AN EVALUATION OF THE PROTECTIVE INFLUENCE OF CULTURE-DERIVED METABOLIC PRODUCTS OF *COOPERIA PUNCTATA* AGAINST *NIPPOSTRONGYLUS BRASILIENSIS* (YOKOGAWA, 1920) IN RATS

by

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EXPLANATORY NOTES

This thesis consists of two parts. Part one is a complete paper to be submitted for journal publication. Part two consists of a more extensive review of the literature, a detailed account of the most critical procedures, detailed results, further acknowledgments, and an abstract.

PART I

PAPER FOR PUBLICATION

AN EVALUATION OF THE PROTECTIVE INFLUENCE OF CULTURE-DERIVED METABOLIC PRODUCTS OF *COOPERIA PUNCTATA* AGAINST *NIPPOSTRONGYLUS BRASILIENSIS* IN RATS

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ABSTRACT

The purpose of this study was to find out if culture-derived metabolic products of Cooperia punctata (Ag.) were capable of conferring immunity against Nippostrongylus brasiliensis infection in rats.

Male Carworth CFE Sprague Dawley rats were inoculated 3 consecutive times at intervals of 15 days each with culture-derived metabolic products of Cooperia punctata prepared according to the methods described by Zimmerman and Leland (1974), and then challenged with 1,200 infective larvae of Nippostrongylus brasiliensis.

Immunodiffusion tests in Ouchterlony plates (serum + Ag.) were positive (precipitin bands) only with sera from the antigen-inoculated rats. Acetate strip electrophoresis of serum showed slight increases in the gamma globulin fraction coupled with corresponding decrease in albumin.

Necropsy 10 days after challenge revealed that 2 of the 8 antigen-inoculated rats harbored fewer adult worms than the controls. Rats receiving adjuvants mixed with physiological saline showed very slight difference in worm burden as compared to normal rats.

The nematode Nippostrongylus brasiliensis and the culture-derived metabolic products of Cooperia punctata were used in this work. Both helminths belong to the Phylum Nemathelminthes, class Nematoda, order Rhabditida and family Trichostrongylidae.

The purpose of the present study was to determine if culture-derived metabolic products of Cooperia punctata, when injected into rats, were capable of inducing protection against subsequent exposure to Nippostrongylus brasiliensis. This would demonstrate the possibility of a cross immunity between nematodes from different vertebrate hosts.

MATERIALS AND METHODS

Experimental Animals

Male, white rats (Carworth CFE Sprague Dawley) weighing between 150-250 grams were used. These were kept in metal rat cages provided with water and commercial rat pellets ad libitum.

Culture-Derived Metabolic Products of *Cooperia punctata* (Antigen or Ag.)

This product was prepared according to the method described by Zimmerman and Leland (1974). The crystallined and lyophilized form of the product was diluted in physiological (0.15M NaCl) saline solution.

The quantity of the metabolic product thus prepared was quantitated in terms of nematode equivalents per ml. A nematode equivalent (Neq.) was the quantity of metabolic product eliminated into culture medium by one nematode from day 21 to 24 (72 hours) after inoculation.

Combination of Ag. with Adjuvant (Adj.)

The dissolved Ag. to be injected was mixed with an equal volume of complete Freunds Bacto Adjuvant code number 0638-59 (Difco Laboratories Inc., Detroit 1, Michigan). The 2 components were pulled into glass syringes and the syringes connected to each other by means of a rubber tube (Williams and Chase, 1967). The contents of the 2 connected syringes were thoroughly mixed until the mixture became milky white and a drop would float undispersed on water. This was the form (Ag. + Adj.) injected into the rats.

Inoculation of Rats

Eight rats were each inoculated three consecutive times at intervals of 15 days with 0.5 ml. of Ag.-Adj. mixture containing 100,000 Neq.

A second group of 10 rats (controls) was inoculated only with 0.5 ml., 50-50 mixture of adjuvant and physiological saline (Adj.-saline) mixed by the two-syringe technique. These also were inoculated 3 times at intervals of 15 days. In both groups of rats, the first inoculation was given intramuscularly in both rear legs. The second inoculation was given subcutaneously on the left side of each of the rats and the third inoculation was given subcutaneously on the right side. All the rats were subjected to the same environmental conditions.

A third group of 3 uninoculated rats was also maintained along with the inoculated groups.

Nippostrongylus brasiliensis

The Nippostrongylus brasiliensis used was originally a mice-adapted isolate and the infective larvae (L_3) were serially passed through several groups of white rats before use in the experiments. Its maintenance in the laboratory was carried out by bimonthly passages through rats.

The Baermann apparatus was used to recover L_3 from animal charcoal or vermiculite-fecal cultures. The L_3 thus recovered were repeatedly washed with distilled water and their number determined by dilution counting.

Challenge of Rats with L_3

Twelve hundred L_3 contained in 0.3 ml. of distilled water were injected subcutaneously.

Determination of Eggs per Gram of Feces (e.p.g.)

Estimation of daily e.p.g. was carried out using the flotation method as described by Leland (1963).

Total worm counts were performed on bulked intestinal contents and a pepsin-hydrochloric acid digest of the intestinal mucosa.

Analysis of Blood

Blood and serum were collected from the rats according to the methods described by Leland et al. (1955). A portion of the blood collected was mixed with EDTA in order to determine the number of red cells, white cells, packed cell volume, quantity of haemoglobin and differential leukocytes.

The quantity of haemoglobin per 100 cc. of blood was determined with the Haden-Hausser Haemoglobinometer (Clinical Model, Hausser and Son of Philadelphia). Packed cell volume (PCV) was determined with an International micro-capillary centrifuge (model MB, International Equipment Company, Massachusetts) operating at a relative centrifugal force of between 13,136 and 22,349 for 5 minutes, and the International micro-capillary reader (model CR).

Analysis of Serum

Immunodiffusion tests

Ouchterlony diffusion plates (International Chemical and Nuclear Corporation, Irvine, Ca.) were used for immunodiffusion tests (Ouchterlony, 1953). Purified electrophoresis agarose (Sigma Chemical Company, St. Louis, Missouri) was used as a 1% solution in glycine buffered saline. The agarose solution was a modification of the formula by Blair et al. (1960)

as recommended by International Chemical and Nuclear Corporation. Plate preparation was as recommended by the manufacturer.

Sera were dispensed by the Eppendorf Microliter pipettes (Eppendorf Gerätbau Netherl + Hinz, Gmbh, Hamburg) size 50. Pooled immune serum of calf 51 was used as positive control. The serum of this calf had experienced 7 consecutive experimental infections with Cooperia punctata totalling 470,000 L_3.

Following loading of sample wells, incubation for the development of precipitin bands was at 4°C for 24 to 72 hours.

Serum electrophoresis

Total protein determinations were made using the TS meter model 1040b (American Optical Company, Buffalo, N.Y.).

Agarose film electrophoresis was conducted using AC1 cassette system, reagents and instructions of the manufacturer (Analytical Chemists, Inc., Palo Alto, Ca.).

Densitometer scans and quantitation were made with the Quick Scan model 1020 densitometer (Helena Laboratories, Beaumont, Texas).

RESULTS

Prepatent Periods and Egg Counts per Gram Feces (e.p.g.)

As shown in table 1, the prepatent period for all the rats infected with 1,200 L_3s was 7 days as determined by the appearance of nematode eggs in their feces. In the Ag.-Adj. group of rats, greater contrasts existed on day 7 in e.p.g. (200-11,000) as compared to the control groups (500-2700 Adj., 4500-5200, normal).

Rats 35 and 36 (Ag.-Adj.) presented a great contrast to the other members of the group. Counts from both rats were very low for 3 consecutive days before necropsy. The other 6 rats in the Ag.-Adj. group had higher counts. Egg counts for the rats that were uninjected or injected with adjuvant only were more uniform than those in the Ag.-Adj. group.

Duration of Egg-Laying Period

Table 1 shows the end of the egg-production period. Rats numbers 29, 30 and 34 (Ag.-Adj.) were observed until no eggs were detected in their feces. The 3 of them still eliminated some eggs on the 16th day after infection but on the 17th day, no eggs were detected.

Rats 21, 22, 25 and 26 (Adj.) still passed eggs 16 days after infection but on the 17th day, eggs were detected in only the feces of rat 25. No eggs were detected in the latter rat on the 18th day. Also no eggs were detected in rat number 39 (normal) on the 18th day after infection.

Table 1

EGGS PER GRAM OF FECES

Days after challenge	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Rat number			ADJUVANT/CHALLENGED											
21	0	0	500	900	500	1700	3000	2100	1800	670	160	20	0	0
22	0	0	900	1300	1000	3200	5900	4700	4800	2500	900	50	0	0
23	0	0	1800	3500	4600	*								
24	0	0	800	1900	1200	*								
25	0	0	2700	2300	2000	1500	2200	1500	1700	970	400	50	10	0
26	0	0	1500	2000	3500	1400	900	1700	1100	680	200	10	0	0
27	0	0	1500	3800	2700	1900	1500	1900	*					
28	0	0	1300	3000	1900	2100	1000	1100	*					
Average	0	0	1375	2337	2175	1967	2417	2167	2350	1205	415	130	10	0
(range)	(0)	(0)	(500-2700)	(900-3800)	(500-4600)	(1400-3200)	(900-5900)	(1100-4700)	(1100-4800)	(670-2500)	(160-900)	(10-50)	(0-10)	(0)
			ANTIGEN-ADJUVANT/CHALLENGED											
29	0	0	3500	4000	3100	3400	4600	2800	1100	650	230	45	0	0
30	0	0	5200	7200	6000	5500	3000	1200	750	420	150	15	0	0
31	0	0	6000	7000	5800	6700	10100	7600	*					
32	DIED													
33	0	0	11000	15100	14300	7000	4200	3700	*					
34	0	0	1800	3300	2700	2100	1200	1500	1000	720	300	60	0	0
35	0	0	200	700	600	*								
36	0	0	500	900	750	*								
Average	0	0	4029	5457	4750	4940	4620	3360	950	597	227	40	0	0
(range)	(0)	(0)	(200-11000)	(700-15100)	(600-14300)	(2100-7000)	(1200-10100)	(1200-7600)	(750-1100)	(420-720)	(150-300)	(15-60)	(0)	(0)
			NORMAL/CHALLENGED											
37	0	0	5200	6700	3900	3000	3500	2100	*					
38	0	0	4500	3800	4900	*								
39	0	0	4700	5100	5600	4900	4100	3700	2900	1800	750	55	15	0
Average	0	0	4800	5200	4800	3950	3800	2900	2900	1800	750	55	15	0
(range)	(0)	(0)	(4500-5200)	(3800-6700)	(3900-5600)	(3000-4900)	(3500-4100)	(2100-3700)						

* = Day of necropsy.

Adult Worm Recovery

Seventeen rats were necropsied at various times in the patency period. Results obtained are presented in table 2. At 10 days after infection, 5 rats were necropsied. Rats 35 and 36 (Ag.-Adj.) had lower worm counts than the other rats necropsied on that day. Rat 35 contained a total of 13 adult worms, rat 36 a total of 20.

Rats 23 and 24 (Adj.) at Day 10 contained 92 and 50 worms respectively while rat 38 from the normal group had 117 worms.

At 13 days after infection, rats 31 and 33 (Ag.-Adj.) were necropsied and 83 and 115 worms were recovered respectively. Rats 27 and 28 (Adj.) contained 105 and 34 worms respectively while rat 37 from the normal group had 54 adult worms.

Five rats were necropsied at 22 days after inoculation of L_3. No adult worms were recovered from any of them. Similarly, no adult worms were recovered from two rats which were necropsied after 30 days of infection.

Analysis of Blood

The data obtained on the analysis of blood is presented in tables 3a and 3b. Average leukocyte counts of the 3 groups of rats were 10,973 cells/cmm. blood for the antigen-inoculated group, 9,420 cells/cmm. blood for the adjuvant/saline group and 10,692 cells/cmm. blood for the group of normal rats. Red blood cells were between 7.1-9.5 millions per cubic milliliter of blood. There was no appreciable difference between the hematological data obtained before and after infection. Packed cell volume

Table 2

ADULT WORMS RECOVERED AT NECROPSY

Rat number	Days from challenge to necropsy	Number male worms	Number female worms	Total worms recovered	% recovery
	ADJUVANT/CHALLENGED				
23	10	40	52	92	7.7
24	10	28	22	50	4.2
27	13	65	40	105	8.8
28	13	14	20	34	2.8
21	22	0	0	0	0
22	22	0	0	0	0
25	30	0	0	0	0
	ANTIGEN-ADJUVANT/CHALLENGED				
32	DIED				
35	10	6	7	13	1.1
36	10	12	8	20	1.7
31	13	43	40	83	6.9
33	13	60	55	115	9.6
29	22	0	0	0	0
34	22	0	0	0	0
30	30	0	0	0	0
	NORMAL/CHALLENGED				
38	10	50	67	117	9.8
37	13	38	16	54	4.5
39	22	0	0	0	0

Table 3a

BLOOD ANALYSIS

Rat number	Days blood analyzed before challenge	Days blood analyzed after challenge	WBC/ Cmm. (thousands)	RBC/ Cmm. (millions)	PCV/%	Haemo-globin/ 100 cc.
	ADJUVANT/CHALLENGED					
+19	1*	-	10.7	7.73	42	15
+20	1*	-	10.8	8.31	46	15
21	-	4	4.8	9.07	43	14
22	-	4	7.9	9.54	48	15
26	-	4	9.9	8.43	46	15
23	-	10	10.8	8.29	44	15
24	-	10	9.7	9.00	48	15
27	-	13	11.1	9.20	43.5	12
28	-	13	9.0	9.30	46	14
25	-	22	9.5	8.60	47	15
	ANTIGEN-ADJUVANT/CHALLENGED					
31	1*	-	13.4	8.20	44	15
32	1*	-	13.3	8.40	45	15
33	-	4	6.8	8.50	39	14
34	-	4	7.3	8.50	41	14
35	-	10	5.2	9.34	50	15
36	-	10	14.7	9.50	48	15
31	-	13	11.7	9.41	48	14
33	-	13	18.1	7.91	44	13
29	-	22	12.4	7.13	47	14
30	-	22	10.5	7.92	45	14
34	-	22	7.4	8.98	42	14
	NORMAL/CHALLENGED					
37	1*	-	11.5	8.24	46	15
37	-	4	8.0	8.73	44	15
38	-	10	10.6	9.16	46	15
37	-	13	12.5	9.50	46	14
39	-	22	11.2	8.31	47	15
	NORMAL/UNCHALLENGED					
17	49	-	9.0	9.07	46	14
18	49	-	12.0	9.41	48	14

* = Determination by the electronic counter.
+ = Unchallenged

Table 3b

DIFFERENTIAL LEUKOCYTE COUNTS

Rat number	Days counts made before challenge	Days counts made after challenge	% cells/cells per Cmm.					
			Baso-phil	Eosino-phil	Band	Seg-mented	Lympho-cytes	Mono-cytes
	ADJUVANT/CHALLENGED							
*19	1	-	1/107	1/107	3/321	26/2782	68/7276	1/107
*20	1	-	0/0	2/216	3/324	30/3240	65/7020	0/0
21	-	4	1/48	2/96	1/48	28/1344	65/3120	3/144
22	-	4	0/0	1/80	1/80	23/1834	72/5742	3/239
26	-	4	1/99	0/0	3/297	28/2772	65/6435	3/297
23	-	10	0/0	0/0	0/0	21/2278	78/8463	1/109
24	-	10	1/97	0/0	0/0	23/2225	75/7160	2/194
27	-	13	1/111	1/111	2/221	31/3426	64/7072	1/111
28	-	13	1/90	1/90	2/180	19/1710	76/6840	1/90
25	-	22	1/95	0/0	3/284	25/2363	69/6521	2/189
	ANTIGEN-ADJUVANT/CHALLENGED							
31	1	-	1/134	4/536	3/402	30/4020	58/7772	4/536
32	1	-	1/133	5/665	2/266	20/2660	66/8778	6/798
33	-	4	1/68	4/272	1/68	28/1904	61/4148	5/340
34	-	4	1/73	5/363	2/145	22/1595	64/4640	6/435
35	-	10	1/52	4/206	2/103	26/1339	62/3245	4/206
36	-	10	0/0	6/881	3/440	21/3082	65/9539	5/734
31	-	13	1/117	2/234	2/234	27/3276	65/7605	2/234
33	-	13	1/181	3/543	3/543	29/5249	61/11041	3/543
29	-	22	0/0	2/248	1/124	25/3094	68/8416	4/495
30	-	22	1/105	3/315	2/210	24/2520	67/7035	3/315
34	-	22	0/0	3/223	2/149	22/2605	66/4901	2/149
	NORMAL/CHALLENGED							
37	1	-	0/0	0/0	1/115	28/3220	70/8050	1/115
37	-	4	1/80	1/80	0/0	37/2967	62/4970	0/0
38	-	10	0/0	1/106	1/106	20/2125	76/8075	2/213
37	-	13	1/125	1/125	0/0	30/3735	66/8217	2/249
39	-	22	1/112	0/0	3/336	29/3248	65/7280	2/224
	NORMAL/UNCHALLENGED							
17	49	-	1/90.3	0/0	4/361	22/1986	71/6408	2/181
18	49	-	1/120.3	1/120.3	2/241	24/2886	70/8418	2/241

* = Unchallenged

ranges were between 39% and 50%. Haemoglobin concentration ranged from 12 to 15 grams per 100 cc. of blood.

In the differential leukocyte counts, results were fairly uniform for all the rats except for higher eosinophil counts in those rats injected with Ag.-Adj. Monocyte counts were also higher in this group than in the others.

Analysis of Serum

Immunodiffusion tests

Table 4 summarizes the results obtained for the immunodiffusion tests performed.

Serum from each of the 8 rats injected with Ag.-Adj. formed distinct precipitin bands in the Ouchterlony gel diffusion test (serum + Ag.-Adj.). Reactions ranged from those of complete coalescence to partial coalescence between the tested rats' sera and the Cooperia metabolic product. Sera from those rats given only the adjuvant, and from those that were given no injections at all, did not form precipitin bands with the metabolite.

Serum electrophoresis

Results obtained from electrophoretic separation of the serum protein components are presented in table 5. Total serum protein varied from 5.3 to 6.5 grams/100 ml. The percentage of gamma globulin varied from 14.8% to 23%.

In the immunized rats, the percentage of gamma globulin varied between 14.8 and 23%. Rats that were injected with adjuvant only, varied from 15.9 to 18.8% and uninjected rats varied from 15.8 to 18.4%.

Table 4

IMMUNODIFFUSION TESTS (SERUM x AG.)

Rat number	Days serum collected before challenge	Days serum collected after challenge	Reaction after 24 hours	Reaction after 48 hours	Reaction after 72 hours
	ADJUVANT/CHALLENGED				
*19	1	-	0	0	0
*20	1	-	0	0	0
21	-	4	0	0	0
22	-	4	0	0	0
26	-	4	0	0	0
23	-	10	0	0	0
24	-	10	0	0	0
27	-	13	0	0	0
28	-	13	0	0	0
25	-	22	0	0	0
—	—	—	—	—	—
	ANTIGEN-ADJUVANT/CHALLENGED				
31	1	-	+	++	++
32	1	-	+	++	++
29	21	-	+	++	++
36	21	-	+	++	++
33	-	4	+	++	++
34	-	4	+	++	++
35	-	10	+	++	++
36	-	10	+	++	++
31	-	13	+	++	++
33	-	13	+	++	++
29	-	22	+	++	++
30	-	22	+	++	++
34	-	22	+	++	++
—	—	—	—	—	—
	NORMAL/CHALLENGED				
37	1	-	0	0	0
37	-	4	0	0	0
38	-	10	0	0	0
37	-	13	0	0	0
39	-	22	0	0	0
—	—	—	—	—	—
	NORMAL/UNCHALLENGED				
17	49	-	0	0	0
18	49	-	0	0	0

0 = No precipitin bands formed.
+ = Precipitin bands slightly visible.
++ = Precipitin bands very distinct.
* = Unchallenged

Table 5

ELECTROPHORESIS OF SERUM PROTEIN

Rat number	Days serum collected before challenge	Days serum collected after challenge	Total protein Grams/ 100 ml.		Albumin Grams/ 100 ml.	%	α_1 Grams/ 100 ml.	%	α_2 Grams/ 100 ml.	%	β Grams/ 100 ml.	%	γ Grams/ 100 ml.
	ADJUVANT/CHALLENGED												
*19	1	-	6.4	46.2	2.9	24.2	1.6	5.9	0.38	6.2	0.40	17.2	1.1
22	-	4	5.8	43.1	2.5	22.2	1.3	9.8	0.57	6.1	0.35	18.8	1.1
23	-	10	6.0	44.6	2.7	20.5	1.2	8.6	0.52	7.8	0.47	18.4	1.1
24	-	10	5.3	42.0	2.2	20.1	1.1	8.7	0.46	12.6	0.67	16.5	0.88
28	-	13	6.0	44.6	2.7	21.8	1.3	8.8	0.53	6.8	0.41	17.9	1.1
25	-	22	6.3	45.6	2.9	21.2	1.3	7.8	0.49	9.5	0.59	15.9	1.0
	ANTIGEN-ADJUVANT/CHALLENGED												
31	1	-	6.3	43.1	2.7	24.8	1.6	8.5	0.53	5.3	0.33	18.4	1.2
32	1	-	6.5	40.4	2.6	25.3	1.7	7.0	0.46	8.1	0.52	19.2	1.3
35	-	10	5.6	43.0	2.4	22.7	1.3	8.1	0.45	6.8	0.38	19.4	1.1
36	-	10	5.9	41.0	2.4	20.4	1.2	7.4	0.44	8.2	0.48	23.0	1.4
31	-	13	6.1	42.3	2.6	20.9	1.3	8.0	0.49	9.9	0.60	18.8	1.2
33	-	13	5.5	44.6	2.5	20.0	1.1	9.8	0.54	9.4	0.52	17.2	0.95
29	-	22	6.1	38.4	2.3	25.9	1.6	7.6	0.46	10.3	0.63	17.8	1.1
30	-	22	5.8	46.9	2.7	19.9	1.2	7.9	0.46	9.7	0.56	16.5	0.96
34	-	22	6.0	44.8	2.7	23.6	1.4	8.1	0.49	8.7	0.52	14.8	0.89
	NORMAL/CHALLENGED												
38	-	10	5.9	42.0	2.5	24.0	1.4	10.0	0.59	6.7	0.39	17.3	1.02
37	-	13	6.2	46.2	2.9	23.8	1.5	6.6	0.41	5.9	0.36	17.6	1.1
39	-	22	5.8	45.6	2.6	19.4	1.1	7.8	0.45	8.8	0.51	18.4	1.1
	NORMAL/UNCHALLENGED												
17	49	-	6.3	46.9	2.9	25.6	1.6	6.0	0.37	5.6	0.35	15.8	0.99
18	49	-	5.3	48.8	2.6	22.7	1.2	6.2	0.33	5.6	0.30	16.6	0.88

* = Unchallenged

The highest values of gamma globulin were obtained from the sera of rats 35 and 36 (Ag.-Adj. group). These values were 19.4% and 23% respectively.

DISCUSSION

From the results of this experiment, it is evident that some of the rats which were inoculated with the Cooperia antigen showed some resistance to subsequent infection with Nippostrongylus brasiliensis. The data obtained on prepatency however does not justify the above statement. The prepatent period in all the rats both antigen-inoculated and controls was 6 days. Similar lengths of time have been reported by other workers on normal rats: 6 to 7 days (Ogilvie and Jones, 1971); 5 to 7 days (Gharib, 1961); 5 to 7 days (Haley, 1962). One of two things was obvious in this case. Either the antigen has not been able to confer any protection on the rats and as such their prepatent periods have shown no difference from the controls or the protection conferred was not strong enough to lengthen prepatency. This latter proposition seems more probable since further results justify some degree of immunity.

E.p.g. counts were generally high in all the rats both antigen-inoculated and controls with the exception of 2 that received the antigen (35 and 36). For 3 consecutive days, e.p.g. counts in these 2 rats were well below a thousand while several thousands were counted in the other rats.

Several authors have determined the daily egg output of Nippostrongylus brasiliensis in the normal rat. Porter (1935) indicated 935 eggs per female per day. Chandler (1935) obtained 50-274 eggs per female per day and Phillipson (1969) obtained 100 eggs per female N. brasiliensis per day. Graham and Porter (1934) reported that egg production of N. brasiliensis varies with the breed of the rat host employed. The same authors found that a 3-year old strain of N. brasiliensis from Texas constantly produced

fewer eggs per worm than either of 2 strains isolated from rats in Baltimore. Similarly, Kameko (1938) isolated and studied N. brasiliensis from each of 5 wild Rattus norvegicus and claimed that a strain from one animal differed from those of the other 4 with respect to length of prepatent period, rate of infection and egg production. The reduction in egg elimination by these 2 rats in the experiment is more likely to be due to inhibition of worm development rather than to any other reasons since all the rats involved belong to the same breed and were all challenged with the same isolate of N. brasiliensis. Several authors (Haley, 1962; Eisen, 1974) mentioned the fact that individuals belonging to the same group or breed may react differently to the same antigen. It is quite possible in view of this fact, that the Cooperia antigen has produced some inhibition to larval development in those 2 rats while other rats have not been affected.

Some of the rats were necropsied along the way in the experiment and all the 8 that were living 16 days after challenge still eliminated nematode eggs. However at 17 days after challenge (i.e. 11 days of egg production), nematode eggs were recovered from the feces of only 2 of the rats. All those inoculated with antigen passed out no more eggs after the 10th day. It is hard to draw any conclusions here as to the difference in the termination of egg-laying periods since the dates are very close and the number of rats involved is quite small. This egg-production period of 10-11 days compares with the data obtained by Africa (1931) in which he claimed that eggs disappeared from the feces of white rats in 11-19 days after infection and those of Oglivie and Jones (1971) who stated that egg production lasts about a week after which the worms are eliminated within 2 days.

The percentage of adult worms recovered in this experiment was generally low and much more so in 2 of the antigen-inoculated rats (35

and 36) where it was 1.1% and 1.7% respectively. The inhibition referred to above may have been responsible for this low worm recovery. It may be that the Cooperia antigen was able to stimulate some protection against larvae at the site of the tissue barrier which the larvae must first penetrate before establishment in the intestine. Mueller (1938) felt that secretions of tapeworms influenced the immunological defenses though he did not use excretory and secretory antigens of Sparganum mansonoides before challenge with infected cyclops. The spargana which developed were all encysted indicating that the somatic antigen stimulated the host's defense system. It may also have been that the Cooperia metabolite produced an inflammatory reaction in the rats and this reaction inhibited growth and development of N. brasiliensis adults. Cox (1952) concluded that the protective effect of Ancylostoma caninum against Trichinella spiralis was due to inflammation of the intestinal mucosa induced by A. caninum.

A more acceptable explanation for this apparent resistance may be due to the production of antibodies with or without inflammatory processes, similar to the mechanism for development of immunity in response to T. spiralis as proposed by Larsh (1953). A series of non-immunological factors that could lead to low worm recovery in N. brasiliensis infected rats have been discussed by several authors (Thorson, 1954(a); Haley, 1962; Wescott and Todd, 1964). Haley (1962) indicated that only about 55% of those L_3 of N. brasiliensis that pass through the lungs of rats reach maturity while Jarrett et al. (1968) contended that 40% to 60% of those L_3 that are injected do not reach the lungs.

The fact that the N. brasiliensis used in our experiment was originally a mice-adapted isolate should not be overlooked since according

to Wescott and Todd (1966) a mice-adapted strain of N. brasiliensis is still infective for rats but its infectivity is much more reduced.

The data obtained on the blood analysis of the tested and control rats were similar except for the increase in the eosinophil and monocyte components of the white blood cells in the antigen-inoculated group. The average eosinophil count in this group is 408 cells per cmm. of blood while the values are 70 cells and 62 cells per cmm. of blood in the groups that received mixture of adjuvant and saline and the normal group respectively. Similarly the average monocyte count for the antigen-inoculated rats was 435 cells per cmm. of blood while it was 148 cells per cmm. for the group that received adjuvant and saline and 175 cells per cmm. for the group of normal rats. These values although still within the normal ranges calculated by some authors are quite remarkable.

The difference between the total leukocyte values of the 3 groups of rats was not appreciable as these are 10,973 cells/cmm. blood for the Ag.-Adj.-inoculated group, 9,420 cells/cmm. for the Adj.-saline group and 10,692 cells/cmm. of blood for the group of normal rats. The marked increase in number of eosinophils and monocytes may have been due to sensitization of the tissues prompted by the injection of foreign proteins of the Cooperia metabolite.

Taliafero and Sarles (1939) described that at the height of immune reaction to the parasite, a mixed inflammatory reaction of the plasma cells, lymphocytes, mononuclear cells, macrophages and eosinophils occur. They also noted large numbers of globular leukocytes.

Yoeli (1956) infected some voles with Plasmodium berghei and 4 to 7 weeks later, exposed the voles to cercariae of Schistosoma mansoni. The Schistosoma infection alerted the reticulo-endothelial system and the

phagocytic power of the host organism got increased. These reactions markedly affected the course of the plasmodial infection.

Although the data obtained on blood analysis in this experiment has varied from rat to rat, all values are still within limits calculated for normal rats by different authors some of whose values are summarized in table 6.

The considerable variations reported by different investigators may have been due to strain differences and the conditions of sampling. Quimbly et al. (1948) compared values of rat blood from tail tip to that obtained by cardiac puncture and found that the leukocyte values were 23,810 cells per cmm. and 6,425 cells per cmm. respectively. Nichols and Miller (1948) showed that the excitement of rats undergoing ether anesthesia led to an increase of leukocytes in heart blood due to redistribution from peripheral blood and an increase in erythrocyte number possibly due to splenic contraction. Kozma et al. (1969) anesthesized rats with ether and withdrew blood by heart puncture. They reported leukocyte counts of 13,479 ± 3,291 in rats 8 months of age while Cameron and Watson (1949) used free-flowing tail blood and obtained mean total leukocyte count of 21,400 in male rats. According to Schalm et al. (1975) total leukocyte counts are greater at all ages in Sprague-Dawley rats than in Long Evans rats.

Serum from each of the antigen-inoculated rats formed distinct precipitin bands with the Cooperia metabolite put in the central well of the Ouchterlony diffusion plates. These varied from reactions of coalescence to partial coalescence with the Cooperia metabolite put in the central well.

Table 6

COMPILATION FROM THE LITERATURE OF HAEMOGRAMS FOR THE RAT

Source	RBC/ Cmm. (millions)	WBC/ Cmm. (thousands)	Hb/ 100 cc.	PCV %	Neutro-phil %	Lympho-cyte %	Mono-cyte %	Eosino-phil %	Baso-phil %
Gardner, 1947	7.4-9.6	6.4-26.2	10.8-17.5	35-51	12-46	53-83	1-7	0-3.4	0-1
Albritton, E.C. 1952	7.2-9.6	5-25	12-17.5	39-53	9-34	65-84	0-5	0-6	0-1.5
Huang and Bondurant, 1956	6-7	7-13	13-14	46-49	18-22	69-82	traces	0-2	0-5
Hulse, 1964	8.2	6.04	15.4	49	19.8	73.9	4.6	1.7	-
Schalm et al., 1975	7.95±0.49	15.33±3.5	15.4±1.1	48.7±2.2	16.12±6.34	81.5±5.7	1.5±1.4	0.8±0.7	0.05±0.15

These reactions indicated the presence of antibodies in the sera of the rats, which was also an indication that there may be a common antigen in the two helminths involved. There were no precipitin bands formed with sera from the 2 groups of control rats, which is a strong indication that their sera contained no detectable antibodies. It can be hypothesized at this point that there is an antigenic similarity between Cooperia punctata and Nippostrongylus brasiliensis so that serum antibodies built up in response to Cooperia metabolites reacts against N. brasiliensis to make these 2 rats more resistant. More experience, however, is needed with larger numbers of rats. The agar-diffusion tests performed in this work supported this hypothesis. Similar hypothesis was put forward by Larsh (1953) to support the results he obtained when he worked with T. spiralis. Similarly, Crandall et al. (1967) after finding out that sera from mice infected with Ascaris only or Nippostrongylus and Ascaris gave a precipitin band in micro-ouchterlony tests with crude saline extracts of lyophilized Ascaris larvae and adults concluded that there was a minimum of one common antigen in the 2 helminths.

The electrophoretic patterns of the serum proteins of the antigen-inoculated rats show very slight difference when compared with the controls. A slight increase in the gamma globulin fraction coupled with slight decrease in the albumin component was observed. The average of the gamma globulin in the antigen-inoculated rats is 18.34% while it is 17.45% in the group that received mixture of adjuvant and saline and 17.14% in the group of normal rats. Similarly the average of their albumin components are 42.7%, 44.4% and 45.9% respectively. The highest gamma globulin values were obtained from sera of antigen-inoculated rats 35 and 36. These values are 19.4% and 23% respectively but their total serum protein values are low

and thus the gamma globulin in terms of grams per 100 ml. was not elevated.

In view of the fact that antibodies have been detected through the Ouchterlony test, it could be stated here that the increase in the gamma globulin fraction of the rats' serum protein was due to the production of antibodies since according to Eisen (1974) antibodies are usually found in the gamma globulin components.

Great disparities exist in the results obtained by different workers on the electrophoresis of the normal rat serum. A compilation of the values is presented in table 7. There are several different reasons for the apparent disparities. Some used rat serum while others worked with plasma. Electrophoresis was carried out in several different media using different apparatus. Laboratories are not precise in defining the breed of a given animal species and physiological conditions varied from one laboratory to the other.

It can be seen from the results obtained in this experiment that immunization with Cooperia antigen was effective in reducing development of N. brasiliensis in 2 of the 8 rats tested. There was important reduction in nematode egg output and worm burden in the 2 rats. Both the Ouchterlony test and electrophoretic serum analysis showed the presence of antibodies. It appears that the antigen was able to stimulate some protective immunity against larvae at the site of the tissue barrier which the larvae must first penetrate before establishment in the intestine. It is very hard however to explain why only 2 of the 8 antigen-inoculated rats were protected especially since all 8 of them showed positive reaction in the Ouchterlony test. In the establishment of an immune response however, individual body constitution and reaction are of prime importance.

Table 7

COMPILATION FROM THE LITERATURE OF ELECTROPHORETIC PATTERN OF RAT SERUM OR PLASMA

Source	Methods of electrophoresis	Type of buffer	Plasma or serum	Total protein gr./100 ml.	γ %	Fibrin-ogen %	β %	α_2 %	α_1 %	Albumin %
Deutsch and Goodloe, 1945	Modified Tiselius with single section cell	Modified Longsworth veronal buffer	Plasma	-	4.8	19.4	-	-	15.4	59.1
Schultz et al., 1954	Tiselius	Veronal buffer	Serum	6.2	13.2±3	-	14.4±1.2	10±1.3	13.7±3	48.6±1.7
Meyers and Lysenko, 1956	Tiselius	Veronal buffer	Plasma	6.3	8.4	14	-	-	32.5	45.1
Koenig, Virgil, 1961	Tiselius	Veronal citrate buffer	Serum	-	4	-	13	5	13	65
Koenig, Virgil, 1961	Tiselius	Phosphate buffer	Serum	-	8	-	13	8	-	74

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PART II

APPENDIX

REVIEW OF LITERATURE

Immunity in helminth infections has been recognized for almost half a century but not until the work of Sarles (1938) with the in vitro test in Nippostrongylus infections in rats was the study of the serology of these infections pursued exclusively. Several studies have demonstrated that antigen extracted from larval or adult worm tissue can be used to stimulate levels of immunity to challenge with tissue-invading larval or intestinal worms (Miller, 1931; McCoy, 1935; Campbell, 1936; Kerr, 1936; Thorson, 1954(b), Kagan and Oliver-Gonzalez, 1958; Sadun and Lin, 1959; Kowalski and Thorson, 1972), in a homologous infection.

There are numerous references to interference phenomena and cross immunity reactions between various parasitic organisms particularly viruses and bacteria but there are relatively few reports concerning cross immunity involving helminths, and most of the works done in this area of research were performed with whole living worms and not their metabolic products.

The resistance of mice to Hymenolepis nana var. fraterna was reduced in the presence of a concurrent infection with Strongyloides or paratyphoid organism (Brumpt, 1933; Hunninen, 1936), while such resistance was elevated in the presence of N. muris (Larsh and Donaldson, 1944). These differences in results were said to be due to the action of different defensive mechanisms or quantitative differences of the same mechanisms.

Larsh and Donaldson offered no suggestion to explain their finding and no attempt was made to ascertain whether Hymenolepis nana had any effect on Nippostrongylus brasiliensis infections.

Cross resistance between schistosomes and different other helminths have been tested by different investigators. It was found by Oliver-Gonzales et al. (1949) and Senterfit (1958) that Schistosoma mansoni and Trichinella spiralis secrete immunologically related substances causing serological cross reactions. Senterfit indicated that the debilitating effects due to T. spiralis infection may have rendered mice more susceptible to subsequent infection with S. mansoni.

In their own experiment with mice, Jackowski et al. (1961) showed that while prior trichina infections did not reduce the numbers of schistosomes developing from moderately light infections (50 cercariae), they did reduce the numbers developing from heavy infections (200 cercariae). The double infection reduces the numbers of schistosomes but did not influence the numbers of trichina larvae.

In certain dual infections, the time and severity of the second infection appear to influence markedly the course of the first infection. An illustration of this is the work of Yoeli (1956) who found that Plasmodium berghei infections given to voles shortly before or after a Schistosoma mansoni infection showed a high proportion of severe infections produced by the former. However, when the P. berghei infection was given as late as 4 to 7 weeks after S. mansoni, the malaria infection showed mild effects, low parasitemia and no fatalities. It is postulated that the Lymphoid-macrophage system, alerted by the schistosome infection, had increased phagocytic power and was therefore able to affect the course of the malaria infection.

In another work on schistosomes, Hunter et al. (1961) found that when mice were challenged with S. mansoni after initial exposures to Schistosomatium douthitti, the number of S. mansoni recovered were not

appreciably different from those in challenge controls. However when mice were first exposed to S. mansoni and then challenged with S. douthitti, there was a marked reduction in the number of S. douthitti in test animals when compared with the challenge control. This result proves a non-reciprocal cross resistance between the 2 helminths. Explanation suggested for this resistance was a greater susceptibility of one Schistosoma species (S. douthitti) to the more or less non-specific host responses induced by infection with the other species.

An infection per os of 3,000 embryonated Ascaris suum eggs increased the resistance of mice to a challenge infection with 75 Schistosomatium douthitti cercariae initiated 1 to 4 weeks after the A. suum infection (Crandall et al., 1966). In another version of this work, they found that an initial infection with S. mansoni increased resistance to a challenge infection with A. suum given 60 days after exposure to 100 cercariae. There is no experimental evidence to support an antibody basis for this differential cross-resistance, although a greater sensitivity of S. douthitti to the direct or indirect action of antibodies or to the presence of antibodies induced by the A. suum infection reactive only with S. douthitti cannot be excluded.

Immunization by infective larvae of N. brasiliensis and a challenge by S. mansoni cercariae resulted in a significantly lower recovery of worms when compared with the controls (Hunter et al., 1967). The reciprocal of this experiment did not yield statistically significant results. Immuno-electrophoresis showed that the 2 helminths shared at least one antigenic component.

Apart from schistosomes, other workers tried cross reactivity between other helminths. Stewart (1952) reported that a dose of L_3 of Haemonchus

contortus superimposed upon an existing infection of H. contortus or Trichostrongylus spp. either eliminated the existing infection or suppressed the egg output of the parasites. Elimination of either H. contortus or Trichostrongylus spp. was not followed by resistance to the development of the superimposed larvae. Nonspecific host responses rather than antibody are believed to be operative in this case.

Cox (1952) showed that a previous infection with A. caninum produced effects that inhibited the development of T. spiralis in the mice. His results allow the conclusion that the demonstrated effect of current infection in increasing resistance to T. spiralis was not the product of cross immunity between A. caninum and T. spiralis. The effect was shown to be operative in the small intestine of mice 2 days after A. caninum infection, which is too brief a period to allow the development of acquired resistance to A. caninum. Furthermore, the effect was shown to be subsiding within 10 days rather than increasing as would be expected if acquired resistance to A. caninum were involved. Cox therefore came to the conclusion that the protective effect of A. caninum against T. spiralis was due to inflammation of the intestinal mucosa induced by A. caninum.

Goulson (1958) showed that an infection with A. caninum given 24 to 48 hours prior to an infection with T. spiralis caused a significant reduction in the number of adult T. spiralis harbored in the small intestine of mice as measured by adult worm counts 7 days after infection. A. caninum given simultaneously or given 12, 96, 144, or 192 hours prior to the T. spiralis infection failed to interfere significantly with the establishment and maintenance of T. spiralis. These results prove that the nonspecific inflammation resulting from the A. caninum infection was responsible for interfering with the establishment and maintenance of T. spiralis.

Herlich (1965) showed that calves given single oral infection of Cooperia oncophora or Cooperia pectinata were immune to subsequent challenge with the heterologous species while Kocan (1974) found that infection of rats with infective larvae but not transplanted adults, of N. brasiliensis protected them against a challenge infection with Angiostrongylus cantonensis.

In 1975, Kevin et al. immunized rats with L_3 of Strongyloides ratti or N. brasiliensis and subsequently challenged them with L_3 of the heterologous species. They noted significant reduction in numbers of adults of the challenge species as compared to unimmunized controls.

From this review, it is evident that the presence of 2 parasitic infections in one host has a varied effect upon the establishment and maintenance of the separate parasites. In some instances, one infection produces effects that tend to inhibit the other infection, while in other situations, the effects tend to favor the development of the other infection.

Both inflammation and tissue sensitivity, induced by initial infections are said to be responsible for enhanced resistance to the heterologous parasitic infections (Cox, 1952; Goulson, 1958; Louch, 1962; Crandall et al., 1966) while Larsh (1953) implicated the role of antibodies.

We could find no record of a heterologous cross reaction involving Cooperia punctata (either as whole living worm or its metabolic products) and Nippostrongylus brasiliensis.

DETAILS OF MATERIALS AND METHODS

Nippostrongylus brasiliensis

A mice-adapted isolate of this nematode was obtained from Washington State University, Pullman. The infective larvae (L_3) thus obtained were injected into white rats. Since then, these nematodes have been maintained in the laboratory by bimonthly passages through white rats.

Culturing of Nematode Eggs

Nematode eggs eliminated with feces of infected rats were cultured in either animal charcoal or vermiculite in petri dishes. From these cultures, under the laboratory conditions, vigorously active larvae were observed in 6 to 8 days from time of culture. The L_3 were usually recovered from the culture medium by the Baermann apparatus.

Those L_3 which were used in the final phase of the experiment were not held for more than 15 days in the fecal culture medium.

The L_3 obtained through the Baermann apparatus were washed several times with distilled water, centrifuging them down to the bottom of the test tube after each wash, at a relative centrifugal force of 159.6G for 4 minutes.

Numeric Determination of Infective Larvae

The number of L_3 to be injected into each experimental rat was determined by dilution counting. Two-tenths ml. of the original larvae-water solution was further diluted with distilled water to 20 ml. in a graduated cylinder. After proper mixing, 0.1 ml. of the 20 ml. dilution was placed on a microscope slide. All the larvae on the slide were counted and the

process repeated several times after which the total number of larvae was summed and divided by the number of counts made to find the average. The average number obtained was then multiplied by 1000 to obtain the number of larvae per ml. of original fluid medium. From the result obtained, the quantity of fluid that would contain the desired number of L_3 was calculated.

Infection of Rats

Twelve hundred L_3 contained in 0.3 ml. of distilled water were injected into the right side of each rat, using a 2 cc. disposable plastic syringe.

Determination of Eggs per Gram of Feces (e.p.g.)

Daily e.p.g. was estimated by using the flotation egg counts technique as described by Leland (1963).

Two grams of feces were weighed, crushed and made up to a volume of 20 ml. in a graduated cylinder by adding distilled water. From this, 1 ml. was pipetted into lip ground centrifuge tube. The tube was then filled with saturated salt solution. A cover slip was placed on the liquid surface in contact with the ground glass surface and the tube was centrifuged at 104.3 r.c.f. for 5 minutes. The cover slip was then lifted and placed directly on a microscope slide for counting. At this dilution of 2 gms. in 20 ml. distilled water, the number of eggs counted in 1 ml. was multiplied by a factor of 10. Other dilutions made were a function of the number of eggs present on the microscope slide. Further dilutions were made when eggs on a slide exceeded 300 with the standard dilution procedure.

Adult Worm Recovery

Worm counts were made on the contents of the small intestine and the small intestine digest to determine the number of worms. The unwashed intestine was digested in a 1% pepsin-1% HCl solution. Overnight incubation at 37°C was used for dissolution of the protein components of the tissue. The digest process was stopped and the materials preserved by adding formalin to make a final concentration of about 10%. The worms were counted in 2 to 3 cc. replicated volumes. The search for the worms was made using a dissecting microscope (10x).

Experimental Animals

Male, white rats (Carworth CFE Sprague Dawley) weighing between 150-250 grams were used for the experiment. They were individually caged and had free access to water and a complete commercial pellet ration. Hygiene of the cages were checked daily throughout the experimental period.

Culture-Derived Metabolic Products (Ag.) of Cooperia punctata

This was prepared according to the method described by Zimmerman and Leland (1974). The crystallined and lyophilized form of the product meant to be used in this experiment was diluted in physiological (0.15M NaCl) saline solution. The quantity of the Ag. thus prepared was quantitated in terms of nematode equivalent (Neq.) per ml. of dilution, an Neq. being the amount of metabolic product eliminated into medium by one helminth between days 21 and 24 (72 hours) into the culture medium.

Combination of Ag. with Adjuvant (Adj.)

The dissolved Ag. to be injected was mixed with an equal volume of complete Freund, Bacto-Adjuvant code 0638-59 (Difco Laboratories, Detroit 1, Michigan). The 2 components (Ag. and Adj.) were pulled into glass syringes and the 2 syringes were then connected to each other by means of a rubber tube (Williams and Chase, 1967). The contents of the connected syringes were thoroughly mixed (400 cycles) until the mixture became milky-white and a drop would float on water. This was the emulsion that was injected.

Inoculation of Rats

Two-cc. disposable plastic syringes were used to pull out the milky white emulsions from the larger syringes and to inject the rats. Eight rats were each inoculated with 0.5 ml., 3 consecutive times at intervals of 15 days between each inoculation.

A second group of 10 rats (controls) was inoculated with 0.5 ml. of a 50-50 mixture of complete Freund adjuvant and physiological saline, mixed by the two-syringe technique as described above. These also were inoculated 3 times at intervals of 15 days. In both groups of rats, the first inoculation was given intramuscularly in both rear legs. The second inoculation was given subcutaneously on the left side of each rat and the third inoculation which was given 30 days after the first, was given subcutaneously on the right side of the rats.

A third group of uninoculated rats was also maintained along with the inoculated groups.

Analysis of Blood

Harvesting of blood from the experimental rats was done by cardiac puncture using 10 cc. disposable plastic syringes, the rats having been sedated by inhalation of chloroform. A portion of the blood collected was mixed with EDTA in order to determine the red cells, white cells and the differential leucocytes while another portion of the blood was coagulated for serum analysis.

The white cells and red cells contained in a milliliter of blood were determined using the Tri-Lyne Dade pipettes and the Spencer Bright-Line improved Neubauer counting chambers.

Quantity of hemoglobin per 100 cc. of blood was determined by using the Haden-Hausser haemoglobinometer (Clinical Model, Hausser and Son of Philadelphia).

Packed cell volume (PCV) was determined by using the Blu-Tip capillary tubes (Biological Research Incorporated, St. Louis) and the international micro-capillary centrifuge, Model MB (International Equipment Company, Massachusetts), operating at relative centrifugal force of between 13,136 and 22,349 for 5 minutes. Reading was done on the International Micro-capillary Reader model CR (International Equipment Company, Massachusetts).

Analysis of Serum

Immunodiffusion tests

Disposable Ouchterlony double diffusion plates (International Chemical and Nuclear Corporation, Irvine, Ca.) were used for immunodiffusion tests (Ouchterlony, 1953).

One percent purified electrophoresis agarose (Sigma Chemical Co., St. Louis, Mo.) solution was prepared using a modification of gel diffusion buffer as formulated by Blair et al. (1960) and recommended by the manufacturer of the plates.

Sera were dispensed by means of the Eppendorf Microliter pipettes (Eppendorf Geräteban Netherl + Hinz, GMbH, Hamburg) size 50. Pooled immune serum of calf 51 was used as the control. Calf 51 acquired strong immunity to Cooperia punctata after receiving several experimental infections of large doses of L_3 of the worm. The calf was brought to Burt Hall the second day after birth in 1972. He was dosed with 5,000 L_3 25 days afterwards and another 5,000 L_3, 26 days after birth. E.p.g. was very low after this first injection. Then on the 62nd day after birth 10,000 L_3 was given and another 10,000 L_3 on the 63rd day. E.p.g. monitoring was negative after this second series of infection. On the 85th day after birth the calf was dosed with 20,000 L_3 and another dose of 20,000 L_3 on the 86th day. Still e.p.g. was negative and a final dosing with 400,000 L_3 was given at 108th day after birth. There were no eggs detected in the calf's feces even after this last dosage. The calf was bled and the serum collected and preserved. This pooled serum contains antibodies to Cooperia punctata and it is being used as control serum in immunodiffusion tests involving the metabolic product of Cooperia punctata.

Following loading of sample wells, incubation for development of precipitin bands was at 4°C for 24-72 hours.

Total protein and electrophoresis

Total protein in each serum was determined using the TS meter, model 10406 (American Optical Company, Buffalo, N.Y.).

Agarose film electrophoresis was conducted using ACI cassette system, reagents and instructions of the manufacturer (Analytical Chemists, Inc., Palo Alto, Ca.). The only modification made was extending electrophoresis for 45 minutes instead of 30. This was done in order to allow proper separation of the α_1 component from the albumin. All samples which were run for 30 minutes ended with the two components completely merged. Densitometer scans were made with the Quick scan model 1020 densitometer (Helena Laboratories, Beaumont, Texas). From the scan, the percentages and quantities in grams of the different protein components of the serum were analyzed and quantitated. The percentages of the different components were calculated by separating the different peaks on the scan by lining. The area under a peak corresponds to a protein fraction. The value of this area is divided by the sum total values of all the areas under the scan and this is multiplied by 100. To obtain the value of the component in grams, the percentage value is multiplied by the value of the total protein (obtained by using the TS meter, model 10406) and dividing the product by 100.

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AN EVALUATION OF THE PROTECTIVE INFLUENCE OF CULTURE-DERIVED METABOLIC PRODUCTS OF *COOPERIA PUNCTATA* AGAINST *NIPPOSTRONGYLUS BRASILIENSIS* (YOKOGAWA, 1920) IN RATS

by

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Male Carworth CFE Sprague Dawley rats were inoculated 3 consecutive times at intervals of 15 days each with culture-derived metabolic products of Cooperia punctata (Ag.) prepared according to the methods described by Zimmerman and Leland (1974). Two weeks after the last inoculation, the rats were challenged each with 1,200 infective larvae of Nippostrongylus brasiliensis.

Sera from the inoculated rats gave positive reactions (precipitin bands) when tested against the antigen in Ouchterlony immunodiffusion plates. Agarose film electrophoresis of the serum proteins using the ACI cassette system revealed slight increase in gamma globulin coupled with a slight decrease in albumin, especially pronounced in 2 of the tested rats (no. 35 and 36). Differential leukocyte counts showed substantial increases in the number of eosinophils and monocytes of the antigen-inoculated rats as compared to the controls. Daily monitoring of e.p.g. before necropsy revealed very low counts in 2 of the antigen-inoculated rats (35 and 36) as compared to the others and necropsy performed 10 days after larval challenge revealed that the 2 of them harbored fewer adult worms in the intestine than the controls. Worm counts from rats inoculated with adjuvant/saline mixture and those not inoculated at all were fairly close.

These results show that immunization with culture-derived metabolic products of Cooperia punctata was effective in reducing the development of Nippostrongylus brasiliensis in 2 of 8 vaccinated rats as compared with unvaccinated controls.