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THE IMMUNITY OF DOGS TO ANCYLOSTOMA CANINUM.[1]

By

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McCoy (1931) has recently published an extensive study of the immunity reactions of the dog against hookworm under conditions of repeated infection. In seven experiments he employed seventeen dogs most of which were about 2 months old when the first infections were given. The several experiments, with one exception, were arranged to include two or three animals of the same litter. Larvae were administered orally in double gelatin capsules at intervals of a few days beginning with doses of from twenty to seventy-five larvae and subsequently increasing the numbers, often in geometric proportion, until eventually doses of several thousand larvae were being given. The course of the infections was followed for several months by means of egg counts, hemoglobin determinations, and the searching of stools for the recovery of adult worms. It was observed that a maximum egg production was reached in from 2 to 4 months from the time of the first larval infection, and that thereafter the egg count usually dropped to a very low level even though large infective doses were given. In general, it was noted that the repeatedly infected animals exhibited first an increase in the hemoglobin level, then a drop in the egg output, and finally a spontaneous loss of worms. This change in the direction of the curve, with the associated changes in hemoglobin and degree of infection, was found to occur in a condition of resistance to superinfection which was interpreted by McCoy as somewhat of a "crisis." In order to determine the extent to which this resistance was acquired (i.e. elicited by hookworm infestation) most of the experiments were controlled by keeping one or more animals hookworm-

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free until such time as the test infections were given. The results of the individual experiments seemed to indicate that in certain cases (i.e. exps. 4 and 5, pp. 278–286) a true acquired immunity developed since the previously infected animals were more resistant to the test infections than were the controls. In other cases (i.e. exps. 1 and 6, p. 271 and p. 286), the uninfected controls appeared to resist the test infections fully as well as did their litter mates which had been exposed to repeated infection over periods of several weeks. There were other animals, apart from those included in these experiments, which failed to survive the exposure to continued infection. One must presume that these dogs were unable to develop a protective resistance due to considerations peculiar to these hosts and to the conduct of the infections. McCoy accepted the view that previous infection apparently conferred some degree of immunity, particularly in the cases of light infections, although he acknowledged difficulty in separating acquired immunity from age resistance.

Further information which shows the character of this resistance, particularly the rapidity with which it develops in mature dogs, has been presented recently by Foster and Cort (1935) in connection with studies on the effect of diet upon resistance. In this paper data are presented on two dogs (D728, D727) which were first infected at about 8 months of age and were given repeated infections for about 9 weeks. During this time a total of 2625 larvae was given in four infections and the peak of egg production was reached 5 weeks after infection. That a high degree of resistance was present at this time is evidenced by the fact that the peak in the egg curve followed the first two larval administrations and that subsequent doses of 625 and 1000 larvae respectively failed to elicit an increase in egg output. Two other dogs (D731, D732), litter mates and about 8 months of age when infected, were at first kept highly susceptible by placing them on a deficient diet for about 8 weeks, but were later given several heavy doses of larvae separated by intervals of a few days or weeks. Each was given two infections of 1500 larvae, and in both cases there was a slight response to the first of these larval doses as evidenced by a temporary rise in egg output about 3 weeks following the infection. The second dose produced no increase in egg count in either case, and during subsequent exposure to a long series of infections of 500 larvae each, the egg production fell to a very low level. These findings add emphasis to the fact that mature dogs can develop a high degree of resistance to *A. caninum* in a comparatively short time, under conditions of repeated, heavy infections. Also there were presented in that paper the

complete infection histories of four other dogs (D703, D708, D717, D720) which varied in age from 7 to 15 months when first infected. All of these animals developed a pronounced resistance to superinfection under an exposure of from 4 to 6 weeks to repeated hookworm infection. It must be emphasized, however, in order that comparisons with the results of the present study be kept in mind, that all eight of these dogs were mature (8 months old or more) and were able, perhaps because of their age, to withstand surprisingly heavy doses of larvae from the start.

The present studies are a continuation of this line of work with particular emphasis upon the rate at which resistance develops in dogs of different ages and under various degrees of infection. Also an effort has been made to throw some light on the extent to which this resistance is effected by the age of the host as opposed to the operation of an acquired immunity, and upon the extent to which it is a resistance to superinfection. The aim, of course, is to establish, in so far as possible, a credible hypothesis for the explanation of this type of resistance—a type which seems not unlike that encountered in many other host-parasite relationships.

With a few exceptions, the technique affecting the experiments was the same as that employed in the studies discussed above (cf. McCoy, pp. 269–271). In certain cases, as will be indicated, infections were administered percutaneously. Due to an inability to procure an adequate and constant supply of hog lungs, it became necessary to make a change in diet, with the result that most of the animals of this series were fed Purina Dog Chow. A few considerations pertaining to the handling of data, which are apparently introduced for the first time in the present paper, will be discussed in connection with the presentation of the results.

It seems desirable to introduce at this point certain considerations which have been regarded as fundamental to an understanding of the resistance which develops in this host-parasite relationship. One may say at the start that logically the results of experimentation on canine resistance to hookworms seem to fall into two superficially incongruous categories: (1) animals which failed to develop resistance when repeatedly infected and (2) animals which developed a marked resistance. Furthermore, of those dogs which did demonstrate resistance to hookworms, it has appeared that in some carefully controlled cases an acquired immunity was operating, while in others, the fact that the resistance was elicited by hookworm infestation was not apparent from the data. Two further considerations merit emphasis at this point.

One is that *A. caninum* is violently pathogenic, which at once suggests why some animals fail to survive repeated infection and which requires that "immunizing" doses of larvae be carefully regulated. The other consideration is that there can be no question about the ability of the dog to exhibit, under certain conditions of age or exposure, a definite and pronounced resistance to *A. caninum*. We believe that it is through an analysis of the factors responsible, in individual cases, for these diverse results that our most significant interpretations are to be made. Our interest, then, centers primarily upon the nature of this resistance and the factors which cause it to be exhibited, and not upon the fact that resistance of the dog to *A. caninum* has been demonstrated.

The data to be presented in this paper were obtained from the study of thirty dogs (23 experimental and 7 controls) which were used in seven different experiments.

Experimental results.

Experiment 1. D777, D778, D779, D780. In this experiment the animals failed to develop a resistance to *A. caninum* when exposed to repeated infection. The data on the individual dogs are given in table 1 A. The four puppies were litter mates which were born in the

Table 1 A.

Egg count data on D 777, D 778, D 779, and D 780 of experiment 1.

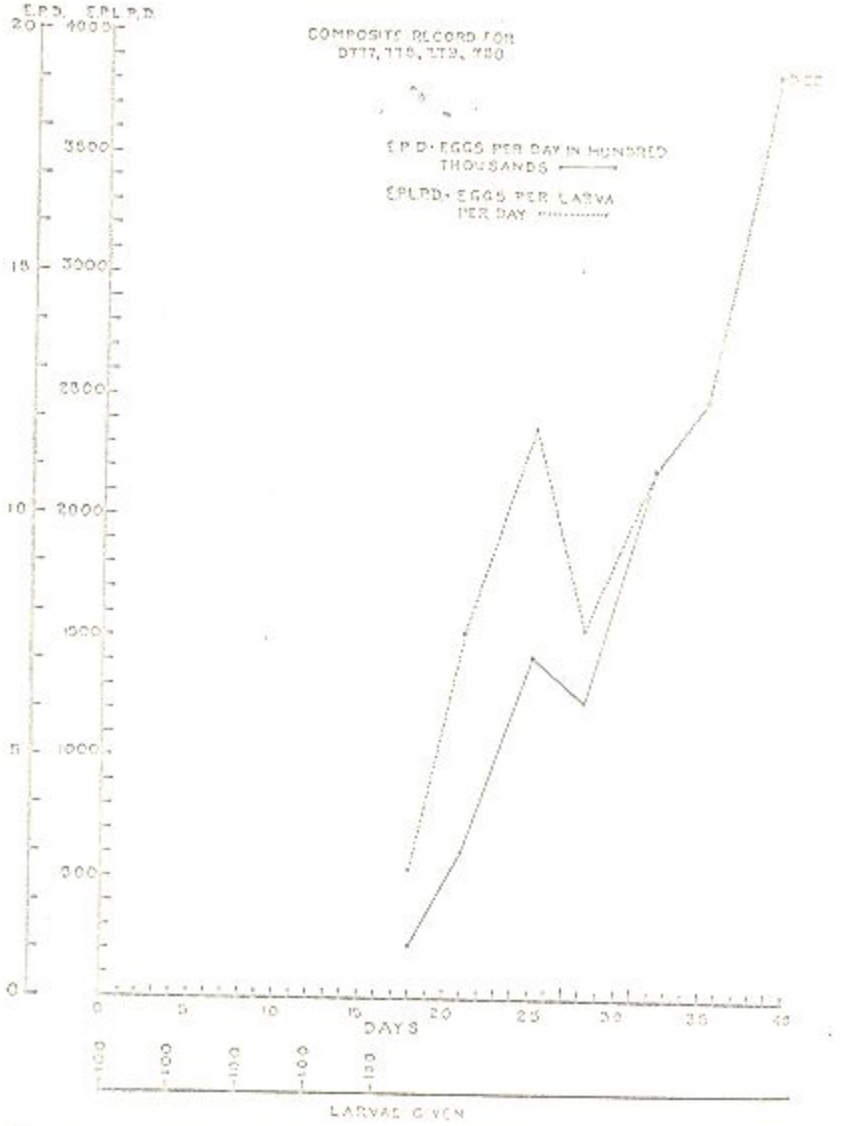
Days on experiment	Larvae given	E.P.D.* in thousands			
		D 777	D 778	D 779	D 780
0	100				
4	100				
8	100				
12	100				
16	100				
18		8	195	83	141
21		162	545	219	286
25		537	637	974	687
28		298	814	545	795
32		785	1590	1120	893
35		1157	1392	831	1591
39		1445	Died on 37th day of experiment	2218	2079
42		960		Died on 39th day of experiment	Died on 41st day of experiment
		Died on 42d day of experiment			

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

laboratory and first infected when 52 days old. Each puppy was given five infections of one hundred larvae each over a period of 16 days, the doses being administered at intervals of 4 days. The larvae were given to all four puppies at the same time and taken from the same culture. The individual variations in egg production were great enough to indicate that puppies of the same litter may vary greatly with respect to natural resistance to hookworm infection, but the general nature of the infestations was alike in all cases. A study of the composite data [2] of E.P.D.[3] in graph 1 shows that the daily egg output increased rapidly from the fourteenth to the twenty-fifth day after infection, at which point there occurred an interruption in the egg curve which may be regarded as of some significance, since all four dogs showed a temporary decrease in the rate of rise of the egg production curve. This occurrence probably indicates that the puppies were beginning to react against the infestations and that they might have been able to compensate for the first three hundred larvae given had the infections been discontinued at that point. With the maturation of the fourth and fifth doses of larvae, however, a considerable and steady increment was registered in the daily egg output, indicating that there was no resistance to the superinfection in these cases. All of these puppies died from acute hookworm disease when from 37 to 42 days on the experiment. The numbers of worms recovered at autopsy ranged from 306 to 428 per dog with an average of 382 (table 1 B). This figure gives an average percentage development of 76.4 per cent, from which it may be concluded that the giving of five hundred larvae in separate doses of one hundred larvae each was little different in its effect from the giving of a single dose of five hundred larvae. There was likewise no indication in these cases that distributing the larval dose over the 16 days of the infection period lessened either the injurious results of the infestation or the degree of parasitism which developed. The rather continuous increase in the larval-

[2] By referring to table 1, one can easily see the method by which the "composite data" on which graph 1 is based were obtained. The individual record gives the average daily egg production for each week. These figures are again averaged in order to determine the average at each point for the four dogs of the series. Although these composite data do not represent an actual infestation history they give the average of a group of animals subjected to the same infections and their legitimate use gives a clearer picture of the results of the experiment than would be found in the data on a single animal.

[3] Eggs per day, weekly average.



GRAPH 1. Composite curves of the daily egg production and of the larval-egg ratios for the four litter mate dogs of experiment 1, D777, D778, D779 and D780, all of which succumbed to the infestations after five separate doses of one hundred larvae each had been administered.

TABLE 1 *B*.

Summary of data on egg count and worms recovered at autopsy on D 777, D 778, D 779, and D 780 of experiment 1.

Dog number	Larvae given	Days infected	Average E.P.D.	Worms recovered	Number females	Per cent females	Average E.P.D.P.A. ♀*	Per cent development
D 777	500	42	1,187,312	336	158	51.07	5,514	61.20
D 778	500	37	1,491,269	426	234	54.22	6,456	85.20
D 779	500	39	1,589,578	368	172	46.71	8,078	73.60
D 780	500	41	1,670,214	428	249	58.16	6,708	85.60
Average		40	1,607,083	382	202	52.86	7,189	76.40

* E.P.D.P.A. ♀ = Average eggs per day per adult female.

egg ratios [3] as shown in graph 1 gives evidence that the puppies were tending to lose resistance during the course of the infestation rather than to gain it. Results of this type emphasize the slowness with which dogs build up resistance to superinfection with the dog hookworm. It is by no means an exceptional occurrence for puppies to acquire fatal hookworm infestations when subjected to repeated infections with relatively light doses of larvae. There is abundant evidence from previous studies and in data which follows to support the belief that had these dogs been allowed to compensate for the injurious effects of their worm burdens by a more spacious distribution of the larval doses they would have been able after a few months to have withstood doses of several thousand larvae. They would also have become able to harbor with relative impunity the numbers of worms which were responsible for their deaths in these instances.

Experiment 2. D670, D672, D673, D675, D676, D678. Control D674. The several animals of this experiment exhibited all of the features which have been recognized above in the introduction. The data are given in tables 2 A and 2 B. The seven dogs of this experiment were of the same litter and were about 4 months old when first infected. D674 was kept as a control of the age resistance, while the

[3] The larval-egg ratio, or the number of eggs per larva per day (L.P.L.P.D.) is a convenient index figure obtained by dividing the number of eggs produced daily (E.P.D.) by the aggregate number of larvae which had been given up to the eggs immediately preceding (larvae 14 days old). It is not intended to imply that the larvae produce the eggs, but it is a recognition of the fact that the egg production of any one day is produced by hookworms which matured from larvae administered 14 or more days earlier, 14 days being the normal prepatent period. The consecutive series of larval-egg ratios are the data from which is derived the larval-egg curve (graph 1).

TABLE 2 *A*.

Egg count data on D 672, D 673 and D 678 of experiment 2.

Days on experiment	Larvae given	E.P.D. in thousands			Worms recovered from D 678
		D 672	D 673	D 678	
0	40				
4	40				
8	40				
12	40				
16	40				
20	40				
24	40				
26		22	62	105	0
28	40				
32	40				
33		51	198	368	0
36	40				
40	80	76	107	138	0
44	80				
47		149	375	458	0
54		256	940	1228	0
57	300				
61	600	364	1095	1151	0
64	640		Died on 61st		
68		382	day of experi-	1489	7
75	3200	556	ment	796	17
82	2560	735		898	15
89		705		875	295
96	2000	Died on 89th		689	186
103		day of experi-		1862	31
106		ment		2044	6
				Died on 100th day of experiment	

others were infected with forty larvae each, every 4 days for ten infections. Then two doses of eighty larvae each were given, followed by a series of much larger doses. All but D678 succumbed to the effects of the worm burdens between 15 and 89 days on the experiment. Three of them D676, D675 and D670 died on the fifteenth, twenty-second and twenty-sixth days while the infective doses were comparatively small, showing again that under conditions of repeated light infection, dogs may not develop enough resistance to combat a gradually increasing worm burden, as would be expected if there had been any appreciable degree of acquired immunity provoked by the

TABLE 2 *B*.

Summary of data on egg counts and worms obtained at autopsy on D 670, D 672, D 673, D 675, D 676, and D 678 of experiment 2.

Dog number	Larvae given	Days infected	Average E.P.D.	Worms recovered	Number females	Per cent females	Average E.P.D.P.A. ♀	Per cent development
D 670	200	26	16,535	23	12	52.17	1,378	11.50
D 672	5360	89	770,000	279	153	54.84	5,033	5.21
D 673	560	61	1,125,268	148	73	49.32	15,414	26.43
D 675	160	22	7,800	11	6	54.54	1,300	6.88
D 676	80	15	——	10	8	80.00	——	12.50
D 678	7920	109	1,953,125	1915	796	40.16	2,546	24.18
Average				397	171	42.79	3,823	14.45

worms. The history of D678 is especially interesting because a considerable degree of resistance seemed to have been developed as a result of the preliminary infections. Subsequent doses of 3200 and 2560 larvae failed to produce any significant increase in egg production. The larval-egg ratio dropped off during the period of these infections to less than 100 E.P.L.P.D. Another strong indication that a marked resistance was becoming operative in D678 is the fact that there was a spontaneous loss of relatively large numbers of worms during this period (table 2 A). After 96 days of repeated infection during which time a total of 7920 larvae was given, this dog and the uninfected control of the same litter (D674) were given two thousand larvae each. The daily egg output of both dogs increased abruptly from this point and there was no evidence at this time of a marked resistance to the invasion and establishment of parasites in either case. The egg production of D678 rose to a maximum of about 2,000,000 E.P.D. and the animal died 10 days after this last infection. The daily egg output of D674, the control which was previously uninfected, rose to a peak of nearly 4,000,000 E.P.D. 3 weeks after infection and rapidly dropped off after the third week.[3] The experimental animal (D678), which had been subjected to this long series of infections, and later succumbed to the injuries of the worms, gave 1915 worms at autopsy, the largest number of worms which has been recovered in our experience from one animal. The marked increase in

[3] The data on D674, the control animal of this experiment, have been omitted after some consideration from the table. Since it was possible to present the very limited but important data affecting this animal in the body of the discussion, and since the animal was later used in another experiment in connection with which its complete infection history is shown (Foster and Cort, 1935), it was considered unnecessary to duplicate this dog's record in the table and graph.

egg output which occurred in this animal during the last 10 days must be accounted for by maturation of worms from the earlier infections, since a prepatent period of 14 days is normally required for the dog hookworm. The nature of the resistance which was manifest previous to the last infection suggested rather definitely, however, that the animal was able to withstand the infections up to that point, and probably would have survived had not the last dose of two thousand larvae been given at an inopportune time. That this dose was followed by a complete collapse of the resistance mechanism and death of the previously infected animal (D678), but not of the uninfected one (D674), suggests certainly that the factor of physiological compensation for the injuries produced by hookworms is an important part of the resistance mechanism. On the other hand, it has been noted that the egg output of the uninfected control (D674) was nearly twice as great as that of the previously infected dog although an aggregate dose of 7920 larvae contributed to the worm infestation of the latter animal. From the fact that D678 was found at autopsy to harbor an abnormally heavy worm burden, one may be permitted to assume that had it been able to withstand the injury from so many worms, its egg production might have equalled or surpassed that of the control. It is of some significance to mention that the marked increase in daily egg count which occurred as a terminal phenomenon resulting in death, a result which was also associated with a terminal increase in the larval-egg ratio, points to a terminal failure of resistance not unlike that which has been observed in dogs whose resistance has been broken by a deficient diet (Foster and Cort, 1935). Furthermore, it appears that a considerable degree of host injury may be attributed to the activity of the immature hookworms from the last infection, the effect of which upon resistance was similar to the effect of a deficient diet.

Three of the animals (D670, D675, D676) of this series died so early in the course of the infections and harbored so few worms at autopsy (ten to twenty-three hookworms) that one hesitates to attribute their deaths to hookworm disease although evidence of other possible causes was not found. It may be said, however, that these were small animals, averaging about 1 kg. in weight, and that there is considerable evidence in the recent work of Foster and Lundsberg (1934) that the injury which hookworms produce is correlated quite definitely with the size of the host, since the anemia-producing capacity of the hookworm is more or less constant. In any event, these animals did not become resistant under continued exposure to hook-

worms. Two others (D673, D672) died after 61 and 89 days of exposure respectively, during which time the total number of larvae given was in the case of D673, 560, and in the case of D672, 5360. Throughout this experiment, there was again constant evidence of marked individual differences among litter mates in natural resistance, in their abilities to combat the infestations, and in the rate at which resistance was developed. Such variations can not be ascribed to differences either in exposure or in age, and one concludes that some host-factor, such as differences in the power of physiological compensation, was responsible for these variations in resistance and susceptibility.

Experiment 3. D746, D747, D748. The discussion of these animals is interposed at this point because they exhibited what has been regarded as the typical picture of a developing resistance against *A. caninum* under conditions of repeated infection and increasing age. The puppies of this experiment were litter mates, born in the laboratory, and first infected when about 2 months old. The data for the individual animals are presented in table 3 and the composite data for the group in graph 2. One puppy, D747, was initially infected with one hundred larvae at 62 days of age, while the other two were first infected 2 weeks later, at which time one hundred larvae were given to D746 (also to D747) and fifty larvae were given to D748. Each was given one hundred larvae on the fifty-eighth day of the experiment, and 366 larvae on the eighty-first day. After 112 days on the experiment, 1071 larvae were given to D746, 714 to D747, and 357 to D748. Beginning on the one hundred and fortieth day and throughout the remainder of the experiment, biweekly infections of five hundred larvae were given to each animal.

While table 3 includes the complete infection histories of all three animals, the individual records are so similar, that the discussion will be based almost entirely upon the composite data given in graph 2.

The peak of egg production in the composite curve (596,000 E.P.D.) was attained 98 days after the first infections were given. At this time an average of 582 larvae in four doses had been given each dog, the animals were approximately 5 months old, and had been infected for 3 months. The course of the infestations was followed for 7 months more while regular biweekly infections were given. For the 3 months immediately following the maximum egg production, the daily egg output fell off very slowly, although an egg count of more than 200,000 E.P.D. still persisted for 6 months after infection. It is apparent, however, that during this time an effective

TABLE 3.

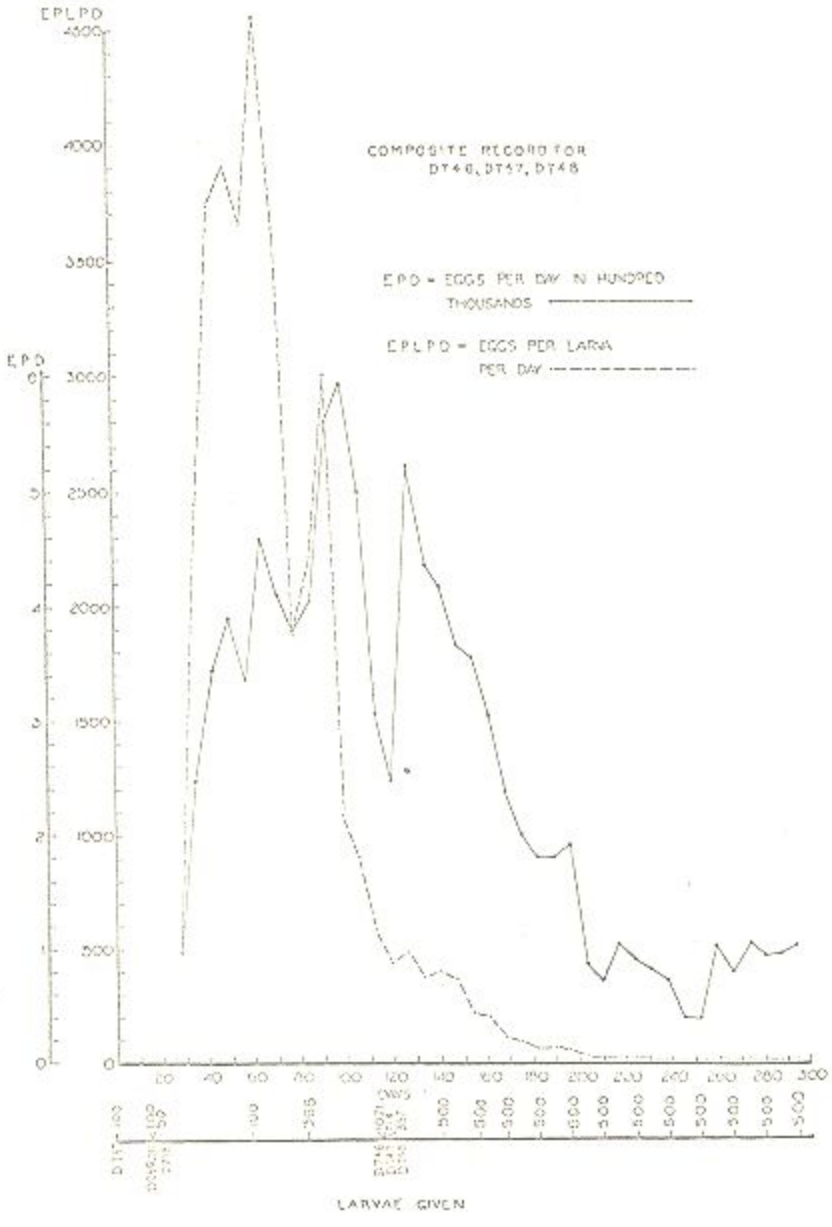
Data on egg count, larvae-egg ratio and hemoglobin for D 746, D 747 and D 748 of experiment 3.

Days on experiment	D 746					D 747					D 748				
	Larvae given	E.P.D. (thous.)	Larvae 14 days old	Eggs per larva per day	Per cent hemoglobin	Larvae given	E.P.D. (thous.)	Larvae 14 days old	Eggs per larva per day	Per cent hemoglobin	Larvae given	E.P.D. (thous.)	Larvae 14 days old	Eggs per larva per day	Per cent hemoglobin
0						100									
14	100				68	100					50				58
28					64		96	200	478	55					60
35		487	100	4870			206	200	1030			72	50	1440	
42		746	100	7460			134	200	670			154	50	3080	
49		927	100	9270			161	200	805			82	50	1640	
56		730	100	7300			127	200	635			151	50	3020	
58	100				73	100				58	100				57
63		944	100	9440			296	200	1480			138	50	2760	
70		804	100	8040			400	200	2000			44	50	880	
77		592	200	2960			285	300	950			257	150	1713	
81	365				70	366				58	366				53
84		594	200	2970			237	300	790			433	150	2887	
91		710	200	3550			356	300	1187			642	150	4280	
98		784	565	1388			221	666	332			784	516	1519	
105		400	565	708			308	666	462			802	516	1554	
112	1071	331	565	586	72	714				72	357				68
119		248	565	439											
126		415	1636	254	57		272	1380	197	66		885	873	1014	60
133		536	1636	328			171	1380	124	72		600	873	687	64
140	500	338	1636	206	73	500	106	1380	77	71	500	811	873	929	70
147		193	1636	118	81		125	1380	90	69		791	873	906	71
154	500	298	2136	140	70	500	179	1880	95		500	586	1373	427	

TABLE 3 (*Continued*).

Days on experiment	D 746					D 747					D 748				
	Larvae given	E.P.D. (thous.)	Larvae 14 days old	Eggs per larva per day	Per cent hæmoglobin	Larvae given	E.P.D. (thous.)	Larvae 14 days old	Eggs per larva per day	Per cent hæmoglobin	Larvae given	E.P.D. (thous.)	Larvae 14 days old	Eggs per larva per day	Per cent hæmoglobin
161		221	2136	103			170	1880	90	73		567	1373	413	58
168	500	191	2636	73	76	500	90	2380	38		500	434	1873	232	63
175		207	2636	78			119	2380	50			295	1873	158	
182	500	225	3136	72		500	120	2880	42		500	198	2373	81	
189		130	3136	42	85		74	2880	26	76		338	2373	142	65
196	500	229	3636	63		500	97	3380	29		500	249	2873	87	
203		98	3636	27			61	3380	18			101	2873	35	62
210	500	57	4136	14	78	500	42	3880	11	71	500	116	3373	34	66
217		71	4136	17			92	3880	24			154	3373	46	
224	500	64	4636	14		500	127	4380	29		500	82	3873	21	
231		53	4636	12	75		140	4380	31	65		52	3873	14	69
238	500	79	5136	15		500	75	4880	15		500	63	4373	14	
245		21	5136	4	60		41	4880	9	57		59	4373	13	
252	500	32	5636	6		500	54	5380	10		500	31	4873	7	
259		154	5636	27	57		80	5380	15	55		74	4873	15	
266	500	104	6136	17		500	56	5880	10		500	76	5373	14	
273		131	6136	21	55		53	5880	9	52		131	5373	24	
280	500	131	6636	20		500	80	6380	14		500	59	5873	10	
287		95	6636	14	68		64	6380	10	56		127	5873	21	64
294	500	174	7136	24		500	49	6880	7		500	87	6373	14	

Continued on other experiments



GRAPH 2. Composite curves of the daily egg production and of the larval-egg ratios for the three litter mate dogs of experiment 3, D746, D747 and D748. This is a rather typical picture of the way in which resistance develops under conditions of repeated exposure and increasing age of the host.

resistance was being built up against constantly maturing worms from the repeated infections. Toward the close of 10 months' exposure to hookworms, when the dogs were about 1 year old, the absolute egg production was at a level of about 100,000 E.P.D., although a cumulative average dose of 6,796 larvae had been given each animal.

In the early history of the infestations (ninth week in graph 2 on the composite data) the egg production approximated four thousand times the number of larvae given. Since the absolute daily egg production fell from 596,000 E.P.D. to 100,000 E.P.D. during the period in which additional infections totalling 6,214 larvae were given, it follows that the figure representing the egg output in terms of larvae given was reduced to a very low level (less than 50). The conditions under which these animals became resistant were those of repeated superinfection and aging of the host. Although the resistance was of a high order, it was far from a complete immunity. The entire picture of the development of this immunity, as detailed above, parallels closely the results obtained by Stoll (1929) and Sarles (1932) in the course of similar experimentation on *Hemonchus* in sheep and *Trichostrongylus* in rabbits. We are reluctant, however, in the light of McCoy's work and of the experiments to follow, to ascribe the resistance observed in this series of animals, in the absence of controls for the effect of increasing age, to the operation of a true acquired immunity.

Experiment 4. D812, D813, Control D814. This experiment includes three litter mates, of which two were infected when 133 days old and one (D814) was kept as a control for the effect of age. All three had harbored light hookworm infestations when very young but were treated to negative about 4 weeks before the present observations were initiated. It may be said, then, that D814 has been regarded as a control animal for this experiment only in the sense that it was kept hookworm-free for a period of 253 days prior to the test infection. For the first 11 weeks D812 and D813 were studied in connection with observations on hookworm anemia, in consequence of which considerable information on the early history of these dogs has previously been published (Foster and Landsberg, 1934, pp. 276–281). The course of their infestations was followed for approximately 27 weeks beyond the point where the anemia studies were terminated. Because of the bearing of their complete infestation records upon the interpretation of the present experiment, the data affecting these animals, exclusive of blood findings, have been presented in full in table 4 and graph 3. In this experiment, D812 was

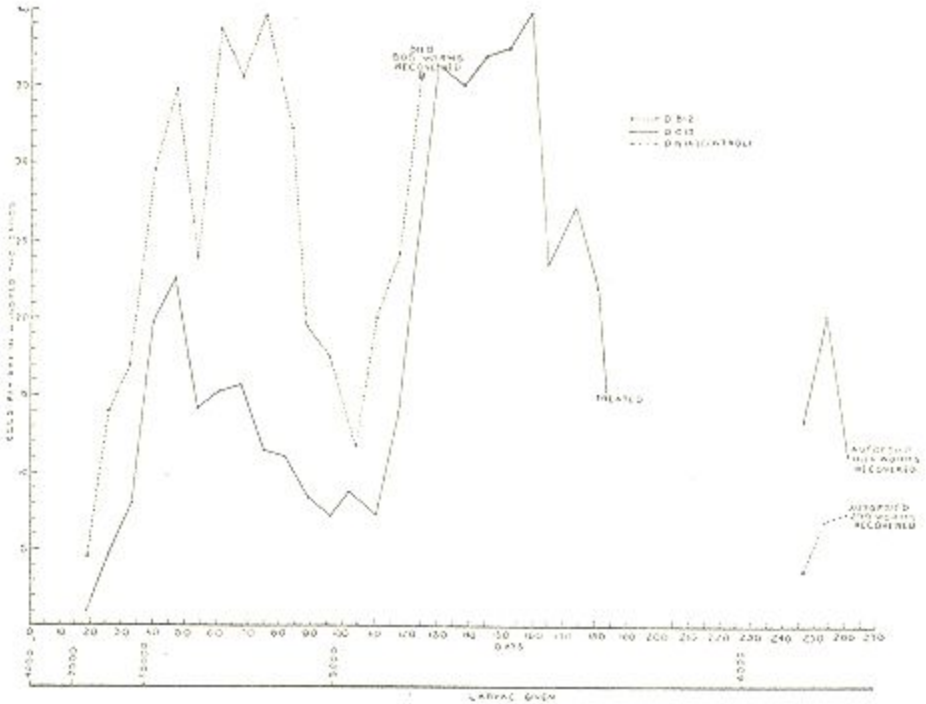
TABLE 4.

Egg counts and data from autopsy for D 812, D 813 and D 814 of experiment 4.

Days infected	D 812		D 813		D 814—Control	
	Larvae given	E.P.D. (thous.)	Larvae given	E.P.D. (thous.)	Larvae given	E.P.D. (thous.)
0	4000		4000			
14	3500		3500			
19		449		93		
26		1392		459		
33		1697		799		
37	15000		15000			
40		2973		1960		
47		3491		2262		
54		2420		1408		
61		3896		1520		
68		3571		1563		
75		3974		1140		
82		3251		1100		
89		1950		836		
96		1756		712		
97	5000		5000			
104		1173		872		
111		2016		714		
118		2424		1492		
125		3607		2712		
132	Died			3684		
139	395 worms recovered			3540		
146				3727		
153				3772		
160				4008		
167				2361		
174				2746		
181				2219		
184				1540		
184			Treated			
227			6000		6000	
247				1356		358
254				2033		694
261				1131		743
			Autopsied 964 worms recovered		Autopsied 299 worms recovered	

infected percutaneously and D813 orally, and the infective doses were large but few in number. Both dogs were infected at the same time and with larvae from the same cultures.

In examining the record presented by these dogs (graph 3) one is immediately surprised by the apparent differences in degree of in-



GRAPH 3. Curves of daily egg production of the three litter mate dogs of experiment 4. The previously infected animal, D813, was not more resistant than its control, D814, when the test infections were given.

festation which resulted from the infections given by skin and by mouth. The percutaneously infected animal showed, in this instance, the greater infestation as indicated by egg-count, although this may indicate only a high-grade individual susceptibility, since orally administered larvae have usually given the higher percentages of development.

During the first 37 days on the experiment, D812 and D813 were given three heavy infections each, totalling 22,500 larvae, in spite of which both animals survived. Although reasonably high egg productions followed, it has seemed that the ability to withstand such

large doses of larvae indicates that an appreciable degree of resistance was present in these dogs when first infected. This interpretation is especially significant when it is noted that a later dose of five thousand larvae, given after the peaks in egg output were passed and at a time when the egg count had fallen to a fairly low figure, resulted in the death of one animal (D812 from which 595 worms were recovered at autopsy), and in an enormously increased egg production of the other (from less than 1,000,000 E.P.D. to about 4,000,000 E.P.D.). The fact that both animals withstood doses of four thousand larvae when the experiment was begun, three thousand five hundred larvae on the fourteenth day, followed by fifteen thousand larvae on the thirty-seventh day, and then finally, on the ninety-seventh day, displayed no resistance whatever to doses of five thousand larvae, can scarcely lead to any other interpretation than that the early infections tended to break down, rather than build up, a resistance to superinfection. Findings of this nature point strongly to the importance of the animal's condition of general well-being as a factor influencing its ability to resist hookworm infection.

In the case of D813, which survived this critical period, observations were continued until the egg curve (graph 3) showed a considerable drop, at which time the animal was treated with tetrachlorethylene. At the time of this treatment the egg output had fallen to about 1,500,000 E.P.D., as compared with about 4,000,000 E.P.D. only 4 weeks earlier. After an interval of 43 days, during the latter part of which D813, as well as its control D814, was consistantly negative to Lane examinations, both animals were given six thousand larvae *per os* in order to determine, if possible, whether or not the long period of previous infestation in the case of D813 had rendered this animal significantly resistant. Following the administration of this test dose, the egg production of D813 rose to a maximum of about 2,000,000 E.P.D., and at autopsy, 34 days after this infection, 964 adult hookworms were recovered. In the case of the control, D814, the egg count rose to about 750,000 E.P.D. at the time of autopsy and 299 worms were recovered. From these data it appears that both animals were fairly resistant to the test dose, although the previously uninfected control, D814, was apparently more resistant than its previously infected litter mate. These findings, although opposed to the operation of an acquired immunity, are in accord with the hypothesis that resistance to hookworms may be influenced materially by the general condition of the host. For example, it is probable that the anthelminthic treatment of D813 led to an improve-

ment in the general health of the animal such that it was able to resist the test infection more effectively than it had the previous infection of five thousand larvae which was given at a time when worms were present. Similarly in the case of the findings on D814, it may be suggested that the absence of exposure to the debilitating effect of hookworms throughout the course of the experiment allowed this animal to enjoy a better state of health and thereby to resist the test dose even more effectively than did D813. While this explanation of the resistance of the dog to hookworms may at first seem unorthodox, it is the explanation which has occurred most frequently to the writer when the attempt has been made to account for the results obtained in a rather extended series of studies upon *A. caninum*, and has been emphasized at this point because the results of the present experiment have seemed to be so satisfactorily explained by this hypothesis.

An additional feature peculiar to this experiment is suggested by a comparison of the results of the test infection of D813 with the terminal findings on the animal which had previously died, D812. It has seemed that the condition of resistance, as exhibited by D813, operated more to suppress the egg production than to limit the establishment of parasites. This interpretation is derived from the fact that D812 showed a terminal egg output of about 3,600,000 E.P.D. and a worm burden of 595 hookworms at autopsy, while D813 showed an egg output of about 2,000,000 E.P.D. and a burden of 964 worms.

Experiment 5. D776, D783, Control D784. The results covering this experiment are presented in table 5 and graph 4. These dogs were litter mates, D776 and D783 being first infected when 200 days old while D784 was kept as a control for the effect of age. Both experimental animals were initially given two series of infections, each series consisting of five consecutive doses over a period of 13 days, the doses within each series being increased geometrically beginning with fifty larvae and ending with eight hundred, a total of one thousand five hundred and fifty larvae being administered in each series. These infections were promptly followed by peaks in egg production which came after about 6 weeks on the experiment and amounted to about 240,000 E.P.D. for D776 and to about half that for D783. Although much smaller doses, as witnessed in experiments 1 and 2, have been found to kill younger animals, there was in these cases no evidence of severe hookworm disease, while also a pronounced resistance to infection appeared to have developed rapidly as compared to the findings on younger animals. Because of the tolerance shown to these preliminary infections which was evidenced also by a steadily

decreasing egg output after the forty-fifth day, a third series of geometrically increasing doses of larvae was administered between the seventy-third and eighty-seventh days on the experiment. In this case the doses were exactly double those given previously. There followed no appreciable increase in egg production in either case, but instead worms began to be lost, as may be observed in the data of table 5. That the loss of worms followed the decrease in egg production fits well the observations of McCoy (1931) who first drew attention to this occurrence. Later in the experiment, in order to determine whether a high grade resistance still prevailed, doses of 1085 larvae were given each dog after 157 days on experiment, and doses of 3100 larvae on the one hundred and eighty-fifth day. In neither case was there any significant increase in egg production. When they had been about 7 months on the experiment, D776 was

TABLE 5.

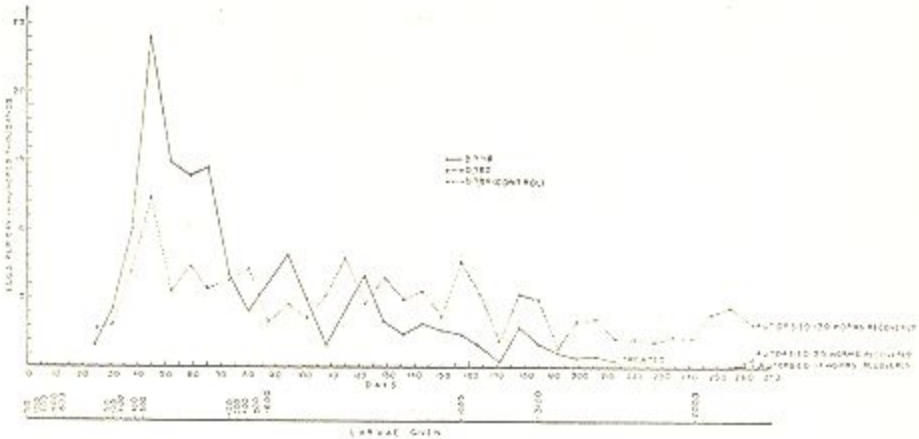
Data on D 776, D 783 and D 784 of experiment 5.

Days infected	D 776			D 783			D 784—Control	
	Larvae given	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)
0	50			50				
3	100			100				
6	200			200				
10	400			400				
13	800			800				
24		152	0		273	0		
29	50			50				
31	100	426	0	100	309	0		
34	200			200				
38	400	1002	0	400	694	0		
42	800			800				
45		2396	0		1232	—		
52		1487	0		552	—		
59		1393	0		729	2		
66		1451	0		586	4		
73	100	673	0	100	630	2		
76	200			200				
80	400	781	3	400	716	—		
83	800			800				
87	1600	593	10	1600	327	—		
94		811	15		445	—		
101		509	14		360	2		
108		163	7		520	8		

TABLE 5 (*Continued*).

Days infected	D 776			D 783			D 784—Control	
	Larvae given	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)
115		421	18		791	2		
122		674	5		468	—		
129		333	10		651	3		
136		246	4		495	2		
143		322	3		552	—		
150		286	5		370	3		
157	1085	242	2	1085	766	0		
164		152	5		527	3		
171		45	8		194	4		
178		300	6		536	6		
185	3100	185	5	3100	491	9		
192		107	—		145	—		
199		83	—		341	—		
206		89	—		358	—		
213		50	—		210	—		
	Treated			Not treated				
220					207	—		
227					191	—		
234					221	—		
241					219	—		
242	2000			2000			2000	
248					397	—		
255		2	—		445	—		neg.
263		84	—		324	—		3
	33 worms recovered at autopsy			139 worms recovered at autopsy			17 worms recovered at autopsy	

treated to negative, while D783 was allowed to retain its worms. The daily egg production at the time of treatment was about 50,000 E.P.D. in the case of D776 and about 210,000 in the case of D783. After an interval of 29 days, both of the previously infected animals and the control, D784, were given two thousand larvae each *per os*. They were autopsied 21 days later, at which time D776 yielded 33 worms after having reached an egg output of 84,000 E.P.D., D783 gave 139 worms after an egg production of 445,000 E.P.D., while the control, D784, had shown only 3000 E.P.D. and gave only 17 worms at autopsy. While these findings again speak against the existence of an acquired immunity, they have seemed in harmony with the explanation given above for the results of the previous experiment.



GRAPH 4. Curves of daily egg production of the three litter mate dogs of experiment 5. The previously infected animals, D776 and D783, were not more resistant to the test infections than was the control, D784.

Experiment 6. D781, Control D782, D788, Control D790. In this experiment are included dogs from two litters all of which were born and reared in the laboratory and which were kept uninfected until used in the present study. D781 and D782 were from the same litter and differed in age from D788 and D790 by only 18 days, the former pair being the older. One of each pair was exposed to a long period of repeated infection, while the other was retained as a control for the effect of age. The two repeatedly infected animals, D781 and D788, were given larvae at the same times and from the same cultures, the only difference being that all of the "immunizing" doses were three times as heavy in the case of D781 as in the case of D788. The data are presented in table 6 and graph 5, the latter showing the infection history of only one pair (D788, D790) since the results from both pairs of animals were essentially alike. The first infections were given at the ages of 109 and 91 days respectively, when D781 was given thirty larvae and D788 only ten. These infections were followed, at intervals of 3 to 4 days, by geometrically increased doses such that on the thirteenth day 480 larvae were given to D781, and 160 to D788. The total larvae given to each dog (table 6) during the first 13 days was, in the case of D781, 480 and to D788, 160 larvae. When it was apparent that these infections were well tolerated, a similar series of doses was again given between 29 and 42 days on the experiment. As a result of this second series of infections, the daily egg production of D781 rose to a well defined peak (2,263,-

(600 E.P.D.) which was reached on the sixty-sixth day. In the case of D788, the peak of the egg output (1,443,000 E.P.D.) was reached on the fifty-ninth day, but the later history of this dog shows that this was really the first maximum point in an essentially bi-modal curve of egg production. During the period from 73 to 87 days on experiment, a total of 1860 larvae was given to D781, in five geometrically increased doses, while one third of this number was given to D788. The number of larvae given in this series of infections was equal to the total number which had been administered previously in each case. Both dogs were quite resistant to these infections since the egg productions did not again rise above the original peaks, although the egg output of D788 was increased to about its former maximum. A few worms were regularly recovered from the feces of both dogs after the original peak in egg production was passed.

It is of interest to point out at this time that the puppies of this experiment were about 40 days older when first infected than were those of experiment 3, and that it took about 1 month's shorter exposure for these slightly older animals to manifest a resistance. The period required for the development of resistance was longer, however, than was required in the case of the older animals of experiment 5.

Additional doses of 651 and 1860 larvae respectively were given to D781 on the one hundred fifty-seventh and one hundred eighty-fifth days of the experiment, while one third of these numbers was administered to D788 on these dates. After about 7 months of exposure and about 4 weeks after the last infections, the egg productions had dropped to quite low levels and both animals were treated with tetrachlorethylene. After an interval of 4 weeks, during the latter part of which they were repeatedly negative to Lane examinations, five hundred larvae were given to D781 and to its litter-mate control, D782, while one thousand larvae were given to D788 and to its litter-mate control, D790. The results of these test infections in both pairs of litter mates were essentially similar (see table 6) in that the previously uninfected controls showed significantly greater egg productions and yielded more worms at autopsy than did their litter mates which had been exposed to previous infection. At the time of autopsy, 35 days after giving the test infection, D781 was showing an egg output of about 31,000 E.P.D. and 27 worms were recovered, while its control, D782, had reached an egg production of 400,000 E.P.D. and 61 worms were recovered. Similarly, D788 had reached an egg production of 237,000 E.P.D. and yielded 29 worms

at autopsy while its control, D790, showed an egg production of 1,054,000 E.P.D. and gave 281 worms at autopsy. These findings immediately suggest that a true acquired immunity was effective in rendering the previously infected animals more resistant to the test infections. It has seemed entirely possible that under certain conditions, not apparent in this experiment, such an immunity may prevail, perhaps because of long continued light exposure to hookworms as McCoy suggested (1931, p. 302). If it be presumed, however, that the previous exposure of these animals was of such a nature as to stimulate a physiological compensation for moderate hookworm burdens, then it is possible that a previously infected animal may be

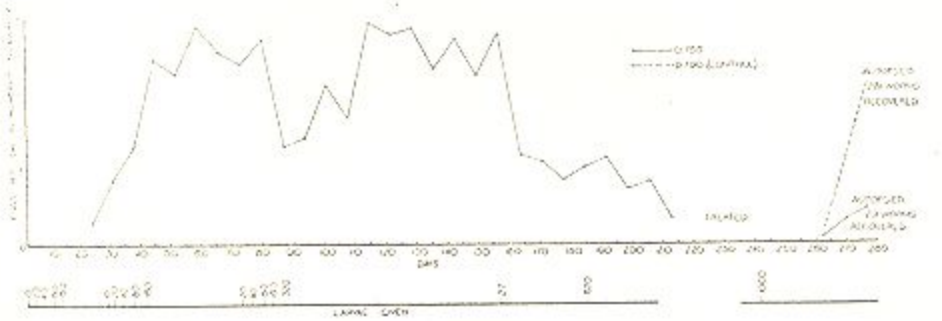
TABLE 6.

Relative resistance to a test infection of two previously infected dogs (D 781 and D 788) after treatment as compared with two previously uninfested litter mate controls (D 782 and D 790) (see experiment 6).

Days	D 781			D 782 (control)		D 788			D 790 (control)	
	Larvae given	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)	Larvae given	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)
0	30					10				
3	60					20				
6	120					40				
10	240					80				
13	480					160				
17		245	0							
24		313	0				142	0		
29	30					10				
31	60	653	0			20	424	0		
34	120					40				
38	240	874	0			80	647	0		
42	480					160				
45		629	0				1223	0		
52		977	0				1124	0		
59		1323	0				1443	0		
66		2263	0				1275	0		
73	60	1297	2			20	1198	2		
76	120					40				
80	240	1028	10			80	1354	0		
83	480					160				
87	960	752	12			320	651	1		
94		577	4				707	5		
101		626	9				1055	1		
108		576	14				841	5		
115		578	11				1470	3		

TABLE 6 (*Continued*).

Days	D 781			D 782 (control)		D 788			D 790 (control)	
	Larvae given	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)	Larvae given	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)
122		432	4				1393	1		
129		1148	0				1432	0		
136		428	0				1161	1		
143		293	0				1361	0		
150		714	0				1114	1		
157	651	714	0			217	1402	0		
164		378	1				594	9		
171		249	4				551	3		
178		454	5				429	5		
185	1860	597	6			620	516	5		
192		129					576			
199		66					363			
206		42					419			
213		27					168			
213	Treated					Treated				
241	500			500		1000			1000	
262		20			38		48			113
269		7			145		160			569
276		31			400		237			1054
	27 worms recovered at autopsy			61 worms recovered at autopsy		29 worms recovered at autopsy			281 worms recovered at autopsy	



GRAPH 5. Curves of daily egg production of two litter mate animals of experiment 5 [D788 and D790 (control)]. There is evidence both by egg count and by worm count of the operation of an acquired immunity.

better able to resist a test infection than would one which has been previously unexposed. That D781 and D788 did compensate for their long-standing infestations, at least in so far as their blood picture was affected, is evidenced by the determinations which were made on the two hundred and ninth day of the experiment. On this date, D781 and D788 were estimated to have 6,620,000 and 6,500,000 red cells per cmm. of blood respectively, while their uninfected controls, D782 and D790 gave readings of 6,820,000 and 6,640,000 red cells per cmm. All four of these dogs were estimated at this time to have 13.4 gms. hemoglobin per 100 cc. blood. Further findings on the blood of these dogs have been presented in table 6, p. 286, of a recent paper by Foster and Landsberg (1934). From these data, it is apparent that, in respect to blood findings, D781 and D788 were in as good condition as their controls even before they were freed of their worms in preparation for the test infection.

In the next experiment, data are presented on six animals where there was likewise an apparent demonstration of acquired immunity, although in that case, it has seemed that this result may be attributed to factors peculiar to the animals used in the experiment.

Experiment 7. D759, D760, Control D763, D761, D762, Control D764. All of the animals used in this experiment were litter mates and were born and reared in the laboratory. Four of them were subjected to repeated hookworm infection, beginning at 8 months of age, while two (D763, D764) were kept as controls for the effect of age. The larval doses were given to all of the animals at the same times and taken from the same cultures, although throughout the experiment D761 and D762 were given three times as many larvae at each infection as were given to their litter mates, D759 and D760. The data relating to these animals and their complete infection histories have been presented in tables 7 A and 7 B and in graphs 6 and 7. It is desired to direct attention to the fact that in graph 6 the data for the similarly infected pairs are given in composite form up to the time when one of each pair was treated to remove its worms prior to giving the test infections, while after this time the data given refer to the infestations of the individual animals. Graph 7 shows the composite larval-egg curves for the two pairs of similarly infected dogs up to the time of treatment (273 days on the experiment).

During the first 10 days, D759 and D760 were given five infections of one hundred larvae each while the other two, D761 and D762, were given five infections of three hundred larvae each. A second

series of four infections of fifty larvae each was given to D759 and D760, at 2-day intervals from 26 to 32 days on experiment, and the other pair were similarly infected with doses of one hundred and fifty larvae. Later infections were given, as indicated in table 7 and graph 7, until, when 273 days on the experiment, a total of 2200 larvae had been given in twenty doses to D759 and D760 and three times this number in as many doses had been administered to D761 and D762.

The composite curves of egg production (graph 6) for both pairs run a nearly parallel course for the first hundred days, in spite of the fact that one pair (D761, D762) had received three times as many larvae as were given to the others. The infestations up to this point were induced by the first two series of infections, as described above, and it was surprising to find that these comparatively few larvae for dogs of this age (8 months) resulted in egg productions of about 1,500,000 E.P.D. Notwithstanding this apparent susceptibility, the egg counts fell off quite rapidly after the sixty-fifth day and following this drop a few worms were recovered from the feces of all four animals. The third series of infections was given at this point and both egg curves (graph 6) showed definite responses to increased infestation, although in this case the more heavily infected pair showed about twice the egg output as was attained by D759 and D760. In the case of the latter two, the egg output dropped off again within about 50 days after this series of infections, although the egg production of D761 and D762 was sustained at a more or less uniform level of about 2,500,000 E.P.D. The fourth series of infections given between 133 and 147 days on the experiment again produced increases of the egg productions, and it was following these infections that the maximum egg counts were obtained on both groups. In the case of the more heavily infected pair, one of them, D762, was killed by the infection and yielded 851 worms at autopsy (170 days on the experiment) while D761, which survived, reached in egg output the high figure of approximately 4,500,000 E.P.D. The dogs which received the lighter infective doses showed a maximum of 2,402,000 E.P.D. on the one hundred and eighty-second day. These counts fell off rapidly to less than 1,000,000 E.P.D. in both cases within a few weeks.

After having been infected for 278 days, and at about 17 months of age, D759 and D761 were given anthelmintic treatment, and D760 was left untreated. At this point, the egg productions were 240,000 E.P.D. in the case of D759, 95,000 E.P.D. for D760, and

905,000 E.P.D. for D761. Twenty-eight days later, at which time all were negative to Lane examination except D760 (which was not treated), doses of two thousand larvae each were given to D759, D760, and to the control D763, while at the same time doses of six thousand larvae were administered to D761 and to the control D764. The last named animal, a previously uninfected control, died 12 days

TABLE 7 *A*.

Data on D 759, D 760 and D 763 of experiment 7.

Days on experiment	Larvae given	D 759		D 760		D 763—Control	
		E.P.D. (thous.)	Worms screened	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)
0	100						
2	100						
4	100						
7	100						
10	100						
21		303	0	143	0		
26	50						
28	50	1104	0	389	0		
30	50						
32	50						
35		770	0	982	0		
42		1077	0	869	0		
49		1963	0	1724	0		
56		1244	0	775	0		
63		1113	0	2117	0		
65	100						
70	100	646	1	1754	0		
73	100						
77	100	407	3	598	5		
80	100						
84		365	8	568	4		
91		494	7	499	3		
98		612	4	1238	0		
105		1467	0	1622	1		
112		909	—	1210	2		
119		1889	—	1284	—		
126		1060	—	1019	—		
133	100	749	—	601	3		
136	100						
140	100	727	—	466	12		
143	100						
147	100	710	3	525	17		

TABLE 7 *A* (*Continued*).

Days on experiment	Larvae given	D 759		D 760		D 763—Control	
		E.P.D. (thous.)	Worms screened	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)
154		615	10	742	4		
161		987	3	925	7		
168		1515	4	560	9		
175		2934	2	1050	3		
182		3057	0	1747	0		
189		1757	0	1759	0		
196		1660	0	1392	2		
203		1286	3	1635	0		
210		915	1	784	2		
217		1269	1	732	2		
224		960	5	366	7		
231		494	3	304	6		
238		657	5	567	3		
245	500	370	8	645	3		
252		208	—	279	—		
259		298	—	284	—		
266		214	—	124	—		
273		240	—	95	—		
273		Treated		Not treated			
280				7	—		
287				62	—		
294				60	—		
301	2000			132	—	2000	
308				58	—		neg.
315		neg.		155	—		neg.
322		16		168	—		135
		Autopsied 21 worms recovered		Autopsied 100 worms recovered		Autopsied 241 worms recovered	

later and 947 hookworms, none of them sexually mature, were recovered at autopsy. His previously infected litter mate was autopsied on the twenty-first day after having shown an egg count of only 33,000 E.P.D. and it was found to harbor only 31 hookworms. Similarly when the other three animals were autopsied on the twenty-first day, the control, D763, was found to have 241 hookworms, while the untreated experimental animal (D760) had 100 worms, and D759, 21 hookworms. The other data are readily ascertainable from the tables and graphs.

It is difficult to account adequately for many of the most striking results of this experiment. Recalling that the four experimental animals of this series were infected twenty times over a period of 245 days, during which time they increased in age to about 16 months, and that they were susceptible to each series of infections as evidenced by responses in egg production following each series of larval

TABLE 7 *B*.

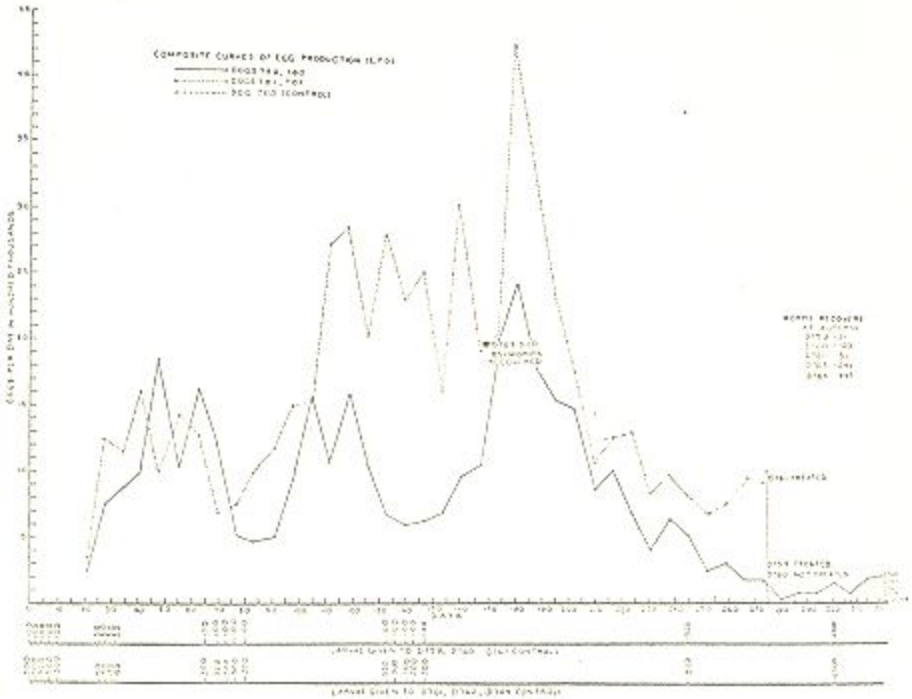
Data on D 761, D 762 and D 764 of experiment 7.

Days on experiment	Larvae given	D 761		D 762		D 764—Control	
		E.P.D. (thous.)	Worms screened	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)
0	300						
2	300						
4	300						
7	300						
10	300						
21		405	0	262	0		
26	150						
28	150	2142	0	325	0		
30	150						
32	150						
35		1810	0	468	0		
42		1562	0	1637	0		
49		1127	0	842	0		
56		1591	0	1253	0		
63		1612	0	922	0		
65	300						
70	300	921	0	415	1		
73	300						
77	300	709	3	789	5		
80	300						
84		745	5	1227	7		
91		1082	2	1248	1		
98		1015	2	1981	1		
105		1003	0	2056	0		
112		1865	—	3545	—		
119		2266	—	3417	2		
126		1702	—	2339	1		
133	300	1871	1	3670	5		
136	300						
140	300	1612	3	2958	—		
143	300						
147	300	2168	2	2814	2		

TABLE 7 *B* (*Continued*).

Days on experiment	Larvae given	D 761		D 762		D 764—Control	
		E.P.D. (thous.)	Worms screened	E.P.D. (thous.)	Worms screened	Larvae given	E.P.D. (thous.)
154		872	3	2322	8		
161		2943	3	3068	5		
168		1903	4	no tube	—		
175		2027	5	Died 170 days on experiment 851 worms recovered			
182		4216	0				
189		——					
196		2301	0				
203		1737	2				
210		1057	1				
217		1249	1				
224		1286	0				
231		823	4				
238		977	2				
245	1500	809	0				
252		676	—				
259		745	—				
266		946	—				
273		905	—				
273		Treated					
280							
287							
294							
301	6000					6000	
308							neg.
315		neg.					neg.
322		33					—
		Autopsied 31 worms recovered				Autopsied 947 worms recovered	

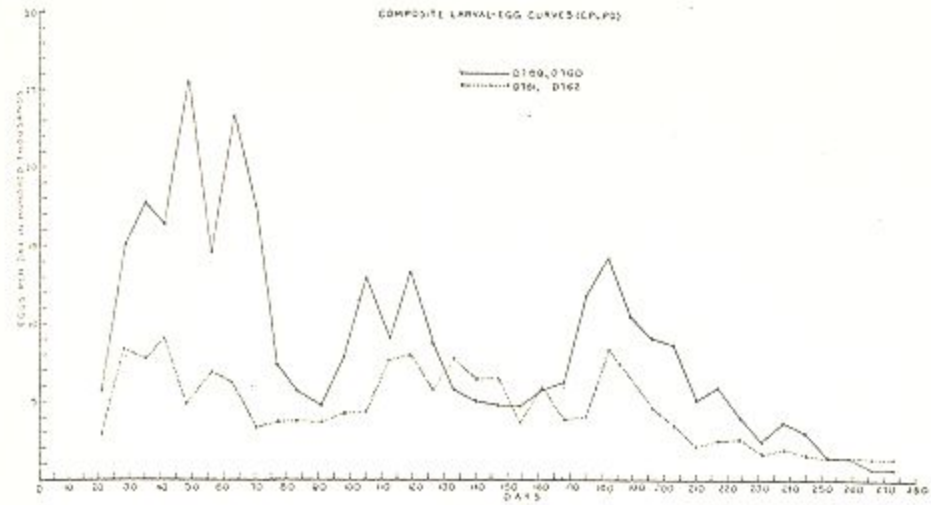
doses, it is surprising indeed to note that this lack of demonstrable resistance throughout the entire course of the infections should finally culminate in an apparently perfect demonstration of acquired immunity. The lack of age resistance, which was constantly apparent during the progress of this experiment, was evidenced not only by the infestation histories of these dogs but also by the size of the worm burdens which were harbored by D762 and D764 at the time of their deaths. It is an exceedingly rare occurrence to en-



GRAPH 6. Curves of daily egg production for the six litter mate dogs of experiment 7. The curves for the differently infected pairs are shown in composite form up to the time when one of each pair was freed of worms prior to giving the test infections. These animals appear to have lacked an age resistance but gave a remarkable demonstration of acquired immunity. This latter was apparently uninfluenced by either the presence or absence of worms at the time the test infections were given.

counter dogs more than a year old which do not show a marked resistance to the dog hookworm. In the experience of this laboratory only one other dog has been found which showed an abnormal lack of developed resistance to *A. caninum* under conditions of increasing age and repeated exposure (viz. D547, McCoy 1931, p. 274 and Foster and Cort 1932a, p. 584). That this dog was the sire of these puppies suggests a solution to this abnormal phenomenon in the dogs of this experiment.

With respect to the composite larval-egg curves, as given in graph 7, it is desired to point out that the larval-egg ratios were, in general, of much lower value for the dogs which received the greater numbers of larvae. This roughly inverse relationship between the number of larvae given and the "number of eggs produced per larva given"



GRAPH 7. Composite curves of the larval-egg ratios for the differently infected pairs of litter mate dogs of experiment 7. Note that the pair which were given the lighter infections (D759 and D760) showed generally a greater egg output per larva given.

may perhaps be partially correlated with the fact that in heavier infestations there are fewer eggs produced per female worm (Sarles, 1929) and with the fact that the percentage development appears to be lower in cases of larger infective doses. With reference to the significance of larval-egg curves, it may be reasoned that, properly interpreted, these data are the most delicate criteria available for assaying the resistance of animals subjected to this type of experimentation. The proper interpretation is the conservative one, namely, nothing should be accepted with certainty as indicating a decreased resistance except an increase in the larval-egg ratio when the conditions are those of superinfection. It is possible, however, that under conditions of heavy infection there may be a decreased resistance which does not permit an increase in the larval egg curve, while also worms maturing from a single infection may produce more eggs as they grow older; hence the limitation of this curve to its application to conditions of superinfection or re-infection. The composite larval-egg curves for D759, D760 and D761, D762 (graph 7) have been regarded as graphically demonstrating the periods of increased susceptibility in the infection histories of these dogs. These periods correspond closely with the maturation of the several series of doses of infective larvae, and indicate that there was a disproportionate

increase in egg output as compared to the numbers of larvae given. These findings add support to data already discussed in demonstrating the absence of an appreciable degree of age resistance or acquired protection during the course of the "immunizing" infections.

Summarily, it may be said that the data of this experiment have demonstrated an absence of age resistance among six litter mate animals, while at the same time there has been presented by these same animals our clearest evidence of the existence of an acquired immunity in dog hookworm infections.

Discussion.

In summarizing briefly the data of the seven experiments discussed above, it may be said that thirty dogs, from eight litters, were used in studies of repeated infection with *A. caninum*, of which twenty-three were subjected to varying degrees of periodic infection while seven were retained as controls for later test infections. At the time they were first infected, the experimental animals varied in age from 2 to 8 months, while the controls, which were kept uninfected until late in the experiments, were from 7 to 18 months old. Of the repeatedly infected dogs twelve succumbed to the effects of the worms, while of those which survived, five were found both by egg count and by worm count at autopsy to have resisted the test infections more effectively than did four controls (experiments 6 and 7), yet the other six previously exposed animals were apparently not more resistant to the test infections than were three controls (experiments 2, 4, and 5). Although some of the previously infected animals were freed of their worms prior to giving the test infections, this did not appear to affect their condition of resistance. A similar approach to the data presented by McCoy (1931) shows that of the repeatedly infected dogs used in the controlled experiments, one died on experiment; while of those which survived, three were found to be more resistant than four controls (experiments 4, 5, and 7), and three others not more resistant than two controls (experiments 1 and 6).

It may be summarized further that the infestation histories of several animals used in the present study which survived the infections, particularly those of experiment 3, represent the normal picture of the manner in which the dog becomes resistant to *A. caninum* under conditions of increasing age and repeated infection. There was at first an accumulation of parasites in the host with a gradual increase in daily egg production, and then a "crisis," after which the egg count fell off in spite of additional infections. In cases where

the feces of the dogs were screened for the recovery of worms, it was observed that the majority of worms were lost after the egg production had begun to decrease.

It is believed that these results offer a complete confirmation of the earlier studies of McCoy to which frequent reference has already been made.

With the completion of the present studies an opportune time is presented for analyzing our information on the resistance of dogs to infection with the dog hookworm, *A. caninum*, in order to formulate, if possible, a hypothesis which shall meet adequately the varied implications of the experimental findings.

This may have its best approach in an unbiased analysis of the extent to which an acquired immunity may be said to prevail in this host-parasite relationship. An acquired immunity is defined as that produced in the host by the presence of the worms. This definition is conservative in its agreement with accepted immunological interpretations, and at the same time is liberal in that it does not exclude from analysis, with respect to acquired immunity, the great bulk of work which has been done on resistance to superinfection. The application of this definition does require, however, that the immunity be elicited by past or present infestation, and that all other causes for the development of the immunity be eliminated.

The fact that repeatedly infected animals have, in several instances, become definitely more resistant to test infections than their previously uninfected controls would be accepted as strong evidence of the existence of an acquired immunity were it not for the equally numerous cases in which the contrary result has been obtained. Unquestionably much of this variation can be explained on the basis of individual difference in natural susceptibilities, and there is considerable evidence in the experiments involving litter mates in the present studies that individual animals may vary greatly with respect to natural resistance. It has seemed, however, that in many of the carefully controlled experiments, there were other factors responsible for these different results such, for example, as the animal's genetic constitution (experiment 7) or its condition of health as affected, perhaps, by previous infection (experiments 1 and 2).

Another possible indication of an immunity elicited by the worms is the apparent suddenness with which the immunity reaction takes place. It has been observed that, under conditions of repeated infection, there is an accumulation of parasites with a gradually increasing egg production, and then a "crisis," after which the egg

count falls off and some of the worms are spontaneously expelled. The importance of this "crisis" as a demonstration of acquired immunity loses much of its significance in the light of certain other findings. In the first place, the suddenness with which this reaction takes place has been found to vary with the age of the host. A 15-month-old dog infected with five hundred larvae each week may fail to develop any infestation at all until several thousand larvae have been given, while the infestation which does develop reaches its maximum egg production in 2 or 3 weeks and falls off rapidly thereafter. On the other hand, young puppies which survive repeated infections, ordinarily reach the peak of egg output in 3 to 5 months, although the egg production does not diminish materially for another 2 or 3 months. It seems entirely possible that the suddenness of this immunity reaction may be due, in large measure, to the reducing effect of increasing age of the host upon the egg production of the worms. It has been noted, also, that under conditions of repeated exposure to moderate infective doses animals may be rendered more susceptible to subsequent infections rather than more immune, and that these animals succumb before the "crisis" is reached.

It should be mentioned also that certain findings have been thought to show an increased resistance of the dog to superinfection with *A. caninum*. Herrick (1928) infected eight dogs and followed the infestations by egg count. Four were treated to negative while the others were superinfected with as many as four doses of larvae. No increased resistance was noted in the treated animals, although it was estimated from egg counts that the number of "eggs per larva" usually fell off as subsequent infections were given in the untreated cases. In summarizing this part of his studies, Herrick stated that "in certain cases where worms were present from previous infections there seemed to be increased resistance to superimposed infection." This interpretation also loses some weight in view of Sarles' (1929) demonstration that the number of eggs produced per worm decreased as the number of worms present increased and in view of data from the present studies which indicate that the number of "eggs per larva" may decrease as the size of the larval dose is increased.

Some attention has been given to a consideration of these factors "in favor of" an acquired resistance in dog hookworm infections, because it was apparent, as shall be pointed out presently, that the weight of evidence is against its occurrence to any considerable extent and that consequently some explanation was necessary to account for the observations which have been interpreted as indicating an acquired immunity.

The following experimental findings have been regarded as strongly indicative of an absence of acquired immunity in the dog against hookworm infection:

In the first place, there is a very general correlation between manifestations of resistance and the age of the host. On this basis an explanation has been offered above for the apparent suddenness of the immunity reaction, and it has been noted also that often when an animal became highly resistant after long continued infection, its litter mate which had never before been infected was equally resistant. Likewise older animals were found to have tolerated heavier initial infections than could be given safely to young animals, while the degrees of infestation which resulted from similar doses of larvae were much reduced in the cases of the older animals. Indeed, throughout these studies, and those of McCoy, there was constantly emphasized the difficulty, and yet the importance, of ruling out *age* as a factor affecting the resistance of the experimental animals.

Secondly, the slowness with which a dog becomes resistant to *A. caninum* points rather against the operation of an active immunity. In the repeated infection of puppies, the reaction is exceedingly slow, with the result that a single infection, or even several infections, confers no measurable degree of immunity to further infection. However, if the animal is allowed to age materially, as has been mentioned previously, a marked resistance is developed, even in the absence of previous infection.

In the third place, there is apparently an absence of demonstrable antibodies, as was shown by Stumberg (1930). This is not regarded as a particularly strong argument, in itself, against an acquired immunity, yet it is wholly in line with the inability to demonstrate consistently an immunity elicited by the worms.

In the fourth place, the evidence has seemed clear that in several instances, the presence of an infestation actually has rendered dogs more susceptible to subsequent infection. Some emphasis has already been given to this finding, in order that the distinction be made between decreased resistance to the injurious effects of parasitism and decreased resistance to the invasion and establishment of parasites. The presence of a heavy infestation has been shown to break down the resistance to increased parasitism, an effect which is similar to that of a deficient diet.

A fifth consideration is the fact that the six exceptional animals of experiment 7 of the present studies failed to become resistant, even after 8 months of continued exposure and when more than 15 months

old. In this instance one can feel certain that the complicating effect of age was eliminated. It was pointed out that the sire of these dogs was considered, up to the time of these experiments, to be the only dog among a large series which was found to lack "age resistance." Of this dog, McCoy wrote: (1931, p. 274) "D547 was an exceptional animal because it did not show the pronounced age resistance exhibited by the other dogs. Although over a year old at the end of the experiment, it was still susceptible to a heavy infection." That the dogs of this litter, which were also unusual in this respect, should have been in the direct line of descent from D547 has seemed to indicate more than a chance occurrence. Although this evidence is admittedly not more than suggestive of the possibility that heredity may be a factor which in some instances may influence the resistance of dogs to *A. caninum*, it has been given some consideration because of its potential significance in the whole field of resistance to helminthic infection. Although these same animals failed to exhibit a resistance to superinfection at any time during the long period of "immunizing" infections, it was surprising to find that in this experiment there occurred, as a result of the test infections, an apparently sound demonstration of acquired immunity. This finding is admittedly the most difficult result to explain adequately among our entire series of experimental studies with the dog hookworm. It may be possible that those animals who, because of their genetic constitution are unable to develop an age resistance, are protected by an ability to acquire an active immunity. If one go so far as to accept this hypothesis, it should not seem unreasonable to take under consideration the possibility that differences in heredity may be more responsible for divergent experimental results than hitherto supposed.

The sixth line of evidence bearing upon this analysis is derived from prenatal infection studies (Foster, 1932). The finding of susceptible puppies born to resistant mothers has seemed to throw some light on the nature of the immunity involved. The absence of protection in the puppies, even prenatally, has been regarded as strong evidence against the possibility that the mothers harbored an active immunity, while, on the other hand, this finding fits in well with the postulation of an age immunity.

Finally, a consideration of the factors which have been shown to affect the resistance of dogs to *A. caninum* makes it apparent that the general condition of the animal is very important in the regulation of the hookworm burden. Those factors which affect the ability of the host to compensate for the injurious effects of parasitism are appar-

ently the factors which reduce the resistance to increased parasitism. We have shown previously that a deficient diet (Foster and Cort, 1932; Foster and Cort, 1935) is one of these factors, while we shall have occasion to point out in later publications that an iron-deficient diet and the production of anemia by periodic bleeding are factors which have seemed to fall in this category. Likewise, the presence of a heavy infestation may militate against the preservation and development of resistance, as has been demonstrated in these experiments. In a recent study upon the anemia of hookworm disease in dogs (Foster and Landsberg, 1934) attention was directed to the apparent correlation which was consistently noted between hemoglobin levels of the blood and the animals' resistance to infection (p. 283). There can be no doubt that the outstanding injury done to the host by hookworms is the production of anemia; hence we may presume to have in this disease a vicious cycle, such that, in dogs at least, the presence of severe hookworm disease predisposes to further infection. This observation, as will be recalled, has already been demonstrated experimentally. Moreover it has seemed reasonable to suppose that age resistance is a manifestation of a non-specific general bodily resistance which, in the case of dog hookworm infestations, is conditioned upon the ability of the host to compensate physiologically for the effects of parasitism. There can be no doubt that the ability of the host to withstand blood loss, and to compensate for this loss, increases with age, at least to a certain point.

In the light of the above considerations, we are inclined to accept the following viewpoint, namely: that animals which, because of over infection, deficient diet, or as a result of the operation of any other factor, become weak, anemic, and poor in general health, may, in consequence, be rendered *less resistant,* not only to the injurious effects of hookworms, but also to the invasion and establishment of these parasites. On the other hand, animals with the advantage of age, or perhaps because of the advantage of a compensation which may have been induced by previous exposure to moderate worm burdens, may become able to resist more effectively the establishment of hookworms as well as their injurious effects.

Barring the possible rôle of heredity, the effect of which would be only to eliminate certain strains of animals from the application of this hypothesis, we submit that this explanation has seemed to us to be the only one which harmonizes the varied results which have been obtained in immunity studies with the dog hookworm.

Summary.

In an experimental study of the resistance of dogs to the dog hookworm, *Ancylostoma caninum*, thirty dogs, representing eight litters, were employed in seven experiments, in which twenty-three of these dogs were subjected to repeated infection while seven were retained as controls. In two experiments, the previously exposed animals were clearly more resistant to test infections than were the previously uninfected controls, while in three other experiments the opposite condition prevailed. Although some "immunized" animals were freed of hookworms prior to test infections, their condition of resistance did not appear to have been affected. Twelve animals died of acute hookworm disease during the course of repeated infections and, from the data presented by several of these, it was concluded that severe infestation may be a factor which predisposes to increased parasitism by hookworms. In other experiments, it appeared that light hookworm burdens sometimes prepared an animal, probably through the mechanism of a physiological compensation, to resist more effectively the test infections, although the distinction was constantly made between resistance to the injuries of worms and resistance to the invasion of worms. On the basis of the results obtained in one experiment, where the animals of one entire litter were unusual in lacking age resistance, it has been suggested that the genetic constitution of animals may be a factor complicating the resistance problem.

From an analysis of our information upon this resistance, it has been suggested that it is neither a true acquired immunity, nor, fundamentally, an age immunity. On the other hand, considerable emphasis has been placed upon the importance of an animal's general condition of health as the regulating factor in its resistance both to the injuries of hookworms and to their establishment within the host. Evidence has been presented to show that factors which militate against an animal's condition of well-being are factors which render that host less resistant to the invasion of hookworms.

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