5015 cc. of blood, giving an average daily blood loss of 35.8 cc. which was equivalent to 0.389 per cent of body weight per day. During this second period only two infections totaling 2200 larvae were given, yet the general trend of the egg curve was materially affected. The egg output increased from an average of 110,000 E.P.D. to a maximum of 944,000 near the end of the bleeding period. The hemoglobin was again reduced to about half of its original level.

Bleeding was discontinued after 368 days on experiment, following which the animal was given five doses of 1000 larvae each within a period of 49 days. In spite of these heavy infections, the egg production fell off to about 200,000 E.P.D. and the hemoglobin returned to its normal level.

Another animal of this series, D730, presented a history which is a little more difficult to analyze. Dog 730 was given an initial infection of 100 larvae when the experiment was started, and the resulting infestation produced a maximum egg output of 131,000 E.P.D., 67 days after infection. At this point the animal was freed of its worms by tetrachlorethylene treatment, and was reinfected on the eighty-first day with another dose of 100 larvae. Meanwhile bleeding had been begun on the forty-ninth day, the infection history of this animal thus being similar to that of D752 up to this point. The bleeding period of D730, however, was continued for 319 days (49 to 368 days on experiment) during which time an average daily loss of 34.4 cc. of blood was sustained. This was equivalent to 0.423 per cent of the body weight per day. In this case, the dose of 100 larvae given during the period of bleeding failed to produce an infestation. Within about 8 weeks the percentage of hemoglobin had dropped about 60 per cent and the animal was again infected with 1060 larvae. The resulting infestation was very light, indicating that this dog was still highly resistant at this point in the bleeding. Two additional doses of 1000 larvae each were given after 158 days and 165 days respectively on experiment. As a result of these infections, there was built up a low grade infestation which persisted throughout the period of bleeding. Subsequent doses of 1200 and 1000 larvae each were given during this period but there was no immediate increase in the daily egg production following these infections. During the last 49 days, the animal was bled more heavily and no additional infections were given. For this interval, an average of 50.7 cc. of blood was taken daily, reducing the hemoglobin index, in one instance, to 24 per cent. The egg production was increased from 118,000 E.P.D. to 299,000, which has been regarded as a substantial increase in view of the high degree of resistance which had been exhibited by this dog. Bleeding was discontinued on the 368th day and the egg count rapidly fell off to 112,000 E.P.D. while the hemoglobin level rose to about 50 per cent. Meanwhile, there were given three infections of 1000 larvae each at weekly intervals. With the maturation of worms from these infections, the daily egg production rose rapidly to a peak of 3,149,000 E.P.D This greatly increased rate of egg output was not sustained, however, and the egg count fell off as rapidly as it had risen, in spite of additional infections.

There are two points in the infection history of this highly resistant dog which merit particular attention. In the first place there was a period of moderate susceptibility near the end of the bleeding period where the egg production increased significantly, in the absence of recent infections. In the second place, there was a period of marked susceptibility, equivalent almost to a complete breaking of resistance, during the 8 weeks immediately following the cessation of bleeding. Heavy infections were given during this period of susceptibility and the egg production was increased more than tenfold over what it had reached at any time previously. It is felt that both of these effects may be correlated with the fact that relatively large quantities of blood were taken during the last 49 days of the bleeding period. The very rapid recovery of resistance which followed this period of great susceptibility appears also to indicate such a relationship.

The data on the third dog of this series, D755, offer evidence which tends to confirm the findings on the other two. This dog was initially infected with 100 larvae, and the resulting infestation produced a maximum of 636,000 E.P.D., 4 weeks after infection. This infestation was expelled by treatment on the sixty-seventh day, and the animal was reinfected with 100 larvae on the eighty-first day. There was no bleeding throughout this second infestation, contrary to the procedure followed in the other two cases. The egg output this time rose to a maximum of only 185,000 E.P.D., this peak being reached 7 weeks after the second infection of 100 larvae. At this point, an additional 150 larvae were given, and bleeding was begun. For a period of 238 days subsequently, the animal was bled regularly, taking an average of 38.7 cc. of blood per day. This was equivalent to 0.426 per cent of the animal's body weight per day. During the bleeding period, a total of 6750 larvae was given in five infections. There was a tendency toward a generally increasing egg production throughout this period, although the average eggs per day did not exceed 675,000 at any time. On the other hand, with the cessation of bleeding (368 days on experiment), the egg count fell to a level of about 100,000 E.P.D., even under conditions of repeated heavy infection.

The discussion thus far has covered an experimental period of 452 days, the data for this period having been presented in table 1. It may be said, however, that these three dogs were followed for an additional 182 days, during which time two of the dogs (D730, D752) were again bled. It seemed unnecessary to add to the table the data for this relatively long period since the pertinent facts, although very significant, may be stated quite briefly. D755, under continued heavy infection, went negative after 494 days on the experiment, was treated on the 508th day, and gave birth to eight puppies on the 520th day. An infection of 1000 larvae given on the 547th day produced a transient infestation, with a patent period of only 4 weeks, and a maximum egg output of only 17,000 E.P.D. D752 went negative after 522 days on experiment and later, during a 31-day bleeding period, reached a maximum egg production of 8000 E.P.D. Forty-two days after cessation of bleeding this animal went negative again. D730 went negative on the 536th day, was bled from the 547th to the 578th days, during which period the egg production increased to a maximum of 43,000 E.P.D. at the close of the bleeding period. During these last bleeding periods, from 400 to 600 cc. of blood were taken from each animal every 5 days.

While such findings do not indicate a striking susceptibility as a result of bleeding, it is nevertheless quite clear that the animals were chronically unable to react so as to throw off their worm burdens so long as the bleeding was continued. It is unfortunate that the nature of these observations precluded any information as to whether the increased egg output for the bleeding periods was due to an increased maturation of worms, or more directly to a removal of the restraint which a high degree of resistance placed upon the function of egg production, although either effect must be interpreted as a shift in the direction of susceptibility.

The effect of a milk diet upon the resistance of dogs and cats to hookwork infection.[4]

Recently, Porter (1935) demonstrated that a higher rate of development of *Nippostrongylus muris* occurred in rats on a whole milk diet than in rats maintained on a good stock ration. This must be interpreted as a change in the direction of susceptibility.

The present studies were conducted on three dogs and five cats. In addition, infections were attempted with the human hookworm, *Necator americanus*, upon two puppies on a milk diet.

During the early part of the experiments the diet consisted of whole milk powder[5] mixed with 7 parts of water. It was soon found necessary, however, to alter this diet so as to ensure fecal samples of proper consistency to make possible an accurate estimate of the daily

[4] The writer is indebted to Dr. H. D. Kruse of the Department of Biochemistry for suggestions upon the composition of this diet. It is a pleasure to acknowledge at this time that the counsel and assistance of Dr. Kruse have been important factors in the execution of this and other dietary studies.

[5] A product of the Mead Johnson Co.

egg production of the infestations. After consideration of the technical difficulties involved, the following diet given in percentage composition by weight, was decided upon: whole milk powder, 72.3; cane sugar, 18.7; and calcium carbonate, 9.0. This was prepared in a dry mixture and combined with 5 parts distilled water for feeding, making a diet equivalent to 95.4 per cent milk, 3.1 per cent dextrose, and 1.5 per cent $CaCO_3$. It was estimated that the addition of sugar up to 3.1 per cent, raised the caloric value approximately 15 per cent per unit of weight, while the $CaCO_3$ fraction effected the desired consistency of the stools. The iron content of this diet, about 0.000225 per cent,[6] compares very favorably with that of fresh milk. An attempt was made to feed a sufficient quantity of this food to satisfy the caloric requirements of the animals. For the most part the experimental animals ate well, although in some instances, the appetites failed after an extended time on this diet and the animals had to be fed artificially with a stomach tube.

Data on D746, D747, D748. These dogs, litter mates reared in the laboratory, were about 1 year old when consigned to this experiment. They had been infected for about 10 months previously in connection with studies upon the immunity of dogs to *A. caninum*. Their complete infection histories during this earlier period have been published (Foster, 1935, p. 75). It may be reviewed that during this earlier 10-month period these dogs had been given 500 larvae each biweekly and had developed a marked resistance to the later infections. Having obtained such a thorough record of the degree of resistance present, these animals were placed upon a milk diet and the standardized larval doses continued.

One animal (D748) died after being on the milk diet only 61 days. At autopsy 66 worms were recovered. Hemoglobin readings made throughout the experiment showed that this dog was not more anemic when on this diet than normally. Likewise, there was no indication by egg count that this dog had lost any appreciable degree of resistance to *A. caninum*. There was, however, a surprising loss of weight, which approached 57 per cent of its original weight. The second dog (D746) survived 173 days and at autopsy 69 worms were recovered. There was a loss of 46 per cent in weight, but again there was no indication either from hemoglobin readings or from observations of the organs at autopsy that any success had been achieved in

[6] Computed from an estimate of the iron content of the whole milk product, the analysis being furnished the writer by Mead Johnson Co. in a private communication.

producing a nutritional anemia. Likewise there was no evidence, either by egg counts throughout the experiment, or by worm counts at autopsy, that the prolonged period on a milk diet significantly altered this dog's resistance to hookworm infection. The third member of this litter (D747) died after 197 days on milk diet, after having lost 51 per cent of body weight during the diet period. This dog became very anemic toward the end, as was strikingly evidenced by the blanched appearance of the liver and lungs at autopsy. A total of 269 worms was recovered, which is significantly more than was obtained from either of the other two dogs. Furthermore, 163 of this number were young worms, a finding which suggests that the resistance of this dog had been broken only a short time before death, at which time a number of worms from recent larval infections were permitted to mature. There was, however, no evidence by egg count of a decreased resistance, the egg count remaining at a level below 50,000 E.P.D. throughout the entire period on milk diet.

In view of the results of other experiments, which are discussed in the following pages, it is important to note the general character of the information which the studies on these three dogs have supplied. Before being placed on the milk diet, these animals had been exposed to periodic hookworm infection for almost a year, and had developed a condition of pronounced resistance to further infection. While on the milk diet, the rate of infection was continued as before, yet the egg output was not materially altered, indicating that the degree of resistance remained unchanged. On the other hand, these dogs lost from 46 to 57 per cent in body weight during this period, and died after from 61 to 193 days on this diet. At autopsy, only one animal, D747, showed evidence of a recently increased worm burden. This dog had also survived the longest (197 days) on the diet and was the only one which had developed a severe anemia.

Attempts to infect puppies on a milk diet with Necator americanus. It is desired to interpose at this point a short discussion of the two puppies which were placed on a milk diet and infected with the human hookworm.

There is apparently no record of the development of *N. americanus* in the dog. The only available report of attempts in this direction is entirely negative (Foster and Cort, 1932, p. 597). In a study by Schwartz and Alicata (1934) it was found that the infective larvae of this parasite given to guinea pigs probably always passed through the lungs, and subsequently developed to the fourth stage in the intestine of that host. With respect to the other important intestinal hookworm of man, *Ancylostoma duodenale,* there appear to be two reports of attempts to infect dogs (Looss, 1911; Nauss, 1921), but in neither of these, as in the cases above, did the worms reach sexual maturity. Nakajima (1931) tried to favor the establishment of *A. duodenale* in rabbits by previously treating the infective larvae with cell emulsions of human organs. It was concluded that these cell emulsions of the normal host (particularly lung tissue) increased the rate of development of this parasite in the rabbit, although no worms reached sexual maturity.

The present experiment involves two puppies which were born in the laboratory, and which were placed on a milk diet when 62 days old. Each was infected the day after being placed on this diet with 4000 larvae of *N. americanus.* In each case 2000 larvae were given by skin and 2000 by mouth. One puppy was autopsied 24 days after infection and 5 worms were recovered. The other puppy was autopsied 31 days after infection and 26 worms were recovered. In both cases the worms were identified as *Necator americanus.* There was no indication of sexual maturity, however, although the worms were otherwise mature and the sexes easily distinguished. It is believed that this is the only record of the establishment of *N. americanus* in the dog host, and the only instance where either of the human hookworms has been reared for as long as a month in an abnormal host. This finding parallels that which has been reported for *A. duodenale,* although in this case (*N. americanus*), the establishment of the parasite may have been favored by the feeding of a milk diet to the dog host.

Experiments with cats, C591, C594, and C595, on a milk diet. These cats were about 10 months old when infected with the cat strain of *A. caninum* (Scott, 1929; Foster and Daengsvang, 1932), a strain of hookworms particularly adapted to the cat. While on a normal diet, each was given a test injection of 11,700 larvae intraperitoneally. One cat (C594) became positive to Lane examination 18 days later, and the other two remained entirely negative until several days after transfer to a milk diet. Since all three cats showed extreme resistance to these initial infections, they were again infected intraperitoneally on the twenty-fifth day with doses of from 3500 to 8500 larvae each (see table 2 and graph 2), and then put on a milk diet. After about 5 weeks and in the absence of additional larval doses, all three cats were showing relatively substantial egg productions (89,000 to 112,000 E.P.D.). In the case of C591, there was a gradually increasing egg output, with a sharp rise just before the animal died (66 days on the

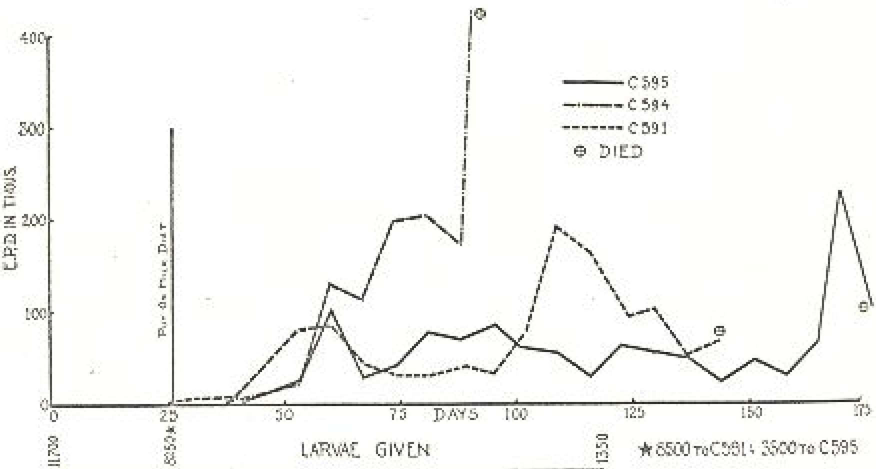
TABLE 2.

Data on the infection histories of cats 591, 594, and 595 which were maintained on a milk diet.

Days on exp.	C 591		C 594		C 595	
	Larvae	E.P.D.	Larvae	E.P.D.	Larvae	E.P.D.
0	11,700		11,700		11,700	
25	8,500	0	8,250	+	3,500	0
26	Put on milk diet					
32		0		3		0
39		0		4		0
53		18		80		25
60		130		89		101
67		112		43		28
74		196		29		42
81		207		28		78
88		168		39		70
91		429				
95	Died			32		85
102	66 days on milk diet			77		61
109				192		57
116				160		29
118			1,350		1,350	
123				93		63
130				100		56
137				51		45
144				65		16
151			Died			43
158			118 days on milk diet			27
165						61
172						232
178						97
					Died	
					142 days on milk diet	

milk diet). At autopsy 324 worms were recovered. During the period on milk diet, this animal lost about 35 per cent in body weight, and it was evident at autopsy that it had become very anemic. The terminal failure of the resistance mechanism, as indicated by the marked increase in egg production just before death, was similar to what had been observed in young dogs on a generally deficient diet (Foster and Cort, 1935). The worm burden at autopsy was unusually large even for young susceptible kittens. The histories of the other two cats (C494, C595) were very similar, as can be seen in graph 2, although there did not occur in either of these cases a marked terminal break-down of resistance, such as was noted in C591. It is evident, however, from the generally increasing proportions of the egg counts throughout the period of milk diet, and from the worm burdens at autopsy (482 and 355 hookworms respectively for C594 and C595), that the resistance of these animals was materially reduced as a result of maintenance on the milk diet.

Experiments with C530, C533. Two cats, which had outgrown their usefulness as culture animals as a result of increasing age and



GRAPH 2. The curves of daily egg production of three cats which were maintained on a milk diet.

more than a year of repeated infection, were placed on the milk diet. This long history demonstrated that they had become almost completely immune to further infection so long as they were maintained on the stock diet. The data covering these cats (C530, C533) are summarized in tables 3 and 4. The infection history of C533 for the first 431 days is not given in detail, since the important aspect of this earlier history has already been mentioned.

The interpretation of the record on C530 has seemed to be perfectly clear, as the findings agree in all important particulars with what has already been discussed for the three previous cases. The animal died after 83 days on the milk diet, the organs appeared extremely anemic at autopsy, and there had occurred a marked terminal increase in the egg output during the 2 weeks before death, with a burden of 223 worms recovered. The case of C533 is not so clear. This animal had been infected for 636 days, and was maintained for

TABLE 3.

Data on the infection histories of cats 530 and 533 which were maintained on a milk diet.

Days on exp.	C 530	
	Larvae	E.P.D.
0	210	
2	125	
30	2,500	
41		11
50	200	
65	2,000	62
163		2
182	1,000	
213	1,000	
240		38
258		53
282	5,000	
303		25
340	400	
359		18
380		13
387		4
395		+
402	Put on milk diet	
408		+
415	11,700	+
422		+
455	3,300	+
471	2,200	27
478		75
485		91
Died 83 days on milk diet		

Days on exp.	C 533	
	Larvae	E.P.D.
9 infections totaling 10,115 larvae. Maximum egg count (120) 119th day. Subsequently as follows:		
432		13
433	Put on milk diet	
436		16
443		28
446	11,700	
450		17
457		14
471		21
486	3,300	2
502	2,200	2
509		14
516		20
523		7
530		11
537		5
544		5
551		3
558		+
564	1,300	+
606	30,000	+
625		6
632		15
636		6
Died 203 days on milk diet		

203 days on the milk diet before it succumbed. This animal was younger than C530, although it had had a much longer infection history, but it survived over two and one-half times longer on the milk diet than the younger animal. This result suggests that C533 may have been getting something which it had been intended to rule out of its diet (i.e., iron). In dietary experimentation, such possibilities must always be recognized, and in this instance it has seemed that this explanation might account for the results obtained. It cannot be said with certainty that the history of this animal is indicative of a reduced resistance while on the milk diet, although there was a

slightly elevated egg production during the 2 weeks before death, and at autopsy 337 worms were recovered. It is to be recognized, however, that in this case this seemingly indicative result may have been influenced by an abnormally large dose of larvae (30,000) which was given only 30 days before the animal died. This dose was prompted by the desire to determine the size of larval doses which this animal could resist. At the time of death this cat was very anemic and had

TABLE 4.

Summary data of the animals used in the milk diet studies.

Animal number	Age in months*	Total days infected	Total larvae given	Days on diet	Larvae given on milk diet	Weight loss on milk diet		Worms recovered
						Gm.	Per cent	
C 530	26	485	29,635	83	17,200	850	38	223
C 533	21	636	58,615	203	48,500	600	35	337
C 591	10	92	20,000	66	8,500	600	35	324
C 594	10	144	21,300	118	9,600	700	35	482
C 595	10	178	16,550	142	4,850	1,100	42	335
Average				122		750	37	340
D 746	12	453	13,136	173	5,500	2,800	46	69
D 747	12	491	13,880	197	6,000	3,100	51	259
D 748	12	341	8,373	61	1,500	3,500	57	66

* Age when put on milk diet.

lost 35 per cent in body weight. Yet in the absence of earlier test infections of similar magnitude, one cannot say that the end result, observed in this animal, was anything other than the effect of an overwhelming massive infection. It is interesting, also, that this latter interpretation not only seems reasonable, but it agrees well with the suggestion that the diet of this animal may not have been successfully restrictive, and accounts for the unsuccessful result as compared with the results on the other four cats of this study.

DISCUSSION.

Briefly summarized, it has been shown that four dogs were rendered more susceptible to hookworm infection by periodic bleeding. In addition two dogs were seemingly rendered more susceptible to the human hookworm, *Necator americanus,* by maintaining them on an iron-deficient (milk) diet. Also 4 of 5 cats and 1 of 3 dogs were quite definitely less resistant to hookworms when kept on a milk diet. The fact that 1 cat and 2 dogs, on milk diet, did not react like their experimental mates, suggested the possibility that, in some uncontrollable way, these animals were getting the substance or substances necessary to meet the needs of the body in maintaining resistance to infection. In general, however, it has been felt that those animals in which the milk diet effected its purpose (the production of anemia) were rendered more susceptible. There remains only the problem of aligning the information obtained on (1) the bleeding experiments and (2) the milk diet experiments into the form of a provisional hypothesis.

It has been assumed that the fundamental effect upon the host of periodic bleeding is an iron-depletion, with a consequent anemia. Likewise, the assumption is made that a lack of iron is the fundamental deficiency of the milk diet used in these studies, and that this deficiency produces a nutritional anemia. It has been shown for the experiments discussed above, that these anemias did follow in the wake of periodic bleeding and the feeding of a milk diet, and that animals were rendered less resistant to hookworm infection by both processes. It has also been explained that those animals which were not rendered anemic on the milk diet were those which failed to become less resistant. While the evidence is admittedly more directional than conclusive, it does appear reasonable to interpret these results as indicative of a positive correlation between iron-deficiency and decreased resistance to infection with the dog hookworm. In turn, an iron-deficiency may be readily translated into terms of an anemia.

There are also other lines of evidence which have pointed to a relationship between the hemoglobin level and the resistance of dogs to *A. caninum*. It was shown by McCoy (1931) that an improvement in the hemoglobin level is the first indication of a developing resistance, and that this was followed by reduced egg output and spontaneous loss of parasites. It was observed by Foster and Landsberg (1934, p. 283) that an increased resistance was practically always evidenced following the improvement of the hemoglobin level of infected dogs by iron therapy.

These considerations strongly suggest that there is a relationship between a condition of anemia and susceptibility to hookworm infection. It has seemed that this view has at least two important applications:

First, it is possible that these findings throw some light upon the explanation of age-resistance, as observed, for example, in the reactions of the dog to *A. caninum*. In the course of hookworm studies, references to several of which have been cited in this report, the writer has often reflected upon the interesting fact that the curve of age-resistance in this host-parasite relationship corresponds to the age-curve of hemoglobin concentration in the blood. Young animals are anemic and susceptible, but over a course of several months, the hemoglobin level gradually improves and the animal becomes gradually more refractory to hookworms. It was felt that if there were an association in this case, certain experimental procedures could be devised to test it. It seemed, for example, that the inducing of an anemia in a resistant animal ought, according to this hypothesis, render him again susceptible to infection. It was partly for this reason that the present studies were conducted, with the result that this hypothesis has been, to some extent, supported. It was also apparent, that since the effect of hookworms upon their hosts is the production of anemia, then the presence of a severe enough infestation to produce anemia in resistant animals ought, by this hypothesis, to render them even more susceptible to subsequent infections (a directly contrary result to what would be expected in the event of an acquired immunity). That overinfestation is a factor which can break down resistance has already been demonstrated (Foster, 1935, p. 101). On the basis of this hypothesis it must follow also, that, were it possible to produce in young dogs (naturally anemic) an abnormally high hemoglobin level, they should then be more resistant to infection. Because Orten *et al* (1932) had demonstrated the production of a polycythemia in rats by the administration of cobalt, this element was tested on young puppies, but it was not possible, in our studies, to produce any significant rise in the hemoglobin level. This has been mentioned, because, with further studies, this result may be achieved, and it is desirable, if possible, that this hypothesis be tested further. It is probable that this hypothesis lacks much of explaining age-resistance, as observed in helminthic and other infections, but it does appear that this is the only experimental attempt which has been made to throw some light on this phenomenon.

Secondly, if it be presumed that these findings are applicable to human hookworm disease, it must be accepted that a heavily infected individual is *ipso facto* an easier prey to increased parasitism. Moreover, it follows that in areas where people suffer from nutritional anemias, which seems to be the case in many endemic hookworm regions (e.g., Porto Rico), one may perhaps attribute part of the hookworm problem to these anemias. In other words, these anemias probably militate against the individuals' resistance to the invasion and establishment of hookworms as well as against their resistance to the injurious effects of these parasites.

Summary.

1. Four resistant dogs were rendered more susceptible to parasitism with *A. caninum* by periodic bleeding.

2. Two puppies were infected with *Necator americanus* when they were maintained on a milk (iron-deficient) diet. The worms did not become sexually mature but reached the fifth stage and survived in the host over a month.

3. In studies upon three dogs and five cats, it was found that one of the dogs and four of the cats became distinctly more susceptible to hookworms when they had been fed for several weeks on a milk diet.

4. It was considered most probable that iron-deficiency, with its consequent anemia, was the responsible factor in each case.

5. It was suggested that age-resistance may possibly be related, in part, to the natural age-curve of the hemoglobin level of the host.

6. The above findings, together with other observations, were interpreted as indicating a probable correlation between anemia and resistance to hookworm infection.

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