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FURTHER STUDIES ON THE EFFECT OF A GENERALLY DEFICIENT DIET UPON THE RESISTANCE OF DOGS TO HOOKWORM INFESTATION.

FURTHER STUDIES ON THE EFFECT OF A GENERALLY DEFICIENT DIET UPON THE RESISTANCE OF DOGS TO HOOKWORM INFESTATION.*

By

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In recent studies an experimental demonstration has been given of a correlation between malnutrition in dogs and susceptibility to infection with the common dog hookworm, *Ancylostoma caninum* (Foster and Cort, 1931; 1932). The malnourished condition was characterized by a lowering of the resistance to infection in animals that had developed a very great resistance due to previous infection and age. There was an increased rate of development of the worms and a greatly increased egg production of the females. When the dogs that had acquired an infestation while on a deficient diet were transferred to a good diet, their recovery of resistance was indicated by a spontaneous loss of worms, which in certain cases amounted practically to a dietary cure, and the development of a resistance to further infection.

The data of the present report have been derived from additional studies on the changes of resistance effected in dogs by the feeding of the same inadequate diet † (Foster and Cort, 1932, p. 242).

The results have exhibited a uniform agreement with our earlier findings; yet their real importance is found in certain new and interesting information which has come in part from the use of younger experimental animals in which the effect of age upon resistance was not so strongly marked as in the case of the animals used earlier, and in part also from the fact that observations were made upon several dogs which were carried to the point of death on the poor diet.

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The twelve dogs upon which these further studies have been made were street dogs of mongrel breed, brought into the laboratory when from 2 to 9 months old. Their ages when first infected in our laboratory ranged from 7 to 19 months. While on a normal diet, they were subjected to repeated hookworm infection to which they responded with the manifestation of a reasonable degree of resistance

TABLE 1.

Data on D 723 and D 727 of group 1.

Weeks	D 723			D 727		
	Larvae given	E.P.D.* in thousands	Per cent hemoglobin	Larvae given	E.P.D. in thousands	Per cent hemoglobin
0	500		72	500		56
1.1			72	500		59
2.3	500		70	500		61
3.4			66	660	141	59
4		139			617	52
4.6	625	333	62	625	824	66
5.9	1,000	312	73	1,500	2,252	62
6.9		215			2,345	64
7.9		307	69		1,437	67
8.9		283	72		1,455	62
9.1	Put on poor diet					
9.9		257			1,011	
10.9		181	74		739	65
11.9		98			594	
12.9		161	77		894	69
13.9		196			759	67
14.9		261	69		1,085	
16	150	239		150	511	72
17		255			1,007	
18	600	349	75	600	990	61
19		282			774	
20		297	59		608	55
21		339			1,186	
22		401	40		1,189	41
23		622			1,670	
23.9	Given liver extract					
24		698			1,265	
25					1,178	47
26		801	44		1,551	
27.4	Returned to normal diet					

TABLE 1 (*Continued*).

Weeks	D 723			D 727		
	Larvae given	E.P.D.* in thousands	Per cent hemoglobin	Larvae given	E.P.D. in thousands	Per cent hemoglobin
28		548	69		1,470	
28.3	300					
29		486			638	
29.4	2,700			2,600		
30		387	58		657	53
31	500	348			379	
32		196	73		414	68
32.4	3,000			3,000		
33		224			294	
34		167	81			69
34.4	13,710			6,855		
35		70			595	
36		199	90		352	78
37		173			368	
38	500	94	83	500	413	72
39		170	84		194	73
40	500	121	86	500	192	73

Continued on other experiments.

* E.P.D. = eggs per day.

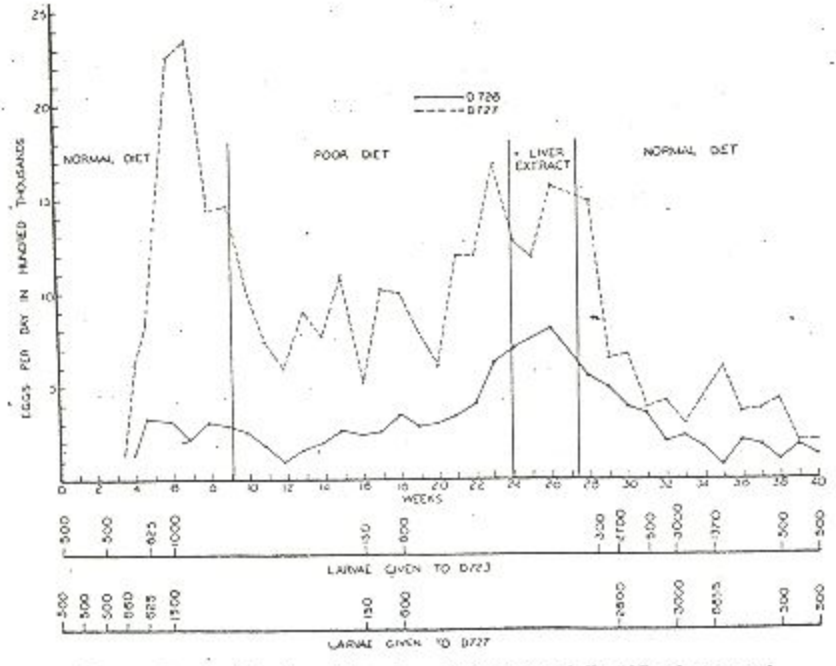
to further infection. In all cases infective larvae were orally administered and estimations of daily egg production were made by the dilution count on 3-day composite fecal samples. The egg count data have been derived from averages of the two egg counts made each week.

In order to facilitate the presentation of the experimental work, the animals have been divided into four natural groups. The data on each will be presented separately.

Group I, comprising D 723 and D 727. The data pertaining to these animals are presented in table 1 and graph 1. Beginning at about 8 months of age, these dogs were given a series of heavy doses of hookworm larvae. Over a period of 6 weeks, D 723 was given four infections, totalling 2,625 larvae; yet the maximum egg output reached during this preliminary period of infection was only 333,000 E.P.D.* This ability to withstand relatively heavy doses of larvae may perhaps be explained by the age of the host. Furthermore, it is significant to note that the peak of egg production (graph 1) followed

* E.P.D = Eggs per day.

the maturation of worms from the first two doses of infective larvae, and that subsequent infections of 625 and 1,000 larvae respectively failed to increase the egg output. After 9 weeks on a good diet, during which time this resistance as manifested by the reduction in the egg count became evident, the animal was placed on the deficient diet. For 3 weeks on this diet, the daily egg production continued



GRAPH 1. Infestation histories of D 723 and D 727 of group 1.

to fall and reached a low level of 98,000 E.P.D., indicating that the resistance which had begun to develop while on the normal diet continued to be effective for some time after the change to the deficient diet. After the third week on poor diet, the egg output began to increase and continued steadily upward throughout the remainder of the period on this diet. This increase occurred in the absence of additional larval doses during the first 7 weeks on the poor diet; therefore it indicates that the breaking of resistance under the influence of the deficient diet resulted in an increased egg production of the worms already sexually mature. It is not impossible, however, that some worms from previous infections may have been retarded in the development on account of a previous condition of resistance, and that with increasing susceptibility of the host they were per-

mitted to mature and thus contribute to the increase in egg production. After the seventh week on poor diet, D 723 was given doses of 150 and 600 larvae separated by an interval of 2 weeks, to test the degree of resistance to superinfection. It has been pointed out that doses of 625 and 1,000 larvae failed to produce an increase in egg production while this animal was retained on the normal diet. Under the influence of the deficient diet, however, the resistance to superinfection had disappeared, since the 5-week period following the administration of these relatively small doses of larvae was characterized by a steadily increasing egg output. After 103 days on the poor diet the daily egg production reached a peak (622,000 E.P.D.), nearly twice that which had been reached when the animal was first infected on a normal diet (333,000 E.P.D.). As a result of the prolonged period on poor diet, the animal lost 37 per cent of its body weight and grew very anemic and emaciated.

The findings on D 727 were essentially similar to those already recited for D 723. This dog was given a preliminary series of six infections totalling 4,285 larvae while on the good diet, during which time it became highly resistant to further infection. It was fed on the deficient diet for 103 days, during which time, it lost its resistance, became anemic and weak, and was reduced 35 per cent in body weight. In this instance, a maximum egg count of 2,345,000 E.P.D. was recorded during the initial period of infection on a good diet. There was no increase in daily egg production which could be attributed to the accession of worms from the last dose of 1,500 larvae which was given during this period. Likewise, in this case the egg count fell off for several weeks after transfer to the deficient diet but began to increase without further administrations of infective larvae. Although the resistance of D 727 was broken down under the prolonged maintenance on a poor diet, the animal did not in this case reach as high a level of egg production under the influence of the deficient diet as was recorded during the early period of heavy infection while on the normal diet.

There is an additional feature in the dietary history of these animals (D 723, D 727) which deserves emphasis. Instead of an immediate transfer from the deficient diet to a normal diet, the period on poor diet was followed by a period of 3 weeks during which the deficient diet was supplemented by the addition of liver extract.* Each dog was given about 5 gm. per day of the powdered extract.

* The liver extract used in these experiments was supplied by the Parke, Davis and Co.

In both cases, this treatment was followed by a return of resistance which was interpreted as being about the same as had occurred in other animals when returned directly to an adequate diet. Although these data are insufficient to warrant conclusions with respect to the restorative power of liver extract, it has seemed worth while to point out that both animals exhibited a "crisis" in egg production during this short interval of liver therapy and that in both cases there was a slight improvement in the hemoglobin level (cf. table 1). This result is of some significance since it suggests that the favorable responses which have followed the administration of liver extract to some cases of human hookworm disease may have resulted more from a general dietary improvement than from a specific effect of liver therapy.

Following this brief period of liver extract administration, D 723 and D 727 were returned to the normal diet. In order to test the effectiveness of the resistance which was being regained rapidly at this time, both dogs were subjected to repeated infection with large numbers of larvae. During a period of 10 weeks, D 723 was given a total of 21,000 infective larvae in six doses and D 727 was given about 13,500 larvae in five doses. In neither case did these administrations of larvae produce any significant changes in the curves of daily egg production, unless, perhaps, in the direction of increased resistance.

Since it was evident that the resistance of these dogs, in so far as could be determined by egg count, was broken down by the deficient diet and restored again by an adequate diet, it was considered unnecessary to sacrifice them for purposes of obtaining worm counts.

Group II, comprising D 731 and D 732. The data on these dogs are given in table 2 and graph 2. They were placed on the deficient diet when about 8 months old, and infected with 1,500 larvae each 3 days later. For a period of 60 days on the poor diet, the egg productions gradually increased, a finding which indicates that the animals were kept highly susceptible to hookworm infection so long as the deficient diet was fed. It cannot be said with certainty, however, that in these cases the failure to develop an immunity was due to poor diet, since no control infections were carried out while the animals were retained on a normal diet. By interpretation of the results on these two animals in the light of the results of other experiments, however, support is given to the belief that the high susceptibility which prevailed while the animals were maintained on deficient diet was the direct effect of this diet. Further support is offered by the fact that a resistance to infection was rapidly developed when the ani-

TABLE 2.

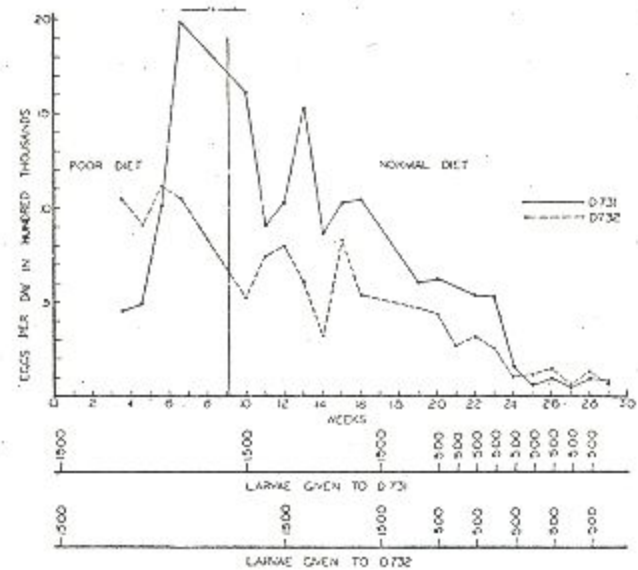
Data on D 731 and D 732 of group 2.

Weeks	D 731		D 732	
	Larvae given	E.P.D. in thousands	Larvae given	E.P.D. in thousands
0	Put on poor diet			
0.3	1,500		1,500	
3.6		456		1,049
4.6		490		913
5.6		1,011		1,116
6.6		1,989		1,050
8.6	Returned to normal diet			
10	1,500	1,614		526
11		903		744
12		1,031	1,500	798
13		1,531		609
14		867		322
15		1,028		823
16		1,040		535
17	1,500		1,500	
19		606		467
20		620		435
20.1	500		500	
21		587		270
21.1	500			
22		534		318
22.1	500		500	
23		532		256
23.1	500			
24		161		103
24.1	500		500	
25		59		111
25.1	500			
26		99		148
26.1	500		500	
27		43		67
27.1	500			
28		92		131
28.1	500		500	
29		78		69

Continued on other experiments

mals were returned to a normal diet. This resistance persisted even under conditions of repeated infection.

Group III, comprising D 674, D 703, D 708, D 717, D 720. The data on these dogs are presented in table 3 and graph 3. The results of this experiment are in many respects more significant and conclusive than any of the experiments previously reported. All of the



GRAPH 2. Infestation histories of D 731 and D 732 of group 2. No examinations made from sixth to tenth weeks.

effects upon resistance to hookworm infection which have been ascribed to the influence of diet in previous experiments were exhibited by these animals with a striking uniformity. The conduct of this experiment was similar to that of other experiments wherein an initial series of repeated infections was given while the dogs were on a good diet and, when a marked resistance became evident, the animals were transferred to the deficient diet. Four of the dogs of this experiment were younger (7 to 9 months old) than any of those studied previously under the influence of deficient diet, and all five of these animals were maintained on the deficient diet until their deaths closed the experiment. It is felt that these considerations added particular significance to some of the responses to the deficient diet which were regularly elicited.

In order to point out the nature of the findings on these dogs, the history of one of them (D 674) will be reviewed in some detail.

TABLE 3.

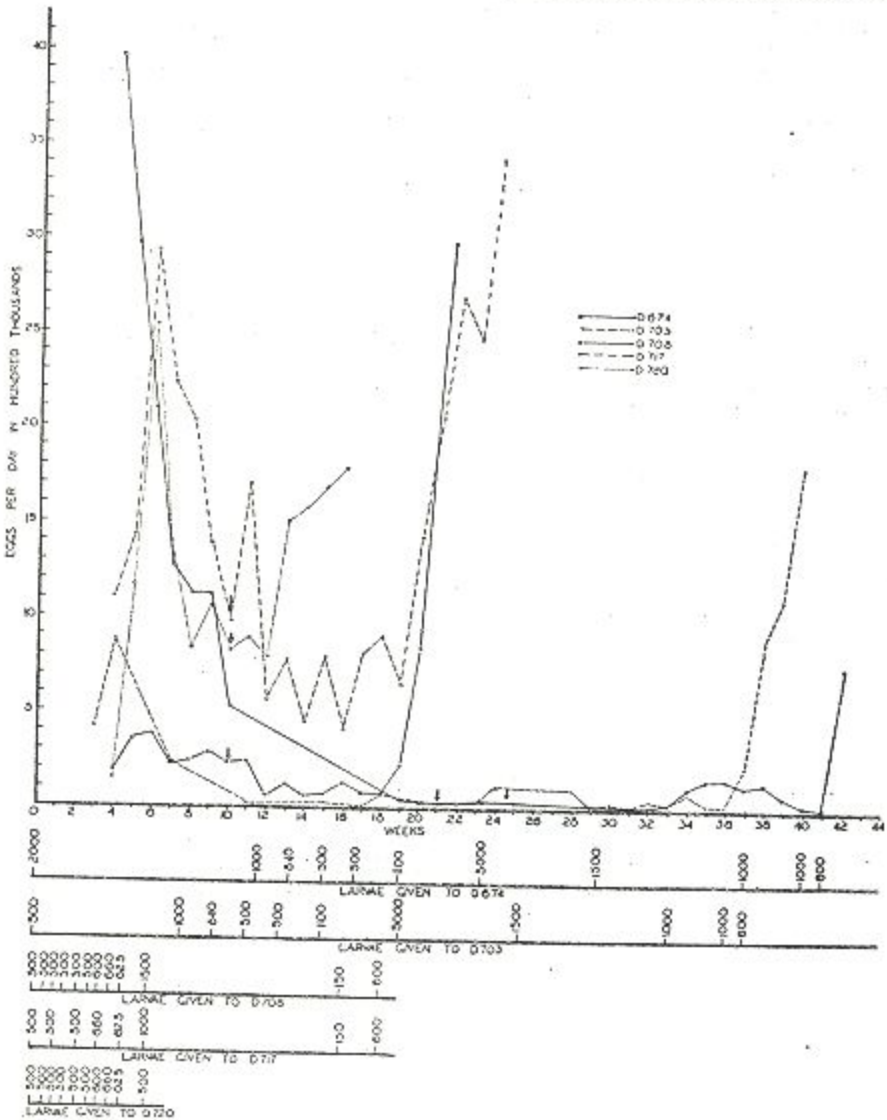
Data on D 674, D 703, D 708, D 717, D 720 of group 3.

D 674			D 703			D 708			D 717			D 720		
Weeks	Larvae given	E.P.D. in thousands	Weeks	Larvae given	E.P.D. in thousands	Weeks	Larvae given	E.P.D. in thousands	Weeks	Larvae given	E.P.D. in thousands	Weeks	Larvae given	E.P.D. in thousands
0	2,000		0	500		0	500		0	500		0	500	
4		3,956	3		420	0.6	500		1.1	500		0.6	500	
5		2,968	4		880	1.1	500		2.3	500		1.1	500	
6		2,091	7		238	1.6	500		3.1	660		1.6	500	
7		1,277	7.6	1,000		2.3	500		4		1,106	2.3	500	
8		1,128	9.3	840		2.9	500		4.6	625		2.9	500	
9		1,127	11	500	19	3.4	660		5		1,429	3.4	660	
10		534	12.7	500		4	600	187	5.9	1,000		4	600	154
11.6	1,000		15	1,100	31	4.6	625		6		2,929	4.6	625	
13.3	840		17		12	5		360	7		2,233	5		1,166
15	500		18		73	5.9	1,500		8		2,032	5.9	500	
16.7	500		19	5,000		6		381	9		1,392	6		2,535
19	1,100	48	21		25	7		229	10		979	7		1,320

TABLE 3 (*Continued*).

D 674			D 703			D 708			D 717			D 720		
Weeks	Larvae given	E.P.D. in thousands	Weeks	Larvae given	E.P.D. in thousands	Weeks	Larvae given	E.P.D. in thousands	Weeks	Larvae given	E.P.D. in thousands	Weeks	Larvae given	E.P.D. in thousands
20		34	21	Put on poor diet		8		246	10	Put on poor diet		8		841
23.3	5,000	43	22		32	9		293	11		1,694	9		1,064
24.6	Put on poor diet		23		39	10		232	12		570	10		824
25		41	24		124	10	Put on poor diet		13		779	10	Put on poor diet	
29.3	1,500		25.3	1,500		11		247	14		450	11		891
32		12	28		112	12		66	15		803	12		798
33		35	29		21	13		127	16	150	418	13		1,507
34		118	30		38	14		65	17		813	14		1,575
35		166	31		20	15		79	18	600	903	15		1,681
36		166	32		54	16	150	139	19		656	16		1,789
37	1,000	127	33	1,000	39	17		87	20		1,432	Died		
38		148	34		91	18	600	82	21		2,057			
39		75	35		35	19		231	22		2,797			
40	1,000	28	36	1,000	34	20		462	23		2,472			
41	600	18	37	600	237	21		844	24		3,424			
42		610	38		894	21.5		3,003	Died					
42.2		754	39		1,117	Died								
Died			40		1,822									
			Died											

When about 7 months old, this dog was given a single infection of 2,000 larvae of the dog hookworm *per os*. This immediately produced a good infestation with a peak of almost 4,000,000 E.P.D., which was reached during the fourth week. From the fourth to the nineteenth week, in spite of additional infections, totaling 8,940 larvae, the egg



GRAPH 3. Infestation histories of D 674, D 703, D 708, D 717, and D 720 of group 3. All of these animals were carried to the point of death on the deficient diet. Arrows indicate the point of change to the deficient diet.

count fell off rapidly to about 50,000 E.P.D., where it appeared to stabilize. When there was no indication that the last series of doses of infective larvae, which had been given, was going to produce increased infestation, the dog was put on the deficient diet. After about 8 weeks on the deficient diet the egg count began to rise, and, for about 8 weeks more there was a sustained increase in daily egg production. For reasons which at present are obscure, the egg count fell back to a very low level 2 weeks before the death of the dog. The last 2 weeks, however, were characterized by a striking rate of increase in egg output, which rose from an average of 18,000 E.P.D. 10 days before death to an average of 754,000 E.P.D. at the time of death, after 124 days on poor diet. At autopsy 155 adult worms were recovered. During the time on the deficient diet, D 674 lost 2900 gm. weight, which was 46 per cent of the animal's former weight.

The initial period of infection was reduced to 21 weeks in the case of D 703 and to 10 weeks in the other three cases (D 708, D 717, D 720). In every case, however, the resistance to superinfection was well established before the animals were placed on poor diet. There was given to D 708, for example, a series of ten infections, totalling 6,385 larvae, over a period of only 6 weeks. The highest daily egg output for this period on normal diet was 381,000 E.P.D. (sixth week), and 4 weeks later, when the animal was put on poor diet, the egg count had fallen to 232,000. The high degree of resistance of this dog while on a normal diet is shown both by the low maximum egg production during this period, and by the fact that the last two of the ten infections, which were of 625 and 1,500 larvae respectively, failed to produce an increased egg output. While on the deficient diet a total of only 750 larvae, given in two doses, produced a terminal egg production of over 3,000,000 E.P.D., which was more than ten times the maximum egg production reached while this dog was maintained on a good diet. When this dog died, after 79 days on the poor diet, 259 worms were recovered, a finding which indicates that in this case also death was probably caused by the combined effects of a poor diet and an increasing worm burden. The findings on D 703 and D 717 were essentially the same in all respects to those already pointed out for D 674 and D 708. These dogs died after 132 and 96 days respectively on poor diet and the number of adult worms harbored was 248 by D 703, and 307 by D 717. The history of D 720 differs only in the fact that no additional infections were given during the period on poor diet, although a total of 5,385 larvae was given during the preceding period on the normal diet. The animal died after 6 weeks on

the deficient diet, during which time its egg production increased from 824,000 E.P.D. to 1,789,000 E.P.D., and at autopsy 321 hookworms were recovered. There was a period of more than 10 weeks between the time when the last infection was given on normal diet, and the time when the terminal increase in egg production was recorded on deficient diet. This effect of the deficient diet upon the infestation (i.e., an increased egg output) which has already been observed in so many cases, has therefore been interpreted in this instance as probably denoting an increase in the number of eggs produced per day per worm rather than an increased maturation of worms from previous infections.

From the above discussion, with the aid of the tables and graphs, we are able to point out quite briefly, the important features of the experiment. The dogs were given a series of repeated infections for from 10 to 23 weeks while on a normal diet. At the end of this initial period of infection, all five of the animals had passed the peak of egg production and were exhibiting a marked degree of resistance to further infection. After they were placed on the deficient diet, and under conditions of relatively light infection, there resulted an initial period during which the resistance fell off very slowly, followed by a terminal period of about 2 weeks during which the egg output increased abruptly and death resulted. The worm burdens were found at autopsy to range from 155 to 321 hookworms with an average of 258 per dog. Death was attributed to the combined effects of deficient diet and increased worm infestations.

Group IV, comprising D 710, D 724A, D 739. The three dogs of this group represent miscellaneous observations which have contributed to our knowledge of the relation of diet to resistance.

The first of these animals, D 710, was the oldest of this series, being about 31 months old when first infected. It was given a single infection of 500 larvae and placed on the deficient diet 59 days later. Meanwhile the daily egg production rose to a peak of 1,545,000 E.P.D. during the fifth week after infection, indicating an abnormal degree of susceptibility for an adult dog. The infection was short-lived, however, as was indicated by average counts of 800,000, 622,000, and 367,000 E.P.D. for the sixth, seventh, and eighth weeks respectively. During the eighth week on experiment, this animal was placed on the poor diet but was continued on it only 20 days because of advanced pregnancy. The successive weekly averages of the daily egg productions for this period were 564,000, 711,000, and 377,000 E.P.D. It is felt that these figures show that the period on deficient diet, although

only 20 days in duration, caused an interruption of the rather rapid rate of decrease in E.P.D. This points to a reduced resistance and since it occurred so soon on the deficient diet, it is considered probable that the condition of pregnancy may have contributed to produce a more rapid weakening of resistance.

D 724A was 19 months old when first infected, and on the basis of previous experience one would have expected a high grade resistance. The animal was put on the deficient diet at the start of the experiment, and after 96 days infections aggregating 4,000 larvae were given orally. The prepatent period was 17 days, about 3 days longer than is encountered in susceptible animals, which indicated that there was some degree of resistance left, even after the extended period on the deficient diet. However, after 120 days on the poor diet, a single composite egg count was obtained which showed a daily egg production of 1,169,000 E.P.D. At this point the animal died. This finding made it appear that there was a complete failure of the resistance of this dog, occurring as a terminal event on the deficient diet. At autopsy 596 worms were recovered.

D 739 was given 1,000 larvae and placed on the poor diet. Three days later an additional 1,800 of larvae were given. The first egg count was obtained 27 days after infection, although there had been a slight passage of eggs for 6 days previously. The average daily egg output for the week ending 31 days on the deficient diet, was 1,601,000 E.P.D. For the following week, an average of 1,634,000 E.P.D. was obtained. The dog died after 39 days on the deficient diet and at autopsy 614 worms were recovered. In this case, and in the case of D 724A, it is probable that the increasing worm burdens, which were built up in the absence of a resistance to the invasion of parasites, contributed significantly to the early deaths of these animals on the deficient diet. A point deserving particular emphasis is that the resistance of these dogs was broken suddenly and terminally, with the result that at the time of the death of the host the infestation was at its maximum egg production.

A summary of certain of the important data on the twelve animals of this series has been presented in table 4. It becomes apparent from a study of this table that the maintenance of animals on the deficient diet produced anemia and loss of weight. Seven of the animals, with ages ranging between 7 and 19 months when first infected, were carried to the point of death on the deficient diet; their worm burdens varied from 155 to 614 worms, figures indicative of the type of infestation usually encountered in young susceptible dogs. The data

TABLE 4.

Summary of important data on the twelve dogs of this report which were studied on deficient diet.

Dog number	Age when infected (months)	Days on experiment	Days on poor diet	Weight loss on poor diet		Per cent drop in haemoglobin	(Av. (1) E.P.D. in thousands	Worms recovered	Per cent females	Av. (2) E.P.D. P.A. ♀
				Grams	Per cent					
D 674	7	296	124	2,900	46	—	658	155	54	7,200
D 703	8	278	132	4,700	49	—	1,351	248	58	9,450
D 708	15	148	79	900	20	50	923	259	54	6,593
D 717	7	165	96	6,200	37	30	2,896	307	64	14,700
D 720	9	112	42	900	26	0	1,735	321	65	8,304
D 724A	19	120	120	6,800	51	—	1,169	596	47	4,175
D 739	8	40	39	1,500	28	—	1,617	614	64	4,146
D 723	8	—	103	2,000	37	44	Not autopsied. Transferred to other experiments.			
D 727	8	—	103	2,500	35	37				
D 710	31	—	20	1,300	12	—				
D 731	8	—	60	2,700	32	15				
D 732	8	—	60	1,600	26	10				
Ave.*	10.4	166	90	3,414	37	—	1,478	357	58	7,897

(1) Average eggs per day in thousands based on counts of last nine days.

(2) Average eggs per day per adult female worm.

* Based on first seven animals all of which died on poor diet after the number of days indicated in fourth column.

indicate also that death was caused by the combined effects of the worm burden and the prolonged feeding of an inadequate diet.

Discussion.

There is evidence in these studies of an effect of dietary deficiency upon resistance to infection which is distinct from the similar relationship which appears also to exist between inadequate nutrition and decreased ability to compensate for the injurious effects of worm infestation. The animals fed on deficient diet exhibited a lowered resistance to the invasion and establishment of hookworms, an increased rate of development of the worms, an increased absolute egg production, and an increase in the number of eggs produced daily by individual female worms.

Of the twelve dogs discussed in this report, seven died while on the deficient diet. In these cases there was uniformly a complete and sudden breakdown of the resistance mechanism which occurred as a terminal phenomenon. Although our attention was not directed to