ABSTRACT OF THESIS

Studies on the Development and Migration of Parasitic Nematodes.

by
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The thesis is divided into two parts. The first part is concerned with the development and migration of *Trichosomoides crassicauda* (Bellingham, 1840), a common nematode parasite of the urinary bladder of rats. The stages in the development of the egg and the experimental data regarding the hatching in various solutions are given. The morphology of the larvae, their migration in the host and the presence of a stylet in the adult worms are described. The incidence of *Trichosomoides crassicauda* and its relationship to the presence of mucoid calculi in the bladder are discussed.

Part II describes an attempt to determine the validity of the theory of retrofection and auto-infection of *Enterobius vermicularis* (Leach, 1853). Details of various methods used for observing the development and hatching out of the eggs of *Enterobius vermicularis* are given.

Owing to difficulties experienced in obtaining an adequate amount of material of *E. vermicularis*, it was decided to determine whether retrofection and auto-infection occur in a closely allied oxyurid *Aspiculuris tetraptera* (Nitzsch, 1821) of mice. The results of

observations on the development and hatching of eggs in various solutions are given. The bionomics of the larvae are described. It was found that the eggs could hatch out on moist cotton-wool swab in the anal opening of a human volunteer and the anal region of mice. Infective eggs were applied in the anal region of clean mice which were immobilised in specially designed cages to prevent their turning. On examination after a few days, adolescent forms were obtained in the colon in four cases. Experiments are now being continued to find out whether mature forms will develop as a result of retrofection. The theory of internal auto-infection is discussed in the light of our own experiments and the data in the literature.

STUDIES ON THE DEVELOPMENT AND MIGRATION OF PARASITIC NEMATODES.

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C O N T E N T S.

SECTION I.

STUDIES ON THE DEVELOPMENT AND MIGRATION OF TRICHOSOMOIDES CRASSICAUDA (BELLINGHAM)

SECTION II.

STUDIES ON RETROFECTION IN ENTEROBIUS VERMICULARIS OF MAN AND ASPICULURIS TETRAPTERA OF MICE.

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1943 - "Human intestinal parasites in Northwest China. A Brief Survey." Chin.Med.J. 61 (4), 292-295.

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1948 - "Malaria in Southern and Western Szechuan with a note on the occurrence of Schistosomiasis japonicum." Chin.Med.J. 66 (6), 319-323.

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The thesis is divided into two parts. The first part is concerned with the development and migration of Trichosomoides crassicauda (Bellingham, 1840), a common nematode parasite of the urinary bladder of rats. The stages in the development of the egg and the experimental data regarding the hatching in various solutions are given. The morphology of the larvae, their migration in the host and the presence of a stylet in the adult worms are described. The incidence of Trichosomoides crassicauda and its relationship to the presence of mucoid calculi in the bladder are discussed.

Part two describes an attempt to determine the validity of theory of retrofection and auto-infection in Enterobius vermicularis (Linnaeus, 1758) Leach, 1853. The results of observations on the development and hatching of the eggs of Enterobius vermicularis in various solutions are given.

Owing to difficulties experienced in obtaining an adequate amount of material of Enterobius vermicularis, it was decided to determine whether retrofection and auto-infection occur in a closely allied oxyurid Aspiculuris tetraptera (Nitzsch, 1821) of mice. Details of various methods used for observing the development and hatching out of the eggs of Aspiculuris tetraptera are given. The bionomics of the larvae are described. It was found that the eggs could hatch out on moist cotton wool swab, in the anal opening of a human volunteer and the anal region of mice. Infective eggs were applied in the anal region of clean mice which were immobilised in specially designed cages to prevent their turning. On examination after a few days, adolescent forms were obtained in the colon in four cases. After 30 to 34 days, eggs of Aspiculuris tetraptera were found in the faeces of three experimental mice. On autopsy, both female and male adult worms were found in the colon of the experimental mice. The experiments prove that retrofection can occur in the mouse Oxyurid, Aspiculuris tetraptera. The theory of internal auto-infection is discussed in the light of our own experiments and the data in the literature.

S E C T I O N I.

STUDIES ON THE DEVELOPMENT AND MIGRATION OF TRICHOSOMOIDES CRASSICAUDA (BELLINGHAM)

INTRODUCTION.

Trichosomoides crassicauda is a common nematode of the urinary bladder of rats and was described by Bellin ham in 1840. Eberth (1863) described the morphology of the dorsal, ventral and lateral cords and the position of the female genital opening. Walter (1866) found this worm harboured in mucoid calculi in the urinary bladder and described the male worm parasitizing the uterus of the female. Von Linstow (1874) carefully described the adult morphology and suggested that the ingested embryos might bore into the wall of the digestive tract and make their way to the pelvis of the kidney. This might occur by the way of the blood stream and in particular by the renal artery. He also found sexually immature individuals in the pelvis of the kidney and in the ureters. Copulation was suggested as taking place in the ureters. In 1882, Von Linstow discovered a stylet in the newly hatched larvae. Hall (1916) found that the embryos escaped from their shells in the vagina of the female after the worm had been in normal salt solution for a brief period. From this, and the fact that the embryos only lived for a short time, he concluded that infection must take place quickly. Yokogawa (1920) studied the migratory route of this worm in the rat. He fed a rat with large numbers of eggs, collected from urine of several infected

rats, and from 16 adult worms containing many eggs in their uteri. Four days later, he killed the rat and found four larvae in the abdominal cavity, two in the pleural cavity and three in the lungs. He described the larvae found in the abdominal cavity as being 820 to 840 μ in length and those in the pleural cavity and two in the lungs as 2,340 μ.

Thomas (1924) carried out a series of experiments to study the life-history of this worm. He stated that "numerous attempts were made to determine the wanderings of the parasite in the body of the host." In 7 experimental rats, from 5 hours to 8 days after infective feedings, a total of 39 larvae were found. Fifteen of them were obtained from the blood, 6 from the crushed liver, 3 from the lung, 7 from the pleural cavity and 8 from the abdominal cavity. Their length varied from 231 to 372 μ. In another series of experiments, he found the eggs of Trichosomoides crassicauda in the urine of experimental rats from the 15th to 47th day after infective feedings.

Recently Smith (1946) infected five groups of rats by feeding them with Trichosomoides crassicauda eggs and obtained serum from the infected rats. He studied the reaction of infected rat serum on the eggs. Rats of group I (Nos. 1-3) each received 11 infections of 25 to 50 eggs over a period of 14 weeks. Rats of group II (Nos. 4-7) received nine similar infections over a period of nine weeks. Rats of group III (Nos. 8-11) received nine infections of 25-100 eggs over a period of 14 weeks. Rats of group IV (Nos. 12-15) received five infections of 100 eggs over a period of 10 weeks. Intervals between

infections were not constant because timing as well as quantity had to depend upon the availability of infective material from wild rats. Control rats in group V (Nos. 12-15) differed in that they received a single infection of 100 eggs, whereas other control groups were kept uninfected. The average number of worms in each rat of the four groups was as follows: 2.7 in group I; 1.8 in group II; 0.25 in group III; and 1.8 in group IV. No parasites were found in any control rats, including group V which had received an infection of 100 eggs each.

Incubation of T. crassicauda eggs with sera of infected rats caused precipitation around the embryonated eggs.

Since the results obtained from Yokogawa and Thomas were somewhat controversial and the stages of the development were not clear, the writer carried out the following observations:

THE MORPHOLOGY AND DEVELOPMENT OF THE EGGS OF T. CRASSICAUDA.

Eggs of T. crassicauda when mature are of a golden colour and have thick shells, capped at both ends with a plug. In a series of measurements involving 11 eggs the size varied from 55.9 to 73.1 μ in length and 32.25 to 51.6 μ in width. Von Linstow (1874) found the eggs to be from 62 to 75 μ long and 29 to 56 μ wide, whereas Hall (1916) reported them to be 62 to 72 μ long and 25 to 56 μ wide. Some eggs in the uterus of the female are in the segmented stage and contain the vermiform embryos. The eggs containing segmenting

embryos (Fig. 1A) were dissected out and transferred to normal saline solution and incubated at 28°C for 20 hours. During this time the segmented embryos in the shells developed into the 8-cell stage (Fig. 1B). After 43 hours incubation, some of the eggs contained about 32 cells (Fig. 1C) and some had developed into the morula stage (Fig. 1D). After a total of 67 hours incubation many larvae had developed in their shells (Fig. 1E). Most of the eggs containing vermiform embryos had thin and colourless shells, only those situated near the vagina having brown and thick shells.

DESCRIPTION OF THE LARVA OF TRICHOSOMOIDES CRASSICAUDA.

The larva to be described is the infective stage, which is contained in its egg shell. It was found that it could hatch out in the uterus when the adult worm was incubated in normal saline. The larvae contained in colourless thin egg shells could also readily hatch out under those conditions. In an attempt to obtain a large number of larvae to study their morphology, various solutions were used to hatch the eggs. They did not hatch out when the brown eggs were incubated in artificial gastric juce, one tenth normal hydrochloric acid, one fifteenth "Milton" solution or Ringer's solution either at 28°C or 37°C. However, hatching occurred when the brown eggs were placed in a hollow slide containing horse serum and incubated at 37°C for 3 hours. The larvae also readily came out of the egg shells when the cover slip was gently pressed with a needle.

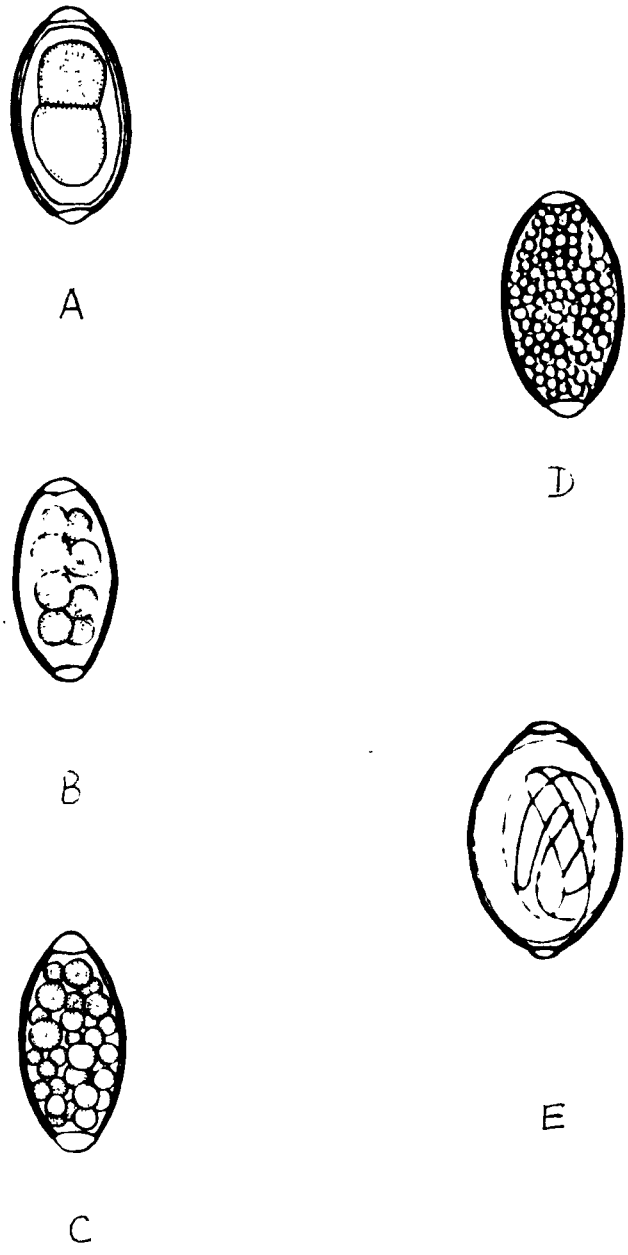

FIG. I.

A. Egg of Trichosomoides crassicauda dissected out from the uterus of a female.

B. Egg of T. crassicauda which has been incubated in normal saline at 28°C for 20 hours.

C. Egg of T. crassicauda which has been incubated in normal saline at 28°C for 43 hours.

D. Egg of T. crassicauda which has been incubated in normal saline at 28°C for 43 hours.

E. Egg of T. crassicauda which has been incubated in normal saline at 28°C for 67 hours. Vermiform embryo in shell is shown.

When pressed out from the eggs, the larvae were so active that it was impractical to draw them under camera lucida; on the other hand, when fixed by heat, alcohol or 10 per cent formalin solution, the internal structure faded. Examination of the larvae in aqueous "polyvinyl alcohol" solution was also unsuitable as they were not completely immobilised. Finally, it was found that the larvae were sluggish or motionless when hatched and ripened in the uterus of the adult worm and their internal structure was still quite clear. In this way, material was obtained for measurement and drawing.

Yokogawa (1920) stated that "the larvae just from the eggs have a very small body of almost uniform thickness, terminating in bluntly rounded ends. They measure about 0.21 to 0.25 mm. in length and 8 to 10 μ in thickness. It was impossible to make out any details of internal structure at this stage."

Thomas (1924) stated that "A morphological study of the larva at this stage shows it fully equipped with a stylet, oesophagus and an intestine. A few cells that possibly might be the beginning of oesophageal cells are shown."

Smith (1926) stated that "lengths of 112 larvae were measured, including a few dead specimens in saline controls. Wide variations occurred within single cultures, and lengths ranged from 254 to 388 μ with a mean length of 308 $\pm$ 3.2" μ

According to the writer's measurements, the length of the larvae varies from 215 to 378 μ. The width of the anterior, middle and posterior end is 9.51 to 12.68 μ, 11.095 to 12.75 μ, and 6.34 to 9.51 μ respectively. Ten larvae were carefully measured and their

dimensions are shown in Table I. The stylet measured from 7.92 to 26.19 μ in length. It is situated at the anterior end and it has the appearance of a hyaline line or two small parallel hyaline lines. Sometimes the stylet protruding from the oral opening resembles a spear head. Some stylets are pointed at the anterior and oval shaped at the posterior end; some are lancet-shaped. In a mounted specimen, a spear head was found at the anterior and a spear shaft at the posterior end. The body of the larva is smooth, elongated and cylindrical with a bluntly rounded anterior and a tapering rounded posterior end (Fig. 2). The region of the oesophagus is 110.95 to 128.38 μ in length and in the newly hatched larva, this part of the body is very transparent. The outline of the oesophagus is indistinct but its lumen can be seen as a shining line. When the larva is obtained after incubation of the adults, many round granules can be seen scattered over this region.

The intestine measures 110.95 to 158.5 μ in length and consists of a row of square, rectangular or oval granular cells. Sometimes two rows of these cells occur, with the lumen of the intestine lying between them. The row of cells ends just anterior to a group of 16-18 globules which occupy a space 22.19 to 38.04 μ long. These globules are round, refractile, scattered irregularly, and can be seen very clearly. Their function is not clear.

The posterior end of the larva just behind the globules measures from 12.68 to 28.53 μ. The internal structure is indistinct. Sometimes two rows of fine granules can be seen extending to the end of the tail.

TABLE I.

MEASUREMENTS OF THE LARVAE OF TRICHOSOMOIDES CRASSICAUDA.

No. of Larva.	1	2	3	4	5	6	7	8	9	10
Length of the larvae (Microns)	339.19	328.02	275.79	329.68	329.68	329.68	329.68	340.775	324.92	324.92
Width of ant. part	12.046	12.046	12.68	9.51	11.095	9.51	12.68	9.51	9.80	11.095
Width of middle part	12.046	11.095	12.68	11.095	11.095	11.095	12.68	11.095	12.2	12.68
Width of tail	9.510	7.925	7.925	7.925	7.925	7.925	9.51	6.34	6.8	6.34
Length of oesophagus	126.80	110.95	110.95	120.46	110.95	120.46	120.46	122.045	128.38	117.89
Length of intestine	158.50	158.5	110.95	152.16	158.5	152.16	158.5	158.5	152.16	158.50
Length of granules area	31.70	34.87	31.70	28.53	31.7	28.53	25.36	38.04	31.7	22.19
Length of stylet	26.19	11.095	9.51	7.92	12.68	7.925	9.51	11.095	11.095	9.51
Length behind the granules area	22.19	23.70	22.19	28.53	28.53	28.53	25.36	22.190	12.68	26.945

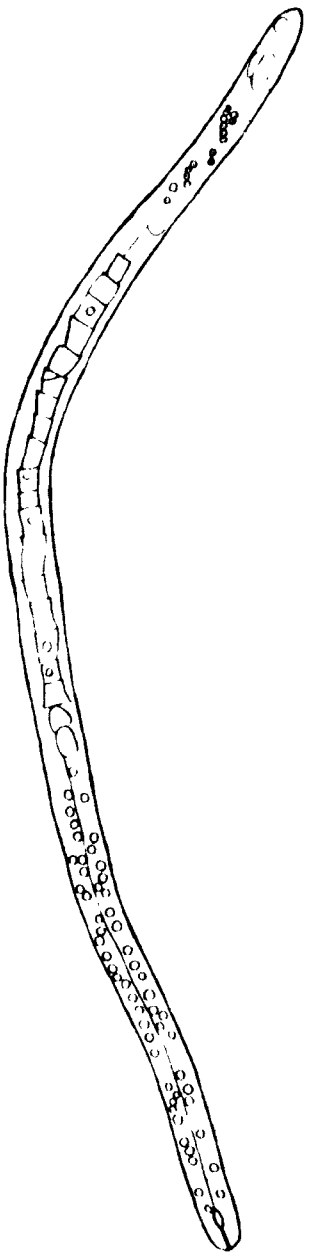

FIG. 2.

Larva of Trichosomoides crassicauda.

THE STYLET OF THE ADULT WORM.

Hoyberg (1907) first suggested that the larvae of Trichinella had a "boring apparatus" and Fulleborn (1920, 1923) showed that the larvae of Trichocephalus trichiurus, T. leporis, a Trichocephalus sp. from monkeys, Trichinella spiralis, Capillaria hepatica and Trichosomoides crassicauda all possessed a distinct onchiostyle. Wehr (1939) found a stylet in the first stage larvae of Capillaria columbae but it was absent in all succeeding stages.

Li (1933) has described a distinct functional stylet in adult Trichocephalus trichiurus and in a Trichocephalus sp. from the macaque, and Chitwood and Chitwood (1937) state that one has been seen in adult T. vulpis.

Von Someron (1939) found a stylet in the buccal capsule of the adult worm of Trichinella spiralis. Thomas (1924) found that young female worms, without males in their uteri, in the renal blood vessels of the kidney were equipped with stylets. He stated that "in all adult egg-laying females situated in the region of the bladder, the writer has been unable to demonstrate the presence of a stylet. On the other hand all males examined from the uteri of such females possessed stylets. Perhaps it may be inferred from this and other facts that the males lead a more roving life than the females, in the sense that the females settle-down and often papillomas completely surround them." In another paragraph he stated that "apparently after a female has settled down as an egg-laying adult she loses her stylet."

In the writer's experiments mature worms were carefully examined to see whether or not there was a stylet in the cephalic region. Mature female worms obtained from the bladder wall of an infected rat were cleaned with water and fixed with alcohol. They were then dehydrated with different concentrations of alcohol and transferred to lactophenol solution for about one week, at the end of which time the specimens were clear and the stylet was found in the cephalic region. The shape of the stylet was like a spear head (Fig. 3A). It was 9.5 μ in length in one specimen, 8 μ and 10 μ in two others. At the posterior end they were 3 μ, 3 μ and 3.5 μ in width respectively. Another spear-shaped specimen (Fig. 3B) was situated in the middle of the oesophagus. It was 7.9 μ in length and 1.5 μ in width. The stylet of male worms was also examined. In one case it was 6 μ in length and 1.5 μ in width and shaped like a spear head. The other case was 9 μ in length and like a pointed hyaline rod. In the first three specimens, the shape resembled that of the mounted specimen of Trichocephalus trichiurus photographed by Li (1933). The writer observed that in the larvae of Trichosomoides crassicauda the shape of the stylet was slightly different. The drawings of Fulleborn (1923) confirmed this view.

THE MIGRATION OF THE LARVAE IN THE HOST.

The cage for collecting urine of rats is illustrated in Fig. 4. The bottom of the cage consists of a frame with a floor of wire gauze to support the rat. Under the floor is a metal funnel to collect the

A

B

FIG. 3.

A and B: Anterior end of a female Trichosomoides crassicauda. Stylet is shown.

FIG. 4.

The cage used for collecting urine of rats.

rat's urine which drops into a glass tube under the cage. To prevent faeces dropping into the tube, a round bulb is hung under the funnel and above the glass tube. In this way, 3 to 5 cc. of urine could be collected in one night. The urine was centrifuged and the precipitate examined for the eggs of T. crassicauda. The cage should be cleaned daily. By examining the urine, it could be decided whether or not the rat was infected.

Eight experiments have been carried out in an attempt to determine the development and migration of the parasite in the body of the host. In five of them, no larva was found either in crushing or in sectioning the internal organs. The successful experiments are described as follows.

EXPERIMENT I.

On February 9th, 1949 at 12 noon, a half-grown male rat was fed with large numbers of T. crassicauda eggs on a piece of bread. Some of the eggs were brown shelled and some were colourless. They had been ripened in normal saline at 28°C for 116 hours. The larvae were pressed out from egg shells and were active. On February 18th, eggs which were collected from the urine of an infected rat were fed to the rat again. The urine of the rat had been examined for T. crassicauda eggs with negative results several times. On March 10th the urine was examined and no egg was found.

On March 11th the urine was again examined and 11 eggs were found. This showed that thirty days after experimental feeding, eggs were found in the urine. Subsequently the urine was examined every day for

more than ten days. Eggs were found each day the number varying from 1 or 2 only to more than 10.

From April 22nd urine was collected from two naturally infected rats and centrifuged to precipitate the eggs for feeding to the rat in an attempt to cause a heavy infection. The rat was fed with the eggs daily for 12 days.

On May 19th, the rat was found dead in its cage. On autopsy the left upper lung was congested. From the upper part of the right ureter, one immature female warm was found and from the urinary bladder, three mature female worms were found. The mature worms harboured male worms in their uteri.

EXPERIMENT 2.

A small male rat, with a body weight of 47 gms. was left without food or water for 5 hours. Then at 12 noon on May 11th, 1949 it was fed with 29 adult T. crassicauda in a bread pellet. These worms had recently been obtained from the bladder of naturally infected rats. The next day, at 12 noon, it was fed with a large number of eggs obtained from the wall of the urinary bladder. At 2p.m. on May 13th, 10 adult worms which had been ripened in normal saline at room temperature for 52 hours were fed to the rat. On May 14th at 10a.m. the rat was fed with 15 adult worms which had been ripened in normal saline at room temperature for 70 hours. (A total of 54 adult worms with large numbers of eggs were swallowed). Signs of tremor were observed on May 14th and on May 16th the rat was found to be dead in its cage.

The rat was examined in the following way. A small incision was made in the abdominal wall and 10 cc. of normal saline was injected several times by means of a pipette and removed again by the pipette. The washings from the cavity were then placed in a tube and centrifuged. The pleural cavity was opened and washed in the same way. It was noticed that the lungs, right middle lobe and left upper lobe were very much congested. The blood in the right auricle was also aspirated for examination. Parts of the left upper lung, heart, liver, spleen, kidney and brain were fixed in 10 per cent formalin solution for sectioning. The remaining parts of these organs were crushed through a fine sieve with normal saline and the resulting fluids centrifuged.

Four larvae were found from the abdominal cavity washings (length 250-278 μ; width 12.75 μ). Two larvae from the pleural cavity washings and one from crushed lungs were found, the latter was 215 μ in length.

EXPERIMENT 3.

On May 12th, 1949 a small male rat with a body weight of 41 gms. was left without food and water for 7 hours. Then at 6p.m. it was fed with 8 adult worms of T. crassicauda which had been ripened in normal saline at room temperature for about 28 hours. Next day at 2p.m. 20 adult worms which had been ripened for 52 hours were fed to it. On May 14th at 10a.m. 14 adult worms which had been ripened for 70 hours were fed to it (in all, 42 adult worms were eaten). All the worms were fed in bread pellets. On May 16th, the rat was found to be dead in its cage and was put in a refrigerator.

Examination was conducted on May 17th by the same technique as described in the previous experiment. From the abdominal cavity washings one live larva was found. Two living larvae were found in the pleural cavity washings.

The right upper and left upper lung was very congested. The right middle lobe was found to have an abscess of a diameter about 3 mm. Parts of the ring upper lung, liver, kidney, heart, spleen, brain and small intestine were fixed for sectioning. The remaining parts of these organs were crushed but were negative for parasites.

The larva found in the abdominal cavity was 306 μ in length and 12.75 μ in width. The larvae found in the pleural cavity were 302 μ and 306 μ in length respectively and both were 12.75 μ in width.

THE PATHOLOGY OF THE LUNG OF RAT OF EXPERIMENT 2.

Histological examination showed a congested condition and signs of consolidation in some areas. The septae had been infiltrated by round cells and polymorphonucleears. Numerous small areas of haemorrhage were very marked. In some areas, the alveolae had been replaced by erythrocytes and leucocytes. The epithelium of the bronchioles was desquamated and there were exudate and some macrophages in the lumen of the bronchioles. Although the larvae of T. crassicauda were not found in any of a series of sections, they were obtained from the crushed tissue of the lung. The rat died after infective feeding, so it is concluded that the pathological condition was caused by the larvae of T. crassicauda.

The lung of the rat of experiment 3 was very much congested at both apices. On histological examination, it had the signs described above. No larva was found in the tissue sections of the internal organs.

THE BIONOMICS OF TRICHOSOMOIDES CRASSICAUDA.

On May 10th, 1949 we collected T. crassicauda from the bladder of brown rats which had been poisoned 5 days previously. We found that one male worm was moving in the uterus of a female worm.

On May 11th, we collected the worms from other rats poisoned at the same time as those above. The worms were put into normal saline and kept at room temperature. 24 hours later, we found some female worms moving in the normal saline.

On May 13th, 10 larvae were found in the anterior part of the uterus of one of these female worms. They hatched out from eggs of the uterus. In that area, there were seven eggs in which the vermiform embryos did not hatch out. On breaking the body of the adult worm, the larvae crawled out and moved actively. Some of the adult worms were put in normal saline and others in Ringer's solution at 28°C. They lived for about 24 hours.

In one instance, four male worms were found living in the uterus of one female worm.

THE INCIDENCE OF TRICHOSOMOIDES CRASSICAUDA AND ITS RELATIONSHIP TO THE PRESENCE OF MUCOID CALCULI.

Incidence.

To carry out the above experiments, a total of 137 rats were examined in order to collect T. crassicauda. (a) 57 of these were obtained from London Docks. Among them 53 were black rats, Rattus rattus and 4 were brown rats, Rattus norvegicus. All were negative. (b) 37 Rattus norvegicus were obtained from Devon. Of these 24 were parasitized, making 64.8 per cent infestation. A total of 127 female worms were found with an average of 5.3 worms per infected rat. The highest number of parasites harboured by one rat was 16. (c) 38 brown rats, Rattus norvegicus, were obtained from Twyford, Lancashire. Nine (23 per cent) were infested with this parasite. The number of worms per infected rat varied from 1 to 10 with an average of 4.2. (d) The other five were white rats, Rattus norvegicus albus, raised in the laboratory. They were all negative. In all 79 brown rats were examined, 33 or 41.3 per cent being infested. The mean number of parasites per infected rat was five.

It is of considerable interest to note that the black rats examined were completely free of this parasite. It seems pertinent to compare our data with that of previous workers. In discussing black rats, Balfour (1922) examined 34 Rattus rattus and stated that, "in addition, Trichosomoides crassicauda Bell was found on three occasions in the urinary bladder, but as only a small number of black rats were examined for the presence of this parasite it would serve no purpose

to state a percentage." Balfour also found Trichosomoides crassicauda Bell in the bladder of 48.3 per cent of 333 brown rats examined. He stated that "The rodents were for the most part obtained from dealers in London and were London rats, but specimens were also secured from such towns as Bedford, Brighton, Chester, Eastleigh (Hants), Horsham, Liverpool, Luton, Maidstone, Preston, Reading and Sandwich. Occasionally rats from country districts were secured and a few were captured from ships at the London docks."

Johnston (1918) found worms in the urinary bladder of only one out of 163 specimens of Epimys ruttus, i.e. 0.6 per cent infestation. In the single case six worms were found. Of 246 Epimys alexandrinus examined only one contained T. crassicauda and that only a single specimen. Ship and house rat varieties of Epimys rattus showed a 0.5 per cent infestation in 409 specimens. 74 out of 205 Ep. norvegicus were parasitized making a 31.2 per cent infestation. He also notes that the greatest numbers of females obtained from any one host were 15 (twice), 11, 10 (twice) and 9 (several times). The usual number was one to three. Of 83 mouse bladders examined, none contained the parasite.

Lowenstein (1910) in his experiments on the formation of tumours in the urinary tract of rats, used 54 rats from four different sources. In the first series of 18 rats all were parasitized; in 16 cases the worms were found in the bladder, once in the kidney and once in the kidney and the ureters. In a second series of 18 rats, no worm was found. In the 9 rats of the third series examined, 5 had parasitized bladders, four of which had papillomas.

Thomas (1924) stated that "from time to time over a period of four years, the author has examined the urinary tract of numbers of rats from Champaign and Urbana, Illinois and vicinity. Of 136 adult rats examined 102 were parasitized; 357 parasites were taken. The average number of parasites per host was 3.5 or a 75 per cent infection. Thirteen half-grown rats averaged 1.5 parasites per host. In all, 149 rats contained 360 worms, or a 69.8 per cent infection with 3.4 parasites per host. The male rats far outnumbered the females taken as only 33 adult females were trapped, of which 20 were infested. The average number of parasites per female was 3.3 per host. Out of 103 adult males, 82 were infested, giving a 79.6 per cent infection with an average of 2 parasites per host." The heaviest infections found by Thomas were 12 (4 times), 11, 10 and 21 in a single host.

Luttermoser (1936) examined 2,636 house rats (Rattus norvegicus) in Baltimore, U.S.A. T. crassicauda was found in 2,259 (90.4 per cent) out of 2,500 adult rats and in 44 (32.35 per cent) out of 136 juvenile rats.

Cram (1928) examined 113 white rats (Rattus norvegicus albus) and found 24 cases (21 per cent) harbouring T. crassicauda in the urinary bladder. The number of nematodes varied from 1 to 7 with an average of 2.4 per infected rat.

Price and Chitwood (1931) found T. crassicauda in 73 out of 100 Rattus norvegicus examined.

Tubangui (1931) dissected a total of 950 brown rats, Mus norvegicus. The incidence of T. crassicauda was 57 per cent. The rats were trapped in different sections of the city of Manila.

Chen (1933) examined 84 rats in Canton, China. Two species of rats were involved in the examination, namely, Mus. rattus Linn and M. norvegicus Erxleben, the ratio being about 9 to 4; some of them were not identified as to species at the beginning of the survey. 10 (11.9 per cent) out of 84 of the rats harboured T. crassicauda.

Chin (1939) examined 129 rats (mainly Rattus norvegicus) with a few Rattus rattus) and found 3 (2.33 per cent) infected with T. crassicauda. The average number of worms per host was 4.33.

Lu (1941) found that T. crassicauda occurred in 13 out of 20 rats (Rattus norvegicus) examined. An average of 7.3 per infected case was found, the maximum being 50 and the minimum 1.

According to this review, most of the rats examined for infection with T. crassicauda were brown rats, Rattus norvegicus and the incidence of infection in this host was rather high. The data obtained by Johnston (1918) had definitely shown that the incidence of T. crassicauda is very small (0.5 per cent to 0.6 per cent) in Epimys rattus and much larger (31.2 per cent) in Epimys norvegicus.

Mucoid calculi.

Smith (1946) published a paper "Are vesical calculi associated with Trichosomoides crassicauda, the common bladder nematode of rats?" He examined 370 rats and of these 32 (8.7 per cent) had vesical calculi and 27 had both calculi and T. crassicauda. He also found that the calculi were much more common in male rats. In 179 males, 30 had calculi, 25 of them had both calculi and worms. The incidence of T. crassicauda of the 370 rats was 65.4 per cent.

During the examination of rats, we also paid attention to the presence of mucoid calculi in the bladder. In the 137 rats used in the present studies, mucoid calculi were found in 11 cases, giving an incidence of 8.03 per cent. In 4 cases, both calculi and T. crassicauda were found. In 7 cases, only mucoid calculi were found, there being no parasites. From an analysis of the incidence of calculi in this series of rats it becomes apparent that the incidence in infected rats is nearly twice as large as in uninfected rats, namely, 12.12 per cent and 6.73 per cent respectively. An analysis of the figures given by Smith (1946) shows that the incidence of calculi in infected rats is more than three times that in uninfected rats, namely, 23.1 per cent and 7 per cent. From these data (see Table II) it is concluded that there is a relationship between the occurrence of T. crassicauda and mucoid calculi.

DISCUSSION.

Yokogawa (1920) stated that "On the method of migration of the larvae of this nematode from the lungs to the bladder of the host there is little definite information. Von Linstow's (1874) finding young worms in the kidneys and ureters suggests that they make their way to the kidney and then pass down the ureters to the bladder. How they make their way from the lungs to the kidney is still an unsolved question. Taking for granted that the migration to the lungs is a necessary phase of the life history of this parasite, there are three possible ways in which it might migrate from the lungs to the kidneys. (1) the larvae might make their way into the small branches of the

TABLE II.

ANALYSIS OF THE INCIDENCE OF CALCULI IN RATS.

Parasite	Calculus	PRESENT AUTHOR			SMITH.		
		With Calculi	Without Calculi	TOTAL	With Calculi	Without Calculi	TOTAL
No. infected		4	29	33	25	83	108
	%	12.12	87.88	100	23.1	76.9	100
No. uninfected		7	97	104	5	66	71
	%	6.73	93.27	100	7.0	93.0	100
Total No.		11	126	137	30	149	179
	%	8.03	91.97	100	16.8	83.2	100

pulmonary veins, be carried to the heart and then pass to the kidney along the aorta and renal arteries; (2) the larvae in the lungs might break into the air cells and, like the hookworm larvae, travel up the trachea and down the oesophagus into the intestine. From here it would be necessary for them to make their way to the kidneys by way of the body cavity; (3) finally, the larvae in the lungs might make their way back into the pleural cavity through the diaphragm and body cavity to the kidney. The course by the blood stream would seem to be very difficult if not impossible on account of the large size of the larvae and the fact that the renal artery is a small vessel which branches at right angles from the dorsal aorta. It is possible of course that the small type found might follow this course. It seems to me that the second course is the most probable, but the solution of this interesting problem must await future investigations."

Thomas (1924) stated that "since larvae have been found in the blood stream it is more reasonable to assume that larvae come into the pleural and abdominal cavities by chance wanderings and the bleeding points on organs is the external evidence of their leaving the blood stream. Furthermore, the author in numerous cases has found blood filled renal tubules in the kidneys of parasitized rats that would indicate larvae had left the blood vessels, and the finding of young forms themselves in blood vessels and renal tubes would bear this out. It might also be well to recall a similar observation by Lowenstein (1910); he, however, found only young forms beside blood vessels with a scattering of red corpuscles near them."

We were of the opinion that if the larvae of T. crassicauda migrated from the lung to the kidney by the circulatory system, they would be dispersed throughout the body by the circulation of the blood. However, we have not been able to demonstrate the larvae in tissue sections. A similar finding is reported by Thomas (1924) who stated that "the left lung, the heart, both kidneys and ureters, the bladder and right lobe of the liver were saved for sectioning" but said that "no larvae were found in the sections of the organs saved."

Concerning the second route of migration, suggested by Yokogawa (1920), it could only be proved if larvae could be demonstrated in the trachea of the experimental rats. Even if larvae were obtained from the intestine of experimental rats, it would be impossible to differentiate between those just hatched and those which had migrated from the lung, since the little growth takes place in the first eight days. Thomas (1924) has shown the larvae in the bronchi, but not in the trachea.

In our opinion, it seems most likely that the migration of the larvae of T. crassicauda from lung to kidney follows the third route suggested by Yokogawa (1920), i.e. the larvae in the lungs make their way back through the pleural cavity, the diaphragm and the abdominal cavity to the kidney.

From our results and the records of previous workers, a total of 58 larvae were found in experimental rats. The distribution of these larvae is listed in Table III. It shows that (a) 15 larvae were found in blood, 5 to 10 hours after the infective feeding; (b) 6 larvae were found in crushed liver, 1 to 4 days after the infective feeding;

TABLE III.

THE RECOVERY OF LARVAE FROM EXPERIMENTALLY INFECTED RATS.

LOCATION IN HOST.	NO. OF DAYS IN HOST.	NO. OF LARVAE RECOVERED BY PRESENT AUTHOR	THOMAS	YOKOGAWA.	TOTAL NO. OF LARVAE RECOVERED
Abdominal cavity	1-4	1	2	4	
	1-5	4			17
	1-8		3, 1		
Pleural cavity	1-4	2	6	2	
	1-5	2	1		13
Lung	1-4		2	3	
	1-5	1			7
	1-8		1		
Liver	1-4		6		6
Right ventricle	10 hours		6		6
Right auricle vena cava	5 hours		9		9

(c) 7 larvae were found in crushed lung tissue, 1 to 8 days after the infective feeding; (d) 13 larvae were recovered from the pleural cavity 1 to 5 days after the infective feeding; (e) 17 larvae were obtained from the abdominal cavity, 1 to 8 days after the infective feeding. It appears from these data that the larvae first migrate from the blood to the lungs; then they traverse the pleural and abdominal cavities and by this route finally penetrate the kidneys.

SUMMARY.

1. The development of the eggs of Trichosomoides crassicauda from the segmented stage to the vermiform embryo in normal saline at 28°C takes about 67 hours.

2. The migration of the larvae in the host is discussed in the light of our own experimental data and the results of previous workers. It is shown that the larvae migrate from the intestine to the lungs in the blood stream. From the lungs they enter the pleural cavity and make their way back through the diaphragm and abdominal cavity to the kidney.

3. The morphology of the larvae and the stylet of the mature female of T. crassicauda is described.

4. The incidence of T. crassicauda in brown rats (Rattus norvegicus) examined in England was found to be 33 out of 79 (41.3 per cent). No infection was found in black rats (R. rattus) examined.

5. The relationship between the presence of T. crassicauda and mucoid calculi in the bladder of rats is discussed. The incidence of calculi is nearly twice as high in parasitized as in unparasitized rats.

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SECTION II.

STUDIES ON RETROFECTION IN ENTEROBIUS VERMICULARIS OF MAN AND ASPICULURIS TETRAPTERA OF MICE.

INTRODUCTION.

Langhans (1926) stated that the larvae of Enterobius vermicularis (Linnaeus, 1758) Leach, 1853, were found in the anal region. He believed that the mature worm migrated downwards from the intestine and laid eggs in the anal opening. If the situation was very good and the temperature was suitable, the larvae might hatch out and migrate into the bowel because in the bowel the temperature was higher than the outside of the anus. Hamburger (1939) supported this view, but he had no experimental evidence. In the last 24 years, however, nobody has found Enterobius vermicularis larvae in the anal region.

Cram, Jones, Reardon and Nolan (1939) examined 1,272 persons at Washington by the NIH swab method and stated that "not only eggs of Enterobius but also those of Trichuris, Ascaris, Necator, Hymenolepis and Taenia have been found on the swab." Mazzotti and Osorio (1942) reported finding eggs of Ascaris, Trichuris, hookworms and tapeworms by Graham's method. Stoll, Chenoweth and Peck (1947) studied the incidence of Enterobius vermicularis in natives of Guam M.I. and found 1 per cent of them infested. They stated that "it is of interest - as

bearing on the efficiency of Scotch tape in removing eggs from the anal skin area and permitting them to be diagnosed by the microscopist later - that while few Enterobius ova were encountered, the eggs of other nematode parasites were frequently found. Altogether, there were 148 hookworm, 36 ascaris and 68 trichuris positives - 8, 6 and 11 per cent, respectively - of the 634 individuals examined. In 21 instances, eggs of two or more parasitic species were present.

Madsen (1945) after rejecting the hypothesis that there is a multiplication of Enterobius vermicularis within the host, also disposed of Langhans' theory. He stated that "Thus it should be possible now to bury this old hypothesis for ever. The same applies to the conception, based upon quite casual findings of larvae in the anal region, that the larvae normally should hatch here and then crawl upwards and develop in the intestine. It haunts as late as in 1939 (Hamburger). See also Langhans (1926)."

Schuffner and Swellengrebel (1949) after citing Langhans' paper, describe how they applied Enterobius vermicularis larvae, hatched from artificial gastric juice, to the sphincter portion of the anus of three volunteers (of whom one was the senior author) and they all became infected. The mode of inoculation, namely retrograde migration of larvae hatched in the anal region, was designated "retrofection." They took care to prevent the possible occurrence of transmission by mouth and daily examined the anal region for eggs by a swab, to study the course of infection. They noticed that "in the second half of the course the appearance changed fundamentally. The intervals

(between finding the eggs in the anal region) became much longer (40 to 50 days), the periods corresponding to the life span of the females. It would appear then that in the earlier phases several generations overlapped, while later on a single generation was responsible for maintaining the continuity." "Although close agreement is most probably due to chance, this periodicity is reminiscent of the fact that 25 years ago, Heubner (1922) found in his own infection, which persisted over a period of four years, a definite rhythm of 40 to 50 days. That makes it very likely that Heubner's infection was also due to retrofection." Then the authors concluded that "The actual proofs for the retrofection have been adduced from two types of evidence, on the one hand the sequels of an acute attack and on the other hand the three parallel experiments."

Although this was the author's conclusion, they still could not explain some of their observations. They stated that "Of special interest is the question, why no retrofection occurs in one individual (vide the Amsterdam experiment), while it does so in others. Even in the same person conditions may change, as is shown in Doctor B's infections. Likewise, in the senior author's case of peranal infection, retrofection developed." In the Amsterdam experiment the senior author fed 8 volunteers with Enterobius vermicularis eggs collected from dust, and six of them became infected. Eggs appeared on the perineum 36-53 days after infection, and persisted for 12-26 days, after which the infection cleared spontaneously.

As shown above, the problem was not yet completely solved. To investigate it further, two methods of approach were possible, (1) directly; by applying infective eggs of Enterobius vermicularis to the anal opening to follow up whether they were hatched and migrate into the bowel, (2) indirectly; by studying animal oxyurids to follow up whether or not retrofection occurred. Since the eggs of Enterobius vermicularis were very difficult to obtain, we studied Aspiculuris tetraptera (Nitzsch, 1821) in mice.

Recently, Prince (1950) found larvae of Syphacia obvelata in the anal region of infected rats. He stated that "a rat was lightly anaesthetized to prevent struggling, and its anal region was washed with 10% alcohol. The washing was caught in a glass container. This liquid was then centrifuged and the sediment examined. A fairly large number of embryonated eggs and some larvae measuring 0.14 to 0.22 mm. in length were obtained in this way. In addition to unhatched embryonated eggs found on the anal region, about 25% of the eggs found were actually empty egg shells. Using water in place of the 10% alcohol, living larvae as small as 0.09 mm. in length have been recovered." Although his study throws some light on retrofection in rats, the evidence is still inconclusive.

THE DEVELOPMENT OF THE EGGS OF ASPICULURIS TETRAPTERA AND ENTEROBIUS VERMICULARIS.

Philpot (1924) stated that eggs contained in the uterus of the mature female of Aspiculuris tetraptera and those from the rectum and faeces of the host were in the segmented stage. The eggs

developed very rapidly when deposited by or pressed from the mature female. Embryos moving within the shell were found after 68 hours incubation in water at 22°C. A similar stage was reached in about 20 hours at 37°C. She found that development also proceeded very readily in saliva.

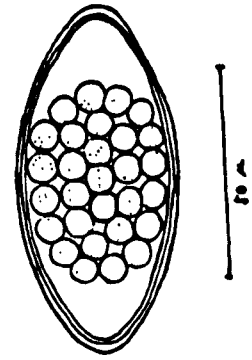
In the case of Enterobius vermicularis, she stated that at 37°C in water, the eggs showed coiled moving embryos when examined after five-and-three-quarter hours.

Zawadowsky and Schalimov (1929) stated that the eggs of threadworms would develop further after leaving the body only if they had reached the tadpole stage. If so, they would develop in normal saline, in water, in saturated copper sulphate solution, in formalin, and other media.

Heller (1944) studied the epidemiology of enterobiasis and showed that the optimal temperature for the development of the eggs was 34 to 36°C, at which temperature, they reached the infective stage in 4-6 hours. The human peri-anal region is very favourable to their development and eggs fixed there in silken bags reached the infective stage in 4-5 hours.

THE DEVELOPMENT OF THE EGGS OF ASPICULURIS TETRAPTERA IN NORMAL HORSE SERUM.

To discover whether eggs in serum discharged by hemorrhoids are readily developed or not, they were cultured in serum. The eggs (Fig. 1A), were dissected out from the uterus of the adult worm and



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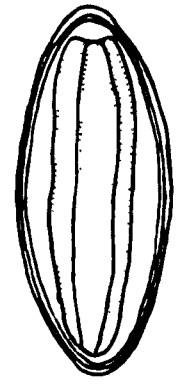

B

FIG. I.

a. Eggs of Aspiculuris tetraptera dissected out from the uterus of a female worm.

b. Egg of Aspiculuris tetraptera which has been incubated in horse serum at 25°C for 17 hours.

incubated in normal horse serum at 25°C. Seventeen hours later, small vermiform embryos were found in their shells (Fig. 1B). After 41 hours, the embryos were longer and after 65 hours incubation, the vermiform embryos were moving in their shells.

COMPARISON OF THE DEVELOPMENT OF THE EGGS OF ASPICULURIS TETRAPTERA IN SALINE SOLUTIONS OF DIFFERENT CONCENTRATIONS.

The principal component of sweat is sodium chloride and its concentration varies from 0.43 to 0.83 per cent (Talbert and Haugen, 1927). To determine, therefore, whether the concentration of saline affects the development of the eggs, they were incubated in different concentrations of saline.

The eggs used for the experiment were isolated from the faeces of six infected mice which were put in a large glass cage for one night. Their faeces were collected and comminuted in a large amount of water, and allowed to settle for half an hour; the top two thirds of the fluid and floating debris were then poured off. The sediment was resuspended with water and allowed to settle again. The upper part of the fluid was poured off and the lower part was strained through a wire basket. The strained suspension was mixed with water, allowed to settle for half an hour and the supernatant fluid was poured off. This procedure was repeated several times, until the supernatant fluid was clear. The sediment was placed in a centrifuge

tube and spun for ten minutes. The fluid was poured off. The sediment was resuspended in saturated saline and spun for another ten minutes. The eggs floating on the surface of the saline were removed by a loop and placed in a tube containing distilled water to wash out the sodium chloride. They had just developed into the segmented stage.

The eggs were transferred to watch glasses (5.3 cm. diameter) for incubation, each watch glass containing about 150 eggs. The watch glasses were sterilized in a dry heat autoclave at 140°C for half an hour and the eggs were incubated in them in about 2 to 3 c.c. of each of the various solutions to be tested. To prevent evaporation each watch glass was kept in a Petri dish containing a few c.c. of water and this was labelled with the name and concentration of the solution, the number of the dish, the temperature and the date. The Petri dishes and their contents were incubated at 28°C or 37°C for various periods after which the numbers of eggs were counted and the percentage developed were calculated.

The eggs were incubated at 37°C for 17 hours in four different solutions, i.e. 0.85 per cent, 0.65 per cent, 0.45 per cent, 0.25 per cent saline and also in pure water. The percentages of developed eggs corresponding to the above solutions were 87.9, 90.4, 93.2, 88.9 and 89.1 in water. It is apparent that below 0.05 per cent, the saline concentration has now great influence on the development of the embryo.

COMPARISON OF THE DEVELOPMENT OF THE EGGS OF ASPICULURIS TETRAPTERA IN HORSE SERUM AND WATER.

Egg were isolated from mouse's faeces which had been discharged within the previous 18 hours. They were in the segmented stage. They were transferred to four watch glasses, two of them containing water, two of them containing horse serum. Two watch glasses (one containing water and one serum) were kept at 28°C and the other two at 37°C for 24 hours. On examination, most of the eggs were fully developed and in some the embryos were moving in their shells. The only difference observed was that the proportion of moving larvae was greater in the watch glasses kept at 28°C (4 in 5) than in those kept at 37°C (1 in 8).

THE COMPARISON OF THE DEVELOPMENT OF THE EGGS OF ENTEROBIUS VERMICULARIS IN HUMAN SERUM AND IN WATER.

Eggs were discharged on a slide by a mature female worm obtained from enema specimens. They were transferred to two watch glasses (one containing water and the other containing human serum) and kept at 37°C for three hours. Some of the eggs were then taken out from each liquid for examination and were found to have developed to the tadpole stage. Five hours later, moving vermiform embryos were found in their shells. From this experiment it is concluded that both serum and water are suitable for the development of the eggs of Enterobius vermicularis.

FACTORS CONCERNING THE HATCHING OF THE EGGS OF ASPICULURIS TETRAPTERA AND ENTEROBIUS VERMICULARIS.

In the investigation of retrofection, the factors affecting the hatching of the eggs of Aspiculuris tetraptera and Enterobius vermicularis require thorough study. Under what conditions can the eggs hatch out? Are there optimum conditions for hatching? To answer these questions, a series of experiments was performed by the writer. Before describing these, it is appropriate to refer to previous work on this subject.

Zenker (1872) (see Cobb, 1890) found the larvae of Oxyuris vermicularis in the duodenum and small intestine. Cobb (1890) hatched out the larvae of Oxyuris vermicularis in the human stomach under normal conditions. He swallowed a "suction capsule" in which was placed a large number of eggs, containing fully developed larvae, which moved actively when warmed. Six hours later, he recovered it by means of purging. On opening the capsule on a warm stage, he found a large number of actively moving larvae. The capsule contained acid fluid. Gordon and Macfie (1924) hatched the eggs of Oxyuris equi in a solution of sodium carbonate (0.1 per cent to 0.5 per cent) after immersion of the eggs in hydrochloric acid (0.1 to 0.2 per cent) for half an hour. The larvae were very active. Philpot (1924) hatched the eggs of Enterobius vermicularis in water at 22°C, 25°C and 37°C and in saliva at 37°C. She tested the following solutions (1) 0.2% hydrochloric acid, (2), 1% trypsin and 0.5% sodium bicarbonate and (3) 0.3% pepsin and 0.1% hydrochloric acid. She found that all of these could kill the tadpole stage of the eggs, but (2) and (3) could

hatch out the eggs containing coiled embryos.

She also hatched the eggs of Aspiculuris tetraptera and stated that embryos could be freed from their shells to a certain extent by stirring the culture quickly or by allowing it to dry and then re-moistening it. The freed embryos showed no movement in water. Emergences from the shell was also induced both in 0.5 per cent and 0.8 per cent sodium bicarbonate.

Lentze (1932) tested the infectivity of the eggs of Enterobius vermicularis by feeding the eggs to mice and later examining the mice intestines.

Jones and Jacobs (1941) used the hatching technique to study the survival of the eggs of Enterobius vermicularis under known conditions of humidity and temperature. They hatched the eggs in a solution of 0.4 c.c. of distilled water and 0.6 c.c. of artificial gastric juice (0.7 per cent HCl and 0.5 per cent pepsin) at 37°C.

In the experiments to be described the eggs used were isolated from mouse's faeces which were collected from its cage. They were hatched in a watch glass by the same technique as that used for study on the development of the eggs of Aspiculuris tetraptera. The technique was gradually developed by us. At first we hatched the eggs in a small Petri dish, but because its bottom was flat, the eggs were not easy to find. Latterly, we used a watch glass. Since fungus was found to grow in the culture, the container was sterilized. After this technique had been developed, however, it was found that Yoshida and Toyoda (1938) had used the same method to hatch ascaris eggs.

THE RESULTS OF EXPERIMENTS ON THE HATCHING OF EGGS OF ASPICULURIS TETRAPTERA.

1. The hatching of the eggs of A. tetraptera in artificial gastric juice.

Three series of experiments were carried out at different times:

(1) The eggs of A. tetraptera were put into two watch glasses containing artificial gastric juice (0.7 per cent HCl and 1 per cent pepsin). One was incubated at 28°C, the other at 37°C. Seventeen hours later, 6 per cent of those eggs incubated at 28°C had hatched out, whereas no hatching occurred in those incubated at 37°C.

(2) The eggs were put into two watch glasses containing 50 per cent artificial gastric juice in distilled water. The results are shown in Table I. It was shown that in these dilute solutions the eggs were readily hatched. The percentage of free larvae and of emerging larvae (Fig. 2) were much higher among those incubated at 37°C.

(3) The eggs were put into a watch glass containing 25 per cent artificial gastric juice in distilled water and were incubated at 37°C. When they were examined 17 hours later, there were 32.5 per cent free larvae and after 65 hours incubation, all the eggs had hatched.

It has been mentioned above that Jones and Jacobs (1941) also hatched eggs of Enterobius vermicularis very successfully in dilute artificial gastric juice which shows that the eggs of these two parasites resemble one another in this respect.

TABLE I.

RESULTS OF HATCHING *A. TETRAPTERA* EGGS IN ARTIFICIAL GASTRIC JUICE.

% Gastric Juice in Water	Temperature.	No. of eggs	Hrs. of incubation	Percentage Hatching
50	28°C	104	14½	FL 17.4 EM 25.4 UE 57.2
50	37°C	130	14½	FL 29.3 EM 36 UE 34.7
25	37 C	160	17	FL 32.5 EM 32.5 UE 35

FL - FREE LARVAE
EM - EMERGING LARVAE.
UE - UNHATCHED EGGS.

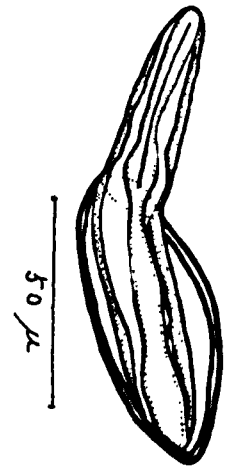

FIG. 2.

Emergence of the vermiform embryo of Aspiculuris tetraptera.

2. The hatching of A. tetraptera eggs in dilute hydrochloric acid.

As it is well known that faeces are sometimes acid and sometimes alkaline, it was of interest to determine whether the eggs of Oxyurids are more readily hatched under acid or alkaline conditions. To investigate this problem, a series of experiments was carried out.

Hydrochloric acid solutions were prepared in two concentrations. Solution (A) consisted of N/50 hydrochloric acid and Solution (B) N/25 hydrochloric acid. Each solution, together with eggs of A. tetraptera, was placed in two watch glasses, one of which was incubated at 28°C and the other at 37°C. (The watch glass which contained solution B and was kept at 28°C was broken by accident). The result of hatching is shown in Table II. The proportions of free larvae in these solutions are rather small. About 60 per cent of the vermiform embryos in their shells appeared to be dead. Some embryos were still moving in their shells. 29 hours later, all the free larvae in these solutions appeared to be dead.

3. The hatching of A. tetraptera eggs in sodium bicarbonate solution.

0.5 per cent and 0.25 per cent sodium bicarbonate solutions were used in these experiments. Two watch glasses were prepared containing each solution, one being kept at 37°C and the other at 28°C. Five hours later, no hatching had taken place in either of these solutions. After 25 hours incubation, both free larvae and emerging larvae were observed in the percentages shown in Table III. In the 0.5 per cent

TABLE II.

RESULTS OF HATCHING A. TETRAPTERA EGGS IN DILUTE HCl.

Concentration of solution	Temperature.	No. of eggs	Hours of incubation	Percentage hatching
N/50	37°C	111	4	FL 10.8 EM 2.7 UE 86.5
N/50	28°C	108	4	FL 5.5 EM 3.7 UE 90.8
N/25	37 C	105	4	FL 8.5 EM 5.7 UE 85.8

FL - FREE LARVAE
EM - EMERGING LARVAE
UE - UNHATCHED EGGS.

solution, incubated at 37°C, 21.2 per cent free larvae, 36.5 per cent emerging larvae and 42.3 per cent unhatched eggs were found, whereas at 28°C, there were 2.5 per cent emerging larvae and 97.5 per cent unhatched eggs. In the 0.25 per cent solution, incubated at 37°C, 19.5 per cent free larvae, 11.3 per cent emerging larvae and 69.2 per cent unhatched eggs were found. When incubated at 28°C, there were 1 per cent emerging larvae and 99 per cent unhatched eggs and there were no free larvae. It is clear that the 0.5 per cent solution is much more suitable for the hatching than the 0.25 per cent solution and the temperature (37°C) is also important. The free larvae found in the 0.5 per cent solution had no movement and those in the 0.25 per cent solution were moving sluggishly.

After 48 hours incubation, in the 0.5 per cent solution at 37°C, 25.2 per cent of the eggs were still unhatched whereas in the 0.25 per cent solution at the same temperature, the proportion was 42.6 per cent. At 28°C, both in 0.5 per cent and 0.25 per cent solutions, the percentage of unhatched eggs was about 95. The free larvae found in the solution appeared to be dead.

After 79 hours incubation, in both 0.5 and 0.25 per cent solution at 37°C, the larvae appeared to be dead.

4. The hatching of the eggs of A. tetraptera in normal horse serum.

Since we conjectured that the eggs might readily hatch out in serum discharged by haemorrhoids and then migrate upwards causing an infection, we incubated the eggs of A. tetraptera in horse serum and followed the results. Two series of solutions were prepared. One

was incubated at 37°C and the other at 28°C. In each series, three watch glasses were prepared. The first contained 10 per cent serum in water, the second contained 20 per cent serum in water and the third contained whole serum.

The eggs of A. tetraptera used in the experiment had just been isolated from mice faeces and had not been ripened. After nineteen hours incubation, segmented embryos had developed into the vermiform embryo stages but no free larva was found in any of the solutions. The result of hatching for 42 hours is shown in Table IV. In the series incubated at 37°C, the percentage of hatching is greater in the dilute solution. In the 10 per cent serum, two emerging larvae were moving actively. The solutions incubated at 28°C had turned red. No hatching had occurred.

After 90 hours incubation, all watch glasses were examined. In the 37°C series, the percentage of hatching, both in the 10 per cent serum and in the 20 per cent serum, had somewhat increased. In the whole serum, 9 per cent emerging larvae and 91 per cent unhatched eggs but no free larvae were found. Some larvae in their shells appeared to be dead. In the 28°C series, small percentages of free larvae were found both in 10 per cent and in 20 per cent serum. In the whole serum, there was no hatching after 90 hours, but after 119 hours, 9.7 per cent emerging larvae were found.

Another experiment was carried out to decide whether we could obtain a high percentage of hatching by continuing to transfer the eggs into fresh horse serum. We used eggs of A. tetraptera which

TABLE IV.

RESULTS OF HATCHING A. TETRAPTERA EGGS IN HORSE SERUM.

Percentage Serum.	Tempera-ture.	No. of eggs		PERCENTAGE HATCHING Hours of Incubation 42	90
10	37°C	129	FL	28.2	64
			EM	11.7	8
			UE	61.2	28
20	37°C	144	FL	8.6	44.1
			EM	8.6	8.9
			UE	82.8	47
100	37°C	121	FL	0	0
			EM	6.3	9
			UE	93.7	91
10	28°C	101	FL	0	3
			EM	0	0
			UE	100	97
20	28°C	113	FL	0	2.8
			EM	0	0
			UE	100	97.2
100	28°C	177	FL	0	0
			EM	0	0
			UE	100	100

FL- FREE LARVAE
EM - EMERGING LARVAE
UE - UNHATCHED EGGS.

had been cultured at 25°C for 3 days. 123 eggs were put into the horse serum and incubated at 37°C. 19 hours later, no free larvae and no emerging larva was found. The eggs were then transferred to a watch glass containing fresh serum and kept at the same temperature. 24 hours later (after a total of 43 hours incubation) 1.2 per cent emerging larvae and 98.8 per cent unhatched eggs were found. The unhatched eggs were transferred to fresh serum again. 24 hours later (after 67 hours incubation in all), 24 per cent emerging larvae and 76 per cent unhatched eggs were found.

5. The hatching of the eggs of A. tetraptera in human sweat.

If the eggs can hatch out in sweat, it is possible that they may give rise to retrofection in this way. To investigate it, 247 eggs were placed in sweat and incubated at 37°C. Three hours later, no hatching was found. Vermiform embryos were moving in their shells. After seven hours incubation, the result was the same. After 26 hours incubation, only 1 emerging larva was found. The sweat had a foetid smell. The vermiform embryos had no movement. After 53 hours incubation, 92 per cent of the larvae had degenerated. The experiment was repeated with the same result. Thus sweat is shown to be unsuitable for the hatching of A. tetraptera eggs.

6. The hatching of the eggs of A. tetraptera in saline.

The failure of A. tetraptera eggs to hatch in human sweat may be due to the fermentation of the sweat inhibiting the process of

the hatching. The principal component of the sweat is sodium chloride. Eggs were therefore incubated in dilute saline at 37°C and 28°C. The results are shown in Table V. This shows that 37°C is more suitable for hatching. The free larvae moving actively in the solution were found.

7. The hatching of the eggs of A. tetraptera in Albumin solution.

Egg albumin was used. Three series of watch glasses were prepared. In (a) two watch glasses were filled with albumin, in (b) two watch glasses were filled with 10 per cent albumin in distilled water and in (c) two watch glasses were filled with 5 per cent albumin in distilled water. Eggs were transferred into each watch glass and one watch glass from each series a, b and c, was incubated at 37°C and the other at 28°C. The results are shown in Table VI.

This shows that the eggs readily hatch at 37°C. At this temperature, the percentage of hatching gradually increased with time. The 10 per cent albumin solution was the most suitable for hatching. After 47 hours incubation, 24.2 per cent of the eggs in this solution had hatched, while in pure albumin only 6.4 per cent had hatched and in 5 per cent albumin only 14.2 per cent. After 118 hours incubation, the percentage of hatched eggs reached its maximum of 89.2 per cent (in 10 per cent albumin solution). At 28°C the eggs did not hatch for the first 4 days; by the fifth day, a few had hatched.

8. The hatching of the eggs of A. tetraptera in the urine of mice.

The eggs of A. tetraptera were isolated from mice faeces and incubated at 25°C for 6 days. 180 eggs were then transferred to mice urine and

TABLE V.

RESULTS OF HATCHING A. TETRAPTERA EGGS IN SALINE.

Percentage Saline	Temperature	No. of eggs	PERCENTAGE HATCHING	Hours of Incubation 19	43	69
0.28	37°C	138	FL	9.8	19.3	21.7
			EM	11	8	6.4
			UE	79.2	72.7	71.9
0.28	28°C	107	FL	0	1	18.7
			EM	6.4	7	6.2
			UE	93.6	92	75.1

FL - FREE LARVAE
EM - EMERGING LARVAE
UE - UNHATCHED EGGS

TABLE VI.

RESULTS OF HATCHING A. TETRAPTERA EGGS IN ALBUMIN.

Percentage Albumin.	Temperature	No. of eggs	PERCENTAGE HATCHING	Hours of Incubation 22	47	70	118
100	37°C	120	FL	0	3.2	10.1	78.1
			EM	1.1	3.2	8.9	6.8
			UE	98.9	93.6	81.0	15.1
10	37°C	83	FL	1.4	13.9	56.4	89.2
			EM	2.9	10.3	11.6	0
			UE	95.7	75.8	32	10.8
5	37°C½	167	FL	1.2	5.4	50	85
			EM	.6	8.8	17.2	0
			UE	98.2	85.8	32.8	14.1
100	28°C	110	FL	0	0	0	7.7
			EM	0	0	0	0
			UE	100	100	100	92.3
10	28°C	181	FL	0	0	0	19
			EM	0	0	0	0
			UE	100	100	100	81
5	28°C	94	FL	0	0	0	13.7
			EM	0	0	0	0
			UE	100	100	100	86.3

FL = FREE LARVAE
EM = EMERGING LARVAE
UE = UNHATCHED EGGS.

incubated at 37°C. 24 hours later, 41.2 per cent free larvae and 38.8 per cent emerging larvae were found. After 48 hours incubation, 91.9 per cent empty shells were found. After 90 hours, in all, 97 per cent empty shells were found. Although the larvae hatched out, in no instance was any movement observed. This indicates that the larvae cannot survive for long in urine.

9. The hatching of the eggs of A. tetraptera in Water.

A large number of A. tetraptera eggs, just isolated from mice faeces, were transfefred to a small Petri dish and incubated at 28°C. In the first three weeks, no free larva was found and the vermiform embryo in their shells had a normal appearance. One month later, free larvae and emerging larvae were found. After two months incubation, they were still being found. The vermiform embryos in the shells appeared to be alive and most of the eggs had hatched. After a total of three months, free larvae were still found. In the shells, the embryos appeared to be normal but some were degenerate.

Eggs of A. tetraptera containing vermiform embryos in their shells were dried for 24 hours. They were moistened by water and incubated at 28°C for 17 hours. On examination, 6.4 per cent free larvae and 12.9 per cent emerging larvae were found.

10. The hatching of the eggs of A. tetraptera in sugar solution.

0.1 per cent aqueous sugar solution was prepared. The eggs of A. tetraptera with developed vermiform embryos were put into the solution. 48 hours later, a few free larvae and emerging larvae were found. The

results are shown in Table VII. After 120 hours incubation, the percentage of hatching was still small. The experiment was repeated with the same result.

11. The hatching of A. tetraptera eggs in Look's solution.

Three series of solutions were prepared; (a) Look's solution, (b) 50 per cent Look's solution in water and (c) 25 per cent Look's solution in water. Each solution was used for preparing two watch glasses. One watch glass was incubated at 37°C and the other at 28°C. Four hours later, only one emerging larva was found in Look's solution incubated at 37°C. After 98 hours incubation, the number of eggs hatching at 37°C was in inverse proportion to the concentration of the solution. In Look's solution, the free larvae and emerging larvae were 3 per cent. In 50 per cent Look's solution, they were 11 per cents in 25 per cent Look's solution they were 32.8 per cent. The eggs which were incubated at 28°C, had 1 per cent emerging larvae in 25 per cent Look's solution. The results are shown in Table VIII.

12. The hatching of the eggs of A. tetraptera in dilute gastric juice and sodium bicarbonate solution.

Eggs of A. tetraptera were incubated in 50 per cent gastric juice in water for half an hour at 37°C. Then they were transferred to 0.5 per cent sodium bicarbonate solution and incubated at 37°C for four hours. No free larva was found. After twenty-one hours incubation, free larvae were found but no movement.

TABLE VII.

RESULTS OF HATCHING A. TETRAPTERA EGGS IN SUGAR SOLUTION.

Percentage Solution	Temperature	No. of eggs	PERCENTAGE HATCHING	Hours of Incubation 48	120
0.1	28°C	110	FL	1	2.6
			EM	0	1.3
			UE	99	96.1
0.1	37°C	100	FL	3.1	10
			EM	14.3	8
			UE	82.6	81.3

FL - FREE LARVAE
EM - EMERGING LARVAE
UE - UNHATCHED EGGS

TABLE VIII.

RESULTS OF HATCHING *A. TETRAPTERA* EGGS IN LOCK'S SOLUTION.

Percentage	Temperature	No. of eggs	PERCENTAGE HATCHING	Hours of Incubation 4	98
100	37°C	100	FL	0	1
			EM	1	2
			UE	99	97
50	37°C	100	FL	0	4
			EM	0	7
			UE	100	89
25	37°C	119	FL	0	27.6
			EM	0	10.2
			UE	100	67.2
100	28°C	100	FL	0	0
			EM	0	0
			UE	100	100
50	28°C	100	FL	0	0
			EM	0	0
			UE	100	100
25	28°C	100	FL	0	0
			EM	0	1
			UE	100	99

FL - FREE LARVAE
EM - EMERGING LARVAE
UE - UNHATCHED EGGS

THE HATCHING OF THE EGGS OF ENTEROBIUS VERMICULARIS

To obtain material for this study, enema specimens from persons infected with E. vermicularis were brought from hospital. The faeces were comminuted and mixed in a large amount of tap water in a tray and after sedimentation the supernatant fluid was decanted. This procedure was repeated several times until the water in the tray was clear. The worms were picked out and washed in normal saline and then transferred to clean distilled water or normal saline. Eggs were obtained after being discharged by the living worms or, in a few cases, the gravid worms were transferred to a clean slide to let them discharge their eggs, which were then used for our experimental work. Most of the worms collected from enema specimens were not fully developed so that the eggs obtained from them were not very satisfactory for the hatching test because most of them were immature. Frequently the eggs did not develop at all and sometimes only a small portion of them developed. In only a few cases did the eggs develop very well. Although this was not altogether satisfactory, it was the only way available for us to obtain material for experimental work. Gravid female worms were selected and used for the following experiments:

(1) The eggs were dissected out from a gravid female E. vermicularis and incubated in water at 23°C for 18 hours. Vermiform embryos developed in all of them and many free larvae were seen moving actively in the water. More than 100 eggs were counted, of which 11.7 per cent were empty shells, 29.1 per cent were in the act

of hatching and 59.2 per cent were unhatched eggs. Five hours after this counting, the free larvae were dead. After 42 hours incubation, there were 85.5 per cent empty shells, 1 per cent hatching and 13.5 per cent unhatched eggs. After a total of 66 hours incubation, 98 per cent empty shells and 2 per cent unhatched eggs remained.

(2) 150 eggs discharged by a female E. vermicularis were incubated in water at 37°C for 16 hours at the end of which time 5.5 per cent free larvae, 22 (30.5) per cent hatching and 64 per cent unhatched eggs were found. After 30 hours incubation, all the eggs had hatched out.

(3) Eggs discharged by a female worm were incubated in normal saline at 28°C for 77 hours. Only a small proportion of the eggs developed vermiform embryos. A few were hatching and there were a few actively moving free larva.

(4) 200 eggs discharged by a female E. vermicularis in normal saline were kept at 37°C. 19 hours later, 31 per cent and, 23 hours later, 58 per cent of the eggs had developed vermiform embryos but no free larvae nor emerging larvae were found. After 122 hours incubation, 3 per cent empty shells, 14 per cent hatching and 83 per cent unhatched eggs were found.

(5) A few eggs containing vermiform embryos were transferred to different concentrations of saline and incubated at 37°C for 23 hours. The results were as follows:-

a) In 0.85 per cent saline, 7 free larvae were found and 3 of them were moving actively.

b) In 0.65 per cent saline, 11 free larvae were found and 2 of them were moving actively.

c) In 0.45 per cent saline, 27 free larvae were found; 3 of them were active.

d) In 0.25 per cent saline, 11 free larvae were found; 3 of them were active.

(6) Eggs containing vermiform embryos were used to carry out the following experiments:

a) The eggs were transferred to 50 per cent artificial gastric juice in distilled water and incubated at 37°C for 17 hours. All of them hatched, producing free larvae which moved actively.

b) The eggs were transferred to N/50 hydrochloric acid solution and incubated at 37°C for 16 hours. None of them hatched out but after 64 hours incubation, 7 free larvae were found.

c) The eggs were transferred to N/25 hydrochloric acid solution. 16 hours later, no free larva was found. After 64 hours incubation at 37°C only one free larva was found.

The above experiments (b and c) on the hatching of the eggs of E. vermicularis in dilute hydrochloric acid solution were repeated. On this occasion, the eggs were discharged in water and incubated at 37°C for one night but no development of vermiform embryos took place. On transferring them to a piece of wet filter paper and incubating for another night, embryos developed in a few eggs. These

were transferred to two dilute hydrochloric acid solutions (N/50 and N/25) and incubated at 37°C. 23 hours later, 17 free larvae were found in the first solution and 34 in the second. None of the larvae were motile.

(7) Eggs containing vermiform embryos were transferred to a 0.5 per cent sodium bicarbonate solution and incubated at 37°C for 17 hours. One free but inactive larva was found and several eggs were in the act of hatching. The experiment was repeated under the same conditions except that the incubation period was 23 hours. 10 free larvae were found but they were not moving.

(8) Eggs containing vermiform embryos were transferred to a 0.25 per cent sodium bicarbonate solution and incubated at 37°C for 17 hours. Five free larvae were found but they had no movement.

(9) Eggs containing vermiform embryos were transferred to human sweat and incubated at 37°C for 48 hours. A few emerging larvae were found but no free larvae.

(10) Eggs containing vermiform embryos were transferred to human serum and incubated at 37°C for 17 hours. No larvae hatched out. After 73 hours and 93 hours incubation, the embryos were still in their shells. The experiment was repeated and gave the same result.

(11) Eggs containing vermiform embryos were transferred to 10 per cent albumin in water and incubated at 37°C for 5 hours. Two free larvae were moving actively. 23 hours incubation produced 9 free and 3 emerging larvae. The larvae lived for 18 hours.

(12) To determine whether eggs contaminated with urine could hatch out or not, we carried out the following experiment. Eggs containing vermiform embryos were transferred to a slide and dried for 3 hours. They were then moistened with several drops of urine and the slide was put in a Petri dish containing several c.c. of water to prevent the evaporation of urine. After incubation at 37°C overnight, three emerging larvae were found. Two days incubation produced one free and three emerging larvae. Many of the eggs did not hatch.

(13) Segmented eggs discharged by a gravid female E. vermicularis were transferred to a piece of cellophane. This was placed on the right inguinal region of the author, so that the eggs were in contact with the skin. The cellophane was then covered by some cotton wool and a piece of paper which were held in place by 3 strips of adhesive plaster and left for 24 hours. The cellophane was then removed and a piece of Scotch tape was applied to the area to recover the eggs from the skin. To prevent infection, the skin was sterilized with alcohol. Eggs containing vermiform embryos were found in both the cellophane and Scotch tape but there was no evidence of emergence nor of free larvae.

OBSERVATIONS ON THE BIONOMICS OF THE LARVAE OF ASPICULURIS TETRAPTERA.

About 30 eggs of A. tetraptera were transferred to 0.25 per cent saline and incubated at 37°C. 17 hours later, 4 free larvae were found in the solution. After 21 hours incubation there were 7 free

larvae, 4 of them moving actively. The unhatched eggs were removed and afterwards the larvae were examined regularly at two-hour intervals. One of the seven larvae lived 44 hours while three of them lived 28 hours. On the first day they moved actively but on the second day they were sluggish. In two other observations, 7 larvae lived for 25 hours but were not very active.

It was observed that larvae emerging from their egg shells have active movements which later become abated. Since we thought that this phenomenon might be caused by the lower temperature during the time of examination, further observations were therefore made on a warm stage.

Larvae hatched in 0.25 per cent saline were transferred to saline on a hollow slide and covered with a cover glass. When the slide was put on a warm stage kept at about 35°C, the larvae were seen to curve and straighten actively but they did not migrate.

Measurements were made at intervals on two larvae with the following results:

First measurement:

No. 1. Length 131.75 ; width 23.375 ;
Length of oesophagus 55.25 .

No. 2. Length of oesophagus 55.25 ;
Length 136.00 ; width 25.5 .

The slide was then put into a Petri dish containing a few drops of water and kept at 37°C for 18 hours after which they were measured again.

Second measurement:

No. 1. Length 148.75 ; width 23.375 ;
Length of oesophagus 55.25 .

No. 2. Length 165.75 ; width 25.5 ;
Length of oesophagus 55.25 .

Their movement at this time was sluggish. They were incubated at 37°C for another 24 hours and again measured.

Third measurement:

No. 1. Length 170 ; width 23.375 .

No. 2. Length 182.75 ; width 25.5 .

These two larvae had died and were somewhat degenerate. The posterior ends of the oesophagus in both were not clear and this structure could not be measured.

Another two larvae were transferred to a hollow slide from 0.45 per cent saline and kept at room temperature. These had very sluggish movements and did not increase in size even after 30 hours.

THE HATCHING OF THE EGGS OF ASPICULURIS TETRAPTERA ON THE MOIST COTTON WOOL SWAB.

The hatching of the eggs of A. tetraptera in different solutions has been demonstrated above. It was of interest to ascertain also whether or not they can hatch out on a moist surface, so a series of

tests was planned to investigate this problem. An NIH swab tube was used in which the cellophane was replaced by a piece of cotton wool at the end of the glass rod. It was moistened with a drop of 0.25 per cent saline and kept moist by about 3 c.c. of water which was poured into the test tube. Eggs of A. tetraptera, just isolated from mice faeces, were transferred to the cotton wool swab which was then replaced in the test tube but was not in contact with the water. In all, four tubes were prepared and incubated at 37°C. After 24 hours about 1 c.c. of normal saline was dropped on a large slide and the cotton wool swab from one of the tubes was gently stirred in it to wash the eggs onto the slide. When examined under a binocular microscope it was found that the eggs had developed to the vermiform embryo stage but no free larvae had hatched. After 48 hours the second tube was examined. 3 free larvae, 17 emerging larvae, 3 empty shells, 29 embryonated eggs and 3 unembryonated eggs were found. After 72 hours, the third tube was examined and 13 free larvae, 30 emerging larvae, 12 empty shells, 6 embryonated eggs and 10 unembryonated eggs were found. After 96 hours the fourth tube was examined and 13 free larvae, 31 emergences, 13 empty shells, 60 embryonated eggs and 3 unembryonated eggs were found.

THE HATCHING OF THE EGGS OF ASPICULURIS TETRAPTERA ON THE SKIN OF A VOLUNTEER.

Although the eggs of A. tetraptera were thus shown to be able to hatch out on a moist cotton wool swab, it was still not certain whether or not they could hatch on moist skin. Three experiments were carried out to ascertain this.

EXPERIMENT I.

About 40 segmented eggs of A. tetraptera were applied to the left forearm of the author and were covered by a piece of cellophane which was overlapped by a piece of cotton-wool. These were held in position with adhesive plaster. 18 hours later embryos had developed in the shells but no free larva was found.

EXPERIMENT 2.

Eggs of A. tetraptera (just isolated from mice faeces) were transferred to a piece of cellophane by means of a loop. In some of them vermiform embryos had formed, while others were still in the segmented stage. No free larvae were present. The cellophane was then attached to the right inguinal region of the author, covered by a piece of cotton wool and held in position with adhesive plaster. 42 hours later, 75 eggs were found on the cellophane but there were no free larvae. Some eggs had a degenerate appearance. Any remaining eggs or larvae adhering to the skin were removed by means of Scotch tape. 40 eggs but not free larvae or empty shells were found in this way.

As a control, other eggs from the same batch as used in the above experiment were applied to a cotton wool swab moistened with saline. They were incubated at 37°C and 42 hours later they had hatched.

A third experiment was carried out using the same method except that the eggs were applied to the skin for an extended period of 67 hours. The results were also negative.

THE HATCHING OF THE EGGS OF ASPICULURIS TETRAPTERA IN THE ANAL OPENING OF A VOLUNTEER.

As shown above, the eggs of A. tetraptera would not hatch on human skin either of the forearm or of the inguinal region. It was thought possible, however, that they might hatch in the anal region. A series of experiments were carried out to determine this.

EXPERIMENT I.

Eggs of A. tetraptera were isolated from mice faeces and cultured in water at 24°C for 4 days. 371 eggs were transferred to a piece of cellophane which was inserted into the anal opening of the author at 12 noon on January 6th, 1950. 22 hours later the cellophane was taken out and put on a drop of water on a slide. Another drop was added to the surface of the cellophane and covered by a coverglass. On examination, three eggs of A. tetraptera were found, one with a vermiform embryo which had degenerated to half the normal size, while the other two had a normal appearance.

EXPERIMENT 2.

In the second experiment, eggs of A. tetraptera were cultured at 25°C for 66 hours. A large number of them was transferred to a piece of cellophane and examined under a binocular microscope. No free larvae or emerging larvae was found. The cellophane was inserted into the anal opening of the author. A small number of the same batch of eggs was transferred to 0.25 per cent saline and incubated at 37°C as a control. 24 hours later the cellophane was

removed. The anal region was swabbed by an NIH swab and any remaining eggs or larvae were removed by a piece of Scotch tape. A few drops of 70 per cent alcohol was put on a slide. The cellophane was put into 70 per cent alcohol on a slide, covered with a coverglass and examined. One larva, two emerging larvae, two empty shells, 13 eggs containing normal vermiform embryos and one egg with a degenerated embryo were found. On the NIH swab, ~~two empty shells and~~ there were two empty shells and two emerging larvae ~~were found~~ and on the Scotch tape swab, two empty shells and 8 eggs. In the control batch of eggs kept in 0.25 per cent saline there were several free larvae.

EXPERIMENT 3.

Eggs of A. tetraptera were isolated from mice faeces and cultured in water at 25°C for one night after which time they developed vermiform embryos. They were transferred to a piece of cellophane and examined to ensure that no free larvae nor emerging larvae were present. The cellophane was inserted into the anal opening of the same volunteer and 24 hours later it was taken out and put in 10 per cent formalin solution. Any other eggs or larvae remaining adhered to the skin were removed by means of Scotch tape. 12 empty shells (Fig. 3) and 30 eggs of A. tetraptera were found on the cellophane and 19 eggs on the Scotch tape.

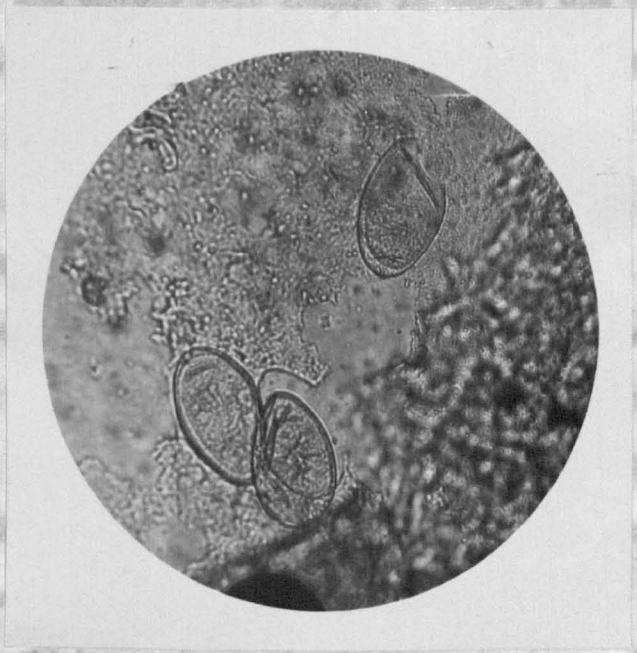

FIG. 3.

Eggs of Aspiculuris tetraptera hatched out in the anal opening of a human volunteer. Empty shells are shown.

EXPERIMENTS ON RETROFECTION IN ASPICULURIS TETRAPTERA.

To investigate the possible occurrence of retrofection of A. tetraptera in a mouse, a special cage was designed (see Fig. 4) to immobilize and to prevent it licking its anal region. The cage was one inch in height and width, four inches in length and was made of perforated zinc with a lide of the same material. At the front part of the lid, two holes were made to supply food and water. A funnel containing wheat was connected with one hole in the cage so that the mouse could eat continually, and a tube of water was connected with the other hole. A hole was made at the bottom of the back of the cage so that the faeces of the mouse could drop out, and another one at the front for cleaning out waste food. At the back of the cage a small hole, through which the tail extended, was made. The cage was fixed down on a wooden base and the tail of the mouse was also stuck to it by a piece of adhesive plaster. In this way the mouse was immobilized but very completely housed.

EXPERIMENT I.

Eggs of A. tetraptera were isolated from the faeces of six infected mice and incubated in distilled water at 25°C for 19 hours, after which time they contained larvae but the larvae had not emerged from their shells. On January 12th, 1950 at 12 noon a large number of these eggs was applied to the anal region and vagina of a mouse (No. 18) whose faeces had been repeatedly examined

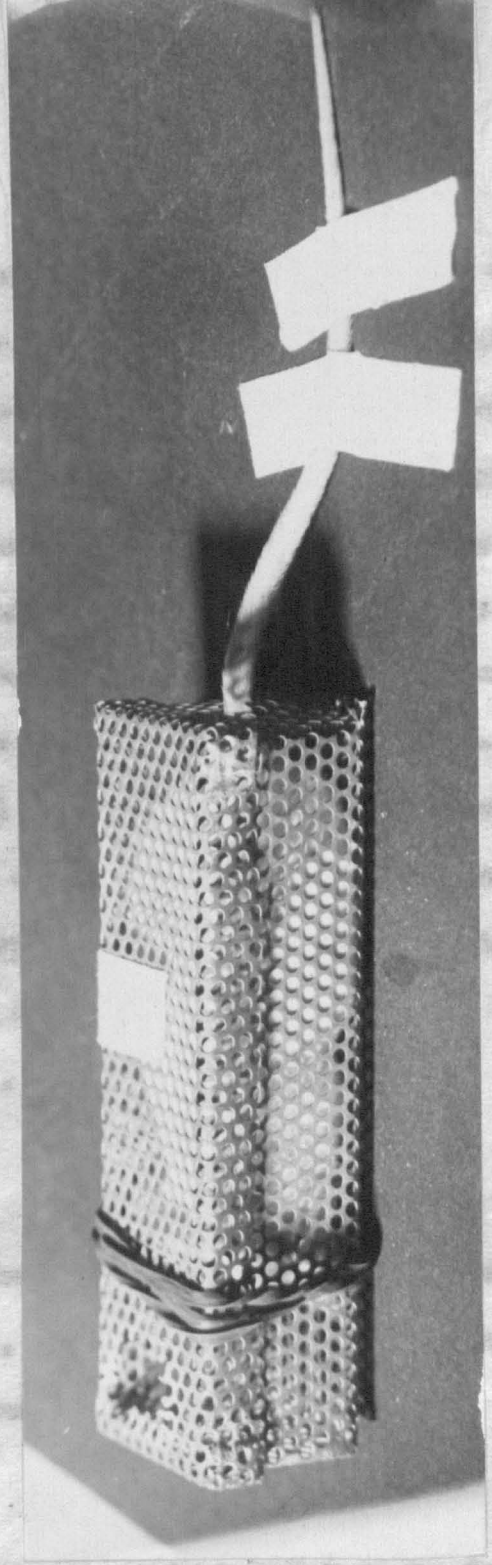

FIG. 4.

The cage used for immobilizing experimental mouse.

both by direct smear and by flotation technique for one month and had proved negative for A. tetraptera eggs. The mouse had been starved for 18 hours to reduce its bowel movement and during the experiment it was immobilized for 46 hours in the special cage described above which had been sterilized in boiling water for 10 minutes. It was then killed and its anal region was dissected out for examination. The anus was washed in a small quantity of 10 per cent formalin which was examined under a microscope. Two free larvae, one empty shell and one egg were found. The colon was opened in a Petri dish containing several c.c. of normal saline. Three larvae of A. tetraptera were found, one of which is shown in Fig. 5. They measured as follows:

1) Length 148 , width 17 .
2) Length 148.75 , width 19.12 .
3) Length 144.5 , width 17 .

Caecum, small intestine, stomach and oesophagus were negative for parasites. Vagina, uterus and bladder were also dissected and examined but all were negative.

EXPERIMENT 2.

Eggs of A. tetraptera were isolated from the faeces of six infected mice and cultured at 25°C for 18 hours.

No. 16 mouse was kept in a round glass cage from December 22nd, 1949 onwards and its faeces were repeatedly examined both by direct

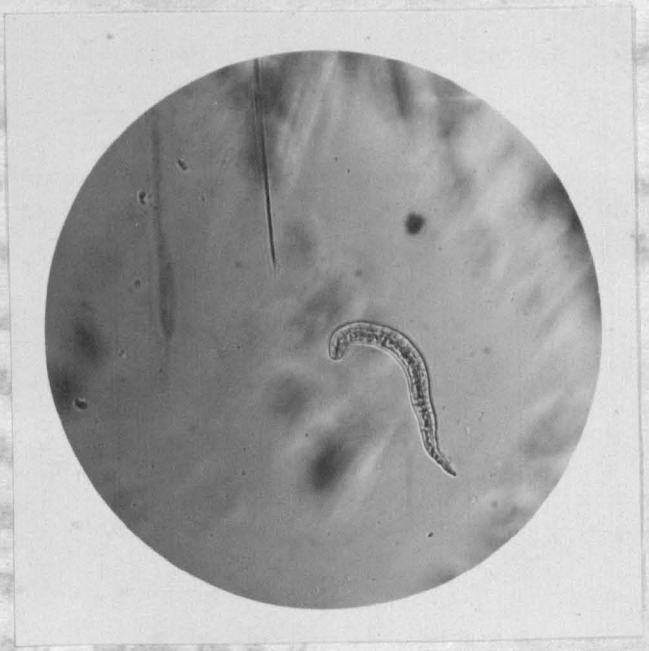

FIG. 5.

Larva of Aspiculuris tetraptera found in the colon of mouse No. 18 (Experiment 1). Infective eggs had been applied on the anal region of the mouse for 46 hours.

smear and by flotation technique. It was proved negative for parasitic eggs. The same mouse was starved from January 16th, 1950 at 5p.m. for 17 hours to avoid the discharge of large amounts of faeces. On January 17th, at 10a.m. a large number of the eggs of A. tetraptera prepared as above were applied by a loop to the anal region of this same mouse which was immobilized in the cage already described. It was fed on small amounts of wheat and water for 74 hours and finally killed by chloroform.

The digestive tract was carefully examined. From the colon, five larvae were found and one of them is shown in Fig. 6. Their sizes were given as:-

1) Length 144.5 μ, width 17 μ.
2) Length 144 μ, width 17 μ.
3) Length 148 μ, width 21.2 μ.
4) Length 157.25 μ, width 21.25 μ.
5) Length 212 μ, width 25.5 μ.

From the caecum one larva was found. Its size was measured as:-

Length 180 μ, width 22.2 μ.

Anus, rectum, small intestine and stomach were examined but all were negative.

EXPERIMENT 3.

No. 20 mouse was kept in a small round cage from January 27th, 1950 onwards. Its faeces were repeatedly examined both by direct smear and by flotation technique. It was proved negative for any

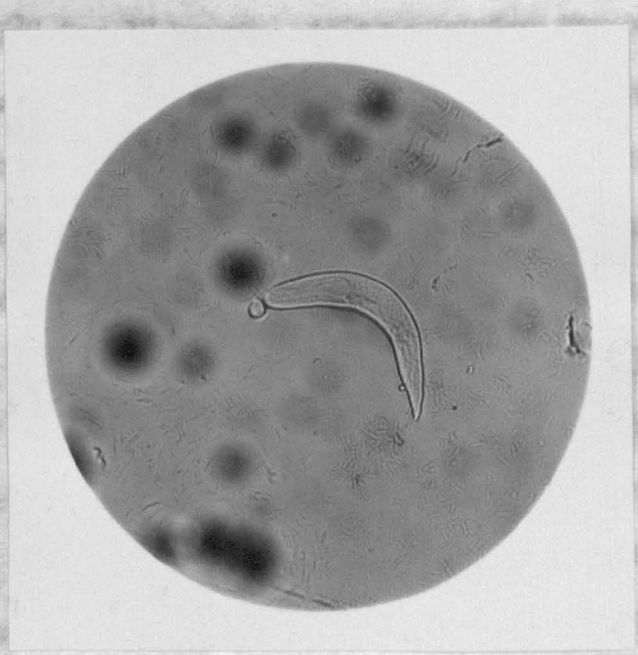

FIG. 6.

Larva of Aspiculuris tetraptera found in the colon of mouse No. 16 (Experiment 2). Infective eggs had been applied on the anal region of the mouse for 74 hours.

parasitic eggs. From 5p.m. on March 6th, 1950 it was starved for 18 hours. Its anal region was then wetted with 0.25 per cent saline and a large number of eggs of A. tetraptera were applied to the anus by means of a loop. The eggs had been isolated from the faeces of six infected mice. Some of them were cultured at 25°C for 130 hours and the others at 37°C for 18 hours. After applying the eggs to the anal region, the mouse was immobilised by the technique described above. It was fed on wheat and water for 48 hours and then killed by chloroform. On examining the digestive tract carefully, two larvae were found in the colon and their measurements were as follows:-

1) Length 135 μ ; width 17 μ ; oesophagus and bulb 51 μ.

2) Length 147 μ ; width 15 μ ; oesophagus and bulb 59.5 μ.

The caecum contained a single larva whose measurements were:

Length 153 μ ; width 19.125 μ ; oesophagus and bulb 57.375 μ.

The rectum, small intestine and stomach were carefully examined but with negative results. From the anus, one egg of A. tetraptera was recovered.

EXPERIMENT 4.

Mouse No. 8 was fed in a small glass cage from September 22nd, 1949 onwards. Its faeces were collected weekly for examination by direct smear and flotation method. It was proved to be free of parasitic eggs. On November 14th, it was immobilised in a cage and three thousand eggs of A. tetraptera which had been cultured in water at 25°C for 6 days were injected into the anus.

On November 15th its faeces were examined by direct smear and one egg of A. tetraptera was found. On November 16th all the faeces discharged by the mouse were collected and examined by flotation method, and eight eggs of A. tetraptera were recovered. On November 18th two eggs were recovered from the faeces by the flotation method. Subsequent examinations were negative. The vermiform embryos of these eggs appeared to be viable and no empty shells were found.

On the morning of November 28th the mouse was found to have jammed itself in the cage in attempting to turn round. When it was released it was in a critical condition. It was therefore removed to a large round glass cage and three hours later it had recovered.

On November 29th it was chloroformed and its abdominal wall was opened. The alimentary canal was removed and the contents of the oesophagus, stomach, small intestine, caecum and colon were examined separately. In the colon two immature A. tetraptera were found which measured as follows:

1) Length 1152 μ; width 102 μ; Length of oesophagus 276.25 μ; Length of tail (from anus to the posterior end) 140.25 μ.
2) Length 1116 μ; width 97.75 μ; Length of oesophagus 165.75 μ; Length of tail 140.25 μ.

EXPERIMENT 5.

Mouse No. 15 was kept in a round glass cage from December 22nd, 1949 onwards. Its faeces were examined every week by both direct smear and flotation technique and proved to be negative. On January

24th, 1950, 430 eggs of A. tetraptera were applied to its anal region. These eggs had been isolated on January 23rd and incubated at 25°C for one night. After being immobilized for four days in the special cage the anal region and the hairs on the buttock, abdomen and posterior legs were sterilized with a 10 per cent formalin solution and the mouse then released and fed in a glass cage. Its faeces were examined but no egg was found up to April 3rd. On April 4th after 18 hours starvation, a large number of eggs of A. tetraptera were again applied to the anal region of the same mouse and it was immobilized in the special cage. The eggs used for this experiment had been isolated from the faeces of six infected mice and incubated at 37°C for 18 hours. The special cage used to immobilize the mouse had been sterilized in boiled water for ten minutes.

In the special cage it was fed with oats and its appetite was very good. On April 6th it was released and fed in the glass cage after being cleansed with formalin as described above. Up to April 28th, its faeces were negative but on May 5th, two A. tetraptera eggs were recovered.

On May 8th, the mouse was chloroformed and the colon, caecum, small intestine and stomach were carefully examined. Six adult female worms of A. tetraptera were found in the colon; no other parasites were present. They measured as follows:-

1) Total length 3726 μ ; width 252 μ ; oesophagus 450 μ.
2) Total length 3726 μ ; width 261 μ ; oesophagus 426 μ.
3) Total length 3672 μ ; width 243 μ ; oesophagus 441 μ.
4) Total length 3366 μ ; width 243 μ ; oesophagus 432 μ.
5) Total length 3600 μ ; width 252 μ ; oesophagus 468 μ.
6) Total length 3078 μ ; width 225 μ ; oesophagus 450 μ.

EXPERIMENT 6.

Mouse No. 40 was kept in a sterilised round glass cage from March 16th, 1950 onwards. Its faeces were examined weekly both by direct smear and by flotation technique and proved negative. On April 12th at 1.30p.m. A. tetraptera eggs which had been incubated at 37°C for 18 hours were applied to the anal region of the mouse which was immobilized for three days. On April 15th, its anal region, the hairs of abdomen, buttock and hind legs were sterilized with 10 per cent formalin and it was then transferred to the round glass cage.

On May 12th, one egg of A. tetraptera was found in its faeces. On May 15th, the mouse was chloroformed and its alimentary tract was carefully examined. From the colon three adult female A. tetraptera were recovered. One of them had been cut into two pieces during opening of the intestine. The other two measured as follows:-

1) Length 3906 μ ; width 180 μ ; length of oesophagus 414 μ.
2) Length 4041 μ ; width 207 μ ; length of oesophagus 441 μ.

EXPERIMENT 7.

On March 16th, 1950 mouse No. 41 was isolated in a sterilized round glass cage. Its faeces were examined weekly by both direct smear and flotation technique and proved negative. On April 12th, eggs of A. tetraptera isolated from mice faeces and incubated at 37°C for 18 hours, were applied to the anal region and the mouse was immobilized. On April 15th, it was released from the special cage and after sterilizing its anal region, the hairs of abdomen, buttock and hind legs with 10 per cent formalin, it was transferred to a round glass cage. On May 16th, four eggs of A. tetraptera were found in its faeces. The mouse was chloroformed and its colon, caecum, small intestine and stomach were examined. From the colon, four females and two male A. tetraptera were recovered. They measured as follows:-

Females:

1) Length 4176 μ; width 199.8 μ; Length of oesophagus 414 μ.
2) Length 4122 μ; width 201.6 μ; Length of oesophagus 395 μ.
3) Length 3906 μ; width 216.0 μ; Length of oesophagus 432 μ.
4) Length 3249 μ; width 162.0 μ; Length of oesophagus 387 μ.

Males:

1) Length 3141 μ; width 144.0 μ; Length of oesophagus 315 μ.
2) Length 3204 μ; width 153.0 μ; Length of oesophagus 360 μ.

CONTROL FOR THE EXPERIMENTS ON RETROFECTION IN ASPICULURIS TETRAPTERA.

From March 7th, 1950 onwards, mouse No. 35 was isolated in a sterilized round glass cage. Its faeces were examined weekly by both direct smear and flotation technique. No egg was found. On May 18th, the mouse was chloroformed and its colon, caecum, intestine and stomach were carefully examined but no parasite was found.

Although only one mouse was used as control in this experiment the following six mice with negative results, in spite of experimental attempts to induce retrofection, may be regarded as additional data. On six occasions eggs in the infective stage of A. tetraptera were applied to the anal region of mice (Nos. 10, 12, 17, 21, 45, 56) which had been examined for a period ranging from 4 to 12 weeks and proved negative to natural infection of A. tetraptera. Three of them were killed to investigate whether larvae of A. tetraptera were present after 2 to 7 days application of eggs, but no larvae or adults were found in the alimentary canal. Subsequent examination of the faeces of the other three mice for eggs were negative. These mice were killed at the end of 5 weeks or more and the alimentary tract contained no larvae or adults.

THE PROBLEM OF INTERNAL AUTO-INFECTION OF ENTEROBIUS VERMICULARIS.

The theory of internal auto-infection was propounded by Vix (1860) (vide Seurat 1920) who had seen some larvae in course of

hatching in the rectal mucosa. Still (1899) studied 200 autopsies of children under 12 years of age. Oxyuris vermicularis was found in the intestine in 38 cases (19 per cent). In 25 out of those 38 cases, the worm was found in the appendix. He concluded that "the generally accepted view that every single ovum of Oxyuris vermicularis must be swallowed before it can be hatched is at least open to doubt and there is a strong probability that the appendix vermiform serves in some cases as a breeding place for threadworms." His theory was based upon the following two findings -

(1) Most of the young worms were found in the colon and caecum and only once were they found in the lower end of the small intestine. He stated, "I have found young threadworms once as high as 45 inches above the ileocaecal valve and two or three times I have seen mature worms either just above the valve or in the last few inches of the ileum, but such a finding has been very exceptional in my experience."

(2) In five heavily infected cases, the hands were left unwashed after the night's rest. The dirt on the finger ends and under the nails of all the fingers was then scraped off and examined microscopically. In three of the cases on 2 occasions and two of them once, the result of the examination was that one solitary ovum was found in the dirt under the nail of one child out of the five. On further investigation, the results were the same. He stated that "It might be added that although there is experimental evidence to prove that the swallowing of the ova is followed after a few weeks by the

appearance of threadworms in the faeces, the proof that every threadworm that is passed from the bowel has been introduced in this way, is yet to seek."

Rodenwaldt and Rockmann (1921) reported the case of a 21 year old girl, from whose diarrhoeic stool young Oxyuris vermicularis larvae were found for 11 consecutive days. The authors proposed two possible explanations -

(1) The patient may have re-infected herself by ingesting an adult female worm and the developing larvae have passed straight through the gut on account of the diarrhoea or (2) development of the eggs may have occurred in the intestine in internal auto-infection without the eggs having first passed to the exterior, the larvae in this case finding their way to the exterior owing to the diarrhoea.

The authors consider that the first explanation is not probable because during the 11 days no adult female was found in the stools and if the adult worm had been ingested before the examination of the faeces was commenced, the larvae in the diarrhoeic stools towards the end of the 11 days would have been more developed than those found at the beginning, which was not the case.

Koch (1925) stated that the eggs of Enterobius vermicularis were able to hatch and produce internal re-infection.

Langhans (1926) found that larvae or dead adults of Oxyuris vermicularis could be found in diarrhoeic stools.

Lubieniecki (1931) stated that there are individual differences in patients so marked that in many, but not in all, Oxyuris multiplies without leaving the gut.

Zawadowsky and Schalimov (1929) studied the development of the eggs of Enterobius vermicularis and concluded that since threadworm eggs cannot develop beyond the tadpole stage without oxygen and since this is absent in the gut, auto-infection was impossible.

Hall and Cram (1930) stated that "Evidence that Enterobius vermicularis may cause auto-infection by reproduction of successive generation within the host is lacking."

Heller (1946) carried out an observation on Enterobius vermicularis and Passalurus ambiguus to find out whether internal auto-infection takes place and concluded that the results obtained seemed to show that in Oxyurids, internal auto-infection is impossible.

Faust (1949) stated in his "Human Helminthology" that, "As previously stated, there is no convincing evidence of internal auto-infection."

Brumpt (1949) who discusses this question has expressed the opinion that the known facts do not permit belief in a multiplication of the parasites in the intestine.

We thought that it would be interesting to tackle this problem experimentally so the following test was carried out.

Six naturally infected mice were transferred to an empty glass cage and they were only allowed to drink water for 17 hours. Faeces were collected from the cage for isolating eggs of A. tetraptera. The eggs were examined under the microscope and it was found that segmented embryos were contained in their shells except 4 of them

containing small vermiform embryos. They were mixed with about 0.2 c.c. of water and injected into the anus of a mouse (No. 25) with a small blunt pipette. The pipette was inserted into the anus about 1 cm. The mouse had been repeatedly examined and it was proved that no egg was found from its faeces. It was starved for 22 hours to reduce the frequency of bowel movement. After injection, the mouse was immobilized in a special cage to prevent its eating its faeces. It was fed with a small amount of food. It died 20 hours later. The anus, colon, caecum, small intestine and stomach were examined and no eggs of A. tetraptera were found. The faeces contained in the intestine were examined by flotation technique. One egg was found but its appearance was like those of injection, except that the shell was lightly shrunken. Five eggs were found from the discharged faeces, two of them like those of injection. Two eggs were slightly developed. One egg developed but had not reached the vermiform embryo stage. According to our previous experiment, the eggs isolated in the above manner could develop into the vermiform embryo stage within 17 hours. But in this experiment, the eggs did not develop in the intestine even 20 hours later. This may be an evidence that the eggs of Oxyuris cannot develop in the intestine.

Another theory of internal auto-infection was propounded by Penso (1932). He studied Oxyuris ambiguus in the rabbit and found that it discharged its eggs under the intestinal mucosa. He stated in Enterobius vermicularis, penetration of the mucosa for purposes

of egg-laying may therefore by assumed to be not unusual. The author concluded that auto-infection is not sufficient to explain the tenacity and duration of Oxyuriasis and that the worms can multiply without at any time leaving the body of the host. On this view the adult phase and the period of coitus occur in the lumen of the intestine and the egg-laying and larval phases are in the intestinal wall. The anal migrations of gravid females are to be explained as ensuring the diffusion of the parasite from individual to individual.

Penso (1933) brings forward, as confirmation of his view that Oxyurids complete their life cycle in one host, a statement by Wetzel (1931) that in Dermatoxys veligera of the hare he has demonstrated larvae in the thickness of the mucosa. But while Wetzel believed that the larvae were entering the mucosa, Penso is satisfied that they were doing just the reverse.

Penso (1935) calls attention to the importance of the life-cycle of Oxyuris, and reiterates his theory.

Wendt (1936) found that the eggs of Enterobius vermicularis could not develop in hydrogen or carbon dioxide. He concludes that oxygen is essential to their development. He admits that sufficient oxygen might be present within the intestinal wall but urges that demonstration of infective eggs and larval stages in the mucosa is necessary before the theory can be accepted.

This theory poses two problems - (1) Does Enterobius vermicularis discharge its eggs in the intestinal mucosa and (2) Can the eggs of Enterobius vermicularis develop and hatch out in the

tissue? Concerning the first point, we think that it is pertinent to review the literature to see whether eggs have been found in the intestinal mucosa. Concerning the second point, we thought that it would be interesting to carry out an experiment to find out whether the eggs can develop or not.

REVIEW OF THE LITERATURE.

Cecil and Buckley (1912) studied 129 cases of appendicitis in children. They found that in 17 of them the appendix contained Oxyuris vermicularis. In two cases, Oxyuris vermicularis was found in the mucosa. In one of them hyaline Oxyuris was found completely encapsulated in fibrous tissue. Invasion of lymph follicle by a male oxyuris was found in another section. There were no inflammatory changes in the tissue surrounding the parasite. No egg was found in the tissue.

Innes and Campbell (1914) studied 100 appendices from cases of appendicitis and found that 17 were infected with Oxyuris vermicularis. Since they did not carry out histological examination, we did not know whether there were eggs in the tissue or not.

Harris and Browne (1925) found 22 cases infected with O. vermicularis in an uninterrupted sequence of 121 cases of operative appendicitis. From the faeces contained in the appendix, they found Oxyuris eggs in five cases. In a number of instances, the worms were found buried in the mucosa. At times only the head of the worm had penetrated and at others from one half to three quarters of the nematode had entered into the mucous membrane. The parasites had

produced marked ulceration. The epithelium was totally destroyed and at times excavations had occurred underneath. The destruction of tissue extended through the submucosa and involved a portion of the musculature. They stated that "Occasionally in a section, an ovum of the Oxyuris is found deep in the mucosa. While it is possible that these may have been expressed from the parasite, the absence of the latter in the adjacent serial structure renders this less likely." We thought that the ovum found in the deep mucosa, may have reached there through the ulcerative lesions. It is well known that the female Oxyurids discharge a great number of eggs within a short time. The finding of a single ovum in the mucosa cannot be reconciled with its being discharged by the female worm there.

Gordon (1933) studied 26,051 appendices received for routine diagnosis at the pathological laboratories of the University of Michigan during the period from July 1st, 1894 to October 31st, 1932. The specimens were obtained from residents of Michigan State. He found 311 appendices (1.19 per cent) containing Oxyurids. The parasites were present in the lumen alone in 256 instances (82.31 per cent). In 22 others, however, while Oxyurids were present in the lumen, they were also intimately associated with the mucosa. In this group the mucosa showed shallow half moon-shaped depressions approximately corresponding in size and shape to the worms. Of the remaining 33 appendices, 17 had pinworms both in the submucosa and in the lymphoid follicles, 12 contained them in the submucosa alone,

and 4 in the lymphoid follicles only. Evidence is adduced to show that in these 33 appendices, penetrations occurred after operations in every instance. He stated that "In no instance was there any sign of a more severe or advanced degree of inflammation at levels containing the worms than at levels free from parasites. This type of invasion is therefore of no pathologic or clinical significance."

Beck (1934) found Oxyurids in 35 out of 1718 (2.03 per cent) appendices. He examined the appendices immediately after their removal to determine whether the penetration of Oxyurids was pre-operative or post-operative. The parasites were found to be present in the lumen of the appendix in all appendices studied. No case were they found in the lymphoid follicles or submucosa, nor intimately related to the mucosa. Sections made at points where the parasites had been found did not show the shallow half-moon shaped depressions or erosions approximately corresponding in size and in shape to the worms; this indicated that they had not been intimately related to the mucosa. He stated that "Examining the appendices immediately after removal by the method I have used tends to confirm this theory of Gordon's."

Goodale (1934) investigated a series of 1639 consecutive appendices, 101 (6.1 per cent) had Oxyuris vermicularis in varying numbers in the lumen. He did not mention whether or not there were eggs in the tissue of appendices.

Ugiie (1935) studied appendicitis connected with intestinal parasites. Among 330 appendices, there were 20 (6.1 per cent) with Oxyuris. The initial symptoms indicating acute appendicitis consisted in irritation of and mechanical injury to the mucous membrane, and were evidently caused by the invasion by the parasites. They used the name "Appendicopathia oxyurica" proposed by Aschoff. This condition was found in 16 of the 20 Oxyuris cases. In the other somewhat more advanced case, there appeared not only slight lesions of the mucous membrane such as fissures and haemorrhages but also, and starting from these points, an invasion by various bacteria, setting up inflammation and thus constituting appendicitis simplex. Among his material, four oxyuris cases belonged to this category. According to his report, he did not find Oxyuris eggs in the appendical wall.

Warwick (1935) examined 2,344 appendices and found 45 (1.9 per cent) containing Oxyurids. In two of them the parasite was buried in the wall of the appendix, in one in the mucosa and in another in the sub-mucosa. The author agreed with Gordon's conclusion that the parasites might have penetrated the wall of the appendix after removal of the organ. She stated, "But in this hospital, it is the custom to place each appendix into an ice-box directly after removal and this may account for the fact that only two parasites penetrated the wall before they were overcome with the cold."

Botsford, Hudson and Chamberlain (1939) found that 71 of 1,343 appendices removed at the Children's Hospital, Boston from 1929 to 1939 were infected with pinworms. In three cases, portions of pinworms were found in the submucosa. These authors did not report finding any eggs in the mucosa.

Wax and Cooper (1941) reported a series of 1,016 cases of appendicitis and found 8 cases (0.007 per cent) infested with Oxyuris vermicularis. They stated that, "The oxyuris is, however, also found in apparently normal appendices, in the submucosa as well as in the lumen. Often little or no inflammatory reaction is found about the oxyuris; but while it may be impossible to demonstrate to demonstrate the point at which the organisms penetrate the mucosa, they are supposed to produce characteristically shallow haemorrhagic ulcers in the mucosa."

Ashburn (1941) studied a series of 2,317 surgically removed appendices. Oxyurids were found in 184 cases (7.94 per cent). In 79 of these they were present in the sectioned material and in 105 Oxyurids were found only in the removed appendical contents. Of the 79 cases showing Oxyurids in the lumen of the sectioned appendix, 13 showed the worm in contact with mucosa; in 7 of these there was pressure atrophy of the epithelium with formation of shallow crescent-shaped depressions. In a few instances there was a little karyorrhectic necrosis, but no inflammation or haemorrhage was found. One appendix showed an Oxyurid in the deep part of the mucosa. The lack of inflammation, necrosis or haemorrhage shows that the invasion occurred post-operatively.

Rector (1943) studied 210 appendices and found 10 or 4.76 per cent were infected with Enterobius vermicularis. He stated, "As to whether Oxyuris vermicularis parasites found embedded in the appendical wall represents an in vivo or a fixation phenomenon, it may be stated that in no instance was such a migration of the parasites seen in this series in which all appendices were opened and emptied in the fresh state before immersion in fixative."

Bijlmer (1946) reported a case of a 46 year-old man who died after four days in hospital in Rotterdam during which time he passed daily 10 to 20 thin stools mixed with blood. The pathological diagnosis was, "Acute ulcerative exacerbation of a chronic entero-colitis throughout rectum and colon and some ulcers in the ileum due to Oxyuriasis." In eight pieces of the colon with a total area of 4 square cm. there were more than 80 worms in aggregation in the submucosa and the subserosa. From a number of such pieces examined, it was estimated that there were roughly 10,000 worms in the rectum and colon. All the worms were very small, in particular the females. The average length of 172 males was 1,550 μ and of 26 females 1,677 μ. Not one female containing eggs was found. In the submucosa the worms were concentrated around the ulcers and accordingly they were often surrounded by large numbers of leucocytes; sometimes the worms were lying in an abscess but very often the tissue did not seem to react at all to the invasion. No egg was found in the tissue. He stated,

"It remains unsettled which was pre-existent, the ulceration or the invasion of the worms. The author himself leans to the first interpretation."

Professor Buckley (1949) demonstrated a slide of Enterobius vermicularis in the intestinal wall. This specimen was a section through the transverse colon of a 56 year old Hausa man who died of gangrene of the lung. A few chronic hypertrophic ulcers were found in the colon and one of these had apparently been invaded by large numbers of adult Enterobius vermicularis. There was no egg or larva in the tissue.

According to these records, the penetration of worms into the intestinal wall is not very common. The finding of an ovum in the intestinal wall was only reported once (Harris and Browne 1925) and it had apparently reached there through ulcerative lesions. We feel that it is unreasonable to use this extraordinarily uncommon phenomenon to explain the frequency and tenacity of Oxyuriasis.

EXPERIMENT ON THE DEVELOPMENT AND HATCHING OF ASPICULURIS TETRAPTERA EGGS IN TISSUE.

To determine whether or not oxyurid eggs can develop in tissue the following experiment was performed. First, we incubated the eggs of A. tetraptera in penicillin solution and proved that they could develop in it. Then an infected mouse was killed. From its colon, nine mature female A. tetraptera were obtained. The worms were washed with distilled water and then with penicillin solution

(100,000 units in 10 c.c. distilled water). Eggs were dissected out in penicillin solution by an aseptic technique and most of them were injected into the abdominal wall of a clean mouse. Next day, the area of injection on the mouse was examined and no inflammation was noticed. 10 days after injection, the mouse was killed and the hairs on the area of injection were shaved. The abdominal wall in the area of injection was fixed in 10 per cent formalin solution for tissue section. Unfortunately, from the tissue sections neither free larvae nor eggs were found.

The remaining eggs dissected out from the female worm above were incubated in penicillin solution at 37°C for control. 48 hours later, 46 eggs had developed into vermiform embryos in their shells. 54 eggs were still undeveloped. After 70 hours, 59 eggs had developed into vermiform embryos and 41 were still undeveloped. In the field observed, five emerging and three free larvae were found. After 94 hours, 60 eggs had developed into vermiform embryos, 40 had not. In this field, 16 emerging and two free larvae were found. 9 days later 69 eggs had developed; 31 eggs had not. Among these eggs, 22 emerging larvae and 3 free larvae were found.

Although we cannot say whether the eggs of A. tetraptera can develop in the tissue or not, four points seem clear; (1) the occurrence of the egg of E. vermicularis in the intestinal wall is very uncommon, (2) the penetration of the worm, E. vermicularis, into the intestinal wall frequently occurs after operation, (3) nobody has reported finding larvae of E. vermicularis in the intestinal wall,

(4) the larvae of E. vermicularis cannot penetrate the skin of a baby mouse. (Philpot, 1924). Consequently it seems that the theory propounded by Penso cannot be accepted.

DISCUSSION.

Rodenwaldt and Rockemann (1921) and Langhans (1926) reported the finding of young larvae of Oxyuris vermicularis from diarrhoeic stools. They assumed that they had hatched in the intestine. According to our experiments, the explanation seems to be that the larvae are swept away with the diarrheic stool after hatching in the anal region.

Langhans (1926) stated that the larvae of Enterobius vermicularis had been found in the labia and vagina. From our experiments, the eggs of Enterobius vermicularis did hatch in urine and albumin. We believe his finding is possible.

According to Schuffner and Swellengrebel's (1949) experiment, the larvae of Enterobius vermicularis can migrate from the anal region to the intestine and grow into adults. It is shown that both the eggs of Enterobius vermicularis and Aspiculuris tetraptera can hatch out in water, dilute acid solution, dilute alkaline solution, dilute artificial gastric juice, albumin solution and saline. The eggs of the former hatch much more readily in any solutions than those of the latter. Since the latter can hatch in the anal region, we therefore believe that the former can as well.

Philpot (1924) fed Aspiculuris tetraptera eggs to mice and 18 hours later found larvae with an average length of 150 in the caecum; 44 hours later she again found larvae with an average length of 184 in the caecum; 90 hours later she found larvae with an average length of 218 in the large intestine. She stated that "A migration from the caecum to the large intestine occurred at about this time."

In our first experiment on retrofection, we found larvae with an average length of 147.08 in the large intestine of a mouse when it was killed 46 hours after applying the eggs in the anal region. In our second experiment, we found five larvae with an average length of 161.15 in the large intestine and one with a length of 180 in the caecum. In view of the size of the larvae and the organ in which they were found it seems unlikely that infection originated from contamination by mouth.

In our fourth experiment on retrofection, four days after the injection of the eggs of Aspiculuris tetraptera into the anus of the mouse (No. 8) only the eggs were found in the faeces. However, no empty shells were found. This indicates that the eggs cannot hatch in the intestine. On examination, however, two immature adult worms were found in the colon. The probable explanation is that during defaecation some of the eggs adhered to the anal region and then hatched out enabling retrofection to take place.

Sawaitz, D'Antoni, Rhude and Lob (1940) stated that "The usual hygienic measures such as frequent house cleaning, change of underwear and bed sheets and frequent bathing did not interrupt the important transmission routes of Enterobius. A cleanliness period of six weeks in one home did not result in any decrease in the infection. The surprising result of an actual increase in incidence from 38 to 51 per cent shows that the measures taken did not reduce the opportunities of infection. Inhalation probably does not play an important part in this infection." If transmission is only by hand and by air, the usual hygienic measures should control the disease, or decrease the incidence. However, the facts are quite different. In this case, if we assume that retrofection is occurring, it is easy to understand why normal hygienic measures do not reduce the incidence of infection.

Recently McMullen (1949) studied the course of a pinworm infection case. The patient was a 43 year old man. The diagnosis was made on 15th January, 1947. Four other members of his family were also infested with pinworms. A Scotch tape swab was taken every morning before bathing and defaecating. On 27th May, 1947 the patient left the United States for Japan. Baths were taken at least once a day and sometimes as many as three were taken in a 24 hour period. Swabs were examined until 31st March, 1948. During the period of 503 days examinations were made on all but nine days.

SUMMARY.

This part of the thesis describes an attempt to determine the validity of theory of retrofection and auto-infection in Enterobius vermicularis. The results of observations on the development and hatching of the eggs of Enterobius vermicularis in various solutions are given.

Owing to difficulties experienced in obtaining an adequate amount of material of Enterobius vermicularis, it was decided to determine whether retrofection and auto-infection occur in a closely allied oxyurid Aspiculuris tetraptera of mice. Details of various methods used for observing the development and hatching out of the eggs of Aspiculuris tetraptera are given. The bionomics of the larvae are described. It was found that the eggs could hatch out on moist cotton wool swab, in the anal opening of a human volunteer and the anal region of mice. Infective eggs were applied in the anal region of clean mice which were immobilized in specially designed cages to prevent their turning. On examination after a few days, adolescent forms were obtained in the colon in four cases. After 30 to 34/40 days, eggs of Aspiculuris tetraptera were found in the faeces of three experimental mice. On autopsy, both female and male adult worms were found in the colon of the experimental mice. The experiments prove that retrofection can occur in the mouse Oxyurid, Aspiculuris tetraptera. The theory of internal auto-infection is discussed in the light of our own experiments and the data in the literature.

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