THE ROLE OF TOXOCARAL LARVAE IN THE TRANSMISSION OF MICROBIOLOGICAL INFECTION

by

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ABSTRACT

The purpose of this research was to study the relationship between the Toxocara canis larvae and the transmission of bacteria in mice. Special attention was given to the attempt to understand the mechanisms by which the larvae could play a role in the dissemination of infection. Radioactive isotopes were used to label the Escherichia coli bacteria. Three isotopes were used, one β emitter (^{32}P) and the two others were ^{59}Fe and ^{51}Cr as γ emitters. Exposure of the larvae to labelled bacteria, both in vitro and in vivo resulted in obtaining evidence that they could carry the bacteria.

When the experiments were done in vivo, the results strongly suggested that the T. canis larvae were able to disseminate the bacteria from the intestine to all the organs of the animals tested. The attempt to check whether the larvae exposed to the bacteria in a test tube could disseminate bacteria to the organs of mice, showed inconclusive results. Confirmatory experiments were carried out by using bacteriological and serological techniques. Evidence was obtained that the larvae were able to carry and might be able to disseminate the E. coli to the organs of the mouse.

The results will be discussed in relation to the possible dangers of these worms in the dissemination of disease-causing micro-organisms from the intestine to other parts of the body.

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INTRODUCTION

The Toxocara canis larvae, after emerging from the ova in the intestine, undergo a somatic migration and are able to travel in the blood to any organ in the body, including the brain. This raises the question of whether these larvae, leaving a heavily contaminated system like the alimentary canal, might be able to transport or facilitate the transmission of microbiological organisms to the other parts of the body, including the brain, thus causing severe damage like encephalitis or poliomyelitis; and some other bacterial or viral infections in other parts of the body to which the larvae might migrate, such as hepatitis or respiratory infections. The results obtained by Woodruff et al.(1966) support the possibility of the toxocaral role in facilitating poliomyelitis. They used the highly specific toxocaral skin test after the antigen was standardized by Woodruff and Thacker (1964). The results demonstrated that there were much higher percentage (13.6) positive results in patients who had had poliomyelitis, than in apparently healthy control persons (2.1%).

It was with this idea in mind that the present investigation was undertaken and the Escherichia coli bacteria were chosen on the grounds that they are normal inhabitants of the human intestine. The aim of this investigation

is to explore the possibility of larval migration facilitating microbiological infection of the central nervous system and other organs of the body and the problem of dual bacterial and helminthic infections, with special reference to the study of the mechanism or mechanisms of transmission.

LITERATURE REVIEW

The parasite T.canis was originally identified by Werner (1782) and was clearly differentiated from its related species, particularly Toxocara leonina by Leiper (1907). The full development of the worm was not studied until done so by Sprent (1958). The adult worms occur in the intestine of various species of the genus canis and in several species of foxes and the male worm measures up to 10 cms and the female up to 18 cms. The worm has special adaptations for transmission. It has long been known that infection may be acquired by ingestion of eggs or by prenatal transfer of larvae from the tissue of the mother, where they may be stored for a year or more (Douglas and Baker, 1959) to those of the foetus (in utero) where they remain in the lungs until birth (Schacher, 1957; Sprent, 1958). The result is that puppies are very commonly infected with Toxocara canis - the older dogs less commonly so. It has been observed by several workers that in older animals, especially sexually mature females for a short time beginning about one month after whelping that they frequently have a patent infection. Sprent (1961) suggested that such infections are acquired by ingesting larvae passed in the faeces of the new-born pups. The other mode of infection is through paratenic hosts, that is animals acquiring infection from contaminated soil and passing the larvae on to other animals and finally

to the dog when eaten as prey. This mode of transmission was demonstrated by Sprent (1958). Dogs and foxes were fed tissues of mice which had been infected with T.canis previously. In foxes adults and larvae were found in the intestine at 2-3 weeks after infection. Larvae were also recovered from the somatic tissues especially the lungs. In dogs very few larvae were found in the somatic tissues. As 3 days after infection second stage larvae were found in the stomach wall, at 9 and 10 days after infection fourth stage larvae were found in the intestine. In puppies under the age of 5 weeks, the larvae can go on to mature into adult T.canis, probably pursuing a cycle of development similar to that of Ascaris lumbricoides in man (Sprent, 1952; Woodruff, 1970); that is being taken to the liver or lung in the blood and there leaving the blood vessels to migrate up the bronchial tree and re-enter the intestine.

In man when infective T.canis eggs are swallowed, the larvae emerge from the eggs in the human intestine, penetrate the intestinal wall and are taken in portal blood to the liver and the lungs, and usually beyond them to other tissues throughout the body. Once in a blood vessel, the larvae appear to leave it at a point at which its body approaches the diameter of the vessel (Sprent, 1955a). The larvae of T.canis have been shown to reach

the lungs of mice at a relatively early stage of infection and to undergo a somatic migration (Sprent, 1952). The minimum diameter of these larvae is more than 0.01 mm and less than 0.02 mm at the time they reach the lungs. Most probably the larvae gain the arterial circulation by breaking the capillaries and entering the pulmonary veins. Observation of the brain of mice infected with T. canis indicated that the lumen of the cerebral arteries approximates the diameter of the larvae at the point at which they enter the surface of the brain (Sprent, 1955b). This was indicated by the occurrence of early haemorrhages at specific sites, mostly near the dorsal surface of the brain in mice experimentally infected with T. canis; later haemorrhages in other parts of the brain indicating that the larvae had begun to migrate through the brain tissues. (Woodruff, 1970), suggested that this is also likely to explain the somewhat uniform position of granuloma caused by larvae of T. canis emerging from retinal blood vessels (Duguid, 1961a and 1961b). He also suggested that it explains further why Ascaris larvae are filtered out of the circulation in man in the liver and lungs and do not pass in the blood beyond these organs to other parts of the body.

Prevalence in dogs:

Toxocara canis is the commonest round worm of the dog,

few dogs escaping infection (Beaver et al., 1952). It has cosmopolitan distribution as the following figures indicate.

Overall % of dogs infected	% of infected puppies	Place
13.5	not reported	Marseilles, France (Solomon, 1933)
82	" "	Calcutta, India (Maplestone and Bhaduri, 1940)
76.5	" "	Phillippines (Yutuc, 1954)
21	36	Indiana, U.S.A. (Ehrenford, 1956)
20	not reported	Boston, U.S.A. (Heiner and Kevy, 1956)
20.7	" "	Home Counties, England (Woodruff and Thacker, 1964)
82.5	" "	Cairo, Egypt (Rifaat et al., 1969)
28	" "	Dar-es-Salaam, Tanzania (Wiseman and Woodruff, 1971)
36.5	" "	Ibadan, Nigeria (Wiseman and Woodruff, 1971)
41	" "	Cairo, Egypt (Khaled et al., 1973)
18.1	" "	Belgium (Vanparijs and Thienpont, 1973)
35.1	63.8	Australia (NG and Kelly, 1975)
7.3	22.2	Great Britain (Jacobs and Pegg, 1976)
7.2	30.5	London, England (Turner and Pegg, 1977)

The figures indicate that there is a very high prevalence of infection in dogs in tropical areas. Although the percentage of dogs infected in the U.S.A. and Europe is much lower than that of the tropical areas, it is still high

in the former areas when taking into consideration the high level of the standard of health services and health education. The figures in the Philippines, India and Egypt could be compared with the previous survey in Cambridge, England, in which 24 dogs were examined and 70.8% were found to be infected (Nuttall & Strickland, 1908). No adequate previous survey had been done in England prior to that done by Woodruff and Thacker (1964). Beaver (1962), stated that "T. canis can be expected to be present throughout the tropics, sub-tropics and much of the cooler regions". This statement goes with the reports from Egypt, Tanzania and Nigeria as African countries, and the Philippines and India as Asian countries. In areas where there have been dependable surveys one is led to the conclusion that there is a large reservoir of infection with toxocariasis in dogs. Bearing in mind the close contact between man and dog, transmission of infection to man would be expected to be common.

Diagnosis in man:

When man ingests the infective toxocaral eggs the larvae will emerge and migrate in the tissues, but do not develop beyond the second stage in the human. Unlike Ascaris they do not mature, enter the gut, and reveal their presence by passing ova. As a result the only method of ascertaining a definite diagnosis is by finding the larvae in the tissues

by biopsy techniques. Whenever the infection has been demonstrated in the liver during life, it has been by open operation and removal of a granuloma visible on the surface (Beaver et al., 1952).

Beaver et al. (1952) reported the first human case infected with T. canis larvae in a girl aged 2½ years who had severe eosinophilia (80,000 WBC/cmm with 50% eosinophils). Laparotomy disclosed an enlarged liver studded with white plaques. Sections from the liver showed portions of a larval nematode that was well preserved and showed no evidence of autolysis or degeneration. It was proved to be a Toxocara canis larva.

The clinical syndromes which toxocariasis is known to cause do not justify biopsy except in very few suspected cases. It was shown by Woodruff (1970) that the diagnosis of Toxocaral infection can be strongly suspected on clinical features of the patient - the presence or absence of eosinophilia - and by using the skin test and fluorescent antibody technhique. Duguid (1961b) observed delayed skin responses in patients with ocular lesions suspected of being toxocaral in origin, but cross-reactions with sera from patients with A. lumbricoides had been obtained. Woodruff & Thacker (1964) were able to standardize the skin test by following the method of Fairley (1931). The standardized antigen was used at a

dilution of 1/1000 and results obtained with this dilution were highly specific. Adult specimens of T. canis were dried and aqueous solution of antigen were prepared from them. The sterile aqueous solutions were used for intradermal sensitivity tests. In carrying out the skin test 0.1 ml of the solution was injected and when the initial weal doubled its size the reaction was considered positive. The test was carried out among 85 persons with evidence of helminthic infections. The helminthic infections proved by demonstration of the parasite, their ova or larvae. Included in this group of helminthic infections were Trichuris trichiura, hookworm, Schistosoma mansoni Ascaris lumbricoides, Strongyloides stercoralis, Onchocerca volvulus, Loa loa and Trichostrongylus colubriformis. Among all this group of patients, using antigen in 1/1000 dilution, the test was positive in only two. The results, therefore, indicated the skin test for T. canis gives no significant number of cross-reactions in other helminthic infections when used in 1/1000 dilution. Further evidence of the value and specificity of the skin test has been demonstrated on animals by Wiseman & Woodruff, 1967; Wiseman et al, 1969. Six mice were infected with 200 - 600 T. canis eggs and the skin test was observed to be positive from the ninth day of infection. No reaction occurred in control non-infected animals. Experiments on monkeys showed the same results with positive skin even in those

animals who were infected with 20 eggs only.

Bisseru & Woodruff (1968) reported the successful use of the fluorescent antibody test as a tool for the diagnosis of toxocaral infection. T.canis larvae exposed to immune serum, obtained from patients proved to have the toxocaral infection either by histological examination or by positive skin test, followed by fluorescein labelled antiglobulin, gave a bright greenish-yellow fluorescence of the cuticle. Best results were obtained when the larvae were alive or used within three days of recovery from mice. Cross-reacting fluorescence was obtained when serum from patients with A.lumbricoides was used. These cross-reactions were removed by using adsorption of the Ascaris antibodies with the extract of Ascaris lumbricoides. Patterson et al. (1975) reported the use of radioimmunoassay systems for toxocariasis, but it was not possible to give a clear diagnosis on whether the parasite was Ascaris or Toxocara. The pathological and clinical damages due to the migration of the Toxocara larvae in man could be responsible for facilitating or provocating microbiological infections. The clinical picture of the type of larva migrans caused by T.canis in man varies widely with the number of larvae ingested and the duration of infection and the presence of larvae in critical locations. Before the application of the skin test and the fluorescent antibody test, toxocariasis used to be thought to be a disease of young children

with a history of dirt eating (pica). Since the application of these two tests together with accumulated knowledge about the clinical features and mode of transmission of the toxocara, it has been shown that the disease is not limited to young children only. Children have a higher incidence than adults due to the fact that they are more liable to get the disease from public places and dooryards, because of their poor hygenic habits. Beaver (1952) reported the presence of Toxocara canis ova in dooryards in the United States. Borg & Woodruff (1973) reported that they were able to find Toxocara canis in 195 out of 800 soil samples collected in public places all over Britain.

Many patients complain of no symptoms. In others, although able on questioning to recall symptoms, these were not severe enough to have led the patients to seek medical aid (Woodruff, 1965). The general symptoms resulting from toxocaral infection in man consist most commonly of hepatic discomfort, asthmatic and bronchitic episodes and fever. Further general symptoms resulting from toxocaral infection in man found on physical examination are hepatomegaly, usually moderate but sometimes extreme.

In many of the cases of toxocariasis, liver enlargement has been noted. Usually the hepatomegaly is moderate, but in some cases extreme, the liver edge reached well

below the umbilicus (Snyder, 1961). In mild infections such as those reported by Smith & Beaver (1953) hepatomegaly was not noticed. Smith & Beaver (1953) infected two mentally defective infants with 200 T. canis eggs. In both cases eosinophilia was a major feature but hepatomegaly was not noticed in one patient; in the other the liver was just palpable during the ensuing month. Splenomegaly is frequent but not of great degree. Leucocytosis and eosinophilia are common (Snyder, 1961; Jung, 1963; Beaver, 1963). Beaver (1962) reported that in four-fifths of selected cases of toxocariasis the eosinophils represented more than 50% of leucocytes in the blood and in approximately 20% of cases the eosinophils represented over 60% of more than 60,000 W.B.C. per cubic mm. In some cases the eosinophilia exceeded 80% (Zuelzer & Apt, 1949; Snyder, 1961).

Signs of pulmonary infiltration, such as cough, dyspnoea, rales, wheezing or even radiological evidence of pulmonary infiltration have been observed in many of the toxocara cases reported (Beaver et al., 1952; Snyder, 1961; Patterson et al., 1975). Woodruff (1970) reported that 17% out of 76 patients with asthma, bronchitis and cough, mostly in association with eosinophilia, have given positive skin tests.

In addition to the above signs and symptoms there are a

number of somewhat less characteristic conditions which have been associated with toxocariasis, like frequent upper respiratory infections, gastro-intestinal disturbances or abdominal pain, pallor or anaemia.

Toxocara larvae migrate widely in the tissues and so may traumatize some important parts of the body and provoke deposition of fibrin and fibrosis, which if it occurs in some vital parts such as the heart and brain, could lead to dangerous complications. The presence of toxocaral larvae in the myocardium was demonstrated by Dent et al. (1956) in their study at necropsy of a child who died from homologous serum hepatitis with overwhelming T. canis infection. Another patient, reported by Friedman and Hervada (1960), who had several attacks of fever, bronchitis, pneumonia, 37% eosinophilia and had been in contact with a dog in whose faeces ova of T. canis was found, there was a marked increase in the size of the heart, which returned to normal as the other clinical features settled down. A liver biopsy was not performed so that a definite diagnosis could not be made, but the circumstantial evidence led to the belief that it was a toxocaral infection. Woodruff (1965) suggested that involvement of the myocardium in this way may play a part in the production of the endomyocardial fibrosis in Africa and elsewhere. Woodruff (1970) reported a patient that had been seen by Professor M.S.R. Hutt in East Africa.

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The patient was a 25 year old sweeper who had had a history of vague substernal discomfort lasting about a month, but this had not been incapacitating and he had carried on with his work until he suddenly developed pain in the chest, became short of breath, produced copious sputum and was admitted to hospital. Pulmonary oedema was present on admission and he died within a few hours. At necropsy his lungs were grossly oedematous, but no parasites of any kind were detectable in the bowel; the heart showed extensive myocardial degeneration with gross eosinophilic cell infiltration. Professor Hutt remarks that the eosinophils in some areas appeared to be arranged around a central region of necrosis, reminiscent of worm tracks and the changes appear to be those of parasitic infiltration.

On a similar basis it has been suggested that granulomatous foci following the death of larvae occurring in the brain, might lead to the possibility of some form of epilepsy (Snyder, 1961; Brain & Allan, 1964; Woodruff et al.,1966; Woodruff 1970). Snyder (1961) reported a case of a two year old boy who had had severe pulmonary involvement in addition to some clinical manifestation of toxocariasis, he had 35/50 thousand leucocytes (mm^3) with 30% eosinophils. Chest roentgenograms showed diffuse mottling of both lungs, Despite antibiotic therapy his condition was deteriorating and he became critically ill. He responded well to cortisone

treatment with oxygen and parenterally fluid. This child, for a few months prior to the onset of his pneumonitis, had suffered repeated mild seizures clinically typical of petit mal epilepsy. Although biopsy was not performed in this case, the circumstantial evidence suggested that it was a toxocara case and that the petit mal epilepsy was due to the parasite.

Brain and Allan (1964) reported epileptic seizures in a 32 year old woman. She had had many, both minor and major, most of them during sleep. Toxocaral infection was suspected on clinical grounds and on positive skin test. The interesting thing about this case is that when she was treated with antitoxocaral drug (Diethylcarbamazine 3 mg per kg body weight, three times daily for three weeks) she greatly improved and then stopped having the convulsions.

Woodruff et al. (1966) found that 26 out of 349 epileptic patients had reacted positively to toxocara skin tests, compared with 7 who did so from among 329 normal persons. Among those with a positive skin test on epilepsy 77% had had a dog or cat in their household for at least three months before the development of their epilepsy. These findings, together with the fact that when a toxocara larva dies it produces a focal area of granulomatous reaction, might well be expected to produce focal epilepsy.

If the infection is heavy many of the larvae die in the cortex, a number of foci and generalized epilepsy could develop.

One of the toxocaral infections that received greater attention than the others is that involving the eye and causing granulomatous reaction in the choroid and retina, with subsequent endophthalmitis and retinitis (Wilder, 1950; Nichols, 1956; Ashton, 1960; Woodruff et al., 1961; Bird et al., 1970; Phillips & Mackenzie, 1973).

The cause of this attention is not because the larvae has a particular predilection for the eye, but because the presence of the parasite in the eye will cause damage which will give rise to symptoms followed by surgical interference and histological diagnosis. The presence of the larvae in other organs, like the liver or lung, would be unlikely to give rise to any specific symptoms or clinically recognizable syndromes. The early lesions are raised as a result of granulomatous tissue (Woodruff & Thacker, 1964; Woodruff, 1970). Involvement of the eye with Toxocara should be suggested if a unilateral raised lesion, eosinophilia or a positive skin test and usually a positive fluorescent antibody test result (Woodruff, 1970).

Transmission of and predisposition to infection

The question arises as to whether T.canis larvae migrating from the contaminated intestinal contents could possibly carry with them bacteria or cause damage in the tissues, especially the brain which could create favourable conditions for the growth of microbiological organisms. Such damage to the tissues was reported by many workers. Sprent (1955b) studied brain sections of mice experimentally infected with T.canis. Haemorrhages were evident on the surface of the brain and the larvae were lying freely in the brain tissues and some of them were associated with areas of haemorrhage.

Bisseru (1969) demonstrated that T.canis larvae in the livers of mice showed persisting inflammatory changes around the portal tracts with liver cell degeneration. In the lungs there were haemorrhages with inflammatory reaction consisting of clumps of lymphocytes with occasional neutrophils and few eosinophils. In the brain there were haemorrhages on the surface of the brain with haemorrhagic tracts and haemorrhages around the larvae.

The microscopical examination of toxocaral lesions in the tissues of man was first reported by Beaver et al. (1952). Sections from a liver of a child who had toxocaral infection showed extensive areas of focal necrosis

and inflammatory reaction around the necrotic foci and in the portal spaces. Portions of the parasite were also found in these sections.

In the case reported by Dent et al. (1956) where a child died of serum hepatitis associated with overwhelming toxocaral infection, a liver biopsy had been performed 7 weeks before the death of the child and numerous focal granulomas discovered. Microscopically these granulomas presented a central area of necrosis, epithelioid cells and a dense infiltrate of eosinophils, neutrophils, lymphocytes and plasma cells with larvae of T. canis in many sections. At necropsy granulomatous lesions with haemorrhages, necrosis and inflammatory cells were found in the heart, lung, liver, spleen, pancreas, kidneys, intestine, mesenteric lymph nodes and brain.

From these studies one can reach the conclusion that the larvae of T. canis could create foci of tissue damage that might create favourable conditions for the micro-biological organisms. On the other hand, it is well known that adult Ascaris lumbricodes are able to cause peritonitis and other septic complications by their migrations from the lumen of the intestine, particularly after operations on the intestinal wall, or by causing perforation. Shope (1941, 1943a 1943b) discussed the role that a parasitic nematode can play as a reservoir for the maintenance of a virus under both experimental

and natural conditions. He found the nematode swine lungworm could act as a reservoir host for swine influenza virus. He did not succeed in his attempts to demonstrate direct transfer of the viruses of swine influenza and hog cholera, but the lungworm as it passes through successive developmental stages in its definitive host - pigs - harbours the virus in what Shope described as masked form. The description was used because of the failure to obtain direct transmission of the infection. Stimuli were needed to produce swine influenza. The pigs were injected with repeated doses of Hemophilus influenzae or were fed embryonated eggs of Ascaris. It was very difficult to explain the role of these stimuli in the provocation of the influenza. Although a high percentage of the experimental pigs produced signs and symptoms of influenza supposedly transmitted to them by the lung-worms, it was evident from the results that the demonstrable transmission of the virus by the nematode was not a regularly or easily reproducible phenomenon.

The relation between the swine lungworm and swine influenza virus needs more organized work than that done by Shope because no control animals were used in the experiments and the infected animals were left together in the same pen so that Shope could not exclude the possibilities of cross-infection. A good demonstration of the ability of nematode larvae to carry microbiological organisms is the interesting work of Syverton et al. (1947). The experimental

work that was done on the guinea pig gave proof that Trichinella spiralis larvae after maturation in the muscles of the guinea pig infected with viral lymphocytic choriomeningitis had acquired the virus and was capable of transmitting it to new susceptible hosts. The other interesting result in the experiment was the proof that the transmission of the virus was not due to mere adherence to the outer surface of the larvae, but that these actually harboured it. An interesting observation in this study was the failure of the T.spiralis larvae to acquire the virus when suspended in fluid containing a large quantity of the virus. From the experimental work that led to these results it seemed that the periods of exposure of the larvae to the virus were very short. In two experiments the period was only 3 hours and in a third experiment the period was 5 hours. No attempts were made to incubate the parasite and the virus for longer periods. Evidence was obtained of the ability of the larvae from animals with co-existent trichinosis and lymphocytic choriomeningitis to act as efficient agents for transmitting the infection to a new host. In these latter experiments the larvae and the virus were in contact for a much longer period than the time of incubation in the test tube.

It is surprising that the studies of Shope (1941) and Syverton et al. (1947) did not lead to an explosion of similar studies of the role of helminth larvae and ova

in other virus and bacteria diseases. The discovery of an ascarid larva in the brain of a child who had died of severe poliomyelitis supported the belief in the possibility that viruses may be transmitted by parasites to the central nervous system. The case was reported by Beautyman and Woolf (1951). In the report Professor J.J.C. Buckley suggested that the larva might be of an ascarid from a dog or cat, because the development of the Toxocara was not then fully described. A great amount of experimental work by Sprent was performed to increase knowledge about the migratory behaviour and development of this nematode (Sprent, 1952, 1953, 1954, 1955a, 1955b and 1958). In this work on experimental animals he showed that invasion of the C.N.S. by T. canis does occur and he drew attention to the possibility of microbiological organisms transmitted by the larvae (Sprent, 1955a).

Woodruff, (1965), stated that "A very uncommon proved manifestation of Toxocariasis, but one of great importance, is encephalitis due to larval migration". He based this statement on the several reports of infection with Toxocara larvae and associated encephalitis and especially on the evidence given by Sprent and on the cases reported by Beautyman and Woolf (1951) and by Van Thiel (1960). Other cases were reported by many workers and were summarised by the excellent review of Beaver (1962). Brain and Allan (1964) reported a case of a 32 year old woman who

complained of headache, convulsions and epileptic seizures. The case was diagnosed as toxocaral infection involving the central nervous system according to the clinical manifestations and toxocara skin test. Since then many cases of encephalitis have been reported.

Other cases of acute meningomyelitis associated with Toxocara were reported by Engel et al. (1971) and Muller-Jensen et al., (1973). In these cases the diagnosis was made according to the signs and symptoms and serological demonstration of specific antibodies to be due to the T. canis larvae.

Schochet (1967) reported a case of toxocariasis in which the lesions in the brain were considered to be significant in causing death. A 2 year old boy was admitted to the hospital with a diagnosis of a possible brain abscess. He had increasing stupor that progressed to coma. He also had one left sided convulsion and later had twitching about his mouth. The W.B.C. count was 20,500 cells per cubic mm with 4% eosinophils. Despite continued antibiotic therapy the child died twelve hours after admission. At post mortem the brain was oedematous. Specimens submitted at the time of autopsy for viral studies were subsequently reported as negative. Histological examination showed the presence of multiple, small, sharply demarcated regions of granulomatous inflammation. Parts of coiled nematode,

represented by cross and oblique sections, were found in the subcortical white matter of the cerebral hemisphere. This was identified by Dr. P.C. Beaver as a larva of T. canis. There were widespread focal inflammatory lesions in the brain. As the nematode was the only etiologic agent identified with no significant pathogenic conditions other than encephalitis to account for the patient's death, the larva must be considered as the probable cause of death.

Although most of the reports concentrated on the presence of the larvae in the central nervous system, they were also found in many parts of the human body. In one case a 19 month old male with toxcariasis died of an incidental homologous serum hepatitis and was reported by Dent et al. (1956). Granulomas, with and without the larvae of T. canis were widely scattered throughout the viscera and central nervous system. Larvae were found in the heart, lung, liver, kidney, small and large intestine, mesenteric lymph nodes and brain. Widespread visceral lesions of identical character were also found in which no larvae could be demonstrated. Beaver et al. (1952) reported the first proved larva of a Toxocara canis in the human liver. Snyder (1961) reported 20 cases of toxocariasis, in 10 of them

the diagnosis was proved by liver biopsy.

Toxocara canis larvae and microbiological infections:

A. Evidence in experimental animals

As infection with toxocara in man has only been proved in the last 25 years, most interest has concentrated and most work has been done on the establishment of facts about the migratory behaviour of the larvae and the most common clinical manifestations. There have also been great efforts to discover diagnostic methods apart from tissue biopsy techniques.

Mochizuki et al. (1954) successfully demonstrated experimentally in mice that T.canis larvae in migrating to the brain are capable of transmitting Japanese B. encephalitis virus. They fed a number of mice about 2000 embryonated T.canis eggs. Four days later they inoculated these mice subcutaneously with the virus suspension. A control group of mice were inoculated with virus suspension only. The results was a clear and a significant difference in mortality was observed between the two groups of mice, higher in the dually infected mice. Even with 500 eggs there was a clearly significant difference between the two groups. Their conclusion was that the provocation of the virus encephalitis was most probably caused by destruction of the blood brain barrier by the prior intracerebral migration

of the larvae, but they did not exclude some other mechanism. Mochizuki and his colleague were only interested in the role of the larvae in producing encephalitis so they did not look for the effect of the larvae on other organs and they could not produce evidence concerning the ability of the larvae, in general, to act as a vehicle or a synergist for the virus.

The experiment was repeated by Pavri et al. (1975) but in addition they examined the lung, liver, spleen, kidney and blood in addition to the brain and they tried to isolate the virus from the specimens collected from the groups of mice given Japanese Encephalitis virus alone and those dually infected. The results showed that in the group receiving dual infections a statistically higher proportion of the mice were affected as compared with the control groups in relation to sickness and death, while there was no significant difference between the rates of virus isolation from the blood or lung. On the other hand, a statistically significant difference was noted in the rates of isolation from the brains of the dually infected mice. They suggested that the larvae, during their passage into the tissues, especially the brain, might either transport the virus or produce conditions favouring the virus entry across the blood-brain barrier, but they also gave other possibilities such as the effect of dual infection on the immune mechanism of the host.

B. Evidence in Man

On the basis of the demonstration of a larvae of T.canis in the brain of a child who had died of poliomyelitis reported by Beautyman and Woolf (1951) it was suggested by Woodruff et al. (1966) that T.canis larvae might carry with them bacteria or viruses or cause damage in the central nervous system and other tissues, which could afford foci for the growth of viruses or bacteria circulating in the blood at the time at which the damage was done. In the case reported by Dent et al. (1956) of a child who had died of homologous serum hepatitis T.canis larvae were found widely scattered throughout the viscera and central nervous system. The hepatitis was considered as incidental and due to one of the several blood transfusions he had received. This conclusion is probably correct, but the mechanical damages caused by Toxocara larvae as indicated by the macroscopical and microscopical findings in the viscera, the larvae could play a role in the provocation of the viral infection by creating favourable conditions for the growth of the virus.

The work of Woodruff et al. (1966) stimulated a wider interest in a possible relationship between T.canis larvae and poliomyelitis. They were able to examine 191 persons who had poliomyelitis in the past. The results showed a much higher percentage (13.6) of those patients reacted positively to the skin test than apparently healthy

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persons (2.1%). The results were consistent with the explanation that the larvae in migrating from the alimentary tract to the brain had carried with them poliomyelitis virus. In Egypt an investigation confirming this study was carried out and reported by Khalil et al. (1971), they followed the same pattern of study by using toxocara skin test in control and in poliomyelitis cases. The work was carried out on 102 infants and children suffering from chronic poliomyelitis affecting different parts of the body, and on 70 apparently healthy controls of the same age group. Of the 102 cases of poliomyelitis, six (5.8%) had toxocaral infection proved by the skin test. In the control group, out of 70 only one had a positive test (1.4%).

These results showed a higher percentage of positive skin tests in the poliomyelitis cases compared to the control. Their findings are similar to and supported by those of Woodruff et al. (1966).

No case has yet been reported implicating T. canis larvae in the transmission of bacteria, but this possibility has been suggested. Other nematode larvae have been reported in relation to bacterial infection. An important report by Brown and Perna (1958) concerns a 58 year old woman who died of Escherichia coli septicaemia and meningitis. In addition, this woman was found to have a massive infection

with Strongyloides. Brown and Perna considered that the strongyloides infection could have played an important part in the development of the septicaemia and meningitis due to the migrant Strongyloides larvae carrying the E. coli, either mechanically, or in their intestinal tracts, to the blood stream and then to the central nervous system.

A similar case was reported by Wilson and Thompson (1964). A 24 year old woman was admitted to hospital with five months history of feeling unwell. On the day after admission the patient developed the symptoms and signs of acute small bowel obstruction. Laparatomy showed enlarged bowel. A gland was removed, divided and sent for histological examination and culture. The culture produced a scanty growth of Escherichia coli. Microscopically in the mesenteric gland there was a collection of eosinophils surrounding two portions of larvae. Unfortunately they were not diagnosed as Strongyloides stercoralis until similar, but more obvious, organisms were found in the post mortem section. The patient died of purulent meningitis which, after necropsy, showed the whole surface of the brain to be involved. A heavy growth of Escherichia coli was obtained from the pus. It is most probable that the larvae of Strongyloides carried with them the coliform bacteria that caused the meningitis.

From the literature reviewed one could reach the conclusion that the Toxocara canis play a role in the transmission of microbiological organisms. The mechanism or mechanisms involved are not clear. There could be more than one mechanism by which the larvae could lead to provocation of microbiological diseases. They might carry the organisms on their bodies from the alimentary canal to different parts of the body. Another possibility is that the mechanical damage caused by their migration could create favourable conditions for the growth and multiplication of the microbiological organisms.

MATERIALS AND METHODS

Introduction

The radioactive isotopes were widely used to study the molecular biology of the Escherichia coli; ^{14}C, ^{32}P, ^{13}C, ^{15}N and ^{3}H isotopes were used by many investigators. ^{14}C and ^{32}P were used by Patel and Kaback (1976) in their study on the role of the carbodiimide-reactive component of adenosine-5'-triphosphatase complex in the proton permeability of E.coli membrane vesicles. Approximately 0.05 mg of membrane protein was incubated with 30 mM Tris-HCl containing 2 mM magnesium sulphate and carbodiimide. Reactions were initiated by addition of 10 µl of (γ-^{32}P)ATP (final concentration 7 mM containing about 60,000 counts/min). Inorganic phosphate was extracted into 0.85 ml of isobutyl alcohol-benzene-aceton, and aliquots of the upper phase were dried on planchets and counted in a nuclear Chicago gas flow counter.

Letsinger et al. (1976) describe the synthesis of 5'-amino-5'-deoxythymidine-5'-triphosphate. Sodium trimetaphosphate hexahydrate and d(NH2)T were dissolved in 50 ml of a solution containing approximately 100 u Ci of ^{32}P labelled sodium trimetaphosphate. After 24 hr the reaction mixture was lyophilized, redissolved in 50 ml of 0.05 M triethylammonium bicarbonate and

precipitated with methanol. Chromatography on a DEAE-cellulose plate with 0.02 MLiCl showed two spots corresponding to d/NH)$_2$T and (5-NH)-dTTP.

An autoradiogram revealed 4 radioactive materials: (5-NH)-dTTP, trimetaphosphate and two unidentified inorganic phosphates. Assay of the radioactivity by liquid scintillation counting showed that 22% of the total radioactivity was in the (5-NH)-dTTP, 33% in trimetaphosphate and 45% in the two unidentified phosphates.

Tang and Guha (1975) used the ^{32}P-ATP and the ^{32}P-GTP to label the E.coli cP78. The bacteria was grown in 20 ml minimal M9 medium. The culture was then spun down at 6,000 r.p.m. for 10 minutes at room temperature. The pellet was resuspended and shaken in 20 ml of a plasmolysing buffer. The cells were then poured into 20 ml prewarmed M9 minimal medium containing 0.5 mCiγ-^{32}P-ATP or 0.5 mCiγ-^{32}P-GTP. This labelling procedure was terminated after 15 min by pouring the culture into 5 ml frozen M9 buffer, and immediately spinning down at 4^{o}C; RNA extracting was then carried out. The RNA was analysed through a 5 cm, 1.2% polyacrylamideagarose gel, and ekectrophoresed at 5 mA and 50 V for 1.75 hr. the gels were sliced and exposed to X-ray sensitive

films and developed. The incorporation of the labellled phosphorus into the entire RNA molecules was demonstrated.

White and Rudolph (1979) used the ^{13}C-^{15}N- and ^{3}H-labelled glycines to incorporate the pyrimidine moiety of thiamin by E.coli. The E.coli B was grown on 100 mL of defined liquid medium containing glucose and all of the amino acids except serine. The labelled glycines were added. The fresh E.coli cells were isolated, separated from the growth medium by centrifugation and resuspended in 8 mL of 0.1 MHCl. The cells were then placed in a boiling water bath for 20 min to extract the thiamin. Gas chromatograph-mass spectrometer was used for the analysis of the isotope.

White (1978) used the ^{13}C stable isotope to study the biosynthesis of the thiozole moiety thiamin in E.coli. The E.coli B was grown on 100 mL of definited liquid medium at 37°C in a 2 L wide-mouthed Erlenmenger flask on a rotary shaker. The medium consisted of 12.1 g of Tris, 9.9 g of K_2HPO_4, 5.3 g of NH_4Cl, 5 g of casamino acids, 2.9 g of sodium citrate, $2H_2O$, 4 g of the labelled compound, 0.5 g of NaCl, 120 mg of $MgSO_4$, 44 mg of $CaCl_2 \cdot 2H_2O$ and 0.8 mg of $FeCl_3 \cdot 6H_2O$ per L of water; the GC-MS were used to analyse the isotope.

Counting of radioactive samples:

The beta particles (^{32}P) and the gamma-ray photons (^{59}Fe and ^{51}Cr) emitted in radioactive disintegrations can be recorded as individual events by suitable detectors (e.g. Geiger or scintillation counters). Any such detectors will record a so-called background count even when there is no radioactive sample present. This results from the detection of gamma-radiation from radioactive material in the ground, buildings, etc., of cosmic radiation from outer space, and to some extent, of beta particles from traces of radioactive materials in the counter itself. Thus in fact two measurements must always be made. First the count rate (N_B) due to this background radiation alone must be determined. Then the measurement must be repeated to give the count rate (N) due to both the test sample and the background together. The actual count rate (N_S) for the sample (which is proportional to the sample activity) is then given by $N_S = N - N_B$.

The beta particles from radioactive material have only a limited range dependent on their energy, so, for the detection of these particles there must be a thin window through which they may enter the counter. The area of window cannot be very large (1-3 cm diameter) so that only small samples can be presented to the counter,

(Oliver, 1971). In the current experiments the beta radiation from the ^{32}P samples were put on planchets. These planchets were then placed on the thin end window. The counter does not need to have a thin window for the detection of penetrating gamma-radiation.

A. Radioactive isotopes for labelling the E.coli bacteria

In order to be able to trace the bacteria to the sites to which they were taken throughout these experiments they were labelled with radioactive isotopes. As the initial experiments did not take more than eight days it was possible to use an isotope with a short half life - thus avoiding the danger of unnecessary contamination. For this reason the β emitter, ^{32}P, having a half life of 14.3 days was chosen. Later experimental work posed a technical problem in measuring the radiation in the organs of mice as will be seen later. During later experiments the γ emitting isotopes ^{51}Cr and ^{59}Fe having half lives of 27.8 days and 45 days respectively were used. The E.coli were subcultured in glucose broth that contained bacteriological pepton (L37) - phosphate as P_2O5 (1.3%) and 75 p.p.m. iron.

Experiment No.1 : ^{32}P (β emittent) for labelling the bacteria

Escherichia coli bacteria were obtained from the Bacteriology Department in the London School of Hygiene and tropical Medicine. The strain was subcultured in MacConkey's media and used in all the experiments. A single colony of E.coli was taken by sterilized loop from the

MacConkey's plate and was inoculated into 4 ml of 1% glucose broth contained in a bijou bottle and incubated at 37°C for 24 hours. This culture was used the next day to inoculate eight bijou bottles each with 4 ml of 1% glucose broth solution and ^{32}P in the form of Na phosphate and then incubated at 37°C. The bijou bottles were divided into four pairs and each pair was marked "day one" to "day four" according to the period of incubation with the isotope. In each of the successive four days one pair was taken from the incubator in order to wash the bacteria. The ^{32}P was used in different doses (0.25 m Cu, 0.5 m Cu and 1 m Cu). To kill the bacteria 0.5 ml of 0.5% formalin solution was added to each bottle, the contents of each bottle were then transferred to a centrifuge tube. From each bottle 0.1 ml was taken by pipette and placed on a planchette for counting the radiation. The planchette was then marked "culture". The cultures were spun for 10 minutes in the centrifuge at a speed of 2400 revolutions per minute (r.p.m.), the bacteria were precipitated to the bottom of the centrifuge tubes and the supernatant was discarded by means of a pasteur pipette. To resuspend the bacterial deposit in the centrifuge tube 4.5 ml of phosphate buffer saline was used, i.e. the same volume of broth plus the 0.5 ml of 0.5% formalin solution. The bacteria were spun again for 10 minutes at 2400 r.p.m. and the supernatant was discarded by means of a pasteur pipette. This procedure of washing the bacteria

MacConkey's plate and was inoculated into 4 ml of 1% glucose broth contained in a bijou bottle and incubated at 37°C for 24 hours. This culture was used the next day to inoculate eight bijou bottles each with 4 ml of 1% glucose broth solution and ^{32}P in the form of Na phosphate and then incubated at 37°C. The bijou bottles were divided into four pairs and each pair was marked "day one" to "day four" according to the period of incubation with the isotope. In each of the successive four days one pair was taken from the incubator in order to wash the bacteria. The ^{32}P was used in different doses (0.25 m Cu, 0.5 m Cu and 1 m Cu). To kill the bacteria 0.5 ml of 0.5% formalin solution was added to each bottle, the contents of each bottle were then transferred to a centrifuge tube. From each bottle 0.1 ml was taken by pipette and placed on a planchette for counting the radiation. The planchette was then marked "culture". The cultures were spun for 10 minutes in the centrifuge at a speed of 2400 revolutions per minute (r.p.m.), the bacteria were precipitated to the bottom of the centrifuge tubes and the supernatant was discarded by means of a pasteur pipette. To resuspend the bacterial deposit in the centrifuge tube 4.5 ml of phosphate buffer saline was used, i.e. the same volume of broth plus the 0.5 ml of 0.5% formalin solution. The bacteria were spun again for 10 minutes at 2400 r.p.m. and the supernatant was discarded by means of a pasteur pipette. This procedure of washing the bacteria

with phosphate buffered saline was repeated three times and on each occasion the supernatant was discarded. At the end of the third wash 0.1 ml of the supernatant was taken on a planchette marked "third wash" and the rest was discarded. The bacterial deposit was resuspended in 0.1 ml of normal saline and the suspension was transferred to a planchette marked "bacteria". The three planchettes, together with a control one to measure the background radiation, were taken for counting to a castle type scintillation counter with air end window.

Experiment No.2 : The Use of ^{51}Cr and ^{59}Fe (γ emitters) for labelling the bacteria

The same methods were used as in Experiment No.1. In this experiment, however, all radiation measurements were made in a well type scintillation counter.

B. The ability of the T.canis larvae to carry E.coli

1. In Vitro:

Experiment No.3 :

The eggs of T.canis were obtained from the uteri of female worms taken from the intestines of dogs, mostly puppies. The worms were dissected under a dissecting microscope. The eggs were placed in a 0.5% formol saline solution in bijou bottles at room temperature and left to embryonate. The formol saline solution was used to

prevent contamination of the ova by microbiological organisms as the ova were incubated for atleast 6 weeks to ensure full maturity.

To each mouse 1,000 embryonated ova were fed directly into the stomach by means of a syringe and plastic tube. Four days later the mouse was killed. The liver, lung and brain were obtained and suspended in 50 ml water. Each organ was placed in a Baermann apparatus for separation of the larvae. Of the T.canis larvae obtained by this technique, five were put in each of six bijou bottles containing 2 ml of normal saline. To each bottle 24 hours incubated ^{32}P labelled E.coli bacteria were added (about 10^6 as estimated by haemocytometer method).

The bottles were divided into three pairs, a pair to be used on each of three successive days. The bottles were incubated at room temperature. To wash the larvae the contents of each bottle were placed in a petri dish placed under a dissecting microscope. 0.1 ml was taken from the dish, placed on a planchette and the radiation in the media containing the larvae counted. The larvae were gathered by means of a curved end Pasteur pipette with as little fluid as possible and were transferred to a small dish containing 2 ml of normal saline to dilute the concentration of bacteria gathered up with the larvae. This small dish was marked "first wash". The larvae were left

in the first wash for 15 minutes to rinse off, as far as possible, bacteria attached to their surface. The larvae were then gathered up and transferred to another small dish containing 2 ml of normal saline marked "second wash". Then after 15 minutes they were similarly transferred to the "third wash" that was again a small dish containing 2 ml of normal saline).

After 15 minutes the 5 larvae were picked up with 0.1 ml of the third wash saline and were placed on a planchette marked "larvae", and the radiation counted. From each wash 0.1 ml was taken on a planchette to count the radiation in the washes. The radiation of each specimen was counted for one minute in a castle type scintillation counter. The experiment was repeated 19 times with the material incubated for one day and two days, and 12 times for that incubated for three days.

The same experiment was carried out, but this time using ^{59}Fe isotope and the larvae were incubated for 24 hours only.

2. In Vivo:

In this experiment (β emitting) ^{32}P labelled bacteria were initially used, a further technical problem arose. It was not possible to count the radiation in the suspension of the entire organ in the well type scintillation

counter. At the same time, smaller samples of 0.1 ml, physically acceptable on the planchette, could not give satisfactory evidence. Being such small fractions of the total 10 ml suspension, the radiation from such small samples would have been negligible; consequently (γ emitting) ^{59}Fe labelled bacteria were used to measure the radiation in the whole organ suspension. Male T.O. strain mice were used in a weight range of 18-25 gm.

Experiment No.4 : Using ^{32}P labelled bacteria

Four mice were used in this experiment. Two of these, I_1 and I_2, were the dually infected mice which had been fed T.canis embryonated eggs in addition to the labelled bacteria. The other two mice, C_1 and C_2, were the control mice that had been fed labelled bacteria only. Each experiment took seven days. E.coli bacteria were incubated in two bijou bottles each containing 4 ml 1% glucose broth with ^{32}P and incubated at 37°C for 24 hours.

Day one: The bacteria were washed as described in experiment No.1. The deposit of bacteria was resuspended in 1.3 ml normal saline. 0.1 ml of this suspension was taken on a planchette to count the radiation in the bacteria. The rest of the suspension was divided into 4 parts, i.e. 0.3 ml a part. A part was given to each of the four

mice, I_1, I_2, C_1 and C_2. The suspension was fed directly into the stomachs by means of syringe and plastic tube. To dose the mice with more labelled bacteria, two more bijou bottles of 1% glucose broth plus ^{32}P were inoculated with E.coli and incubated at 37°C for 24 hours to be labelled and then fed to the four mice on day two.

Day two: The same procedures were followed as on day one. Two more bijou bottles were used to cultivate E.coli with ^{32}P. The cultures were to be fed to the mice on day three.

Day three: The bacteria that were incubated on day two were washed and then fed to the four mice as in the first two days. The two mice I_1 and I_2 were fed 1,000 embryonated ova of T.canis each directly into the stomach. The two control mice, C_1 and C_2, did not receive ova.

Day seven: i.e. Four days after the infected mice I_1 and I_2 received the ova, the mice were killed. The livers and brains were obtained. Each of these organs was emulsified in 10 ml of water. 0.1 ml sample was taken from each emulsion, placed on a planchette to measure the radiation in each organ. The emulsions of the infected mice, I_1 and I_2, were diluted by adding 50 ml of water to each organ emulsion and by using Baermann technique it was possible to detect the T.canis larvae. From each emulsion 5 larvae were placed on a planchette

to count the radiation.

Experiment No.5 : Using ^{59}Fe labelled bacteria

The same methods were used as in experiment No.4, but ^{59}Fe isotope was employed to label the bacteria. The use of the γ emitter isotope made it possible to measure the radiation in the whole 10 ml of the organ's suspension instead of 0.1 ml specimen as in using the β emitter ^{32}P in the previous experiment. In this experiment the lungs were also obtained in addition to the livers and brains.

C. The ability of the T.canis larvae to disseminate the labelled bacteria

The purpose of this experiment was to determine whether the larvae cultivated with labelled bacteria in vitro could transmit the bacteria when fed to mice. Thus it was the continuation of experiment No.3, but ^{59}Fe was used instead of ^{32}P to label the bacteria as the initial experiments with ^{32}P posed the technical problem previously explained.

Experiment No. 6 :

Day one: A mouse marked "A" was fed 2,000 T.canis embryonated ova directly into the stomach by means of a syringe and plastic tube.

Day three: Two bijou bottles of 1% glucose broth and 1 μ Ci of ^{59}Fe were inoculated with E.coli bacteria and incubated at 37°C for 24 hours.

Day four: Mouse "A" was killed and the larvae were obtained from the lung, liver and brain by using Baermann technique. The bacteria in the two bijou bottles were washed as described in experiment No.2. The radiation in the bacteria was measured for one minute with the well type scintallation counter. The larvae obtained from mouse "A" were put into a bijou bottle of 2 ml normal saline, the labelled bacteria were added and the bottle was incubated at room temperature for 24 hours.

Day five: The larvae were washed by using the same methods described in experiment No.3, then the larvae were fed directly into the stomach of a mouse marked "B".

Day ten: Mouse "B" was killed. The liver, lungs and brain were obtained and each organ was emulsified in 10 ml of water then the radiation was measured in the well type scintillation counter. Each organ's emulsion was put in a Baermann apparatus for the detection of larvae. Five larvae were taken from each emulsion placed on a planchette and radiation was counted. The radiation count time was one minute for each specimen. The larvae

obtained from each organ were counted.

The experiment was repeated nine times using various numbers of larvae; 32 - 1,250 larvae were used. In seven of these experiments 0.5 ml of blood was obtained and placed on a planchette to determine whether there was any effect due to the larvae labelled by the E.coli bacteria.

The same experiments were carried out using 320 and 370 T.canis larvae, but ^{32}P was employed instead of ^{59}Fe.

RESULTS

Experiment No.1 : ^{32}P (β emitter) for labelling the bacteria

For four successive days four duplicate experiments were carried out for labelling the E.coli bacteria with the (β emitter) ^{32}P. In each of these experiments the dose of the radioactive isotope was increased to discover whether the bacteria were able to accept the isotope, and if so, to determine the period of incubation and the dose of ^{32}P that would lead to better labelling. The results are summarized briefly and are simplified in Table 1.

The Table shows the period of incubation in days and the radiation counted in 0.1 ml of the broth that contain the isotope, and the E.coli bacteria had 0.5 ml of 0.5% formol solution added to kill the bacteria. The Table shows the radiation counted in 0.1 ml of the 2 ml third wash saline and not the radiation in the first and second washes. The radiation in these two washes were estimated to evaluate the process of washing and they showed that most of the isotope was washed away in the first washing, then the second and third washings were just to remove traces of the isotope, bringing the radiation count in the 0.1 ml of the third wash down to about the value of the background. For this reason there was no need to wash the bacteria more than three times.

On the first day of incubation the mean amount of radiation in 0.1 ml broth culture was 1071 count/minute, while the mean amount of radiation in 0.1 ml third wash supernatant was 2 count/minute only. The mean count in the bacteria was 2630 count/minute. This gave clear indications that the bacteria were able to take the isotope and that the process of washing was successful. On the second day of incubation the mean amount of radiation in 0.1 ml third wash supernatant was 2 count/minute, while the mean amount of radiation in 0.1 ml broth was 1117 count/minute. The mean amount of radiation in the bacteria that were resuspended in 0.1 ml saline was 2307 count/minute. On the third day the mean amount of radiation in 0.1 ml broth culture was 1072 count/minute, while the mean radiation in 0.1 ml third wash supernatant was one count/minute only, and the bacteria were able to take a mean of 1760 count/minute. On the fourth day of incubation the mean amount of radiation in the broth was 1127 count/minute and the mean in 0.1 ml third wash supernatant was one count/minute only, and the bacteria were able to take 1357 count/minute.

The results show that the E.coli is able to accept higher doses of ^{32}P in the first day and this acceptance decreases during longer periods of incubation. The amount of radiation in the bacteria is much higher than that in the total volume of the third wash, which could be calculated

by multiplying the radiation counted in 0.1 ml by the 2 ml volume of the third wash solution.

There is clear evidence that the E.coli bacteria were labelled with ^{32}P in all the periods of incubation in all doses of the isotope to such a degree that it is obvious without a significance test.

Experiment No.2 : The use of ^{51}Cr and ^{59}Fe (γ emitters) for labelling the bacteria

The results of the experiments with the (γ emitters) ^{51}Cr and ^{59}Fe are summarized and are shown in Tables 2 and 3 respectively. The Tables show the radiation counted in 0.1 ml of the third wash solution and compared to the radiation count in the E.coli bacteria resuspended in 0.1 ml saline.

As these radioactive isotopes are γ emitters, it was possible to measure the radiation in the total volume of the cultures and the three washes. The radiation measured in the total volumes of the third wash were much less than the radiation in the bacteria. The radiation in the first and second washes are not shown in the Tables because they were done just to evaluate the process of washing, and as in the case of ^{32}P it was possible to get rid of most of the isotopes in the first wash. The

mean amount of radiation in 0.1 ml of the third wash supernatant was one count/minute, which is much smaller than the mean amount of radiation in the bacteria (283.62 count/minute) that were resuspended in 0.1 ml saline.

In the experiments with ^{51}Cr the bacteria were incubated for eight days because the results for the first four days showed that the isotope had a greater chance to adhere to the bacteria with longer periods of exposure, as shown in Table 2. The radiation in the E.coli was less after the fifth day of incubation. Although there was clear evidence that the bacteria were accepting the ^{51}Cr, the amount of radiation was not high as in the case of ^{59}Fe, where the radiation in the bacteria was very high. For this reason the γ emitter ^{59}Fe was used in the further experiments.

The amount of radiation in the E.coli bacteria takes the same pattern as with ^{32}P and bigger amounts of the isotope were incorporated in the bacteria in the first day, after which it decreases (Table 3). The radiation in the third wash is higher than that in the case of ^{32}P. This might be explained by the high amount of radiation in the background (around 400 count/minute) in the ^{59}Fe experiment, while it was around 10 count/minute in the ^{32}P experiments. On the first day of incubation the mean radiation count in 0.1 ml third wash was 14 count/minute while the mean amount

of radiation in the bacteria was 12,446 count/minute. The mean amount of radiation in 0.1 ml of the third wash supernatant on the second day was 13 count/minute, while in the bacteria it was 7,396 count/minute. With longer periods of incubation the radiation in the bacteria was gradually decreasing, so that on the sixth day of incubation the amount of radiation was 2,086 count/minute; while in 0.1 ml of the third wash it was 10 count/minute.

Experiment No.3 : The ability of the T.canis larvae to carry the labelled bacteria (in vitro)

In this experiment attempts were made to discover whether the larvae can carry the labelled bacteria. This was done by measuring the radiation in T.canis larvae exposed to ^{32}P labelled bacteria, and then washed to get rid of the isotope in the culture and in the washing solution. Groups of five larvae incubated with ^{32}P labelled bacteria for one day, two days and three days, were washed three times with saline. The radiation in five larvae was compared with the radiation in 0.1 ml of the third wash. The results are summarized and are shown in Tables 4, 5 and 6.

The Tables show the radiation measured in 0.1 ml of the media containing the labelled E.coli plus the five larvae and 0.1 ml of each wash to evaluate the process of washing.

Table 1.

RADIATION COUNT IN BROTH CULTURE - THIRD WASH AND BACTERIA AFTER INCUBATION WITH ^{32}P

Incubation period	Count in 0.1 ml broth	Count in 0.1 ml third wash supernatant	Count in bacteria resuspended in 0.1 ml saline
One Day	459	2	1664
	477	0	1426
	478	3	1768
	527	1	1378
	1566	2	3283
	1914	3	4070
	2076	0	4824
Mean	1071	2	2630
Two Days	473	0	1147
	500	7	1440
	503	0	1184
	507	0	1403
	1650	4	2800
	2054	0	3684
	2130	0	4494
Mean	1117	2	2307
Three Days	403	4	1354
	426	1	1390
	492	0	914
	494	0	1041
	1628	0	2372
	1968	0	3056
	2097	0	2195
Mean	1072	1	1760
Four Days	503	0	846
	504	0	1245
	517	0	889
	579	0	1212
	1663	1	1306
	1930	4	1977
	2195	0	2021
Mean	1127	1	1357

Table 2.

RADIATION COUNT IN THE THIRD WASH AND E.COLI BACTERIA AFTER INCUBATION WITH ^{51}Cr

Incubation period	Radiation count in 0.1 ml third wash	Radiation in bacteria resuspended in 0.1 ml saline
One Day	1	51
Two Days	1	368
Three Days	1	380
Four Days	1	282
Five Days	1	432
Six Days	1	285
Seven Days	1	280
Eight Days	1	191
TOTAL	8	2269
MEAN	1	283.62

Table 3.

RADIATION COUNT IN THE THIRD WASH AND E.COLI BACTERIA AFTER INCUBATION WITH ^{59}Fe

Incubation period	Radiation count in 0.1 ml third wash	Radiation in bacteria suspended in 0.1 ml saline
One day	14	12,446
Two days	13	7,396
Three days	5	2,918
Four days	6	2,642
Five days	8	2,183
Six days	10	2,086
TOTAL	56	29,671
MEAN	9.33	4,945.16

It is clear that it was possible to get rid of most of the bacteria by the first wash; and by the third wash it was possible to reach a level of very small amounts of radiation. After 24 hours of incubation the mean amount of radiation in 0.1 ml of the first wash was 35.8 count/minute and the mean in 0.1 ml in the second wash was 15.7 count/minute, while in 0.1 ml of the third wash it was only 4.6 count/minute. The mean amount of radiation in the larvae was 12.4 count/minute. On the second day of incubation the mean amount of radiation counted in 0.1 ml of the first wash was 85 count/minute and in 0.1 of the second wash was 20.2 count/minute and only 5 count/minute in 0.1 ml of the third wash. The mean amount of radiation in the larvae was 12.3 count/minute. After 72 hours of incubation the mean amount of radiation counted in 0.1 ml of the first wash was 55.41 count/minute and in 0.1 ml of the second wash was 24.58 count/minute, while in the third wash it was 4.5 count/minute. The mean radiation in the larvae was 12.50 count/minute.

Statistically the probability for each of the three successive days of incubation is less than 0.01 ($p < 0.01$) which means that the results are highly significant.

This suggested that the larvae were able to carry the E.coli bacteria. The larvae were less active in the second and third days of incubations. Attempts were made to incubate

Table 4.

RADIATION IN FIVE T.CANIS LARVAE AFTER 24 HOURS INCUBATION WITH E.COLI BACTERIA LABELLED WITH ^{32}P

Number of experiment	0.1 ml media containing labelled bacteria	0.1 ml first wash	0.1 ml second wash	0.1 ml third wash	Five larvae suspended in 0.1 ml saline
1	304	28	1	1	5
2	718	44	20	14	13
3	233	16	9	5	22
4	454	30	12	0	7
5	349	17	7	1	9
6	873	66	31	2	21
7	699	43	25	9	5
8	675	36	15	2	15
9	717	67	31	8	14
10	290	11	6	4	13
TOTAL	5312	358	157	46	124
MEAN	531.20	35.80	15.70	4.60	12.40

Table 5.

RADIATION IN FIVE T.CANIS LARVAE AFTER 48 HOURS INCUBATION WITH E.COLI BACTERIA LABELLED WITH ^{32}P

Number of experiment	0.1 ml media containing labelled E.coli	0.1 ml first wash	0.1 ml second wash	0.1 ml third wash	Radiation in 5 larvae suspended in 0.1 ml saline
1	39	8	9	8	31
2	62	6	3	4	14
3	1148	326	20	2	13
4	3352	303	47	6	11
5	519	23	19	2	13
6	451	20	15	4	7
7	684	49	24	9	9
8	730	53	30	8	7
9	710	43	25	3	10
10	718	19	10	4	8
TOTAL	8413	850	202	50	123
MEAN	841.30	85.00	20.20	5.00	12.30

Table 6.

RADIATION IN FIVE
T.CANIS LARVAE AFTER 72 HOURS INCUBATION WITH E.COLI BACTERIA
LABELLED WITH ^{32}P

Number of experiment	0.1 media containing labelled E.coli	0.1 ml first wash	0.1 ml second wash	0.1 ml third wash	Radiation in 5 larvae suspended in 0.1 ml saline
1	21	7	2	2	12
2	35	21	9	7	13
3	2169	118	32	6	9
4	1346	74	50	13	14
5	1004	37	22	2	5
6	959	48	28	1	17
7	1901	98	17	3	13
8	508	41	20	3	15
9	1209	66	27	6	17
10	619	72	41	5	14
11	268	21	12	3	13
12	648	62	35	3	8
TOTAL	10687	665	295	54	150
MEAN	890.58	55.41	24.58	4.50	12.50

the larvae for longer periods, but all five larvae were dead after four days of incubation. This means that radiation in the groups of larvae were around the same for the different days of incubation, which means that the larvae probably carry the same amount of bacteria whether the incubations were for one, two or three days. For these reasons the larvae used in continuing further experiments were incubated for one day only.

The same methods were followed in the experiment with ^{59}Fe and the groups of larvae were incubated for one day only (Table 8). The mean of radiation in the five larvae (10.71 count/minute) is about four times more than that of 0.1 ml third wash (2.85 count/minute). Statistically there are significant differences between the third wash and the larvae with $p < 0.05$. The mean of radiation in the larvae are less than that for the ^{32}P experiments.

Experiment No.4 : The ability of larvae to disseminate the ^{32}P labelled bacteria in the body of mice In Vivo:

Both the infected and control mice were given the same dose of 24 hours labelled E.coli bacteria orally. Each mouse received about 1,000 embryonated ova of T.canis. The infected mice were killed four days after having received the ova and the control mice were killed at the same time. 0.1 ml samples were taken from suspensions of the liver and

brain of each mouse to see whether the larvae had some effect on the transmission of the labelled bacteria from the intestine to these organs. The results are simplified in Table 7. The mean amount of radiation in 0.1 ml of the livers suspensions of the dually infected mice was 9.21 count/minute which is higher than that of 0.1 ml obtained from the livers suspensions of the control mice (4.91 count/minute). The mean amount of radiation in 0.1 ml of the brains suspensions of the dually infected mice was 4.91 count/minute, while the mean for 0.1 ml of the control group was 2.78 count/minute.

The radiation in both the liver and brain is higher in the mice that received the ova than in the control mice and statistically highly significant. For the liver $p < 0.02 > 0.01$ and for the brain $p < 0.01$. In this experiment in spite of the statistical significance between the two groups of mice, it was clear that a valid conclusion could not be reached concerning the role of T.canis ova in the dissemination of bacteria due to the small volume of the sample. For this reason the γ emitter ^{59}Fe was used in further experiments.

Experiment No.5 : The use of ^{59}Fe labelled bacteria

The use of the γ emitter ^{59}Fe made it easier to measure the radiation in the whole suspensions of the liver and brain. In this experiment the radiation in the lung was also counted to obtain more accurate information of the

distribution of the labelled E.coli in both infected and control mice. The results, summarized in Table 9, show the radiation in these three organs in the two groups of mice. In the livers the mean amount of radiation was 463.25 count/minute for the dually infected group, while it was 189.93 count/minute for the control group. In the lungs the mean amount of radiation for the dually infected group was 152.58 count/minute, while it was 52.83 count/minute for the control group. In the brain the mean amount of radiation was 40.75 count/minute for the dually infected mice and 16.31 count/minute for the control group.

For both the liver and brain there are highly statistically significant differences and $p < 0.01$. For the lung $p < 0.05$, which might be due to the smaller number of samples. The results showed that the T.canis larvae played a big role in the dissemination of the E.coli bacteria to the three organs of mice. The radiation in the infected mice was about three times greater than that in the control.

Five larvae were obtained from each organ to discover whether they were able to carry some labelled bacteria. The results are simplified and are shown in Table 10. The larvae had obtained the same amount of radiation from the intestine of mice as from the in vitro suspension. The means of radiation in the groups of five larvae obtained from the liver, lung and brain of mice were 8.25 count/minute, 8.12 count/minute

Table 7.

RADIATION IN THE LIVER AND BRAIN OF MICE TO WHICH HAD BEEN ADMINISTERED FIRST ^{32}P LABELLED E. COLI BACTERIA AND SECOND 1,000 T. CANIS OVA

Number of experiment	Infected mice		Control mice	
	0.1 ml liver	0.1 ml brain	0.1 ml liver	0.1 ml brain
1	3	7	4	0
2	1	6	2	2
3	5	0	1	2
4	3	1	2	3
5	4	3	0	0
6	5	3	0	0
7	3	4	0	3
8	0	2	1	1
9	2	0	1	2
10	2	4	1	2
11	0	0	1	0
12	3	2	1	2
13	14	7	15	11
14	41	15	10	10
15	18	12	9	1
16	11	2	15	2
17	6	0	1	1
18	12	0	4	0
19	6	7	13	4
20	11	2	4	0
21	25	8	14	8
22	26	8	15	3
23	26	8	13	4
24	15	12	5	0
25	6	8	2	2
26	16	7	15	6
27	20	9	3	5
28	5	7	0	5
29	2	3	3	4
30	2	1	2	0
31	7	8	2	3
32	0	1	1	3
33	4	5	2	3
TOTAL	304	162	162	92
MEAN	9.21	4.91	4.91	2.78

Table 8.

RADIATION IN FIVE LARVAE AND IN THIRD WASH AFTER 24 HOURS INCUBATION WITH ^{59}Fe

No. of experiment	Larvae	Third wash
1	15	02
2	12	12
3	05	00
4	21	02
5	13	02
6	05	00
7	04	02
TOTAL	75	20
MEAN	10.71	2.85

and 8.5 count/minute respectively. The means of radiation in the groups of five larvae incubated in vitro with labelled bacteria were between 10.71 - 12.5 count/minute. The mean of radiation in the five larvae incubated in vitro is the count in the larvae plus the count in 0.1 ml third wash solution. The means of the radiation in 0.1 ml third wash solution were between 2.85 - 5.0 counts/minute. So the actual radiation obtained by the larvae is the same in vitro and in vivo.

Experiment No.6 : The ability of larvae exposed to labelled bacteria in vitro to disseminate them to the tissues of mice

In this experiment the T.canis larvae were incubated with ^{59}Fe labelled E.coli in bijou bottles for 24 hours, then fed to the mice. The radiation in the larvae was estimated by measuring the count in five larvae to be sure that they were labelled. Various numbers of larvae were used (32 - 1,250). The mice were killed five days after they received the larvae. The liver, lung and brain and 0.5 ml blood were used for counting the radiation that was compared with the background in the surrounding air. The means of radiation counted per minute were, from liver 4.44, lung 8, brain 2 and blood 10.16 (Table 11). This amount is small compared with the amount of radiation obtained from experiment No.5, (liver 463,25, lungs 152.58 and brain 40.75 counts/minute).

Table 9.

RADIATION IN THE LIVER, LUNG AND BRAIN EMULSIONS OF INFECTED AND CONTROL MICE AFTER 3 - 5 DAYS INFECTION WITH 1,000 T.CANIS OVA AND SUSPENSION OF ^{59}Fe LABELLED E.COLI BACTERIA

Number of experiment	Infected with ova and bacteria			Control with bacteria only		
	Liver	Lung	Brain	Liver	Lung	Brain
1	920	-	96	68	-	6
2	264	-	41	64	-	17
3	184	82	59	183	52	47
4	92	63	4	35	11	1
5	1208	131	42	55	43	3
6	479	148	41	238	54	21
7	46	-	26	368	-	5
8	194	-	46	75	-	62
9	184	97	14	133	27	13
10	118	47	13	109	2	4
11	1209	184	84	442	15	28
12	378	449	25	230	287	5
13	199	75	30	164	44	5
14	399	15	67	181	12	18
15	810	256	14	395	72	2
16	728	284	50	299	15	24
TOTAL	7412	1831	652	3039	634	261
MEAN	463.25	152.58	40.75	189.93	52.83	16.31

Table 10.

RADIATION IN FIVE LARVAE OBTAINED (IN VIVO) FROM THE ORGANS OF MICE FED WITH ^{59}Fe LABELLED E.COLI BACTERIA

Number of experiment	Liver	Lung	Brain
1	9	7	8
2	3	9	8
3	10	9	4
4	10	10	13
5	7	7	10
6	13	9	11
7	8	5	10
8	6	9	4
TOTAL	66	65	68
MEAN	8.25	8.12	8.50

These results could be explained by the small number of larvae obtained from the organs of mice (Table 12). Only a small percentage of larvae were able to reach the mice organs. Out of 3,382 larvae fed to the nine mice, only 259 larvae were obtained from the organs of corpses. The average number of larvae obtained from the livers was 4.67, from the lungs 15.78 and from the brains 8.33.

Two mice were given 320 and 370 T.canis larvae incubated with ^{32}P (Table 13). The same methods were followed as in the experiments with ^{59}Fe. The results are not much different from the ^{59}Fe experiments. The means of radiation count/minute in the liver (1.5), lung (2), brain (1.5) and blood (2) are negligible. In the two mice (Table 14), the percentage of larvae obtained from the liver, lung and brain were 3.32, 2.32 and 2.17 respectively.

Table 11.

RADIATION IN DIFFERENT ORGANS OF MICE FIVE DAYS AFTER RECEIVING 32 - 1250 T.CANIS LARVAE INCUBATED WITH ^{59}Fe LABELLED BACTERIA

Number of experiment	No.of larvae	Liver	Lung	Brain	0.5 ml blood
1	32	3	9	1	No sample
2	45	4	8	0	No sample
3	100	2	10	4	No sample
4	200	5	7	4	10
5	250	11	14	0	4
6	250	5	0	5	33
7	355	6	16	2	11
8	900	4	2	0	2
9	1250	0	6	0	1
	TOTAL	40	72	16	61
	MEAN	4.44	8.00	2.00	10.16

Table 12.

NUMBER OF LARVAE COLLECTED FROM DIFFERENT ORGANS OF MICE FIVE DAYS AFTER GIVING VARIOUS NUMBERS OF ^{59}Fe LABELLED LARVAE TO MICE

No. of experimental mouse	No. of larvae fed	No. of larvae collected		
		Liver	Lung	Brain
1	32	9	3	11
2	45	0	6	3
3	100	1	16	5
4	200	0	5	3
5	250	9	6	7
6	250	0	5	5
7	355	0	5	7
8	900	10	42	16
9	1250	13	54	18
TOTAL	3382	42	142	75
Mean	375.77	4.67	15.78	8.33
Percentage		1.24	4.20	2.21

Table 13.

RADIATION IN DIFFERENT ORGANS OF TWO MICE FIVE DAYS AFTER RECEIVING 320 AND 370 LARVAE INCUBATED WITH ^{32}P LABELLED BACTERIA

Number of larvae received by mice	Radiation in 0.1 ml Liver	Radiation in 0.1 ml Lung	Radiation in 0.1 ml Brain	Radiation in 0.1 ml Blood
320	3	1	1	4
370	0	3	2	0

Table 14.

NUMBER OF LARVAE COLLECTED FROM DIFFERENT ORGANS OF TWO MICE FIVE DAYS AFTER RECEIVING 320 AND 370 LARVAE INCUBATED WITH ^{32}P LABELLED BACTERIA

Number of larvae received by mice	Number of larvae collected		
	Liver	Lung	Brain
320	8	6	7
370	15	10	8

Further experimental work on the ability of Toxocara canis larvae to carry and disseminate E.coli bacilli in vitro and in vivo.

These experiments were carried out to ascertain the veracity of the results obtained from the previous work in which radioactive isotopes (^{32}p and ^{59}Fe) were used to label the E. coli. In the current work the bacteria were detected by bacteriological and serological methods instead of counting the radiation from them after labelling. The non-pathogenic E. Coli strain No. 8196 was used. The larvae were exposed to the bacteria in bijou bottles and incubated then stained with Gram stain after having been washed three times with sterile saline.

The Gram stain was preferable to the fluorescent stain because of the fact that in order to get rid of the autofluorescence, i.e. pepsin digestion would get rid of the bacteria. In vivo the aim of the experiment was to collect evidence indicating whether the larvae were able to carry the E. coli to the organs of the mice whether the bacteria were coming from the test tubes or the intestine. For this reason a

group of control mice fed on larvae that were not exposed to the bacteria was not included; but when the bacteria were isolated from the majority of the dually infected mice, efforts were made to determine whether they were carried by the larvae from the test tubes or the intestine.

The bacteria were isolated from the organs of mice three days after the animals were fed T.canis larvae that had been exposed to E.coli in bijou bottles. Two groups of 10 mice each were included in the experiment as controls. In the first control group each mouse was fed 0.1 ml suspension of E.coli broth culture. A group of 10 normal mice was also included in the experiment. The control mice were also killed and the same methods were applied to isolate bacterial growth from the organs. When Gram negative lactose fermenter bacilli were isolated from any organ, serological tests were carried out to find whether the bacteria were of the same strain (No.8196) of E.coli that the larvae were exposed to in the bijou bottles. Aseptic precautions were followed to avoid contamination.

Materials and Methods

Experiment No.7

I. In Vitro:

Stock E.coli culture (strain no. 8196) on nutrient agar were inoculated to 4 ml nutrient broth contained in a bijou bottle and were incubated at 37°C. After 24 hours about 1,000 T.canis larvae were added to the bacteria in the bijou bottle. The bottle was incubated at room temperature for 24 hours. The larvae then were picked up by a bent Pasteur pipette. They were washed three times with sterile saline as described in the radio-active isotopes experiments. The larvae were suspended in sterile saline then were fixed with 10% formalin and put on slides. The slides were fixed by heat and were stained with Gram stain method as follows:

1. Aniline gentian violet for 2-3 minutes. Five grams of gentian violet were ground in 10 ml absolute alcohol in a mortar and 88 ml of distilled water with 2 ml aniline were added. Then they were ground until the stain was dissolved. The mixture was allowed to stand before it was filtered.
2. Rinsed in running water for 2 minutes.
3. Gram's iodine solution for 2-3 minutes. Iodine 1 gram, potassium iodide 2 grams with 300 ml distilled

water.

4. Rinsed in running water for 30 seconds.
5. Decolourised with absolute ethyl alcohol until no more colour came away.
6. Counterstained with safranin. Safranin 1 gram with 200 ml water.
7. Rinsed in running water and dried.

A Gram stained smear was obtained from the second wash to detect the Gram negative bacilli that the larvae were exposed to in the bijou bottle. At the same time sub-culture on nutrient broth was obtained from the second wash supernatant and was incubated at 37°C for 24 hours. The bacteria grown in the nutrient broth were plated to McConkeys media that were incubated at 37°C for 24 hours. Gram stained smears were obtained from the McConkey's plate. When Gram negative lactose fermenter bacilli were isolated, a single colony was inoculated to agar slope and incubated at 37°C. Serological and biochemical (API) tests were then carried out on the bacilli to find out whether they were of the strain of E.coli used in the beginning of the experiment. These tests are described in experiment number 8.

Experiment No.8

In Vivo:

Three groups of ten mice were used in this experiment.

The first group was fed larvae that were exposed to E. coli. A control group was fed bacilli only. A second control group of 10 normal mice was also included in the experiment. The same non-pathogenic E.coli (strain number8196) was inoculated into 4 ml nutrient broth contained in a bijou bottle and incubated at 37°C for 24 hours. About 5,000 T. canis larvae were then added to the culture and the bijou bottle was incubated at room temperature for 24 hours. The larvae picked up by bent Pasteur pipette were washed three times with sterile saline as described before. Then the larvae were suspended in 0.5 ml sterile saline and fed to a mouse directly into the stomach by means of a syringe and plastic tube. Three days later the mouse was killed. The liver, lungs and brain were obtained. The left lobes of the lung were emulsified in a sterile tissue grinder with 2 ml sterile saline. The emulsion was halved to 2 nutrient broth tubes that were incubated at 37°C for 72 hours. From the right lung paraffin sections were obtained. The sections were put on slides, fixed and stained with Hematoxylin and eosin. The slides were examined for the presence of T. canis larvae in order to be sure that they were able to migrate from the intestine to the organs of the animals. A portion of the liver approximately 1 gram in weight was emulsified in a sterile tissue grinder with 2 ml sterile

isotonic saline. As in the case of the lung, this emulsion was halved into 2 nutrient broth tubes and incubated at 37°C for 72 hours. The brain was emulsified in a sterile tissue grinder with 2 ml sterile saline. The emulsion was transferred to a nutrient broth tube and incubated at 37°C for 72 hours.

After the 72 hours of incubation all the broth cultures were plated to McConkey's agar plates and incubated at 37°C for 24 hours. When lactose fermentation was detected a single colony was taken on a slide. Gram stained smear was prepared to find whether the bacteria was Gram negative. When Gram negative, lactose fermenting bacilli were detected; one colony was inoculated to nutrient broth and incubated for 6 hours at 37°C. From the broth culture the bacilli were plated to McConkey's agar and incubated at 37°C for about 24 hours. A single colony from the McConkey's agar was inoculated to a nutrient broth slope for serotyping of the bacilli. The serotyping was done by using polyvalent E. coli antisera (supplied by Colindale Laboratories, 1978), for I, II and III. When no agglutination with the strain of bacteria by the above antisera was detected, the conclusion was that the E. coli were non-pathogenic with a strong possibility that they were of the same strain of bacteria that was used in the beginning of the experiment.

isotonic saline. As in the case of the lung, this emulsion was halved into 2 nutrient broth tubes and incubated at 37°C for 72 hours. The brain was emulsified in a sterile tissue grinder with 2 ml sterile saline. The emulsion was transferred to a nutrient broth tube and incubated at 37°C for 72 hours.

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The micromethod multitest system (API) (supplied by API Lab. Products Ltd.,) was then used to identify the isolated bacteria. This was carried out by comparing the biochemical activity of the bacteria isolated from the organs of mice with that of the original stock culture of E. coli (strain no. 8196). The API System is one of several devices commercially available for the identification of Enterobacteriaceae. A plastic strip holding 20 miniaturized compartments, or cupules, each containing a dehydrated substrate for a different biochemical test (Smith et al., 1972). This device is based on work by Buissiere and Nardon (1968) who established many of the physical and chemical requirements of such micromethods.

To each mouse of the first group of 10 control mice a suspension of 0.1 ml E. coli broth culture was fed directly into the stomach by means of a syringe and a plastic tube. The 0.1 ml bacterial suspension was obtained from a 24 hours nutrient broth culture of E. coli (strain no. 8196). A second group of 10 untreated mice was also included. When the control mice were killed the same methods were applied to isolate the bacteria from the agars.

Results

Experiment No.7 : In Vitro

When the Gram stained slides of T. canis larvae were

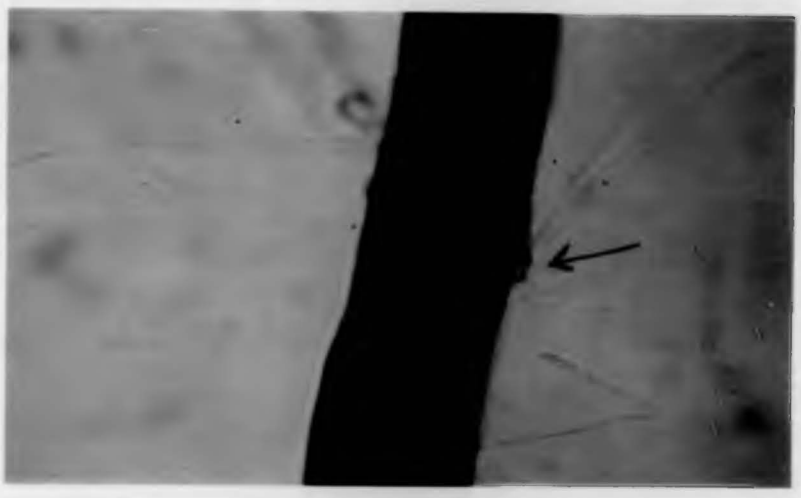
examined, very few Gram negative bacilli (presumably E. coli) were seen adhering to the external surface of some larvae (Figure 1). The bacilli could not be seen adhering to the external surfaces of the majority of the larvae. Subcultures obtained from the second wash supernatant were plated onto McConkey's agar. The Gram negative bacilli were proved to be E. coli when they were found to be a lactose fermenter on the subcultures obtained from the second wash supernatants. The bacilli obtained from all the subcultures were Gram negative lactose fermenters. The bacteria were strongly suspected to be of the same strain used in the beginning of the experiment, because no agglutination was observed with the strain of the bacilli by the polyvalent E. coli antisera for I. II and III.

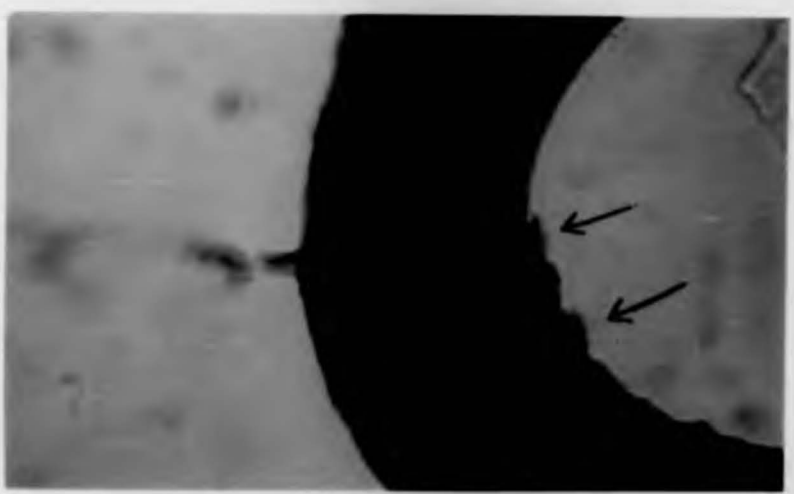
Experiment No.8 : In Vivo

Gram negative bacilli probably of the same strain that the T. canis larvae were exposed to in vitro were isolated from the lungs of 7, the livers of 4 and the brains of 2 mice out of the 10 mice that were fed the larvae that were exposed to the bacilli (Table 15). The bacilli were isolated from the brain of Mouse 3 while the lung and the liver of this mouse were sterile. In the case of Mouse 5, the bacilli were isolated from the lungs in addition to the brain. In the case of all the mice, where isolation of the bacilli were obtained from the

Figure 1.

GRAM STAINED T. CANIS LARVA SHOWING THE E.COLI BACILLI ADHERING TO THE EXTERNAL WALLS





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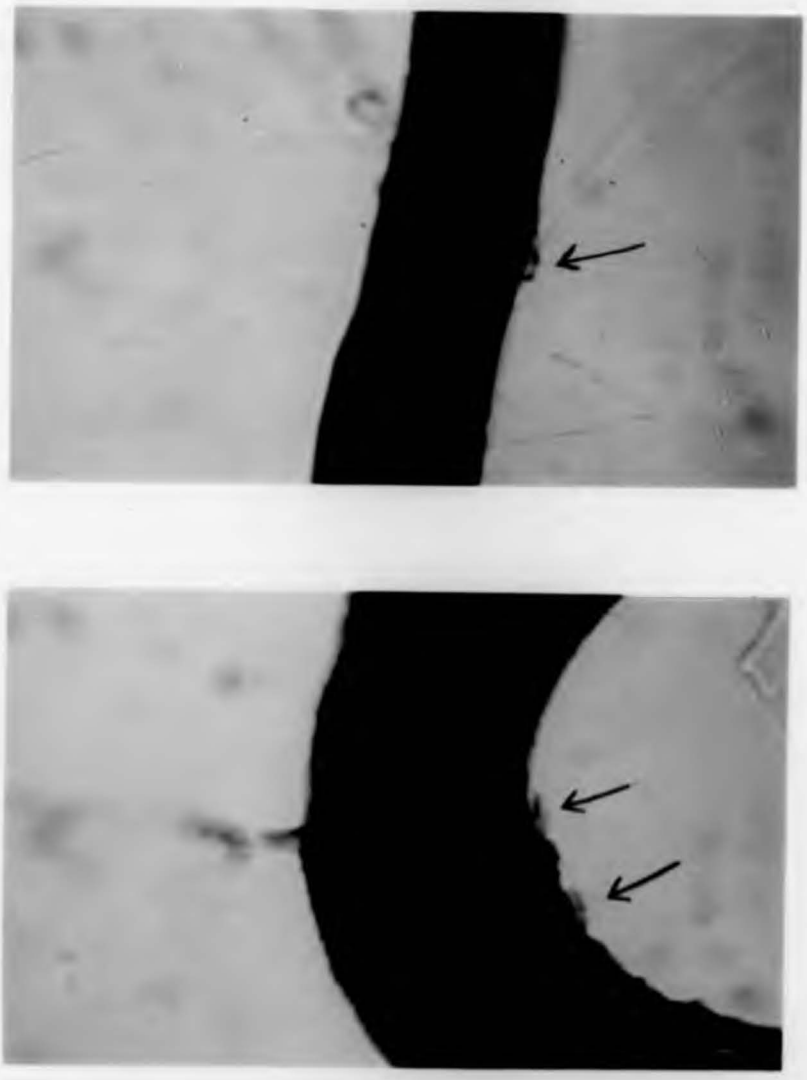
GRAM STAINED T. CANIS LARVA SHOWING THE E.COLI BACILLI ADHERING TO THE EXTERNAL WALLS



livers (Mice 2, 6, 8 and 9), the bacteria were also isolated from the lungs. It was not possible to isolate the bacteria from both the liver and brain of any single mouse.

The bacilli that were isolated from all the organs (marked +) in Table 15 were proved to be E.coli and probably of the same strain used in the beginning of the experiment. They were Gram negative lactose fermenting bacilli. There was no agglutination with the polyvalent E.coli antisera for I, II and III. This finding gave a strong possibility that the E.coli isolated were non-pathogenic and probably of the same strain of bacteria that the T.canis larvae were exposed to before the larvae were fed to the mice.

The results of the API test showed that the overall agreement in the biochemical activities of the isolated and the original cultures of E.coli was 100%.

Portions of the larvae were seen on all the Hematoxylin and eosin slides prepared from the sections of the lungs (Figure 2). All the organs of the two groups of the control mice were sterile.

Table 15

ISOLATION OF E.COLI BACILLI FROM THE ORGANS OF TEN MICE EACH FED WITH 5,000 T. CANIS LARVAE THAT WERE EXPOSED TO THE BACILLI IN VITRO

Mouse no.	Lung	Liver	Brain
1	-	-	-
2	+	+	-
3	-	-	+
4	-	-	-
5	+	-	+
6	+	+	-
7	+	-	-
8	+	+	-
9	+	+	-
10	+	-	-
Total +	7	4	2
10 controls fed E.coli	neg	neg	neg
10 normal animals	neg	neg	neg

Figure 2.

LUNG SECTIONS FROM 2 MICE SHOWING PORTIONS OF T. CANIS LARVAE

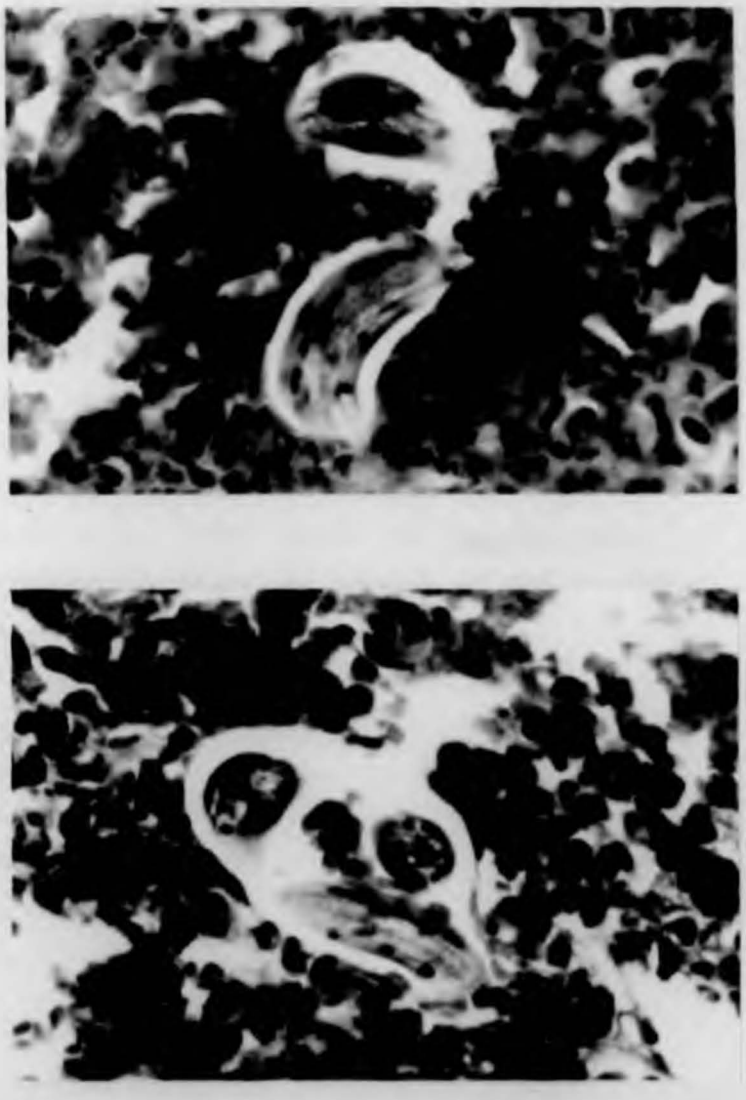
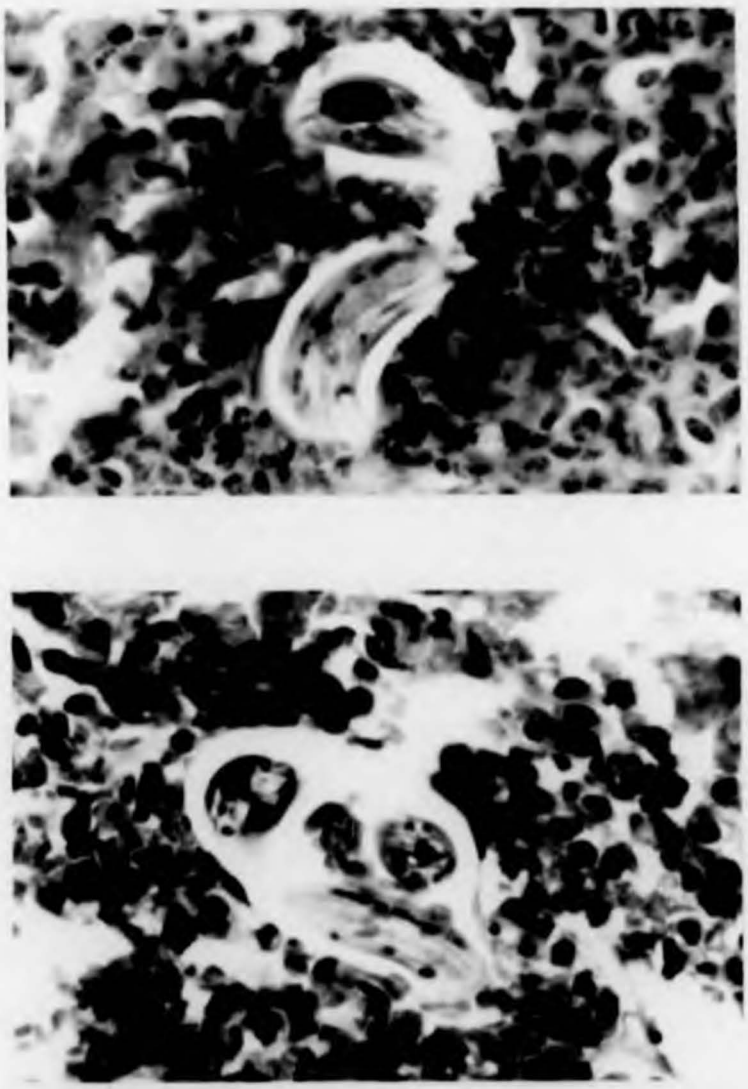


Figure 2.

LUNG SECTIONS FROM 2 MICE SHOWING PORTIONS OF T. CANIS LARVAE



DISCUSSION

The previous attempts to study the relationship between the migrating larvae and the microbiological organisms are few when the importance of the subject from the medical point of view is taken into consideration. In general the previous studies were in two categories.

In the first category are the studies of Shope (1941, 1943a and 1943b) on the transmission of swine influenza virus by the swine lung worm under both experimental and natural conditions. The study reported by Syverton et al. (1947) was a very good indication of the ability of Trichinella spiralis to carry the virus of lymphocytic choriomeningitis experimentally in Guinea pigs. Also in this first category is the experimental work reported by Mochizuki et al. (1954) where the Toxocara canis larvae were used to study the ability of the nematodes to act as a provoking factor in Japanese encephalitis. The results showed that there was provocation of the virus encephalitis in the dually infected mice and there was little pathogenic effect on the brain when the strain of virus used was injected alone. Repeat work by Pavri et al. (1975) confirmed these findings and demonstrated that the T.canis were able to provoke Japanese encephalitis.

In all these studies on animals the experimental approaches depended on detecting the clinical and pathological changes after dual infection of the animals with microbiological organisms and parasites. The only exception was the study done by Pavri et al. (1975). In that study attempts were made to isolate the virus from different organs of mice. The other observation to be made on these studies is that in most of them viruses that cause clinical and pathological changes in the central nervous system were used. It seems that the reason for using these viruses is most probably due to the fact that in experimental animals the clinical manifestations of the pathological changes in the nervous system are clearly presented and easily detected.

In the second category there are two important studies that also involve the central nervous system by viruses. The first study was in England and was reported by Woodruff et al. (1966) pointing to the relationship between poliomyelitis and Toxocara canis. In Egypt repeat work confirmed the same relationship and was reported by Khalil et al. (1971). The methods employed in both studies were the same. The Toxocara skin test was used for the diagnosis of toxocariasis in patients who had had poliomyelitis. The results were compared to a

group of apparently healthy controls. These retrospective studies are the only experimental work for exploring the relationships between the nematode larvae and the disease-causing micro-organisms.

From both categories of experiments, whether in animals or in man, it became clear that the nematode larvae used, i.e. Trichinella spiralis and T.canis, probably played a role in the transmission or dissemination of the viruses, but the mechanisms or mechanism involved were not clearly evident.

The ability of ^{32}P isotope to label the E.coli bacteria

In this study Escherichia coli bacteria were used. The use of these bacteria, which are a natural inhabitant of the intestine of both mice and man, could give clear answers about the ability of T.canis larvae to carry microbiological organisms. The use of this bacteria gives more practical clinical applications than by using uncommon micro-organisms. The use of radioactive isotopes to label the bacteria made it possible to detect their presence in the bodies of the very small T.canis larvae. On the other hand, evidence of the presence of the bacteria in different organs of mice could be sought, whether there were pathological changes in the tissues of the mice or not. For these reasons, the use of the labelled bacteria

gave a better chance to study the mechanisms involved in the relationships between the larvae and the bacteria.

The bacteria were first successfully labelled with ^{32}P. This isotope, with a half-life of 14.8 days, was considered suitable because the initial experiments were planned to take less than ten days. There is no doubt that the bacteria were able to take up the radioactive isotope. In the four days of the experiments, the means of radiation counted per minute in 0.1 ml of the third wash supernatant were 1 - 2, while the means of radiation counted in the same volume of bacterial suspensions were 2630 - 1357 (Table 1). Less radiation was counted when the periods of incubation increased. The radiation counted on the second day of incubation was about 12% less than that counted on the first day, 24% less on the third day than the second day, and 23% less on the fourth day than the third day. These percentages are much higher than the percentages obtained from the isotope decay tables, which showed that the ^{32}P loses about 5% of its radioactivity daily. This suggests that a large amount of the bacteria accept the isotope in high

levels on the first day of exposure, then lose some of it as the period of incubation lengthens. The washing of the bacteria was very successful, for after three washes the amount of radiation counted in the supernatant solution (1 - 2) was negligible. This leads to the conclusion that the amount of radioactivity was due to the actual radiation in the bacteria only. These findings were very important for continuing the experimental work, because they demonstrated that the radiation counted in the T.canis larvae and mice tissues was most probably due to the presence of the labelled bacteria.

^{51}Cr to label the E.coli bacteria.

The use of ^{51}Cr indicated that the bacteria took up some of the isotope, but to a much less extent than they did with ^{32}P. The maximum count of radiation in the bacteria (432 count/minute) was about twice the background which was sometimes about 400 count/minute (Table 2). For that reason the use of this isotope was discontinued.

The ability of ^{59}Fe isotope to label the E.coli bacteria

For ^{59}Fe the bacteria were labelled in the same way as ^{32}P, i.e. more radiation was counted on the first day of exposure and the amount with which they became labelled

was less after longer periods of incubation. On the first day the mean amount of radiation counted per minute in 0.1 ml bacterial suspension was 12,446; while the mean in the same volume of bacterial suspension was 2,086 count/minute on the sixth day of incubation (Table 3). This is different from the ^{51}Cr where the amount of radiation was gradually increased during the first three days of incubation, then less radiation was counted with longer periods of incubation. The percentage of daily decrease in the level of radiation was high compared with the percentage level for natural decay. In the isotope decay table the percentage is about 2% daily, while there was a 40% decrease in the second day of exposure and about 60% in the third day. As with ^{32}P this suggested that a large amount of the bacteria accept the isotope in high levels on the first day of exposure, then lose some of it as the time of incubation lengthens. The washing of the bacteria was very successful, because the mean amount of radiation counted in 0.1 ml of the third wash supernatant was about 10 count/minute, which is negligible in comparison with radiation in the background, which was sometimes about 400 count/minute.

There is a clear indication that the E.coli accept the ^{32}P isotope to a much more stable degree than they do with the other isotopes used in this study. It is known that both phosphorus and iron are required for the growth of

nearly all organisms (Stanier et al. 1971). To grow, organisms must draw from the environment all the substances which they require for the synthesis of their cell materials and for the generation of energy. In E. coli, phosphorus is approximately 3% of the dry weight of the cell, while iron is about 0.2% only. By using the ^{32}P and ^{59}Fe there is a good reason to assume that the amount of radiation in the larvae and mice was due to the bacteria, for fluid in which the bacteria had been washed produced a negligible mean radiation count.

The ability of the Toxocara canis to carry the bacteria in vitro

The use of radioactive isotopes in this study helped in demonstrating that the larvae were able to carry the E. coli bacteria. The mean amount of radiation counted in the larvae was about three times more than that counted in the supernatant after three washes ($p < 0.01$ for ^{32}P labelled bacteria and $p < 0.05$ for ^{59}Fe labelled bacteria). Results obtained with the use of both ^{32}P and ^{59}Fe isotopes indicated that the larvae were able to keep their loads of bacteria even after three washes (Tables 4, 5, 6 and 8).

This observation suggested that the bacteria were

either adhering to the external walls of the larvae or they were carried in their alimentary canal. Examinations of the Gram stained slides of T.canis larvae that were exposed to the E.coli (strain no.8196) in test tubes showed that few E.coli were seen to be adhering to the external surfaces of some larvae. (Figure 1.)

Dr J. Grant of the Department of Microbiology, London School of Hygiene and Tropical Medicine (personal communication) stated that the object shown in Figure 1 cannot be stated with certainty to be E.coli bacilli. The other tests that were carried out, however, (i.e. Gram stain, polyvalent antisera and API System) gave strong evidence that they were E.coli and possibly of the same strain that were used in the beginning of the experiment.

Although the very few bacteria that were seen adhering to the external walls of the larvae could explain the ability of the larvae to carry the E.coli to the organs of the mouse, the possibility of transference of E.coli from the alimentary canals of the larvae cannot be overlooked. This possibility was not proved because it was not possible to obtain sections of the

larvae in the current work. Attempts were made to obtain paraffin sections from 30,000 larvae. About 200 slides were prepared and stained with Gram stain, but it was not possible to detect any transverse section of the larvae.

In the studies carried out by other workers, prior to this work, it had only been possible to find whether the larvae were capable of carrying the organisms by feeding them to experimental animals and observing whether the animals developed signs of the infection. The methods that were employed to demonstrate the ability of the larvae to carry and transmit the organism were not always conclusive because of the involvement of the host animals.

It is clear that the clinical and pathological changes depend not only on the disease-causing organism, but to some extent on the resistance of the individual host to that particular infection and also on the dose of organisms that reaches the organs affected. This means that small doses of the organism transmitted by the larvae may not necessarily cause a clinical or pathological change and could lead to the possibly incorrect conclusion that the larvae were not able to carry the organism.

The work of Shope (1941) could not be conclusive because two factors were involved in addition to the larvae and the virus, i.e. pigs and the Haemophilus influenzae, making four variables as contrasted with two in the experiments discussed here. Shope's attempts to demonstrate swine influenza virus by direct means have failed. Other stimuli were used to provoke swine influenza; in some of the experiments 30,000 - 50,000 normal embryonated ascaris ova, in addition to the H. influenzae. Although the animal's temperature rose and it appeared ill, there was a chance that this clinical picture could be due to the ingestion of Ascaris ova. It is difficult to find any clinical application behind the use of 100 c.c. of 95% ethyl-alcohol mixed in the ground grain mash of the swine to provoke the influenza. The use of these complicated experimental approaches, however, gives some indication of the role of the worm in transmitting the influenza virus, but gives no clear idea about the mechanism involved. Also the use of some expressions by Shope, such as "the virus carried in a masked form" were not well understood.

The life span of the T. canis larvae in bacteria contaminated media

In the current study the use of the radioactive isotopes

helped in demonstrating that the T. canis larvae were able to carry E. coli bacteria. One important observation was the inability of the larvae to live for more than three days in the test tubes; this prevented the work from being extended for a longer period.

In all these experiments the activity of the larvae could be judged by their mobility. On the first day of incubation with the labelled bacteria, all the larvae were active. This activity was much less on the second day and on the third day some of the larvae were immobile. On the fourth day all larvae were immobile.

There is no report of the life span of the T. canis larvae in bacteria-contaminated media. The fact that the larvae were able to live for three days at room temperature gives some information about the possibility that the toxocaral infection might be transmitted to an animal if it eats the carcass of another animal infected with the T. canis larvae. Sprent (1953) reported the results of a study on the longevity of various ascarid larvae and their resistance to freezing and putrefaction. The study showed that T. canis larvae kept in a refrigeration unit at -20°C could not live for six days. To study the resistance of the ascarid larvae to putrefaction, the bodies of infected mice were allowed to undergo putrefaction at about 27°C. At various intervals the

mice were digested and mobility of the recovered larvae was observed. The results showed that although the larvae of A. columnaris, A. devosi and T. transfuga were evidently able to withstand putrefaction for at least six days, the larvae of T. canis were immobile at the end of the same period of six days, but Sprent does not actually give the survival time of T. canis in these conditions. It may have been less than six days. As there are no more reports on the subject of longevity of the T. canis larvae, the results obtained from the experiments in vitro could be considered as a good indication of their life span in media contaminated with bacteria in addition to the radioactive isotope.

These results might lead to the conclusion that there is a possibility that toxocaral infection could be transmitted if the carcass of the animal that received the infection is eaten by another animal during the first three days after death. Because the mobility of the larvae decreased with longer periods of exposure to the labelled bacteria, it is logical to conclude that the shorter the period that elapses between the death of the infected animal - usually rodent - and the ingestion of its carcass, the greater the chance for the infection to be transmitted. This means that if

the body of the infected rodents are eaten immediately as prey by another animal, there will be every possibility that the infection will be transmitted.

These observations are in agreement with the results demonstrated experimentally by Sprent (1958) who fed dogs and foxes the carcasses of mice which had ingested the embryonated toxocara eggs and harboured the second stage larvae in their tissues. He found that in some instances development of these larvae proceeded in the stomach and intestine. Few larvae were found in the somatic tissues of dogs, but three days after infection fourth stage larvae were found in the stomach wall and 9 and 10 days after infection fourth stage larvae were found in the intestine. In foxes, fourth stage larvae and adults were found in the intestine 17 and 21 days after infection; larvae were also recovered from the somatic tissues, especially the lungs.

The role of the T. canis larvae in the transmission and dissemination of the bacteria in the tissues of mice

The use of the isotopes in this study led to a better understanding of the role of the T. canis larvae in the transmission and dissemination of the bacteria in the tissues of mice. As mentioned earlier in this paper, those methods of testing the role of the larvae in the dissemination of the bacteria were probably able to demonstrate

even the small amount of the micro-organism present in the organs of the mice, which usually does not lead to the occurrence of clinical or pathological signs and symptoms.

In the experiment with ^{32}p labelled bacteria the results showed that T.canis larvae probably played a role in the dissemination of the E.coli. The amount of labelled bacteria in the two organs tested - the bacteria in both liver and brain - of dually infected mice was twice that found in the control animals (Table 7). Significantly more radiation was present in the livers of the infected mice than in the controls ($p < 0.02$). The statistical difference between the brains of the 2 groups was even more significant with $p < 0.01$. Obviously these statistical differences gain in importance from the small volume of the organ's suspension used in this experiment. It was mentioned earlier that it was not possible to measure the radiation for the β emitting ^{32}p in volumes larger than 0.1 ml due to the nature of the scintillation counter. This small number was 1/100 fraction of the total suspension of each organ. The evidence suggests that the larvae did help in the dissemination of the bacteria in the mice tissues.

When the same experiment was repeated by using the γ

emitter, ^{59}Fe, it was possible to detect the presence of labelled bacteria in the total suspension of the mice organs. In this experiment the differences in the amount of labelled bacteria in the organs tested were greater than those of the ^{32}p experiment. There is about three times as much radiation in the tissues of the dually infected group of mice (Table 9). This ratio of 3/1 is applied to the individual organs and also to the total radiation in the three organs tested. The statistical differences were highly significant for both liver and brain ($p < 0.01$). They were less striking but still significant at the 5% level for the lung ($p < 0.05$). The results in the case of the lungs would probably have reached greater statistical significance had it been possible to take readings on a larger number of lungs.

It is interesting to notice that the amount of radiation in the liver is more than the lung and more in the latter than in the brain. It is well known that most of the T.canis larvae after hatching in the intestine migrate to the liver, then the lungs and from there to different organs and tissues, including the brain. There appears to be a good indication of a relationship between the number of larvae reaching an organ and the amount of radiation in that particular organ. Thus there is a greater chance of microbiological disease being acquired if a larger number of parasites are ingested.

The findings of two to three times more radiation in dually infected mice than in the control group are in agreement with the demonstration reported by Pavri et al. (1975) in which 20 mice were infected with 700 - 1,000 embryonated eggs of T. canis and in addition these mice were infected subcutaneously with sublethal doses of Japanese encephalitis virus. A control group of 30 mice were given the virus only. Of the dually infected mice 57% showed morbidity (no signs of the sickness were given), while 20% of the control group showed the same pattern of sickness - a ratio of nearly 3/1 which is similar to the results summarised in Tables 7 and 9.

Mochizuki et al. (1954) obtained about the same ratio when ten mice were fed each with about 2,000 embryonated T. canis eggs. Four days later five of the mice were inoculated subcutaneously with a suspension of Japanese B. encephalitis in 10-1 dilution. All of the dually infected mice died in about six days after the virus inoculation, against two mice that received the virus suspension only.

The amount of radiation measured from the groups of five larvae obtained from each organ gives an indication that they most probably carry the labelled bacteria (Table 10). The amount of radiation in each group of larvae was about the same amount as that found in those exposed to the labelled

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The amount of radiation measured from the groups of five larvae obtained from each organ gives an indication that they most probably carry the labelled bacteria (Table 10). The amount of radiation in each group of larvae was about the same amount as that found in those exposed to the labelled

bacteria in the test tubes. The means of radiation in the groups of five larvae obtained from the liver, lung and brain of mice were 8.25, 8.12 and 8.5 counts/minute respectively (Table 10). The means of radiation in the group of five larvae exposed in vitro to labelled bacteria were between 10.71 and 12.5 counts/minute (Tables 4, 5, 6 and 8). The radiation count obtained in vitro represented the radiation in the larvae plus the radiation in 0.1 ml of the third wash solution. The means of radiation in 0.1 ml third wash solution were between 2.85 and 5 counts/minute. The actual radiation counted in the larvae, therefore, is about the same in vitro and in vivo.

These results strongly suggested that the larvae, if exposed to the E.coli, do carry the bacteria whether the exposure is in a test tube or the intestine. The amount of bacteria carried by an individual larva is small, and this supports the suggestion that in order to transmit a microbiological infection from the intestine to other organs of the body, a very large dose of larvae is necessary. On the other hand, in the case of a highly pathogenic organism, like the poliomyelitis organism, a small number of larvae might be able to transmit the infection.

These observations might explain why the larvae of T.canis were incriminated in the transmission of

viruses but not bacteria. All the reported cases that related the T. canis to microbiological diseases involved viruses that cause severe signs and symptoms. Mochizuki et al. (1954) obtained evidence in experimental animals that the larvae were able to transmit Japanese B. encephalitis virus and cause death and morbidity in mice infected with larvae and virus. The conclusion obtained by Pavri et al. (1975) confirmed that of Mochizuki et al, by repeating the experiments. They obtained results that gave a clear and significant difference between the dually infected mice and those that received the virus only, in relation to sickness and death.

In man, the reported cases were also of viral infection. The case reported by Dent et al. (1956) of a child who died of homologous serum hepatitis where T. canis was found widely scattered throughout the organs, including the central nervous system, is an example. Another case was that reported by Beautyman and Woolf (1951) where a T. canis larvae was found in the brain of a child who died of poliomyelitis. This leads to the conclusion that serious disease could be occasioned if a few larvae could carry small doses of a highly pathogenic organism to important organs like the liver and brain. In addition the two cases reported by Brown and Perna (1958) and by Wilson and Thompson (1964) where the patients died of E. coli

septicaemia and meningitis in relation to overwhelmimg strongyloides, indicated that a large number of larvae could carry big doses of microbiological organisms causing severe disease.

The possibility that more than one mechanism is involved in the transmission of the E. coli

The amount of radiation carried by each larva does not explain the high amount of radiation in the mice exposed to larvae and bacteria, so some other mechanisms must be involved in the transmission of the bacteria from the intestine to other organs. It has been shown that larvae carry bacteria, but an anomaly was also found that there was radiation in the organs, more than that which could be accounted for by the larvae present with their known loads of radiation. This observation led to the conclusion that some labelled bacteria or loose label from bacterial disintegration or metabolism find their way to the tissue damaged by larvae such as the liver, lung and brain. A possible explanation is that the larvae, by their migration and by their active life in these organs, might create favourable conditions for the bacteria to settle and multiply. Sprent (1955b) in the study of the invasion of the central nervous system by nematodes, showed that T.canis larvae in mice were able to cause haemorrhages and necrosis in the brain tissues and he suggested that the damage might create favourable conditions for the pathogenic micro-organisms circulating

in the blood at that time.

Bisseru (1969) published a detailed study of the microscopic changes in the livers, lungs and brains of mice due to the migration of T. canis larvae. The study demonstrated that there were scattered foci of inflammatory cells, haemorrhage and fibrin deposit. In all three organs studied, the larvae appeared to be moving at the time of fixation. The results in these studies, together with the fact that the larvae are well tolerated by experimental animals and may stay alive in their tissues for a considerable time (Beaver, 1962), suggest that it is possible that the damage they cause is responsible for the large number of bacteria in the dually infected mice, indicated by the amount of radiation counted.

The results specified in experiment number 5 indicated that after feeding the mice T. canis larvae that were exposed, in test tubes, to ^{59}Fe labelled E. coli, they were probably able to transmit a small amount of the bacteria to the organs of the mice. The results are interesting in spite of the very small amount of radiation counted in the mouse tissue. The count per one minute in a single mouse was 24. The number of larvae found in the three organs was very small, even when 1,250 larvae were ingested. The small number of larvae collected from each

organ could be responsible for the small amount of bacteria found in the tissues (Table 11). This observation is supported by the higher amount of radiation in the lungs, where a higher percentage of the larvae were found. On the other hand, the higher amount of radiation counted in the liver, where a smaller number of larvae were obtained, compared to the brain where a larger number of larvae were found, could be explained by the possibility that the larvae during the portal migration left some of their bacterial loads in the liver. The large number of larvae found in the lungs five days after the ingestion of the larvae could lead to two suggestions:

1. The larvae, after reaching the intestine acted in the same way as when they hatch from ingested embryonated eggs, and will follow the liver-lung pattern of migration. This kind of migration was demonstrated by many workers (Beaver et al., 1952; Sprent, 1952, 1955b, 1958; Tiner, 1953; Wiseman et al., 1971). Sprent (1958) demonstrated that in a mouse five days after the ingestion of 5,000 embryonated T. canis ova, 539 larvae were obtained from the liver, while only 63 were obtained from the brain. No figure was given for the lungs. Sprent's results differ from those obtained in this study where the number of larvae obtained from the brain is three times more that that obtained

from the liver. However, Wiseman et al.(1971) reported that in mice, five days after the ingestion of 1,000 embryonated T. canis, there was a wide variation in the numbers of larvae recovered alive from the livers. The numbers were varied to the degree that from the livers of two mice 141 larvae were obtained from one, while only one larva was obtained from the other. These findings suggested that the observed small numbers of larvae recovered from the livers occurred by chance alone.

2. The larvae, in addition to the hepatic migration, might follow another pattern of migration, most probably directly to the lung, then to other organs, including the brain.

The amount of radiation counted in the lungs of mice was twice that counted in the livers. The mean of radiation in the lungs was 8 count/minute, while the mean in the liver was 4.44count/minute (Table 11).This observation supported the second pattern of migration rather than the first. The other observation that supported the second route of migration, was the higher percentage of larvae obtained from lungs than from the liver. The percentage of larvae obtained from the lungs was 4.20, while only 1.24 per cent were obtained from the liver, (Table 12). The small percentage of larvae found in the liver, together with the low radiation

counted from the liver suspensions, indicated that some larvae followed the normal portal migration to the liver then to the lungs.

When the experiment was repeated with ^{32}P, it was obvious from the beginning that there were less chances of success in getting clearer answers than the ^{59}Fe experiments, due to the fact that less volume of samples could be used for radiation counting. The results summarised and shown in Table 13 did not lead to any definite conclusion.

The small number of larvae reaching the three organs of mice is most probably due to their weakening during the period of incubation in the test tubes. This weakness was observed in experiment Number 3. Another possible explanation is that the larvae might lose some of their ability to penetrate the intestinal wall after their first time penetration of these walls before they were incubated in the test tubes.

The results obtained from experiment Number 8 by isolating the E. coli from the organs of the mice after they were fed T. canis larvae that were exposed to the bacteria in vitro, confirmed the results obtained when the radioactive isotopes were used to trace the bacteria. Out of the 10 mice that received the larvae, the E. coli were isolated from the lungs of 7, the livers of 4 and

the brains of 2 (Table 15). No E. coli growth was isolated from any mouse out of the 20 mice of the two control groups. The migration of the T. canis larvae from the intestine to the organs of the mice were proved and demonstrated by the hematoxylin and eosin stained slides of the sections obtained from the lungs (Figure 2).

The bacteriological and serological tests that were performed in this experiment strongly suggested that the bacteria that were carried by the larvae and isolated from the organs of the mice were of the same strain (8196) that were used in the beginning of the experiment. The observation that the larvae carried more bacteria to the lungs than to the livers or the brain, which was proved by the higher rates of isolation, is similar to that obtained when the experiments were carried out by using the ^{59}Fe isotope (Table 11). In the experiment in which ^{59}Fe was used as a tool to trace the E. coli, the mean radiation counted in the organs of 9 mice was 8 in the lungs, 4 in the livers and 2 in the brains (Table 11). In both experiments there was more chance for the bacteria to be detected in the lungs, then in the liver and then in the brain.

In the absence of a quantitative evaluation of the larvae in the different organs tested in this experiment, it is difficult to comment on the effect of the number

of larvae in each organ on the rates of bacteria isolation from these organs, but in the ^{59}Fe experiment the number of larvae found in the lungs of the mice was more than that found in the livers or brains (Table 12). For this reason it is possible to suggest that in the isolation experiment there could be larger number of larvae in the lungs than in the livers or brains. In the ^{59}Fe experiment it was suggested that the T. canis larvae in addition to their hepatic migration might follow another pattern of migration, most probably directly to the lungs, then to other organs, including the brain. This pattern of migration was suggested only in the cases when larvae and not ova of T. canis were fed to these mice. The results obtained from the isolation experiment, where the bacteria were isolated from the lungs of 7, the livers of 4 and the brains of 2 out of the 10 mice that received the larvae, supported the same suggestions, because the 10 mice were fed larvae and not eggs.

In spite of the fact that 5000 larvae are considered to be a very big dose to an animal like a mouse, the isolation experiment demonstrated that the T. canis larvae could carry and disseminate microbiological organisms from the alimentary canal to different organs of the body, including the brain. It also suggested that proportionally smaller doses of the larvae that

might be ingested by man could lead to dangerous health hazards if highly pathogenic micro-organisms were involved. This could be true in the case of a small number of T. canis larvae that might carry poliomyelitis virus reaching the central nervous system.

The results from this study showed that there are relationships between T. canis larvae and the bacteria. The relationship of the larvae to the dissemination of the bacteria might not be different from that of Trichinella spiralis in the transmission of the lymphocytic choriomeningitis virus (Syverton et al., 1947) as far as the nematode larva's ability to carry the microbiological organism is concerned. In the case of the Trichinella spiralis the virus was harboured by the larvae throughout its life cycle. This possibility was not explored for T. canis in the current study.

It was clear from the experiments that the larvae played a major role in the dissemination of the bacteria to all the organs tested. These findings are in agreement with the results reported by Mochizuki et al. (1954) and by Pavri et al. (1975).

The amount of the bacteria carried by the larvae as indicated by the radiation counted after their exposure to

the labelled bacteria - whether in the test tubes or in the intestine of animals - was very small. This leads to the belief that a large dose of the larvae is needed in order to carry sufficient numbers of the micro-organism to cause disease. This belief is supported by the high rate of isolation of E. coli bacteria from the organs of the 10 mice, each infected with 5,000 larvae.

In the case reported by Beautyman and Woolf (1951) only a single T. canis larva was demonstrated in the brain. This could have been responsible for carrying the virus to the central nervous system. In that case a child of six years died of acute anterior poliomyelitis because a small dose of poliomyelitis virus could multiply in the brain tissues and lead to poliomyelitis. At post mortem in all the organs examined, other than the brain, no larvae were demonstrated, even after careful histological search of the liver, kidneys and lungs. The absence of larvae from these places where T. canis larvae are commonly found, provides a good reason for believing the explanation given by Beautyman and Woolf, that the presence of the larva was accidental. Such an interpretation would be in tune with the conclusions reached from the current study.

The two roles played by the larvae in the carrying and

dissemination of the bacteria indicate that the dissemination of disease-causing micro-organisms may be more widespread than has hitherto been suspected. This is exemplified by the results obtained and reported by Woodruff et al. (1966). Of 191 patients who had had poliomyelitis, 13.6% had a positive toxocaral skin test, against 2.1% in 329 apparently healthy controls. Confirmatory results were reported by Khalil et al (1971), who found that 5.8% out of 102 children who had had poliomyelitis had a positive skin test, compared with 1.4% out of 70 apparently healthy controls.

CONCLUSIONS

The final conclusions that might be reached from this work were:

1. The larvae did carry the bacteria when they were exposed to them in test tubes and probably when exposed in the intestines of mice, but this awaits final confirmation.

2. There is a strong possibility that the larvae were able to carry and to disseminate the E.coli during their migration from the intestine of the mice to the lungs, livers and brains. The bacteria were seen adhering to the external surfaces of some larvae. It was not possible to demonstrate whether the larvae were able to carry the bacteria in their alimentary canals. However, this possibility should not be excluded.

3. The amount of bacteria carried by T.canis larvae, as was indicated by the amount of radiation counted in each individual larva, did not explain the large differences between the amount of bacteria in the infected and control groups of mice. This led to the possibility that some extra mechanisms might be involved. Most probably, the mechanism is the damage to the tissues caused by the migration of the

larvae, which created favourable conditions for the settlement and growth of the bacteria.

4. When the larvae were exposed to the labelled bacteria in vitro then were fed to the mice, the results strongly suggested that they were able to carry and disseminate the bacteria to the tissues.

5. The small percentage of larvae (1.24% in the liver, 4.2% in the lungs and 2.21% in the brain) found in the tissues of mice after the larvae were fed instead of embryonated eggs, led to three possibilities. It could be due to the fact that wide variations in the numbers of larvae obtained from the organs of mice have been demonstrated (Wiseman et al., 1971). The second possibility is that the larvae might be weakened by their exposure to the bacteria and the radio-active isotopes. The third possibility is that as the larvae were obtained by giving embryonated eggs to mice, they might lose some of their ability to penetrate the intestinal wall due to the expiry of some enzymes that were used in the first migration. This explanation could be tested by using artificially hatched larvae.

6. Finally, one will conclude that the T. canis larvae are of real danger to man, not only because of their

migration to the organs, especially vital ones like the eyes, heart and brain. The cosmopolitan distribution of this parasite in addition to the close relationship between man and dog, whether in rural or urban areas, puts greater responsibilities for the control of the parasite in dogs.

SUMMARY

This research concentrated on an attempt to understand the mechanism or mechanisms by which the larvae could play a role in the provocation and dissemination of infection, in addition to the establishment of more facts about this role.

The use of radioactive isotopes made the identification of the bacteria easier, whether they were in the larvae or in the tissues of mice. Escherichia coli bacteria were used on the grounds that they are a normal inhabitant of the human intestine. Three radioactive isotopes were employed, one was a ^{32}P as a β emitter and the two others were ^{51}Cr and ^{59}Fe as γ emitters.

The bacteria were labelled with the isotopes then they were exposed to the T.canis larvae in the test tubes. They were washed thoroughly and the radiation was counted to find whether they were able to carry the labelled bacteria. The results obtained were in support of their ability to carry the bacteria, when both isotopes ^{32}P and ^{59}Fe were used to label the bacteria.

The work was extended to discover whether the larvae do play a role in the dissemination of bacteria. The

Escherichia coli were labelled and then fed to two groups of mice, one group received at the same time about 1,000 embryonated T.canis eggs in addition to the bacteria. The mice were killed later and their organs were emulsified and radiations in these emulsions was counted. The results obtained showed that when using both isotopes ^{32}P and ^{59}Fe there was a strong possibility that the larvae disseminated the bacteria from the alimentary canal of mice to the liver, lung and brain. Some larvae were obtained from the dually infected animals to find whether they were able to carry the bacteria as they did when exposed in vitro. The results obtained showed that they most probably did carry the bacteria and in amounts approximately similar to those acquired in vitro.

To obtain more information about the mechanisms involved, the larvae were exposed to the labelled bacteria in vitro, then were fed to the animals. Various numbers of larvae were used. The mice were killed and their organs were emulsified and the radiation in the emulsions was counted. Very small amounts of radiation were counted, which suggested a small number of bacteria, together with few larvae abtained from the organs tested.

Further experimental work was carried out using bacteriological and serological techniques. A known

non-pathogenic strain of E.coli was fed to mice, together with T.canis larvae. The results obtained showed that there is a strong possibility that the same strain was isolated from the liver, lung and brain of the infected animals, but not from controls. These findings supported the results obtained by using the radio-active isotopes in the previous experiment.

REFERENCES

ASHTON, N. (1960). Larval granulomatosis of the retina due to Toxocara. Brit. J. Ophth., 44, 129.

BEAUTYMAN, W. and WOOLF, A.L. (1951). An Ascaris larva in the brain in association with acute anterior poliomyelitis. J. Path. and Bact., 63, 635.

BEAVER, P.C. (1952). Observations on the epidemiology of ascariasis in a region of high hookworm endemicity. J. Parasitol., 38, 445.

BEAVER, P.C. (1962). Toxocarosis (visceral larva migrans) in relation to tropical eosinophilia. Bull. Soc. Path. exot., 55, 555.

BEAVER, P.C. (1963). Mimeographed document WHO/Helminth, 45.

BEAVER, P.C., SNYDER, C.H., CARRERA, G.M., DENT, J.H. and LAFFERTY, J.W. (1952). Chronic eosinophilia due to visceral larva migrans, report of three cases. Pediatrics, 9, 7.

BIRD, A.C., SMITH, J.L. and CURTIN, V.T. (1970). Nematode optic neuritis. Am. J. Ophth., 69, 72.

BISSERU, B. (1969). Studies on the liver, lung, brain and blood of experimental animals infected with Toxocara canis. J. Helminth., XLIII, 267.

BISSERU, B. and WOODRUFF, A.W. (1968). The detection of circulating antibody in human Toxocara infections using the indirect fluorescent antibody test. J. Clin. Path., 21, 449.

BORG, O.A. and WOODRUFF, A.W. (1973). Prevalence of infective ova of Toxocara species in public places. Brit. Med. J., 4, 470.

BRAIN, L. and ALLAN, B. (1964). Encephalitis due to infection with Toxocara canis. Report of suspected case. Lancet, 1, 1355.

BROWN, H.W. and PERNA, V.P. (1958). An overwhelming strongyloides infection. J. Am. Med. Assoc., 168, 1648.

BUISSIERE, J. and NARDON, P. (1968). Micromethode d'identification des bacteries. I. Interet de la quantification des caracteres biochimiques. Ann. Inst. Pasteur, Paris, 115, 218.

DENT, J.H., NICHOLS, R.L., BEAVER, P.C., CARRERA, G.M. and STAGGERS, R.J. (1956). Visceral larva migrans with a case report. Am. J. Path., 32, 777.

DOUGLAS, J.R. and BAKER, N.F. (1959). The chronology of experimental intrauterine infections with Toxocara canis (Werner 1782) in the dog. J. Parasitol., 45, Suppl. 43.

DUGUID, I.M. (1961a). Chronic endophthalmitis due to Toxocara. Brit. J. Ophth., 45, 705.

DUGUID, I.M. (1961b). Features of ocular infestation by Toxocara. Brit. J. Ophth., 45, 789.

EHRENFORD, F.A. (1956). Canine ascariasis - a potential zoonosis. J. Parasitol., 42, Suppl. 12.

ENGEL, H., SPIECKERMANN, D.A., TISMER, R. and MOBIUS, W. (1971). Akute meningomyelitis durch Toxocara larven. Dtsch. Med. Wschr., 96, 1498.

FAIRLEY, N.H. (1931). Serological and intradermal tests in filariasis. A Preliminary Report. Trans. Roy. Soc. Trop. Med. Hyg., 24, 635.

FRIEDMAN, S. and HERVADA, A.R. (1960). Severe myocarditis with recovery in a child with visceral larva migrans. J. Pediatrics, 56, 91.

HEINER, D.C. and KEVY, S.V. (1956). Visceral larva migrans. Report of the syndrome in three siblings. New Engl. J. Med., 254, 629.

JACOBS, D.E. and PEGG, E.J. (1976). Gastrointestinal nematodes of elite show dogs in Great Britain. J. Helminth., 50, 265.

JUNG, R.C. (1963). Mimeographed document WHO/Helminth, 27.

KHALED, M.L.M., KHALIL, H.M. and RIFAAT, M.A. (1973). Incidence of Toxocara canis infection among pet dogs in Cairo. Ain Shames Med. J., 24, 273.

KHALIL, H.M., KHATTAB, A.K., EL-FATTAH. S.M.A., KHALID, M.L., AWAAD, S. and RIFAAT, M.A. (1971). Inter-relationship between poliomyelitis and Toxocara infection. Trans. Roy. Soc. Trop. Med. Hyg., 65, 599.

LEIPER, R.T. (1907). Two new genera of nematodes occasionally parasitic in man. Brit. Med. J., I, 1296.

LETSINGER, R.L., WILKES, J.S. and DUMAS, L.B. (1976). Incorporation of 5'-amino-5'-deoxythymidine 5'-phosphate in polynucleotides by use of DNA polymerase I and a ØX 174 DNA template. Biochemistry, 15, 2810.

MAPLESTONE, P.A. and BHADURI, N.V. (1940). The helminth parasites of dogs in Calcutta and their bearing on human parasitology. Indian J. Med. Res., 28, 595.

MOCHIZUKI, H., TOMIMURA, T and OKA. T. (1954). Cerebrospinal nematodiasis as a provoking factor in Japanese B. encephalitis : an experimental approach. J. Infec. Dis., 95, 260.

MULLER-JENSEN, A., SCHALL, J., WEISNER, B. and LAMINA, J. (1973). Eosinophile meningo-enzephalo-myelitis und viszerales syndrom durch Askaridenlarven beim Erwachsenen. Dtsch. Med. Wschr., 98, 1175.

NG, B.K.Y. and KELLY, J.D. (1975). Anthropozoonotic helminthiases in Australasia. Part 3 : Studies on the prevalence and public health implications of helminth parasites of dogs and cats in urban environment. Int. J. Zoon., 2, 76.

NICHOLS, R.L. (1956). The etiology of visceral larva migrans. II. Comparative larval morphology of *A.lumbricoides*, *N.americanus*, *S.stercoralis* and *A.caninum*. J. Parasitol., 42, 363.

NUTTALL, G.H.F. and STRICKLAND, C. (1908). Note on the prevalence of intestinal worms in dogs in Cambridge. Parasitology, 1, 261.

OLIVER, R. (1971). Principles of the use of radio-isotope tracers in clinical and research investigations. The Commonwealth and International Library of Science, Technology and Liberal Studies.

PATEL, L. and KABACK, H.R. (1976). The role of the carbodiimide-reactive component of the adenosine 5'-triphosphatase complex in the proton permeability of Escherichia coli membrane vesicles. Biochemistry, 15, 2741.

PATTERSON, R., ROBERTS, M., HART, R.J. and HUNTLEY, C.C. (1975). Evaluation of visceral larva migrans by radio-immunoassay systems. Pediatrics, 56, 417.

PAVRI, K.M., GHALSASI, G.R., DASTUR, D.K., GOVERDHAN, M.K. and LALITHA, V.S. (1975). Dual infections of mice : Visceral larva migrans and sublethal infection with Japanese encephalitis virus. Trans. Roy. Soc. Trop. Med. Hyg., 69, 99.

PHILLIPS, C.I. and MACKENZIE, A.D. (1973). Toxocara larval papillitis. Brit. Med. J., I, 154.

RAISTRICK, E.R. and DEAN HART, J.C. (1976). Ocular toxocariasis in adults. Brit. J. Ophth., 60, 365.

RIFAAT, M.A., KHALIL, H.M. and KHALID, M.L.M. (1969). Incidence of Toxocara canis infection among stray dogs in Cairo. Ain Shames Med. J., 20, 345.

SCHACHER, J.F. (1957). A contribution to the life history and larval morphology of Toxocara canis. J. Parasitol., 43, 599.

SCHOCHET, S.S. (1967). Human Toxocara canis encephalopathy in a case of visceral larva migrans. Neurology, 17, 227.

SHOPE, R.E. (1941). The swine lungworm as a reservoir and intermediate host for swine influenza virus. II. The transmission of swine influenza virus by the swine lungworm. J. Exp. Med., 74, 49.

SHOPE, R.E. (1943a). The swine lungworm as a reservoir and intermediate host for swine influenza virus. III. Factors influencing transmission of the virus and the provocation of influenza. J. Exp. Med., 77, 111.

SHOPE, R.E. (1943b). The swine lungworm as a reservoir and intermediate host for swine influenza virus. IV. The demonstration of masked swine influenza virus in lungworm larvae and swine under natural conditions. J. Exp. Med., 77, 127.

SMITH, M.H.D. and BEAVER, P.C. (1953). Persistence and distribution of Toxocara larvae in the tissues of children and mice. Pediatrics, 12, 491.

SMITH, P.B., TOMFOHRDE, K.M., RHODEN, D.L. and BALOWS, A. (1972). API system : a multitube micromethod for identification of Enterobacteriaceae. Applied Microbiol., 24, 449.

SNYDER, C.H. (1961). Visceral larva migrans. Ten years experience. Pediatrics, 28, 85.

SOLOMON, S.G. (1933). The helminth parasites of dogs in Marseilles. J. Helminth., 11, 157.

SPRENT, J.F.A. (1952). On the migratory behavior of the larvae of various Ascaris species in white mice. I. Distribution of larvae in tissues. J. Infect. Dis., 90, 165.

SPRENT, J.F.A. (1953). On the migratory behavior of the larvae of various Ascaris species in white mice. II. Longevity of encapsulated larvae and their resistance to freezing and putrefaction. J. Infect. Dis., 92, 114.

SPRENT, J.F.A. (1954). The life cycles of nematodes in the family Ascarididae Blanchard 1896. J. Parasitol., 40, 608.

SPRENT, J.F.A. (1955a). On the invasion of the central nervous system by nematodes. I. The incidence and pathological significance of nematodes in the central nervous system. Parasitology, 45, 31.

SPRENT, J.F.A. (1955b). On the invasion of the central nervous system by nematodes. II. Invasion of the nervous system in ascariasis. Parasitology, 45, 41.

SPRENT, J.F.A. (1958). Observations on the development of Toxocara canis (Werner 1782) in the dog. Parasitology, 48, 184.

SPRENT, J.F.A. (1961). Post-parturient infection of the bitch with Toxocara canis. J. Parasitol., 47, 284.

STANIER, R.Y., DOUDOROFF, M. and ADELBERG, E.A. (1971). General microbiology. Macmillan, London.

SYVERTON, J.T., McCOY, O.R. and KOOMEN, J. (1947). The transmission of the virus of lymphocytic choriomeningitis by Trichinella spiralis. J. Exp. Med., 85, 759.

TANG, P.W. and GUHA, A. (1975). Determination of 5 Triphosphate terminus of 16S ribosomal RNA precursor. Nature (Lond.), 257, 157.

TINER, J.D. (1953). The migration, distribution in the brain and growth of Ascarid larvae in rodents. J. Infect. Dis., 92, 105.

TURNER, T. and PEGG, E. (1977). A survey of patent nematode infestations in dogs. Veterinary Record, 100, 284.

VANPARIJS, O.F.J., THIENPONT, D.C. (1973). Canine and feline helminth and protozoan infections in Belgium. J. Parasitol., 59, 327.

VAN THIEL, P.H. (1960). Larva migrans visceralis. Ned. T. Ceneesk., 104, 1104.

WERNER, P.C.V. In vermium intestinalium, p.144, 1782. Quoted in Chitwood and Chitwood (1937).

WHITE, R.H. (1978). Stable isotope studies on biosynthesis of thiazole moiety of thiamin in Escherichia coli. Biochemistry, 17, 3833.

WHITE, R.H. and RUDOLPH, F.B. (1979). Biosynthesis of the pyrimidine moiety of thiamin in Escherichia coli : incorporation of stable isotope-labelled glycines. Biochemistry, 18, 2632.

WILDER, H.C. (1950). Nematode endophthalmitis. Tr. Am. Acad. Ophth., 55, 99.

WILSON, S. and THOMPSON, A.E. (1964). A fatal case of strongyloidiasis. J. Path. Bact., 87, 169.

WISEMAN, R.A. and WOODRUFF, A.W. (1967). Toxocara skin sensitivity tests and other observations in animals experimentally infected with Toxocara canis. Trans. Roy. Soc. Trop. Med. Hyg., 61, 827.

WISEMAN, R.A. and WOODRUFF, A.W. (1971). Toxocariasis in Africa and Malta. The frequency of infection in host animals and its incidence and distribution in humans as revealed by skin sensitivity tests. Trans. Roy. Soc. Trop. Med. Hyg., 65, 439.

WISEMAN, R.A., WOODRUFF, A.W. and PETTITT, L.E. (1969). Further Toxocara skin sensitivity tests in animals experimentally infected with Toxocara canis. Trans. Roy. Soc. Trop. Med. Hyg., 63, 246.

WISEMAN, R.A., WOODRUFF, A.W. and PETTITT, L.E. (1971). The treatment of toxocaral infection : some experimental and clinical observations. Trans. Roy. Soc. Trop. Med. Hyg., 65, 591.

WOODRUFF, A.W. (1965). Pathogenicity of intestinal helminthic infections. Trans. Roy. Soc. Trop. Med. Hyg., 59, 585.

WOODRUFF, A.W. (1970). Toxocariasis. Brit. Med.J., 3, 663.

WOODRUFF, A.W., ASHTON, N. and STOTT, G.J. (1961). Toxocara canis infection of the eye. Trans. Roy. Soc. Trop. Med. Hyg., 55, 13.

WOODRUFF, A.W., BISSERU, B. and BOWE, J.C. (1966). Infection with animal helminths as a factor in causing poliomyelitis and epilepsy. Brit. Med. J., I, 1576.

WOODRUFF, A.W. and THACKER, C.K. (1964). Infection with animal helminths. Brit. Med. J., I, 1001.

YUTUC, L.M. (1954). The incidence and prepatent period of Ancylostoma caninum and Toxocara canis in prenatally infected puppies. J. Parasitol., 40, Suppl. 18.

ZUELZER, W. and APT. L. (1949). Disseminated visceral lesions associated with extreme eosinophilia. Am. J. Dis. Child., 78, 153.