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THE DEVELOPMENT OF *HYDATIGERA (TAENIA) TAENIAEFORMIS* (CESTODA, CYCLOPHYLLIDEA) *IN VIVO*.

THESIS

for the

Degree of Doctor of Philosophy

in the

University of Glasgow

by

William M. Hutchison, B.Sc.

January, 1959.

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THE DEVELOPMENT OF HYDATIGERA (TAENIA) TAENIAEFORMIS (CESTODA, CYCLOPHYLLIDEA) IN VIVO

CONTENTS

I N T R O D U C T I O N

INTRODUCTION

Research on tapeworm metabolism is desirable since a knowledge of cestode growth requirements and metabolic pathways would permit the rational development of more effective anthelminthic agents. Such agents would be designed to block essential metabolic reactions of the parasite. Apart from this practical application, a greater understanding of cestode metabolism would be of interest from the academic standpoint because knowledge of the morphology and life cycles of this group of animals is out of proportion to what is known about their physiology.

Current research on cestode physiology can be divided into two main categories, i.e. *in vivo* and *in vitro*. The *in vivo* approach, used extensively by Chandler and his co-workers, subjects the cestode within the intestine to different conditions by altering the metabolism of the host. This can be done by the removal of certain glands of the host or by the use of abnormal or deficiency diets. Using especially *Hymenolepis diminuta* and *H.nana*, workers in this field have made important contributions to the

knowledge of the nutritional and endocrinological relationships of tapeworm and host.

On the other hand, several leading authorities on experimental parasitology (Smyth 1947, von Brand 1952) are agreed that a complete understanding of the metabolism of parasitic helminths can only be obtained by *in vitro* experimentation. A considerable initial difficulty with *in vitro* work resulted from the cestode being in contact with the bacterial flora of the intestine. When such an adult tapeworm is removed to culture media, these bacteria soon produce changes which result in the death of the worm. Smyth (1946) avoided the difficulty of sterilising the outside of the worm by using the plerocercoids of pseudophyllidean cestodes. These develop under aseptic conditions in the body cavity of fish which are the intermediate hosts. Removing the plerocercoid by an aseptic technique and transferring it to culture media, Smyth succeeded in obtaining maturation. It was felt that such *in vitro* techniques could also be profitably applied to the Cyclophyllidea and it was the original intention of the writer to use this

approach to study the metabolism of this group of cestodes.

The suitability of different species of cyclophyllidean tapeworms for experimental work was considered and Hydatigera (Taenia) taeniaeformis, the broadnecked tapeworm of the cat, was selected. Unlike the cysticerci of the Taenia, the larval form of this tapeworm (sometimes called Cysticercus fasciolaris) is strobilated and is consequently referred to as a strobilocercus (Sambon 1924). It is found in the liver tissue of various rodents, rats and mice being the most common intermediate hosts of natural infections. The larva lies within a cyst on the surface of the liver and is seldom deeply embedded. The host capsule surrounding it can undergo a malignant transformation into a sarcoma and thus has attracted considerable attention. The mature worm is most frequently found in the small intestine of the domestic cat.

H. taeniaeformis was selected for in vitro cultivation for the following reasons. Firstly, it can be easily maintained in vivo in the laboratory so that experimental material is available at all

times. Secondly, the larval form exists aseptically within the host capsule in rodent liver and can easily be transferred to culture media without bacterial contamination. Thirdly, the strobilocercus is large enough to be weighed and subjected to chemical analysis for comparison with later stages of development. Fourthly, the strobilocercus is well developed with proglottids already demarcated and it is possible that development of the genitalia might commence without an initial somatic growth phase. This is known to occur when the more highly differentiated pseudophyllidean plerocercoids of Schistocephalus and Ligula are cultured at 39°C.

This combination of advantageous features can be found in no other cyclophyllidean and it seemed probable that H. taeniaeformis was the most suitable cyclophyllidean species for in vitro work.

During the initial experimental work, examination of the literature revealed that, although the broad outlines of the life history of H.taeniaeformis were well known, most of the work had been directed at the pathological and immunological

aspects of the larval infections. Little detailed work had been done on the development of the larval or mature worm and virtually nothing was known about the rate of growth, changes in chemical composition or the time scale of morphological development, i.e. age at which strobilocerci become infective and the period required for maturation in the cat etc. Moreover, where work had been done on such aspects, it was soon apparent that considerable errors existed. Consequently, it was necessary to modify the thesis to an investigation of the _in vivo_ development of _H.taeniaeformis_. Once the physical and chemical changes taking place during the life of the tapeworm have been determined, they could be used as standards with which _in vitro_ results could be compared.

The work has been grouped into five Sections. The first Section consists of a historical review of the work performed on _H.taeniaeformis_. Section 2 deals firstly with the world incidence of the tapeworm in the intermediate and definitive hosts, and secondly with a more narrow aspect, namely, the incidence of the tapeworm in the

definitive host in Scotland. Section 3 deals firstly with the growth of the strobilocercus in mouse liver, and, secondly, with the growth of the adult worm in the intestine of the cat. Section 4 consists of an investigation into the chemical composition of the tapeworm, in particular the changes which take place during development in the water, carbohydrate and nitrogen content.

For clearness of presentation, each sub-section is treated as a separate unit with a discussion as to the significance of the results obtained. A general discussion of the work as a whole and its application is given in Section 5.

Accounts of this work have been published or accepted for publication under the following titles:-

(a) "The Incidence and Distribution of Hydatigera taeniaeformis and other Intestinal Helminths in Scottish Cats". Journal of Parasitology, 43, 318-321.

(b) "Studies on Hydatigera taeniaeformis.
1. Growth of the larval stage". Journal of Parasitology. (Accepted for publication)

(c) "Studies on Hydatigera taeniaeformis.
2. Growth of the adult phase".
Experimental Parasitology.
(Accepted for publication. Vol. 8 (5) Sept.1959).

(d) "Studies on Cestode Metabolism. IV. The Nitrogen Fraction in the Large Cat Tapeworm". Experimental Parasitology, 7, 349 - 365. (with C. A. Hopkins).

Pages 127 to 154 of this thesis, dealing with "Nitrogen Metabolism" is an account of joint work carried out with Dr. C. A. Hopkins.

SECTION 1

HISTORICAL REVIEW

HISTORICAL

According to Rudolphi (1810), the first mention of Hydatigera taeniaeformis was made in 1684 by Francesco Redi, the Florentine physician, in his "Observations on the Parasites of Living Animals". It was next described and named by Pallas (1767). During the next 100 years, many descriptions of this tapeworm were published, all of which are fully reviewed by Loveland (1894).

Since observations made on the morphology of H.taeniaeformis provided the main clues which led to the discovery of the cestode life cycle, it seems appropriate that a brief outline of this 'landmark of parasitology' should be given at this point.

Until 1852 nothing was known about the life history of any cyclophyllidean tapeworm. Rudolphi (1809, 1810) recognised 5 classes of parasitic worms which he named Nematoidea, Acanthocephala, Trematoda, Cestoidea and Cystica. The Class Cystica consisted of what are now known to be larval cestodes or bladder worms, but were, at that time, thought to be an entirely different group of parasites. Each bladder-

worm possessed its own specific name, for example, Cysticercus bovis (=Taenia saginata), Cysticercus cellulosae (=T.solium),and Cysticercus fasciolaris (=H.taeniaeformis). It is still a common practice to use these names when referring to the larval worms. Parasitologists (Goeze 1782) (Von Siebold 1850) were impressed by certain features, such as the hooks and suckers, which the Cystica had in common with certain members of the Class Cestoidea. The suspicion that a closer relationship existed between these two groups was strengthened by observations made on C.fasciolaris. This cystic species showed definite affinities to the cestodes since it was strobilated and had a similar external appearance. Closer examination showed that C.fasciolaris possessed a short neck and was similar in many other respects to the cat tapeworm. With this key to the problem, Kuchenmeister (1852) performed the feeding experiments which proved, beyond all doubt, that C.fasciolaris in the liver of the mouse was the juvenile form of H. taeniaeformis in the intestine of the cat. Later, Kuchenmeister (1857) recalled that many research workers such as Von Siebold in Germany, and Thompson in Glasgow, were

aware of the affinities of the Cystica to the Cestoidea but their opinion at that time seemed to be that the Cystica were cestodes which had become "dropsically degenerate" as a result of developing at a site other than the intestine. Kuchenmeister (1852b) is perhaps better known as a result of a later experiment which was concerned with the feeding of Cysticercus cellulosae to a condemned murderer. The post mortem examination of this person revealed maturing Taenia solium thus establishing the identity of the cysticercus from pork muscle. These findings gave the study of parasitology a tremendous impetus and the life histories of many other species of Taenia were rapidly elucidated. It is generally agreed (Wardle & McLeod 1952, Chandler 1955 and Reinhard 1958) that, from this work and from concurrent research on Trichinella spiralis by Herbst (1851), the experimental phase of parasitology commenced.

Bartels (1902) described the anatomy of the strobilocercus in detail and also outlined genital development in the adult worm. Janicki (1907) gave a full account of the development of the embryo within

the uterus of the mature worm. The discovery of cancer in the rodent liver (Borrel 1906) resulting from infection with C.fasciolaris resulted in extensive research into the pathology of the larval infections by Bullock and Curtis (1920). A series of studies on the immunological aspects of larval infections was commenced by Miller (1930) and by Campbell (1936). An investigation of premunition in the domestic cat was also undertaken by Miller (1932). Mendelsohn (1936) attempted to culture a 15 day larval worm aseptically and Wilmoth (1945) also used aseptic techniques to study respiration of the larval worm. The most recent work on H.taeniaeformis is that of Rees (1951, 1952) who re-investigated the anatomy of the strobilocercus and mature worm.

SECTION 2

INCIDENCE

WORLD INCIDENCE AND DISTRIBUTION

Rats, mice and domestic cats are the major hosts of *Hydatigera taeniaeformis* and since they are cosmopolitan it is not surprising that this cestode has a world wide distribution. Although records of the presence of this cestode are common, only the more extensive reports, which give details of incidence, are quoted in Table 1.

There is little information on the extent to which the mouse is parasitised in nature since the main interest has been focused on the rat for two reasons. Firstly, because of its association with plague, and, secondly, because some rats which are infected with the larval form of this cestode develop cancer of the liver.

In the American plague research laboratories, extensive examinations of rat populations were carried out, e.g. McCoy (1909) autopsied 100,000 *Rattus norvegicus* in San Francisco, but, unfortunately, the total number of rats parasitised by *Cysticercus fasciolaris* is not mentioned. Due to the large number of animals examined and the speed

TABLE 1

Location	Species of Rodent	No. of Rodents Examined	% Infected	No. of Cysts per Rodent	Investigator
Tunis	R.norveg R.rattus	2,000	40	-	Bridre & Conseil 1909
Algiers	R.norveg R.rattus	1,970	20	-	Bridre & Conseil 1910
Tunis	R.norveg R.rattus	3,800	31	-	
Caracas	R.norveg	750	38.4	1-5	Brumpt 1934
Panama City	R.norveg	400	40	-	Calero et al 1950
Colombo	R.rattus	2,583	15	-	Crutz 1947
U.S.A. (Ohio)	R.norveg	50	10	1-2	Forbes 1942
U.S.A. (Maryland)	M.musculus	78	1.3	-	Hall et al. 1955
Taiwan	R.norveg	358	46.3	-	Li & Hsu 1951
U.S.A. (Baltimore)	R.norveg	2,636	18.1	1-8	Luttermoser 1936
U.S.A. (Washington)	R.norveg	100	54	-	Price et al 1932
Wales	R.norveg	52	15.4	1-2	Rees 1951
U.S.A. (St.Louis)	R.norveg	100	72	-	Tsuchiya et al. 1936.
U.S.A. (Illinois)	R.norveg	532	80.5	1-13	Wantland 1957
China (Soochow)	R.norveg R.rattus	250	44.8	3-8	Wu 1930-31

Table 1. The incidence of the strobilocercus of H.taeniaeformis in the intermediate hosts.

deemed necessary, the search for tapeworm larvae was a cursory one. However, the presence of the cestode was mentioned when it was associated with a liver tumour.

Information on the incidence of this larval cestode has been amassed mainly by Cancer Research workers who were investigating the hypothesis that parasites were responsible for cancer. Borrel (1907, 1909) who was concerned with this aspect, examined the livers of wild rats for the presence of C.fasciolaris and found that there was a wide variation in the incidence of this parasite in different localities. Examination of the findings of other workers not only confirms that this is the case but also that considerable variations in the incidence can occur in one locality from year to year. (Bridre & Conseil 1909, 1910).

Table 2 summarises information on the incidence of H.taeniaeformis in domestic cats and it is evident that the results are as variable as they were for the intermediate rodent hosts. Detailed reports, the majority of which come from U.S.A., show that the number of cats examined is not so numerous,

TABLE 2

Location	No. of Cats INfected	% Infected	Numbers per Cat	Investigator
India (Calcutta)	250	40	-	Chandler 1925
Denmark (Copenhagen)	100	7	-	Christensen et al (1946)
U.S.A. (Tennessee)	12	75	-	Ciordia & Jones (1956)
U.S.A. (Chicago)	51	3.9	1-3	Cross & Allen (1948)
Poland (Lodz)	50	9	-	Gluszkowska (1955)
U.S.A. (Oklahoma)	30	85	-	Guberlet (1923)
Scotland (Glasgow)	1707	7.7	1-23	Hutchison (1957)
U.S.A. (Ohio)	83	1.2	-	Koutz & Rebrassier (1942)
Poland (Warsaw)	66	30	-	Lukasiak (1937)
U.S.A. (New Jersey)	100	8	-	Mann & Fratta (1952)
U.S.A. (Illinois)	50	80	1-7	Wantland (1957)

Table 2. The incidence of H.taeniaeformis in the domestic cat.

presumably because there are fewer opportunities for large scale autopsies. Moreover, apart from rodent control, the cat is of little significance to public health authorities.

Populations of cats and rats do not migrate far from their home area (Sawitz, 1939: Barnett 1958) and thus, although the incidence of this tapeworm in one region of a city may give a true index of its endemicity for that particular region, this may not hold for the rest of the city. For example, it is difficult to believe that Wantland's (1957) figure of an 80% infection in cats of the city rubbish dump at Bloomington, Illinois, holds for the whole city. Gluszkowska (1956) and Hutchison (1957) are of the opinion that the incidence in cats is dependent on the degree of rodent control in operation. Wantland's high figures were probably obtained from a region of the city where no such control existed, nevertheless, his results are of considerable interest since he is the only worker who has investigated the incidence in both intermediate and definitive hosts from the same locality.

THE INCIDENCE OF HYDATIGERA TAENIAEFORMIS IN SCOTTISH CATS

The estimation of the incidence of H. taeniaeformis was based on the direct examination of the small intestine of 1,707 stray city cats which had been killed by local authorities. This total is comprised of a series of random samples taken at monthly intervals from 1952 to 1955. Although this appears to be a large number, it is, in fact, only 2% of the total number of stray cats humanely destroyed in this period. The incidence of the other intestinal helminths also recorded in this section is based on a more detailed examination which was carried out on 1,104 of the cats.

H. taeniaeformis

H. taeniaeformis was found in 132 cats giving an incidence of 7.7%. The number of cestodes per cat varied from 1-23; 47.7% harboured a solitary cestode, 25.7% harboured 2-3 cestodes, while the remainder were infected with burdens varying from 4-23 as indicated in Figure 1.

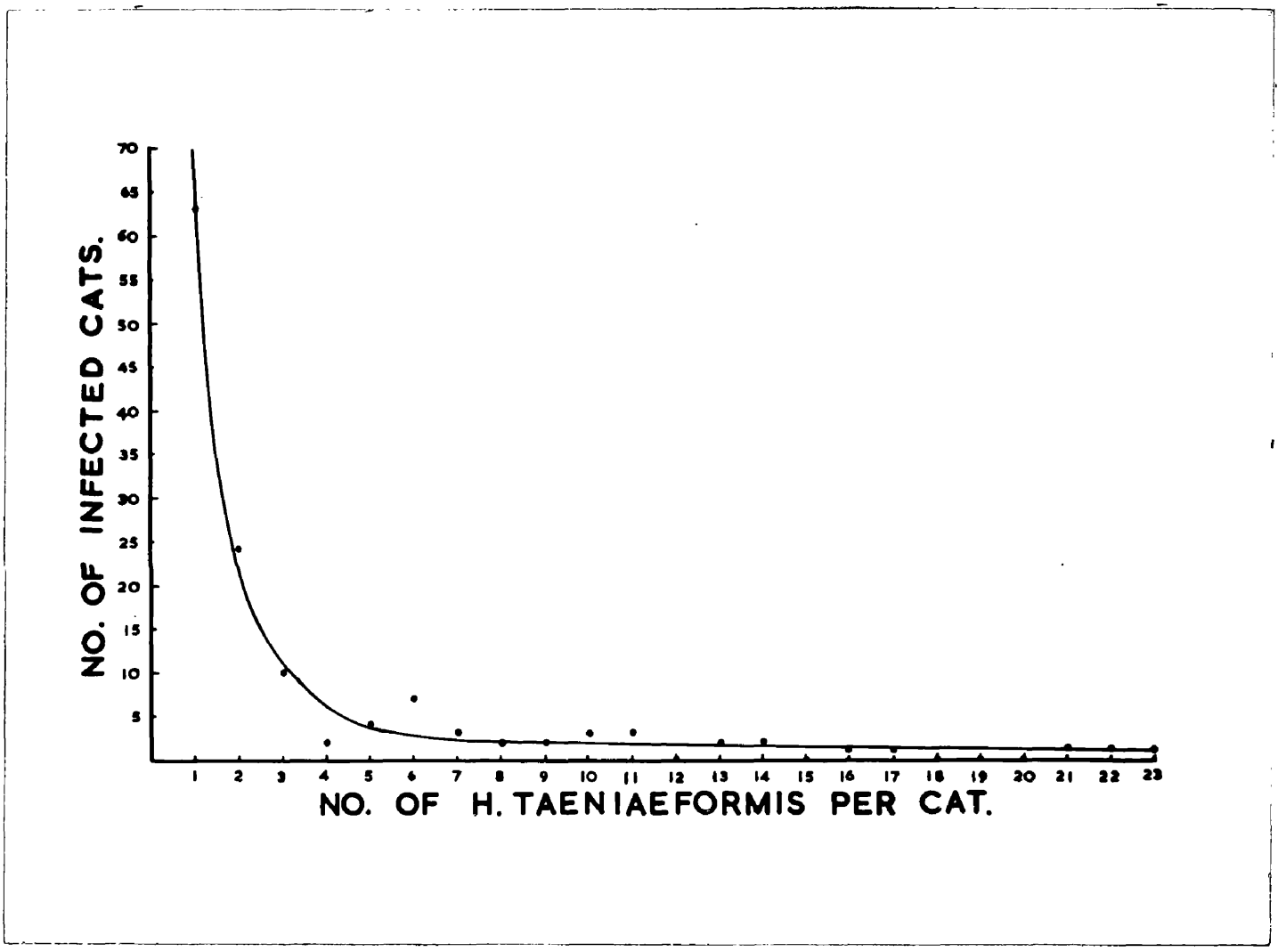

Figure 1. The intensity of infection with Hydatigera taeniaeformis.

Number of H.taeniaeformis per cat	Number of Cats Infected	Total No. of Cestodes Recovered
1	63	63
2	24	48
3	10	30
4	2	8
5	4	20
6	7	42
7	3	21
8	2	16
9	2	18
10	3	30
11	3	33
12	0	0
13	2	26
14	2	28
15	0	0
16	1	16
17	1	17
18	0	0
19	0	0
20	0	0
21	1	21
22	1	22
23	1	23
TOTAL	132	482

Table 3. The intensity of Hydatigera taeniaeformis infection in cats.

The distribution of H. taeniaeformis in the small intestine was influenced by the total number of worms present (Table 4).

The stomachs of the stray cats were not examined but of 42 cats infected in the laboratory, 2 had H. taeniaeformis attached to the stomach walls. The complete strobila of these worms lay within the stomach but it is possible that they had been regurgitated from the small intestine. Grassi and Parona (1879) and Stiles (1894) described similar cases.

Dipylidium caninum

This was the commonest cestode and was found in 50.5% of 1,104 cats. Infections were usually heavy, frequently consisting of masses of more than a hundred worms. D. caninum invariably occurred far back in the intestine: in 96% of the infections, all the worms were in the posterior third. Even within this region, distribution was sharply delimited. The last 10 cm were always free of worms, presumably because of the lack of food.

TABLE 4

NOS. OF *H.taeniaeformis* PRESENT PER CAT	*H.taeniaeformis* PRESENT IN THE SMALL INTESTINE						TOTAL
	Anterior Third		Middle Third		Posterior Third		
	a	b	a	b	a	b	
Light Infection (1-3 worms)	13	9%	128	91%	0	0%	141
Medium Infection (4-9 worms)	30	24%	91	73%	4	3%	125
Heavy Infection (10 or over)	54	25%	146	68%	16	7%	216
TOTAL	97	20%	365	76%	20	4%	482

Table 3 shows the effect of increased worm burden on the distribution of *H.taeniaeformis* in the small intestine of cats.

a = Number of worms

b = Number of worms found in the region expressed as a percentage of the total recorded (last column).

Toxocara mystax

T. mystax was present in 13.7% of the cats examined. It was found in the first two thirds of the small intestine. It was also observed in the stomach of 3 out of 20 naturally infected laboratory cats which were examined within 10 minutes of being killed.

Toxascaris leonina

This nematode was recovered from the intestines of 15.3% of the cats. It had the same distribution as T. mystax but infections were usually heavier. A worm burden of 10-20 was common compared with 2-5 for T. mystax.

Diphyllobothrium sp.

An unidentified species of Diphyllobothrium was found on 2 occasions. In each case the cestode was established at the beginning of the middle third of the small intestine. This genus has not previously been recovered from Felis domesticus in Great Britain although numerous cases have been reported from other parts of the world.

DISCUSSION

The low incidence of *H. taeniaeformis* compared with that of *D. caninum* is probably due to the less favourable conditions which exist in cities for the completion of its life-cycle. Whereas fleas, the intermediate host of *D. caninum*, remain common on city cats, extensive rodent control has reduced the chances of the *H. taeniaeformis* cycle being completed.

The predominance of solitary *H. taeniaeformis* in the cat may suggest the existence of an immunity due to premunition. However, this is improbable as super-infections have been established on many occasions in this laboratory and also by Miller (1932). It is more probable that this low level of infection in cats is due to the random catching of rats and mice which carry a single strobilocercus. This latter suggestion is supported by the observations of Luttermoser (1936) who found that the majority of wild rodents examined harboured only a single strobilocercus.

The distribution of *H. taeniaeformis* (Table 4) indicates that these cestodes show a clear

preference for the middle of the small intestine. This is particularly marked in the light infections where over 90% occurred in this region. The colonization of the anterior, and, to a much less extent, posterior third, is probably a result of population pressure. It is interesting to find that even with very heavy infections, worms are rarely found in the posterior part of the ileum, although this region often supports masses of D. caninum. This difference in the ecological distribution of these two cestodes suggests an underlying difference in their physiology.

Unfortunately, there is remarkably little information about the physical conditions or amounts of unabsorbed foodstuffs that are to be expected in the different regions of the intestine. Without such information it is difficult to suggest the significance of this distribution. Archer and Hopkins (1958a) record that, in rats, Diphyllobothrium sp. establishes itself in the posterior part of the ileum, in a position comparable to that occupied by D.caninum in cats, but does not grow until it migrates to a more

anterior position. They conclude that the posterior part of the rat's ileum is too depleted to support the growth of Diphyllobothrium, although this region is commonly occupied by the small cestode Hymenolepis fraterna. It is possible that a similar difference in physiological adaptation exists in the case of D. caninum and H. taeniaeformis. All that can be concluded from the present results is that the distribution of the 2 species of cestode in the cat shows sharp specific limits, the very existence of which indicates a response to an unidentified stimulus.

Concurrent infections with both D. caninum and H. taeniaeformis were observed on many occasions but, because of their different distribution, little interspecific competition is to be expected. H. taeniaeformis were rarely present in sufficient numbers to affect helminths farther down the intestine, and it is unlikely that cestodes occurring in the posterior part of the intestine would affect adults of another species occupying a more anterior region.

SUMMARY

The incidence of cestodes and nematodes in the small intestine of stray cats living in Scottish cities has been estimated to be: *Hydatigera taeniaeformis* 7.7%; *Dipylidium caninum* 50.5%: *Toxocara mystax* 13.7%: and *Toxascaris leonina* 15.3%. The distribution and intensity of infection of these helminths within the gut is recorded and their inter-relations discussed.

SECTION 3

GROWTH

(a) LARVAL GROWTH

LARVAL GROWTH

No detailed studies have been made of the life and growth of *H. taeniaeformis* although the outlines of its life history are well known. The results obtained from a study of the morphological development and growth of the larval worms in the liver of Strain A mice are presented in this section. Knowledge of larval growth is a prerequisite of *in vitro* cultivation because it is essential to know at what stage worms become infective if it is hoped to induce maturation *in vitro*. The only information available on the age at which larvae become infective is given by Wilmoth (1945) who stated that he had received information from Bullock Curtis and Dunning to the effect that strobilocerci became infective to cats after 7 months of development in the rat.

Materials and Methods

The mice which were infected were offspring of an inbred strain known as Strain A obtained originally from the Imperial Cancer Research Fund,

London. It had been found previously that they were highly susceptible to infection with H.taeniaeformis. Only mice between the ages of 45 - 75 days were used as they were refractive to infection outside these limits. (cf. age resistance to infection with larval H. taeniaeformis shown by albino rats, Greenfield 1942). Measured amounts of H.taeniaeformis eggs suspended in water were administered by stomach tube. The latter consisted of a No.17 hypodermic needle with the tip covered by a short length of narrow bore (0.5 mm) polythene tubing.

Eggs were obtained from the gravid proglottids of adult H. taeniaeformis which had been recovered from the intestine of cats. After washing these worms in tap water, the posterior six proglottids were removed and the stage of development of the contained embryos checked. If the eggs were found to be fully embryonated, the proglottids were stored in balanced saline at 4°C. Frequent changes of saline were necessary due to bacterial growth. Material stored in this manner for 5 months was found still to be infective.

Usually the gravid proglottids were used

within a few days of removal from the cat. The proglottids were halved longitudinally along the main axis of the uterus and the eggs pressed out of the lateral branches. An aqueous suspension was then prepared for administration to mice. As the eggs settle rapidly in water, the suspension was shaken thoroughly before being drawn into the syringe. A bubble of air was also drawn in, which facilitated mixing within the syringe between administrations to each mouse.

Proglottids stored for longer than two weeks before use tended to degenerate and become brittle. After this change had occurred, the eggs were not readily released and the proglottids had to be homogenised at low speeds. The released eggs were unaffected by this treatment but the proglottids disintegrated and the resulting fragments were small enough to pass through the stomach tube without causing blockage.

The growth rate was estimated by killing the mice at varying intervals after infection and comparing the dry weight of the larvae which had been recovered and dried for 24 hours at 105°C.

Although large strobilocerci can be surface dried satisfactorily by blotting in order to obtain the fresh weight, it becomes progressively more difficult to surface dry the smaller specimens and weigh with accuracy. Estimations of growth were based, therefore, on increase in dry weight.

Results

(1) Morphological Development

In order to determine the stage of development, the larvae were removed from their host capsules at intervals after infection. The cysts were clearly visible to the naked eye, being 0.5 mm in diameter, at the end of the 7th day. The contained larvae were in the form of undifferentiated bladders. By the end of the 14th day, the cysts were 2 mm in diameter and a cephalic rudiment had developed by an inpushing of the bladder (Figs.2 & 3).

Larvae examined on the 30th day were found to have developed into invaginated cysticerci complete with suckers and hooks (Fig.4). Too few larvae were

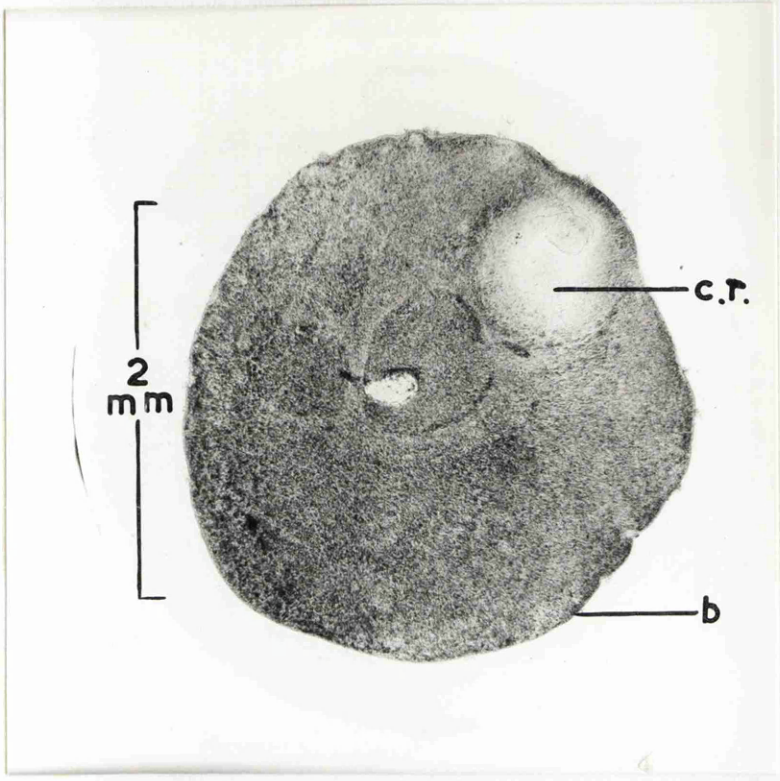

Figure 2. Larva removed from mouse liver 14 days after infection consisting of a simple bladder (b) and an inverted cephalic rudiment (c.r.)

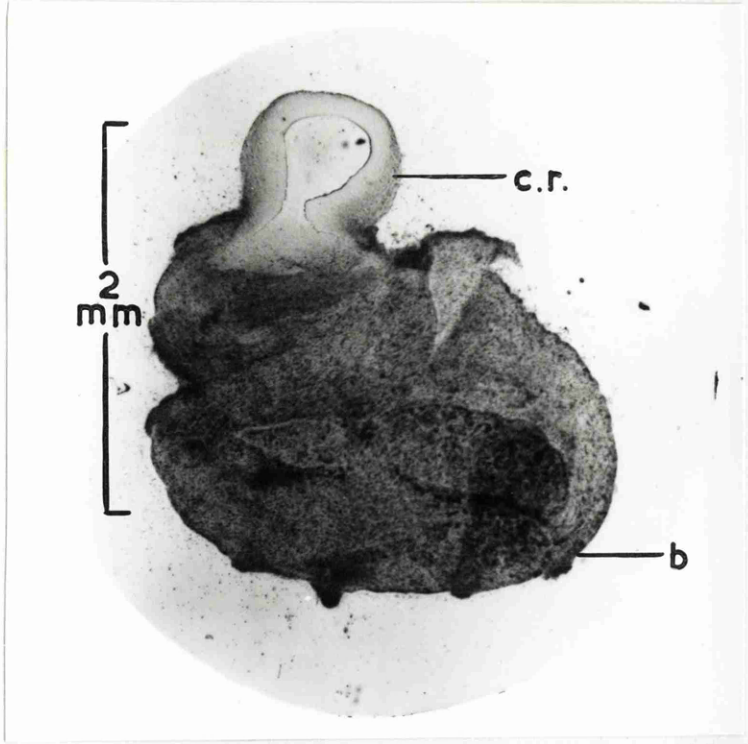

Figure 3. The bladder (b) of the same larva ruptured to show the cephalic rudiment (c.r.)

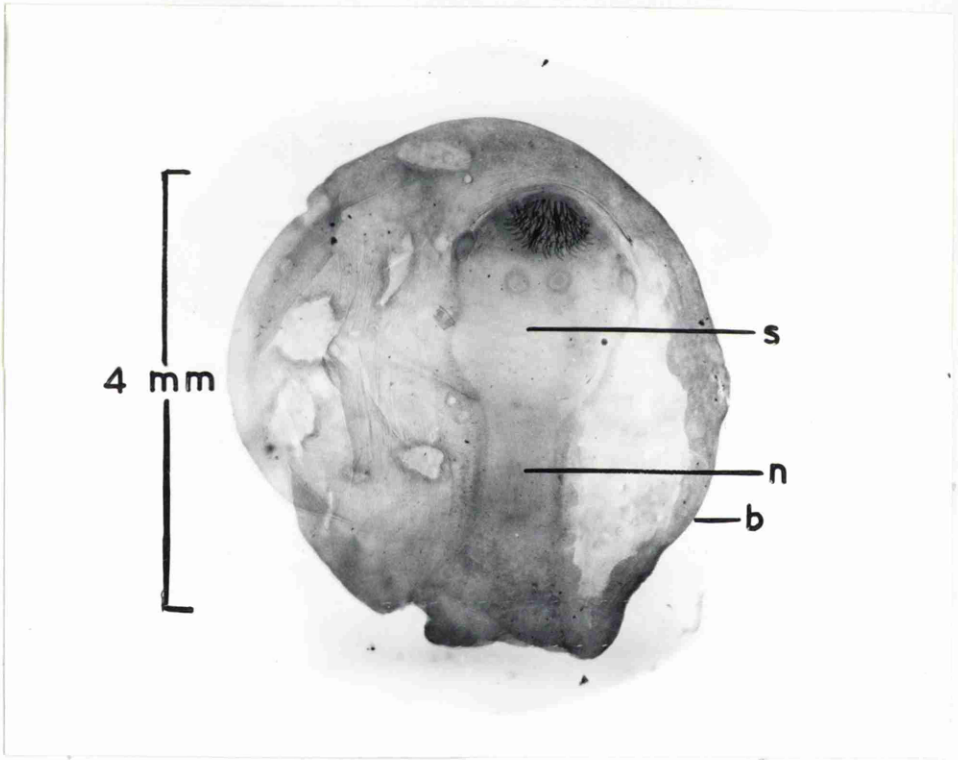

Figure 4. The "invaginated cysticercus" stage 30 days after infection of the mouse showing bladder (b), invaginated scolex (s) and neck (n).

obtained from light infections to be accurately weighed, but 200 larvae at the same stage of development were obtained from heavily infected mice. From these it was found that the average dry weight of the invaginated cysticercus was 0.87 mg. Feeding experiments indicated that worms at this stage of development were not infective to cats.

On the 42nd day a precocious evagination of the complete cysticercus was observed (Figs. 5 & 6). Strobilated larvae were recovered on the 48th day (Fig. 7). The weight and age at which they became infective to the definitive host was determined by feeding a series of larvae to cats. The results showed that larvae had to undergo a minimum period of 60 days in the mouse before becoming infective. The condition and appearance of a 62 day infective strobilocercus is shown in Fig. 8. The average fresh weight of such larvae is 20 mg.

Examination of the complete strobila of 169 strobilocerci failed to reveal any trace of genital anlagen. This was confirmed by sectioning

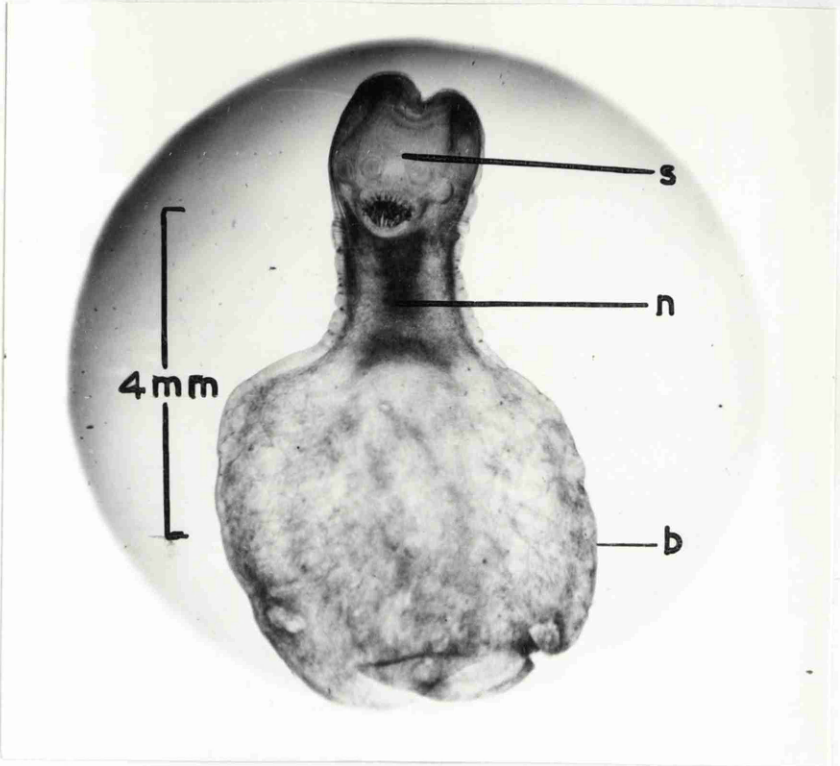

Figure 5. The invaginated cysticercus undergoing a precocious evagination on the 41st day.

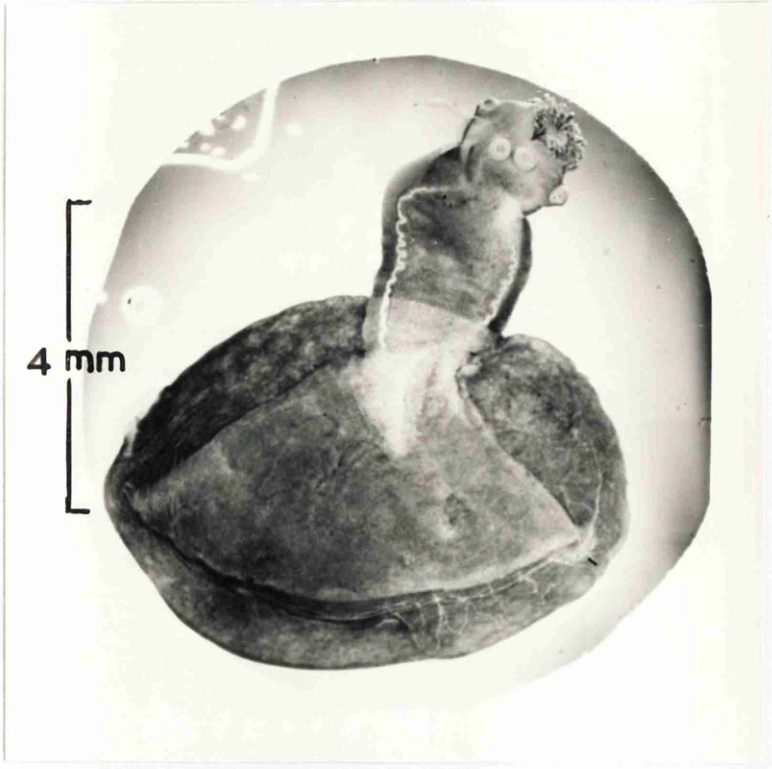

Figure 6. An evaginated cysticercus on the 42nd day.

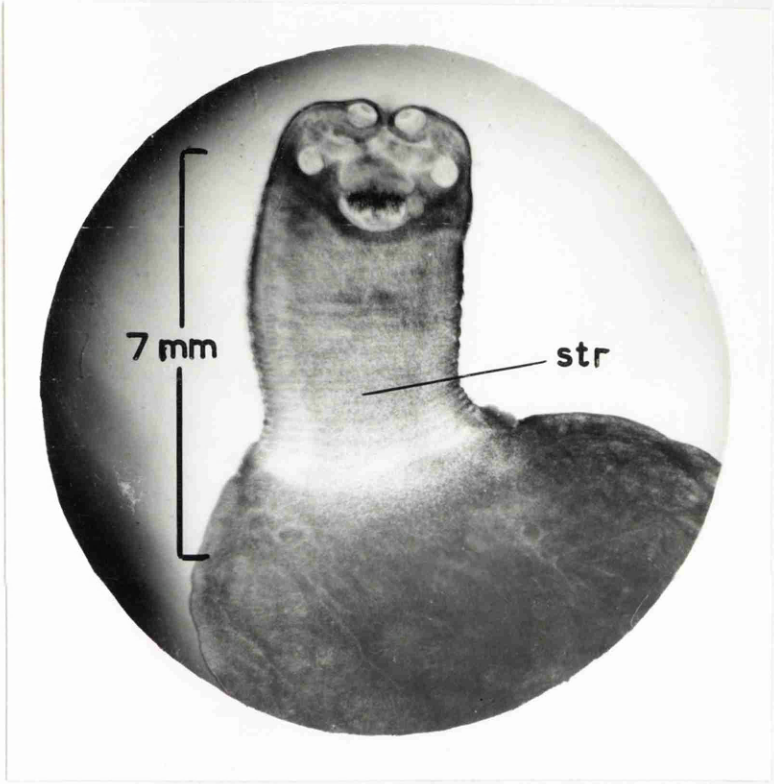

Figure 7. A young strobilocercus after 48 days of development in mouse liver. The strobilated region (str) can be seen to the posterior.

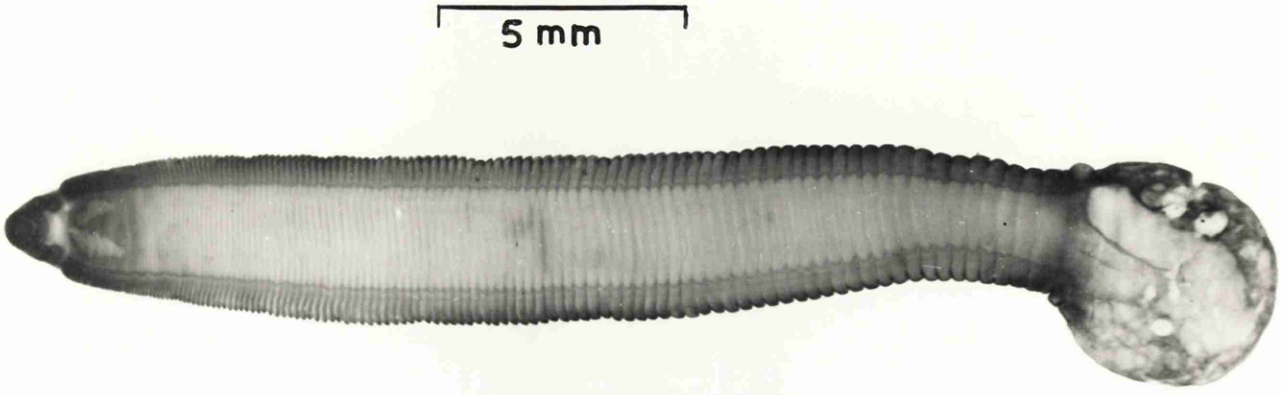

Figure 8. A mature strobilocercus 62 days after infection of the mouse. Infective to cats.

7 individuals.

(2) Degeneration of Larvae during Development.

During the course of infection in the mouse it was frequently observed that, when the hexacanths were established in the liver, degeneration occurred within the first 14 days and the cysts were replaced by small yellow scars. Sometimes well developed strobilocerci which had been in the liver for periods up to 4 months were dead on removal from the cyst. Bullock and Curtis (1924) report that the death of the older larvae is followed by atrophy of the cyst and sometimes complete replacement fibrosis.

Although the cysts and their contents are normally aseptic, bacteria sometimes gain entry. There is a consequent suppuration of the cyst accompanied by death and degeneration of the contained larva. Bacterial examination of such cysts from mice revealed a Gram positive pleiomorphic bacillus, Corynebacterium murium.

The host capsule is normally transparent and the presence of the strobilocercus gives the cyst a white appearance. Black cysts are frequently

encountered in the heavier infections exceeding 6 months duration. This abnormal colour is due to the fact that an internal haemorrhage has occurred, flooding the cyst with blood. The contained strobilocerci are distinctly yellow in colour but are morphologically normal and capable of infecting cats.

(3) Growth of Larva

Figure 9 shows the growth rate of larval H.taeniaeformis in mouse liver. The abscissa represents the age of the larva in the mouse and the ordinate, the dry weights of the larval cestodes. Since strobilocerci develop from identical cellular masses, and, since the stage at which growth commences is the same in each case, weight can be employed as a direct measure of growth. No estimations of the dry weight were made earlier than 35 days because the larvae were so small that the removal of the large numbers required for an accurate weight was impracticable.

Points represent the mean dry weight of cestodes from lightly infected mice bearing not more than 12 cysts per liver. Although up to 12 worms per mouse was considered as a light infection, 66%

of the points were obtained from mice bearing less than 6 worms. A closed point (●) indicates that the estimation is based upon material recovered from several mice while an open point (○) indicates that only a single mouse was examined.

The mean dry weights of cestodes recovered from heavy infections in single mice are plotted individually and are shown on the Figure as solid squares (■). In each case the total number of cestodes present in the liver of the heavily infected mouse is recorded as an index to the right of the square. Heavily infected mice were easily distinguished from lightly infected ones due to the tremendous distension of the abdomen (Figs. 10 & 11). One mouse, having been infected for 36 weeks, weighed 66.9 gm and contained 100 worms in the liver. The fresh weight of the worms when removed was 24.4% of the weight of the mouse. This is by no means an exceptional case as involvement of the liver to this extent was common. The condition of the liver in one particular mouse, carrying a burden of 233 worms, is shown in Figure 12.

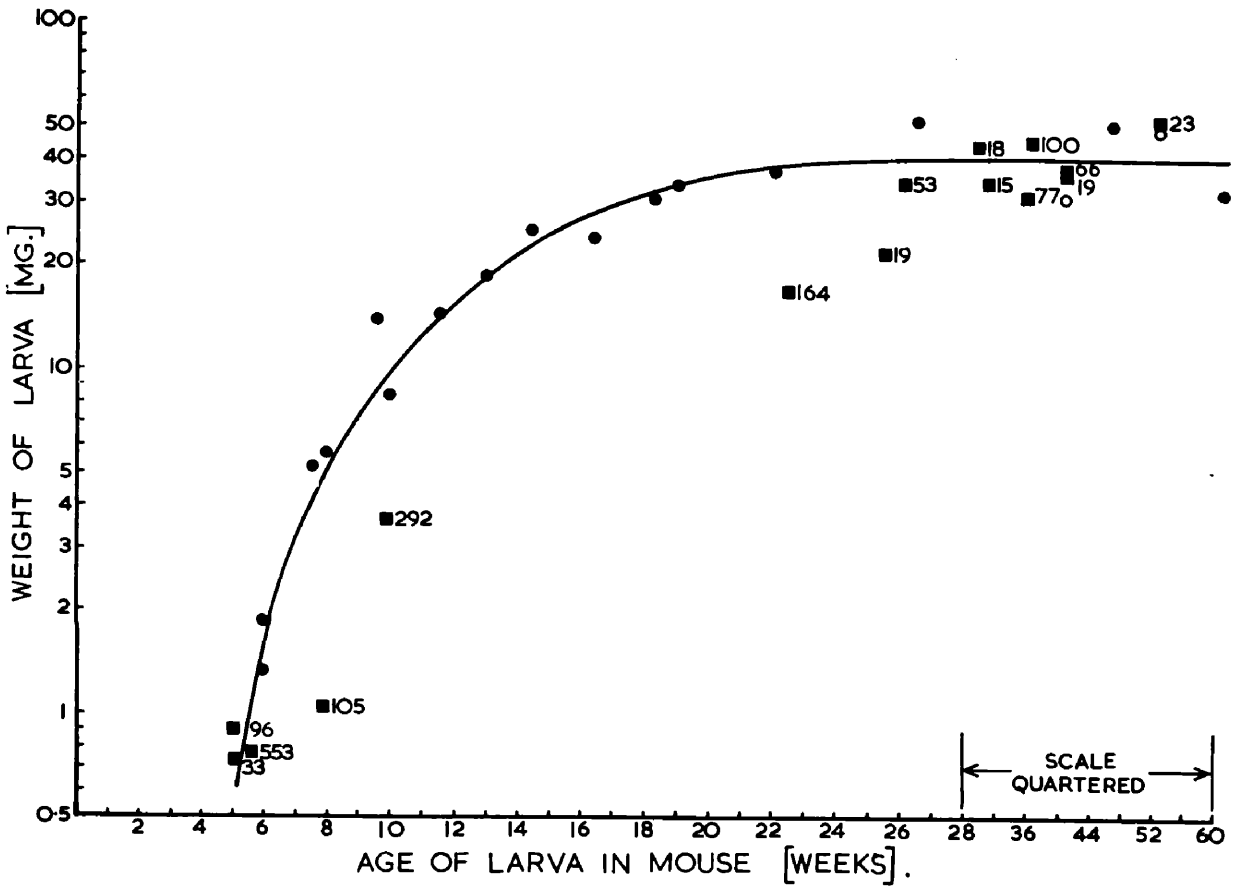

Figure 9. Growth of larval H. taeniaeformis is plotted on a logarithmic scale and shows the increase in dry weight at successive intervals of time.

■ = The average dry weight of worms from one heavily infected mouse. The index represents the worm burden.

● = The average dry weight of light worm burdens (1 - 12 worms/mouse). Result based on worms recovered from several mice.

○ = The average dry weight of a light worm burden recovered from a single mouse.

(See also Table 5.)

TABLE 5

Age of Strobs. in weeks	No. of mice killed	No. of Strobs. in liver	Number Weighed	Mean dry Weight
5.1	1	96	96	0.87
5.1	1	33	33	0.74
5.6	1	553	380	0.77
6	5	10	10	1.34
6	4	17	17	1.86
7.6	2	6	6	5.18
7.9	1	105	97	1.05
8	5	11	11	5.7
9.6	2	8	8	13.9
9.9	1	292	280	3.65
10	5	10	10	8.37
11.6	3	24	24	14.3
13	3	12	12	18.4
14.4	3	14	14	24.76
16.4	5	26	26	23.59
18.3	9	62	62	30.5
19	3	14	14	33.4
22.1	2	11	11	36.9
22.6	1	164	164	16.5
25.4	1	19	14	21.7
26.1	1	53	53	34.17
26.4	2	6	6	51.3
29.4	1	18	17	44.3
30.7	1	15	13	34.5
35	1	77	75	31.14
36.3	1	100	88	44.55
40	1	66	66	37.73
40	1	19	19	36.29
40	1	12	12	30.97
46.3	2	25	25	50.18
52.4	1	23	23	51.5
52.4	1	8	4	50.2
60.4	1	12	12	31.2

Table 5. The mean dry weight of different age groups of larval H. taeniaeformis.

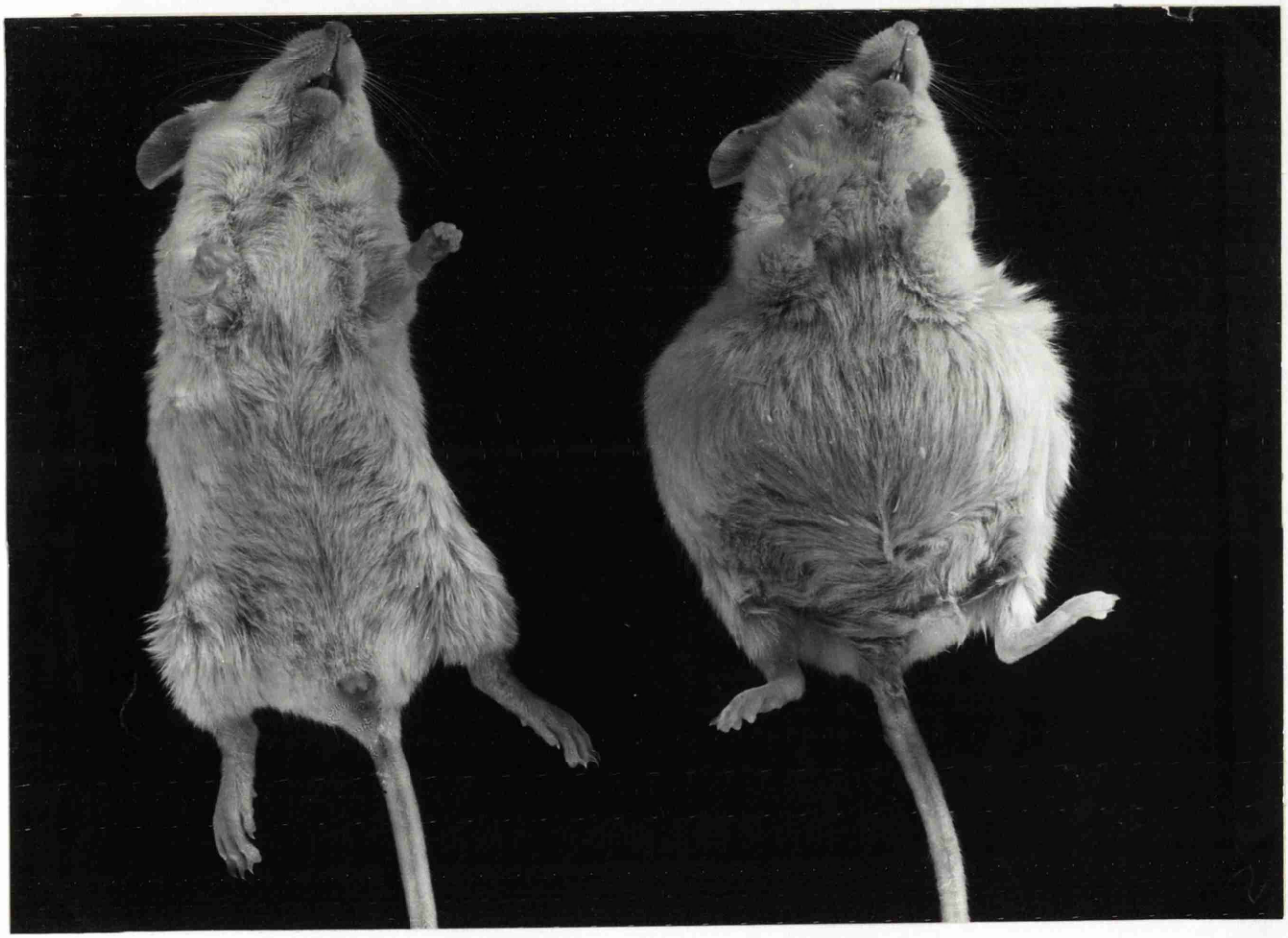

Figure 10. Control (left) and experimental male mice 87 days after infection.

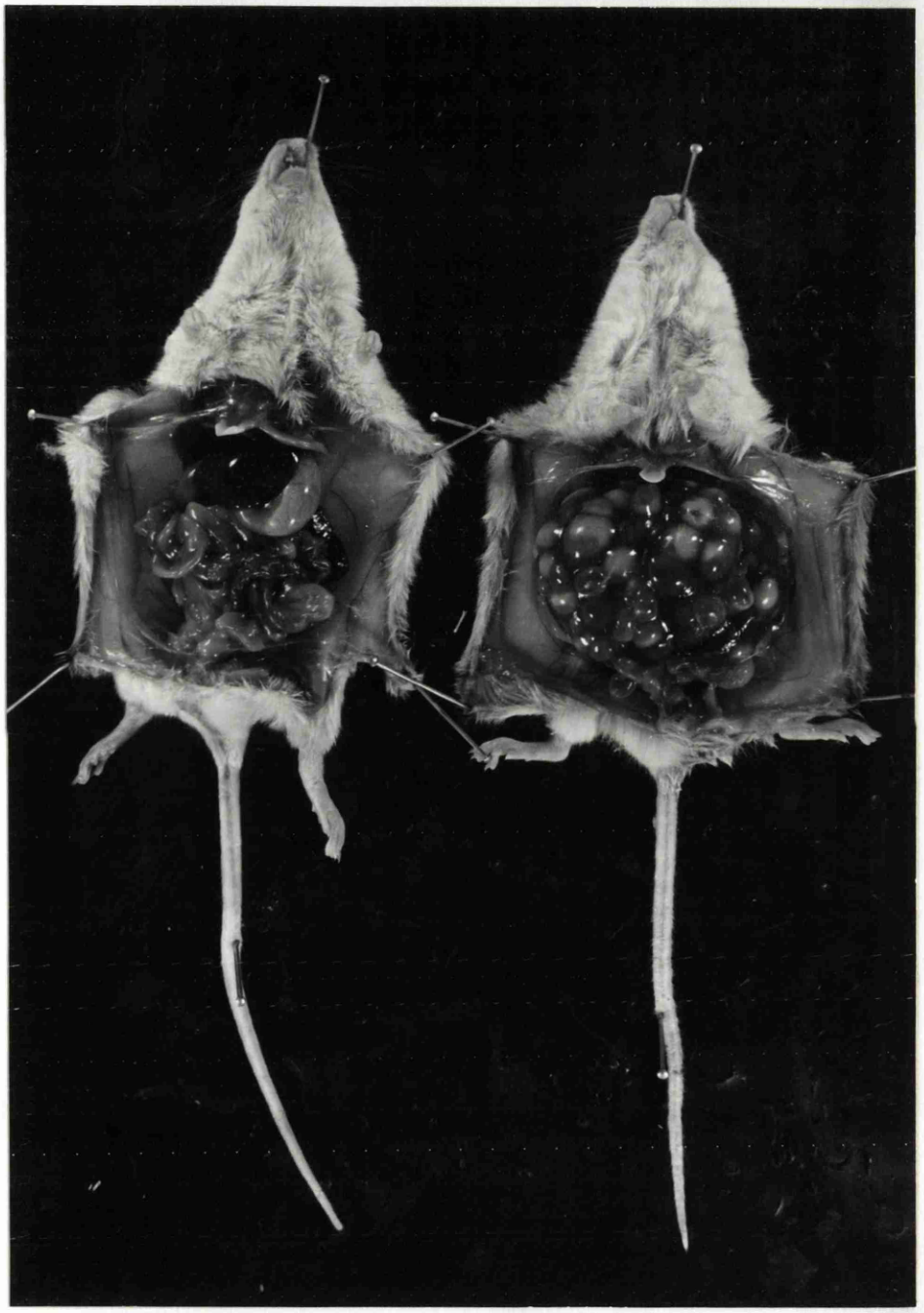

Figure 11. The same mice opened to show the numerous strobilocerci established in the liver.

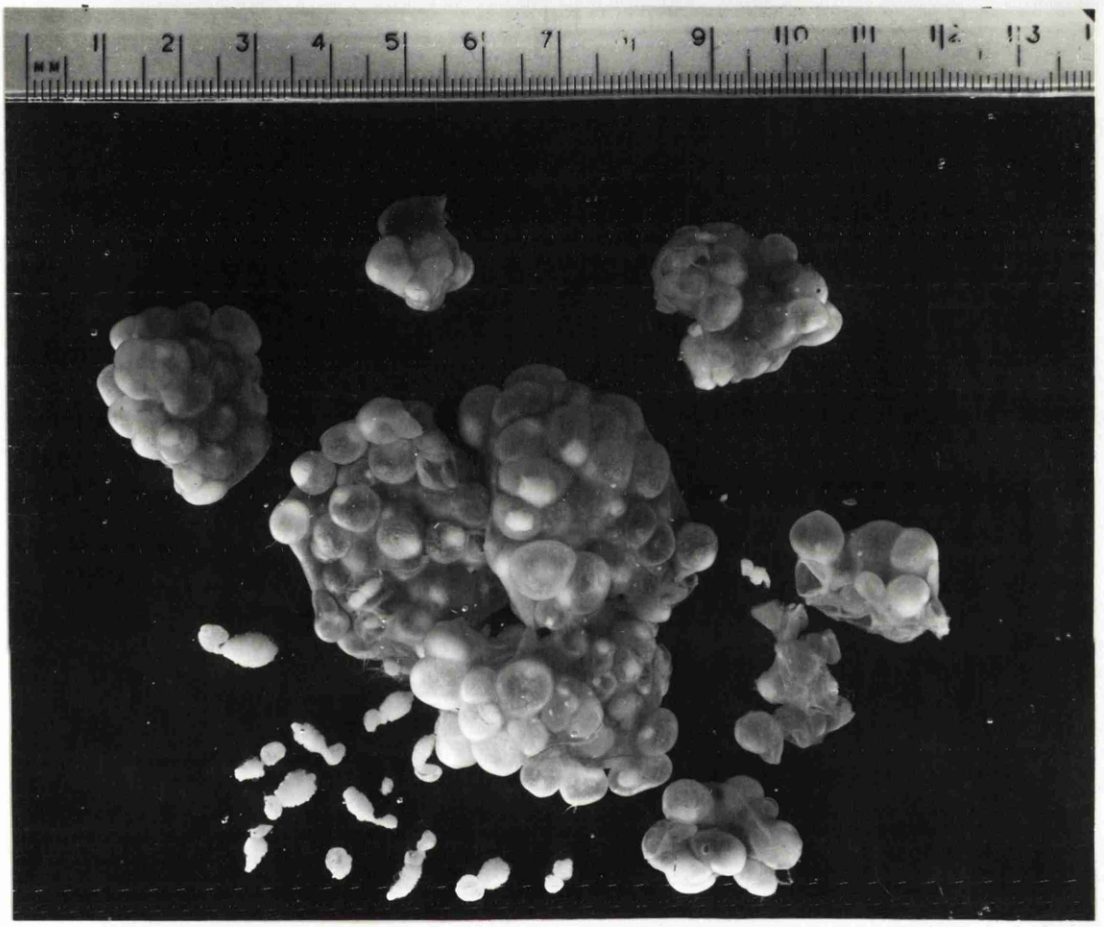

Figure 12. The complete liver of another laboratory infected mouse from which 233 larvae were recovered. Several contracted strobilocerci which have been removed from their host capsules are shown below the infected liver. The scale is in cm.

(4) The Weight of the Oncosphere

The oncosphere is the stage at which growth commences in the mouse liver. If the dry weight could be estimated and compared with the weight of larva recovered after 6 weeks, it would give an indication of the rate of growth during this early phase of larval development. Direct estimations cannot be made on the weight of the oncosphere but an approximate dry weight in gm can be obtained by multiplying the volume of the oncosphere in cc by the specific gravity (s) and the dry/fresh weight ratio (w).

Since the oncosphere can be seen through the embryophore, it can be measured. Some are ovoid having a length of 21 microns and a breadth of 18 microns while others are spherical with a diameter of 20 microns. From this latter measurement, the volume can be most easily obtained.

The eggs of H. taeniaeformis, being dense, settled rapidly in a solution having a specific gravity of 1.19. When the embryophores were removed with hatching fluid (Silverman 1954) and the oncospheres placed in a graded series of NaCl solutions,

it was observed that they sank in a solution of specific gravity 1.04 and floated in another of specific gravity 1.07. The specific gravity of the oncospheres lies between these two limits and was taken as being 1.055.

The dry/fresh weight ratio of the oncosphere of any tapeworm is unknown but in the following calculation its value is taken as being 0.27 which is equivalent to the average dry/fresh weight ratio of the larval worm in the liver of the mouse.

The dry weight of the oncosphere is, therefore, $4/3\pi r^3$ w.s. $= 1.33 \times 3.14 \times (10^{-2})^3 \times 0.27 \times 1.055$

$= 1.193 \times 10^{-6}$ mg

i.e. 1.193 millimicrograms.

Even allowing for an error of $\pm 50\%$ in the dry/fresh weight ratio, the weight of the oncosphere lies in the range 0.6 - 1.8 millimicrograms.

Discussion

The weight of an oncosphere, when it becomes

established in a mouse liver, is approximately one millimicrogram. This weight increases 1 million times during the first 40 days resulting in a larva of 1 mg (Fig. 9). As no intervening figures have been obtained during this period, the shape of the growth curve is not known. After the 40th day, the rate of growth (by which is meant the length of time taken to double the dry weight) decreases rapidly, and by the 22nd week has virtually stopped. This conclusion is different from that of Sweatman & Plummer (1957) who found that growth in Taenia hydatigena, developing in a lamb, was constant throughout the period of investigation (9 months). This comparison, however, is not necessarily valid as these workers measured growth as increase in "linear dimension" of the cyst. Whether the scolex and neck region, or even the total dry weight of the whole larva, continued to increase was not determined.

At least two physiological reasons can be advanced to explain the slowing and ultimate cessation of growth of H. taeniaeformis occurring in light infections. The first is that the host capsule,

which forms around the larva, gradually limits the rate of entry of metabolites. Stoner & Hankes (1955) have shown that this occurs during the development of Trichinella spiralis larvae. Secondly, Lewart & Lee (1956) who investigated the physiological processes taking place during the early growth phase of larval H. taeniaeformis by means of histochemical techniques, have suggested that the production of a collagenase-like enzyme, which reaches a maximum in the early larva, facilitates the rapid absorption of nutrients by softening the intercellular material and basement membranes of the liver cells. The decreased production of this enzyme by older larvae may in turn lead to a decrease in available food.

While either or both of these processes may be contributory factors, it seems probable that this slowing of growth is not just a chance result of starvation due to a thick host cyst or low collagenase production. If it were, a much greater variation in the size of larvae would be expected. The smoothness in the fall off in the rate of growth (Fig.9) suggests a more precise control mechanism. Although there is

no direct evidence of such an intrinsic control mechanism, the similarity between this growth curve and that of many other animals suggests that growth is being limited as a result of genetically controlled changes in hormone concentration rather than as a pathological response to extrinsic factors.

A considerable population of larvae can be supported by a single mouse (Fig.9) but individual mice bearing more than 200 larvae were never observed beyond the 11th week. There is little difference in the amount of growth which has taken place in the widely differing worm burdens of 553 and 33 worms plotted between the 5th and 6th weeks. This shows that competition for available growth substances is absent at this stage. Moreover, both these results lie on the graph of the light infections, and so, during the initial rapid growth phase, the growth rate is constant and is not affected by the worm burden. Beyond the 6th week, it is evident that some limiting factor is depressing the growth rates of the heavier infections. No permanent stunting

of the worms was observed and no cases were recorded in which the average maximum size was not reached by the 26th week after infection.

Conflicting reports have appeared as to whether or not there is a genital rudiment present in the proglottids of the strobilocercus of H. taeniaeformis. Nelson (1924) reported the presence of an anlagen in the larvae of H. taeniaeformis recovered from a musk rat. Rees (1952) in her extensive investigations on the anatomy of the strobilocercus, did not observe any trace of an anlagen in 9 individuals from 8 rats - a result confirmed in the present work. Rees suggested that its presence may be a variable feature but it is extremely doubtful whether the structures described by Nelson (1924) were, in fact, genital rudiments. In his paper, photographs are shown of the "rudiments" which do not in any way resemble the true genital rudiments making their first appearance in the cestode after 4 days within the definitive host.

The infective strobilocercus of H.taeniae-formis (See Figure 8) consists of two distinct

portions. In the anterior, there is a robust and highly contracted region called the true larval strobila which survives digestion in the cat. The remainder of the worm, which is digested, is a flimsy and relaxed region called the pseudostrobila. The exact demarcation of these two portions cannot be determined by external examination. Internally, according to Joyeux & Baer (1938) differences in the musculature exist and there are less calcareous corpuscles in the pseudostrobila. It was the opinion of Leuckhart (1886) that the complete larval strobila, with the exception of the neck and scolex, was digested away and that the growth of the adult commenced from this point. Miller (1932) disproved this, and by counting the proglottids of the strobilocercus in order to compare them with the number observed after a period of 24 hours in the intestine of the cat, he was able to show that only 25% of the proglottids had been digested. A more detailed account of the relationship between strobila and pseudostrobila is given in the

following sub-section (Adult Growth).

Finally, from the information presented in this paper, a conclusion can be drawn as to the most suitable size of strobilocercus for cultivation. Mendelsohn (1935), who was the first to attempt cultivation of larval H. taeniaeformis aseptically, selected the undifferentiated bladder which he removed from rat liver 15 days after infection. After culturing in roller tubes for 35 days, he observed that the cephalic rudiment had developed. Successful cultivation of this stage will give information concerning the metabolism of the larval worm. If, however, the object is to obtain in vitro the development of the adult worm, then it is apparent that the larva should be at least 60 days old as this is the minimum age of larva capable of maturing in vivo. This applies only to larvae from light infections as growth in the heavy infections is retarded and the larvae take longer to reach the infective size. Therefore, a better criterion than age is fresh weight, the critical size being about 20 mg.

Summary

Investigations on the development and growth of larval Hydatigera taeniaeformis in Strain A mice have shown that, on the 30th day after infection, an invaginated cysticercus developed. This evaginated on the 42nd day and by the 48th day a strobilocercus had formed. The youngest strobilocerci capable of infecting cats had a fresh weight of 20 mg. and were recovered from mice after 60 days. Rapid growth occurred during the first 6 weeks. Thereafter, the growth rate slowed down and with light worm burdens growth stopped 22 weeks after infection. Heavy and light worm burdens grew at the same rate initially, but beyond the 6th week the growth rate of worms in heavy burdens was retarded.

(b) ADULT GROWTH

ADULT GROWTH

There is little information about the rate of growth of tapeworms and most of what there is, (Wardle and Green 1941, Archer and Hopkins 1958) is concerned with pseudophyllidean worms. Chandler (1939) outlined the normal growth rate of the cyclophyllidean, Hymenolepis diminuta, by measuring its length after fixed periods in albino rats. Apart from the work on Hymenolepis, the papers on the growth of the Cyclophyllidea are indirect, such as that by Penfold et alia (1937) who counted the number of proglottids shed per day by Taenia saginata. It was considered that a more detailed investigation of the growth of H. taeniaeformis was desirable and the object of the present work was to determine its rate of growth in the definitive host.

In addition to determining the rate of growth during the pre-patent period, an attempt has been made to settle an old controversy, namely, how much of the strobilocercus is digested on entry into the cat's intestine?

Materials and Methods

The strobilocerci used to infect cats were removed from the livers of laboratory infected Strain A mice. The cats used in these experiments were mostly immature but older than 10 weeks. Animals of this age were used as they were the easiest to obtain. Faecal examinations were carried out for H. taeniaeformis but such examinations could not detect the presence of worms during their pre-patent period. For this reason the cats were kept for at least 7 to 10 days prior to infection so that natural infections could be distinguished easily, by their size and stage of development, from those induced in the laboratory. Only one natural infection occurred in the 130 cats used.

A strobilocercus occurs within a host capsule embedded in the liver tissue of an infected rodent. The worm was removed to determine its condition and size for comparison with the later stages recovered after infection of the definitive host. On removal from the host capsule, the larval worm was washed in balanced saline and then dried by blotting lightly on

filter paper. After determining the fresh weight, it was transferred to a tube of balanced saline containing worms of a similar weight group. A weight group consisted of a number of strobilocerci whose weights lay within a 20 mg. range. When sufficient strobilocerci of a particular weight group were obtained, they were fed simultaneously to a cat. Doses were usually of 5 strobilocerci, though a few received as many as 10. The period of time elapsing between the removal of strobilocerci from a freshly killed mouse and infection of the cat never exceeded 3 hours.

In nature, it is possible that many strobilocerci are damaged in the cat's mouth. Such damage was avoided during experimental feeding by placing worms directly into the cat's stomach by means of a 12 inch polythene stomach tube (bore 2 mm). In order to get the worms into the narrow bore of the stomach tube, the temperature of the balanced saline, in which they had been temporarily stored prior to feeding, was raised to 38°C. The anterior proglottids which had remained contracted after removal from the host capsule, then relaxed as the worm became active

and proceeded to elongate. The consequent reduction in width and depth of the anterior enabled the worm to be drawn easily into the stomach tube provided that the scolex entered first. Larger bores of stomach tube had proved unsuitable as they were difficult to pass down the oesophagus, especially when kittens were being infected.

In the experiments performed to determine the growth rate of H. taeniaeformis in the definitive host, cats were autopsied at intervals after infection and the cestodes were rapidly washed, blotted and weighed.

Results

(1) The Shedding of the Pseudostrobila

The strobilocercus sheds a portion of its strobila on entering the intestine of the cat. The fraction of the strobilocercus which survives is referred to as the true larval strobila and that which is shed, the pseudostrobila.

Figure 13 shows the percentage of the fresh weight of the strobilocercus which survived a period of 24 hours in the intestine of the cat. The ordinate is the weight of a worm, recovered from a cat, expressed

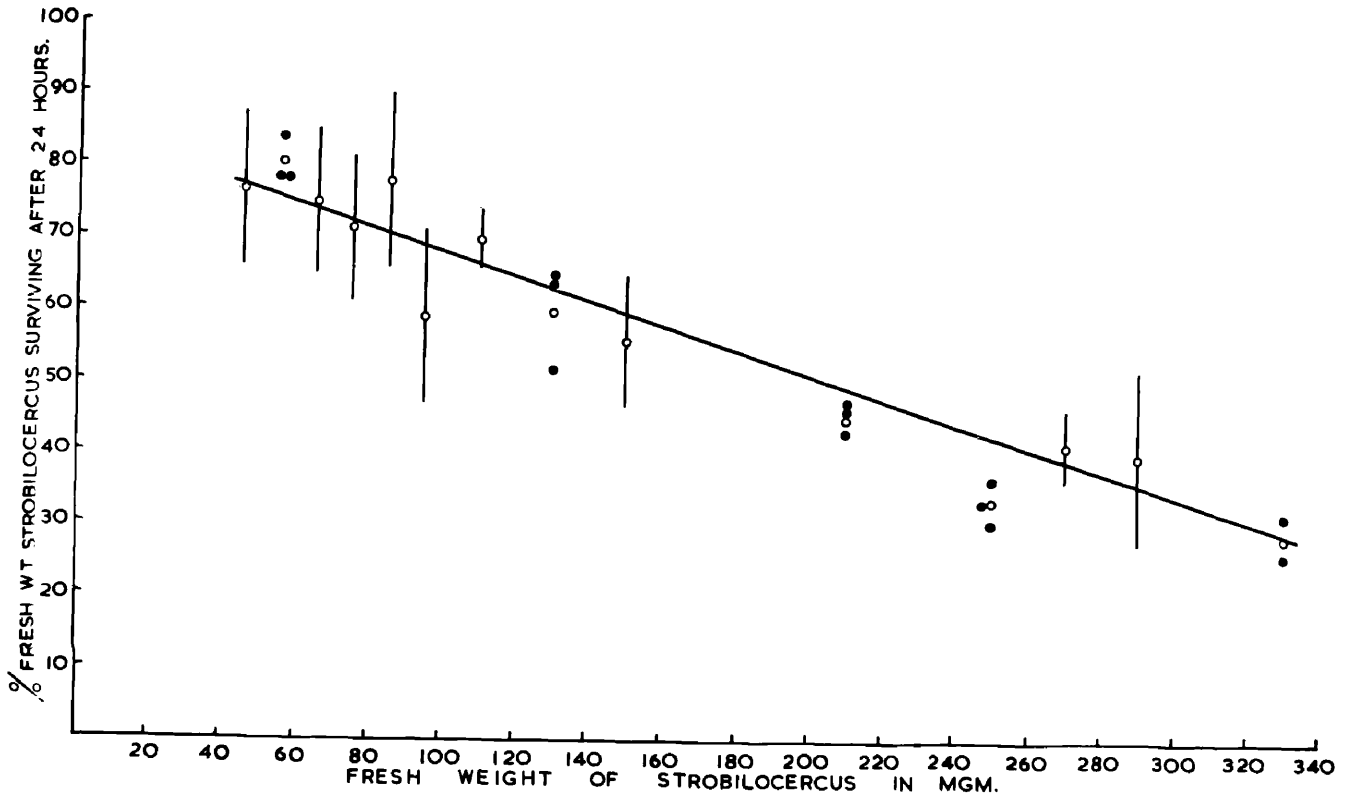

Figure 13. The percentage of the fresh weight of the original strobilocercus which survived digestion after 24 hours in the intestine of the cat.

● = The fresh weight of a worm recovered from a cat expressed as a percentage of the mean fresh weight of infecting strobilocerci.

○ = Mean values. Where 4 or more worms were present, the standard deviation is represented by a vertical line.

Table 6. The amount of the strobilocercus which survives after 24 hours in the cat's intestine.

The mean fresh weight of worms recovered (A) is expressed in Column 6 as a percentage of the mean fresh weight of the strobilocerci used in the infection (S).

TABLE 6

Cat No.	No. of Strobs. Fed	Mean Fresh Wt. of Strobs in mg (S)	Number Recovered	Mean Fresh Wt. of Worms Recovered in mg (A)	$\frac{A}{S}$ %
93	5	45	5	32.06	76.6
94	4	45	4	34.46	
95	3	55	3	44.13	80.2
96	8	65	6	48.53	74.66
97	10	75	7	53.9	71.9
98	5	85	5	65.8	77.4
99	7	95	5	55.78	58.7
100	7	110	7	75.97	69.1
101	3	130	3	77.7	59.76
102	4	150	4	82.85	55.23
54	4	210	3	95.6	45.5
55	4	250	3	84.33	33.23
57	5	270	5	115.92	41.45
60	5	290	4	115.6	39.87
61	5	330	2	95.25	28.86

as a percentage of the mean weight of the infecting strobilocerci. (The comparison had to be made with the mean weight of the infecting larvae as it was not possible to associate a worm recovered after 24 hours with a specific larva.) The abscissa is the mean weight of the strobilocerci fed to a cat (see methods for weight range and number of larvae in a doze). Mean values of the results, in all cases, are plotted as open points; where 4 or more worms were recovered the standard deviation is shown by a vertical line, but with less than 4, results are plotted for individual worms as closed points.

Figure 13 indicates that the amount of pseudostrobila lost in the cat varies and is governed by the weight of the infecting strobilocercus. Strobilocerci within the range 45-330 mg. fresh weight showed a progressive increase in the amount of pseudostrobila lost. Worms beyond this range were uncommon but experiments with 3 weight groups of larger strobilocerci resulted in a loss of 70% of the original fresh weight, suggesting that beyond 330 mg. the graph levels off at approximately 30%.

The value of the true larval strobila can be calculated for any weight group of strobilocerci between the limits shown in Figure 13. In order to obtain this result, the mean weight of infecting strobilocerci is multiplied by that fraction which would survive digestion as indicated by Figure 13. It is important to be able to obtain the value of the true larval strobila because it is this portion of the strobilocercus from which growth commences. In the following section, estimations of growth of the tapeworm in the cat's intestine are based on the weight of the true larval strobila and not on the whole strobila.

(2) Growth in the Cat

The growth pattern of H. taeniaeformis during the first 42 days in the cat is shown in Figure 14. The abscissa represents the number of days which the tapeworm has been in the cat. The ordinate represents, on a log. scale, the amount of growth which has taken place. If all the cats had been infected with larvae of closely similar weight, growth could have been expressed in terms of increase in absolute weight, as the results would have been directly

comparable with each other. However, the wide range in the fresh weights of the strobilocerci recovered from mice made this procedure impracticable. The means of the weight groups which were used for infecting cats varied from 35 - 270 mg. Consequently, instead of expressing the amount of growth taking place in terms of absolute weight, a relative or comparative value was calculated by dividing the average weight of worms recovered from the cat by the value of the true larval strobila. The latter was derived from the weight group of infecting strobilocerci as explained in Part 1 of this sub-section. Thus the ordinate, which represents the amount of growth, is the number of times that the maturing worms have increased their fresh weight from the time that the pseudostrobila was shed 24 hours after infection.

In Figure 14 growth is represented as commencing 1 day after infection but this is an indication of the time taken for the shedding of the pseudostrobila rather than expressing a time lag before growth starts. Since the amount of pseudostrobila

shed varied according to the weight group of strobilocerci used, the drop in weight could not be represented by a single line in Figure 14. Thus, this is omitted from the graph.

The average amount of growth which has taken place in worms recovered from individual cats is plotted either as a solid point or triangle. An arrowed point (♂) represents the average growth of worms which has taken place in immature male cats while the plain points (●) are the corresponding results for immature females. Triangles represent the average growth of worms in mature cats. As can be seen from the distribution of points in Figure 14, several cats were autopsied after having been infected for the same period of time. A comprehensive mean value for the growth of all worms recovered is shown as an open point (○). Its position is not symmetrically disposed between the solid points but biased towards the result obtained from the cat bearing the heaviest infection.

After shedding the pseudostrobila, H. taeniaeformis commences growth immediately with no

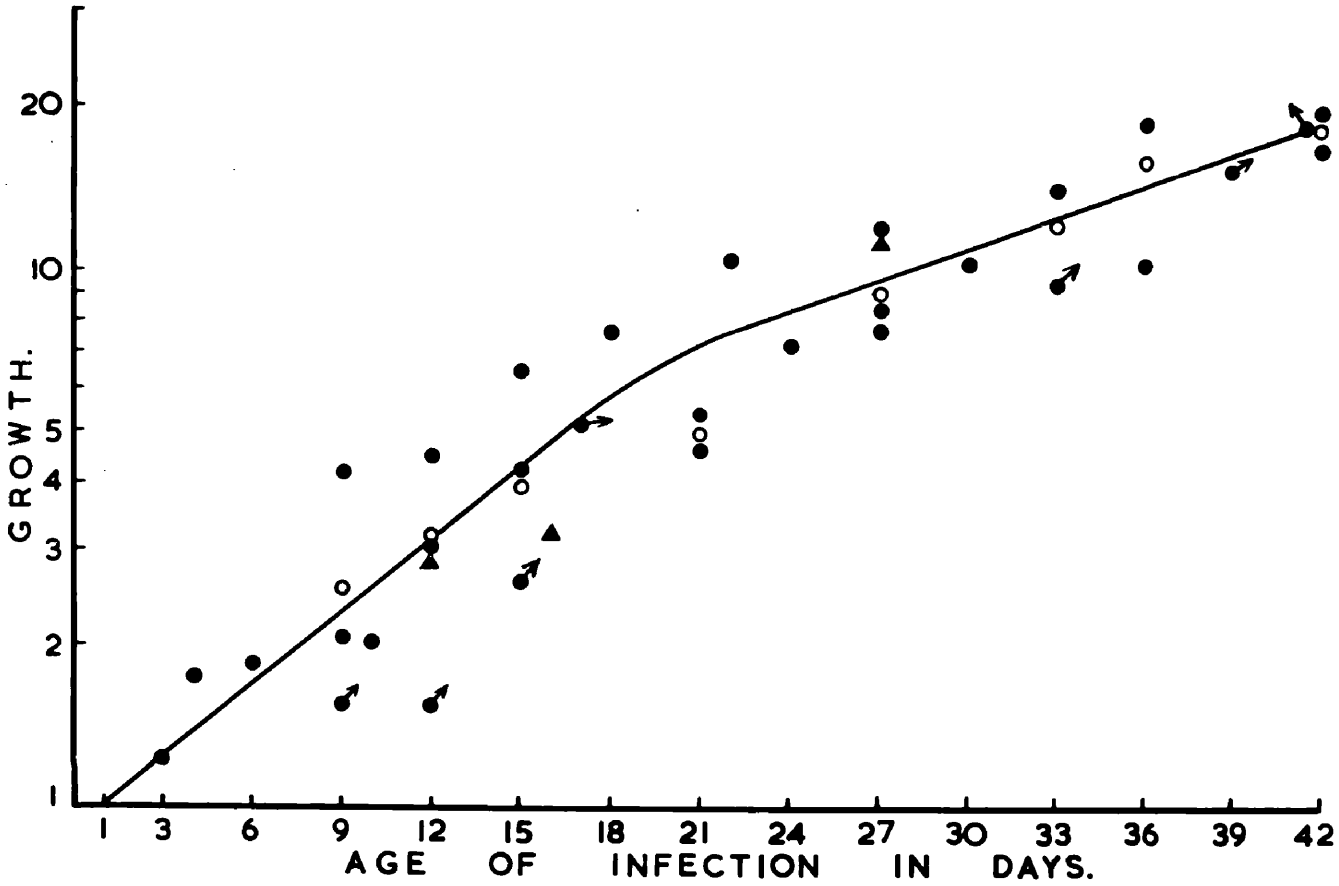

Figure 14. The growth of H. taeniaeformis in cats is plotted on a logarithmic scale and is calculated by expressing the fresh weight of the cestode recovered as a multiple of the fresh weight of the true larval strobila (See text).

● = The mean value for growth of the cestodes in the intestine of an immature female cat.

♂ = The mean value for growth in an immature male cat.

▲ = The mean value for growth in a mature cat.

○ = A comprehensive mean value for the growth of all worms recovered from cats which had been infected for the same period.

Table 7. The growth of *H. taeniaeformis* in *Felis domesticus*.

Each of the 34 results shown was obtained from a single cat.

Column 3 was calculated from Figure 13 as described in the text.

Column 5 shows the amount of growth which has taken place. The mean values shown were obtained by expressing the mean fresh weight of the worms recovered as a multiple of the mean fresh weight of the true larval strobila (Col.3). The number in parenthesis in Col.2 represents the number of strobilocerci fed to a cat while that in Col. 4 represents the number of worms recovered.

TABLE 7

Age of Cestodes Recovered (days)	Mean Fresh Weight of Infecting Strobs. (mg)	Mean Fresh Weight of True Larval Strobila (Calculated) (mg)	Mean Fresh Weight of Cestodes Recovered (mg)	Growth
3	135 (5)	81	99.4 (4)	1.23
4	130 (5)	81.9	151.5 (1)	1.84
6	170 (5)	95.2	176 (3)	1.85
9	35 (8)	27	102.5 (4)	3.80
9	150 (7)	90	139.7 (2)	1.55
9	105 (5)	70.35	145.0 (3)	2.06
10	115 (4)	74.75	152.0 (3)	2.03
12	130 (5)	81.9	245.1 (5)	2.99
12	250 (4)	107.5	329.7 (3)	3.07
12	270 (4)	108	167.4 (3)	1.55
12	125 (4)	80	361.3 (4)	4.52
15	45 (6)	34.2	222.5 (3)	6.51
15	170 (6)	95.2	405.2 (4)	4.26
15	210 (6)	105	277.2 (5)	2.64
16	130 (7)	81.9	265.3 (5)	3.24
17	110 (6)	73.7	374.4 (5)	5.08
18	170 (5)	95.2	736.7 (5)	7.74
21	230 (6)	108.1	494.6 (6)	4.57
21	145 (5)	87	469.3 (5)	5.39
22	55 (7)	41.25	435.4 (2)	10.55
24	150 (5)	102	741.9 (4)	7.27
27	250 (7)	107.5	835.1 (7)	7.77
27	110 (6)	73.7	881.3 (4)	11.96
27	110 (6)	73.7	855.0 (2)	11.60
27	90 (7)	63	533.2 (3)	8.46
30	150 (5)	102	1061.5 (3)	10.41
33	270 (7)	108	1014.5 (3)	9.39
33	140 (4)	85.4	1214.1 (4)	14.22
36	190 (5)	100.7	1041.5 (1)	10.34
36	130 (5)	81.9	1526 (2)	18.63
39	125 (6)	80	1204.4 (6)	15.05
42	110 (5)	73.7	1350.5 (5)	18.32
42	70 (5)	51.5	850.6 (4)	16.52
42	130 (5)	81.9	1610.9 (4)	19.67

intervening lag period. The first phase of growth is exponential and hence gives a straight line when plotted on a log scale. This phase continues to the 18th day at which time the growth rate is seen to decelerate. A straight line plot is still obtained on the logarithmic scale indicating that the cestode has entered a second slower phase of exponential growth. Beyond 42 days no results are available, since the present investigation is mainly concerned with outlining the normal growth pattern from the time that the strobilocercus enters the cat to the point at which gravid proglottids are shed. This may occur at any period from 36 to 42 days.

Examination of the posterior proglottids of the maturing worms revealed that ova appeared in the uterus between the 16th and 18th day (Figs. 15 & 16). Fully developed hexacanth embryos were not observed until the 33rd day.

(3) Site of Development in the Small Intestine.

Examination of 20 cats, 24 hours after infection with H. taeniaeformis, showed the following distribution of cestodes in the small

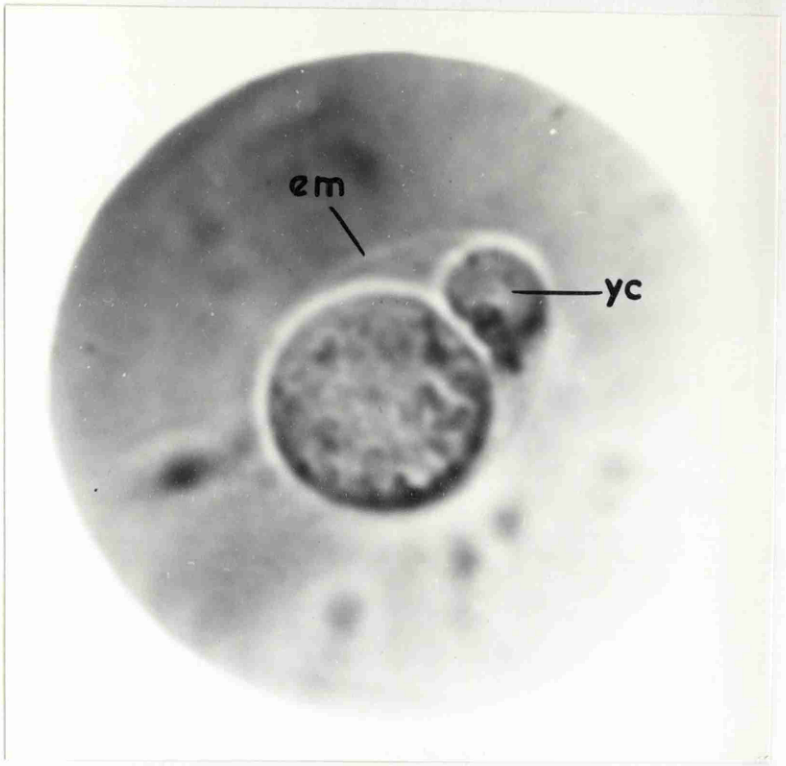

Figure 15. Intra-uterine egg from a 16 day adult H. taeniaeformis.

em = extraembryonic membrane : yc = yolk cell

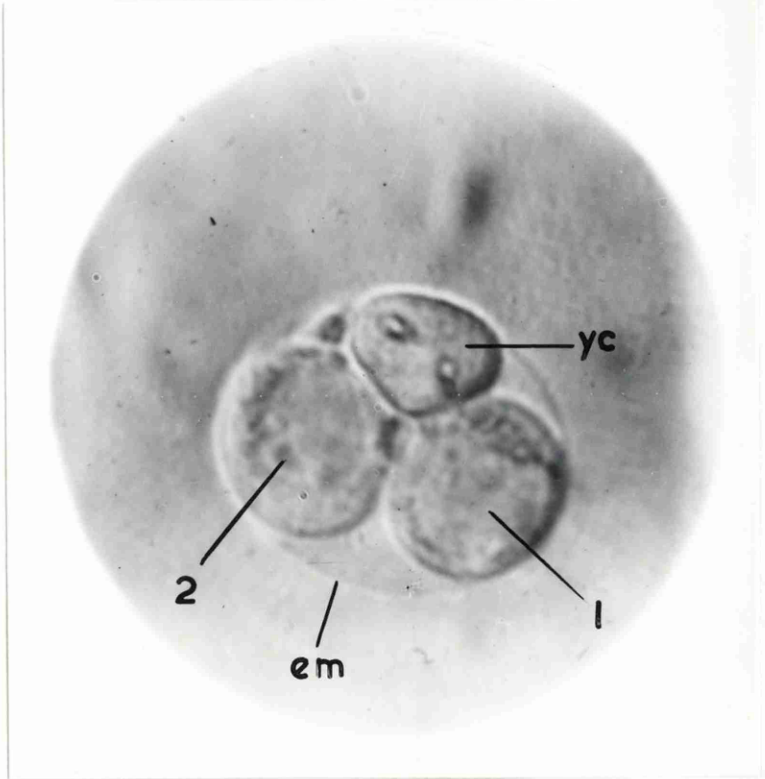

Figure 16. Two-celled intra-uterine embryo from 17 day adult H. taeniaeformis.

em = extraembryonic membrane : yc = yolk cell
1 & 2 = macromeres.

intestine: - Anterior Third - 22%: Middle Third - 72%: Posterior Third - 6%.

The distribution of cestodes in laboratory induced infections of durations varying from 6 to 42 days was: Anterior Third - 16%: Middle Third - 75%: Posterior Third - 9%.

In natural infections (Section 2) the distribution was: Anterior Third - 20%: Middle Third - 76%: Posterior Third - 4%.

Since the cestodes recovered after 1 day in the cat have the same distribution as those in older infections, it is concluded that H. taeniaeformis establishes itself immediately in the region of the small intestine where growth occurs.

(4) Abnormal Hosts

In the search for more easily maintained laboratory animals to act as definitive hosts for H. taeniaeformis, it was found that this cestode would survive for 48 hours in the intestine of the albino rat. After the first 24 hours, the worms were recovered from the middle third of the small intestine. The pseudostrobila had been shed in the

normal manner and the cestode was normal morphologically. There was, however, a marked difference observed in the glycogen content and the dry/fresh weight ratio when they were compared with worms which had been in cats for a similar period. After 48 hours in the rat, the tapeworms were generally found in the posterior third of the small intestine, and, by this time, morphological abnormalities particularly in the scolex region, had become apparent. The scolices were markedly inactive. Estimations of the glycogen showed that the content had dropped still further.

Rabbits, dogs and ferrets proved refractive to infection.

Discussion

In common with other Cyclophyllidea, there are three distinct phases of development in the life history of H. taeniaeformis, namely, embryonic, larval and adult development. Embryonic development, taking place within the uterus of maturing H. taeniaeformis, has already been fully described by Janicki

(1907). This phase differs from the others in that development is not accompanied by increase in size. The growth and development of the larval form, which in H. taeniaeformis is a strobilocercus, has been described in the previous sub-section.

The final phase of development, involving growth and maturation, starts when the strobilocercus is ingested by the definitive host. It was the opinion of Leuckart (1878) that the complete larval strobila was digested leaving the neck and scolex to grow into the mature tapeworm in the intestine of the cat. Miller (1932) stated that 25% of the strobilocercal proglottids were lost in the cat. Joyeux & Baer (1938) showed, firstly, that the pseudostrobila was shed by the 20th hour after infection of the cat, and, secondly, that the amount of pseudostrobila which was shed was variable. Both of these findings have been confirmed by the present series of experiments and the relationship existing between the weight of the strobilocercus and the amount shed in the cat is given in Figure 13.

By extrapolation of the graph in Figure 13, the abscissa is cut at approximately 500 mg. This

would suggest that strobilocerci of 500 mg consist almost entirely of pseudostrobila and hence that Leuckart's hypothesis might be true in heavy worms. But even this is extremely doubtful, as the 3 sets of results obtained with heavy larvae indicate a levelling out of the graph in worms over 300 mg at a proportion of 70% pseudostrobila to 30% true larval strobila. In addition to the loss of the pseudostrobila, it seemed possible that other factors, such as changes in the glycogen and water content, could be responsible for alterations in the fresh weight of the worm during the first day in the cat. In Section 4, it is shown that no major change occurs in the glycogen content during this period. It is also shown that the water content of the anterior portion of the strobilocercus which survives digestion does not alter appreciably. Therefore, it is evident that the change in fresh weight which occurs during the first 24 hours in the cat can be attributed solely to the loss of the pseudostrobila.

One feature, which became apparent from Figure 13, is that the pseudostrobila is formed at a faster rate than the true larval strobila during the

growth of the strobilocercus. This follows from the fact that the ratio of the true larval strobila to the whole strobilocercus decreases as the weight of the strobilocercus increases. No function can be attributed to the pseudostrobila and it is difficult to account for its existence. A developing larval worm is recognisable for the first time as a strobilocercus on removal from the mouse liver on the 48th day after infection. The strobilocerci which are formed at this time continue to develop in mouse liver, but, if they are fed to cats before the 60th day, they are digested. It is possible that some of the early proglottids formed at this time, which are incapable of survival in the cat, are pushed to the posterior after the 60th day to form the pseudostrobila.

The way in which the pseudostrobila increases in size after the 60th day is even more hypothetical, at least two methods are possible. Firstly, the proglottids of the pseudostrobila may divide. A complete series, from the commencement to the termination of a "division" has been observed but it was

difficult to decide whether they were actual stages of division or whether they were abnormally formed proglottids. Secondly, a more likely explanation is that, as the true larval strobila increases in size, proglottids in its posterior degenerate and are incorporated in the pseudostrobila.

The pseudostrobila was completely shed within 20 hours, but, for convenience, estimations of the amount shed were made 24 hours after the infection of the cat. As already explained, growth is based on a comparison of the estimated weight of the infecting worm after 24 hours in the cat with the actual weight of the worm observed at a later date. Consequently, any growth which may take place in the true larval strobila during the first day is not taken into account in Figure 14. Since the doubling time during the initial phase of growth is 8 days, it is not considered that this introduces an appreciable error.

Archer and Hopkins (1958) found that an initial lag period existed in the case of Diphyllobothrium and attributed this fact to the tapeworm establishing itself initially in the

posterior part of the small intestine. Migration to the mid region coincided with the commencement of growth. A comparison of the position of H. taeniaeformis recovered from cats 24 hours after infection with the position of worms recovered from infections of longer duration, showed that this cestode becomes established initially in the middle third of the small intestine and remains subsequently at this site. The fact that the majority of cestodes were observed in the middle third is evidence that this is the most favourable position for development. That few cestodes were recovered from the posterior third, suggests that conditions in this region were unsuitable for normal metabolism and development. Moreover, worms recovered more posteriorly had an abnormally low glycogen content (Section 4 - Glycogen Metabolism). Having established itself in a favourable position initially, it is to be expected that H. taeniaeformis commences growth immediately without an intervening lag period.

In these experiments to determine the rate of growth of H. taeniaeformis, 20.6% of the cats used were immature males. The sex of the cats is shown (Figure 14) because Addis (1946) has presented evidence that the sex of an immature host has a bearing

on the growth of tapeworms. His findings were that Hymenolepis diminuta was partially stunted after 14 days in immature male rats on a complete diet but that growth was normal in similarly treated immature females. As Addis used the length of the tapeworm as a basis for estimating growth, whereas weight has been used in the present series of experiments, the results are not altogether comparable. But reference to Figure 14 shows little evidence that growth of H. taeniaeformis was affected by age or sex of the cat.

Figure 14 indicates that there are two distinct exponential phases of adult growth. The first phase has a doubling time of 8 days and terminates on the 18th day after infection. There follows a period of deceleration and growth enters a second exponential phase with a doubling time of 16 days. It is significant that the transition from one phase to another coincides with the commencement of egg production between the 16th and 18th day.

During the first phase, somatic growth occurs and genitalia are elaborated in the posterior

proglottids. With the commencement of egg production, large numbers of fertilised ova pass into the uterus to commence embryonic development. It is suggested that, when eggs start to appear in the uterus on the 18th day, further somatic growth of the proglottid ceases because all growth substances are being utilised for the further production of sperm, ova and vitellaria.

The second phase of exponential growth could be considered as the summation of two processes which are taking place simultaneously in the anterior and posterior part of the worm respectively. In the anterior region, proglottids are continuing to double their weight every 8 days in the same way as the whole immature worm grows during the first 18 days. Growth of a proglottid in a mature worm continues in this manner from the point at which it is formed to the point at which egg production commences. After a short transition period of deceleration, growth of the somatic cells in the proglottid ceases. In the posterior region, although the proglottids have stopped growing, their weights are increasing due to the production of eggs which are retained in the uterus.

But it is suggested the rate of weight increase is now much slower. The time taken therefore to double the total body weight increases and gives rise to the second exponential growth phase with a doubling time of 16 days (Fig. 14). No results are available beyond the 42nd day after infection but it is probable that increase in size will cease when production of proglottids in the anterior is balanced by the shedding of those which are gravid from the posterior. Alternatively, there may be periodic fluctuations due to irregular loss of the gravid proglottids.

Summary

The amount of pseudostrobila shed by the strobilocercus of H. taeniaeformis on entering the cat's intestine varies from 20 - 70% of the total weight depending on the size of the infecting strobilocercus. The tapeworm commences growth immediately on entering the small intestine. No preliminary lag period could be detected and this was attributed to the fact that initial establishment occurs at a favourable site for development. There was no evidence of forward

migration in the intestine after establishment.

The growth pattern for the first 42 days of development (prepatent period) was found to consist of two distinct exponential phases. The transition from one to the other coincided with the commencement of egg production. The possible factors causing this transition are discussed.

SECTION 4

CHEMICAL COMPOSITION

General Introduction

At the present time there is insufficient data to decide whether the chemical composition of helminths, developing normally, follows any specific pattern. Reid (1942) showed that the percentage glycogen composition varied extensively in the course of 24 hours. But the fact that he could show a diurnal rhythm is evidence of the constancy of composition at any particular time.

Before commencing the investigations described here, two questions were posed. Firstly, was it likely that two worms of the same species at the same stage of development would be of similar composition, and secondly, even supposing worms did follow a fixed pattern, of what value would such information be to the helminth physiologist?

As an answer to the first, reference should be made to Needham's (1950) discussion on heterauxesis. This concept was first developed by the morphologist as an expression of the rate of growth of a part compared to the rate of growth of the whole. More recently it has been found to apply to many biochemical

ratios, e.g. rate of increase in percentage water, glycogen, fat, phosphorus etc. in the body compared with the rate of increase in total body weight. It is not yet possible to say of what fundamental significance the plots of these ratios are, but an examination of the immense array of data collected by Needham shows that the percentage composition of animals is normally remarkably constant at any specific stage in their life. Furthermore, changes in percentage composition come about slowly, and usually at a constant rate throughout life, that is, a substance does not increase at one stage and decrease at another. There are exceptions to this rule but they are usually associated with a major change in the life of the animal such as the period of metamorphosis of an insect.

The establishment of the existence of biochemical heterauxesis in helminths would be of value for several reasons. If the percentage composition of protein, glycogen, water etc., follows a specific pattern during growth, then it can be used as a standard for the comparison of results obtained from

the analysis of worms developing *in vitro*. In addition to the establishment of the normal levels of macro-constituents at various stages of development, there exists the possibility that helminths, like other animals, may show major changes in composition at certain stages, for instance, at the time of transfer from one host to another. It was this aspect in particular that was of interest, as it might reveal a quick method for determining the suitability of culture media. Thus if there is a specific change in the chemical composition of a parasite when it changes hosts, then any suitable culture medium should initiate the same change.

The existence of such a sudden specific change has already been shown to occur in the glycogen level when *Schistocephalus solidus* is transferred from the coelom of a fish (larval habitat) to the small intestine of a bird (adult habitat) (Hopkins, 1950). This information was later used as a criterion of in *vitro* development (Hopkins, 1952).

There are several other ways in which a detailed knowledge of the chemical composition through-

out life may be of value. Such information would give more precise understanding about the rate of growth than the dry or fresh weight does, as from it can be calculated the rate of synthesis of proteins, nucleic acid, etc. It would also give more specific information about the amount of metabolites absorbed from the host.

(1) WATER CONTENT

WATER CONTENT

The water content and dry matter have been recorded for many different species of cestode (reviewed by von Brand 1952). The reports, with the exception of that on Diphyllobothrium sp. (Archer & Hopkins 1958b), deal with the water content of the cestode at one particular stage of its life cycle or record averages from worms of different and unknown ages. Very little is known about the fluctuations in water content which may occur during the life cycle. This aspect has been investigated for both the larval and adult stages of H. taeniaeformis.

Materials and Methods

In this section the dry weights of the cestodes are expressed as percentages of the fresh weights. From this percentage, which is referred to as the dry/fresh (D/F) weight ratio, the dry matter and water content of the cestodes can be easily derived e.g. a D/F weight ratio of 30% means that 30% of the fresh weight of the worm consists of dry matter and the remaining 70% represents the water content.

(1) Larval cestodes.

Larval H.taeniaeformis were removed from Strain A albino mice which had been killed at intervals after infection. The worms were immediately washed in balanced saline after removal and then pressed between filter paper to remove surface moisture and weighed (fresh weight). In this process the bladder bursts and often a small part is lost, but, in larvae aged 8 weeks and older, the bladder wall is a negligible fraction of the total mass. Immediately after weighing, the larvae were placed on porcelain slabs at 110°C and dried for 18 hours, then reweighed (dry weight). They were then stored in desiccators over silica gel and were subsequently used in estimations of the glycogen content and nitrogen fraction. In desiccators no difference in composition was detected up to 1 year later, except for a slight gain in weight due to the reabsorption of a small quantity of water.

In order to investigate the possible existence of a gradient in the water content along the length of the strobilocercus, worms were removed from mouse liver and each was divided into anterior and posterior portions

which were estimated separately for dry/fresh weight ratios. The point of division in the 48 strobilocerci examined was varied so as to form a series in which the dry weight of the anterior portion varied from 5 - 95% of that of the whole worm. Since the reactions to this parasite vary from host to host, it was considered advisable to employ a population of cestodes from the liver of a single mouse. This ensured that conditions exerted by the host were uniform and that all strobilocerci had developed under identical conditions.

(2) Adult cestodes.

Adult worms were obtained from the small intestine of laboratory infected cats immediately after killing. The worms were treated in the same way as the larvae, i.e. washed in balanced saline, blotted, fresh weight determined, and then after drying at 110°C for 18 hours, reweighed.

RESULTS AND DISCUSSION

(1) D/F Weight Ratio during Larval Development

In Figure 17, the mean D/F weight ratio of each age group of strobilocerci recovered from mice over a period of 75 weeks is plotted as a solid point. The number of strobilocerci on which each mean is based together with the number of mice killed to provide each result is given in Table 8.

Although larvae can be seen and removed from the liver after the second week, the difficulties involved in weighing such small organisms coupled with the difficulty in getting rid of surface moisture made it impossible to obtain an accurate D/F weight ratio before the end of the 8th week. With the larger larvae obtained on and after this period, the presence of small quantities of cystic fluid remaining after blotting did not affect the D/F weight ratio greatly.

From the 8th to the 26th week (Figure 17) the dry weight increases at the same rate as the fresh weight. This is similar to what occurs in the plerocercoid of the pseudophyllidean tapeworm,

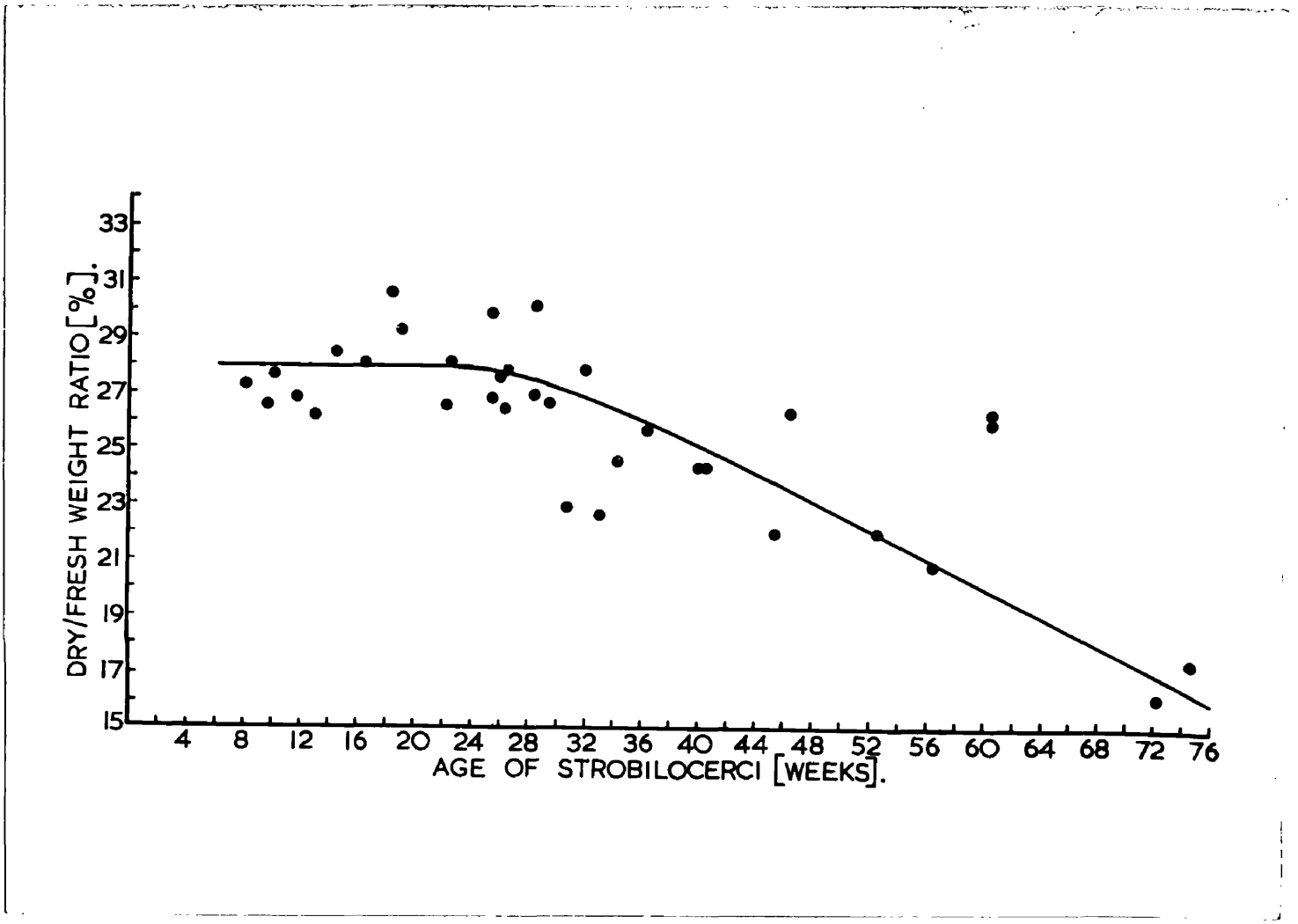

Figure 17. The mean dry/fresh weight ratios of larval H. taeniaeformis removed from the livers of Strain A mice which had been infected for periods varying from 8 - 74.6 weeks.

(See also Table 8).

Table 8. The D/F weight ratio of strobilocerci aged from 8 - 74.6 weeks.

Column 3 shows the number of strobilocerci found in each mouse liver. Where 3 or more mice were examined, the range in numbers of strobilocerci is shown.

TABLE 8

Age of Strobs. in Weeks	No. of Mice killed	No. of Strobs. per Liver	No. of Strobs. Estimated	Mean D/F Wt.Ratio (%)
8	5	1-6	11	27.26
9.6	2	4 & 4	8	26.58
10	5	1 - 6	10	27.62
11.6	3	1 - 12	24	26.83
13	3	1 - 6	12	26.12
14.4	3	1 - 6	14	28.5
16.4	5	1 - 12	26	28.06
18.3	9	1 - 12	62	30.5
19	3	1 - 6	14	29.24
22.1	2	6 & 5	11	26.55
22.6	3	28 - 164	232	28.13
25.4	1	19	19	26.8
25.3	1	110	14	29.9
26	4	1 - 8	15	27.58
26.1	1	53	53	26.47
26.4	2	3 & 3	6	27.82
28.6	1	12	3	26.9
28.6	1	20	2	30.19
29.4	1	18	17	26.6
30.7	1	15	15	25.3
31.9	1	16	13	27.86
32.9	2	6 & 12	18	22.66
34.3	2	3 & 15	18	24.67
36.3	1	100	88	25.74
40	3	12 - 66	68	24.40
40	1	66	29	24.93
45.3	1	28	25	22.0
46.3	2	12 - 13	25	26.37
52.4	1	23	23	22.48
56.4	2	15 & 20	35	20.87
60.3	1	12	12	26.03
60.3	1	9	9	26.36
72.1	1	17	17	16.19
74.6	1	12	11	17.42

Diphyllobothrium sp. (Archer and Hopkins 1958b). The majority of animals cited by Needham (1950), however, show a tachyauxetic increase in the dry weight during development and the only parallel mentioned in which the rate of increase is isauxetic, is in the early development of the avian embryo.

The increase in the water content which takes place after the 26th week is difficult to explain as it is the opposite to what happens in most animals (Needham loc. cit.). Within the host capsule, the strobilocercus lives in a homeostatically controlled environment, and, for this reason, the increase can not be attributed to changes in environmental conditions. In addition, growth of the strobilocercus has stopped and so this change in the water content is not associated with any morphological development. It is concluded that changes in the dry/fresh weight ratio after the 26th week may be associated with the degeneration of the worm and further evidence to support this is presented in the following sub-section (glycogen metabolism).

(2) D/F Weight Ratio during Maturation

In view of the variation in the D/F weight ratio found in very young and very old larvae, only strobilocerci between 10 and 26 weeks were used to infect cats. In Figure 18, mean dry/fresh weight ratios of worms recovered are plotted against the period of time which the worms spent in the small intestine of the cat.

During the first 18 days, the dry weight increases bradyauxetically with respect to the fresh weight, i.e. the water content of the worm increases. After maturation about the 18th day, this increase in the water content slows and the dry/fresh weight ratio is stabilised at 21% about the 32ND day.

A comparison with Figure 17 shows that there appears to be a decrease in water content from 72% to 68% when the worm enters the small intestine of the cat. This decrease is connected with the loss of the pseudostrobila rather than an actual change in water content as is evident from the following results which deal with the water gradient in the strobilocercus.

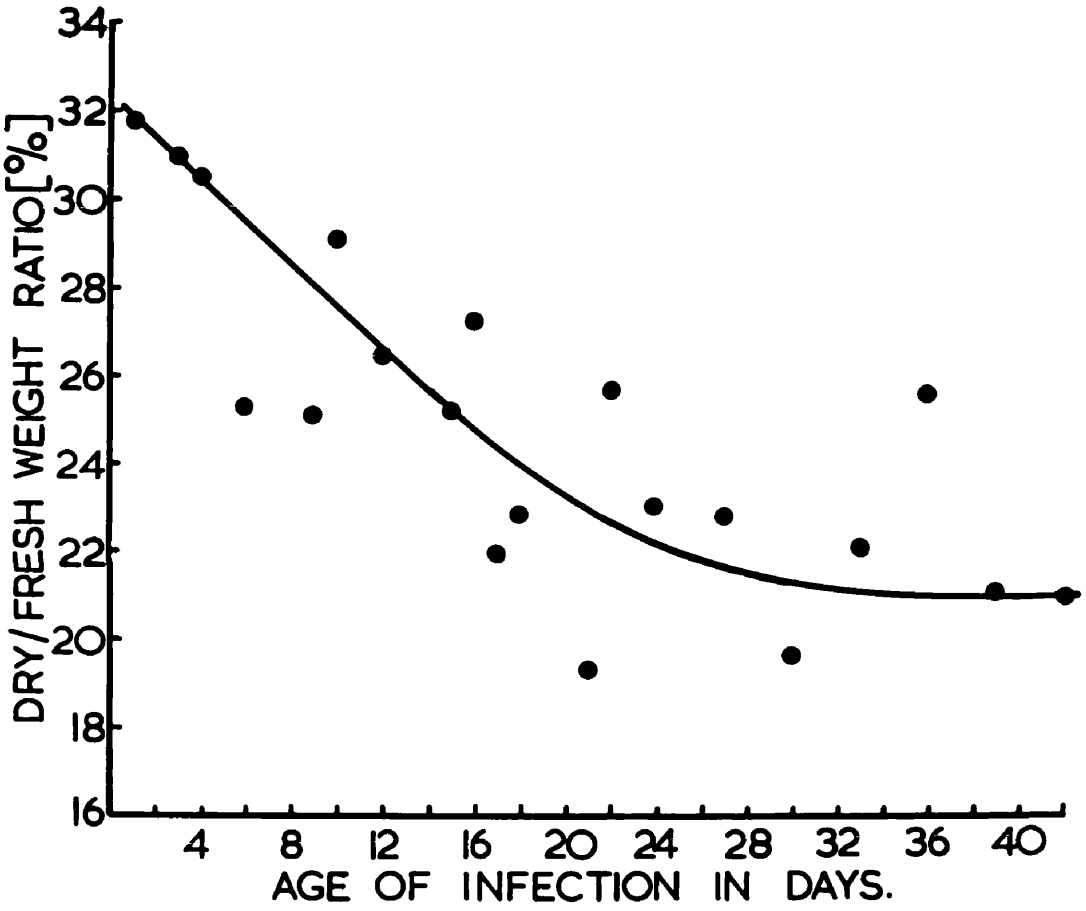

Figure 18. The mean dry/fresh weight ratios of H. taeniaeformis removed from the intestines of cats.

(See also Table 9)

TABLE 9

Age of Adult Cestodes in days	No. of Cats Examined	No. of Cestodes Recovered	Mean D/F Weight Ratio (%)
1	16	67	31.79
3	1	4	30.79
4	1	1	30.52
6	1	3	25.25
9	3	6	25.05
10	1	3	29.12
12	4	16	26.44
15	3	12	25.18
16	1	5	27.25
17	1	5	21.98
18	1	5	22.84
21	1	6	19.29
22	1	2	25.69
24	1	4	23.06
27	4	15	22.84
30	1	3	19.63
33	2	7	22.10
36	2	3	25.54
39	1	6	21.10
42	3	12	20.99

Table 9. The D/F weight ratio of H. taeniaeformis recovered from the small intestine of domestic cats.

(3) The Water Gradient in the Strobilocercus

To investigate the water content of different regions of the larval strobila, 48 strobilocerci from a single mouse were each divided into anterior and posterior portions (see methods).

In Figure 19, the ratio of the dry weight of each posterior portion to the total dry weight of the corresponding strobilocercus is expressed as a percentage and plotted against the D/F weight ratio of the posterior portion. The points represent the results obtained for single worms while the open square represents the mean D/F weight ratio for the complete population of strobilocerci removed from the liver of this particular mouse. In Figure 20 the results obtained for the anterior portions of the strobilocerci were plotted similarly (see legend to Figure 20).

Figure 19 shows that the water content of the posterior portion decreases as the point of division is moved anteriorly. Thus, along the length of the strobilocercus, there is a water gradient i.e. the water content increases towards the posterior. This is confirmed by the results plotted in Figure 20.

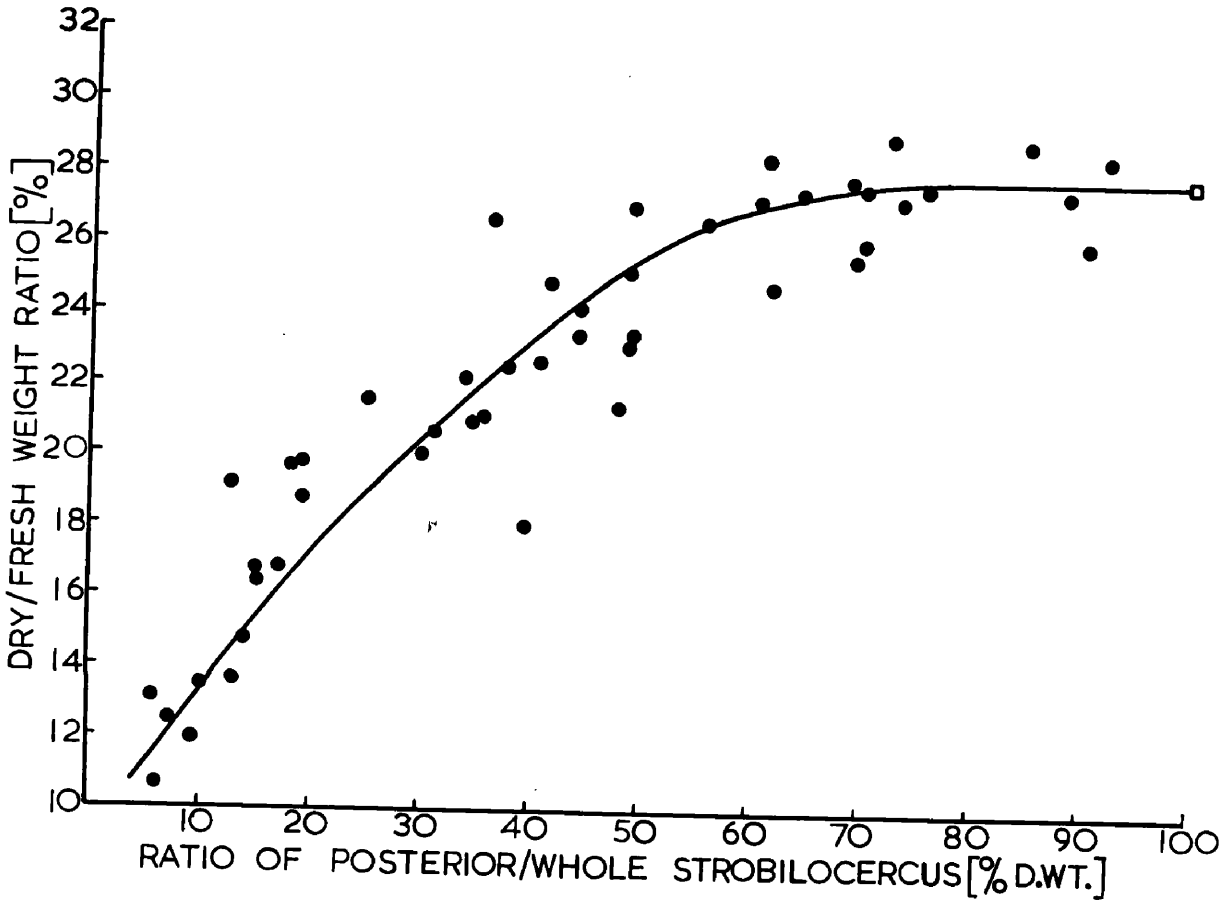

Figure 19. The dry/fresh weight ratio of different portions of the strobilocercus.

The dry/fresh weight ratio of the posterior portion of each strobilocercus is plotted against its dry weight expressed as a percentage of the dry weight of the complete worm. (See also Table 10).

□ = The mean D/F weight ratio of the 48 complete strobilocerci.

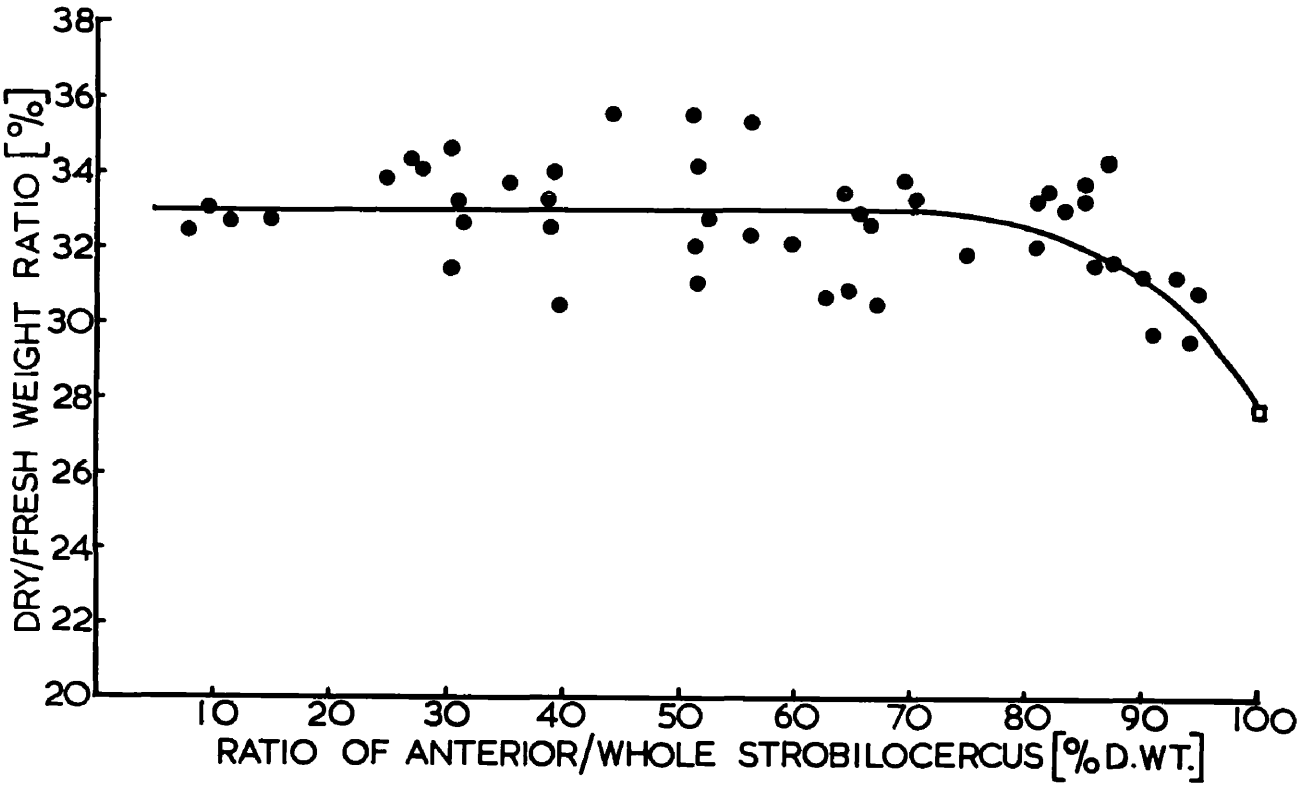

Figure 20. The dry/fresh weight ratio of different portions of the strobilocercus.

The dry/fresh weight ratio of the anterior portion of each strobilocercus is plotted against its dry weight expressed as a percentage of the dry weight of the complete worm (See also Table 10).

□ = The mean D/F weight ratio of the 48 complete strobilocerci.

Table 10. The D/F weight ratio of the anterior and posterior portions of *H. taeniaeformis*.

TABLE 10

Dry Wt. of Strobs. (S)	ANTERIOR PORTION		POSTERIOR PORTION	
	$\frac{A}{S}$%	D/F Wt. Ratio	$\frac{P}{S}$%	D/F Wt. Ratio
30.6	7.8	32.43	92.2	28.48
35.4	9.6	33.01	90.4	26.08
45.2	11.5	32.70	88.5	27.45
23.3	15.0	32.71	85.0	28.86
44.9	24.7	33.84	75.3	27.55
41.5	26.8	34.36	73.2	27.14
37.0	27.8	34.10	72.2	28.99
33.4	30.3	34.67	69.7	27.51
34.7	30.3	31.44	69.7	26.02
42.2	30.8	33.25	69.2	25.52
37.0	31.4	32.68	68.6	27.73
31.2	35.3	33.74	64.7	26.47
34.4	38.7	33.28	61.3	24.87
21.6	38.9	32.56	61.1	28.33
30.2	39.1	34.00	60.9	24.86
40.0	39.8	30.46	60.2	27.14
39.5	44.1	35.58	55.9	26.59
37.9	51.2	35.53	48.8	23.33
33.3	51.4	34.20	48.6	27.04
30.9	51.5	31.05	48.5	23.08
40.9	51.6	32.07	48.4	25.19
40.6	52.5	32.77	47.5	21.32
35.3	56.1	35.36	43.9	24.18
23.7	56.1	32.36	43.9	23.37
39.2	59.7	32.10	40.3	22.64
29.0	60.7	30.50	39.3	18.01
29.2	62.7	30.65	37.3	22.47
39.7	64.0	33.51	36.0	26.58
20.6	64.6	30.86	35.4	21.04
31.0	65.5	32.95	34.5	20.90
33.6	66.4	32.65	33.6	22.16
30.9	69.3	33.81	30.7	20.61
50.2	70.1	33.30	29.9	20.00
22.3	74.9	31.81	25.1	21.54
35.3	80.7	32.06	19.3	18.73
33.1	81.0	33.29	19.0	19.75
31.9	81.8	33.50	18.2	19.79
34.4	83.3	33.01	16.7	16.80
35.0	84.9	33.26	15.1	16.46
32.0	85.0	33.75	15.0	16.72
33.4	85.9	31.54	14.1	14.78
37.7	87.0	34.31	13.0	13.61
32.3	87.3	31.68	12.7	19.16
28.0	90.0	31.26	10.0	13.46
31.0	90.7	29.73	9.3	11.93
27.7	92.8	31.23	7.2	12.50
39.0	94.1	29.52	5.9	10.65
45.6	94.7	30.83	5.3	13.19

Although from the shape of the curve in Figure 19 the posterior 75% of the worm appears to have a higher water content, examination of Figure 20 shows that the increase in water actually occurs in the last 20 - 25%. It is the high water content in this region which leads to an apparently low D/F weight ratio in the whole of the posterior 75% (Figure 19).

The main point of interest is that the D/F weight ratio of the anterior part of the strobilocercus corresponds with that observed initially in the adults from cats, indicating that there is no sudden change in water content when the cestode arrives in its new environment. The difference observed between the D/F weight ratio of complete strobilocerci fed to cats and that of worms recovered during the next 4 days is explained by the fact that there exists a water gradient down the larval worm. The high water content of the posterior lowers the overall D/F weight ratio of the strobilocercus. When this posterior part, the pseudostrobila, is digested, the resultant worm has the D/F weight ratio of the anterior portion.

Summary

From the 8th to the 26th week, the water content of the strobilocercus of Hydatigera taeniaeformis is constant having a mean value of 72.3% of the fresh weight, but, thereafter, an increase occurs until, by the 74th week, the amount of water present is 82.6%. It is suggested that this increase is due to degenerative changes occurring in the larval cestode. The water content of the anterior and posterior of the strobilocercus has been shown to differ. There is little difference in the anterior 75% of the larval worm which has a water content of 67%. The water content of the posterior 25% is 82%. This posterior portion with the higher water content is shed as part of the pseudostrobila within the intestine of the cat. The D/F weight ratio of the cestode after 1 day in the cat is 32% and is similar to that observed in the anterior of the strobilocercus. This indicates that the transfer from intermediate to definitive host does not affect the water content of this tapeworm.

During the first 18 days in the cat, the water content increases from 67% to 78%. Stability in the D/F weight ratio is reached by the 32nd day.

(2) GLYCOGEN METABOLISM

GLYCOGEN METABOLISM

The greater proportion of the carbohydrate fraction of tapeworms consists of glycogen which is stored in the tissues for energy metabolism. The actual metabolic processes involving the utilisation of the glycogen have been studied in Hymenolepis diminuta by Read (1951).

Current knowledge on the glycogen content of different species of cestode has been reviewed by von Brand (1952). In several cases quoted, a progressive increase in the endogenous glycogen of larval stages has been demonstrated, but, in most cases, histochemical techniques were used which are only semi-quantitative. Hopkins (1950) described variations in the glycogen content of Schistocephalus solidus during maturation in pigeons. More recently, Archer & Hopkins (1958 b) investigated the glycogen metabolism of Diphyllobothrium sp. during development in the albino rat.

In this section, the fluctuations in the glycogen content at various stages in the life history of H.taeniaeformis are described. It is shown that the glycogen reserves in the strobilocercus may represent as much as

45% of the dry weight. It follows that glycogen metabolism must be of great importance to the cestode and for this reason demands special attention.

Materials and Methods

The material used in these experiments was dried and weighed as described in the previous subsection (Water Content). All glycogen estimations were based on dry weight.

Representative specimens were selected for estimation from each age group of larval worms. The mean dry weight of these age groups had been calculated and the worms whose dry weight lay closest to this mean were selected. Because of the small size of the larvae recovered during the first 8 weeks in mice, estimations had to be based on groups of worms. All other worms, both larval and adult, were large enough to be estimated individually.

The fewer number of adult cestodes in each age group made it possible for all to be estimated. However, adults which had been longer than 24 days in the cat were

too large to have their glycogen content determined by a single estimation. As an alternative to dividing these worms and estimating the portions, it was decided to grind the entire worm and estimate a sample of the homogeneous powder.

The dried material to be estimated, having been weighed, was hydrolysed at 100°C with 2 ml of 30% KOH for 8 minutes. After this time, 3 ml of 95% ethanol was added to precipitate the glycogen. To ensure that all the glycogen had been precipitated the solution was heated to boiling and allowed to stand for 15 minutes. The tubes were centrifuged for 5 minutes at 1,500 r.p.m. to compact the glycogen, and the supernatant was poured off. After draining, the tubes were kept at 4°C until used.

The glycogen was next converted into glucose by hydrolysing at 100°C for 3½ hours in $\bar{N}$. H_2SO_4. After cooling, the pH of the solution was altered to between 5.5 and 6.5 by the addition of NaOH. Bromo cresol purple was used as an indicator because it had been found that the few drops used did not interfere with the subsequent estimation (Hopkins 1950). The glucose

solution was then diluted to a convenient strength (10.0 - 40.0 mg %) and either estimated at once or after storing overnight at 4°C in a refrigerator.

The glucose was estimated according to the technique described by Shaffer and Somogyi (1933) in which Reagent 50 with an additional 5 gm of KI was used. The glass balls used by Shaffer to seal his reduction tubes were replaced by rubber bungs fitted with bunsen valves as recommended by Hopkins (1950). The reduction tubes were boiled in a constant level water bath for exactly 15 minutes and then placed in a pail of cold water for 105 seconds to bring the temperature to approximately 30°C. The iodine, released on addition of 2 ml of a solution of 2.5% KI + 2.5% potassium oxalate and 5 ml of $\bar{N}$. H_2SO_4 was measured after 10 minutes by titration against 0.005 N sodium thiosulphate. A batch of 3 tubes was estimated from each glucose solution and, if the variation in the titres exceeded ± 0.1 ml, another batch was estimated.

Conversion of the glucose figures into equivalent glycogen was not undertaken due to doubts as to the correct factor.

Results and Discussion

(1) Glycogen Content of Strobilocercus

In Figure 21, the glycogen content of larval worms, expressed as a percentage of the dry weight, is plotted against their age in weeks. For the first 8 weeks the worms were too small to be estimated singly and so glycogen estimations had to be carried out on groups of worms. The number of worms in each group, estimated during this period, is represented by an index adjacent to the solid point. Points without indices represent estimations performed on single worms. A hollow point (O) shows the mean glycogen content of a particular age group of worms.

One feature of interest is the low results obtained from estimations based on groups of 96 and 33 worms recovered after 5 weeks in mouse liver. In the section on larval growth, it was shown that growth was rapid in the first 6 weeks of larval life and it seems probable that, during this period, the food absorbed by the parasite is utilised mainly for growth. This hypothesis is supported by the low glycogen figures at

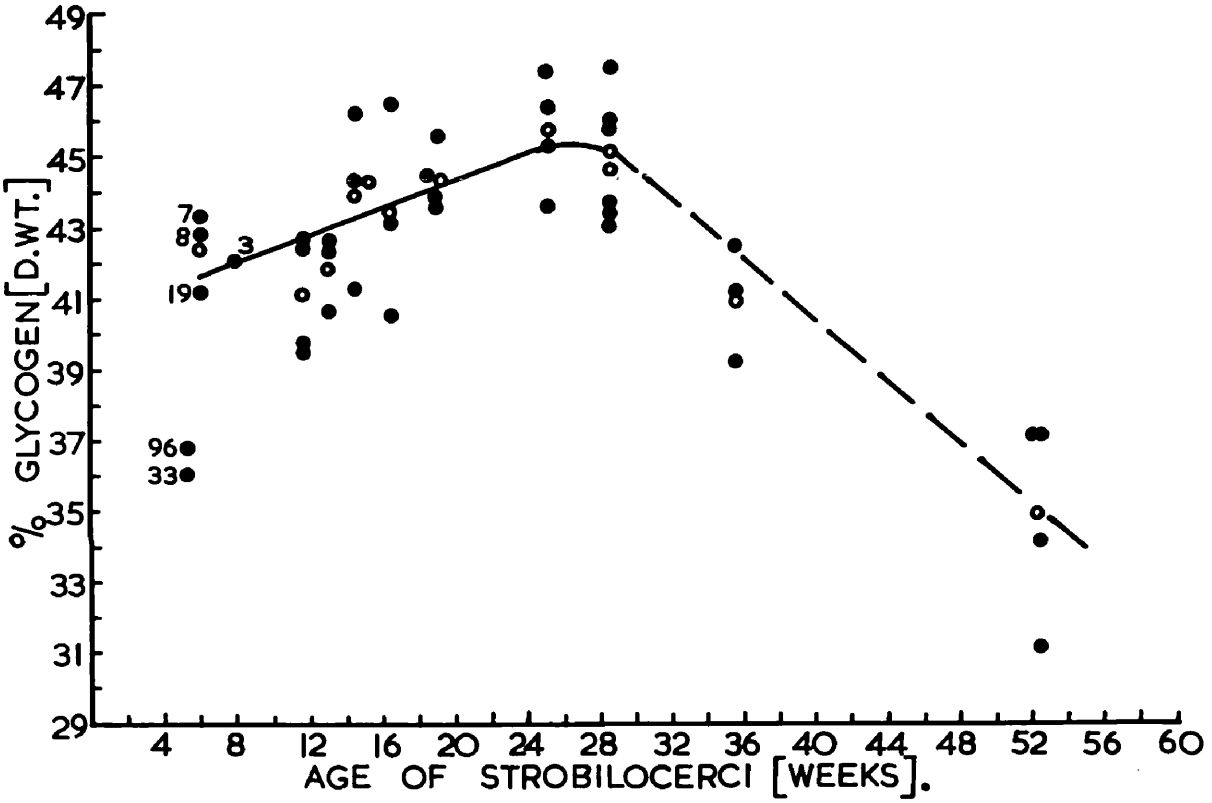

Figure 21. The glycogen content of larval H.taeniaeformis removed from the livers of Strain A mice is expressed as a percentage of the dry weight.

● = Estimations performed on single worms. Where the smaller worms have been grouped for estimation, the numbers in each group is represented by an index to one side of a solid point.

○ = The mean glycogen content of worms estimated.

(See also Table 11).

TABLE 11

Estimation Number	Age of Larval Cestodes in weeks	Numbers Estimated	Mean Glycogen Content (% D.Wt.)
330 A	5	96 T (1)	36.84
330 B	5	33 T (1)	36.08
331	6	34 T (3)	42.43
322	8	3 T (1)	42.09
316	11.6	4	41.12
317	13	3	41.84
318	14.4	3	43.96
28	15.3	15	43.56
319	16.4	3	43.42
320	19	4	44.33
35	25.3	3	45.73
32	28.6	3	44.61
33	28.6	3	45.16
323	35.6	3	40.96
321	52.4	4	34.88

Table 11. The glycogen content of larval H.taeniaeformis removed from the livers of Strain A mice. T (Column 3) indicates that the worms being too small for individual estimations, have been estimated in a group. Where larvae were grouped the number of separate estimations is shown in parenthesis.

the 5th week which, when compared with subsequent results, indicate that glycogen is not being so extensively stored during the early rapid growth phase.

Between the 5th and 6th weeks, the glycogen content increases rapidly from 36% to 42% but thereafter the rate of increase is very much slower. Needham (1950) has shown that sudden changes of this nature in the metabolic rates of animals are rare and occur at times of major body change e.g. insect metamorphosis. In Section 3 it was shown that a considerable morphological change takes place in the larval worm between the 40th and the 42nd day. It is suggested that the change in glycogen metabolism is closely associated with this change in morphogenesis.

The gradual increase in the glycogen content of the larva which commences at the 6th week and terminates at the 26th, is in accordance with Needham's findings, namely, that changes in the percentage composition of animals come about slowly and at a constant rate. During this period, it can be seen from Figure 21 that the glycogen is increasing more rapidly than the dry weight, or, in other words, the glycogen is increasing

tachyauxetically in relation to the dry weight. In this respect the glycogen metabolism of the strobilocercus resembles that of embryonic vertebrates where the glycogen content also increases tachyauxetically.

After the 26th week, the results show that the glycogen level in the strobilocercus drops. The most likely explanation for this is that the worm is undergoing degenerative changes. The decrease in the glycogen can not be a result of metabolic activities, because, firstly, the strobilocercus is inactive in a homeostatically controlled environment, and secondly, growth has stopped (Section 3, Figure 9), and no morphological changes are taking place.

The suggestion that degenerative changes are taking place is supported by evidence presented in the water content section in which it was shown that abnormalities occur after the 26th week. Further support is given by the fact that the change from tachyauxesis to bradyauxesis does not fit in with the patterns of change in percentage composition normally observed in animals. Needham (1950) has shown that

such changes are either bradyauxetic or tachyauxetic during specific phases of development.

The only other estimations of the glycogen content of H. taeniaeformis larvae are those by Salisbury and Anderson (1939) on material obtained from albino rats.

The age of the larvae used (which were removed from rats not mice) was "8 months or more". (These larvae were provided by cancer research workers, Dunning and Curtis, and as most sarcomata appear on the 13th month after infection of rats with H. taeniaeformis, it is highly probable that the majority of larvae given to Salisbury & Anderson were approximately 13 months old).

The glycogen content of aqueous extracts of dried powdered larval material was found to be 28%. However, their technique measured only the water soluble or lyo-glycogen and did not take into account the insoluble or desmo fraction.

Using the same extraction technique on strobilocerci aged 11 months from mice, the writer found that the glycogen content of the aqueous extract

was 27.78%, while that of the residue was 9.4%. The total glycogen content of the strobilocerci, i.e. 37.18% is low because the larvae are in the degenerative phase. It is quite clear, however, that Salisbury & Anderson's figure of 28% for the total glycogen content of the larva is erroneous.

(2) Glycogen Gradient in the Strobilocercus.

Evidence for the existence of a glycogen gradient along the length of the strobilocercus of H. taeniaeformis is presented in Figures 22 and 23. Estimations were based on strobilocerci which had been obtained from a single mouse. Each worm was divided into an anterior and a posterior portion, and, by varying the position of the dividing cut, the amount of the total weight of the worm in the anterior or posterior was varied by approximately 10% (see Table 12).

In Figure 22, the glycogen content of the anterior portion is plotted against its dry weight expressed as a percentage of the dry weight of the complete strobilocercus. The results obtained for the posterior are plotted in a similar manner in Figure 23.

Figures 22 and 23 show the glycogen content of different portions of 13 strobilocerci from a single mouse.

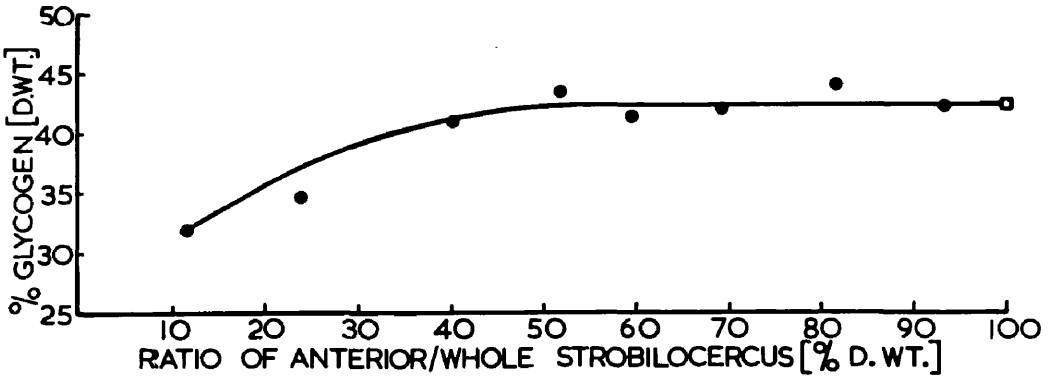

Figure 22. The glycogen content (% dry weight) of the anterior portion of each strobilocercus is plotted against its dry weight expressed as a percentage of the dry weight of the complete worm. (See also Table 12).

□ = The average glycogen content of 6 complete strobilocerci from same liver.

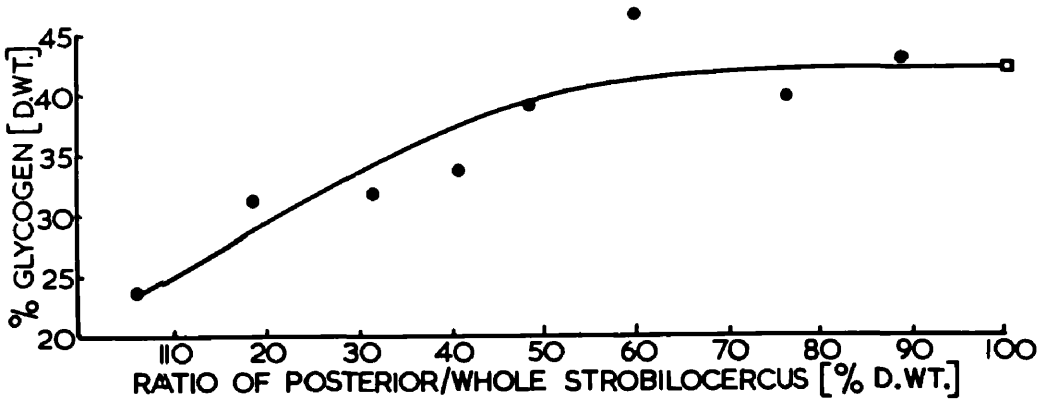

Figure 23. The glycogen content (% dry weight) of the posterior portion of larval H. taeniaeformis. The abscissa of each point was calculated as in Figure 22. (See also Table 12).

□ = as in Figure 22.

TABLE 12

No. of Strobs. Estimated	Total Dry Weight (mg)	ANTERIOR PORTION		POSTERIOR PORTION	
		% of Total Dry Wt.	% Glycogen	% of Total Dry Wt.	% Glycogen
4	138.9	93.53	42.36	6.47	23.8
2	64.1	81.59	44.19	18.41	31.26
2	80.4	69.65	42.13	30.35	31.8
1	38.7	59.68	41.61	40.32	33.7
1	40.7	51.84	43.7	48.16	39.04
1	40.3	40.19	41.13	59.81	46.76
1	43.1	23.89	34.66	76.11	39.89
1	44.7	11.63	32.21	88.37	43.27

Table 12. The glycogen content (% Dry Weight) of anterior and posterior portions of the strobilocerci of H. taeniaeformis.

When the weight of a portion of a strobilocercus was too small for estimation, several worms had to be grouped (Table 12 indicates where this has been done).

From Figures 22 and 23, it is apparent that the glycogen rises in the anterior 40% of the strobilocercus and falls in the posterior. The lower glycogen content of the posterior is not surprising since this region is shed on being ingested by the cat. The conclusion drawn from the results is that the portion of these larvae which later forms the adult (the anterior 60 - 80%) has a glycogen content which does not differ to any appreciable extent from that of the complete strobilocercus.

Although no previous quantitative estimations have been made on the distribution of glycogen in larval cyclophyllideans, differences in its regional distribution in adult Cyclophyllidea have been observed. Read (1956) has shown that the anterior quarter of mature Hymenolepis diminuta has less glycogen than any other quarter of the strobila.

Daugherty & Taylor (1956), by analysing 10 cm lengths of the mature worm, found that, with the exception of the scolex region, the glycogen content of the anterior and posterior strobila was lower than the centre portion.

(3) Glycogen Content of the Adult Worm.

Only strobilocerci between the age limits of 10 - 26 weeks were used to infect cats. The mean glycogen content of all worms removed from mouse liver and estimated during this period was 43.4%. (Calculated from Table 11). After 24 hours in the cat, the glycogen content was 42.4% (Table 13), suggesting that little change occurs during this period. By 72 hours, a fall equal to 14% of the original glycogen content has occurred. Between 6 and 42 days, the mean glycogen level is 34.5% which is equal to 79.5% of the larval content. However, the variation is considerable and it is, therefore, not possible to decide whether the 3 day result marks a transition from the high larval to the low adult level, or whether it is part of the normal fluctuation which adult worms show irrespective of age.

Reid (1942) observed similar variations in the amount of glycogen present in Raillietina cesticillus, and found that the fluctuation was diurnal, depending on the time of day at which the birds were fed. It is probable that variations in the glycogen content of H. taeniaeformis are caused similarly, being the result

TABLE 13

Cat No.	Age of Adults (Days)	No. of Cestodes in Intestine	Number Estimated	Complete (C) Powdered (P)	Mean Glycogen Content (% D.W.)
100	1	7	3	C	42.41
127	3	4	2	C	37.51
40	6	10	10	C	30.23
106	9	3	3	C	33.39
41	12	22	13	C	33.42
124	16	5	2	C	38.91
42	20	40	12	C	31.45
47	24	4	1	C	39.12
48	30	3	3	P	27.66
120	36	2	2	P	42.98
88	42	3	3	P	33.38

Table 13. The glycogen content of H.taeniaeformis recovered from the small intestine of cats.

of fluctuations in the amount of carbohydrates present in the cat's intestine.

In contrast to what was observed to happen to the water content of the worm, the loss of the pseudostrobila does not affect the % glycogen content. Nor was this to be expected, as the part of the strobilocercus which survives to form the adult worm has a closely similar glycogen content to that of the whole strobilocercus (Table 12 and Figure 22).

The main points of interest shown by these estimations is the absence of a fall in the glycogen content during the first day [which is confirmed by the absence of a rise in % nitrogen composition (Figure 27)] and the maintenance of an "adult level" approximately 25% lower than the "larval level". These two points, taken in conjunction, indicate that the fall in glycogen occurs as a result of a definite change in metabolic balance, and is not merely a result of starvation during the period of adaptation to the new host.

(4) The Glycogen Level and Position in Intestine.

In Figures 24 & 25 the glycogen content of H.taeniaeformis is plotted against the distance of the

worm from the pyloric sphincter, expressed as a percentage of the total gut length. This method of calculating the value of the abscissa was necessary because of the variation in the gut length of different cats.

A complete population of 10 worms was removed from Cat 40 on the 6th day after infection. The results (Figure 24) showed that there was little variation in the glycogen content of the 7 worms in the anterior 60% of the small intestine. The glycogen content of the worms posterior to this was significantly lower.

Results shown in Figure 25 were obtained from an extremely heavy infection of 40 cestodes which were recovered from Cat 42, 20 days after infection. The complete population of cestodes was not estimated. Individual worms, including the first and last, were selected at regular intervals along the infected region of the cat's small intestine. From the estimations, it was again apparent that the glycogen content of the majority of worms in the anterior two thirds of the small intestine was higher than that of the worms established

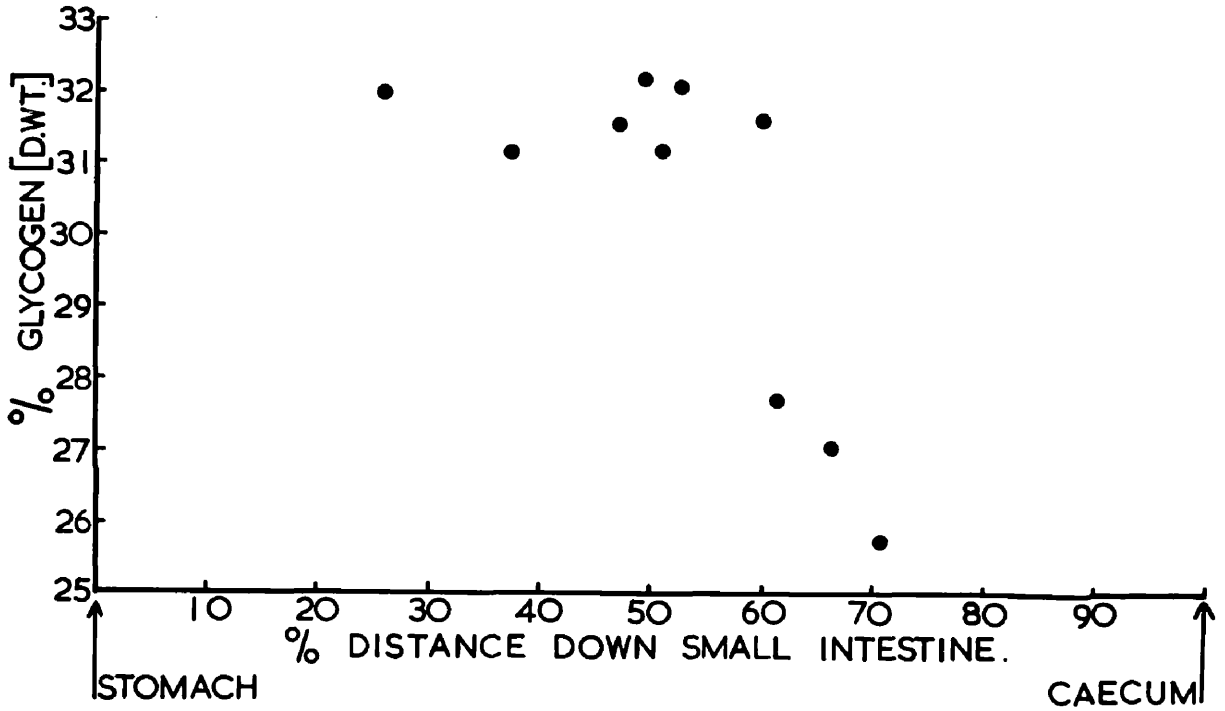

Figure 24. The glycogen content of a complete population 10 worms recovered from the small intestine of Cat 40. The abscissae of the points plotted were obtained by expressing the distance of each worm from the pylorus as a percentage of the total gut length of the cat. (See Table 14).

TABLE 14

% Distance down Small Intestine	% Glycogen (Dry weight)
25.7	31.94
37.1	31.16
47	31.54
49.1	32.18
50.9	31.24
52.6	32.06
60	31.62
61.1	27.71
66.3	27.09
70.85	25.79

Table 14. The glycogen content of a complete population of worms recovered from different positions within the intestine of Cat 40. Column 1 was obtained by expressing the distance of the cestode from the pyloric sphincter as a percentage of the total gut length.

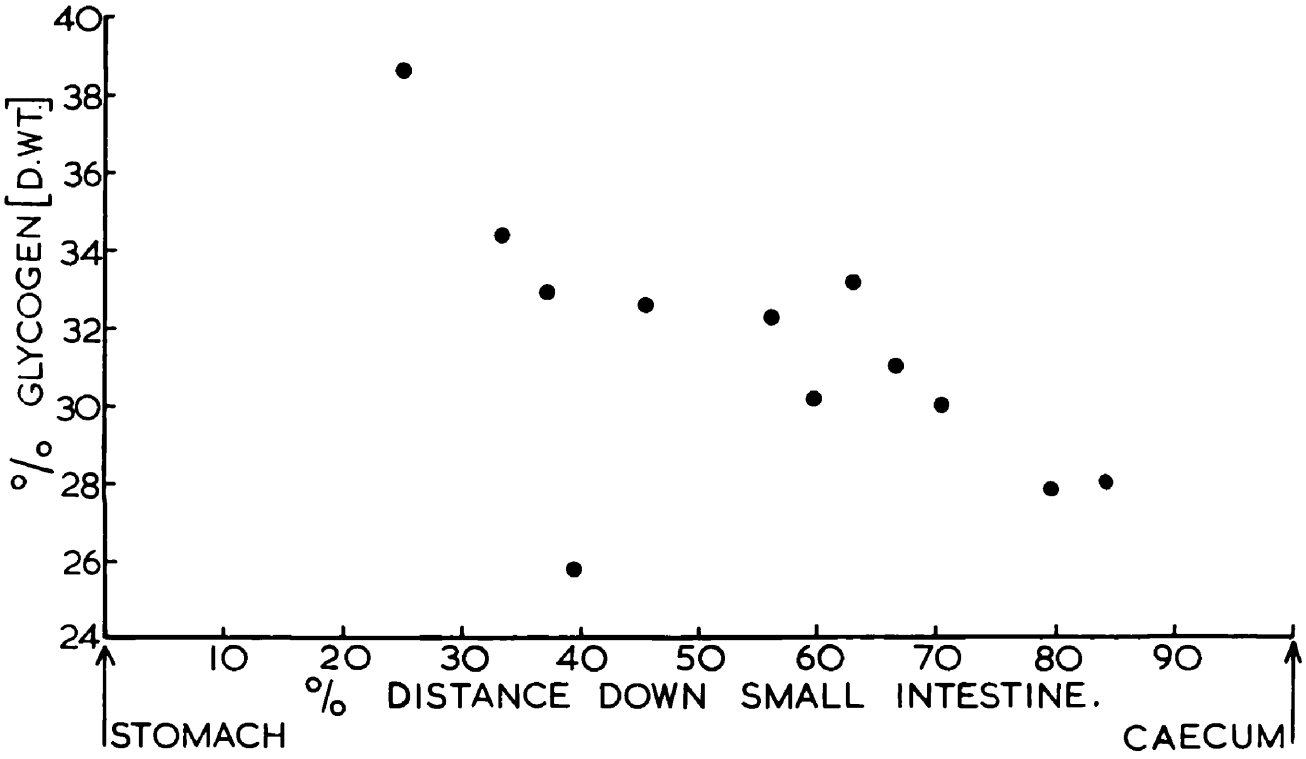

Figure 25. The glycogen content of 12 of the 40 H. taeniaeformis recovered from Cat 42. The abscissae of the points plotted were calculated as for Figure 24. (See Table 15).

TABLE 15

% Distance down Small Intestine	% Glycogen (Dry Weight)
25	38.68
33.3	34.42
37.1	32.93
39.4	25.81
45.4	32.61
56.1	32.31
59.8	30.21
62.9	33.26
66.6	31.03
70.4	30.09
79.5	27.96
84.1	28.16

Table 15. The glycogen content of 12 of the 40 H. taeniaeformis recovered from Cat 42. The position of each cestode in the small intestine (Column 1) is obtained by expressing its distance from the pyloric sphincter as a percentage of the total gut length.

posteriorly. These results confirmed earlier observations that the posterior third of the small intestine of the cat was least favourable for the normal metabolism of H. taeniaeformis (Section 3, Page 75).

As can be seen in Figure 24 and Table 14, the glycogen content of the cestodes in the anterior 60% of the small intestine of Cat 40 lies within the range 31.1 - 32.2%. The absence of extreme variation suggests that, in light infections, there is little competition for available carbohydrate food materials in the gut.

In the heavier infection of Cat 42, the variation in the glycogen content of worms from the same region of the small intestine is much greater (Figure 25 and Table 15). Moreover, in this latter case, the glycogen content of the worms decreases progressively from the anterior to posterior of the small intestine. This lower glycogen content in worms situated in the anterior region of the gut, but behind other worms, may be due to the combined effect of host and more anteriorly attached worms absorbing

most of the glucose. However, it is equally probable that it is due to worms over-lapping each other and so reducing the area for absorption.

Summary

After an initial increase from 36% - 42% between the 5th and 6th weeks, the glycogen content of the strobilocercus of Hydatigera taeniaeformis increases tachyauxetically with respect to the dry weight but after the 26th week, the increase becomes bradyauxetic. Since such a change is contrary to what happens in the majority of animals, it is suggested that larval degeneration is occurring. A comparison of the glycogen content of the strobilocercus obtained in the present series of experiments is made with that obtained by previous workers. A glycogen gradient is shown to exist in the strobilocercus. The percentage composition is higher in the middle of the worm than it is at the extremities.

The glycogen content of the adult worm removed from the cat was variable, but, in any one

infection, the glycogen level of worms in the posterior 40% of the small intestine was lower than those in the anterior regions. This is thought to indicate that conditions in the posterior third of the small intestine are unsuitable for the normal metabolism of H. taeniaeformis.

(3) NITROGEN METABOLISM

NITROGEN METABOLISM

In this subsection, the changes in total nitrogen composition during the larval and adult phases of Hydatigera taeniaeformis are described. This, together with the previous subsections on water and glycogen metabolism, is an attempt to correlate biochemical changes in the chemical composition of helminths with specific developmental changes.

Materials and Methods

Larval H. taeniaeformis were recovered from the livers of Strain A albino mice, immediately after killing. Small larvae, i.e. ones judged to be less than 2 mg fresh weight, were dissected from the liver, washed free of tissue debris in balanced saline and dried in batches of 10-50 on small squares of filter paper. The determination of their fresh weight (F.W.) was impractical, as even after the cyst is broken the presence of small quantities of cystic fluid greatly affects the dry/fresh weight ratio. Larvae judged to be in the range of 2 mg-10 mg F.W. were dried individually and sorted, after their dry weights had

been determined, into weight groups. Larvae over 10 mg F.W. were handled individually throughout the analysis. These larger larvae and the adults recovered from the intestine of laboratory infected cats were washed, blotted, weighed and dried as described previously.

Total nitrogen was determined by micro Kjeldahl using enough material to contain a minimum of 0.4 mg nitrogen and a maximum of 6.0 mg. With glycine, between these limits the method gave an accuracy of ±2%. Digestion of dried worm was done by boiling for 75 minutes, with 1.6 ml nitrogen-free H_2SO_4 using an anti-bump glass bead and a small bead of mercury as a catalyst. Normally the digest was colourless but even in those tubes in which slight darkening of the digest still remained, further digestion (up to 3 hours) did not increase the amount of ammonium sulphate formed. The digest, after cooling and diluting, was mixed in the distillation tube with 8 ml of 40% NaOH and 1 ml of saturated $Na_2S_2O_3$. Steam distillation was carried on for 2 minutes 45 seconds, for the first 90 seconds of which the tip of the condenser was kept below the 2% boric acid in which the ammonia was trapped. Back

titration to the original colour was done with the appropriate strength HCl, usually N/100. The indicator used was that recommended by Ma and Zuazaga (1942). In the calculation allowance has, of course, to be made for the small amount of alkali that comes over in addition to the ammonia.

As stated, small larvae were estimated in groups so as to obtain the required weight of material; the presence of a small square of filter paper in the digest did not interfere as tests showed it contained no detectable amount of nitrogen. Adult worms, the dry weights of which were often several hundred milligrams, were either divided by transverse cuts into sections of suitable weight and each section estimated, or the whole worm was powdered and 30-50 mg samples were estimated.

Details of the methods used to infect mice and cats, the age at which larvae become infective, adults mature etc. have been given in Section 3 (Growth).

Results

Part 1. Total Nitrogen Composition of Larval H.taeniaeformis.

Strobilocerci were removed from the livers of

mice in which infections ranged from 1-300 larvae (Table 16, column 3). Their fresh (wet) weight, dry weight and total nitrogen was determined. The purpose of the experiments described in this section was to determine whether the percentage nitrogen ($\%N_2$) in relation to dry weight followed a fixed pattern during development, or whether it fluctuated widely in different larvae.

a. Nitrogen level in sibling larvae. Variation in total nitrogen content was determined by estimating the nitrogen in not less than 7 larvae from each of 8 different infections (Expts. 15A and B, 12,300, 33, 301A, 304A and B, Table 16). The total number of larvae estimated was 84. The variation within each group of siblings is shown by the standard deviation (column 7). In all cases this was small, never exceeding 5% of the mean nitrogen content.

b. Nitrogen level in larvae of the same age in different hosts. Having established that the nitrogen level varied little in larvae of any one infection, the next point was to determine whether there was any significant variation between larvae of the same age

TABLE 16

The Nitrogen content, expressed as a percentage of dry weight, during the growth of H. taeniaeformis larvae.

Data.

Each experiment is based on larvae taken from the liver of a 'Strain A' mouse. Column 4 shows the number of worms estimated. Where larvae were grouped, the number of separate estimations is shown in brackets.

For an explanation of the correlation coefficient, (column 8), see text.

S = significant at 5% level.

H.S.= highly significant at 1% level.

N.S.= statistically not significant at 5% level.

TABLE 16

Expt. No.	Age of larvae (days)	No. of larvae in mouse	No. estimated	Mean dry wt. (mg)	% Nitrogen		Correlation coefficient
					Mean	σ	
306A	42	300	200(4)	0.87	7.14	0.20	-
B	42	11	8(1)	1.93	5.64	-	-
C	42	9	9(1)	0.87	6.04	-	-
D	42	4	4(1)	1.62	5.83	-	-
E	42	7	7(1)	2.5	5.63	-	-
307A	53	2	2	4.65	5.17	-	-
B	53	4	4	5.45	5.35	0.25	-
309	55	105	77(4)	1.19	5.99	0.44	-
312A	67	4	4	13.5	4.48	0.08	-
B	67	4	3	13.5	4.37	-	-
313	69	292	280(9)	3.7	5.04	0.29	-.984 H.S.
102	69	258	151(4)	3.0	4.97	0.14	-
15A	107	40+	8	13.2	4.25	0.16	-.061 N.S.
B	107	120	10	19.4	4.28	0.08	-.033 N.S.
12	139	-	17	11.6	4.21	0.18	-.499 S.
300	178	19	14	21.7	4.42	0.18	-.820 H.S.
33	200	20	9	34.5	4.06	0.14	-.283 N.S
302A	269	4	4	39.1	4.39	0.27	-
B	269	1	1	32.7	4.20	-	-
C	269	1	1	40.5	4.08	-	-
D	269	1	1	19.8	4.21	-	-
301A	360	7	7	42.7	4.40	0.22	-.900 H.S.
B	360	5	5	30.3	4.04	0.30	-
C	360	2	2	51.5	4.24	-	-
304A	423	9	8	31.2	4.35	0.19	-.708 S.
B	423	12	11	31.2	4.28	0.13	-.775 H.S.

developing in different mice. Larvae were examined from infections of six different ages. In Expts. 307A and B, 15A and B, and 304A and B, groups of larvae were compared from two mice. Statistical analysis of the data (t test) indicated in each case that there was no reason to suppose that the samples drawn from the two mice were not from a single homogeneous population.

In Expts. 306, 302 and 301 groups of mice were examined. The results (column 6) again show that the variation between larvae from different mice is small. The standard deviation calculated by grouping all the nitrogen estimations at a particular age is no greater than that expected from a similar number of larvae recovered from a single mouse.

Further evidence that the nitrogen level shows little variation between larvae developing in different mice will be discussed later when it is shown that between certain ages there is no change in percentage nitrogen composition and hence it becomes valid to compare the larvae from all the mice within this age limit. Even without this additional evidence, there seems little doubt that development as measured by total nitrogen content is similar in larvae of the

same age, whether they are in the same mouse or different mice.

c. Nitrogen level in larvae of different ages. The nitrogen contents of larvae aged 42-423 days are shown in Figure 26. Each group of estimations is of larvae from one mouse; where 4 or more larvae have been estimated the mean (open circle) is shown and the vertical line indicates the standard deviation. The graph shows that there is a fall in percentage nitrogen composition during the early developmental period, which ceases when a level of approximately 4.25% is reached. After this point, which occurs about the 65th day in light infections, the total nitrogen level remains remarkably steady.

The shape of the graph is confused during the early stages by the considerable difference shown in the nitrogen level of larvae developing in heavy and light infections. There is no doubt that in both cases the nitrogen level is falling but in heavy infections the stable level is not reached as quickly as it is in light infections. An interesting point about this graph is that the line showing the nitrogen content of the most advanced larvae, i.e. those in light infections,

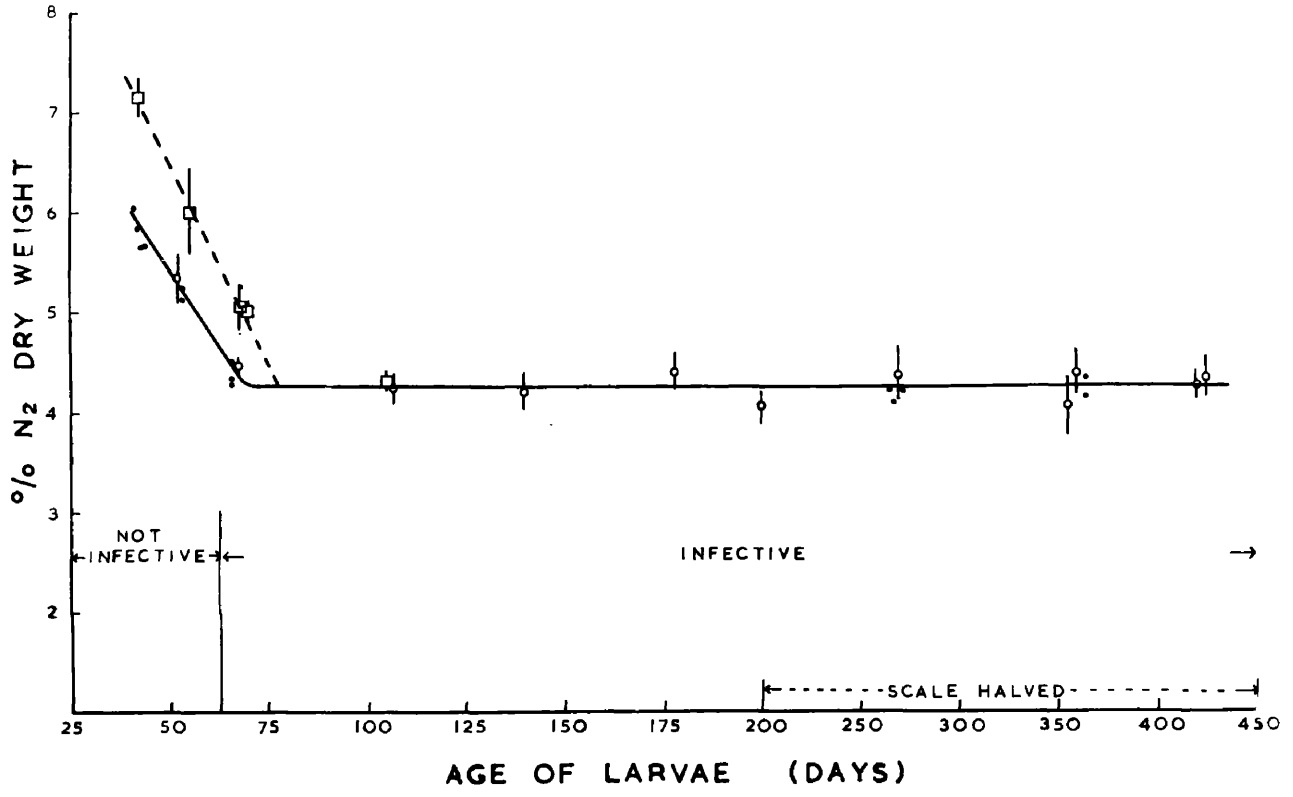

Figure 26. Shows the % N_2 in relation to dry weight of larval H. taeniaeformis from 42 - 423 days.

● = single estimation.

○ = mean of 4 or more estimations (larvae from light infection in mouse liver).

□ = mean of 4 or more estimations (larvae from heavy infection).

| = standard deviation.

For further details, see Table 16

cuts the upper limit (4.5% N_2) of the stable level about the 65th day. This is almost exactly the same as the earliest time (60 days) at which larvae have been found to be infective (Section 3 - Larval Growth).

d. Nitrogen level in larvae of the same age from heavy and light infections. Larvae from heavy and light infections were examined from 42, 53/55, 67/69 and 107 day infections (Table 16). In all except the 107 day infections, a clear difference existed, the nitrogen level was considerably higher in the larvae developing in the heavy infections. Extrapolating the line connecting the 42,55 and 67 day figures from heavy infections cuts the 4.25% level about the 80th day and hence after this age no significant difference between the nitrogen levels of larvae developing in heavy and light infections is to be expected. The 107 day results are in keeping with this interpretation.

It seems reasonable to associate the higher nitrogen level in larvae developing in heavy infections with the retardation which occurs in growth. Thus for instance, in Expt. 309 the mean dry weight of 55-day old larvae (from a 105 larvae infection) was only 1.2 mg. This is similar to the mean weight of the larvae

developing in two light (9 and 4 larvae) infections for 42 days (Expts. 306C and D), and as can be seen the nitrogen levels are nearly the same. A similarity also exists between the nitrogen levels of larvae developing in heavy infections for 69 days (Expts. 102 and 313) and larvae of the same weight in light infections. No direct comparison can be made between the total number of larvae in Expts. 102 and 313 as no light infections with larvae weighing 3-3.7 mg were estimated, but 71 (Expt. 313) and 15 (Expt. 102) of the larvae, which had been sorted into groups of approximately equal weight larvae, had an average weight of 5.2 mg. The nitrogen level of these larvae, which were estimated in 3 groups, was 4.9%, compared with 5.26% in larvae of the same mean weight developing in light infections (Expts. 307A and B). The fit is not perfect but close enough to suggest that the nitrogen level is correlated with the amount of growth that has occurred, i.e. to the <u>size</u> of the larva, not its <u>age</u>.

It should of course be realised that although heavy and light infections have been referred to and in Figure 26 two corresponding lines have been drawn, these represent boundary limits rather than specific and distinct pathways. Larvae developing in infections of intermediary

numbers will, presumably, give intermediary values for $\%N_2$. The level, however, should be predictable if the age and weight of the larvae are known.

e. Nitrogen level in relation to the weight of the larvae in an infection. In Part 1a it was shown that the variation in the nitrogen level of sibling larvae was quite small. It was noticed, however, that light larvae tended to have higher than average nitrogen contents and vice versa. This relationship of weight to nitrogen content is shown in all experiments in which 7 or more larvae were estimated individually by a correlation coefficient (Table 16 column 8). A negative correlation coefficient was always obtained, and, in 5 of the 8 experiments, a significant or highly significant relationship exists. In only two experiments (15A and B) was there a near random relationship. In both these experiments the larvae estimated were only a small proportion of the whole and were all in a narrow weight range. This relationship of a low nitrogen level in the largest larvae was apparent in nearly all the other experiments for which no correlation coefficients have been calculated, when the results were plotted on a graph.

f. Nitrogen level in the true larval strobila and pseudostrobila of the larva. H. taeniaeformis larvae evaginate about the 42nd day of development and thereafter a strobila is formed. This larval-formed strobila may reach at least 70 cm in length, but on entering the definitive host up to 70% is lost (Section 3 - Adult Growth). The larval body may be described as consisting of true larval strobila in the anterior and pseudostrobila in the posterior. It was apparent, therefore, that if comparisons were to be made between larvae and adults the proportion of the total nitrogen occurring in the true larval strobila as distinct from the whole larva must be determined. It is not possible to do this exactly as there is no morphological demarcation between true larval strobila and pseudostrobila, but In Expt. 8, worms were divided approximately into anterior and posterior halves. The % N_2 in anterior halves of 5 worms was 4.6, and of the posterior halves, 4.2. The mean of the total percentage agrees with estimations in which whole worms were analysed. In Expt. 312, worms were divided unevenly and it was found that although, as in Expt. 8, the anterior part usually contained more than the posterior portion, this was not the case if the division of the body was such that the anterior 75% was

compared with the posterior 25%. In these conditions the posterior part had an equal or higher % N_2 level.

From this data it is concluded that there is a gradual fall in nitrogen level down the anterior 60-80% of the larva and then a sharp rise in the posterior. This change in the nitrogen gradient in the posterior portion has not been further investigated as it does not affect the general conclusion that the part of the larva which later forms the adult (the anterior 40-60%) differs only slightly in % N_2 from the whole larva.

Part 2. Total Nitrogen in Adult H.taeniaeformis

a. General pattern. The nitrogen content of worms at various ages between 1 and 42 days is shown in Table 17 and Figure 27. A rapid rise occurs in percentage composition following establishment in the cat; this change probably occurs on the second day not the first, a point which will be referred to later. By the end of the 2nd day the nitrogen level has increased to between 6 and 7%, a level which appears to be characteristic of the adult worm, at least up to the end of the prepatent period (36th-42ND day).

b. Nitrogen level in relation to the size and number of worms in an infection. As was the case in

TABLE 17

The Nitrogen content, expressed as a percentage of dry weight, during the growth of H. taeniaeformis adult.

Data

Each experiment is based on worms taken from the small intestine of a cat.

The correlation coefficient (column 7) is the relationship between size of worm and % N_2 content. For further explanation, see text.

S., H.S., and N.S. as in Table 16.

TABLE 17

Expt. No.	Age of infection (days)	No. of worms in cat	No. estimated	Mean dry wt. (mg)	Mean % N_2	Correlation coefficient
101	1	3	3	26	4.43	-
102	1	4	4	28	4.68	-
38	2	6	3	7	6.45	-
35	3.5	19	17	7	6.50	-.699 H.S.
2	4	-	6	18	6.85	-.631 N.S.
32	4	8	6	28	6.15	-.841 S.
33	4	10	9	15	6.51	-.320 N.S.
45	6	3	2	42	6.54	-
27	8	17	4	35	6.39	-
41	12	25	8	19	6.46	-.528 N.S.
65	15	3	2	62	5.71	-
72	15	5	2	59	5.79	-
42	20	39	10	31	6.92	-.373 N.S.
73	21	6	4	88	5.85	-
110	21	4	2	136	5.83	-
112	25	3	1	145	5.98	-
86	27	4	2	232	6.65	-
75	27	7	2	136	6.15	-
29	36	17	7	146	6.85	-.755 S.
28	38	4	1	106	6.13	-
50	42	5	3	302	6.56	-

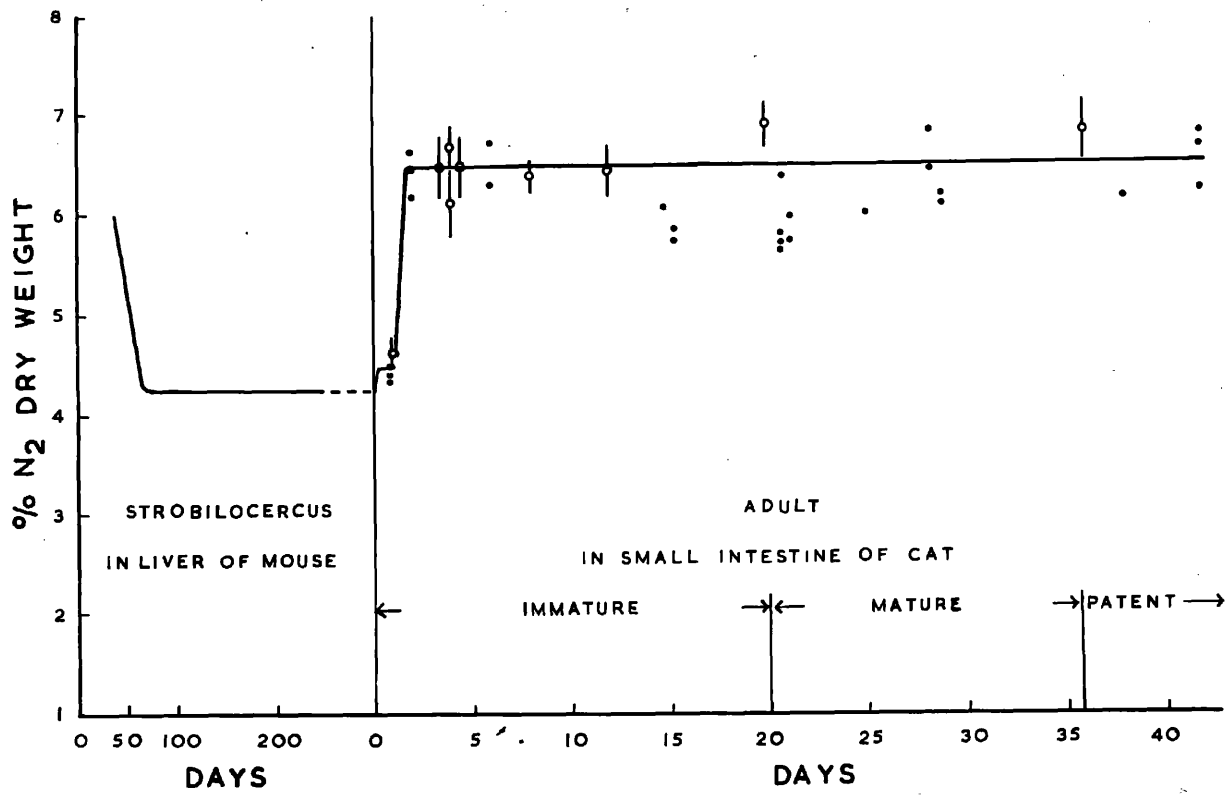

Figure 27. Shows the changes which occur in the % N_2 composition of H. taeniaeformis in the larval and adult stages.

● = single estimations

○ = mean of 4 or more estimations.

| = standard deviations.

For further details see Table.17.

larvae, the greater the amount of growth that had occurred, the lower the % N_2 level. Thus, in all the infections in which 6 or more worms occurred, a negative correlation coefficient (Table 17) was obtained when the % N_2 was correlated with dry weight. Although only three of the results are significant at the 5% level, two others are nearly significant, and taken as a whole the seven figures suggest a relationship, though not necessarily a direct one.

The effect of heavy worm burden was not investigated as it was desired to measure optimum growth rates. In only five of the cats was the worm burden more than 12 worms. Of these five, worms in three cats killed after $3\frac{1}{2}$, 8 and 12 days respectively showed no difference in rate of growth or nitrogen level to normal, but in the other two (Expts. 42 and 29, Table 17) growth had been retarded and as always in these circumstances a higher than average nitrogen content existed.

Discussion

The only previous work on nitrogen in H. taeniaeformis is that of Salisbury and Anderson

(1939), who found that the nitrogen level in (infective) larvae was 4.95% of the dry weight. This figure is higher than that from Strain A mice, but the results are not strictly comparable, as the larvae used by Salisbury and Anderson were taken from rats not mice. In order to check on this possible source of variation, attempts were made to infect four strains of rats but all were highly resistant, and only occasional infections were obtained. As most of the larvae to reach the liver were dying at a very early stage (cf. Campbell (1936) late immunity), the conditions for growth were obviously unsuitable, and hence the few nitrogen estimations carried out are of dubious value. The result obtained (mean of six estimations was 4.6% N_2) is in fact about midway between Salisbury and Anderson's figures and the results from 'Strain A' mice. It seems probable, therefore, that the nitrogen level is affected not only by the factors described in this paper but also by the species and even strain of host. This is not surprising in view of the fact that conditions in the livers of rats and mice differ so greatly as to give total immunity in one strain and no immunity in another.

No estimations on the adult <u>H. taeniaeformis</u>

have been carried out previously but several adult cyclophyllidean cestodes have been estimated. The results, as shown in summary (v. Brand, 1952) are all lower than H. taeniaeformis, but examination of Reid's (1942) paper on Raillietina cesticillus (he is the only worker to have shown the range of diurnal scatter) shows that the mean nitrogen level varied from 5.83% to 7.87%, depending on when the host was last fed. This range almost exactly corresponds to the limits observed in H. taeniaeformis, although the means (Figure 27) are, of course, less than this. The lower scatter shown by H. taeniaeformis is in keeping with the slightly different experimental conditions. Reid's figures are means calculated from worms recovered from birds at the highest point of a 24 hour feeding cycle and after 16-20 hours starvation, whereas our figures were calculated from worms recovered from cats which had never been starved for more than 14 hours, but also were, probably, rarely killed when the glycogen level was at its peak in the worms.

This illustrates well the importance of correlating analytical figures with a particular stage and time in the helminth and host's life. Comparisons

of analytical data which are divorced from such an association are likely to give the impression that great variability in chemical composition exists, whereas, in fact, the contrary may be the case.

The much wider fluctuations which occur in the nitrogen level of adults than of larvae are to be expected, as the gut environment is much less stable than the liver. Although the total amount of protein, or other nitrogen-containing substances in the worm, is not likely to vary much during the normal period when there is no food in the gut, the glycogen content varies considerably (Table 13), and this will cause a reciprocal rise or fall in the percentage nitrogen content.

The principle object of this investigation, however, was to determine whether a "normal" nitrogen level existed at a particular age or size, and if so, whether there were any changes which could be correlated with developmental phases. The results (Figs. 26 and 27) show clearly that this is the case, particularly in the larval stage.

During early development of the larval stage in the liver of a mouse, the nitrogen level falls steadily until about the 65th day. At this point a

change occurs in the relative rates of metabolism; nitrogenous products, which previously were accumulating in a bradyauxetic relationship to dry weight, increase isauxetically. Examination of H. taeniaeformis before and after this period show no morphological differences. The evertion of the scolex and start of strobilization (which occurs in the larval host in H. taeniaeformis) might be expected to mark a change to a new developmental pathway, but this occurs about the 42nd day (Section 3 - Larval Growth).

Physiologically, however, a major change does occur; the larvae become infective about the 60th day. It seems reasonable to assume therefore that the two, infectivity and nitrogen level, are linked. If this assumption is made it becomes interesting to speculate on the way in which the nitrogen level can be associated with infectivity.

It is improbable that any direct relationship exists; rather it must be that the fall in nitrogen occurs at the same time as some other change is taking place. At least two possibilities exist. Firstly,

the common factor may be size. The nitrogen level, as was discussed earlier (Part 1 d) is related to the weight of the larva, not its age, and therefore if infectivity is reached when a certain size is attained, this will also be associated with a specific nitrogen level. The difficulty about this interpretation is that it offers no explanation, other than a coincidence, as to why the critical mass at which the larva becomes infective is also the one at which the synthesis of nitrogenous substances alters.

A second possibility is that the falling nitrogen level is merely the reciprocal of an important metabolite. The most likely one is glycogen since it is known to occur in cestodes in large quantities and hence variations in its percentage composition would be sufficient to account for the considerable changes in percentage nitrogenous matter. Unfortunately, only one series of glycogen estimations was made of larvae at this age (Figure 21) and this indicates that the glycogen rise occurs during the 6th week, i.e. before the fall in nitrogen level. However, without more estimations, it is impossible to be certain whether the

changes in these two major chemical constituents are reciprocal effects or are independent.

So far only the change which occurs at the time when the larva becomes infective has been considered. Reference to Figure 27 shows that a change in level occurs when the larva enters the definitive host, but this change is of a different nature to that which occurred in the larva. Nitrogenous substances increase isauxetically in the infective larva and, within the accuracy of the measurements, they continue to do so in the adult. The rise which occurs in the first 48 hours in the cat is not due to synthesis alone, but also probably to a fall in glycogen. If, after a few days, the nitrogen content returned to the larval level, the change would be of little significance or value as a criterion for cultivation, as it would merely indicate a period of starvation during the switch from a liver habitat to attachment in a suitable region of the intestine [cf. migration of Diphyllobothrium in the small intestine during the first 2 days of adult life (Archer and Hopkins, 1958a)] . The fact that the nitrogen level rises and remains high, around 6.5%,

suggests that the fall in glycogen is not due to utilization of reserves during a starvation period, but rather a change in metabolic balance in the worm. This is important as it means that here is a possible criterion for judging normality of development in vitro. In culture experiments carried out no such change in nitrogen level occurred if glucose was provided in the medium, and the worms, although remaining alive for several weeks, showed no signs of adult development or growth.

It is concluded, therefore, that in the in vitro experiments the conditions were such as to maintain larval life, but not to cause the enzymatic change which is the forerunner to the rapid growth phase and maturation which occurs when the larva enters the cat's intestine. Another indication that the rise in nitrogen is due to a change in metabolic balance and not to glycogen utilization during a period of starvation, such as occurs in Diphyllobothrium sp. (Archer and Hopkins, 1958 b), is that little rise occurs on the first day and a large increase on the second. This, if the change is due to enzymatic adaptation, is easily explainable, but if due

to starvation the reverse might be expected.

The desirability of further investigation into the metabolic changes which occur in the first 48 hours in the cat is clearly indicated. Such an investigation might well reveal information which would help to explain host specificity and also serve as a guide for *in vitro* studies. The importance of changes during this period on host specificity is shown by the observation that *H. taeniaeformis* larvae, when given by stomach tube to rats, become established and survive well for 24 hours, but are lost on the 2nd day (Adult Growth - 4).

SUMMARY

1. The % N_2 composition of larval and adult Hydatigera (Taenia) taeniaeformis has been determined at frequent intervals during development.

2. In larvae of the same age the % N_2 composition was closely similar, whether they had developed in the same mouse or different mice. The only exceptions were larvae from heavy infections of over 100 worms; in these the % N_2 was considerably higher during the non-infective period.

3. During growth of the larva the nitrogen level falls from above 6% (dry weight) at 42 days to less than 4.5% at 67 days. After this age the nitrogen level remains constant at 4.25% $\pm$ 0.25. The change from a bradyauxetic to an isauxetic rate of nitrogen increase in relation to dry weight occurs at the same time as the larva becomes infective.

4. Estimation of the % N_2 level in adult worms also showed a specific pattern exists. During the first 2 days in the cat the nitrogen level rises rapidly to 6.5% ± 0.7, and thereafter remains within these limits throughout the 36 day pre-patent period.

5. The following aspects are discussed:

a. the probability that variation in % N_2 level is an apparent rather than a real change, resulting from absolute changes in the glycogen content.

b. the causal relationship between nitrogen level and infectivity of the larvae.

c. the greater fluctuation of % N_2 composition in adults than in larvae reflects the greater variability of the gut environment compared with that of the liver.

d. the value of the nitrogen level as a criterion for judging normality of <u>in vitro</u> development.

SECTION 5

CONCLUSION & APPLICATIONS

CONCLUSION AND APPLICATIONS

1. Development.

2. Correlation of stage in development with change in chemical composition.

   (i) Larval Development

      (a) Changes observed between the 6th and 26th week.

      (b) Changes subsequent to the 26th week.

   (ii) Adult Development

      (a) Changes resulting from loss of pseudostrobila.

      (b) Initial changes occurring due to new environment.

3. Applications.

1. Development

Larval Hydatigera taeniaeformis, unlike Taenia spp. is not infective at the invaginated cysticercus stage but requires to grow and develop into a strobilocercus before reaching infectivity. However, Wilmoth (1945) was in error when he stated that the larval worm required 7 months in the rodent's liver before becoming infective. It has been shown in the present work that a developmental period of 60 days in mouse liver is sufficient for larvae to reach infectivity.

Growth of larval H. taeniaeformis in the mouse liver was rapid during the first 6 weeks; the larvae, in both light and heavy infections, increased their dry weight approximately 1 million times. In light infections, growth started to slow after the 6th week and larvae reached their maximum size by the 22nd week. In heavy infections, growth was not so rapid and the maximum size was not reached until approximately the 26th week.

The infective larval worm consisted of two distinct portions, firstly, an anterior portion or true larval strobila which survived digestion in the cat's intestine, and secondly, a posterior portion or

pseudostrobila which was digested. This observation that an anterior strobilated portion survived [as distinct from the scolex region only (Leuckart, 1878)] confirmed the earlier work of Miller (1932) and Joyeux & Baer (1938). Further investigations showed that the percentage of the fresh weight of the original strobilocercus which survived to commence growth in the small intestine of the cat, decreased as the strobilocercus grew. Consequently, the amount of true larval strobila present in a strobilocercus depends on the fresh weight of the larval worm.

The true larval strobila usually established itself in the middle third of the small intestine and growth commenced immediately, in contrast to what was observed in Diphyllobothrium sp. (Archer & Hopkins, 1958a). The first phase of exponential growth had a doubling time of 8 days and terminated at the start of egg production between the 16th and 18th day. There followed a period during which growth slowed and then a second phase of exponential growth, having a doubling time of 16 days, commenced on the 21st day after infection of the cat. Shed proglottids appeared in the faeces between the 36th and 42nd day. Ova in the terminal proglottids were fully embryonated by the 33rd day.

2. Correlation of Stage in Development with Change in Chemical Composition.

Attempts were made to correlate stages in the development of *H. taeniaeformis* with specific changes which occurred in the chemical composition.

(i) Larval Development.

(a) Changes observed between the 6th and 26th week.

Up to the 42nd day, there was evidence of a rapid increase in the percentage glycogen content. From the 42nd day to the 26th week, the glycogen level increased more slowly. This change in the rate of glycogen storage occurred at the same time as evagination and it seems possible that they are related.

Between the 42nd and 67th day, the increase in nitrogen was slower than the increase in dry weight, i.e. there was a decrease in the percentage nitrogen level from above 6% of the dry weight to 4.25%. About the 67th day a change occurred in the rate of synthesis of nitrogenous compounds, which resulted in the total nitrogen increasing, thereafter, at the same rate as the dry weight, and hence, the percentage nitrogen

remained constant (at 4.25%) for the remainder of larval life. Examination of the larval worm before and after this change in rate of nitrogen increase revealed no morphological differences. However, a major physiological change occurred, in that larvae became infective about the 60th day. There can be little doubt that these two changes, rate of synthesis of nitrogenous compounds, and infectivity, are correlated, though not necessarily directly.

(b) Changes subsequent to the 26th week

The only other changes in percentage chemical composition observed during larval life occurred about the 26th week. The percentage glycogen decreased, and the water content, which had remained constant since the 8th week, started to increase. Apart from the fact that larval growth stopped about the 26th week, no other change in morphology or physiology occurred which could be associated with these changes in chemical composition. It is suggested that the changes were the result of degenerative processes which commenced after the cessation of growth.

(ii) Adult Development

As it was intended to use the results of this work as a guide to *in vitro* studies, in which it was hoped to start with infective larvae and induce development into a mature worm, particular attention was paid to the changes which occur when the larval worm enters the cat's intestine.

(a) Changes resulting from loss of pseudostrobila.

The pseudostrobila is shed in the cat within 24 hours and it had to be determined whether this loss affected the percentage chemical composition of the worm. Estimations of the distribution of water, glycogen and nitrogen along the length of the strobilocercus showed that the removal of the posterior portion of the larval worm corresponding to the pseudostrobila, resulted in only slight changes in the percentage glycogen and percentage nitrogen. Thus, any changes occurring in these two constituents after ingestion of the larva by the cat could be attributed solely to environmental conditions within the intestine. This was not the case with the water content since its value was lowered by

the removal of the posterior portion of the larval worm. Consequently, any change observed in the water content of the worm after 24 hours in the intestine of the cat would be the result of the loss of pseudostrobila plus environmental conditions.

(b) Initial changes occurring due to new environment.

The water content of worms recovered from the intestine of cats after 24 hours was 68% of the fresh weight, and that observed in the anterior of the larval worm was 67%. Thus, little change occurred initially but a slow increase in the water content followed during maturation. Stability, at a level of 79% was reached about the 32nd day.

On the first day after infection of the cat, the nitrogen and glycogen levels were 4.5% and 42.4% of the dry weight respectively. Since the larval nitrogen level was 4.25% and the mean glycogen content of larval worms used for infection (age range = 10 - 26 weeks) was 43.4%, it is obvious that little change had occurred during the first 24 hours.

The considerable fluctuations in the glycogen content, which occur in worms during development in the cat's intestine, make it impossible to be certain when the mean adult level of 34.5% is reached. Thus, the glycogen level can not be used to indicate when metabolism changes from a larval to an adult course. This limits its employment as a criterion for *in vitro* studies although an abnormally low level in a cultured worm would indicate that conditions in the medium had been unfavourable. However, the nitrogen level, which increases from 4.25% to 6.5%, is a very distinct and clearly demarcated change which always occurs shortly after the worm enters the cat's intestine. This rise in percentage nitrogen is, *in part*, the result of a fall in glycogen content, but it is also evident that, *in addition*, a change in total nitrogen from a larval to an adult level is occurring. A fall in glycogen from 43.4% (mean larval content) to 34.5% (mean adult content) would only account for a reciprocal rise in nitrogen from 4.25% (larval level) to 4.66%. A rise to a level of 6.5% was actually observed and this can only be accounted for, if a rapid synthesis of nitrogenous material has taken place. No comparable change occurred during the subsequent prepatent period; the nitrogen content remained relatively constant at 6.5% ± 0.7.

3. Applications

The main object of this thesis was to investigate fully the life cycle of Hydatigera taeniaeformis in terms of growth, development and chemical composition. Having outlined the conclusions reached, consideration is now given to the ways in which the knowledge gained can be applied to the cultivation of this worm in vitro. As was explained in the "Introduction", the larva is the most suitable stage for the commencement of cultivation even though the aim is to obtain adult worms. It is apparent from the results reported here that larvae used for such experiments must be at least 60 days old as this is the minimum age at which larvae can undergo maturation in the definitive host. This applies only to larvae from lightly infected mice because growth in the heavy infections is retarded and the larvae take longer to reach the infective size which is about 20 mg (fresh weight). Thus, the suitability of larvae for cultivation should be based on their fresh weight rather than their age.

As the pseudostrobila is shed in the cat during the first 24 hours and hence, forms no part of the adult worm, it is probably desirable that this region should

be removed prior to cultivation. The exact demarcation between true larval strobila and pseudostrobila can not be detected by external inspection [though Joyeux and Baer (1938) claim that internal histological differences exist] . Exact division does not appear to be essential as Smyth (1958) has cultured successfully 1 - 2 mm fragments of larval Diphyllobothrium dendriticum.

Comparison of the chemical composition of worms matured in vivo with those cultured in vitro is one of the established techniques for judging the success of cultivation experiments. This biochemical approach was first adopted by Hopkins (1950, 1952) who used the percentage glycogen composition of Schistocephalus solidus, matured in pigeons, as a standard with which to compare the results obtained from worms cultured in vitro.

The percentage water, glycogen and nitrogen content in H. taeniaeformis has been determined throughout larval and adult development and it only remains to decide which of these are of value as standards with which to compare in vitro results. The true larval

strobila of the strobilocercus had the same water content as the worm which was recovered after 1 day in the cat and the subsequent increase occurred slowly. Due to the absence of a sharp change, the water content was considered of little value in indicating the change from larval to adult metabolism. However, the constancy of the water content at any specific age, in both larvae and adults, makes it a useful indicator for detecting degenerative changes in cultured worms. The suitability of the percentage glycogen level as a criterion has already been discussed on page 162.

As already mentioned, the most characteristic change which occurs in chemical composition after the larval worm enters the definitive host is the 50% increase in percentage nitrogen content. This change occurs within 2 - 3 days. Presumably, if *in vitro* conditions are accurately reproducing the *in vivo* conditions, then a similar change should occur in cultured worms. This criterion has the advantage that it refers to a large and easily measured change which should occur quickly and, therefore, the

suitability of media for promoting adult development can be determined within the space of 2 - 3 days.

The use of chemical composition as a criterion further restricts the age of larvae which can be used for experimental purposes because degenerative changes occur after the 26th week.

The rate of development is one of the factors which must be considered when selecting a species for *in vitro* cultivation. Rapidly developing species seem to be the most satisfactory because the suitability of different media is quickly apparent. All the cestodes employed for *in vitro* cultivation to date develop rapidly, e.g. *Schistocephalus solidus*, *Ligula intestinalis* and *Diphyllobothrium dendriticum* produce eggs in 36 hours, 60 hours and 7 days respectively. The section, in this thesis, on growth and development shows that *H. taeniaeformis* compares most unfavourably with these pseudophyllidean tapeworms. Ova which are produced between the 16th and 18th day, are not fully embryonated until the 33rd day, and, by this time, the worm has reached a length of several feet. Apart from the major problem of providing a chemically and physically suitable medium,

the provision of the necessary volume required for the successful cultivation of a complete worm of this size would be a project of considerable magnitude. The successful growth to maturity of a complete H. taeniaeformis adult must be regarded as a problem which is unlikely to be solved for several years. However, one of the first steps in the solution of this problem of growing H. taeniaeformis in vitro is the determination of the factors which cause the change from larval to adult metabolism. Age and size of larvae capable of maturing have been determined and criteria suggested which should indicate whether such a change is occurring.

Finally, it may be pointed out that, although the slow rate of development of H. taeniaeformis is a disadvantage in cultivation from the point of view that cultures must be kept for several weeks, there are certain compensatory advantages. The rate of growth, i.e. increase in mass, of H. taeniaeformis is much slower than that of Diphyllobothrium sp., and, therefore, the amount of metabolites needed in a day is much less. Thus, it is possible that the early stages of the adult development of H. taeniaeformis may, in fact, be less exacting to grow in culture than some pseudophyllideans.

ACKNOWLEDGMENTS

The experimental work described in this thesis was performed in the Department of Zoology, Glasgow University and I am grateful to Professor C. M. Yonge for the many facilities which were placed at my disposal. I am also extremely grateful to Dr. C. A. Hopkins whose advice and criticism at all stages were invaluable.

My thanks are also due to Professor J. P. Todd of the School of Pharmacy, Royal College of Science & Technology, Glasgow, for encouragement and for providing animal house facilities and other opportunities to work on this project. I am also indebted to all members of the staff of the Department of Biology, R.C.S.T. In particular, I would like to thank Dr. Blodwen Lloyd and Miss M. S. Provan for many courtesies extended to me.

In experimental parasitology, sources of materials sometimes cause serious problems. Such crises rarely arose during the course of the present work, largely due to the co-operation of Arthur Morel & Co.

The Director of the Imperial Cancer Research Fund, London, provided the original breeding pairs of Strain A mice. Dr. P. H. Silverman supplied the

hatching fluid for the H. taeniaeformis ova. Mrs. Jean Lindsay typed the manuscript. I gratefully acknowledge the help received.

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