IMMUNITY TO HISTOTROPIC ASCARIASIS IN GUINEA PIGS DUE TO INOCULATION OF METABOLIC PRODUCTS OF LARVAL ASCARIS SUUM

by

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ABSTRACT

IMMUNITY TO HISTOTROPIC ASCARIASIS IN GUINEA PIGS DUE TO INOCULATION OF METABOLIC PRODUCTS OF LARVAL ASCARIS SUUM

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Excretory-secretory (ES) products elaborated into ambient culture medium during axenic cultivation of Ascaris suum through second larval molt were injected into guinea pigs to evaluate their antigenic properties in inducing functional immunity to histotropic ascariasis. Culture medium which had supported developing larvae of A. suum was concentrated by dialysis and injected subcutaneously into guinea pigs- two injection equivalent to 25,000 and 50,000 infective larvae respectively, were given fourteen days apart. Fourteen days following the second injection immunity was tested by orally administering 10,000 infective eggs of A. suum to each animal. Guinea pigs which had received two previous oral immunizing inoculations of infective eggs at fourteen day intervals as above (Immunized Group) and non-treated animals (Control Group) were challenged simultaneously with the ES Antigen Injected Group.

Injection of ES antigen reduced the number of worms migrating (average total 578.7, 733.3, and 2641.7 for ES Antigen Injected, Immunized and Controls respectively); inhibited growth rate (average size of worms were 1.13mm, 0.53mm, and 1.61mm in the ES Antigen Injected, Immunized and Controls respectively); slowed migration rate (0.61, 0.69, and 0.16 in the ES Antigen Injected, Immunized and Controls respectively); and reduced pathology. Clinical, histologic and hematologic findings further affirm the immunizing properties of ES antigen.

These findings support the supposition of others who have concluded that metabolic products of worms serve as antigens to induce functional immunity in vertebrate hosts (Soulsby 1962, Taffs 1964b,Guerrero, 1969).

ACKNOWLEDGMENTS

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CHAPTER I

INTRODUCTION

The larger nematodes have been known for many years. The "fiery serpent" mentioned in Genesis is probably the Guinea worm, Dracunculus medinensis. This worm and the human ascarid, Ascaris lumbricoides, were referred to in the Ebers Papyrus, which was written about 1553-1550 B.C. Aristotle (384-332 B.C.) referred to the human ascarid, pinworm and the tapeworm in his Historia Animalium. The ancient Greeks knew of dog ascarids. Columella (100 A.D.) reported seeing ascarids in calves and Vegetius (400 A.D.) in horses. Avecenna (981-1037 A.D.) knew of the human ascaris and D. medinensis. In 1684, Redi compiled accounts of known helminths. In 1800, Rudolphi recorded 603 species of nematodes, 460, of which were intestinal worms. Grassi, in 1887, found that human and dog ascarids had direct life cycles. Stewart described the migration of Ascaris lumbricoides through the lungs of pigs in 1916 (above historical references are cited from Levine, 1968).

The class nematode: (phylum Aschelminthes) are round bodied worms having a straight tubular intestinal tract and tubular reproductive organs suspended in a noncoelomic body cavity. Worms in this class are non-segmented and covered by a secreted cuticle. Nematodes have an extremely wide distribution. They can be found in many different habitats. Some are parasitic in vertebrates and invertebrates, and plants; others are free-living found in soil, fresh or sea water, bottom sediments, etc. They can be found in almost all ecological surroundings except air and the open sea. They are among the most significant invertebrate parasites of animals. Kates (1965 cited from Levine 1968) estimated that there were more than seven billion helminth infections in pets and farm animals in the United States. Stoll (1947) estimated that there were 2.25 billion human helminth infections in the world population.

Ascaris suum, the nematode considered in the following study is probably the most important parasite of domestic swine. It is similar in appearance and life history to A. lumbricoides, the large round worm of man. In fact the only morphological distinction between the two species is a minor difference in denticles bordering the lips (Sprent 1952).

Females of A. suum may reach 40 cm in length and 6 mm in diameter. Males can be distinguished from females by a dorsally curved hook at their posterior end. The worms are pink when recovered from the small

intestine of pigs, but turn white when preserved in formalin solution.

As with other parasites, female ascarids are prolific egg layers- Kelley and Howe (1962) reported a daily egg production exceeding one million eggs per female. These eggs with a thick brown shell, pass through the gastrointestinal tract and leave the host via the feces. The eggs are highly resistant to chemical agents and undergo larval development in the soil.

Embryonic development is related to environmental conditions and under high humidity and optimum temperature of 31.1 degrees C, larva in each of the eggs will have developed to the infective stage within ten to fourteen days (Levine 1968). Eggs at this stage have lengthy longevity and may remain infective in the soil for at least four years (Spindler, 1940).

When these infective eggs are ingested by a mammalian host they hatch in the small intestine. The larvae penetrate the intestinal wall and migrate into mesenteric venules, passing by way of the hepatic portal system through the liver. The larvae develop in the liver, molting to the third stage, four to five days after infection. They go from the liver entering the right atrium of the heart, then through the pulmonary artery to the lungs, eight to nine days after infection, where they develop further. The larvae break through the alveoli into the bronchioles. If the infection is too severe there is massive lung hemorrhage and the animals may die of verminous pneumonia. The

larvae travel through the bronchi into the trachea, they are coughed up, swallowed and reach the intestine, about the fourteenth or fifteenth day after infection, where they become adult. A. suum will only reach maturity in the proper host-swine (Belding, 1958).

Hosts react to nematode parasites by displaying varying degrees of resistance (Sprent 1963). Genetic resistance is referred to as natural or racial immunity - particular hosts harbor specific parasites. Resistance that develops from exposure to the parasite or its products is referred to as acquired immunity. This immunity may be active or passive. An active acquired immunity is a direct response made by the host to an infection or antigen - a build up of antibodies by the host to combat the antigen dispensed by the infection. A passive acquired immunity is the result of introducing into the host antibodies prepared in the body of some other host. A mother may pass on antibodies to her newborn in the colostrum etc. (LaPage 1963).

Immunity to A. suum has been demonstrated by many investigators (Kelley 1957, 1960, Ransom 1920, 1921, Fallis 1942, 1943, 1948, Taffs 1960, 1964a, Soulsby 1961, 1962).

Soulsby (1962) proposed the target of immunity to be third stage larvae of A. suum. He implanted third stage larvae into the peritoneal cavity of immune rabbits. These larvae became covered with white blood cells, the majority being eosinophils. This reaction occurred when third stage larvae were implanted, but did not occur with second stage larvae. Precipitates formed about the mouth, anus, and

excretory pore of third stage larvae that were placed in immune serum, but did not form on second stage larvae. Soulsby concluded from these results that substances elaborated between the second and third molt served as antigen to stimulate production of antibodies against the third stage larvae.

Rogers (1960) reported that exsheathing fluid excreted when third stage larvae of Trichostrongylus colubriformis were ingested, served as an antigen, leading to the production of antibodies which produced cuticular precipitates as described above. These studies indicate that a substance excreted or secreted between second and third stage larvae function as antigen leading to antibody production.

The following experiment was devised to test the hypothesis that a substance elaborated by axenically maintained larvae of A. suum will stimulate production of immunity to histotropic A. suum when injected into guinea pigs. This material referred to as excretion- secretion (ES) antigen was prepared from media which supported artificially hatched larvae from second stage to third stage of development. It was injected subcutaneously and immunity was compared to immunity by oral infections.

CHAPTER II

MATERIALS AND METHODS

Twenty-two healthy guinea pigs averaging 450 gm each were used in the experiment.

Each of eight guinea pigs were given infective eggs of A. suum by gavage to produce immunity as in previous studies (Kelley et al 1957, 1960, Taffs 1960, 1964, 1968, Sprent 1953). Details of this procedure are described on page 8. I will refer to these animals as the Immunized Group.

Another group of eight guinea pigs received two subcutaneous injections of ES antigen of A. suum (see page 7 for description of the antigen). I will refer to these animals as ES Antigen Injected Group.

Six animals had no contact with infective eggs of A. suum or ES antigen prior to the challenge. I will refer to these as the Control Group.

Immunity in all three groups was challenged by giving each guinea pig 10,000 infective eggs of A. suum orally two weeks following the last experimental treatments.

Infectious eggs of A. suum were prepared by Reginald Galloway of Eli Lilly Research Laboratories, Greenfield, Indiana by incubating eggs which had been obtained from female worms collected at an abattoir. The distal one to one and a half inches of the uteri from these worms were excised and placed in a container in a small amount of water. An equal volume of 1N NaOH was added and with occasional stirring, this mixture was held at room temperature until most of the uterine tissue was broken down (about four hours). The suspension was ground in a tissue grinder and the eggs were washed with water until the supernatant became clear. These washed eggs were then suspended in 0.5 percent formalin solution and placed in a Salvarsen tube which is an enlarged burette. Filtered air was bubbled slowly into the bottom of the tube while the suspension was held at 28 degrees C. After thirty days in this incubation chamber the fertilized eggs had developed into second stage infective embryos. For storage, they were washed several times and resuspended in fresh 0.5 percent formalin solution.

Second stage larvae were artificially hatched from the infective eggs of A. suum using the method of Rogers (1960). These tiny worms were placed into the culture media which contained calf serum and salt solution and were maintained for six days at 37 degrees C. The larvae grew in the medium and when exceeded 300 microns they were considered as third stage larvae. These third stage larvae were removed

from the media by centrifugation. The resulting supernatant, containing metabolic products which had been elaborated by the living, growing, molting larvae in the media, was concentrated four and one-half times by removing water by dialysis at 4 degrees C. This final material contained 50,000 larvae equivalents per ml of solution. Merthiolate (1:10,000) was added to the antigen as a preservative.

ES antigen was administered subcutaneously in the abdominal area. The first injection consisted of one half ml antigen and one half ml adjuvant. The second inoculation (1 ml antigen and 1 ml adjuvant) was given fourteen days after the first.

The adjuvant consisted of two percent aluminum hydroxide in gel form and served to hold the antigen in contact with immunogenic tissue of the animals for a longer time to exacerbate the development of an immune response.

The number of eggs for oral immunizing inoculations and challenge infections was determined by performing aliquot counts on dilutions of stock supply of eggs. These dilutions of worm eggs were made by mixing one drop of stock supply of eggs of A. suum with 49 drops of water. One drop of the resulting suspension was placed on a microscope slide and examined beneath the microscope to determine the total number of eggs in the original drop. The number of eggs in this drop of 49:1 dilution was multiplied by 50 to determine the number of eggs per drop of stock suspension. The procedure was repeated at least fifteen times

to eliminate error of particle counting. After the number of eggs per drop of stock suspension was established the proper number of drops were administered via an intraesophageal tube for respective treatments.

Oral inoculations were given at fourteen-day intervals to induce immunity. The Immunized Group consisted of only three guinea pigs because the initial oral inoculation killed the remaining five animals in the group.

Clinical signs were observed three times daily as infection progressed. Respiration, general-behavior, general condition, morbidity and other signs of well being were noted.

Blood samples were collected from ear veins three times weekly. Grams of hemoglobin per 100 ml of blood, total number of leucocytes per cubic millimeter, differential white cell counts and bilirubin determinations were done by conventional methods (Davidsohn 1969). Serum proteins were determined electrophoretically by the Microzone Method (Beckman 1963).

Ether was used to euthanize the animals. At necropsy lesions on the lungs and livers were observed and rated. Lesions on the lungs were categorized according to a rating devised by Brown and Chan (1955). This rating grades the extent of lung pathology from zero to five. Lungs showing no petechiae were rated 0; those with fewer than six were rated 1; with scattered petechial spots and a few confluent ecchymotic areas were rated 2;those with ecchymotic areas of moderate

size were rated 3. A rating of 4 had large confluent ecchymotic areas; and complete hepatization of both lungs resulted in a rating of 5.

Samples of lung, liver and intestine were collected for histological analysis. These tissues were fixed in ten percent formaldahyde, imbedded and cut at four microns thickness by usual procedure and stained with Hematoxylin-Eosin (Wintrobe 1967). Tissue sections were examined for the presence of larvae, cellular infiltration and tissue damage.

To determine the number of larvae, one lung from each animal was suspended in 0.8 percent solution of NaCl and ground in a Waring Blender for 25-30 seconds. Resulting tissue fragments and larvae were concentrated by centrifugation (three minutes at 2,000 rpm). After centrifugation, the supernatant was discarded; the residue resuspended and the volume adjusted to 10 ml. One ml aliquots of this suspension were examined to determine the number of larvae in the original sample. After thorough mixing one ml was withdrawn and placed on a microscope slide. Total number of larvae in this one ml aliquot was determined by counting the total number of larvae with the aid of a microscope. After a sufficient number were counted to obtain a reliable average, this average number per ml was multiplied by 10 (the total volume containing the residue) to arrive at the total number in the original samples . This represented only one lung therefore the figure was doubled to include both lungs.

The entire liver was ground in the same manner as the lungs, but the resuspended substance was too opaque to allow observation of a 10 ml volume. Thus it was diluted to 40 ml and totals were determined by aliquot counting as above. Larvae were measured by ocular micrometer and average length was based on measurement of three to five larvae per specimen. Student's t-test was used in all tests for significant differences.

CHAPTER III

RESULTS

Immunized Group- The initial inoculation of infective eggs of A. suum induced severe respiratory complications in all animals in this group. By the sixth day after they received their first immunizing infection, they exhibited anorexia, watery eyes, increased and difficult respiration and ataxia. The animals were thumping severely and had obviously lost weight. Five of the animals in this group died on the eighth and ninth day of severe verminous pneumonia (confirmed by postmortum examination of the lungs).

The second immunizing inoculation (given 14 days after the first) did not affect the guinea pigs. The challenge inoculation of 10,000 eggs likewise had no effect on these animals.

ES Antigen Injected Group - The subcutaneous injections of ES Antigen had no effect on the guinea pigs.

Typical signs of histotropic ascariasis appeared within seven days after a challenge dosage of 10,000 infective eggs of A. suum was given- animals were thumping and lethargic. These signs were

less severe than those observed in the initial inoculations of the Immunized Group above or in the challenge infection of the Control Group below.

Control Group - Received only 10,000 infective eggs at the time of challenge. Seven days later, at necropsy, they were severely affected- coughing, thumping, anorexic and this degree of morbidity indicated that the animals would have surely died of verminous pneumonia had the experiment been extended.

Total white cell counts per cubic millimeter (WBC) varied from 6,000 to 9,500 -- within normal range for guinea pigs (Schermer 1967). Table 1.

Total number of eosinophils per cmm of blood of Immunized Group increased slightly following the initial gavage of infective eggs -- initial count of 50 increased to 588 on day seven (Table 2). There was no increase following the second immunizing egg inoculation. Following the challenge inoculation the total eosinophil count increased twelve fold -- 130 eosinophils per cmm prior to challenge increased to 1235 eosinophils per cmm two days following challenge inoculation.

Eosinophil counts remained within normal range for guinea pigs in ES Antigen Group until after challenge. At necropsy, seven days after the oral challenge inoculation of 10,000 eggs, eosinophils per cmm had increased from 340 to a high of 760 (Table 2). Eosinophilia in the Control Group remained within the normal range following the

Table 1

AVERAGE NUMBER OF LEUCOCYTES PER CUBIC MILLIMETER OF BLOOD

Day	Immunized	ES Antigen	Controls
0[1]	5800	6000	--
2	5500	5900	--
4	5600	5200	--
7	8400	7000	--
9	8900	5500	--
11	---	5900	--
14[2]	7500	6400	--
16	8100	8200	--
18	7700	8500	--
21	6400	8700	--
23	7100	8500	--
28[3]	6500	6100	8700
30	9500	7600	6500
32	8900	7800	6300
35[4]	7800	6500	6900

[1]First oral immunizing inoculation of eggs of A. suum and first ES Antigen injection.

[2]Second egg and ES Antigen inoculation.

[3]All animals received 10,000 infective eggs of A. suum as challenge of immunity.

[4]All animals necropsied.

Table 2

AVERAGE NUMBER OF EOSINOPHILS PER CUBIC MILLIMETER OF BLOOD

Day	Immunized	ES Antigen	Controls
0[1]	50	60	--
2	165	118	--
4	224	104	--
7	588	280	--
9	534	55	--
11	--	59	--
14[2]	450	256	--
16	324	246	--
18	154	425	--
21	128	261	--
23	213	340	--
28[3]	130	122	87
30	1235	760	130
32	801	468	252
35[4]	312	325	207

[1] First oral immunizing inoculation of eggs of A. suum and first ES Antigen injection.

[2] Second egg and ES Antigen inoculation.

[3] All animals received 10,000 infective eggs of A. suum as challenge of immunity.

[4] All animals necropsied.

challenge inoculation -- did not exceed 300 eosinophils per cmm (Table 2).

Polymorphonuclear neutrophils and lymphocytes fluctuated within normal range in both the Immunized and ES Antigen Injected Groups. However following challenge on day 28 a marked reversal occurred between these two groups. Neutrophils in Immunized Group averaged 4,539 per cmm four days following challenge, whereas on the second day following challenge, an average of 2,356 neutrophils per cmm occurred in the ES Antigen Injected Group. An opposite situation was found with lymphocytes on day two -- Immunized Group 3,895 per cmm versus 4,180 per cmm is ES Antigen Injected Group (Table 3).

Fluctuations of hemoglobin were within normal range for guinea pigs (Table 4).

An average of 0.2 mg bilirubin per 100 ml of serum was detected in Immunized animals on the ninth day following inoculation and 0.5 mg per 100 ml of serum on the fourteenth day. No detectable bilirubin was present in Controls throughout the experiment.

Serum protein levels, determined electrophoretically, are presented in Table 5. There were no differences between treatments.

The initial immunizing inoculation of worm eggs produced severe pathology in the Immunized Group. As the larvae of the initial inoculation migrated through the animals, histological changes in the lungs, liver and intestine were noted. The lungs of the animals severely infected

Table 3

AVERAGE NUMBER OF POLYMORPHONUCLEAR NEUTROPHILS AND LYMPHOCYTES PER CUBIC MILLIMETER OF BLOOD

Day	Immunized		ES Antigen		Controls	
	Poly.	Lymph.	Poly.	Lymph.	Poly.	Lymph.
0[1]	2842	2784	3000	2940	--	--
2	2145	3190	2891	2891	--	--
4	2240	3024	1810	3120	--	--
7	4200	3444	2660	3850	--	--
9	3738	4489	2200	3025	--	--
11	--	--	2596	3068	--	--
14[2]	2025	4725	3264	2752	--	--
16	2835	4941	3116	4510	--	--
18	3619	3542	2550	5355	--	--
21	3392	2816	3567	4698	--	--
23	3195	3621	4080	3825	--	--
28[3]	3315	2925	4623	3233	4269	4176
30	4275	3895	2356	4180	2730	3510
32	4539	3382	3432	3686	2709	3150
35[4]	4446	2886	3770	2275	4623	2001

[1] First oral immunizing inoculation of eggs of A. suum and first ES Antigen injection.

[2] Second egg and ES Antigen inoculation.

[3] All animals received 10,000 infective eggs of A. suum as challenge of immunity.

[4] All animals necropsied.

Table 4

AVERAGE GRAMS OF HEMOGLOBIN PER 100 MILLIMETERS OF BLOOD

Day	Immunized	ES Antigen	Controls
0[1]	16.7	13.9	--
2	15.5	13.5	--
3	13.6	13.9	--
7	12.6	14.5	--
9	12.0	14.6	--
11	--	16.0	--
14[2]	15.0	15.6	--
16	16.1	14.8	--
18	16.0	15.0	--
21	16.3	15.3	--
23	14.0	14.0	--
28[3]	15.2	15.0	14.6
30	16.8	13.9	14.5
32	15.3	14. 0	13.0
35[4]	14.7	13.2	13.5

[1] First oral immunizing inoculation of eggs of A. suum and first ES Antigen injection.

[2] Second egg and ES Antigen inoculation .

[3] All animals received 10,000 infective eggs of A. suum as challenge of immunity.

[4] All animals necropsied.

Table 5

AVERAGE PERCENTAGE OF COMPONENTS OF SERUM PROTEINS DETERMINED ELECTROPHORETICALLY

Group	Day	Albumin	Alpha Globulin	Beta Globulin	Gamma Globulin
Immunized	0	49	32	12	8
	35	41	27	24	8
ES Antigen	0	49	32	12	8
	35	40	35	16	8
Control	0	52	29	12	7
	35	45	35	14	6

(those that died after the first inoculation) were massively hemorrhagic and larvae were found (Figure 1). Foreign- body giant cells, eosinophils and an increased number of white blood cells were present in the lungs (Figure 2). Lung tissue was hemorrhagic and edematous.

Small white lesions appeared on the livers of the animals in this group. Microscopically, many lymphocytic cells infiltrated the portal areas of the liver sections, where the larvae had passed through (Figure 3).

Sections of intestine displayed massive infiltration of white cells. Comparable numbers of eosinophils and plasma cells were evident in these sections (Figure 4).

ES Antigen Injected Group - Histologic study of tissues of this group at challenge were similar to effects observed in the Immunized Group. The lungs were not as hemorrhagic as in the Immunized Group however. There were comparable numbers of eosinophils, plasma cells and lymphocytic cells present. One animal in this group had larvae visible in the lung (Figure 5), one had larvae visible in the liver sections. Another had large deposits of calcium in the liver. Figure 6 represents liver tissue with lymphocytic infiltration similar to that observed in the initial inoculation of the Immunized Group. Liver lesions were evident in this ES Antigen Group. The walls of the intestinal tissue were infiltrated with white cells similar to that observed following the initial inoculation in the Immunized Group (Figure 7).

Control Group- Larvae were present in all sections of lung in this group following the challenge inoculation. The lungs were massively hemorrhagic and consolidated (Figure 8). The liver showed necrotic areas where larvae had passed through (Figure 9). No larvae could be found in the liver and intestine. Gross liver lesions were absent in this group. Microscopically, necrosis and inflammation were present where larvae migrated on the way to the lungs. The intestinal walls were mildly infiltrated with white cells. Rating of lung pathology is presented in Table 6.

Table 6 lists the number of larvae found in the lungs and livers of all animals and presents average length of worms found in these organs. The liver-ratio, an indication of rate of larval migration, is presented in Table 6 as well.

There were more larvae recovered in the livers of the Immunized Group than in the lungs (506.6 in the liver compared with 226.6 in the lungs). In the ES Antigen Injected Group, the number of larvae found in the livers was slightly higher than the number found in the lungs (356.2 in the liver and 222.5 in the lungs). Whereas, the number of larvae recovered from the livers of guinea pigs in the Control Group was below the number found in the lungs (420.0 in the livers and 2221.6 in the lungs).

FIGURE 1

MICROSCOPIC PATHOLOGY OF GUINEA PIGS

Eight Day Post Infection (25,000 Eggs)

Lung Tissue (x 430)

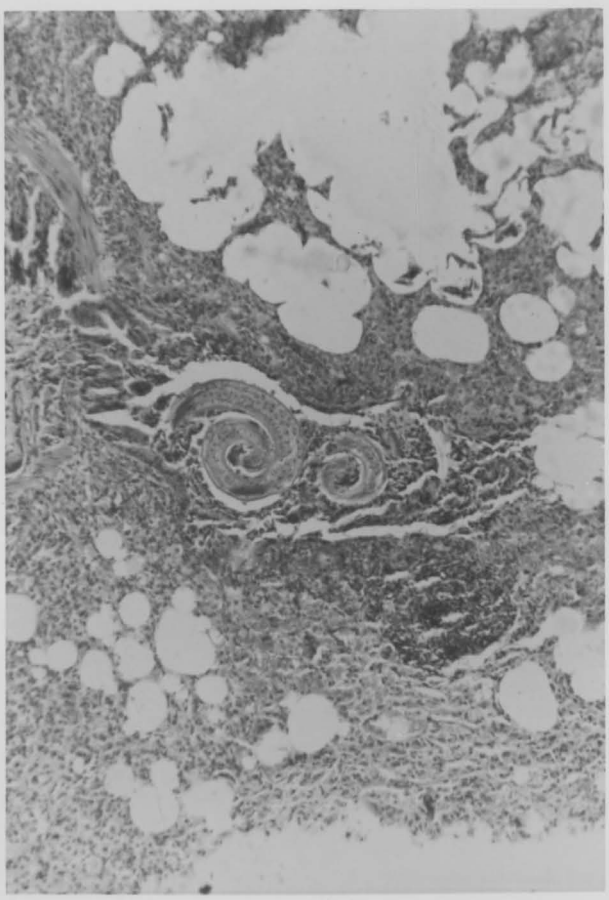

FIGURE 2

MICROSCOPIC PATHOLOGY OF GUINEA PIGS OF THE
IMMUNIZED GROUP
AT NECROPSY
Lung Tissue (x 430)

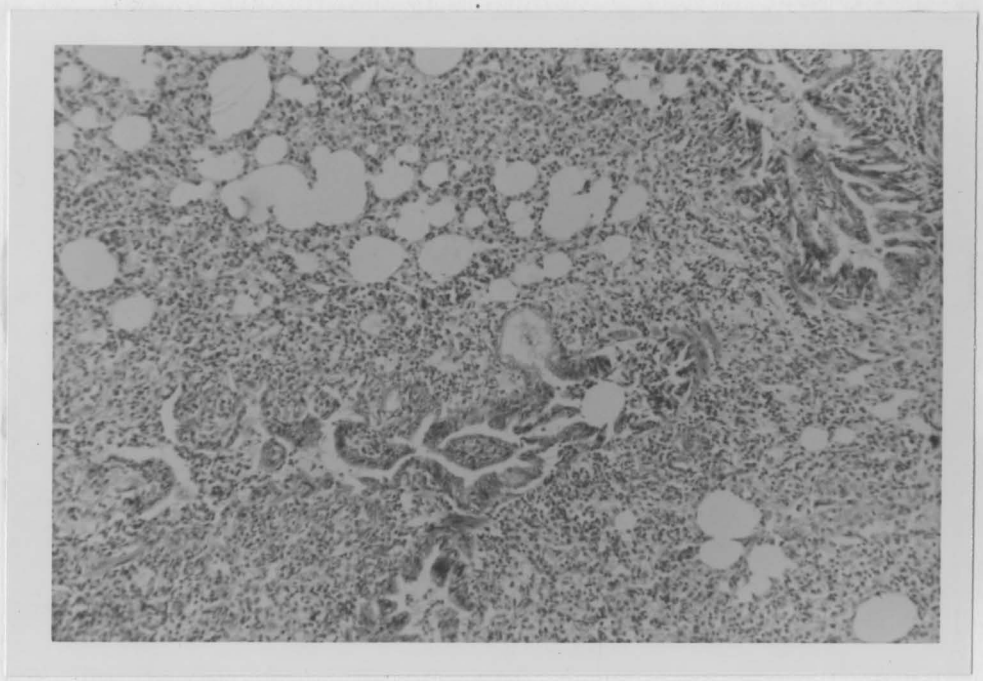

FIGURE 3

MICROSCOPIC PATHOLOGY OF GUINEA PIGS OF THE
IMMUNIZED GROUP
AT NECROPSY
Liver Tissue (x 430)

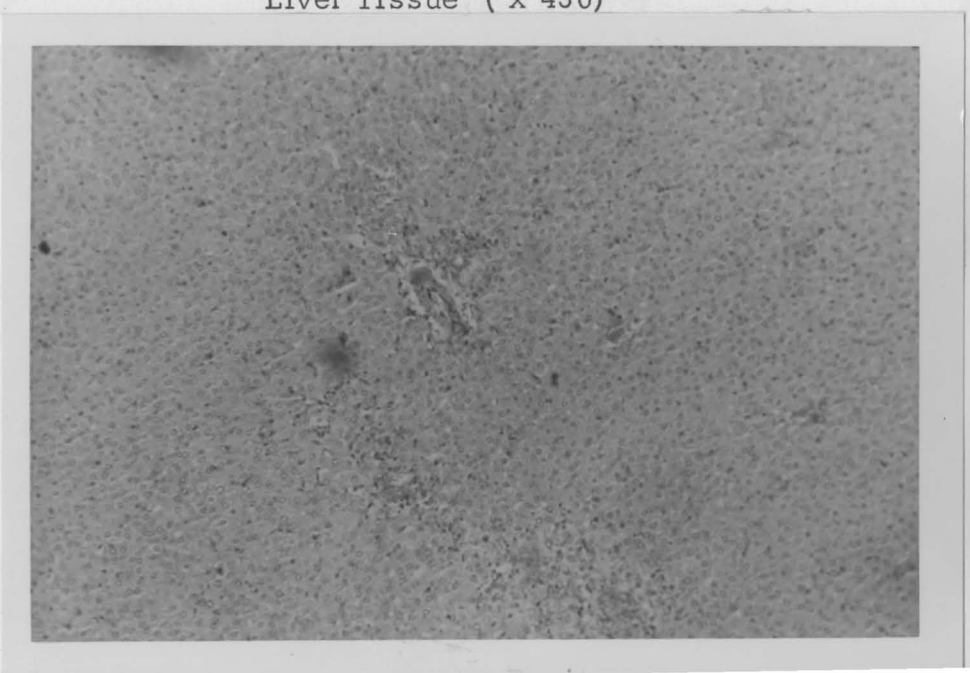

FIGURE 4

MICROSCOPIC PATHOLOGY OF GUINEA PIGS OF THE
IMMUNIZED GROUP
AT NECROPSY
Intestinal Tissue (x 430)

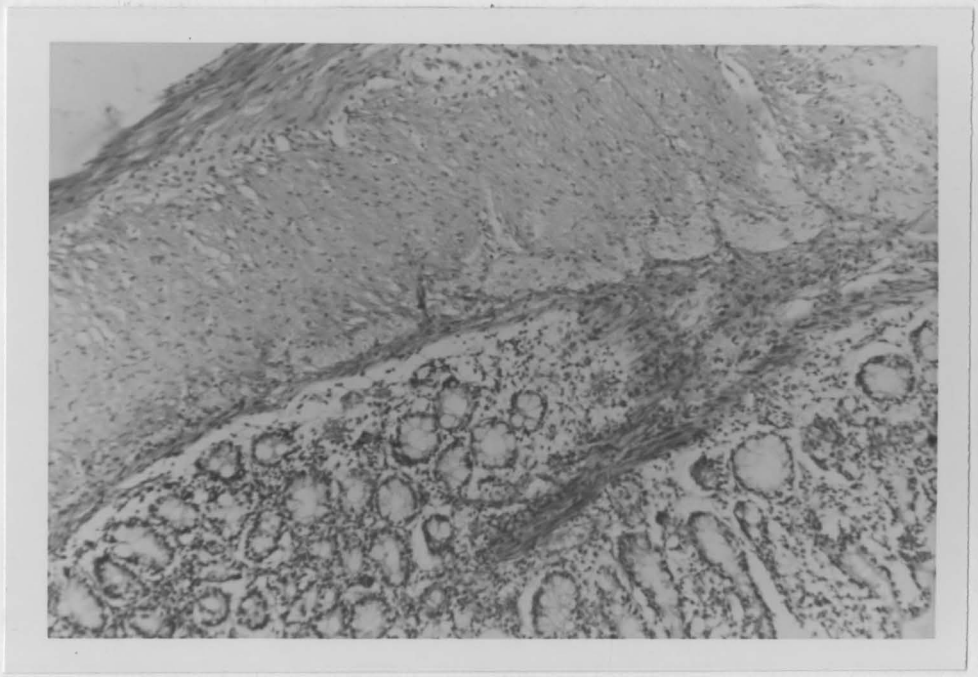

FIGURE 5

MICROSCOPIC PATHOLOGY OF GUINEA PIGS OF THE
ES ANTIGEN INJECTED GROUP
AT NECROPSY
Lung Tissue (x 430)

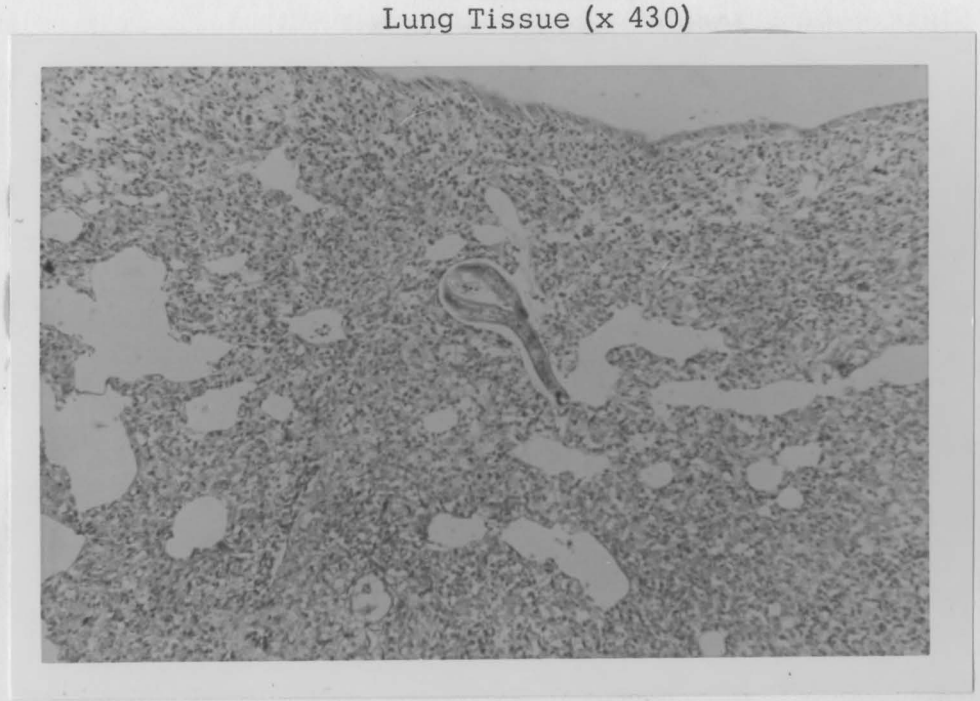

FIGURE 6

MICROSCOPIC PATHOLOGY OF GUINEA PIGS OF THE
ES ANTIGEN INJECTED GROUP
AT NECROPSY
Liver Tissue (x 430)

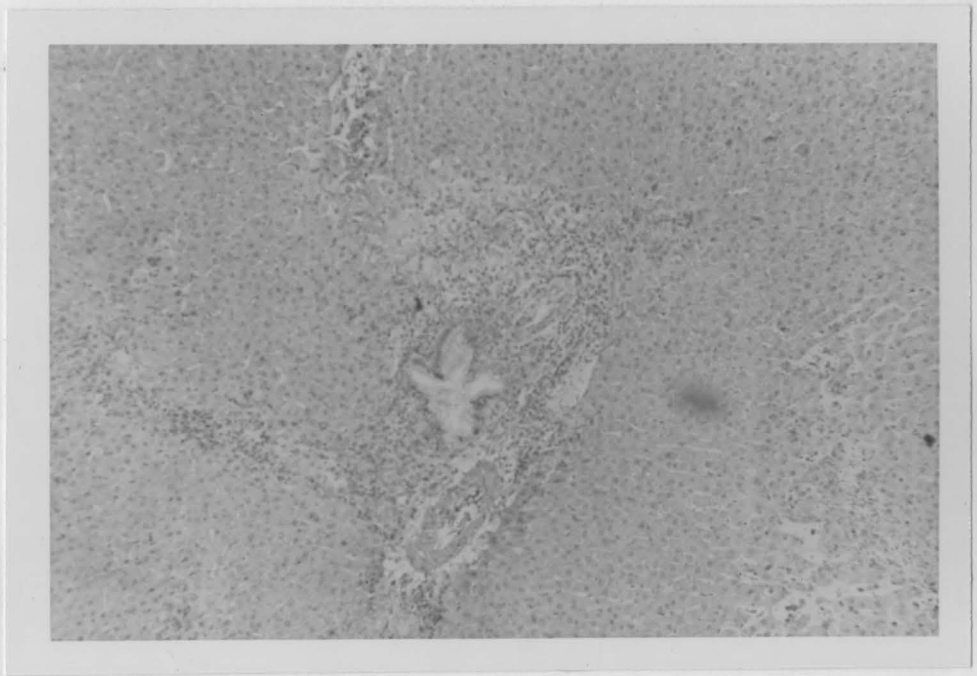

FIGURE 7

MICROSCOPIC PATHOLOGY OF GUINEA PIGS OF THE
ES ANTIGEN INJECTED GROUP
AT NECROPSY
Intestinal Tissue (x 430)

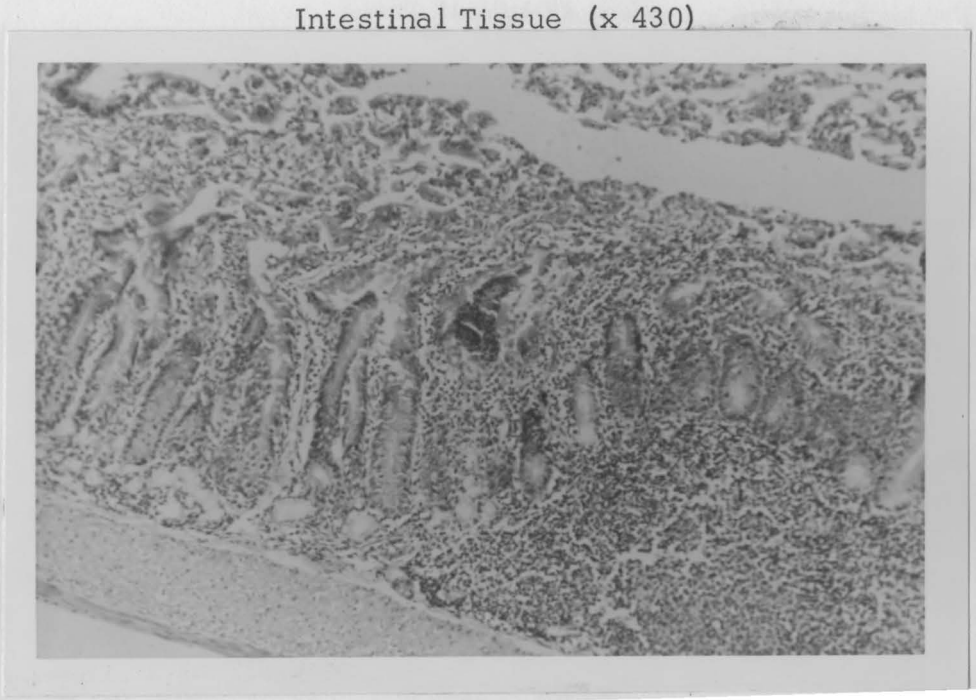

FIGURE 8

MICROSCOPIC PATHOLOGY OF GUINEA PIGS OF THE CONTROL GROUP AT NECROPSY
Lung Tissue (x 430)

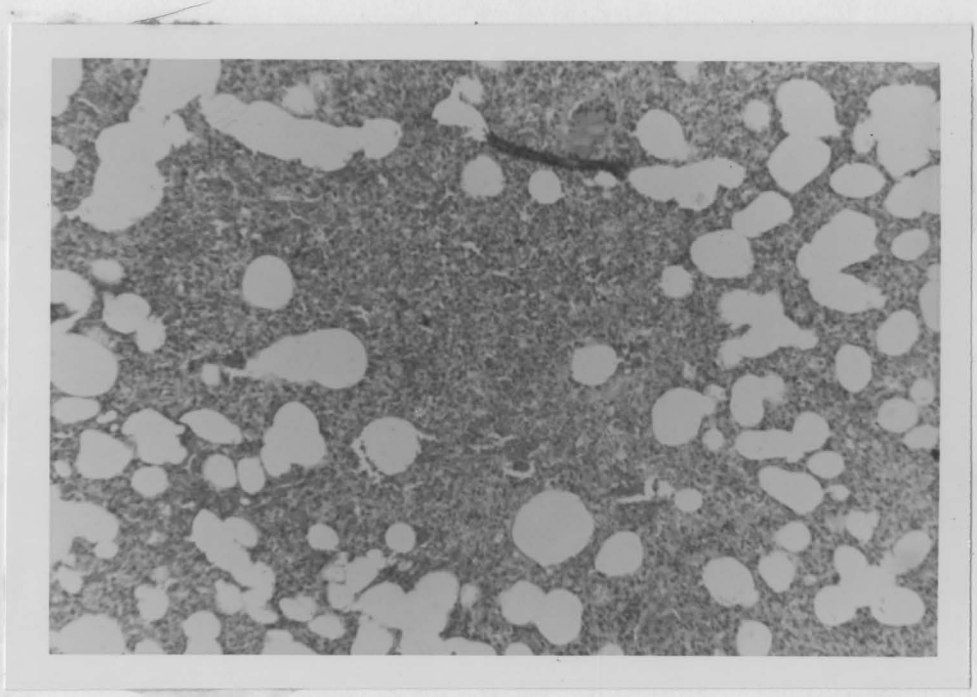

FIGURE 9

MICROSCOPIC PATHOLOGY OF GUINEA PIGS OF THE CONTROL GROUP AT NECROPSY
Liver Tissue (x 430)

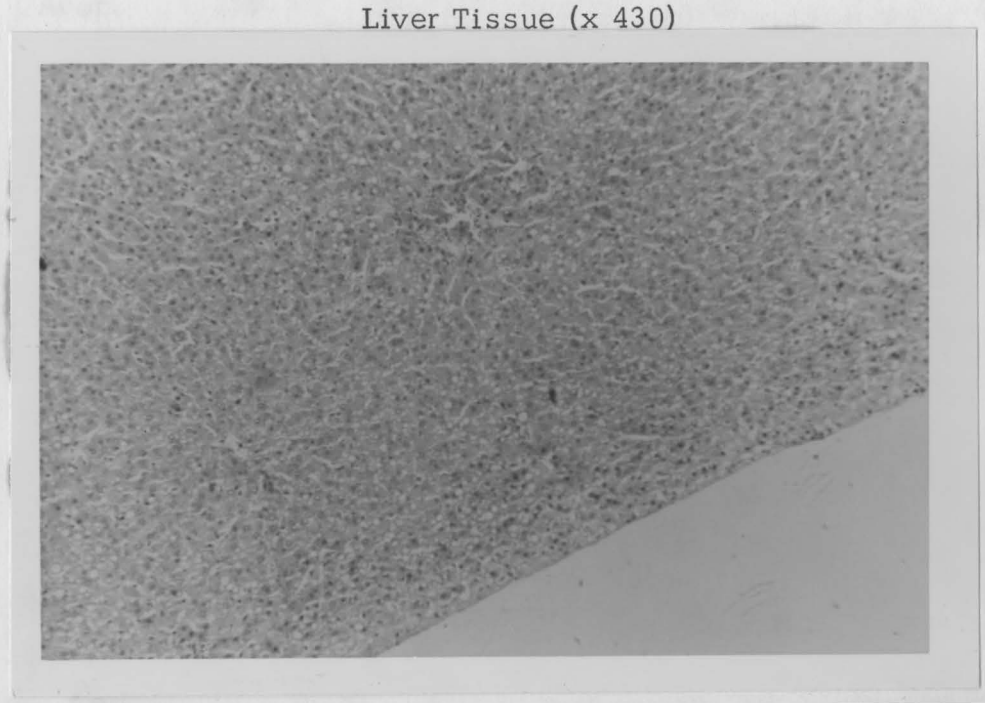

Table 6

NUMBER OF LARVAE RECOVERED AND RATING OF LUNG PATHOLOGY

Group	No. in Liver	Size in Liver	No. in Lungs	Size in Lungs	Total No.	Liver Ratio	Path Index
			Immunized Group				
I_1	480	1.35mm	160	.52mm	640	.75	1
I_2	520	1.30	200	.60	720	.72	2
I_3	520	1.20	320	.48	840	.62	3
Aver.	506.7	1.28	226.7	.533	733.3	0.69	2.0
			ES Antigen Injected				
A1	230	.90mm	120	.50mm	350	.66	1
A2	320	.97	120	1.15	440	.73	2
A3	360	1.20	280	1.05	640	.56	1
A4	420	1.10	240	1.20	660	.64	2
A5	440	1.30	160	1.40	600	.60	0
A6	360	.80	220	1.45	580	.62	3
A7	320	1.20	320	1.20	640	.50	2
A8	400	1.10	320	1.09	720	.56	2
Aver.	356.2	1.07	222.5	1.130	578.7	0.61	1.85
			Control Group				
C1	420	1.5mm	2600	1.50	3020	.11	3
C2	360	1.3	2000	1.65	2360	.15	4
C3	600	1.4	1250	1.45	1850	.33	4
C4	320	1.3	1800	1.70	2120	.10	3
C5	300	1.4	2680	1.50	2980	.10	4
C6	520	1.5	3000	1.85	3520	.15	3
Aver.	420	1.4	2221	1.61	2641.7	.16	3.5

CHAPTER IV

DISCUSSION

ES Antigen Injected animals had fewer worms, smaller worms, retarded rate of migration and reduced pathology than were found in the non-treated Control Group. These results support the hypothesis tha metabolic products elaborated during the development of larvae of A. suum produce functional immunity to histotropic ascariasis when injected into guinea pigs.

When equal numbers of infective eggs are administered to various groups of animals the total number of larvae recovered from the animals at necropsy should be similar. If immunity is induced, antibody production is stimulated and migrating larvae are affected, resulting in the number of larvae recovered being reduced. Therefore, the total number of worms recovered from the ES Antigen Injected and the Immunized guinea pigs should have been comparable; whereas more worms should be found in the Control Group since little antibody producing stimulant was present. The results obtained corroborate the above statement. The total number of migratory larvae recovered

indicated a comparable level of immunity in the Immunized and ES Antigen Injected Groups (averaging 733.3 and 587.7 larvae respectively); whereas, an average of 2641.7 larvae was found in each animal of the Control Group ($p < 0.05$). Thus the difference in total number of migrating larvae was evidence that the excretory-secretory products stimulated an immune reaction.

The size of the larvae is another criterion of immunity. In a primary infection- such as the challenge infection of the Control Group - the second stage larvae molt in the liver and migrate on into the lungs. By the time the larvae reach the lungs they have probably molted again (to third stage) and now average 1.61 mm in length. (Levine 1967). Fallis (1948), Sprent (1949) and Taffs (1964) reported a difference in size (growth of larvae was retarded) in immune animals. They attributed this effect to resistance imposed by the antibodies produced during the initial infection. In my study larvae from lungs of the ES Antigen Injected Group averaged 1.13 mm and 0.533 mm from the Immunized Group, whereas, larvae from the Controls averaged 1.61 mm. There was a statistically significant ($p < 0.05$, student's t-test) difference between size of worms from Controls and ES Antigen Injected Group (average 1.61 mm and 1.13 mm) and between the Immunized and Controls (0.533 mm and 1.61 mm). There was, however, an unexpected difference in size between larvae recovered from the ES Antigen Injected Group and the Immunized Group (1.13 mm and 0.533 mm).

Sprent (1949) studying the migration of Ascaris larvae in immunized and non-immunized mice applied a statistical measurement, which he designated "liver-ratio", to determine the progress of migration through the animals. The "liver-ratio" is determined by dividing the number of larvae recovered from the liver by the total number of larvae recovered from the lungs and liver. It is apparent that the "liver-ratio" will decrease as the histotropic phase progresses. In the early stages a much greater proportion of total number of larvae are in the liver (high "liver-ratio"), midway in the stage an equal number should be found in liver and lungs (a "liver-ratio" approaching 1); in the latter stage of migration almost all of the larvae will be found in the lungs (a low "liver-ratio"). Thus "liver-ratio" is a measurement of rate of migration. If larvae are retained or impeded by the liver, a high "liver-ratio" will be maintained. The number of larvae recovered from the lungs and livers of individual animals may vary considerably, therefore this ratio is valuable since it measures the degree to wh ich the liver hinders migration regardless of the number of larvae involved. In my study animals in the Control Group had an expected "liver-ratio" of 0.16 when necropsied on the seventh day following infection (almost all of the larvae had migrated to the lungs). The rate of migration was impeded in the treated animals. The ES Antigen Injected Group and the Immunized Group had an average "liver-ratio" of 0.61 and 0.69 respectively, seven days following the challenge infection. These results indicated

that the ES antigen was nearly as effective as the oral infection in retarding migration. It probably indicated that the liver is a site where antigen-antibody reaction occurs.

Because the "liver-ratio" indicated larvae were trapped in the liver (see above), I expected to find greater numbers of larvae in the livers of the guinea pigs in the ES Antigen Injected and Immunized Groups than in Control animals. This was not the case. All three groups had similar numbers of worms in their livers- averaging 420.0, 356.2, and 506.7 larvae per liver of each of the animals in the Control Group, ES Antigen Injected Group and Immunized Group respectively. Perhaps this unexpected similarity does not reflect a discrepancy but is a statistical artifact instead. An average of 2641.7 larvae were estimated present in each of the guinea pigs from the Control Group. Of this total, 16 percent occurred in the liver (see Table 5). This 16 percent, although much smaller than the 61 and 69 percent that occurred in the ES Antigen Injected and Immunized Groups respectively, when applied to the larger total (2641.7) produced a number in the liver comparable to the number found in the other two groups.

Immunity moderated pathology of histotropic ascariasis. The ES Antigen Injected Group and Immunized Group had an average lung pathology index of 1.86 and 2.0 respectively, whereas, pathology rating of the Control Group averaged 3.5. This moderation is probably due to two factors, (1) fewer larvae present in "immunized" animals

and (2) many of the larvae present in "immunized" animals were trapped in livers. Lung damage results when larvae rupture capillaries of alveoli breaking into the air sacs of the lungs. Hemorrhage and other mechanical damage results. Thus the damage is directly proportional to the number of worms penetrating the lungs. Fewer larvae were present in "immunized" animals - hence less damage.

Taffs (1964b)studying antibody response in guinea pigs, concluded that when Ascaris larvae return to the intestine (after travelling through the wall of intestine, to the liver and lungs) they release antigenic materials which are absorbed by the tissue intestinal wall and cause antibody production. The presence of large numbers of eosinophils and plasma cells in the intestinal tissue are evidence of an immune response, since within the cytoplasm of plasma cells, immunoglobulins are synthesized and released into the plasma (Davidsohn, 1969).

Histological sections of the intestinal wall of animals in the ES Antigen Injected and Immunized Groups exhibit a similar cellular response- massive infiltration of a comparable number of eosinophils and plasma cells (see Fig. 7 and 4). The animals in the Control Group were necropsied before the larvae of A. suum could return to the intestine. The intestinal walls of these animals were mildly infiltrated with white cells. It appears from the results that the mechanism of immunity operates in the intestine also.

Although my research supported the hypothesis that ES antigen induced functional immunity in guinea pigs, it would be further supported by additional experimental investigation. In the first place differences would be more valid if the number of experimental animals were increased. The three animals in the Immunized Group exhibited a variation in numbers of migrating larvae from 350.0 to 720.0 in the ES Antigen Injected Group.

In addition, the degree of immunity may be tested by varying the number , interval and site (for example the footpads of guinea pigs) of ES antigen inoculations- the effects of other systems of administration of immunizing agents.

The Microzone Method of electrophoresis used in this study determined the total albumin, alpha, beta and gamma globulin. Although the results of this procedure were inconclusive, since the variation within the groups was so insignificant, further immunoelectrophoretic analysis would determine the abonormal fraction of the various protein groups. However, more specific procedures of immuno-diffusion employing the Ouchterlony gel-diffusion method- a qualitative study forming a precipitin arc in a response to specific antigen to specific immunoglobulin, or a quantitative study of immuno-diffusion by the use of quantitative standards, will reveal the specific identity of the immunoglobulins that make up the total gamma globulin which thereby induce functional immunity. Further, serological studies using agglutination

or precipitin techniques, may help determine qualitative and quantitative antibody production.

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