TAENIA CRASSICEPS : STRUCTURE, DEVELOPMENTAL BIOLOGY, AND HOST-PARASITE RELATIONSHIP

by

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ABSTRACT

The structure and developmental biology of Taenia crassiceps is described for the following stages : the egg, the cysticercus, and the adult. The techniques used included light microscopy, histochemistry and scanning and transmission electron microscopy. Emphasis was placed on some important features of the parasite that are especially adapted to its mode of life, i.e. the scolex and tegument, which comprise the parasite component of the host-parasite interface, and the reproductive system, including the egg.

Attempts were made to obtain an efficient in vitro culture system in order to study the developmental biology of the parasite under axenic conditions. Various combinations of culture vessels, culture media, media supplements, pretreatments, and agitation were used to determine the optimal conditions for development. Cysticerci developed in vitro up to the stage of early genital primordia formation. A 17-day adult worm from an artificially infected dog was cultured for another 14 days after which fully formed eggs were obtained.

The host-parasite relationship of adults in dogs and metacestodes in mice with regards to the pathogenesis and kinetics of infection was investigated. Although metacestodes reproduced rapidly in mice, the host showed remarkable tolerance to the resulting parasite burden, and the only pathogenic changes were chronic peritonitis, slight hepatic necrosis and amyloidosis, and slight pulmonary emphysema.

Some preliminary studies established an immunological response to an intraperitoneal challenge infection of metacestodes following a primary subcutaneous inoculation of parasite homogenate. This response appeared to be biphasic in nature, with an initial humoral

mediated attack which caused tegumental damage, followed by a cellular mediated one which resulted in cellular adherance and, subsequently, encapsulation of the parasite.

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CONTENTS

ABSTRACT 2

ACKNOWLEDGEMENTS 4

CONTENTS 6

LIST OF FIGURES 10

LIST OF TABLES 10

LIST OF PLATES 10

ABBREVIATIONS 11

GENERAL INTRODUCTION 13

Distribution and Hosts 13

Life-cycle 15

Terminology 15

Strains 17

T. crassiceps as a Laboratory Model 18

Scope of this Study 19

GENERAL METHODS 22

Routine Maintenance of *T. crassiceps* Metacestodes 22

General Procedures in Sterile (Aseptic) Work 23

SECTION I. GENERAL STRUCTURE AND DEVELOPMENT OF TAENIA CRASSICEPS

A. INTRODUCTION 24

1. The Importance of Structural Studies in Parasitology 24

2. Review of the Literature on the Structure and Development of *Taenia crassiceps* 28

B. MATERIALS AND METHODS 30

1. Source of Parasite 30

2. Morphology and Histology 31

3. Histochemistry 33

(i) Rostellar Hooks and Eggs 33

(ii) Nervous System 33

(iii) Calcareous Corpuscles 34

(iv) Others 34

4. Ultrastructure 35

(i) Scanning Electron Microscopy 35

(ii) Transmission Electron Microscopy 36

C. RESULTS 38

1. The Egg 38

2. The Cysticercus 43

3. The Adult 59

D. DISCUSSION 130

E. SUMMARY 155

SECTION II. IN VITRO CULTIVATION OF TAENIA CRASSICEPS

A. INTRODUCTION 159

1. Review of Literature on Cultivation of Cestodes 159

2. The in vitro Cultivation of Taenia crassiceps 161

B. MATERIALS AND METHODS 162

1. Source of Parasite 162

2. Physical Conditions of Culture 163

3. Culture Media 164

4. Standard Procedure for in vitro Culture 167

5. Assessment of Worm Development 169

6. In vitro Cultivation of the Oncosphere and Adult 169

C. RESULTS 170

1. Hatching and Activation of Oncospheres 170

2. *In vitro* Cultivation of Cysticerci 171

3. The Ultrastructure of *in vitro* Cultured Cysticerci 186

4. The Ultrastructure of *in vitro* Cultured Adult Worms 190

D. DISCUSSION 217

E. SUMMARY 226

SECTION III. PATHOGENESIS AND KINETICS OF *TAENIA CRASSICEPS* INFECTION

A. INTRODUCTION 229

1. Review of Literature on Pathogenicity of Taeniid Infection 229

2. Review of Literature on Pathogenicity of *Taenia crassiceps* 235

B. MATERIALS AND METHODS 239

1. Maintenance of Parasite and Infection Regime 239

2. Assessment of Parasite Burden 239

3. Pathological Examination 240

C. RESULTS 240

1. Larval Stages 240

(i) Oncospheres 240

(ii) Metacestodes 241

(iii) Pathology 243

2. Adult Worms 246

D. DISCUSSION 253

E. SUMMARY 260

SECTION IV. PRELIMINARY STUDIES ON THE IMMUNOLOGY OF *TAENIA CRASSICEPS*

A. INTRODUCTION 262

1. Development of Parasite Immunology 262

2. Immunology of Cestode Infections 262

3. Review of the Literature on the Immunology of *Taenia crassiceps* 267

B. MATERIAL AND METHODS 271

1. Source of Parasite 271

2. Experimental Protocol 271

C. RESULTS 272

D. DISCUSSION 291

E. SUMMARY 296

GENERAL DISCUSSION 297

SUMMARY 306

REFERENCES 310

APPENDIX 355

LIST OF FIGURES

1. The Life-cycle of Taenia crassiceps 16

2. Oncosphere and Early Post-oncosphere Stages of T. crassiceps metacestodes 42

3. Morphological Forms of T. crassiceps Metacestodes 44

4. Rostellar Hooks in T. crassiceps Cysticerci 47

5. Diagrammatic Representation of a Transverse Section through the Scolex of T. crassiceps 60

6. The Female Reproductive System in a Mature Proglottid of T. crassiceps 64

7. Stages of Development in the in vitro Cultivation of T. crassiceps 173

LIST OF TABLES

I. In vitro Culture of Taenia crassiceps Cysticerci 177

II. Kinetics of Metacestode Population 242

LIST OF PLATES

Plates 1 - 108 Section I - Structure and Development 72

Plates 109 - 161 Section II - In vitro Culture 193

Plates 162 - 170 Section III - Pathogenesis 248

Plates 171 - 197 Section IV - Immunology 277

ABBREVIATIONS

af	axial filament
b	bud
ba	base
bb	basal body
bl	basal lamina
c	cilium
ca	capsule
cc	cytoplasmic connection
cf	cytoplasmic fold
cm	circular muscle
co	central core
ct	conjoined tubule
d	desmosome
db	disc-shaped bodies
dc	distal cytoplasm
e	embryophore cell
eb	embryophoral block
eg	egg
em	embryophore
ep	epithelium
er	endoplasmic reticulum
esv	excretory/secretory vesicle
ex	excretory canal
f	'flame' of flame cell
fc	flame cell
fi	fibrous zone
fp	filamentous process
gc	golgi complex
gly	glycogen
h	hook
hc	host cell
hca	host cell capsule
ho	hook organ
hoc	hook organ canal
ie	inner envelope
la	lamella
le	leptotriches
li	lipid droplet
lm	longitudinal muscle
lyb	lysosomal body
m	mitochondria
ma	macrophage
mg	Mehli's gland
mi	microthrix
mt	microtubule
mu	muscle
n	nucleus
nm	nuclear membrane
np	nerve process
nu	nucleolus
o	oncosphere
od	oviduct
oe	outer envelope
oec	outer envelope cell

om	oncospheral membrane
on	oncoblast
ov	ovary
p	parasite
pb	periphery of microthrix base
pc	parenchymal cell
pcy	peripheral cytoplasm
pd	pair of doublet elements
pec	perinuclear cytoplasm
rb	ribosomal bodies
rc	residual cytoplasm
rp	rostellar pad
rs	radial spokes
rt	rootlet
s	sheath
sd	septate desmosome
sg	secretory granule
sh	shaft
so	sensory organelle
sp	sperm
sr	seminal receptaculum
st	spermatids
te	testis
tm	transverse muscle
ts	tube-like structure
ut	uterus
v	vacuole
ve	vas efferens
ves	vesicle
vi	vitellaria
vid	vitelline duct
vs	vitelline secretory body
vv	vitelline vesicle

GENERAL INTRODUCTION

Distribution and Hosts

Taenia crassiceps (Zeder, 1800) Rudolphi, 1810 is a fairly common cestode parasite in Northern Europe and North America. The adult worm is found in the intestine of canids and felids while the larval stages, conveniently termed metacestodes, parasitize the body cavities and subcutaneous regions of rodents and moles.

Little work has been carried out on the biology of this tapeworm since the initial investigations by Leuckart (1856) and Braun (1896, 1897) in which the life-history was broadly outlined. Rausch (1952) first reported the adult from North America, collecting adults from the Arctic fox, Alopex lagopus innuitus from Alaska, and the metacestodes from the lemmings, Dicrostonyx groenlandicus richardsoni and Lemmus trimucronatus trimucronatus, from Manitoba and the Melville Peninsula, respectively.

In a relatively extensive series of studies by Freeman et al (1961) and Freeman (1962), adult worms of T. crassiceps were recorded in naturally infected red foxes, Vulpes fulva, a wolf, Canis lupus, and a coyote-dog hybrid in Southern Ontario. Subsequently, adult T. crassiceps were recorded from red foxes in New Brunswick and Nova Scotia (Smith, 1978), and Arctic foxes in North-West Territories (Eaton & Secord, 1979) in North America. In Europe, T. crassiceps adults were reported from red foxes in France (Joyeux & Baer, 1936), domestic cats, Felis domestica, in Czechoslovakia (Mituch, 1968) and wild cats, F. silvestris, in Bulgaria (Yanchev & Genov, 1978). Natural infections of the metacestodes have been recorded in a number of mammalian hosts. Apart from those hosts mentioned earlier that were reported by Rausch (1952), metacestodes of T. crassiceps have

also been found in Microtus pennsylvanicus, Tamias striatus, Ondatra zibethicus and Marmota monax in Southern Ontario (Freeman, 1962), and in Dicrostonyx torquatus in North-West Territories (Webster, 1974) in Canada. Occurrence of the larval stages in the United States of America has been reported by Fleming et al (1979) who found them in Marmota monax in central New York State and Albert et al (1972) who recorded the same host species in Maryland. M. monax was also recorded as a host for the parasite in Switzerland by Fatzer et al (1976), while Sharpilo (1976) reported Cricetulus migratorius harbouring the metacestodes in the Ukrains in Russia.

Adult T. crassiceps worms were established by artificial infection in red foxes and the domestic dog, Canis familiaris, by Freeman (1962). In the same study, a variety of small mammals were fed eggs of T. crassiceps. Cysticerci developed in Ondatra zibethicus, Tamias striatus, Peromyscus maniculatus, Tamiasciurus hudsonicus, and Sciurus carolinensis.

Studer and Baer (1949) first showed that T. crassiceps metacestodes can be maintained by intraperitoneal inoculation. It is possible to maintain a stock of T. crassiceps metacestodes by intraperitoneal passage in laboratory mice, mainly due to the fact that the parasite has the peculiar ability to reproduce asexually by exogenous (endogenous, very rarely) budding within this intermediate host. Budded outgrowths increase in size and can either be pinched off from the "parent" or remain attached and develop scoleces. Laboratory rats have also been reported to be used to maintain T. crassiceps metacestodes (Chernin, 1974; Anderson & Griffin, 1979 a,b).

Life-cycle

From records of host species and infection studies the life-cycle of T. crassiceps has been established (Fig. 1). Eggs passed out of definitive hosts such as foxes, wolves, dogs and cats, on ingestion by secondary hosts such as rodents and voles hatch in the intestine. The activated oncospheres penetrate the intestinal wall, enter the blood stream and are then passed to the other parts of the body. Once established at a particular site, usually the peritoneal cavity or subcutaneous axilla, the metacestode develops into a cysticercus which can reproduce asexually by vegetative budding. When the secondary host falls prey to a predator, such as an Arctic fox, the cysticercus evaginates, attaches to the intestine by means of suckers and rostellar hooks, and begins to mature into a strobilate tapeworm.

Terminology

To facilitate subsequent discussions, the following terminology adopted by Freeman (1962) is used. "Bud" includes all metacestodes which are predominantly a hollow ball with little or no scolex differentiation beyond the presence of a scolex canal. "Cysticercus" includes all metacestodes with detectable development of suckers and rostellum, but which lacks obvious attached buds. "Budder" includes those cysticerci with attached buds. The "parent budder" is the original source of buds in the site of an infection, usually resulting from the infection of a metacestode. A "daughter cysticercus" is one produced by the parent budder. While the above terms should suffice in general descriptions of the metacestodes of T. crassiceps, more descriptive terms will be used when necessary.

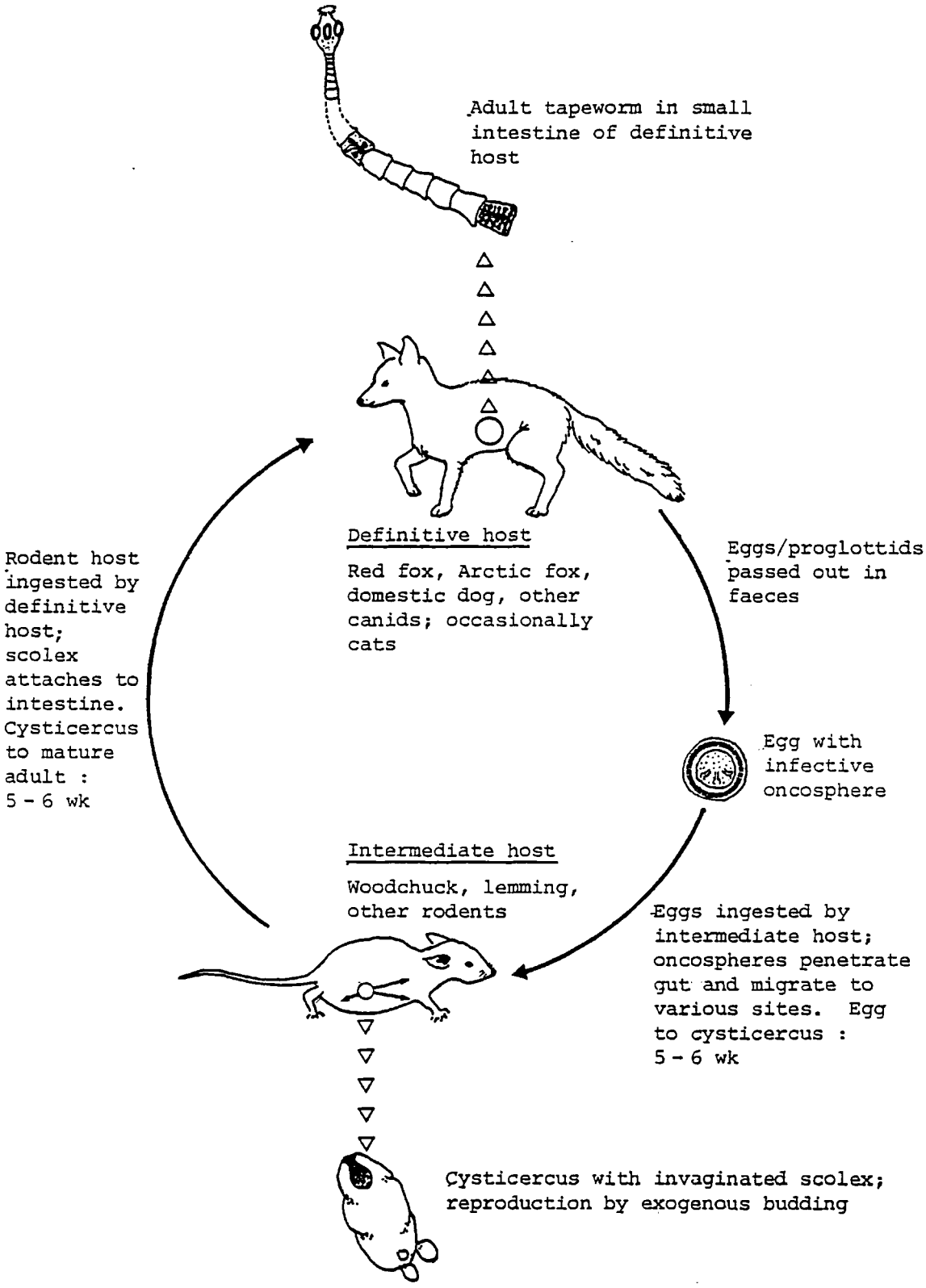


Fig. 1. The Life-cycle of Taenia crassiceps

Strains

In the literature, various strains of T. crassiceps have been used by different workers. The ORF strain was first isolated in 1952 at the Ontario Research Foundation, Toronto, Canada by Freeman (1962) who also recorded a new TOI strain in 1961 found in naturally infected woodchuck, Marmota monax. In 1965, another distinct strain was reported by Dorais and Esch (1969) from the W.K. Kellogg Biological Station, Michigan, USA; this was called the KBS strain. Most of the work in the literature has been done on these main strains and in particular the KBS and ORF forms. Subsequently, more strains have been isolated and identified by biochemical as well as antigenic differentiation, e.g. the ERS strain (Chernin, 1974,1975a) and the HYG strain (Chan & Freeman, 1976). However, these later strains have not been universally accepted. It is generally accepted that the ORF is an abnormal strain having arisen from an initial adult infection in the fox from which the eggs were fed to mice and the subsequent metacestodes passaged continually in mice; these metacestodes were then found to be non-infective to the definitive canid host (Freeman, 1962). Mount (1968) investigated the development of hooks and rostellum in the ORF strain and found them incomplete and irregular. Other differences between the ORF and KBS strains that have been reported include reproductive capacity (Dorais & Esch, 1969), generation time (Beavers et al, 1971), rate of RNA and DNA synthesis (Kuhn & Esch, 1970; Smith et al, 1972b), rate of incorporation of leucine (Culbreth et al, 1972), and antigenic composition (Fox et al, 1971). There are reasons to believe that these differences may be the result of aneuploidy in which the diploid chromosome number for the ORF strain is 14 while that in KBS is 16 (Smith et al, 1972a). In the present study the KBS

strain, being the normal or wild-type strain, is routinely used, though occasional use is made of the TOI strain.

T. crassiceps as a Laboratory Model

T. crassiceps offers many advantages for the study of cestode biology. Exogenous budding, as occurs in T. crassiceps cysticerci, is confined to 3 families, viz. the Taeniidae, Dibothriocephalidae and Hymenolepididae. Because of the prolific rate of vegetative reproduction (Freeman, 1962), T. crassiceps metacestodes can be easily and inexpensively maintained in large quantities in laboratory mice. The availability of easily maintained and well-developed metacestodes provides opportunities for various kinds of biochemical, physiological and morphological studies, including in vitro work, since they are present in the sterile cavity of the peritoneum. In immunological studies, direct intraperitoneal inoculation of the metacestodes avoids the problem of assessment of the effect on the host immune response by earlier, tissue penetrating and migratory larval stages. This problem is frequently a major difficulty in the interpretation of studies on the generation of the immune response to tissue-dwelling larval cestodes (Gemmell & MacNamara, 1972). A short life-history in which cysticerci grow to egg-producing adults within 5 to 6 weeks in the gut of foxes and dogs (Freeman, 1962) makes T. crassiceps an ideal tapeworm for developmental studies, particularly in vitro cultivation work as shown by Esch and Smyth (1976). Lastly, T. crassiceps offers practical advantages for experimentation due to the fact that the metacestodes are well-developed and large enough for easy handling and manipulation, and are only rarely known to be pathogenic in man.

Scope of this Study

The study covers broad aspects of the biology of *Taenia crassiceps* so as to present a comprehensive understanding of the parasite and its relationship with the host. The suitability of *T. crassiceps* as a laboratory model for basic research offers the parasitologist immense scope for experimentation and training. It serves to exemplify how knowledge of parasite-host systems might be extrapolated from basic to applied research in attempts to control cysticercus and adult tapeworm infections.

Section I deals with the structure and development of the tapeworm, an important discipline which reveals basic knowledge of the worm. Detailed studies have been undertaken to reveal the gross morphology and fine structure of the parasite in all stages of the life-cycle. Attempts were made to elucidate the structure and nature of the embryophoral blocks and oncospheral membrane in order to understand more fully oncosphere hatching and activation.

The body-covering of cestodes, now generally referred to as the tegument, is a metabolically active surface which plays an important role in their physiology. Much of this section, therefore, deals with a detailed study of the tegument of the worm in the developmental stages from cysticercus to adult. Comparisons made between the external and internal anatomy of the tegument from the scolex, neck, bladder wall and mature segments contribute to our understanding of the nervous, digestive-absorptive and immunological relationship at this region of host-parasite contact. By studying the internal histology of proglottids at progressive maturation levels along the length of the adult worm it is possible to get some ideas regarding the morphogenesis of organs in the reproductive system.

Difficulties encountered in the various disciplines of parasitology are often associated with the extrinsic nature of the host-parasite relationship. Problems in isolating the parasite completely from the host and host products have prevented or discredited many research findings. This is particularly so in areas of biochemistry, physiology and immunology, and even in morphology since it is very difficult to remove all traces of host material from the tegument and external structures of parasites. Again, T. crassiceps provides a useful model for in vitro work due to the short time it takes for maturation (Esch & Smyth, 1976). Section II is a study on the in vitro cultivation of T. crassiceps in an attempt to find out the most efficient and reliable way to culture the parasite in defined or semi-defined culture systems, and maintain its life-cycle in the laboratory. Histological and ultrastructural comparisons were made between in vitro cultured worms and in vivo worms to assess the efficacy of the culture system.

The pathogenicity and kinetics of infection by metacestodes in mice and adults in dogs is the subject of investigation in Section III. Emphasis was placed on the larval infection for two main reasons - the easy availability of the larvae-mouse host system, and the fact that the cysticercosis that results from such infections is more important as a disease than that caused by the adult worm in dogs. The particular form of cysticercosis caused by rapid proliferation of T. crassiceps metacestodes in the peritoneal cavity of the mouse is different from most other forms in which organs, muscles or other tissues are invaded. The pathology and pathogenesis of an infection such as that caused by T. crassiceps, therefore, warranted some investigation. In particular, external observations of the mouse as a

whole and histological examination of the internal organs were made to assess the effects of the parasite burden.

While the metacestodes appear to reproduce asexually quite readily, the mouse host shows a remarkable resistance in its ability to tolerate the parasite burden for long periods. The location of the metacestodes in the peritoneal cavity presents a situation in which there is constant and intimate contact between parasite and host tissue. The immunology of such an association could be unique - the host's tendency to protect and rid itself of foreign organisms competes against the parasite's ability to proliferate rapidly and/or devise means of escape from the immune response; all this at a site constantly bathed in host serum and where macrophages and other peritoneal cells abound. Preliminary studies on the immunology of larval T. crassiceps infections are reported in Section IV.

GENERAL METHODS

Routine Maintenance of T. crassiceps Metacestodes

Both the TOI and KBS strains of T. crassiceps have been maintained in inbred mice in the Animal Unit, Department of Zoology, Imperial College, South Kensington, London, for a number of years. The metacestodes propagate readily when infected intraperitoneally into several strains of white mice of which the TF1, LACA and NMRI are most frequently used.

For routine maintenance, an infected mouse was killed by cervical dislocation and its abdominal cavity opened. All the metacestodes were removed and transferred to Hanks' Balanced Salt Solution (HBSS) at about 38°C. After a brief rinse in HBBS, between 20-30 metacestodes were drawn up a No. 16 needle and recipient mice were then inoculated with the parasites by intraperitoneal injection using aseptic procedures. When exact number of larvae had to be inoculated, they were prevented from rising into the barrel of the syringe by a piece of sintered glass-filter affixed to the base of the syringe barrel.

No discrimination was made between the different morphological forms of metacestodes used for inoculation nor between the sexes in recipient hosts for such routine maintenance work. Freeman (1962) reported that the pattern of post-infection development and reproduction was similar regardless of whether a budder or a cysticercus was injected. He also reported that the sexes of mice were almost equally susceptible to infection via the intraperitoneal route. Though Freeman (1962) recommended that serial subinoculations be standardized by inoculating cysticerci at 50-day intervals, that regime was not strictly adhered to here in routine maintenance of the parasite.

4 - 12 week old mice were normally used for passage of the metacestodes. The mice were kept in cages in light and temperature regulated rooms, and fed with mouse pellets and water ad libitum.

General Procedures in Sterile (Aseptic) Work.

Aseptic procedures and preparations were employed largely in the in vitro culture of parasites. However, most of these were also used in some of the other aspects of this study.

Glassware for sterile work was washed thoroughly in detergent and rinsed repeatedly in distilled water before being autoclaved within autoclave bags. The ends of pipettes and any unstoppered exits on a piece of apparatus were plugged with non-absorbent cotton wool before sterilization. Autoclaving was carried out at 2 kg/cm^2 pressure at 135°C for 7 minutes for pipettes, and at 121°C for 20 minutes for other glassware. Dissection instruments were boiled in a sterilizer immediately prior to use. All solutions were made up in triple-distilled water, filtered through 0.2 µm mesh filters by suction pressure and then incubated at 38°C for 48 hours to check for sterility before the addition of the antibiotics penicillin and streptomycin at a concentration of 2 ml / 100 ml . All procedures were carried out subsequently in a laminar flow cabinet.

SECTION I. GENERAL STRUCTURE AND DEVELOPMENT OF TAENIA CRASSICEPS

A. INTRODUCTION

1. The Importance of Structural Studies in Parasitology

It is indisputable that the study of structure and morphology is crucially important in the field of the biological sciences both in the structure of organs, cells and organelles, and the gross morphology of the organism. This is even more so in the study of parasites. Much of the understanding of parasite taxonomy, life-cycle, physiology, biochemistry and immunology stems from basic structural studies. As biological structures are elucidated, their functions are better understood.

Progress in the understanding of structure is always limited by optical parameters. Before the advent of the microscope, parasitologists were predominantly naturalists using the resolution power of the naked eye. Parasites were then understandably confined to visible worms, and much of their life-cycles virtually unknown owing to the fact that many of the larval stages were undetected. The knowledge of parasitic protozoa was non-existent. With the advent of Leeuwenhock's microscope, the science of parasitology saw a dramatic improvement. Threadgold (1976a) stated : "The use of microscopes in studies of parasitic helminths is almost as old as the field of microscopy itself, for Leeuwenhock, the 'father of microscopy', published works on the liver fluke in 1700 and 1704.". An early example of the role played by morphological studies in tapeworm physiology are observations on the surface of tapeworms by Schiefferdecker in 1874. He reported the presence of fibrils on the surface and interpreted these fibrils as projections of sub-cuticular

cells and aided in the absorption of nutrients. Recently, his structural interpretation was shown to be inaccurate after conclusive ultrastructural studies revealed that the "fibrils" are microtriches which form an integral part of the tapeworm tegument (Rothman, 1963; Threadgold, 1962, 1965). Nevertheless, his physiological speculation was proved correct and receives support from many recent studies on the tapeworm tegument (Arme, 1975; Lee, 1972; Smyth, 1969a,1972).

At the turn of the century more and more work was published which described parasitic helminths in greater structural detail. Besides gross morphological studies, workers began to look more into cellular and sub-cellular structures as the optical properties of light microscopes improved. Present understanding of structure and function in the liver fluke, Fasciola hepatica, has its origins in the early work by Schubman (1905).

The use of histochemical techniques in conjunction with the light microscope early in the century had profound effects on parasitology. The nervous system of cestodes, for example, had until recently been described using morphological criteria only. The first description of the nervous system of a cestode appeared in 1836 when Mueller described what appeared to be ganglia in the scolex of Tetrarhynchus attenuatus. This was followed by some commendable descriptions of transverse commissures, ganglionic swellings and nerve fibres in some taeniids, bothriocephalids and cystecerci by Blanchard (1847). Little was added to this knowledge until the 1880's when crude histochemical techniques were used. Niemiec (1885) resolved many of the controversies using Golgi impregnation techniques, thus providing a basis for further work. Following an increased awareness and understanding of histochemistry and cytochemistry the information

that could be obtained from the light microscope became extensive. Shield (1969) and Leflore & Smith (1976) employed various modern techniques based largely on the demonstration of cholinesterases to describe in great detail the structure and physiology of the nervous system in several species of cyclophyllidean cestodes. During the 1940's and 50's, intelligent applications of histochemistry and cytochemistry contributed to a wealth of knowledge that has proved to be of immense importance for a deeper understanding of cellular physiology in helminths. Axmann (1947) identified glycogen in the parenchymal cells of some trematodes, and Romanini (1947) and Stephenson (1947) identified proteins and polyphenols in the vitelline cells of Fasciola hepatica. More sophisticated studies were subsequently employed by Smyth (1954), Johri & Smyth (1956) and Smyth & Clegg (1959) to identify phenolase and the protein-tanning system in the vitelline cells of trematodes and cestodes. The revelations of light microscope studies, though significant in the cytology and functional morphology of helminths, are often limited by the low resolving power of the light microscope itself. Further, many organs and organelles in helminths are extremely small, e.g. in Hymenolepis diminuta, the mitochondria in the tegument measure only 0.3 - 0.4 μm in diameter, barely resolvable in light microscopes. Parasitic helminths have an intrinsic anatomical and histological organization that bears little resemblance to that in higher groups of animals. The organ and tissue systems are loosely delineated largely due to the lack of well-defined connective tissues. The intricate inter-relationship of cells and tissues is often obscured by the abundance of parenchymal tissues consisting of branching cells resembling a massed epithelium (Threadgold & Gallagher, 1966; Threadgold

& Read, 1970a). It was left to the ultrastructuralists to exploit the higher resolving capability of the electron microscope to try to reveal the fine structure of parasites.

Perhaps in consequence of the very high resolution obtainable from the electron microscope, improved tissue fixation and processing had to be devised. The early days of ultrastructural studies saw vast and rapid improvements both in techniques and instrumentation. Smyth (1969a) stated "As with most ultrastructural studies, each year has seen improvements in technique.....". As in light microscopy, electron microscopy progressed from being used solely for morphological studies to a whole range of accompanying techniques being adapted to reveal fine structure, e.g. histochemistry, cytochemistry, autoradiography and immunology. As a result of ultrastructural studies and their increasingly accepted reliability, there have been changes, in the last two decades, in the attitude and approach to various aspects of parasitology. The helminths are now considered to be organisms of extreme specialization with ingenious adaptations to live and reproduce in a particular environment, which in many cases may be rigidly restricted within narrow physiological ranges. But ultra-structural research had an even more profound influence on modern considerations at the level of parasite anatomy and histology. One good example of this is the present acceptance of a living, syncytial tegument as opposed to a dead "cuticle" covering trematodes and cestodes. The first descriptions of the ultrastructure of the cestode tegument by Read (1955), Threadgold (1962) and Kent (1957) initiated numerous investigations. Consequently, a whole new concept was born in which the "covering" of trematodes and cestodes is universally accepted as being an area of great physiological activity, very much involved in

the secretion and absorption of materials (Lee, 1966; Smyth et al, 1967; Smyth, 1969a; Featherston , 1972). So remarkable has been the revelation of the helminth tegument by the electron microscope that Smyth (1969a) could remark : "electron microscopy has virtually revolutionised our knowledge of this region." when less than 20 years prior to that the cestode tegument was thought to be made up of inert material secreted from subcuticular cells (Hyman, 1951; Wardle & McLeod, 1952). Threadgold (1976a) put it most elegantly when he stated that ".... it is probably not unjustified to claim that the entrance of transmission electron microscopy on to the stage of helminth parasitology reinvigorated the microscopical study of this group and indeed gave a new impetus to, and a renewed and wider interest in, the whole field of parasitology."

The ability to observe, and the level of such observations, determines the parasitologist's depth of understanding with regards to parasite biology. Incorporating the valuable tools provided by biochemistry, histochemistry, cytochemistry and immunology the elucidation of parasite structure by light and electron microscopy has, and will continue to, contribute tremendously to our knowledge of parasites; with increasing knowledge, and application, comes an increasing degree of success in the control of parasitic diseases.

2. Review of the Literature on the Structure and Development of *Taenia crassiceps*

The first major study on *T. crassiceps* was that of Freeman (1962), who reported growth and development patterns of the parasite at various loci in several intermediate hosts. He described in relative detail the process of development from the point of egg ingestion to fully formed cysticerci. From his studies on the pattern of development

following ingestion of metacestodes it was found that the abdominal cavity of mice presents the parasite with a site where asexual reproduction rate was highest. Though Freeman (1962, 1964) did some preliminary observations on the structure of the oncosphere, cysticercus and adult stages, very little was done to reveal the histological structure of the worm. This was left to Bilqees (1966) who, in a series of studies under the light microscope, studied various aspects of the growth, histogenesis, and differentiation of larval T. crassiceps. She investigated the nature of abnormal development in oncospheres and metacestodes, and suggested several factors that might cause this, among which was the repeated transfer of metacestodes in routine passage from mouse to mouse which resulted in abnormal hooks being formed (Bilqees, 1967). Taking advantage of the fact that from a single mouse may be obtained metacestodes at different stages of development, she looked at the phenomenon of budding and found discernible budding gradients in the cysticerci (Bilqees, 1968 a). She also carried out some histological studies on the budding process to demonstrate the importance of the subtegumental cells in the growth and differentiation of buds (Bilqees, 1970 a). Her descriptions of the oncosphere (Bilqees, 1968 b) resemble those of T. saginata and T. pisiformis described by Silverman (1954). The histogenesis of the rostellum and the development of hook organ and hooks were the subject of further research by the Toronto group, and fairly comprehensive accounts have been published (Bilqees & Freeman, 1969; Bilqees, 1970 b). These studies indicated that hooklets develop de novo, which, interestingly, was the conclusion of Leuckart in 1886. This received support from ultrastructural work by Baron (1968) whose interpretations suggested de novo development. However, this was contradicted by

Mount (1970) who suggested that rostellar hook blades originate from a single microthrix, and not by de novo synthesis (Baron, 1968; Bilqees & Freeman, 1969).

Using a series of histochemical techniques in conjunction with the light and electron microscope, Baron (1968) described in great detail the histology and ultrastructure of the bladder wall and scolex of T. crassiceps cysticerci. He found the development of the scolex and rostellum similar to that of Cysticercus fasciolaris and C. pisiformis, and suggested the probable chemical nature of the hooks to be keratin, or that they at least contain keratin. Other than this major contribution on the structure of larval T. crassiceps by Baron (1968) there has been little additional work until the recent studies by Belton (1977) and Prokopic & Hulinska (1979).

The structures revealed in studies with both the light and electron microscope on the bladder wall of T. crassiceps cysticerci are comparable with those of other adult cestodes (Baron, 1968) but there had been no in-depth study of the structure of the adult of T. crassiceps. Freeman (1962) only mentioned very briefly the development of the adult worm in dogs and other hosts. In the current study, detailed investigations have been performed to reveal the external and internal structures of T. crassiceps from the oncosphere to adult stages, using light and electron microscopy and with the aid of histological and histochemical techniques.

B. MATERIALS AND METHODS

1. Source of Parasite

The larval stages of the parasite, the metacestodes, are routinely passaged in mice giving rise to a readily available source of the

parasite. Details of the technique followed can be found under General Methods on Page 22.

Adult worms were obtained by feeding cysticerci to dogs. Four 8 - week old beagles obtained from an acredited supplier of laboratory animals were fed with 20 well-developed cysticerci in a gelatine capsule. The pups were kept in a fenced compound at the London School of Hygiene & Tropical Medicine Field Station at Winches Farm, St. Albans, and kept under normal maintenance conditions. Three of the pups were autopsied 10, 17 and 29 days post-infection respectively and the worms carefully removed from the small intestines under warm normal saline.

Eggs were obtained from gravid segments at the lower ends of worms obtained 29 days post-infection. These were washed thoroughly and stored in distilled water at 4°C. Two attempts to collect gravid proglottids and/or eggs from faecal matter from the fourth pup up to 60 days post-infection were unsuccessful. This pup was then re-infected, but on inspection 32 days later there were no eggs in the faeces nor any worms in the intestines on autopsy.

On removal from the host,worms were routinely washed at least 3 times with Hanks Balanced Salt Solution (HBSS) (see Appendix I) before being processed for structural studies. Although cysticerci remain alive and viable after several days storage at 4°- 6°C, fresh worms were always used for such studies.

2. Morphology and Histology

Whole mounts of cysticerci and adults were prepared by gentle squashing of the worms under formal-saline between a microscope glass slide and a cover slip held together by a little vaseline. The whole

preparation was then kept under the fixative for 4 hours to overnight. After fixation, the worms were freed from the slide and washed in 2 changes of distilled water before staining in Gower's Carmine for at least 12 hours. Following differentiation in acid alcohol, the squash preparations were dehydrated in an ascending series of alcohol, cleared in xylene and mounted in Canada Balsam or DPX.

Tissues were variously fixed in a number of fixatives but the usual ones were Bouin's and formal-saline. These were then dehydrated in alcohols, cleared in xylene and infiltrated with parafin wax in the usual way. Sections were cut at 5 - 8 μm and stained with Ehrlich's Haematoxylin and Eosin. To study the scolex/gut interface, pieces of dog gut with a portion of attached adult worm strobila were fixed in Zenker's fixative for 4 - 8 hours. They were then washed in running tap water overnight, treated with alcoholic iodine and sodium thiosulphate before dehydrating in alcohols, clearing in cedar-wood oil, and infiltration with fibrowax. Serial sections were made at 5 - 8 μm and stained with a number of different stains, viz. 1. Haematoxylin and Eosin, 2. Casson's trichrome, 3. Modified Maximow's, 4. Masson's Haematoxylin-Ponceau's-Fuchsin Light Green, and 5. Modified Giemsa. For details of protocol, see Appendix II. All sections were mounted in Canada Balsam or DPX.

Some adult worm segments were fixed and processed in a way similar to that used for transmission electron-microscopy, details of which are given below. Sections, some serial, were cut on an ultratome at 0.25 - 0.5 μm and transfered onto drops of distilled water on glass slides. Chloroform vapour was used to release the sections from distortion due to compression. When air-dried the sections were stained with a number of stains, viz. 1. Azur II, 2. Modified Giemsa,

3. Sudan Black, 4. Acid Fuchsin, 5. Methyl-Green Pyronin Y, and 6. Methylene Blue. For details of protocol, see Appendix II. The sections were air-dried and mounted in non-drying immersion oil. Semi-permanent preparations were made by sealing the edges of the cover-slips with nail-varnish.

3. Histochemistry

Histochemical tests were carried out to determine the nature and composition of certain structures in the eggs and metacestodes. The following structures were examined :-

(i) Rostellar Hooks and Eggs

The Performic Acid Schiff (PFAS) test for keratin in the eggs and hooks was carried out for squash preparations of whole worms. The theory behind the staining is the oxidation of cystine, which is a major component of keratin, to cysteic acid which in turn reacts with Schiff's reagent. Keratin-containing structures appear bright pink to deep magenta and the amount of colour reflects the amount of available -SS- groups. Further details of this procedure are given in Pearse (1960) and Lyons (1966).

(ii) Nervous System

Histochemical techniques involving the localisation and identification of cholinesterases (ChEs) were used to study the structure of the nervous system in whole mounts of cysticerci. Cysticerci were removed form the peritoneal cavity of mice, washed in warm HBSS, and incubated in an evaginating medium (details in Section II) for 1 hour. Evaginated worms were then fixed in cold buffered formalin for 2 - 6 hours as squash preparations as described earlier. Flattened worms were washed briefly in distilled water and placed in the medium of Holt & Withers (1952) as given by Pearse (1960), using

5-bromoindoxyl acetate (5-BIA) as the substrate for 2 - 4 hours at 37°C., and the medium of Gomori (1952) as given by Pearce (1960), using acetylthiocholine iodide (AThChI) and butyrylthiocholine iodide (BuThChI) as substrates for 2 - 4 hours at 37°C.

Characterisation of the enzyme was attempted using the following inhibitors (Pearse, 1960) : For AChE - BW284C51 (1, 5-bis (4-allyldimethylammoniumphenyl) pentan-3-1-dibromide); for BuChE - Mipafox (N,N-diisopropylphosphorodiamidic fluoride) and iso-OMPA (tetramonoisopropylpyrophostetramide); for both ChEs - eserine (physostigmine). Whole mounts were pre-incubated in the appropriate medium containing inhibitor, but not substrate, for 30 minutes before being transferred to medium containing substrate and inhibitor.

(iii) Calcareous Corpuscles

Formalin-fixed paraffin sections of cysticerci were stained for calcium deposits by the silver method of von Kossa (1901) as given by Pearse (1960). This depends on the presence of insoluble phosphates and carbonates which, in animal tissues, are nearly always those of calcium. Briefly, the sections were immersed in 1% aqueous silver nitrate for 10 - 15 minutes under sunlight or an ultra-violet lamp to convert the calcium salt into its silver equivalent and subsequently reduce this to black metallic silver.

(iv) Others

The Periodic Acid-Schiff (PAS) reaction for PAS positive materials was carried out on paraffin sections of cysticerci and mature worm segments according to the method recommended by Pearse (1960). A technique which involved the Methyl Green-Pyronin Y technique of Brachet (1942) and Kurnick (1955) as given by Pearse (1960) was used to demonstrate the presence of RNA and DNA.

4. Ultrastructure

(i) Scanning Electron Microscopy

Cysticerci were removed from mice, washed thoroughly in 3 changes of warm HBSS, and incubated at 37°C for 1 hour in evaginating medium. After a brief wash in warm HBSS the worms were fixed at 4°C for a minimum of 12 hours in a 4% glutaraldehyde solution made up in Sorenson's phosphate buffer at pH 7.3. On some occasions, a 25 mM HEPES buffered solution was used. At the conclusion of fixation, the worms were washed thoroughly in 3 changes of buffer for at least one hour and dehydrated in an ascending series of acetone (The stages used were : 25% - 15 min ; 50% - 15 min ; 75% - 15 min ; 100% - 2 x 30 min). Critical point drying was carried out in a Polaran E3000 Critical Point Drying Apparatus using liquid carbon dioxide as medium. The specimens were then stuck on to aluminium stubs using double-sided sellotape and coated with gold for 2 - 3 minutes in a Polaron E5000 Diode Sputter Coater. Treated specimens were then viewed with a Cambridge Stereoscan IIA at 10 kV or a Philips 500 scanning electron microscope at 25 kV.

After removal from the dog's gut and washing several times in warm HBSS on a roller, adults were fixed, dehydrated and coated in the manner similar to that described for larvae.

Eggs in the gravid segments were exposed by gently teasing apart the body wall of the proglottids after fixation, dehydration and critical point drying. The exposed eggs and other internal structures were then coated. On occasion, squashed whole mount specimens were released from slides with xylene, washed thoroughly in xylene and split apart along the lateral margin with the aid of fine instruments. This opened up the segments into 2 halves to expose eggs, ovary,

excretory canal, vas deferens, vaginal ducts and other organs. These were then allowed to be air dried, stuck onto stubs and coated before observation under the scanning electron microscope. This relatively crude method was necessary as there was an acute shortage of adult worms for study. Though reservations would have to be made regarding artifactual inaccuracies, the technique did reveal a useful amount of detail regarding the internal structure and organisation of mature and gravid segments.

Photomicrographs were taken on Ilford Pan F 35 mm negative films on the Cambridge Stereoscan IIA and 120 mm films on the Philips 500.

(ii) Transmission Electron Microscopy

The methods used were based on those of Lumsden (1970). Worms were fixed in 3% glutaraldehyde (TAAB) in Sorenson's 0.1M phosphate buffer at pH 7.3 containing 3% sucrose and 0.5 mls of 1% $CaCl_2$ per 100 mls, for at least 2 - 3 hours at 4°C. They were then rinsed in 3 changes of cold buffer containing 5% sucrose and Ca^{++} for at least 30 minutes each and then post-fixed in 1% osmium tetroxide (TAAB) in Sorenson's buffer containing 3% sucrose for at least 1 hour at 4°C. The fixed worms were washed briefly in 2 changes of cold distilled water and dehydrated for 10 minutes at each grade of alcohol from 50% to 90% at 4°C and then left to reach room temperature. Dehydration was completed at room temperature in absolute alcohol using 2 changes of 15 minutes each. The alcohol was replaced with a 1 : 1 mixture of absolute alcohol and propylene oxide (EMscope) for 5 minutes. (This step was left out in later preparations as it was unnecessary). The worms were then cleared in propylene oxide using 2 changes of 5 minutes each and transfered to a 1 : 1 mixture of propylene oxide and araldite resin (TAAB). Details of araldite resin

composition are given in Appendix III. They were left covered on a revolving stage for one hour for infiltration, then uncovered to allow gradual evaporation of the propylene oxide overnight. The following morning the specimens were transferred to fresh resin and allowed to be infiltrated for a further 1 - 3 hours. The worms were then orientated in fresh resin in rubber moulds. On some occassions, when the worms, or parts thereof, were too large, trimming was performed under the resin with a sharp razor blade before embedding. The resin was polymerised at 60°C for 48 hours. The blocks were trimmed and thin sections (silver and gold) were obtained on a Cambridge Huxley Mark 2 ultramicrotome and a Reichart-Jung OM4 Ultracut ultramicrotome. Glass knives, made on an LKB 7800 B knifemaker, were used for fine-trimming and section cutting. The sections were flattened with chloroform vapour and mounted on Formvar coated copper grids of 150 or 200 mesh size. The grids were stained with saturated uranyl acetate in 50% ethanol for 10 - 20 minutes followed by 0.1% lead citrate (Reynold, 1963) made up in boiled distilled water and CO_2-free sodium hydroxide (TAAB) for 5 - 10 minutes. When staining with lead citrate, great care was taken to exclude carbon dioxide to reduce precipitation of lead carbonate on the sections. The sections were examined under an AEI 6B electron microscope at 60 kV and a Philips EM 301 electron microscope at 80 kV. Photographs were taken on Agfa-Gevaert F 071E plate films.

C. RESULTS

1. The Egg

The uniform terminology proposed by Rybicka (1966) for the various envelopes which invest the oncosphere of the Cyclophyllidea is largely adhered to in this account.

Scanning Electron Microscopy

The egg of T. crassiceps is subspherical in shape and measures about 22.5 x 18.5 µm (Plate 1). Under the scanning electron microscope (SEM), a thin "covering", or remnants of it, could be observed loosely attached to the embryophore. This covering consists of 2 components. The "capsule" has a relatively smooth surface and overlay the outer envelope which takes on a reticulate appearance with randomly spaced pits (Plate 2). These pits have a diameter ranging from 0.07 - 0.14 µm. The embryophore, with a thickness of about 0.3 µm, has a smooth surface (Plate 3). In the matrix of the embryophore are small subspherical cavities which measure about 0.2 - 0.3 x 0.1 - 0.2 µm. The infective embryo, the oncosphere, can be located within the embryophore (Plate 3). The surface of the oncosphere is covered by the oncospheral membrane which appears as an undulating membrane under the SEM.

Transmission Electron Microscopy

Transmission electron microscope (TEM) studies reveal further the fine structure of the egg. Closely associated with the capsule are groups of lipid bodies or droplets (Plates 4,6) which appear to be enclosed within it. Mitochondria are present within the two thin membranes which make up the capsule (Plate 7). The origin of the outer envelope appears to be an outer envelope cell, or cells, from

which issue strands of cytoplasmic extensions and folds which spread out to enclose the embryophore resulting in the irregularly pitted surface appearance evident in SEM observations (Plates 4,6). The capsule and outer envelope are closely applied to each other and together form an outermost covering which is in intimate contact with the embryophore (Plates 6,7). The outer envelope has a cytoplasm rich in granular bodies, possibly ribosomal, and mitochondria (Plates 5,7).

The beginning of embryophore formation can be noted beneath the outer plasma membrane of the inner envelope which originates as an embryophore cell (Plate 4). The embryophore is made of blocks that are first observed as discrete granules (Plate 4) and which appear to increase in size by accretion, and come to line the plasma membrane (Plate 5). The embryophore cell has a large nucleus, cytoplasm rich in fine granules, and numerous mitochondria (Plate 5) which become incorporated in the interstices of the embryophoral blocks as the dense block material surrounds them (Plate 8). The spaces occupied by these mitochondria is probably the reason for the presence of the cavities in the embryophoral matrix observed under the SEM (Plate 3). The embryophore stained positively to performic acid - Schiff treatment, suggesting that the embryophoral blocks are made up of, or contain, keratin as reported in other taeniid eggs by other workers (Smyth, 1969a). A dense, homogenous material can be seen between the embryophoral blocks (Plate 11) and this possibly represents some kind of cementing substance holding the blocks together.

The oncosphere membrane consists of a pair of laminae, the outer one being thicker and more electron-dense. The laminae are separated by a layer of closely spaced vesicles (Plates 7,8) and appear to be

the result of the folding in of the membrane (Plate 12). Between the oncospheral membrane and the basal lamina is a layer of cytoplasm of varying thickness which extends around the periphery of the oncosphere (Plate 8). This cytophasmic layer, hereby termed the "peripheral cytoplasm", is modified at some regions into a sheath-like structure in which can be found the distal portion or blade of the oncospheral hooks. Within this "sheath" (Nieland, 1968), the hook is surrounded by folds of membrane projecting from the peripheral cytoplasm (Plate 8). There are numerous dense bodies along the plasma membrane of the peripheral cytoplasm and these appear to be more abundant near the sheath (Plate 8). The basal lamina appears as a low-density zone which lies beneath the peripheral cytoplasm and extends around the entire oncosphere.

The oncospheral cells have generally large nuclei with dense chromatin material (Plate 6). Strands of muscle fibres traverse the oncosphere among the cells and appear to be anchored to the basal lamina by desmosomes or by specialized bulbous endings (Plates 6,10). Other than the somatic cells, two other cell types can be recognized. At the peripheral region of the oncosphere there are one or two largely nucleated cells with dense disc-shaped bodies which are possibly secretory in nature (Plate 7). These cells may represent the penetrating glands that are present in the oncosphere of other species (Silverman & Maneely, 1955; Lethbridge & Gijsbers, 1974). These secretory granules are closely packed together and do not appear to be membrane-bound. The nuclei of these gland cells are less electron-dense compared to those found in the somatic cells or the hook-forming cells or oncoblasts (Ogren, 1955). The oncospheral hooks are present during the early development of the embryophore (Plate 4). The hook lies

intracellularly within the oncoblast. Clusters of mitochondria can be observed next to the hook in the oncoblast (Plate 10). The distal portion of the hook blade lies within a sheath in the peripheral cytoplasm as described earlier. Hook muscles do not appear to terminate directly on the hook, but insert in the basal lamina at the "collar" (Swiderski, 1973) region (Plates 9,10). The mature oncosperal hook is a heterogenous, bipartite structure. Its two components consist of an outer sheath or cortex and an inner core. These show a marked difference in electron density with the cortex, measuring about 0.08 μm in thickness, comparatively more electron-dense.

Attempts at "hatching" and "activating" the eggs of T. crassiceps in vitro were only marginally successful (details in Section II). After treatment with pepsin, trypsin and other enzymes and bile salts, the oncospheres were liberated from the embryophore but none of these appeared to be free from the oncospheral membrane. Under the light microscope, hatched oncospheres were observed to be moving all 3 pairs of hexacanth hooks, possibly in an attempt to break free from the oncospheral membrane. It was possible to distinguish circular regions near the hooks and at the posterior end. These appear to be associated with the secretory activity of the penetration gland(s) (Fig. 2a). A globular protrusion or "bleb" was observed at the anterior end of some oncospheres. This could be a secretory droplet issuing from the penetration gland(s). The bleb increased in size and remained attached to the oncosphere during culture.

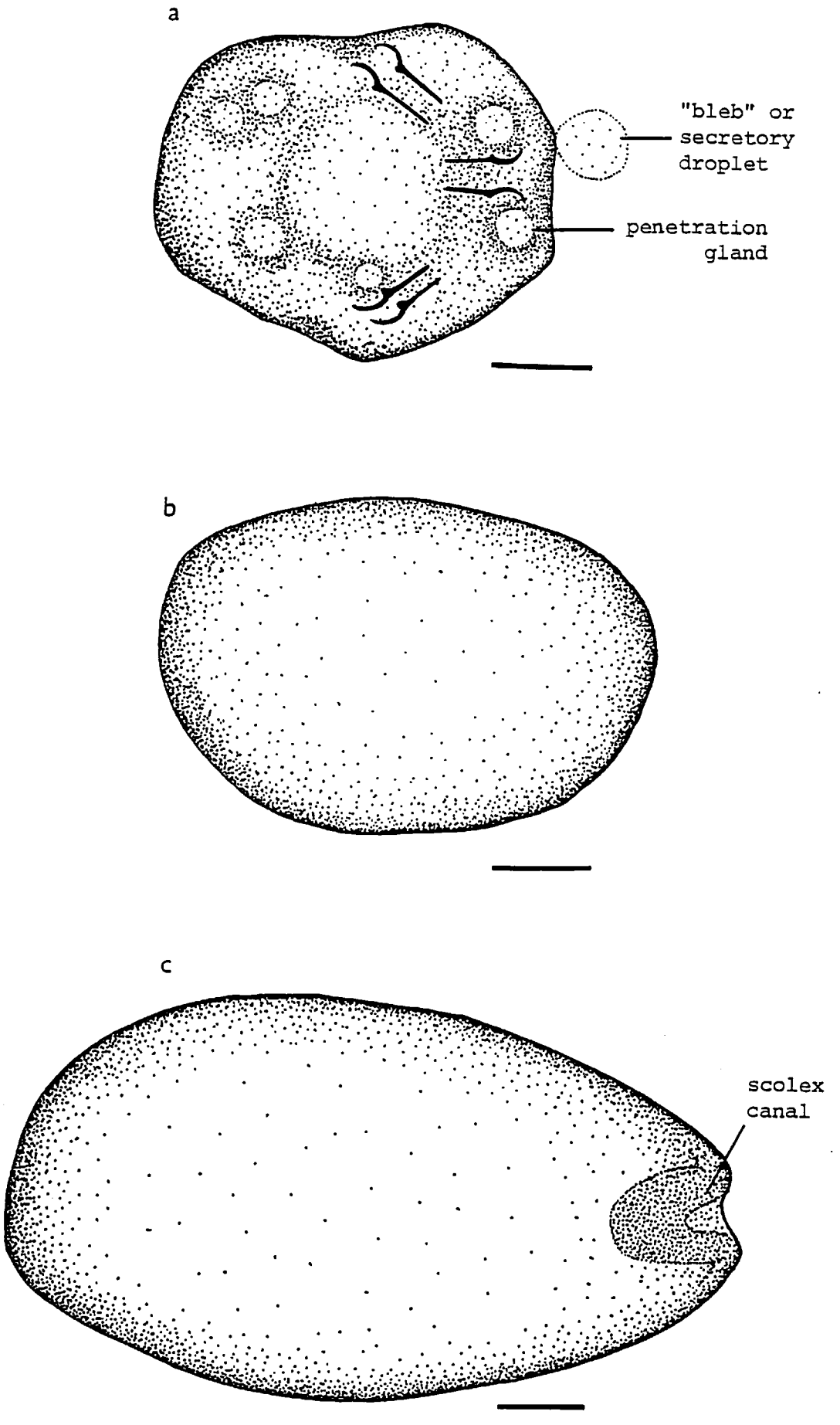


Fig. 2. Oncosphere and Early Post-oncosphere Stages of T. crassiceps Metacestodes

(a) oncosphere (scale bar = 5 μm) (b) detached bud (100 μm)
(c) bud with developing scolex canal (200 μm)

2. The Cysticercus

While there are many forms of post-oncospheral metacestodes, probably representing the progressive stages of development from the oncosphere to a fully formed bladder worm, only the cysticercus stage with a fully formed scolex will be described in detail.

Light Microscopy and Histochemistry

Cysticerci, presumably, arise from the oncosphere; however, in the model system employed in this work, the pre-cysticercus stages are derived by external budding from parent budders. In a population of metacestodes from.any one mouse the proportion of free buds varied from about 15% in KBS strain to about 12% in TOI. When budders without scoleces were included, the figures were 18% and 62% respectively (Appendix IV). Early, or young buds were subspherical in shape, and less than 1 mm in diameter with no signs of scolex development but with a well-developed central cavity or lacuna. Older buds were generally oval shaped and the first signs of scolex development appeared where the tegument on one end showed an indented thickening which would develop eventually into a scolex canal (Fig. 2 b,c).

There are several mophological forms that are commonly encountered in a population of metacestodes in the peritoneal cavity of a mouse infected by the inoculation of metacestodes, and the prominent types are illustrated in Fig. 3. The general appearance of the population of these larval stages was different for the 2 strains, KBS and TOI. The KBS larvae appeared more uniform and regular in shape and size, with relatively less budders or evaginated cysticerci (Plate 13). In contrast, there was considerable variation in the shape and size of TOI metacestode populations where there were relatively more evaginated cysticerci, buds of various sizes, and many budders (Plate 14).

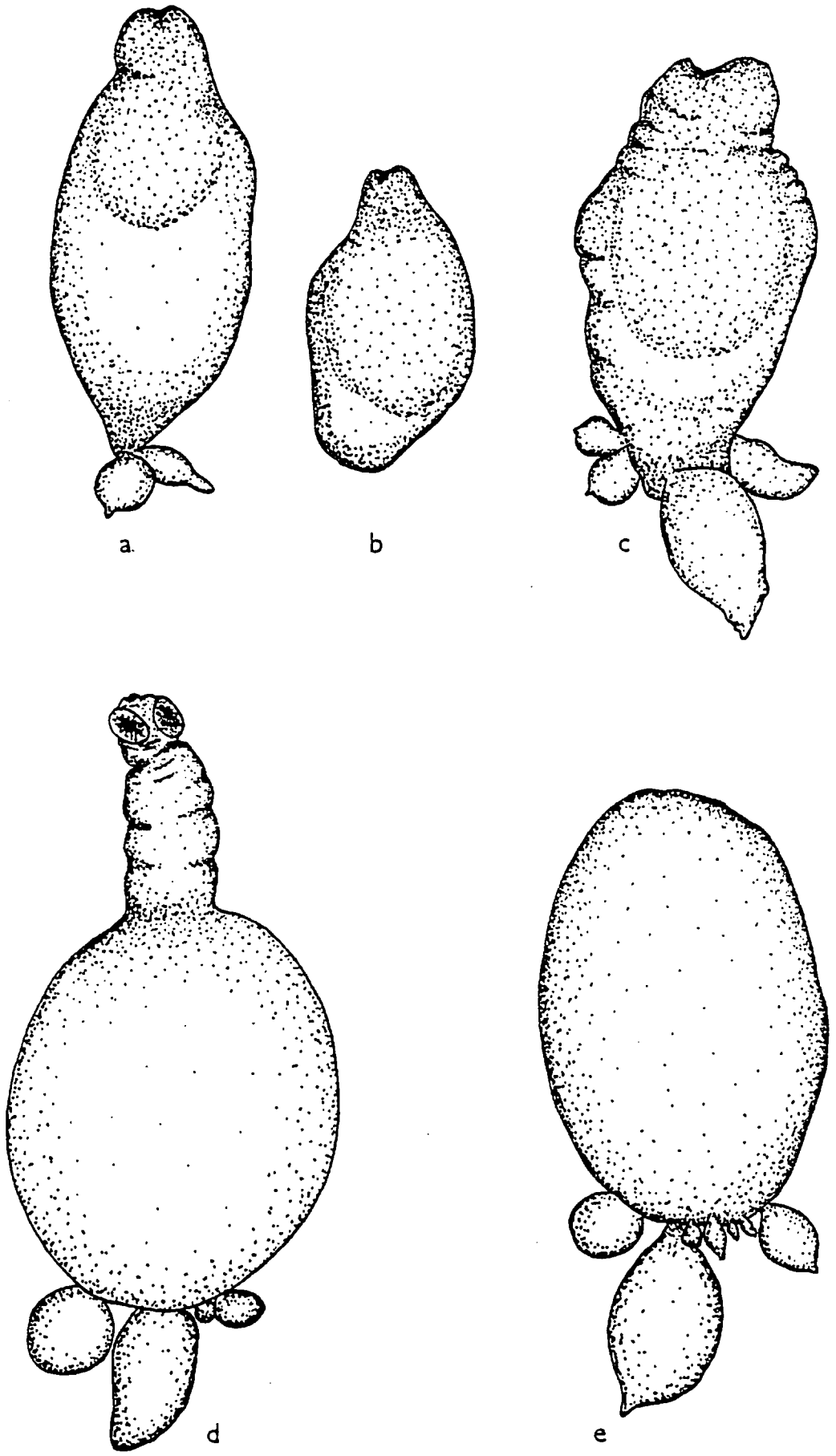


Fig. 3. Morphological Forms of T. crassiceps metacestodes

(a) budding cysticercus with invaginated scolex and large lacuna (b) cysticercus with invaginated scolex and small lacuna (c) budding cysticercus with invaginated scolex and small lacuna (d) budding cysticercus with evaginated scolex (e) budder without a scolex. (Magnification : x 20)

Detailed comparative studies of the various forms and their prevalence within and between strains are not the subject of this study but some relevant data are given in Appendix IV. Common to both strains, there were generally 4 distinct morphological types of metacestodes :- (1) bud, (2) budder, either with or without a scolex, (3) cysticercus with an invaginated scolex, and (4) cysticercus with an evaginated scolex. The most apparent differences between the 2 strain populations are that a high proportion of TOI metacestodes were without a scolex (approximately 62%) and of those with scoleces the majority had them evaginated (approximately 73%). Advanced cysticerci have a deeply invaginated scolex canal at the bottom of which is a well-developed scolex. In the KBS strain, a majority of these forms take on a "solid" appearance due to the absence of a large bladder cavity or lacuna. Conversely, "cystic" forms have a large lacuna, and these were more commonly found in TOI populations. Evaginated scoleces bear 4 large suckers and a protrusive rostellum but hooks were rarely observed to evert from the rostellum completely. Budders were common in both strains but in the TOI, there were more budders without scoleces. All the morphological forms may or may not have buds attached to them at the abscolex end. The prevalence of free buds in a population was noted but it would be difficult to determine how many of the buds became detached from the parent budders as a result of unavoidable manipulation or storage.

In old infections there were usually several caseous opaque bodies which vary in shape and size (Plate 14). Occasionally, these caseous masses showed roughly the shape of a cysticercus with an evaginated scolex. While most of these were free in the peritoneum, some were found adhering to various organs and the mesenteries in the

abdomen. Histological examination revealed recognisable remnants of cysticerci, especially hooks, surrounded by an eosinophilic, amorphous mass, presumably of host origin. There were faint indications that this "capsule" material was laid down in layers. A few scattered nuclei were observed among the capsule material and more in the surface. Sections of these encapsulated parasitic remnants when stained by the silver method of von Kossa (Pearse, 1960) for calcium revealed dense collections of calcium deposits, possibly representing breakdown residues of calcareous corpuscles originally present in considerable number in cysticerci (Plates 19,20).

Whole mount preparations of evaginated cysticerci revealed typical taeniid scolex and hook patterns. There are 4 large muscular suckers, and the rostellum bears 32-36 hooks arranged in 2 concentric rows. In a fully everted rostellum the more anterior circle of large hooks each has an average chord length of 165 μm (range 151 - 185 μm) as compared to the lower circle of small hooks which average 125 μm (range 110 - 137 μm). The hooks showed up well in preparations stained in Gower's carmine (Plate 15). In preparations treated with performic acid-Schiff (PFAS), the hooks took on a bright magenta colour indicating the presence of keratin, or keratin-like proteins (Plate 16). Normal hooks have a long narrow blade in relation to the handle; however, in small hooks, the handle portion is relatively large (Plate 15, Fig. 4). Most KBS cysticerci had the normal hook arrangement, quantity, shape and size. Among TOI larvae there were frequent variations in number, size and shape of rostellar hooks. In PFAS preparations, abnormal hooks could be easily observed. Some of these worms had no hooks at all, or only fragments or minute stumps of hook material. In several cases hooks were much shortened, and

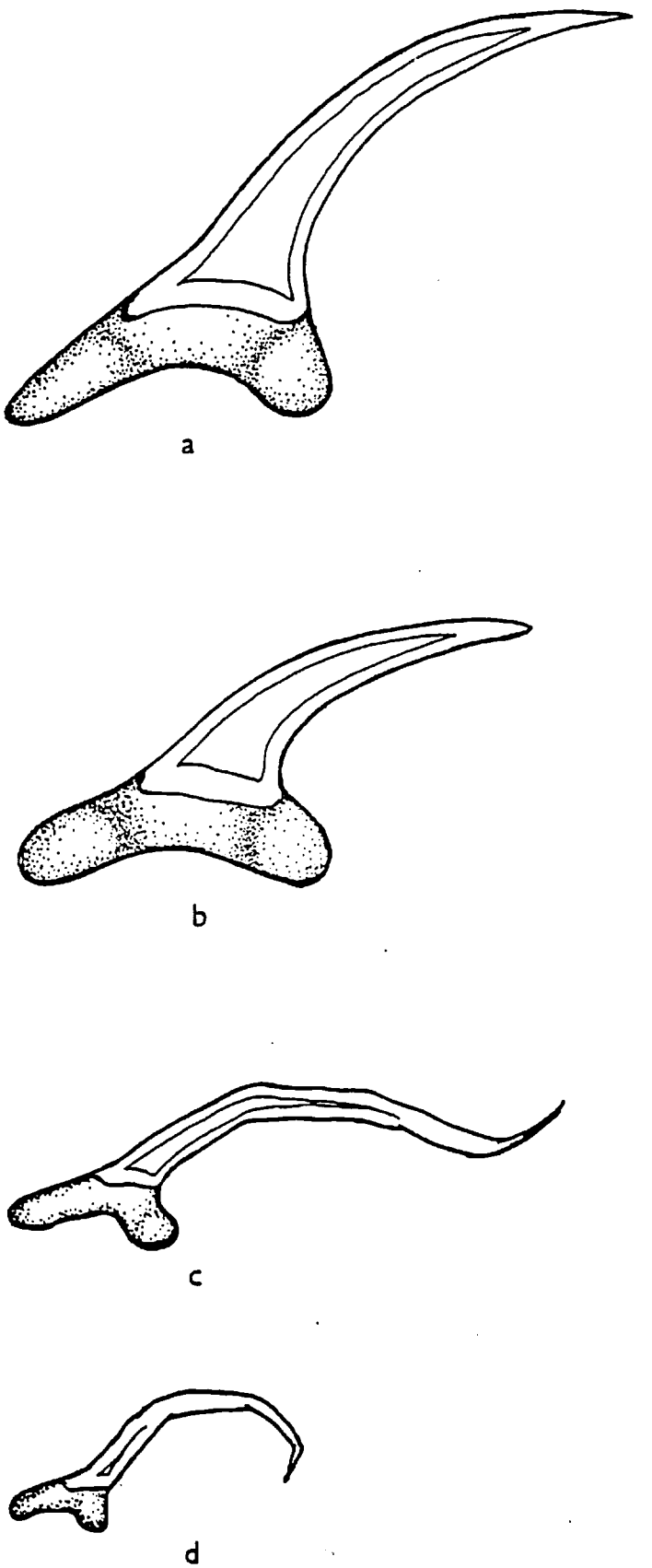


Fig. 4. Rostellar Hooks in T. crassiceps Cysticerci

(a) and (b) large and small hooks of KBS strain (c) and (d) abnormal hooks commonly found in TOI strain (Magnification : x 570)

kinked in shape (Fig. 4). A frequent observation was the presence of a globular protrusion at the rostellar tip of evaginated worms. These protrusons were tongue-like in shape and were retractable into the rostellum. Histological sections revealed that these protrusions were continuous with the cytoplasm of the rostellum indicating that it was intrinsically part of the parasite, and probably not of precipitated material.

Using histochemical localization and characterization of specific esterases, a network of nerve chords and fibres could be revealed (Plates 17,18). The general staining for esterases with the indoxyl-acetate technique revealed a dense wide-spread network of esterase activity, and was far too non-specific. There was intense staining concentrated in the rostellum and suckers but it was difficult to obtain clearly defined areas of esterase activity because of the intensity and non-specificity of the stain. When the thiocholine iodide method was used, there was much better definition. The technique clearly demonstrated the gross nervous system with sharply defined localization. There was intense activity at each sucker region where huge ganglia at the suckers were connected by commissures beneath the rostellum, forming a central nerve ring. There were numerous longitudinal nerve trunks extending down the trunk of the neck to the base of the bladder. These descending nerve trunks were interlinked by an intricate network of fine transverse commissures which ramify throughout the tegument and parenchyma, innervating the entire structure of the bladder (Plates 17,18). Characterisation by inhibitors of specific cholinesterases implicate the utilization of both acetylcholinesterase and butyrylcholinesterase in the mediation of nervous activity.

Observations of corpuscles, presumed to be calcareous, in live worms were further substantiated by histochemical means. Using the silver method of von Kossa (Pearse, 1960), as before, these corpuscles were intensely stained in formalin fixed sections (Plate 19). Though concentrated in the anterior portion of the bladder and neck, the corpuscles were located throughout the general length of the larvae even to the scolex region. The corpuscles were located throughout some sections and not confined to the tegument suggesting that they were also present in the parenchyma of the larvae, especially in the strobila region and scolex. The corpuscles appear to break down into an amorphous ground substance after the parasite is killed and encapsulated by host tissue (Plate 20).

There were positive reactions in varying degrees to PAS treatment in the hooks, rostellum, sub-tegumental cytons and muscles of T. crassiceps cysticerci; however, positive reactions were also obtained in the cytons in the absence of periodic acid pre-treatment resulting in ambiguity in the results. The sucker muscles, tegument and microthrix layer, especially in the scolex region, showed intense β-metachromasia to toluidine blue, suggesting a mucosubstance presence, but the tegument and microthrix border in the bladder wall reacted less strongly. In the bladder region, the presence of PAS-positive contents indicate rich glycogen deposits in the cytoplasm of parenchymal cells.

Scanning Electron Microscopy

The scolex of T. crassiceps, KBS strain, as observed by scanning electron microscopy (SEM), is presented in Plate 21. The scolex bears prominent suckers and a cylindrical flat-topped rostellum which is armed with 32 hooks arranged in 2 rows. The tegumental surface is

thrown into folds giving a wrinkled appearance which may be the result of specimen processing. The rostellum and surrounding region of the scolex including the suckers is densely covered by microtriches giving the appearance of a carpet pile. The microtriches at different regions of the scolex and rostellum vary slightly in their external morphology. Microtriches at the scolex tegument have a broad basal portion and an attenuating shaft (Plate 22). This cannot be revealed very clearly by SEM as the basal portion of the microtriches cannot be observed in its entirety, but transmission electron microscope (TEM) observations, to be described later, confirms this description. There are obvious differences among microtriches found in the region of the rostellar rim (Plate 23), sucker rim (Plate 24), and the inside of a sucker (Plate 25). Microtriches at the rostellar rim are long and thin, with a diameter of about 0.06 μm. At the rim of the sucker, the microtriches appear shorter and are about 0.09 μm in diameter. Both the microthrix forms in the rostellar rim and sucker rim regions have a gently attenuating distal position with the shaft ending in a fine tip. In contrast the microtriches found in the central region of the sucker have rounded tips; further the microtriches are much thicker, the diameter measuring about 0.14 μm. It is inexpedient to ascribe differences in density value for the microtriches at the various regions because it is often impossible to isolate individual microtriches in a count; furthermore, the manner in which the microtriches fall in any observed area varies, depending on the way that particular region of the tegument is folded at the point of fixation. There are hardly any morphologically specialized or modified tegumental projections. Along the sucker rim, what appears to be sensory projections can be seen. These sensory projections or

cilia are usually located in the centre of a rosette-like cavity formed by an outward curvature of microtriches (Plate 26). This is the only modification found throughout the whole surface of the cysticercus. Microtriches on the neck region lower down the worm appear to be very long thin structures which inter-weave among themselves resulting in a dense network appearance (Plate 27). The wall of the bladder portion of a cysticercus (not subjected to enzyme digestion) is thrown into deep folds (Plate 28). Again, this need not be a normal appearance of the bladder, but could be due to fixation or other preparatory procedures. Microtriches on the bladder wall are thin cylindrical structures with shafts that curve downwards at the tips (Plate 30). Rostellar protrusions, encountered often at the tip of the rostellum, have extremely fine microtriches, measuring less than 0.02 μm in diameter (Plate 29). At the abscolex end of the bladder, budded outgrowths are usually found in groups. These buds vary in size and are probably of different age. In larger older buds, slight indentations can be observed at the site of the future scolex canal (Plate 28). The surface of these buds is covered with microtriches similar in appearance to those found on the wall of the parent budder.

In very old infections many large budders are present. These do not possess a scolex, but have many small buds projecting throughout the general body wall (Plate 31). These buds vary in size and shape; while a few look like normal rounded buds, most are conical in shape and resemble tiny cysticerci with a bladder-like base and a neck (Plate 31). At the tip of some of these buds are indentations which could represent a scolex or sucker primordium (Plate 32). Another peculiar aspect of these buds is that they are attached to the parent

budder along a large base, unlike normal buds which attach to parent budders by a narrow stalk (Plate 33). The microtriches on the surface of these large budders are extremely long; they are not erect but are arranged in a dense mat of inter-weaving structures (Plate 34). In contrast, the microtriches on the wall of the buds are generally short and erect, and are interspersed with a few long ones (Plate 35).

Abnormal scoleces are occasionally encountered in metacestode populations. A scolex with more than one set of suckers and rostellum is shown in Plate 36. Polycephalic forms are also present in which the 2 or more scoleces are attached to each other by very short necks. More frequently encountered are worms with abnormal hooks. These hooks are often kinked and bent, and are few in number (Plate 37). In SEM observations, it is often difficult to tell whether hooks are absent since they could have been introverted into the rostellum; however, sections or whole mount worms examined under the microscope would reveal the abnormalities (Fig. 4).

Transmission Electron Microscopy

Transmission electron microscopy (TEM) observations revealed that the tegument of T. crassiceps is basically similar to that of many other cestodes studied (Morseth, 1966, 1967 ; Specian et al, 1974; Anderson, 1975). The tegument can be broadly divided into 3 main regions, viz. the microthrix border, the distal cytoplasm, and the perinuclear cytoplasm. The microthrix border consists of surface projections arising from the distal cytoplasm which, in turn, is an anucleate syncytial layer that maintains continuity with cell-bodies or cytons of the perinuclear cytoplasm via cytoplasmic connections (Plate 38,46). The microtriches are covered by a bilaminate membrane which is continuous over the surface of the distal cytoplasm (Plate 40).

The basal part of the distal cytoplasm is limited by a cytoplasmic membrane, the upper lamina of which occasionally projects into the distal cytoplasm to form 'microtubular' structures (Plate 65). This cytoplasmic membrane is continuous with that of the cytons in the perinuclear cytoplasm (Plates 38,46). This cytoplasmic membrane which covers the external and internal limits of the distal cytoplasm is also termed the external plasma membrane and internal plasma membrane respectively (Beguin, 1966). Immediately beneath the lower membrane, separating the distal cytoplasm from the muscle fibres and the perinuclear cytoplasm, is a fibrous zone (Morseth, 1966) consisting of a matrix of small fibrous-like filaments which are loosely and randomly arranged (Plates 45,47,63). This fibrous zone is given the term basement membrane by other workers (e.g. Beguin, 1966). It is clear that though the whole tegument is a continuous entity; it is only for convenience of description that it is considered in its 3 main "components".

The microtriches have a basic structure from which 2 regions can be distinguished. The proximal part of each microthrix consists of a relatively wide cylindrical "base" (terminology from Jha & Smyth, 1969) with thick electron-dense peripheral walls and a central core of lower density (Plate 43). This central core has a matrix that is continuous with that of the distal cytoplasm but none of the cytoplasmic inclusions that are abundant in the distal cytoplasm are found in the core matrix (Plates 40, 43). A high magnification of the microthrix base, seen in transverse section revealed a double laminated membrane surrounding the very dense peripheral wall. The core matrix is moderately dense and has a granular appearance (Plate 44). The distal part of a microthrix consists of a very dense "shaft" (Jha &

Smyth, 1969). Shafts vary in shape with the majority taking on a gently attenuating appearance (Plates 40,41) while others are modified into filamentous processes with or without a swollen tip (Plates 40, 43). The inside of the sucker cup have a tegument bearing microtriches with short round-tipped shafts (Plate 47). Also in this region are microtriches that have filamentous bases and bulbous shafts. At the rim of the rostellum the microtriches are thin and narrow, without the obvious base and shaft demarcation. Some of these microtriches appear to be joined at the base (Plate 42). High magnification of the shaft revealed the presence of microtubules that run along the longitudinal axis (Plate 41). The junction of the base and the shaft is marked by a tube-like structure with electron-dense walls (or possibly membranes) that curves upwards into the shaft (Plate 43).

The distal cytoplasm varies in thickness depending on the way the body wall is folded at fixation, and also, obviously, on the plane of section; this thickness can vary from 1.5 μm to 10.0 μm when measured on TEM sections. At low magnifications the distal cytoplasm appears granular and electron-dense with small, clear vacuolated spaces and dense bodies (Plate 38). At higher magnifications, the major constituents of the cytoplasm can be observed. These cytoplasmic inclusions consist largely of prominent disc-shaped bodies of diameter 0.12 - 0.15 μm which may or may not be membrane-bound, and many vesicles of various shapes and sizes, each bounded by a membrane, and containing varying amounts of electron-dense material (Plate 39). The disc-shaped bodies are revealed either as round dense structures or bi-concave rhabditiform structures depending on the plant of section. Mitochondria are seldom observed in the distal cytoplasm of the scolex region but are more numerous in the bladder tegument. They are usually

found in the basal part of the distal cytoplasm (Plate 63). Sensory structures or organelles can be located in the distal cytoplasm as bulbous "terminal processes". Septate desmosomes connect the apposed membranes on either side of the terminal process where the membranes of the bulb and the distal cytoplasm are in conjunction at the tegumental surface (Plate 39). These terminal processes usually contain single sensory elements (Plate 39), but occasionally there may be 2 sets of sensory processes (Plate 38). The sensory organelle sends out an elongated distal process or sensory cilium which extends beyond the tegumental surface. The cilium has at its base dense basal bodies from which run microtubules of filaments towards the tip (Plate 39) and rootlets down along the terminal process (Plate 38). There appears to be a continuity of the terminal process with the perinuclear cytoplasm by a specialized cytoplasmic connection or nerve process (Plate 38).

Lying below the distal cytoplasm, but maintaining continuity with it through cytoplasmic connections, are the cell bodies or cytons that make up the perinuclear cytoplasm. Also at this level are two layers of muscle; the outer layer consists of circular muscle fibres and the inner longitudinal. Less obvious are transverse muscle fibres which are more prevalent in the scolex region than in the bladder (Plates 38, 45, 46). Muscle fibres are more abundant and prominent in the scolex region than in the bladder. Round electron-dense granules, presumably excretory, occur in groups in spaces between the muscle blocks (Plates 38,45). The perinuclear cytoplasm of the tegument lies in the parenchyma below the muscle blocks (Plates 45,46). These cell bodies contain a large nucleus, with a prominent nucleolus, golgi complexes, granular glycogen deposits and mitochondria. In the scolex and

rostellar region there are very densely-stained parenchymal cells, probably due to a rich glycogen presence. Lower down at the neck, region of the cysticercus, the perinuclear cytoplasm has numerous lipid droplets of various sizes usually conglomerated into groups (Plates 46,58). The typical perinuclear cytoplasm structure does not extend into the sucker region. Sections of this region reveal the muscular sucker separated from the distal cytoplasm by an electron-lucid fibrous zone (Plate 47) which also extends around the entire muscular sucker internally (Plate 48), separating the sucker from the scolex parenchyma. The sucker muscles are connected to the distal cytoplasm by dense desmosome-like structures that traverse across the fibrous zone (Plate 47). All around the sucker, the muscles terminate at the fibrous zone and are attached by desmosomes (Plates 49,50). Along the edge of the sucker these muscles are observed in alternate longitudinal and transverse arrangement (Plate 49). The spaces in between the muscle fibres are filled with cellular extensions packed with dense rosettes of glycogen granules (Plates 47,48).

Among the closely packed parenchymal cells in the rostellum are the numerous muscle fibres that traverse the region and the occasional flame cell (Plate 51). Rostellar gland cells, abundant below and around the rostellar pad, are easily recognized by their much lobulated pseudopodia-like cytoplasmic processes and large amounts of biconcave disc-like secretory granules (Plates 51,52). These granules have diameters of 0.2 - 0.3 mm. The cytoplasm of these secretory cells contain much ribosomes, a prominent nucleus, numerous mitochondria and granular endoplasmic reticulum. Another feature of the internal structure of the rostellar region is the extensive areas of densely packed disc-shaped bodies and vesicles. This structure has been

variously named the "hypertrophied tegument" (Baron, 1968; Mount, 1970) and "hook organ" (Bilqees & Freeman, 1969). For reasons to be discussed later, the latter terminology will be adopted. In the hook organ there are several canals lined by a membrane which occasionally has microthrix-like projections (Plate 53). The hook organ does not appear to be a discrete structure delineated by a limiting membrane separating it from the rest of the rostellar tissues. In fact, numerous disc-shaped bodies are found scattered among the neighbouring cells, suggesting a gradual transition between the hook organ and rostellar parenchyma (Plate 54). The disc-shaped bodies, though resembling those that occur in the distal cytoplasm of the tegument, are larger, measuring 0.2 - 0.3 mm in diameter. These bodies and the vesicles in the hook organ can either be membrane bound or free (Plate 55). Numerous membrane-bound vesicles with inclusions of varying electron density are present in the lumen of the hook organ canals (Plates 53,57). These vesicles may have a secretory or excretory function. There are electron-dense material in the lumen of the canals, possibly following the breakdown of the membrane of these vesicles. Further, membrane-bound vesicles with similar staining material are present in the hook organ, some of which appear to be in the process of being expelled into the lumen (Plates 53,57). Whorls of lamellae are frequently observed in the canal lumen (Plate 53). These lamellae occur either as an open-ended or continuous band (Plate 56). Rostellar hooks are frequently present in the hook organ canals (Plate 57).

At the neck level of the cysticercus numerous muscle blocks can be found among glycogen-rich parenchymal cells. These parenchymal cells contain relatively large lipid droplets which are usually found

in groups (Plate 58). Typical flame-cells are easily recognised; these have a flame region made up of ciliary filaments arising from basal bodies and striated rootlets (Plate 59). At one end, the large nucleus with a dense nucleolus and chromatic material is prominent. Internal and external leptotriches can be observed at the wall of the flame canal leading to the conjoined tubule. Multi-lamellated corpuscles, conveniently termed calcareous corpuscles, are fairly common in the general scolex and body parenchyma of cysticerci, though more concentrated in the neck region. The calcareous corpuscle consists of densely stained concentric circles in an electron-lucid matrix (Plate 60). The outermost lamella has extensions that permeate along inter-cellular spaces. It is not a continuous ring but interrupted by discrete gaps. It is difficult to ascertain if these corpuscles originate intracellularly since only fully formed ones have been studies by which stage the cell of origin might have degenerated. However, the corpuscle is often observed in intimate contact with glycogen-rich parenchymal cells. Excretory canals frequently occur in the sub-tegumental region. These have a darkly stained wall which extends bleb-like projections into the lumen (Plates 61,62). A continuous plasma membrane covers the wall and projections. The wall appears to be formed from modified cells with a densely stained granular cytoplasm containing a large nucleus with prominent nucleolus and numerous mitochondria.

While the above descriptions provide a generalized account of the ultrastructure of the cysticercus as observed by TEM, it must be pointed out that the picture may be markedly different at various regions of the worm. Thus, the bladder wall tegument (Plate 63) have a relatively sparse distribution of fine micrtriches and a distal

cytoplasm containing mitochondria which are larger and more numerous in number when compared to the scolex region. Further, the muscle blocks beneath the distal cytoplasm are less prominent and not closely packed together. Similarly, the cytons in the perinuclear cytoplasm are not closely packed among parenchymal cells and transverse muscles. Higher magnigications reveal darkly-stained whorls of closely lamellated membrane bodies in the perinuclear cytoplasm (Plate 64). The membrane limiting the basal border of the distal cytoplasm is thrown into folds (Plate 65).

From the above account of the ultrastructure of the cysticercus of T. crassiceps, a generalised picture can be obtained. Figure 5 illustrates a diagrammatic representation of the tegument and associated structures at the scolex region. This representative diagram does not include features found in the central region of the scolex and rostellum, nor the differences in the bladder wall.

3. The Adult.

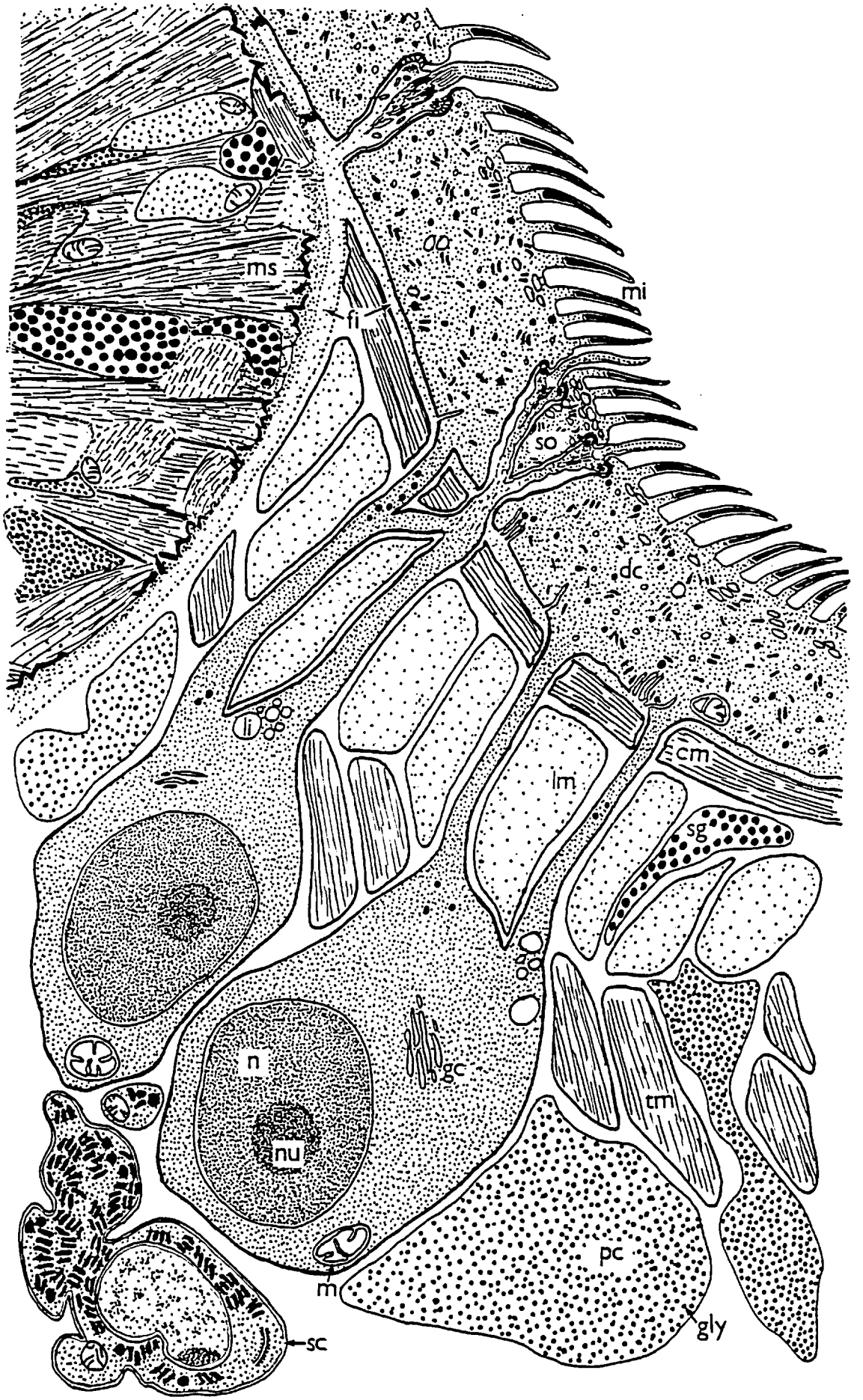
Adult worms from infected dogs were obtained 10, 17 and 29 days post-infection. By examining representative segments along the length of the worm from the scolex to the mature and gravid segments, the developmental biology of the worm could be studied.

Light Microscopy and Histochemistry

The scolex of the adult worm is generally similar to that of the cystecercus. Details of some of the aspects of its structure and histochemistry have been given earlier. The observations that follow were obtained from histological studies on the scolex of the adult worm, and it is assumed that most would be similar in the cysticercus scolex, and thus, add to the picture. In transverse section, the muscular rostellar pad appears to be sharply delineated from the

Fig. 5. Diagrammatic Representation of a Transverse Section through the Scolex of T. crassiceps.

(cm - circular muscle; dc - distal cytoplasm; fi - fibrous zone; gc - golgi complex; gly - glycogen granules; li - lipid droplets; m - mitochondrion; mi - microthrix; ms - muscular sucker; n - nucleus; nu - nucleolus; pc - parenchymal cell; sc - secretory cell; so - sensory organelle)


ms
fi
mi
so
dc
li
lm
cm
sg
n
nu
gc
tm
pc
m
gly
sc

rest of the scolex parenchyma (Plate 66). Internally, short strands of eosinophilic fibres, presumably muscular, are arranged radially along the circumference of the pad. The hooks lie in a homogenous anucleate matrix arranged in radial lobes or pockets (Plate 67). This matrix is probably equivalent to the hook organ observed in the rostellum of the cysticercus with the TEM. In the middle of this matrix are numerous darkly stained cells which probably represent a 'rostellar gland'. With modified Giemsa-Colophonium, methylene blue and toluidine blue staining techniques, what appear to be secretory cells in the rostellar gland were observed to be fairly dispersed in the rostellar region and not confined to the area anterior to the rostellar pad (Plate 68). These gland cells exhibit β-metachromasia, suggesting the presence of acid mucopolysaccharide constituents. Areas of β-metachromasia are also found at the tip of the rostellum especially in the areas immediately surrounding the hooks (Plate 68) which indicates secretory activity in the hook organ. Sections stained with Masson's trichrome revealed "pockets" of mucin containing material around the rostellar pad next to the tegument (Plate 69). Whether these pockets are an intrinsic part of the worm or simply an invagination of the tegument at the plane of section cannot be determined. Further, the origin of the mucin material is unknown, though it appears likely that it is of host origin. The fibrous zone which extends throughout the worm just beneath the basal lamina of the distal cytoplasm stains positively with acid-fuchsin, suggesting that this fibrous zone is collagenous in nature. PAS positive reactions were obtained from the scolex tegument, particularly around the rostellum and the suckers.

Segments at progressive levels of maturity can be studied for

gross morphology by squash preparations of whole worms stained with Gower's carmine. The first signs of sexual maturation, that of a genital streak, occurs about 9 - 10 segments from the first true proglottid. The single genital streak eventually divides into the cirrus and vagina. Cells that form the testes appear as discrete clumps about 17 segments below the first genital streak. Uterus-forming cells occur 1 or 2 segments later, and the distinguishable ovary and vitellaria are obvious about 2 segments posterad. The mature proglottid, each consisting of a fully formed uterus, vas deferens, cirrus, vagina, testes, ovary and vitellaria, extends into several segments, sometimes up to 25, before the first gravid proglottid. A typical mature segment is shown in Plate 70. Sperms from the many dispersed testes (Plate 71) pass along a fine network of vas efferens which lead into the main vas deferens. The vas deferens extends from the middle of the segment laterally and terminates in a coiled cirrus with a cirrus pouch (Plate 71) which is continuous with the vaginal opening just posterior to it. There is a common genital pore which opens to the exterior. From serial sections, it is possible to reconstruct the rest of the female reproductive organs (Plate 72, Fig. 6) which follows the well-known taeniid pattern. The terminology used is adapted from Wardle & McLeod (1952). The vagina leads posteriorly to the dilated seminal receptaculum, seminal canal, and turns anteriorly to become the muscular oocapt before joining the ovary at the "isthmus" or "median ovarian bridge". The oviduct branches off from the seminal canal posteriorly until it meets the common vitelline duct. From this junction arises the long narrow ootype which runs anteriorly to open into the uterus. Mehli's gland cells are located around the ootype

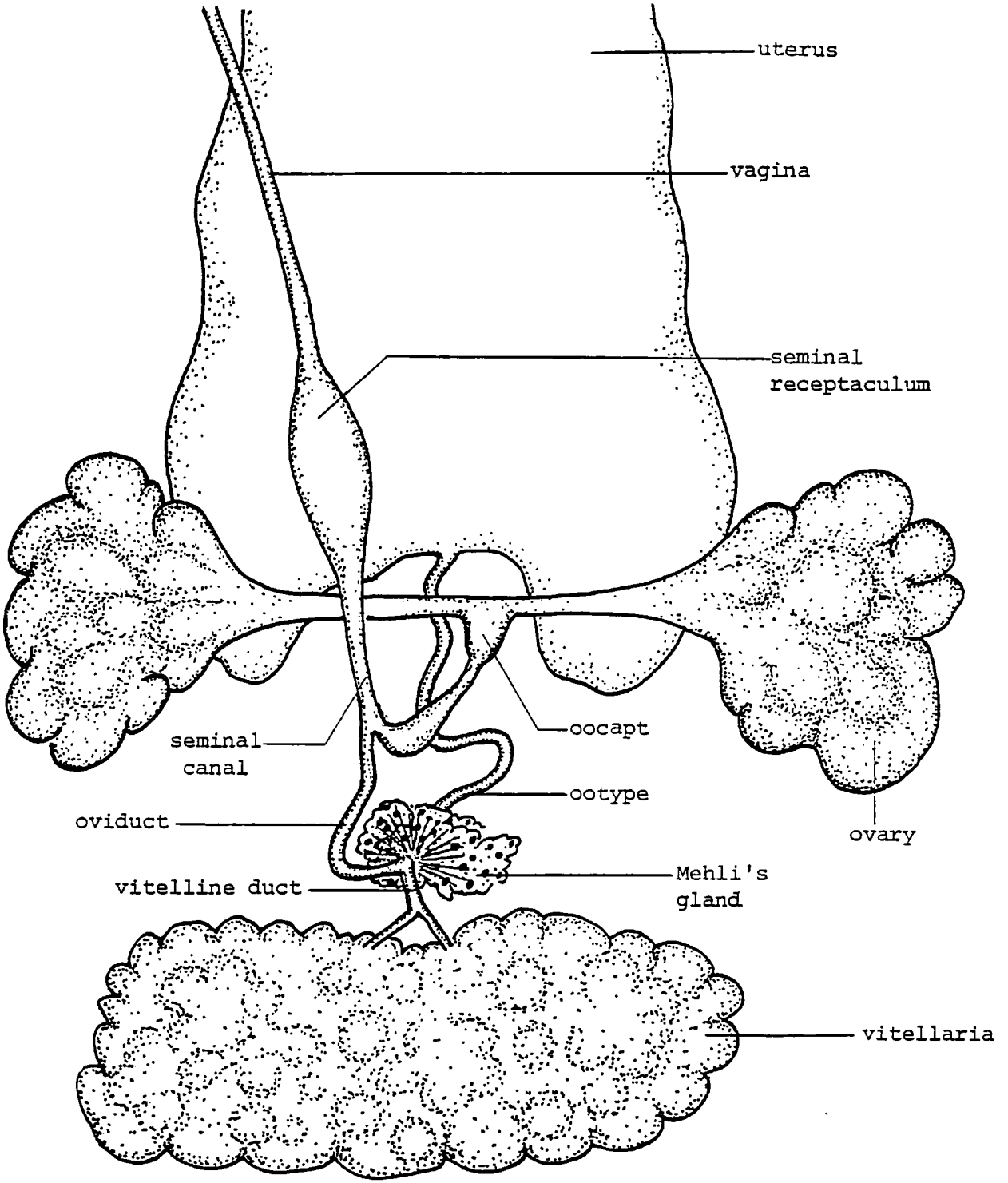


Fig. 6. The Female Reproductive System in a Mature Proglottid of T. crassiceps (Not drawn to scale)

just beyond the junction of the oviduct and vitelline duct. The ovary is conspicuously bilobed, with each lobe consisting of many interconnected chambers giving an alveolar appearance (Plate 72). Within each chamber are closely packed cells which are probably ova. The vitellaria appear as a multichambered conglomeration at the posterior region of the proglottid. Two main ducts lead from the vitellaria anteriorly and join as a common duct through which vitelline cells pass into the ootype.

Lipid droplets, as shown by Sudan Black staining, are found in large concentrations, in the cortical parenchyma. These droplets are more numerous in gravid proglottids than in non-gravid ones. The collagenous fibrous zone below the basement lamina of the distal cytoplasm extends around all the proglottids. This collagenous material is also present in the medullary parenchyma, lining the clear intercellular spaces, and in the matrix of the uterus among the ova. The tegument and subtegumental cells contain a mucoprotein and/or a mucopolysaccharide as suggested by a positive reaction and β-metachromasia to PAS and methylene blue, toluidine blue or Azur II respectively. Whole mounts of gravid proglottids treated with PFAS revealed intensely stained eggs confirming the keratin nature of the embryophoral blocks. The embryophoral blocks and the embryophore cell are rich in RNA as shown by methyl green-pyronin Y staining. There were no positive reactions to the methyl green component of the staining which could be due to the absence or low level of DNA or the incompatibility of the fixative used (in this case, Zenker's) with the stain. Vitellaria cells containing secretory bodies stained β-metachromatically with toluidine blue indicating an acid mucopolysaccharide presence.

Scanning Electron Microscopy

SEM reveals a typical adult tapeworm structure. Worms obtained from 10 - day old infections have a normal scolex with 4 suckers and a strobila with 30 - 35 distinct segments (Plate 73). None of the proglottids have a genital pore at that stage of development. The worm has a tegumental surface generally similar to that found in cysticerci with a fur-like covering of microtriches. However, it was difficult to obtain a clear view of the microtriches as they were obscured by material, presumably of host origin, as a result of the mucous covering over the intestinal villi and digested food in the gut lumen of the dog (Plate 74). The rostellar hooks of both the 10 - day and 17 - day old worms were not observed (Plates 73,75), presumably retracted when the worms were placed into fixatives. On each proglottid at the posterior part of the strobila of 17 - day old worms there is a genital pore (Plate 76) which may be retracted into an atrium (Plates 77,78). Filamentous sperms may be observed in the genital pore (Plate 79). Under high magnification the microtriches appear similar to those on the surface of the neck region of the cysticercus. No modified sensory microtriches or cilia were observed on the adult tegument.

It was possible to use the SEM to observe the internal structures of mature and gravid proglottids by carefully teasing them apart. The large, lateral excretory canal has an internal wall that has small bleb-like surface projections into the lumen (Plate 80). There are also irregularly spaced pits on the wall, possibly representing places where small excretory ducts open into the main canal. Sperms can be observed densely packed together in the testes (Plate 81). The sperms do not appear under SEM to have distinct head, mid-piece and tail

regions but are seen simply as long filamentous structures. The ovary consists of many interconnected ovoid chambers within each of which are the ova (Plate 82). The ova appear irregular in shape and covered by a wrinkled membrane but this may well be due to the processes involved in preparing the specimens for examination. The ova are attached onto thin folds or flaps that extend from the chamber wall (Plate 83). The vagina and much-coiled cirrus are still prominent in very mature proglottids in which can be observed the uterus and its lateral branches filled with eggs (Plate 84). Gravid proglottids have a central uterus "trunk" and numerous lateral branches filled with eggs, but the rest of the reproductive structures are regressed completely (Plate 85).

Transmission Electron Microscopy

Tegument The general plan of the tegument and its associated structures in the adult worm is basically similar to that which occurs in the larval forms (Plate 86). It is thus not necessary to describe in detail except for those structures that appear differently. The microtriches have a relatively narrower and longer basal position (Plate 87). The distal cytoplasm is thicker, usually 0.3 - 0.6 μm, and has a very granular appearance (Plates 86,89). There appears to be many more cytoplasmic connections between the distal and perinuclear cytoplasm (Plate 86). There are numerous sensory organelles (Plates 87-89) with basically similar structures as those occuring in the scolex of cysticerci, but none of the nerve terminal processes were observed to have more than one sensory organelle. Due to the thick distal cytoplasm, the rootlets are longer. Mitochondria were more numerous and located at the basal part of the distal cytoplasm. These had few cristae and appeared very lightly stained (Plate 89). In the

distal cytoplasm, the inclusions are mainly electron dense round bodies and membrane-bound vesicles with material of varying density (Plate 90). There are relatively few disc-shaped bodies; these were found to occur frequently in the cysticerci tegument. Along the surface in many regions of the adult tegument the distal cytoplasm is heavily vacuolated (Plate 90). These vacuoles appear to be the result of surface invaginations, possibly indicating pinocytotic activity.

Vagina The vaginal canal has a structure resembling the surface tegument (Plate 91). The vaginal wall extends fine microthrix-like projections into the lumen. Beneath the wall are layers of circular and longitudinal muscles and beneath these mescles are cell-bodies which, like tegumental cytons, extend cytoplasmic connections to the vaginal wall. The parallel between the components of the vaginal wall and the surface tegument is, therefore, obvious. The wall appears to be a syncytium with a "distal cytoplasm" in contact with the "perinuclear cytoplasm" via cytoplasmic connections and muscle layers in between. For the sake of clarity, the terms "epithelium", and "sub-epithelial cytons" will be used for these components of the vaginal wall. The numerous projections from the epithelium into the lumen of the canal each have a basal portion with an electron lucid central core and a distal shaft portion which is electron dense (Plate 93). They, therefore, bear a close resemblance to tegumental microtriches with the exception that the distal shaft is proportionately much longer than the base. The vaginal wall has traditionally been described as "ciliated" (Gough, 1911) but modern terminology tends to associate cilia with the presence of an axial filament complex or axoneme (Threadgold, 1976 b).

The term "epithelial projections" would be more appropriate and will be used in this case. The epithelium, about 0.5 - 1.0 μm in thickness, is an electron dense cytoplasm containing numerous disc-shaped bodies (Plates 91,93). Many vacuoles are present in the distal region. The basement lamina of the epithelium is thrown into prominent folds or invaginations. Disc-shaped bodies also occur in the sub-epithelial cytons and the cytoplasmic connections (Plate 91). The sub-epithelial cytons have a very prominent nucleus with dense chromatin material along the inner aspect of the nuclear membrane. Between the epithelium and the sub-epithelial cytons are the muscle fibres in alternate longitudinal and circular arrangement (Plate 93). Occasionally, sperms can be observed in the lumen of the vaginal canal (Plate 92).

Vasa efferentia; vas deferens. The vasa efferentia is a network of ducts through which sperms from the testes are collected into a main vas deferens which terminates at the cirrus. Each vas efferens is a thickly walled duct that opens into the testis (Plate 94). Fine filamentous microvilli project from the duct wall into the lumen. The vas deferens, or sperm duct (Wardle & McLeod, 1952), is a long tubular thin-walled structure (Plate 95). The duct wall has filamentous projections into the lumen, similar to the vas efferens but relatively fewer in number. Fully formed sperms are often observed in the vas deferens, concentrated along the sides of the duct.

Uterus. The uterus is a fairly large and conspicuous structure in the medullary parenchyma of mature proglottids. The uterus wall is of moderate thickness and is thrown into expansive folds of lamellae that project into the lumen (Plate 96). A few large muscle fibres are found around the uterus among the parenchymal cells.

Spermatogenesis The earliest stage of spermatogenesis that was observed was that of spermatid formation. Within the thin-walled testes, groups of spermatids are associated in cytophores, attached to a residual cytoplasm by a slender stalk (Plates 97,98). A spermatid has a large nucleus with a prominent nucleolus in a mitochondria-rich cytoplasm. The main part of the spermatid cell body which contains the nucleus appears to be more densely stained than those regions towards the central residual cytoplasm. More advanced spermatids tend to have condensed chromatin material dispersed within the nuclear membrane. The cytoplasm is rich in ribosomal granules, endoplasmic reticulum and mitochondria with few cristae (Plate 99). The early stages of spermiogenesis begin with a protrusion of cytoplasm which eventually grows to form the tail and axial filament of the developing sperm. This consists of an axial filament complex surrounded by an outer layer of microtubules (Plate 100). The axial filament complex is assumed to arise from basal bodies in the spermatid, though pictorial evidence of the connection was not obtained in the present study. At this stage, when the centriole has become the basal body (an assumed origin of the basal body; detailed discussion in Threadgold, 1976b), the nucleus of the spermatid is still intact. A prominent nucleolus and condensed chromatin can be seen within the fully intact nuclear membrane. After further development (not observed), a fully formed sperm is obtained. None of the sections of sperm revealed a sharp morphological distinction between sperm head and tail (Plates 101,102). On rare occasions part of a compressed spiral-like structure of intense electron density is found in a homogenous matrix. This may represent part of the nucleus in the head portion of the sperm. It appears then that the sperm is

basically a long, cylindrical structure with a small head portion and a long tail. The nucleus is modified into a long spiral, coiled round the central axial filament complex or axoneme, perhaps more compressed in the head region than in the tail. Many sections of the sperm tail do not contain nuclear material, indicating that the nuclear spiral does not extend the entire length of the tail. The fine structure of the sperm-tail reveals an outer "membrane"/sheath (whether this is actually membranous or simply an acellular sheath is unresolved) with microtubules along its internal aspect (Plate 103). The microtubules on this outer membrane are arranged in a helical pattern around the axial filament complex. This outer microtubule-membrane covering is not observed around the axial filament complex in some sections (Plate 102). This suggests that the axial filament complex is not enclosed by the outer membrane along its entire length. The central component of the axial filament complex is composed of a set of 9 peripheral elements and a central one (i.e. a 9 + 1 arrangement) enclosed within a striated sheath (Plates 102 - 105). Each of the nine peripheral elements is made up of a pair of microtubules (referred to as "filaments" by some, e.g. Morseth, 1969), designated, according to established nomenclature, as "A" and "B" (Plate 105). The "A" component has a dense core and 2 tail elements, while the "B" component of the pair has a less dense core and no tail elements. Radial spokes connect each of the "A" components of the peripheral doublet pairs to the central core (Plate 105). In all the sections examined, mitochondria, which could be unequivocally identified as such, were not observed in the sperms.

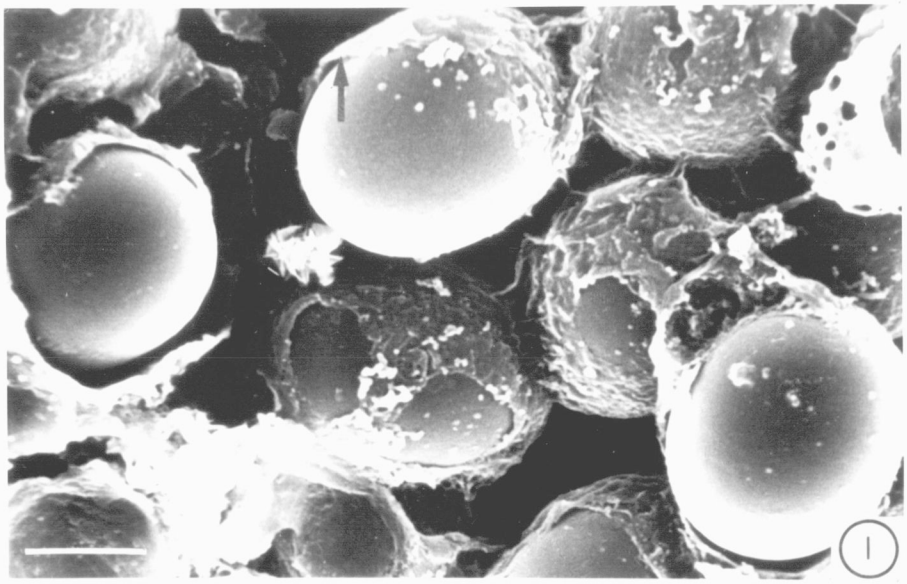
Ovary and Vitellaria Numerous attempts were made to examine the ovary in mature segments. However, these attempts were

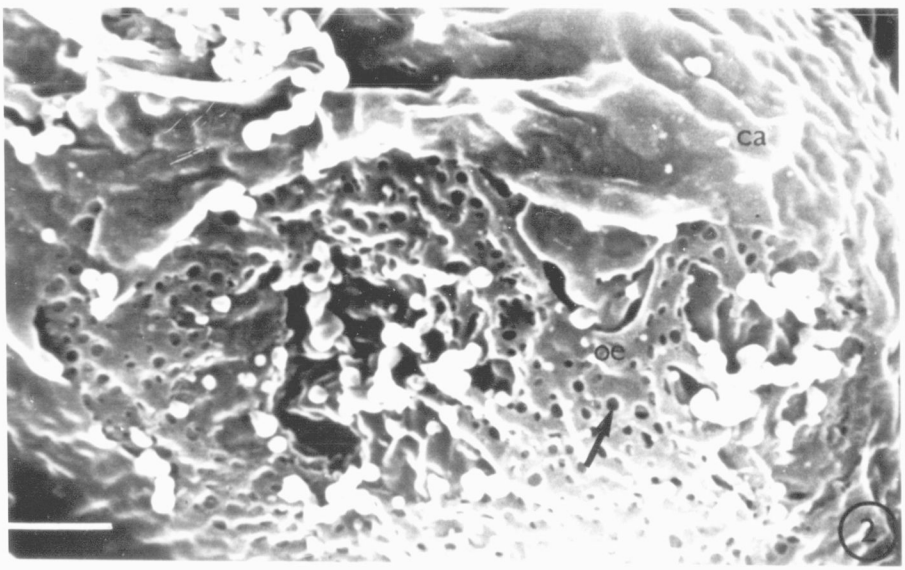
Plates 1 - 3. Scanning electron micrographs of fully-formed, mature eggs of T. crassiceps observed in situ in the uterus of gravid segments.

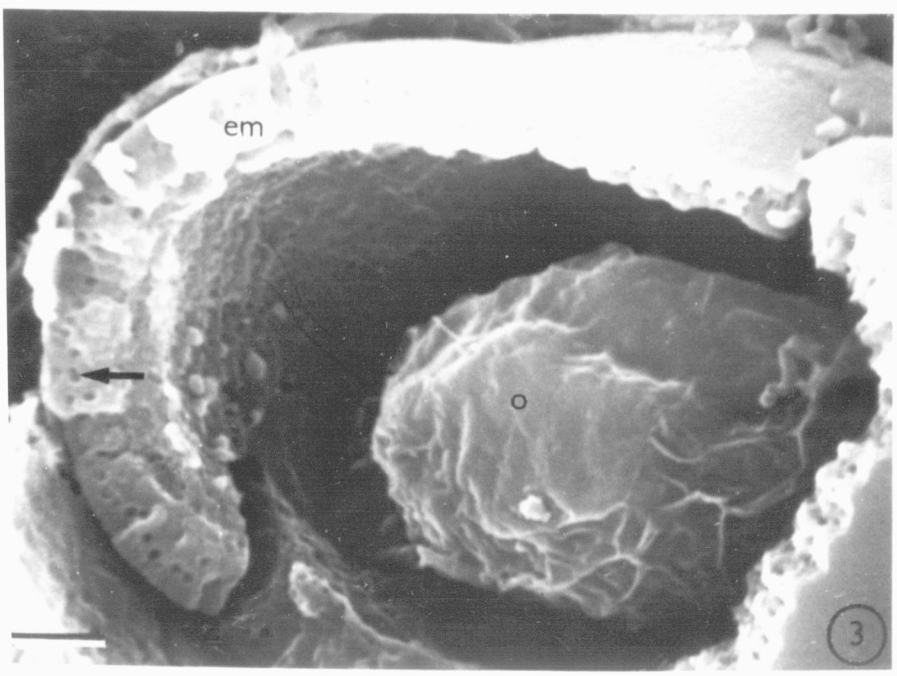
1. Low magnification of eggs. The remains of the capsule and outer envelope (arrow) can be observed on the smooth embryophore surface. (Scale = 10 μm)

2. High magnification of the surface of the outer envelope showing a relatively smooth capsule (ca) over the outer envelope (oe) with a reticulate surface as a result of randomly spaced pits (arrow). (Scale = 2 μm)

3. An egg split open to reveal the embryophore (em) and the cavities (arrow) among the embryophoral blocks. The oncosphere (o) can be seen inside the embryophore. (Scale = 2 μm)


1


ca
oe
2


em
o
3

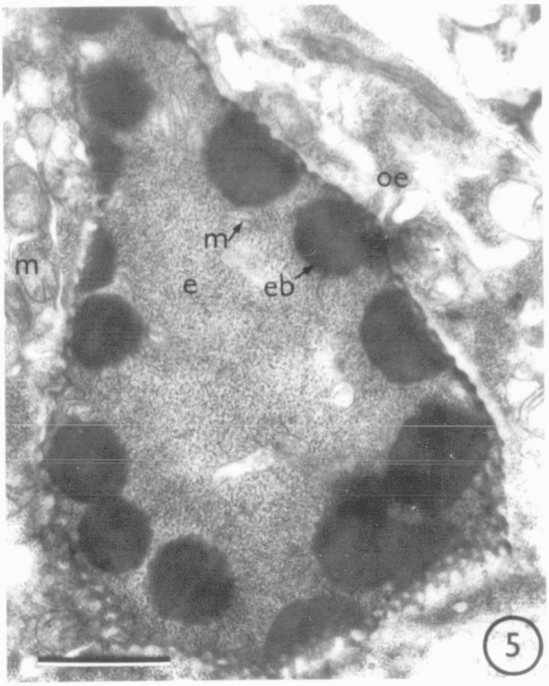
Plates 4 - 7. Transmission electron micrographs of the egg.

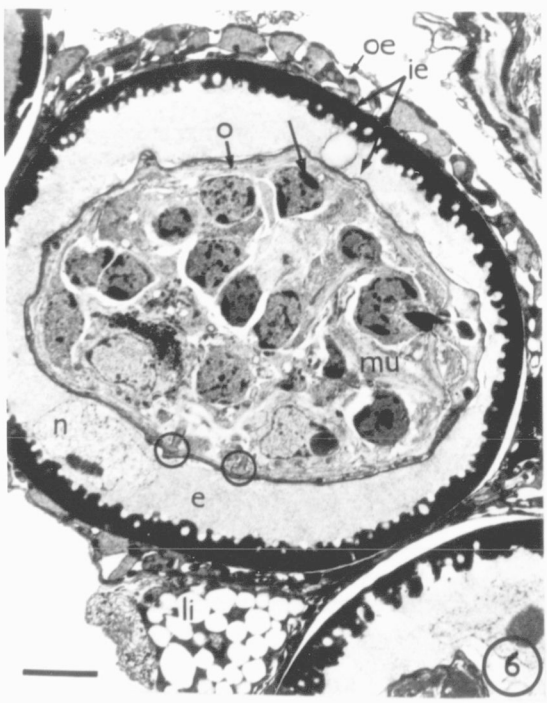
4. Early formation of the embryophore. Embryophoral blocks (eb) line the outer membrane of the embryophore cell (e). The outer envelope cell (oec) projects cytoplasmic extensions which are rich in mitochondria (m). Oncospheral hooks (h) are already present in the oncosphere. A group of lipid droplets (li) is closely associated with the outer membrane of the fully-formed embryophore (em). (Scale = 2 μm)

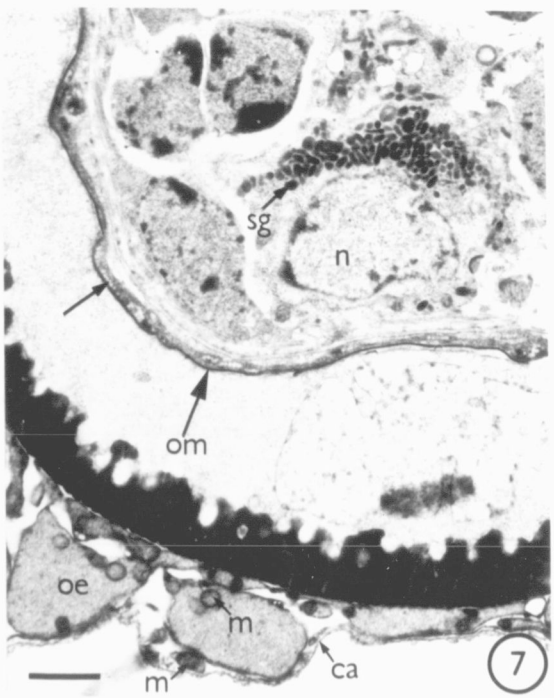
5. An embryophore cell (e) showing the embryophoral blocks (eb) lining the cell membrane and mitochondria (m) in the granular cytoplasm. The outer envelope (oe), has granular cytoplasm rich in mitochondria (m). (Scale = 1 μm)

6. A fully-formed egg with the outer envelope (oe) closely applied onto the inner envelope (ie), and the oncosphere (o) with cells containing nuclei with dense chromatin material (arrow). Note the nucleus (n) of the embryophore cell. Muscles (mu) traverse the oncosphere and some are inserted at the oncospheral membrane by modified bulbous endings (circled). Lipid droplets (li) are closely associated with the outer membrane. (Scale = 3 μm)


em
li
h
oec
e
m
eb
4


oe
m
m
e
eb
5


oe
ie
o
mu
n
e
li
6


sg
n
om
oe
m
m
ca
7

7. High magnification of the oncosphere. Both the capsule (ca) and the outer envelope (oe) contain mitochondria (m). Note the granular cytoplasm of the outer envelope. There are small closely packed vesicles (arrow) associated with the oncospheral membrane (om). Note the secretory gland cell in the oncosphere with a large nucleus (n) and prominent secretory granules (sg). (Scale = 1 μm)

Plates 8 - 12. Transmission electron micrographs of the egg.

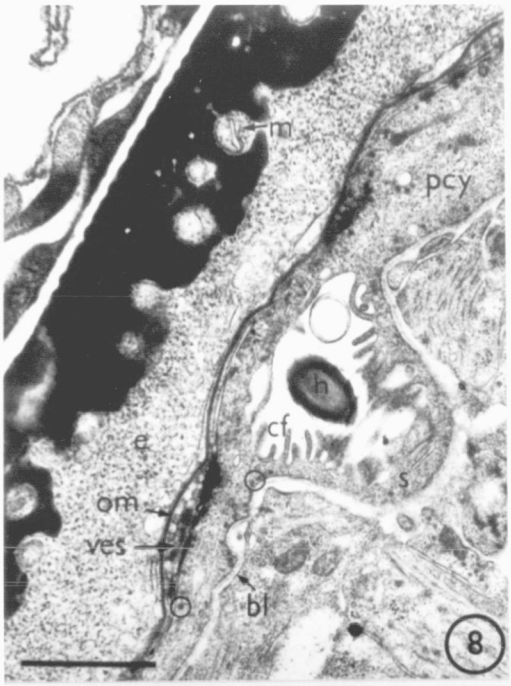
8. High magnification of the egg showing the darkly stained embryophore with cavities occupied by mitochondria (m). The embryophore cell (e) has a cytoplasm rich in granules possibly ribosomal. Note the vesicles (ves) between the 2 laminae of the oncospheral membrane (om). An oncospheral hook (h) lies among cytoplasmic folds (cf) at the core of a sheath (s) which is a modified region of the peripheral cytoplasm (pcy). Dense granules (circled) can be located in the peripheral cytoplasm particularly near the sheath. Below the peripheral cytoplasm is the basal lamina (bl) that extends round the whole oncosphere. (Scale = 1 μm)

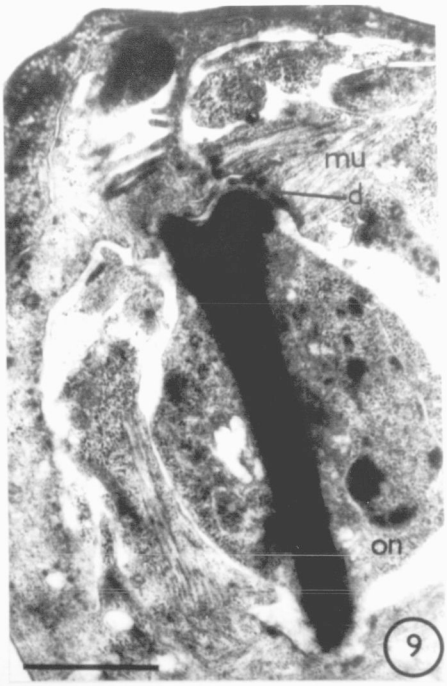
9. An oncospheral hook located intracellularly in a hook-forming cell or oncoblast (on). Muscle fibres (mu) are attached to the hook by desmosomes (d). (Scale = 1 μm)

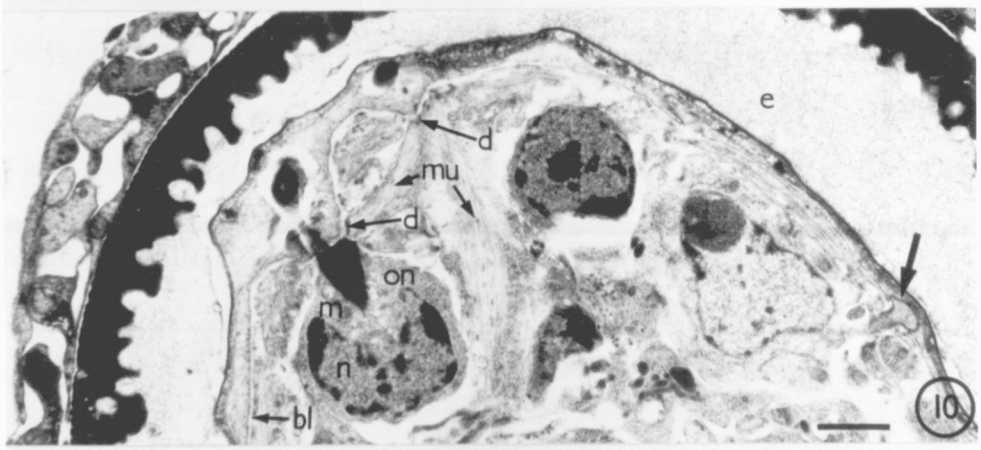
10. The oncospheral hook has its base in the oncoblast (on) which has a large nucleus (n) and numerous mitochondria (m) . Muscle fibres (mu) traverse the embryo and are attached at the hook and basal lamina (bl) by desmosomes (d) or a special bulbous ending (arrow). (Scale = 1 μm)

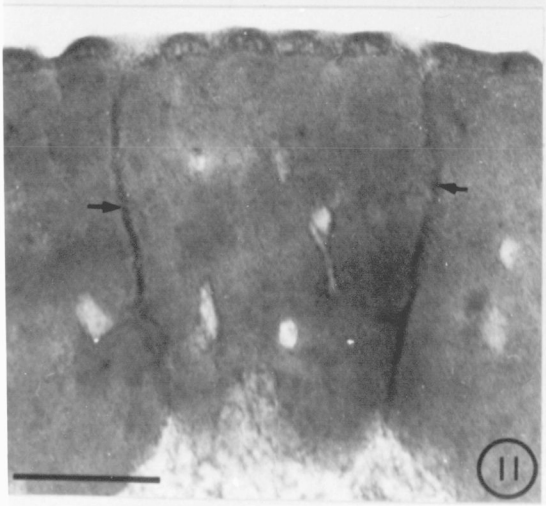
11. High magnification of the embryophore showing an amorphous material, possibly representing a cementing substance, between the keratin blocks.(Scale = 0.25 μm)

12.. An infolding of the cytoplasm (arrow) may be the origin of the double laminated oncospheral membrane.(Scale = 2μm)


m
pcy
h
cf
e
s
om
ves
bl
8


mu
d
on
9


e
d
mu
d
on
m
n
bl
10


11

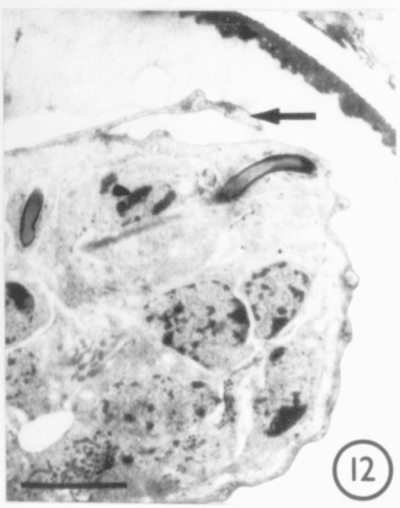

12



Plate 13. Photograph of T. crassiceps metacestodes of the KBS strain. Most of these cysticerci have an inverted scolex. (x 10)

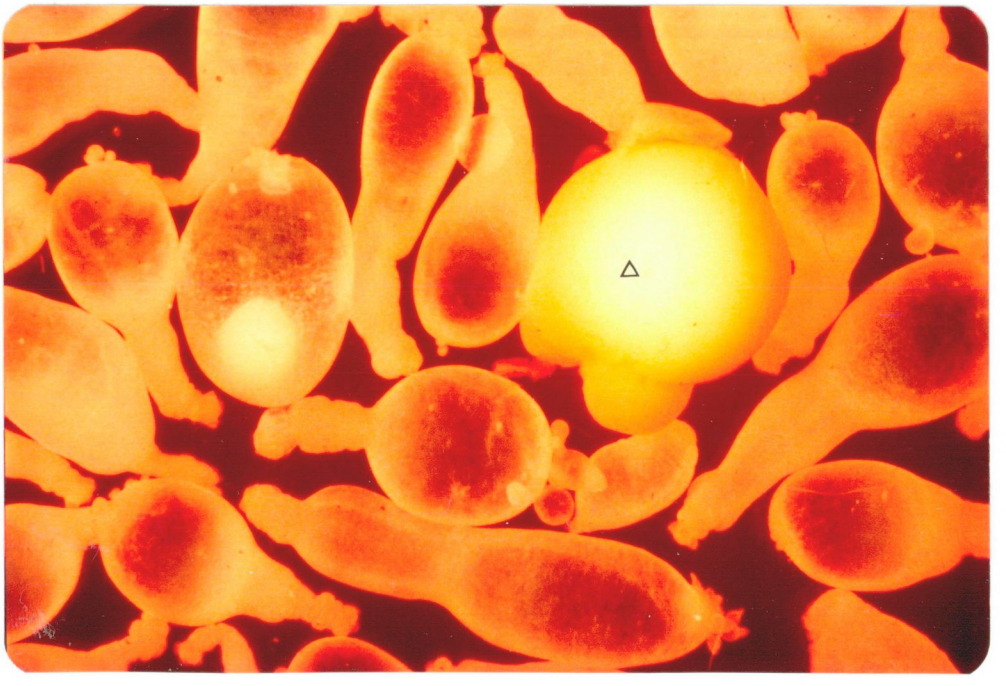


Plate 14. Photograph of T. crassiceps metacestodes of the TOI strain. Most of these cysticerci have an evaginated scolex. Note the opaque caseous mass (△) which results from encapsulation of larva by host material. (x 10)



Plate 15. T. crassiceps (KBS) scolex showing 2 rows of everted rostellar hooks. (Whole mount; formal-saline; Gower's Carmine) (x 160)



Plate 16. T. crassiceps (KBS) scolex showing non-everted rostellar hooks stained for -ss- proteins. (Whole mount; formal-saline; performic acid-Schiff) (x 200)

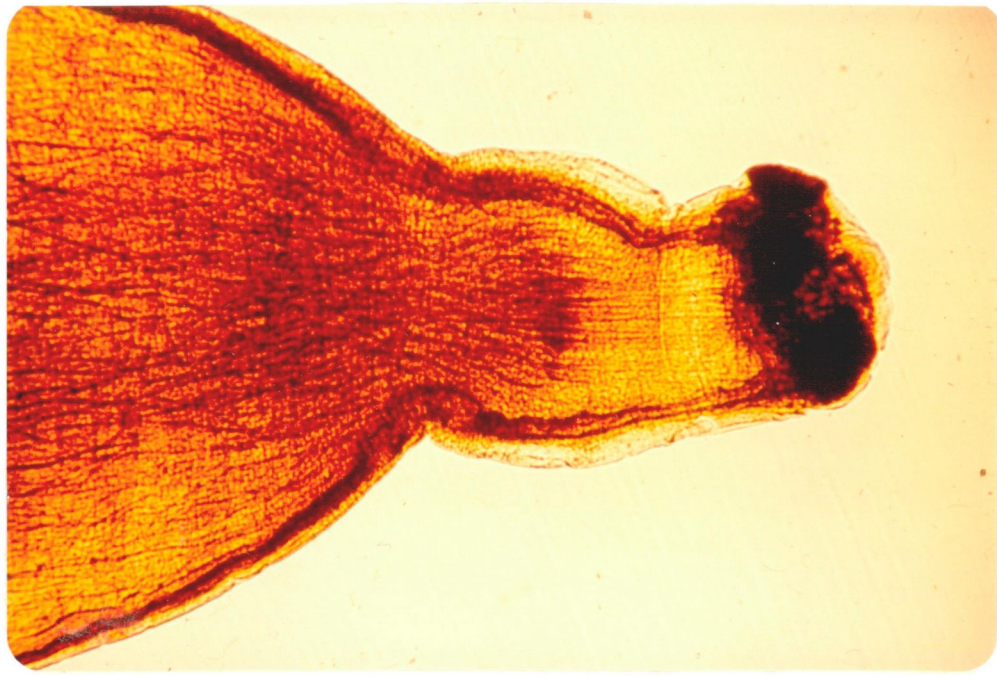


Plate 17. The gross nervous system of T. crassiceps cysticerci revealed by localization of specific cholinesterases. Note the ganglion mass at the scolex and in particular the heavily stained suckers. (Whole mount; cold formal-saline; BuThChI method) (x 50)

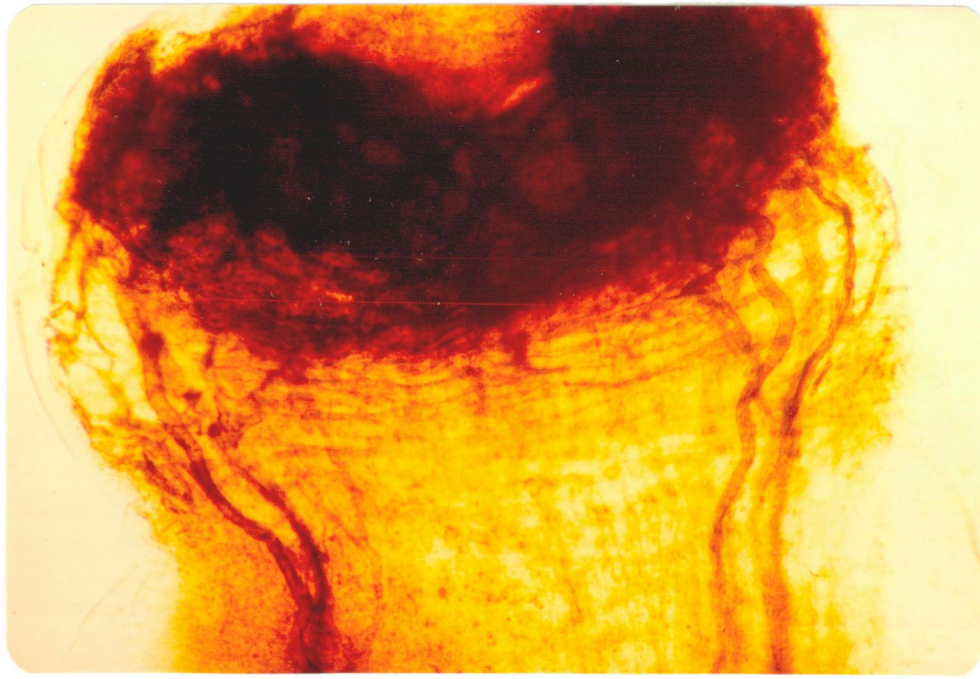


Plate 18. Higher magnification of scolex region to show nerve trunks running from the ganglion down along the neck of the cysticercus. (Whole mount; cold formal-saline; BuThChI method) (x 200)

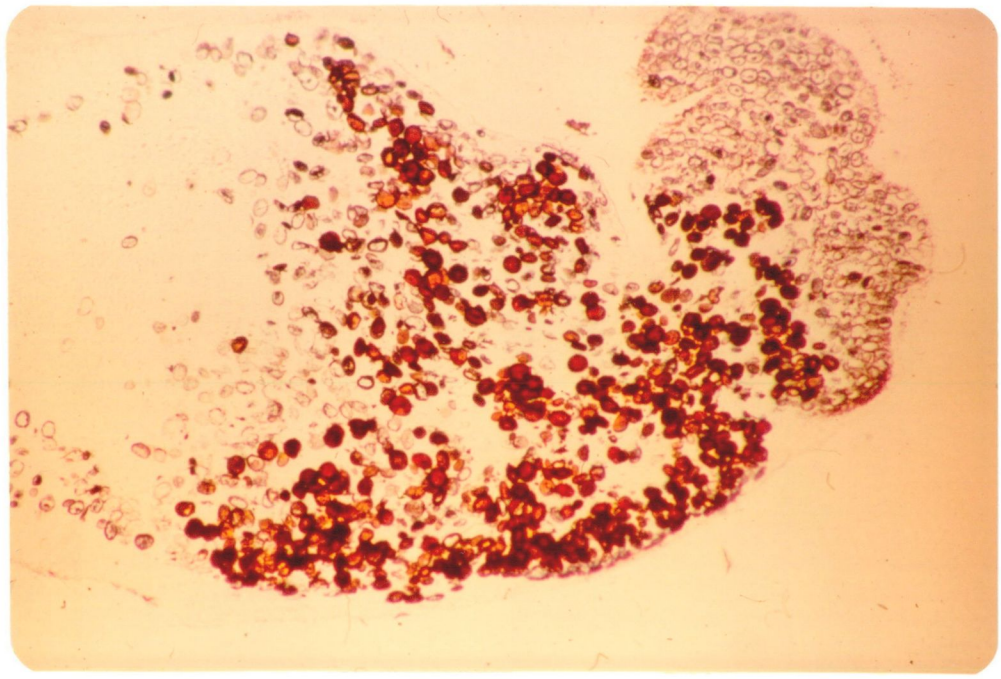


Plate 19. Light photomicrograph of L.S. of a cysticercus showing intensely stained calcareous corpuscles. (Formalin; $AgNO_3$/von Kossa method) (x 200)

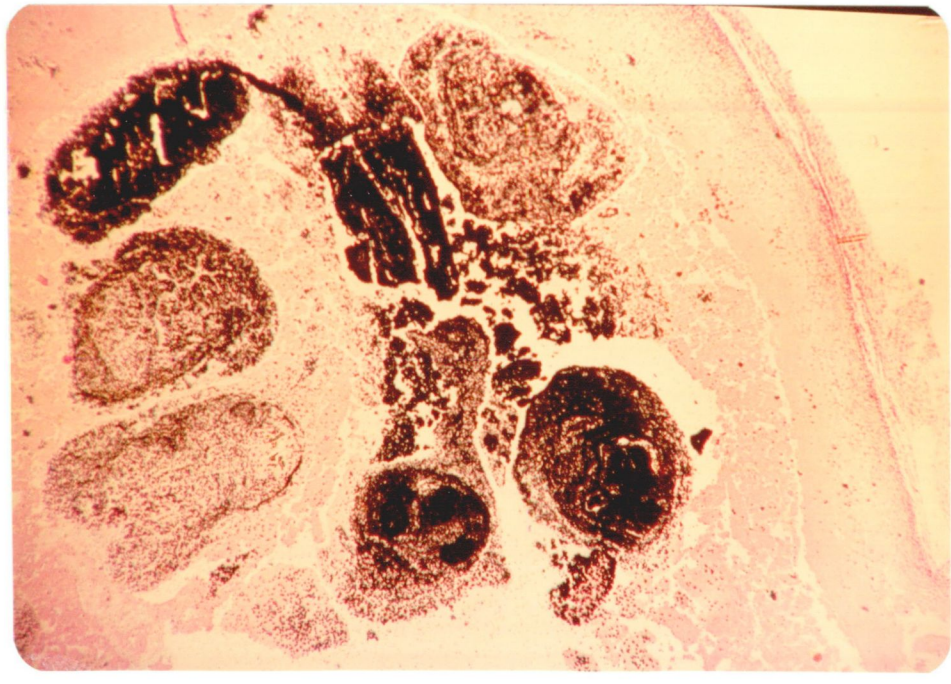


Plate 20. Light photomicrograph of T.S. of an encapsulated cyst. The outline of the worm(s) can hardly be determined. Insoluble calcium compounds, presumably from the calcareous corpuscles, are stained as an amorphous ground material. (Formalin, $AgNO_3$/von Kossa method) (x 50)

Plates 21 - 25. Scanning electron micrographs of the scolex of T. crassiceps cysticercus.

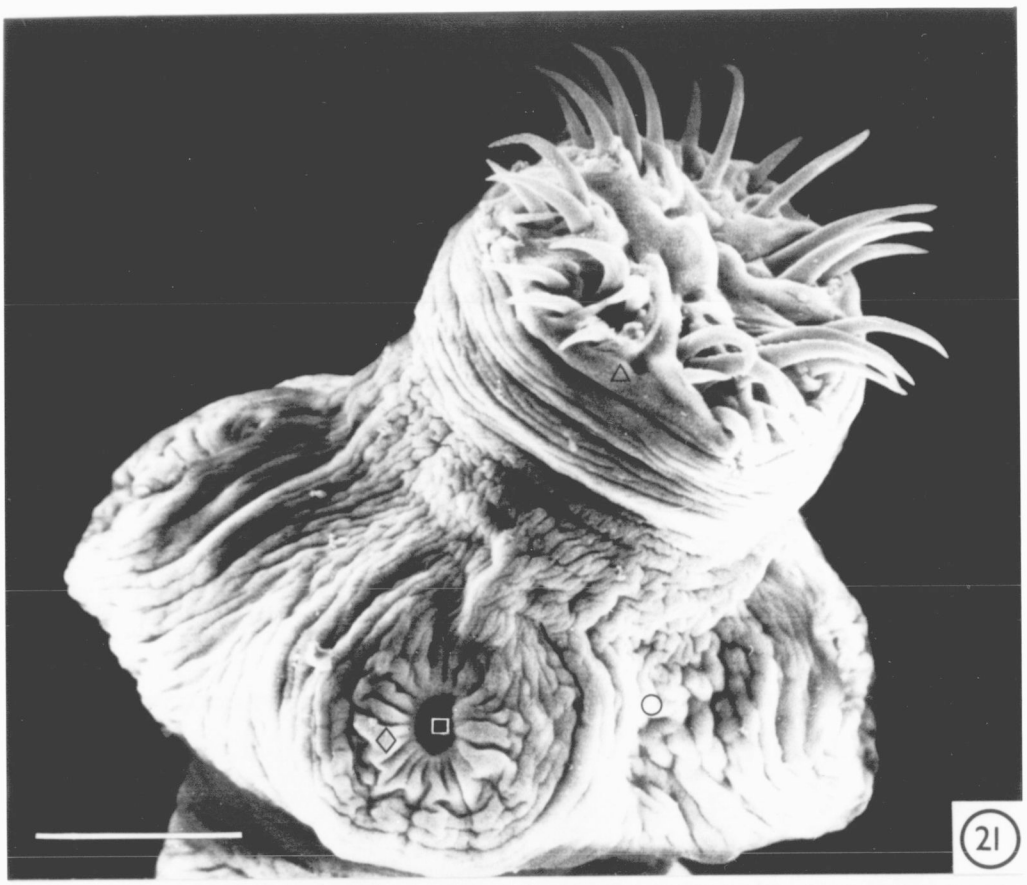
21. General view of the scolex showing typical taeniid features of 4 suckers and 2 rows of rostellar hooks. Higher magnifications of the surface microtriches at the scolex (○), rostellar rim (△), sucker rim (◇), and inner sucker area (□) are given in Plates 22 - 25. (Scale = 100 μm)

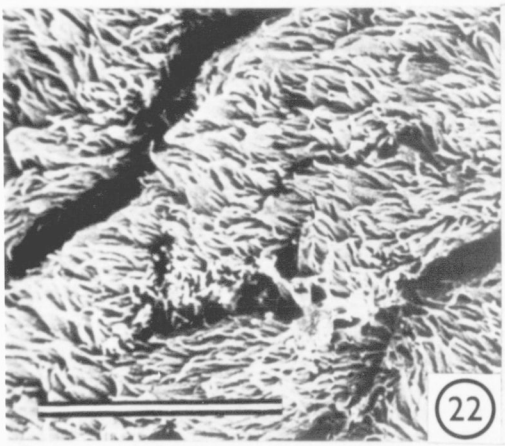
22. Microtriches on the surface of the scolex between the suckers. (Scale = 10 μm)

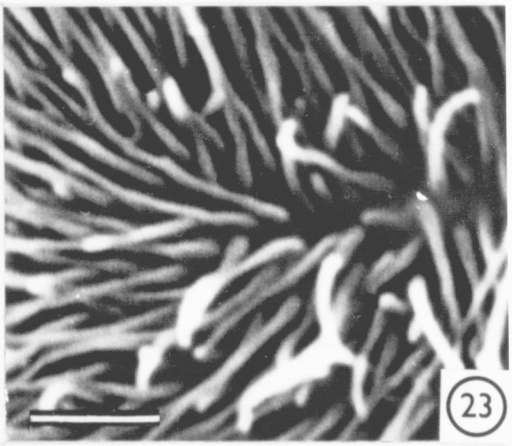
23 Microtriches at the rostellar rim. (Scale = 1 μm)

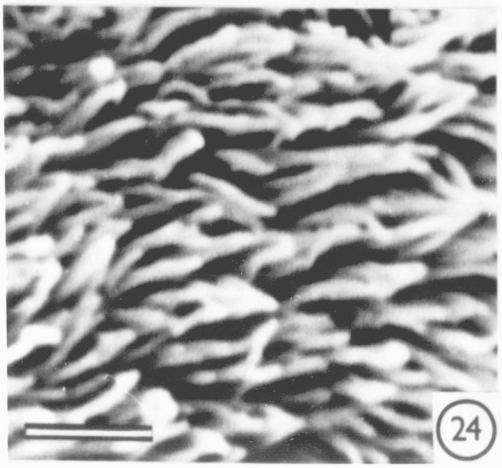
24. Microtriches at the sucker rim. (Scale = 1 μm)

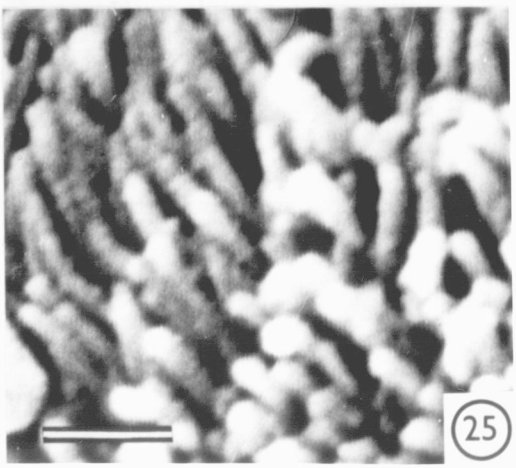
25. Microtriches on the inner cup surface of the sucker. These microtriches are thicker than those found elsewhere on the scolex, and appear to have round-tipped shafts. (Scale = 1 μm)


△
□
◇
○
21


22


23


24


25

Plates 26 - 30. Scanning electron micrographs of the surface structures of T. crassiceps cysticerci.

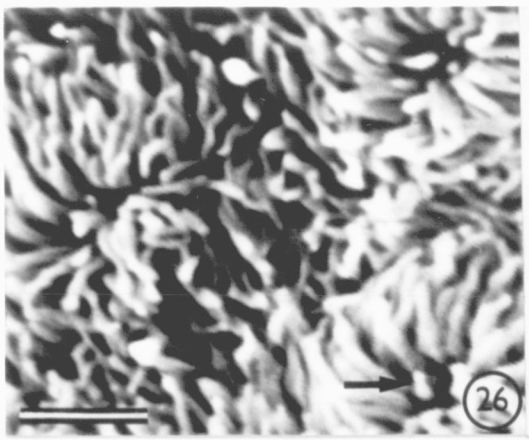
26. Rosette-like formations occur on the sucker rim as a result of a circle of outward curving microtriches surrounding an erect projection (arrow). This structure is presumed to be sensory in function. (Scale = 1 μm)

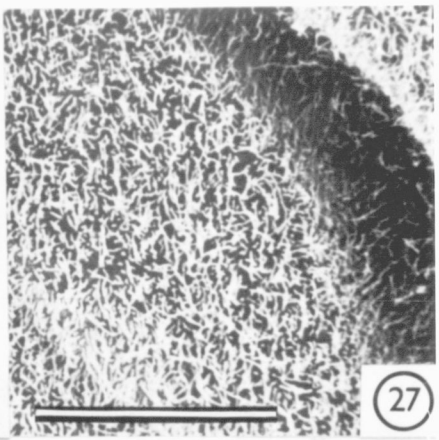
27. A mesh-work of fine microtriches found at the neck surface of the cysticercus. (Scale = 10 μm)

28. A budder with a group of attached buds (b) some of which possess the indentations representing scolex-canal primordia (arrow). (Scale = 100 μm)

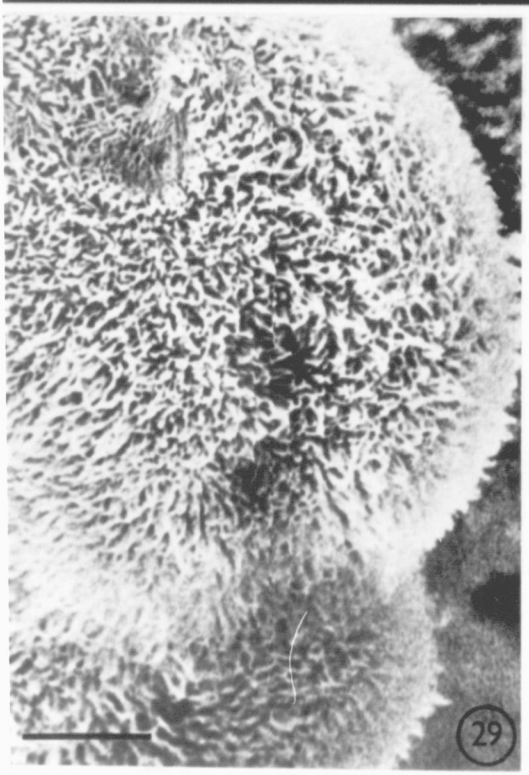
29. Rostellar protrusions with a dense microtrichal surface. (Scale = 1 μm)

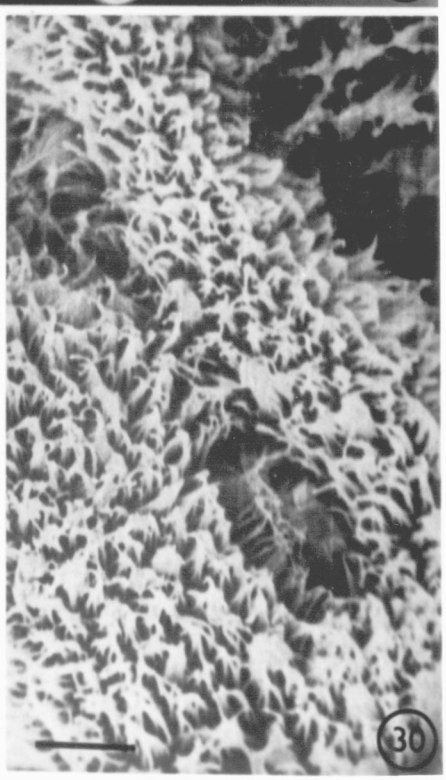
30. Microtriches on the bladder wall of the cysticercus. Note the bent distal regions of the microtrichal shafts. (Scale = 3 μm)


26


27


b
28


29


30

Plates 31 - 35.. Scanning electron micrographs of an aged budder from an 8 - month old infection.

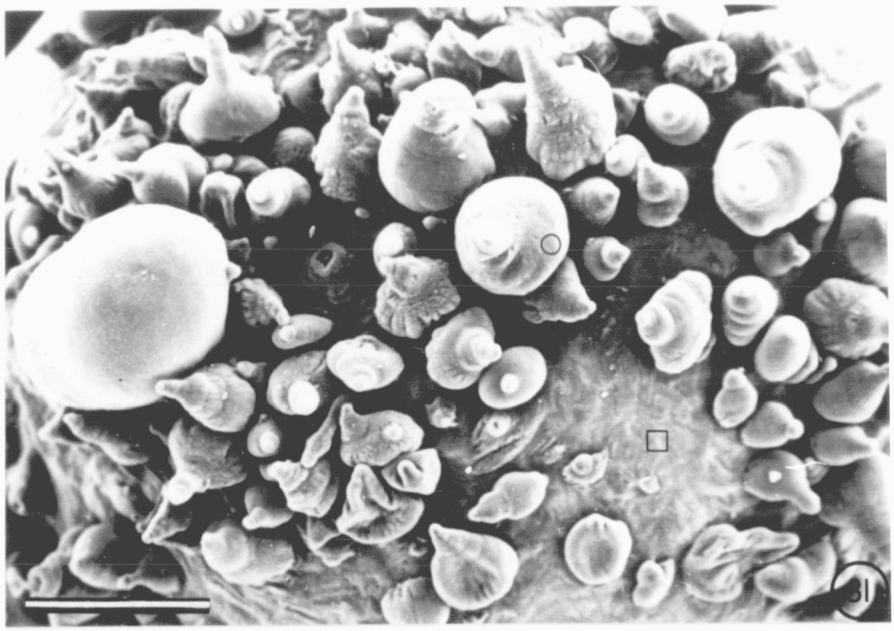
31. The surface of the budder is covered with conical buds of varying shapes and sizes. Higher magnifications of the microtriches on the budder surface (□) and the bud surface (○) are shown in Plates 34 & 35 respectively. (Scale = 500 μm)

32. A bud with what appears to be a scolex primordium where tegumental indentations occur (arrow). (Scale = 30 μm)

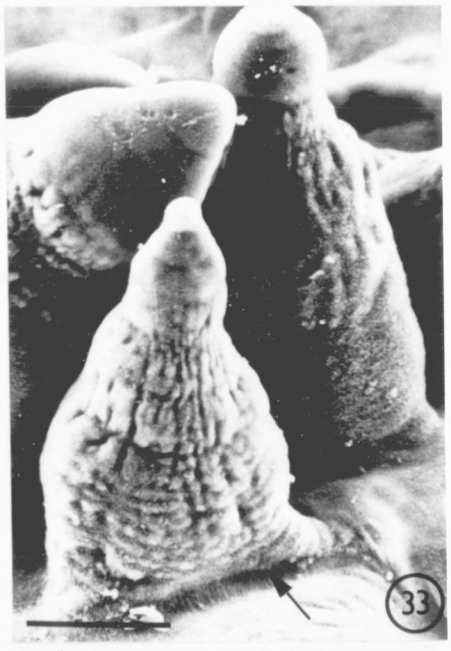
33. The buds are attached to the parent budder along a broad base (arrow. (Scale = 100 μm)

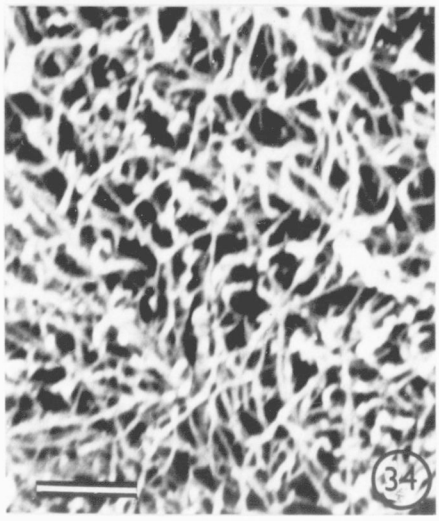
34. Microtriches on the tegumental surface of the budder are long and filamentous. (Scale = 2 μm)

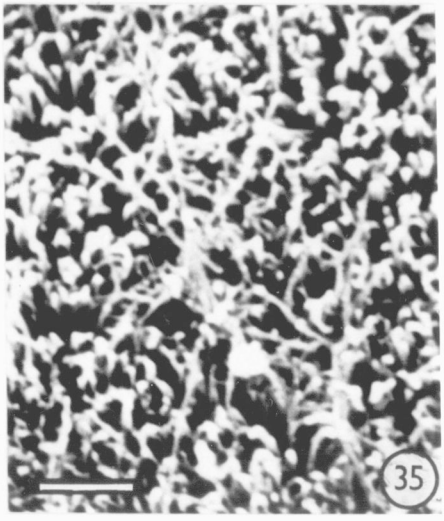
35. Microtriches on the tegumental surface of the buds are generally shorter and erect, with a few which are long and filamentous. (Scale = 2 μm)


31


32


33

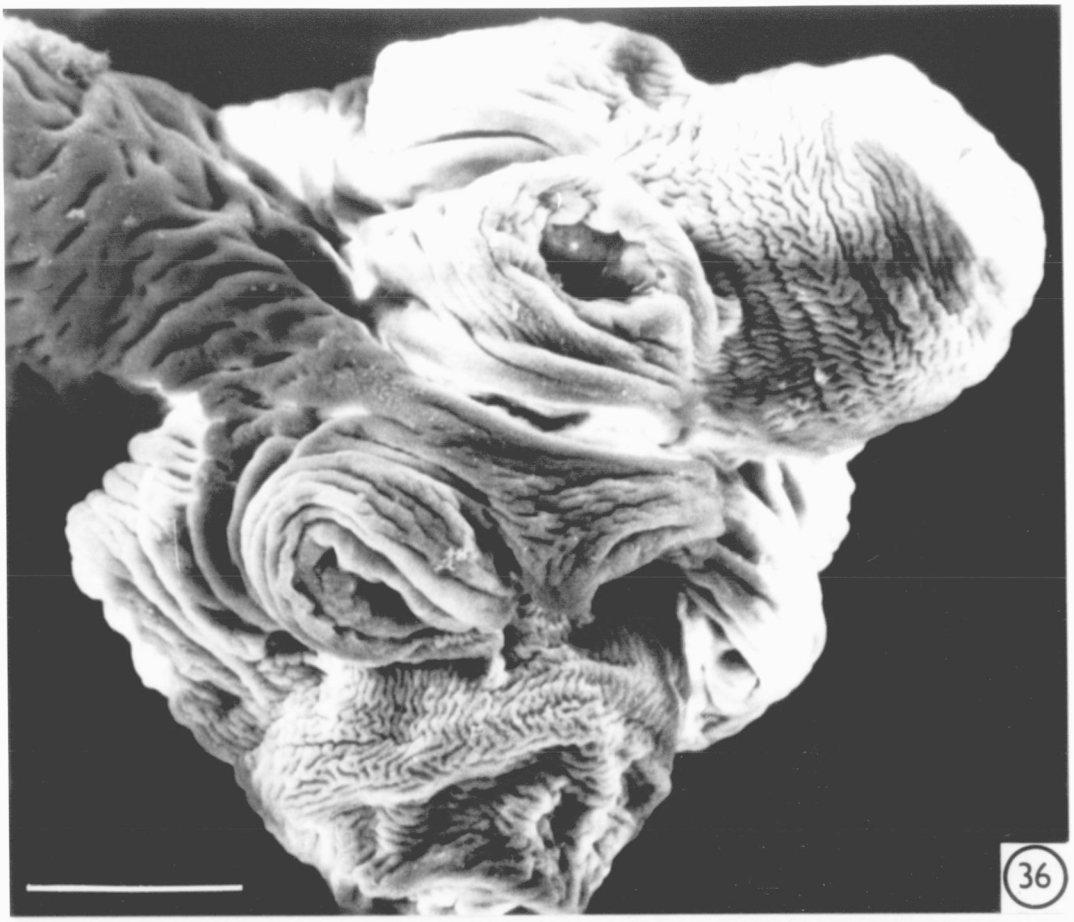

34

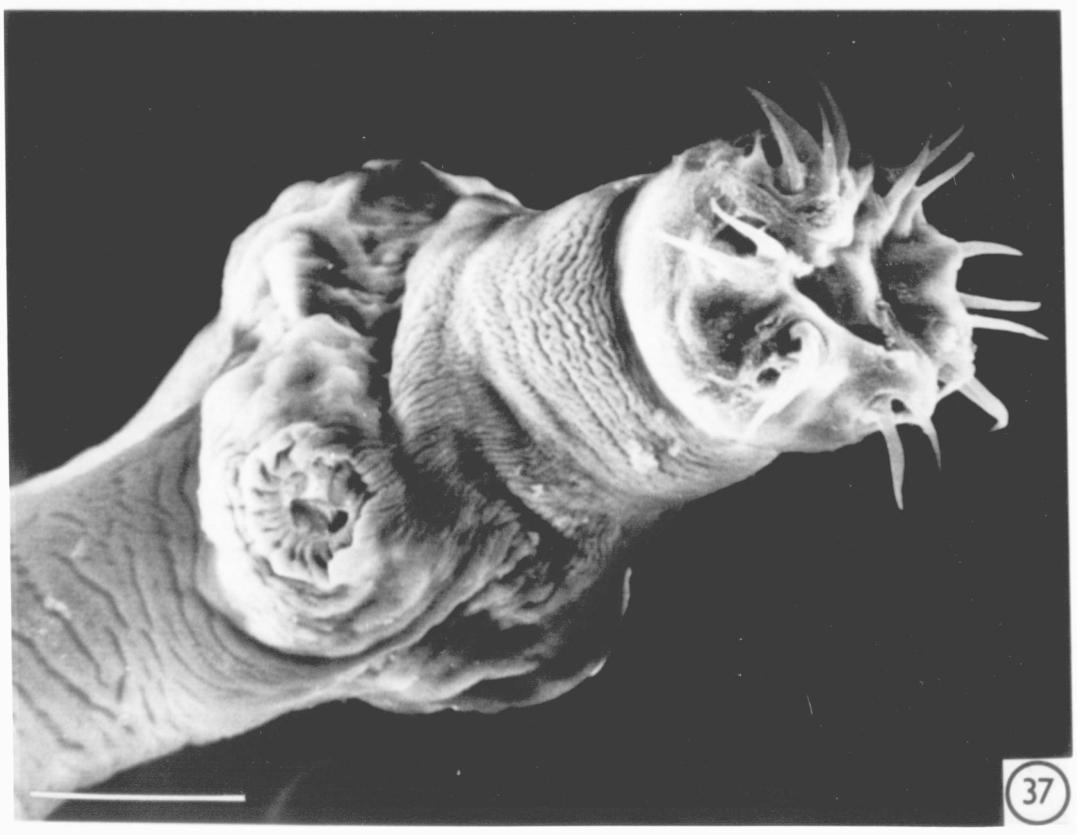

35

Plates 36 & 37. Scanning electron micrographs of abnormal scoleces of T. crassiceps cysticerci.

36. A double set of scolexes on one cysticercus resulting in 8 suckers and 2 rostella. Hooks are either absent or not everted from the rostella. (Scale = 100 μm)

37. A rostelum with only 18 hooks, about half the normal number. Note some of the irregularly shaped hooks. (Scale = 100 μm)


36


37

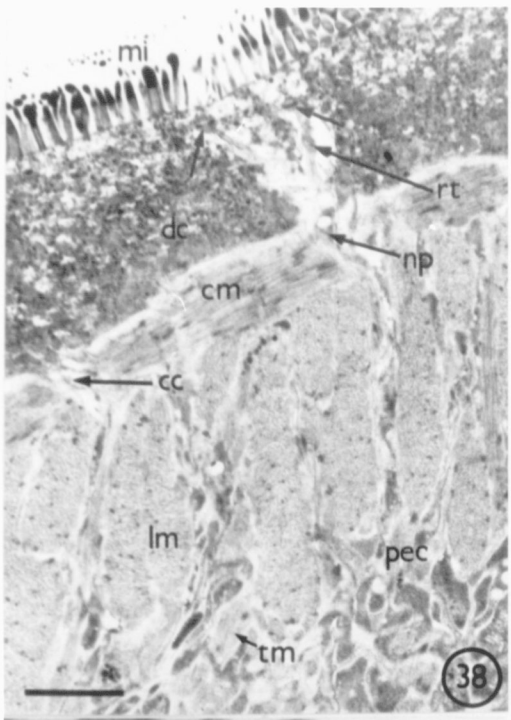
Plates 38 - 44. Transmission electron micrographs of the tegument and associated structures in T. crassiceps.

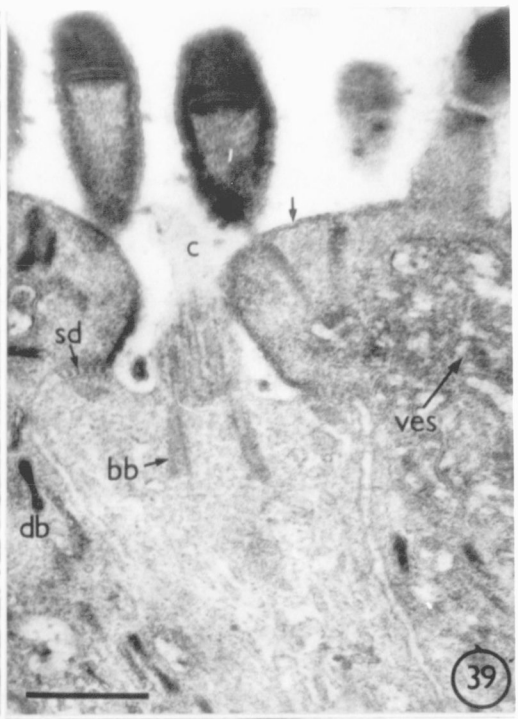
38. A context view of the tegument showing the microthrix border (mi), granular distal cytoplasm (dc) with its cytoplasmic connections (cc) to the perinuclear cytoplasm (pec). Circular (cm), longitudinal (lm) and transverse (tm) muscles are prominent. Note the nerve process (np) terminating in a sensory organelle containing a pair of receptors (arrow) with rootlets (rt) within the distal cytoplasm. (Scale = 3 μm)

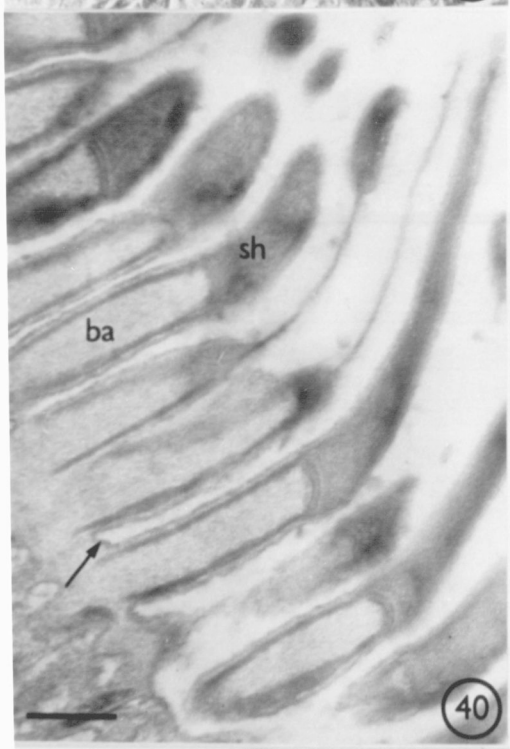
39. A terminal process with a sensory cilium (c) projecting beyond the tegument. Microtubules in the cilium appear to arise from basal bodies (bb). Note the septate desmosomes (sd), the membrane bound disc-shaped bodies (db), vesicles (ves) and the plasma membrane (arrow) covering the distal cytoplasm. (Scale = 0.25 μm)

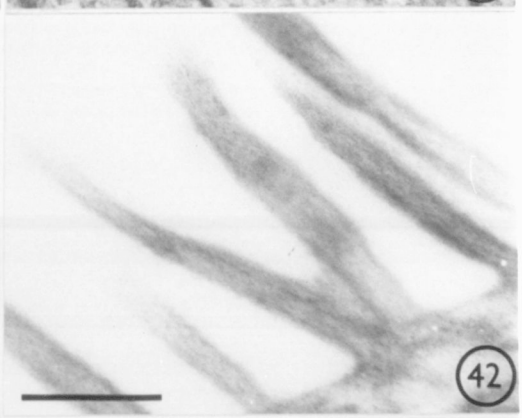
40. Microtriches basically consist of a base (ba) and shaft (sh) which may be attenuating, filamentous, or filamentous with a bulbous tip. A continuous membrane (arrow) covers the microtriches and distal cytoplasm. (Scale = 0.25 μm)

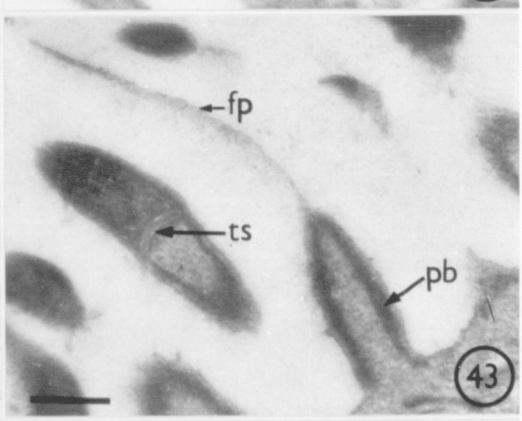
41. The base (ba) of the microtriches has an electron dense wall surrounding a less dense one. Microtubules (mt) run parallel along the longitudinal axis of the electron dense shaft (arrow). (Scale = 0.05 μm)

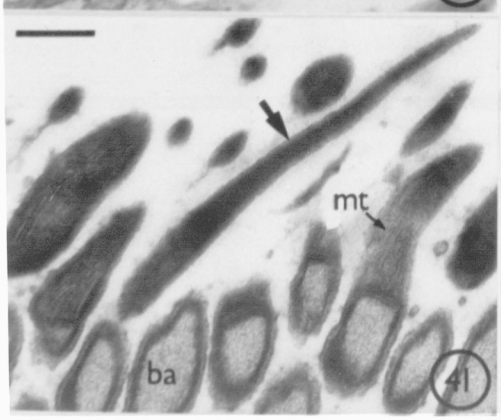

mi
rt
dc
np
cm
cc
lm
pec
tm
38

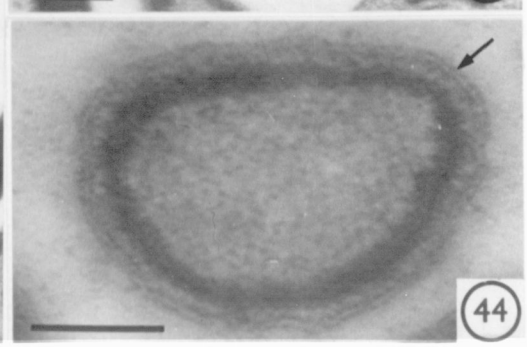

c
sd
ves
bb
db
39


sh
ba
40


42


fp
ts
pb
43


mt
ba
41


44

42. Thin microtriches located at the rostellar rim. Some are joined at the base. (Scale = 0,5 μm)

43. A microthrix with the shaft modified into a filamentous process (fp). Note the darkly stained peripheral wall of the microthrix base (pb). A tube-like structure (ts) marks the boundary between the base and the shaft in each microthrix. (Scale = 0,25 μm)

44. T.S. through the base of a microthrix. An electron lucid zone lies between the bilaminate membrane (arrow) and the dense wall of the base. Note the granular appearance at the core. (Scale = 0.1 μm)

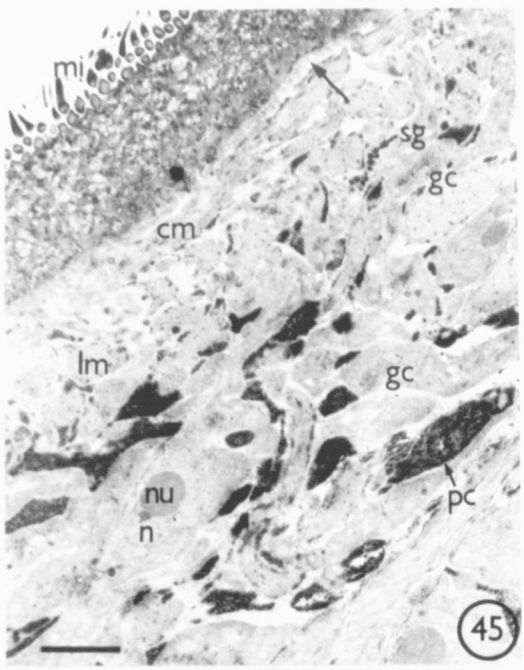
Plates 45 - 50. Transmission electron micrographs of the scolex region of T. crassiceps cysticerci.

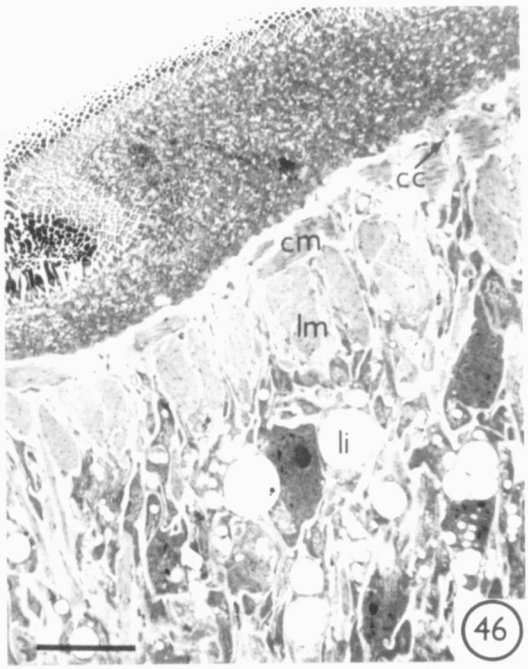
45. Section through the scolex region. Note the fibrous zone (arrow) between the distal cytoplasm and the circular muscles (cm). The cytons have large nuclei (n) with prominent nucleolus (nu) and golgi complexes (gc). Round secretory granules (sg) are found in groups among the longitudinal muscles (lm). Note also the glycogen-rich parenchymal cells (pc). (Scale = 2 μm)

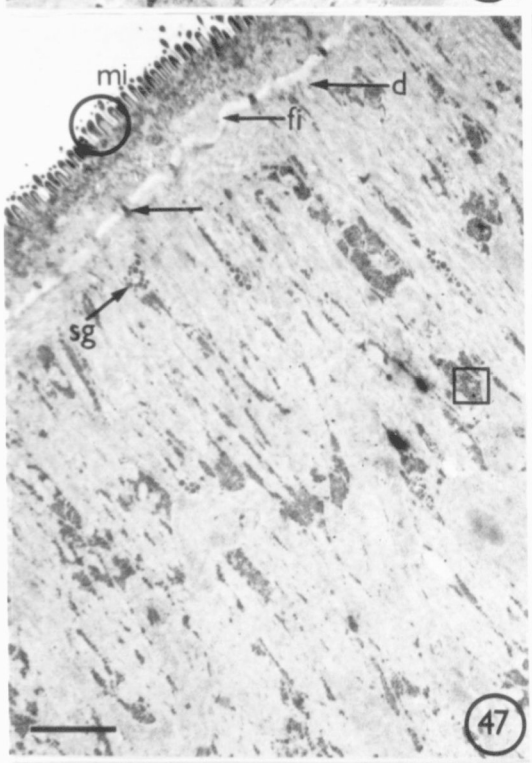
46. Section through the neck region. Cytoplasmic connections (cc) extend from the perinuclear cytoplasm through the muscle layers (cm, lm) into the distal cytoplasm. Note prominent groups of lipid droplets (li). (Scale = 5 μm)

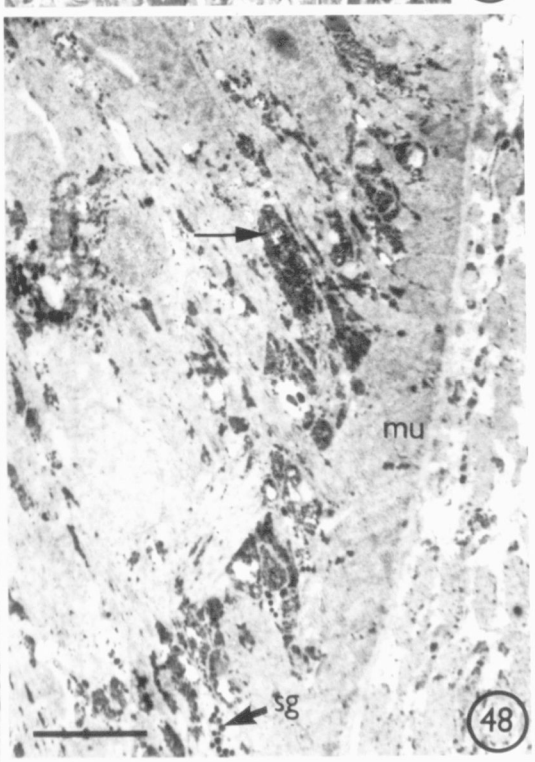
47. Section through the muscular sucker. Microtriches may be modified with filamentous bases and bulbous shafts (circled). The muscular sucker has desmosome-like points of attachment (arrow) with the distal cytoplasm while within the sucker, muscle fibres are attached to the fibrous zone (fi) by desmosomes (d). The sucker is rich in large granules (sg), and intermuscular spaces are filled with glycogen-rich cells (squared). (Scale = 2 μm)

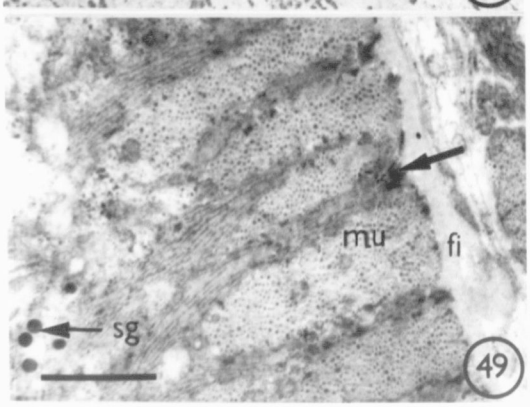
48. Section to show the muscle blocks (mu) along the sucker edge where it meets the scolex parenchyma. Note the abundance of glycogen granules (arrow) and the prominent round secretory granules (sg). (Scale = 3 μm)

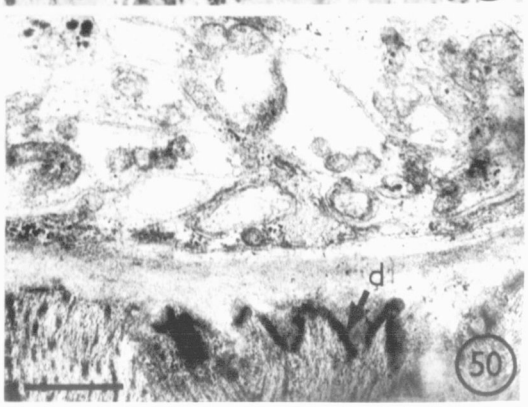

mi
sg
gc
cm
lm
gc
nu
n
pc
45


cc
cm
lm
li
46


mi
d
fi
sg
47


mu
sg
48


mu
fi
sg
49


d
50

49. Muscle blocks (mu) arranged along the inner border of the sucker. These muscles are attached by desmosomes (d) at the fibrous zone (fi) which surrounds the whole sucker. Note the electron dense secretory granules (sg). Scale = 1 μm)

50. Higher magnification of the desmosomes (d) mentioned above. (Scale = 0,5 μm)

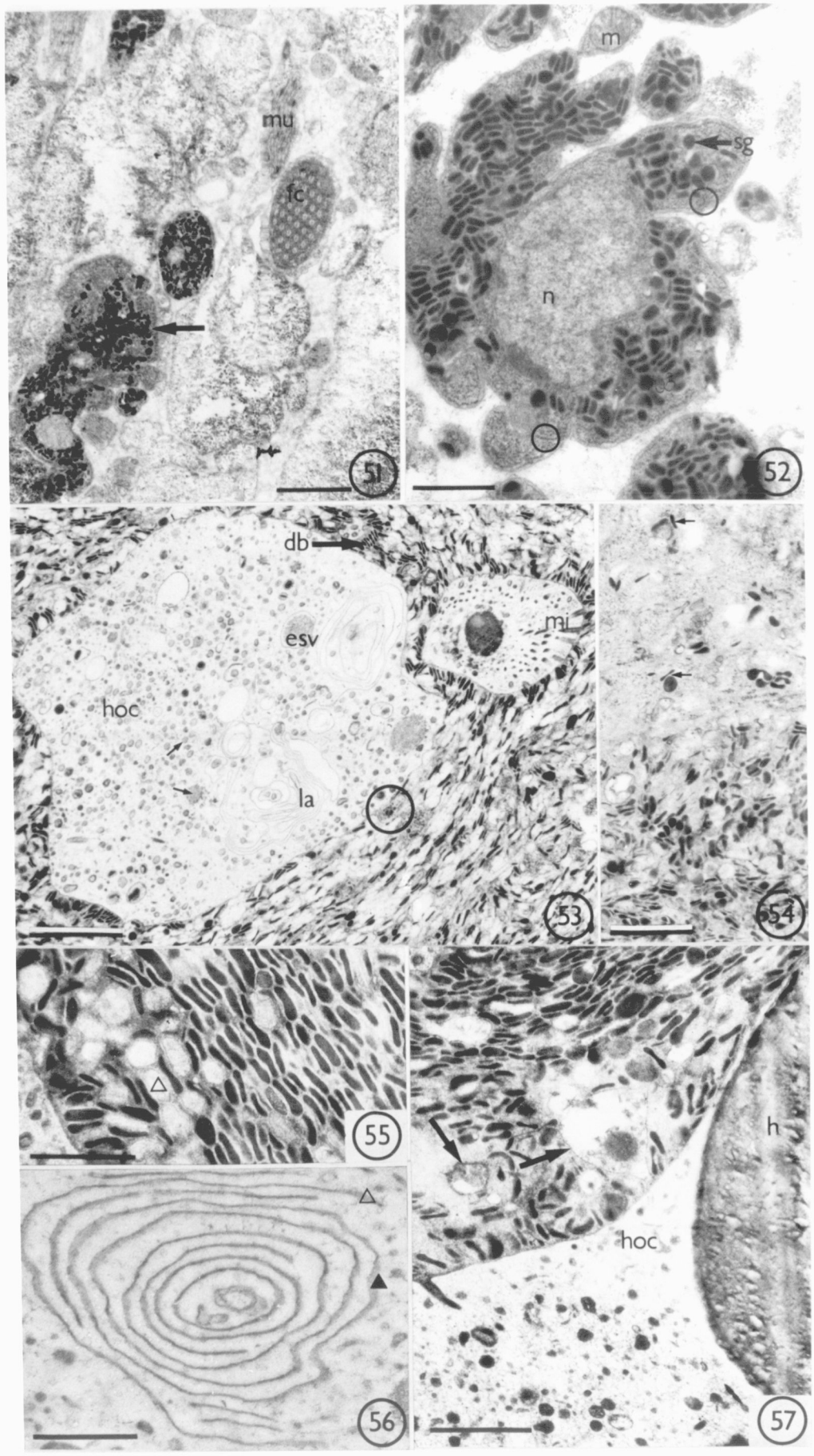
Plates 51 - 57 Transmission electron micrographs of the internal structures of the scolex and rostellar region of T. crassiceps

51. Among the parenchymal cells below the rostellar pad are secretory cells (arrow) with their dense disc-shaped granules, muscle fibres (mu) and flame cells (fc). (Scale = 2 μm)

52. High magnification of a secretory cell. Dense secretory granules (sg), granular endoplasmic reticulum (circled), and large mitochondria (m) are found in the irregular extensions of the ribosome-rich cytoplasm. Note the large nucleus (n). (Scale = 1 μm)

53. An area of dense, closely packed disc-shaped bodies (db) and vesicles, called the "hook organ", in the rostellum. Excretory/secretory material appear to be extruded into the lumen of canals (hoc) from the hook organ (circled). These excretory/secretory materials may be in membrane-bound vesicles (esv) or naked (arrow). Note the whorls of lamellae (la) in the lumen of the canals and the microthrix-like projections (mi). (Scale = 2 μm)

54. Region between the hook organ and the rest of the scolex parenchyma. Some disc-shaped bodies (arrow) are found among the parenchyma. (Scale = 1 μm)


mu
fc
51
m
sg
n
52
db
esv
mi
hoc
la
53
54
55
56
h
hoc
57

55. High magnification of the hook organ to show the disc-shaped bodies and vesicles (△) some of which are membrane-bound. (Scale = 0.5 μm)

56. High magnification of the lamella whorls to show their single laminated membrane-like structure. Note that some are continuous bands (▲) while others are not (△). (Scale = 0.5 μm)

57. Excretory/secretory material may be eliminated into the canal lumen in membrane-bound vesicles (arrow). Such material are abundant in the lumen of the canal (hoc). Note part of a rostellar hook (h). (Scale = 0.5 μm)

Plates 58 - 62. Transmission electron micrographs of T. crassiceps.

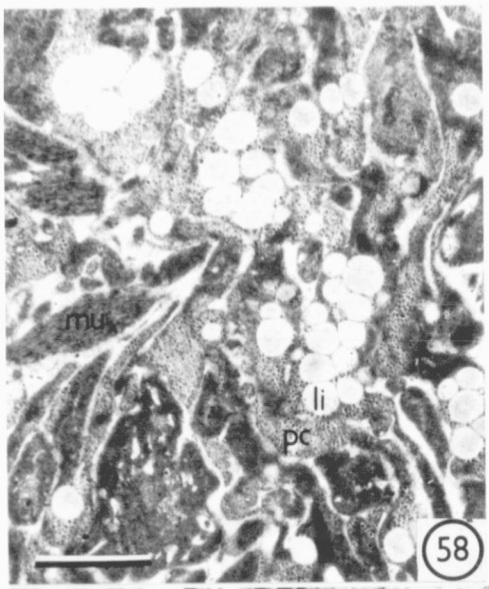
58. Section through the neck region to show muscles (mu) among closely packed glycogen-rich parenchymal cells (pc) with groups of lipid droplets (li). (Scale = 2 μm)

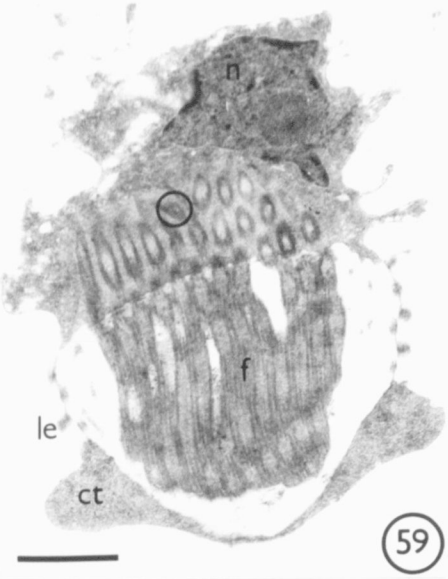
59. The flame cell body consists of 2 parts - one contains the large nucleus (n), and the other contains striated basal bodies (circled) of the cilia which comprise the "flame" (f). ct = conjoined tubules; le = leptotriches (Scale = 1 μm)

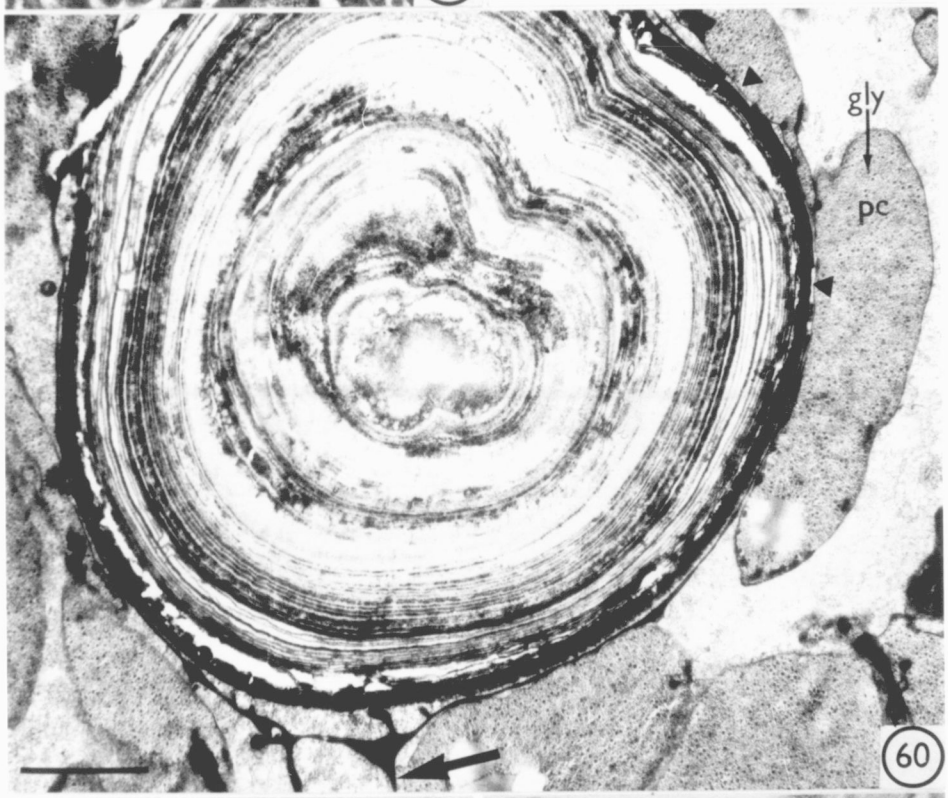
60. A calcareous corpuscle with concentric rings of dense material. The outermost ring is broken by gaps (▲) and has extensions into intercellular spaces (arrow). Note the glycogen-rich (gly) parenchymal cell closely applied to the corpuscle. (Scale = 2 μm)

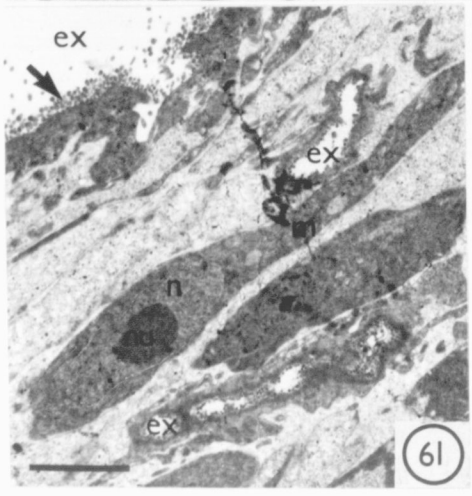
61. Excretory canals (ex) have bleb-like projections (arrow) into the lumen. The canal wall appears to be formed from darkly stained cells with a large nucleus (n) and nucleolus (nu), and numerous mitochondria (m). (Scale = 5 μm)

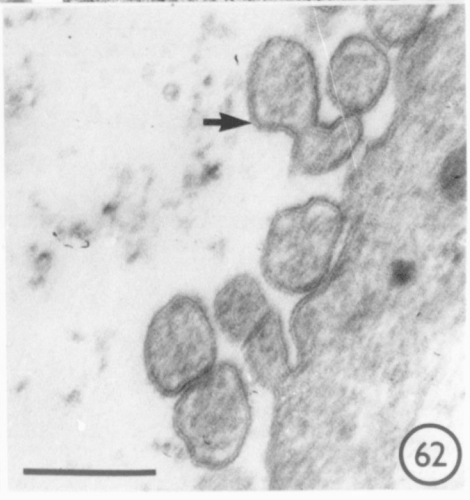
62. High magnification of the wall of the excretory canal showing the bleb-like projections. A plasma membrane (arrow) covers the wall and the projections. (Scale = 0.25 μm)


mu
li
pc
58


n
f
le
ct
59


gly
pc
60


ex
ex
n
ex
61


62

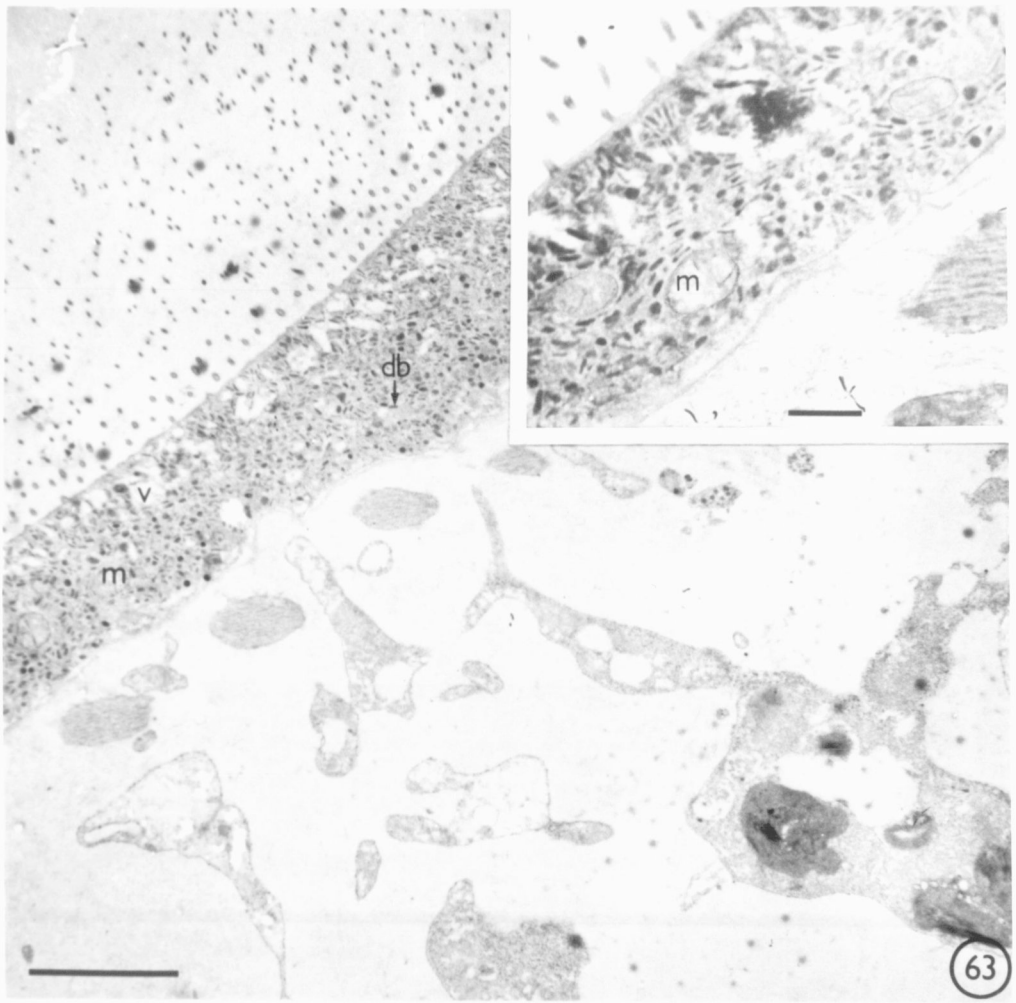
Plates 63 - 65. Transmission electron micrographs of the tegument and its associated structures at the bladder region of T. crassiceps cysticerci.

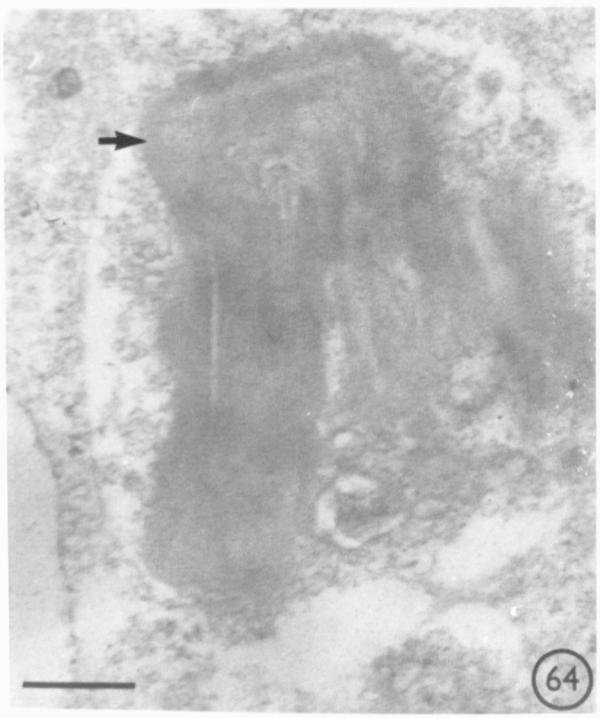
63. The distal cytoplasm resembles that in the scolex region with disc-shaped bodies (db) and vacuoles (v). However, the microtriches are finer and fewer in number, and mitochondria (m) are more numerous and larger. Note the dispersed distribution of muscles and tegumental cell bodies. (Scale = 3 μm)

63 (inset). High magnification of tegument to show the large mitochondria (m) .
(Scale = 1 μm)

64. Whorls of electron dense, closely packed lamellae are occasionally encountered in the perinuclear cytoplasm. (Scale = 0.25 μm)

65. The inner plasma membrane is often thrown into folds (arrow). (Scale = 0.5 μm)

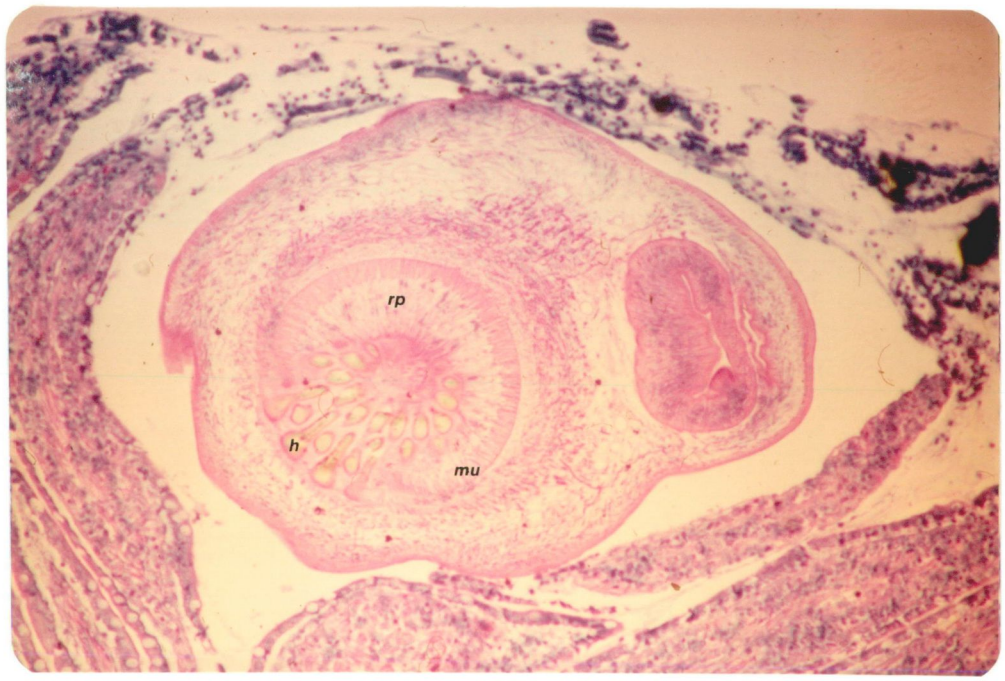

db
v
m
m
63


64


65

Plate 66. Transverse section of the scolex and rostellum of adult T. crassiceps showing part of a sucker, rostellar hooks (h), and the rostellar pad (rp). Note the muscle fibres (mu) arranged radially along the circumference of the pad. Note also the numerous mucous cells (bottom left) which might indicate metaplasia of epithelial cells. (Zenker's; Maximow) (x 200)

Plate 67. Transverse section of the scolex and rostellum of adult T. crassiceps. The hook organ (ho) is stained homogenously and appears as ovoid pockets within which lie the hooks (h). In the centre of the hook organ are the densely stained cells (▲) of the rostellar gland. Note the disruption of the villi epithelium around the hooks (left). (Zenker's; Maximow) (x 320)


rp
h
mu

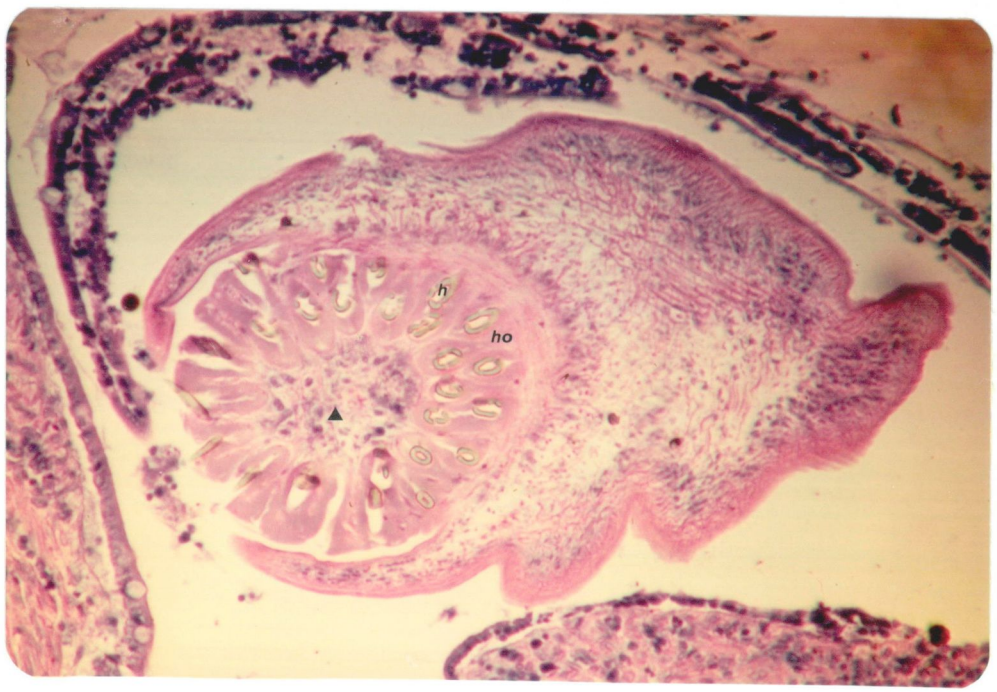
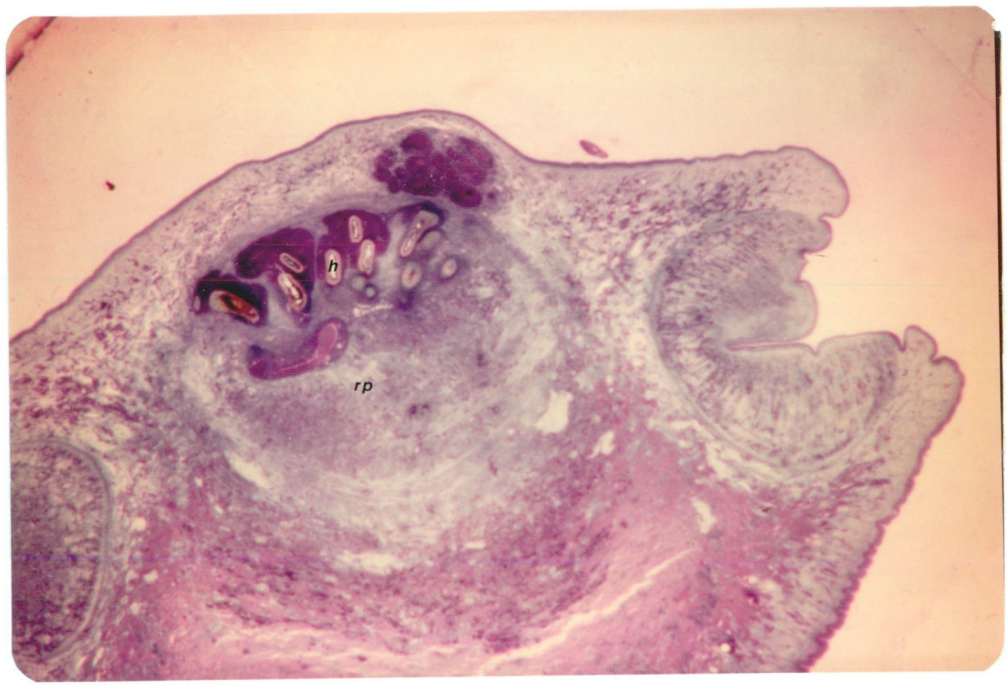

h
ho

Plate 68. Longitudinal section through the rostellum of adult T. crassiceps. Note the extensive areas of ß-metachromasia beneath and around the rostellar pad (rp) and closely associated with the hooks (h). These may represent the mucopolysaccharide secretory activity of rostellar gland cells and the hook organ. (Glutaraldehyde; Toluidine blue) (x 200)

Plate 69. Longitudinal section through the scolex of adult T. crassiceps showing the muscular rostellar pad (rp) and part of a sucker. Around the rostellar pad are cavities containing a mucin material (arrow). Note the flattening of the epithelial cells next to the scolex. (Formalin; Masson's) (x 200)


h
rp

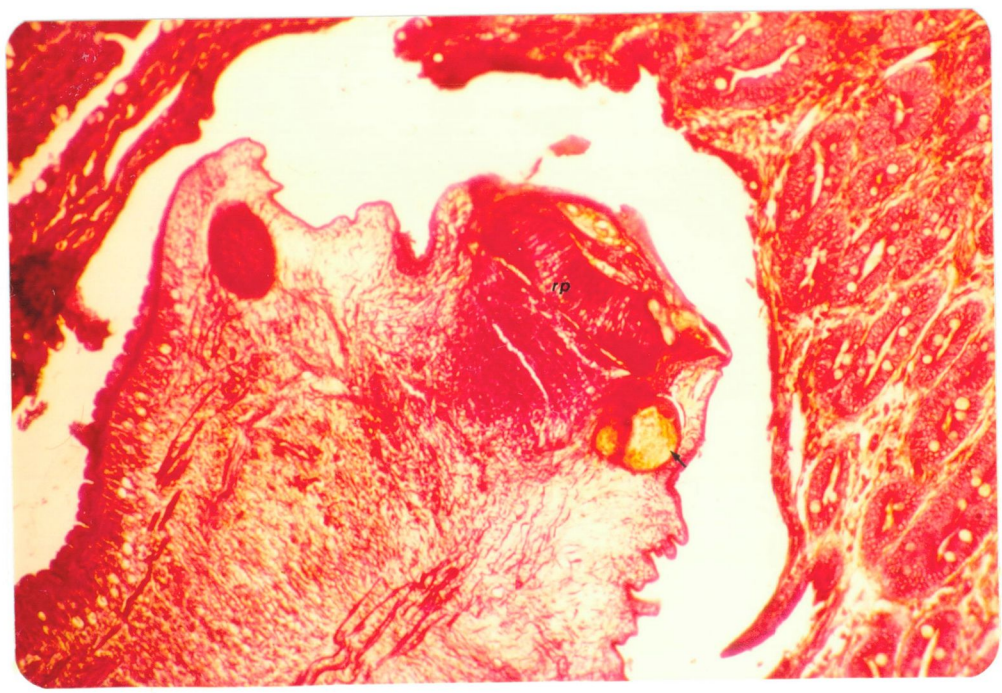
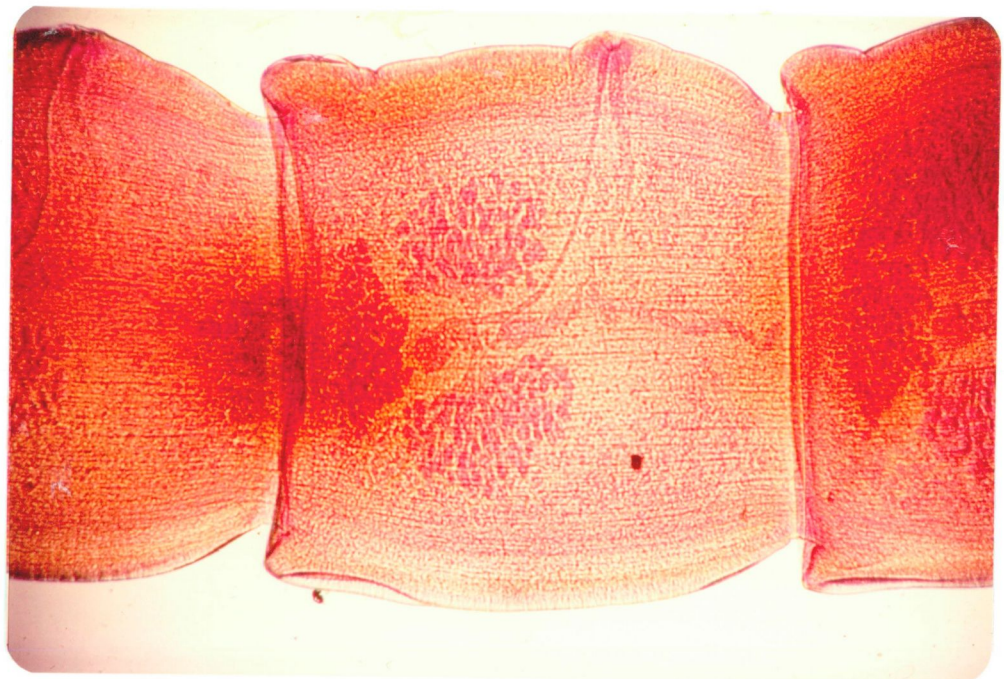

rp

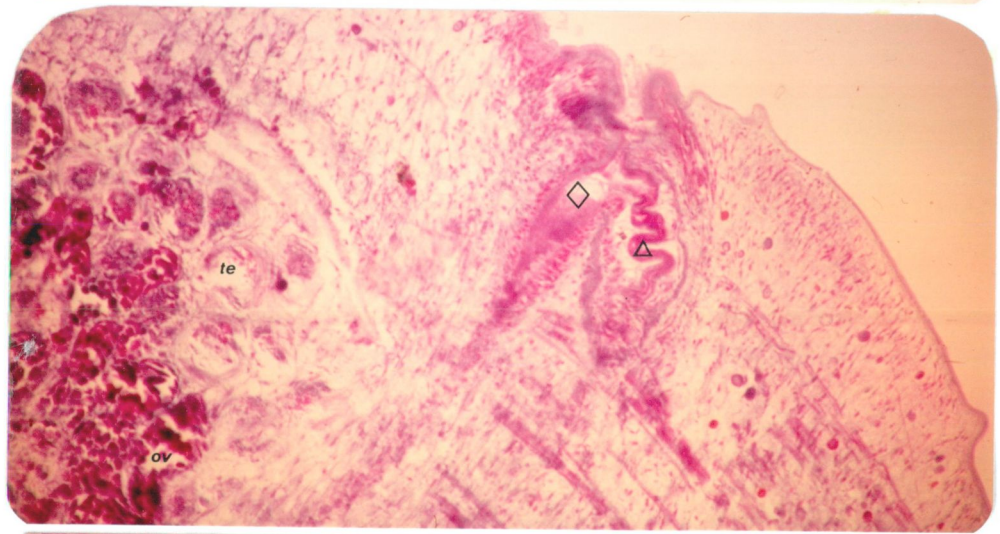
Plate 70. Whole mount of T. crassiceps adult worm showing a mature proglottid containing the reproductive structures. (Formal-saline; Gower's carmine) (x 50)

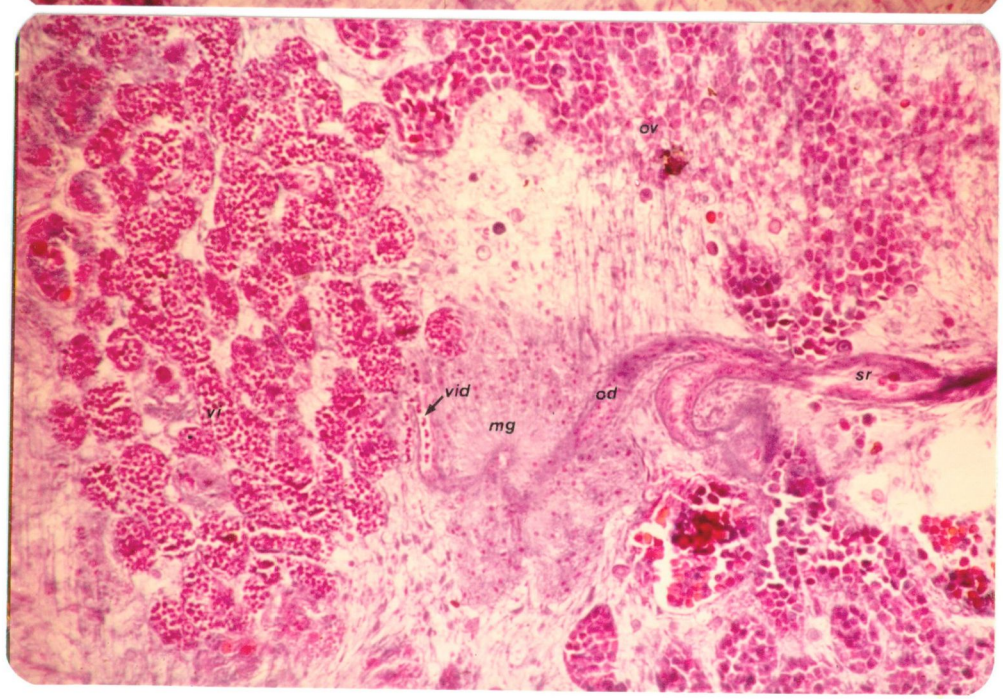
Plates 71 & 72.. Whole mount worms were released from slides and embedded into wax before serial sections were obtained and stained with Casson's trichrome

71. Section through the area of the genital pore revealing the coiled cirrus (△) within the cirrus pouch and the vagina (◇). Note the testes (te) and part of the ovary (ov). (x 200)

72. Section through the female reproductive system. mg = Mehli's gland, od = oviduct, ov = ovary, sr = seminal receptaculum, vi = vitellaria, vid = vitelline duct. (x 200)




te
ov


ov
sr
vid
od
mg
vl

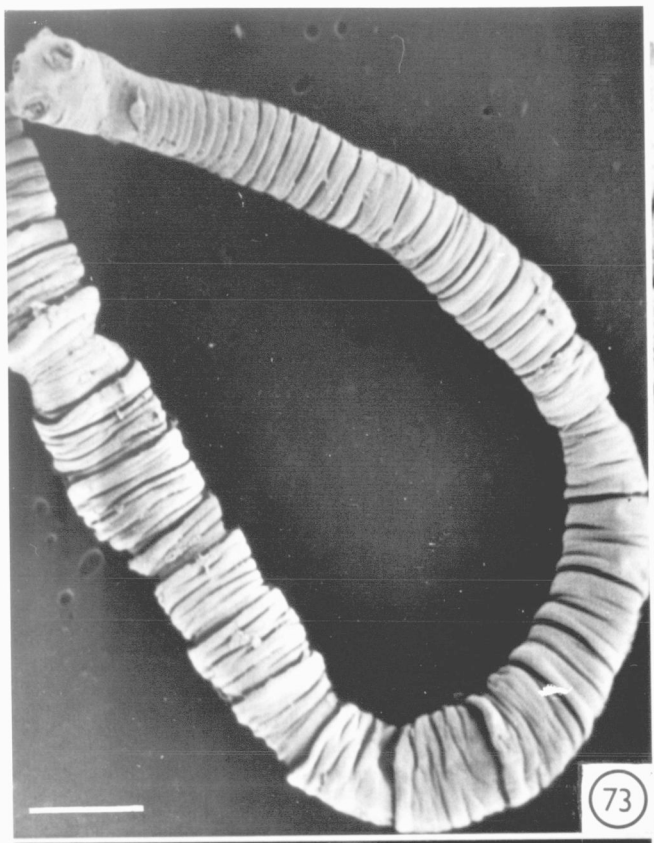
Plates 73 - 76. Scanning electron micrographs of adult T. crassiceps.

73. A 10-day old worm with a normal sucker but retracted rostellum and segmented strobila. (Scale = 500 μm)

74. Part of the sucker showing the typical microtrichal surface of the tegument. Note the amount of host material (arrows) that may obscure finer details. (Scale = 10 μm)

75. Scolex with retracted rostellum (▲) on a 17-day old worm. (Scale = 100 μm)

76. The terminal proglottid of a 17-day old worm with a genital pore (arrow). (Scale = 100 μm)


73


74


75


76

Plates 77 - 79. Scanning electron micrographs of a mature proglottid from a 17-day old worm.

77. The genital pore is often observed within a wider atrium (arrow). (Scale = 100 μm)

78. A higher magnification of the genital atrium within which is the genital pore (arrow). (Scale = 20 μm)

79. Sperm tails (arrow) extending from the genital pore. (Scale = 10 μm)



77



78

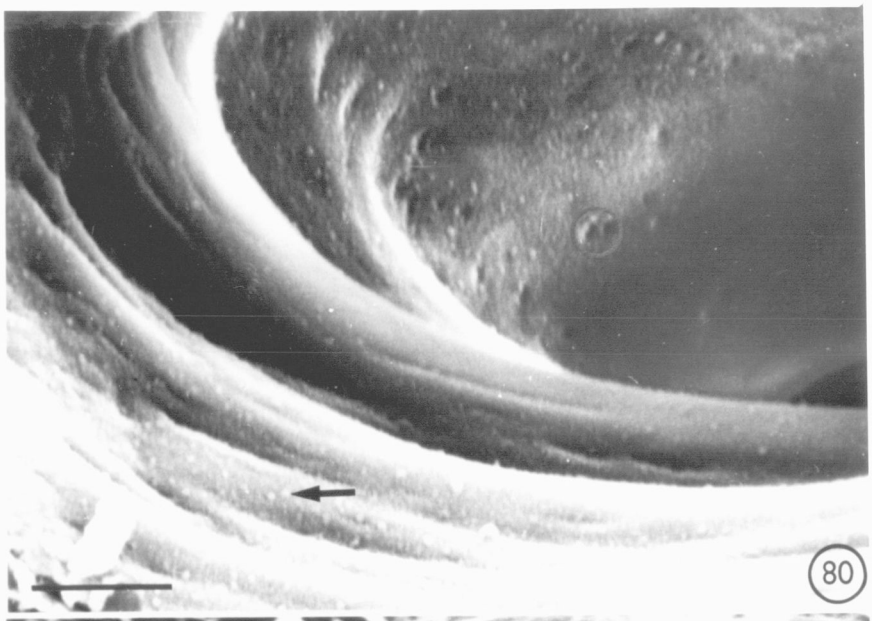


Plates 80 - 85. Scanning electron micrographs of some of the internal structures of an adult T. crassiceps. Plates 80, 81 & 83 were obtained from teased apart mature segments of whole worms that had been processed previously for normal SEM observations. Plates 82, 84 & 85 were obtained from squashed worms previously processed for light microscopy but teased apart.

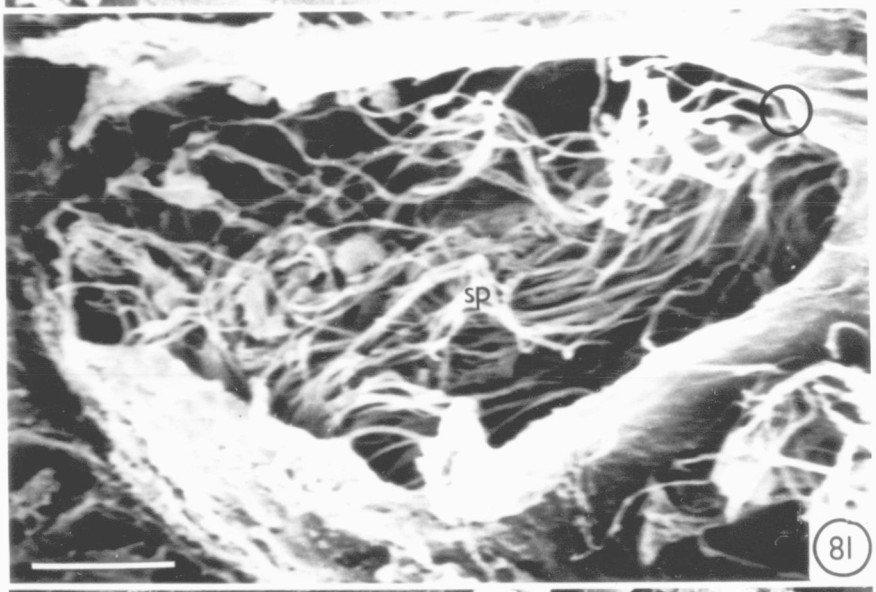
80. A view of the lumen and wall of a large excretory canal. There are small bleb-like projections (arrow) on the wall. Larger pits or openings (circled) could indicate the places where small canals drain into the large canal. (Scale = 10 μm)

81. A testis with the thin-walled tunica propria (circled) teased open to reveal a bunch of sperms (sp). (Scale = 10 μm)

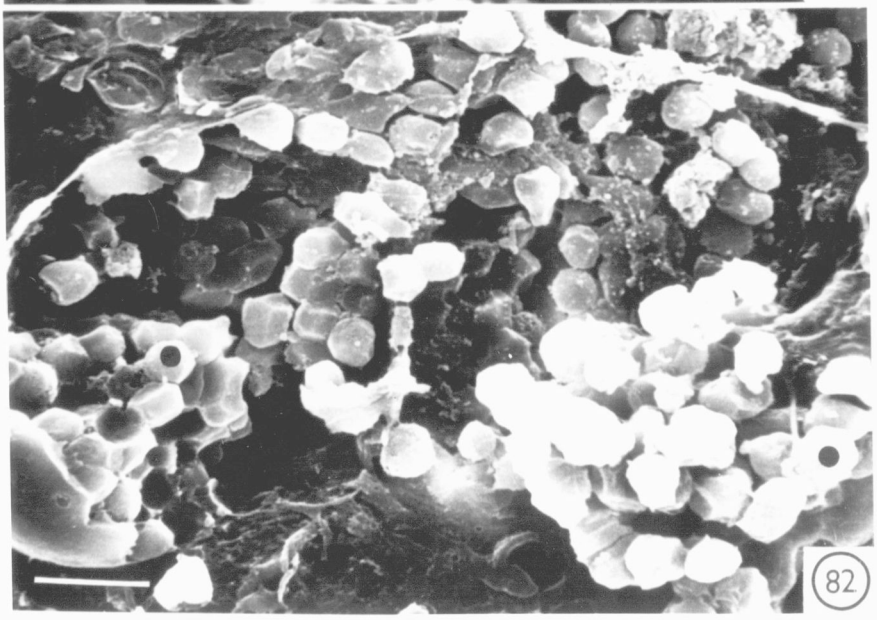
82. Ova (●) are closely packed within each"chamber" of the ovary.



80



81



82

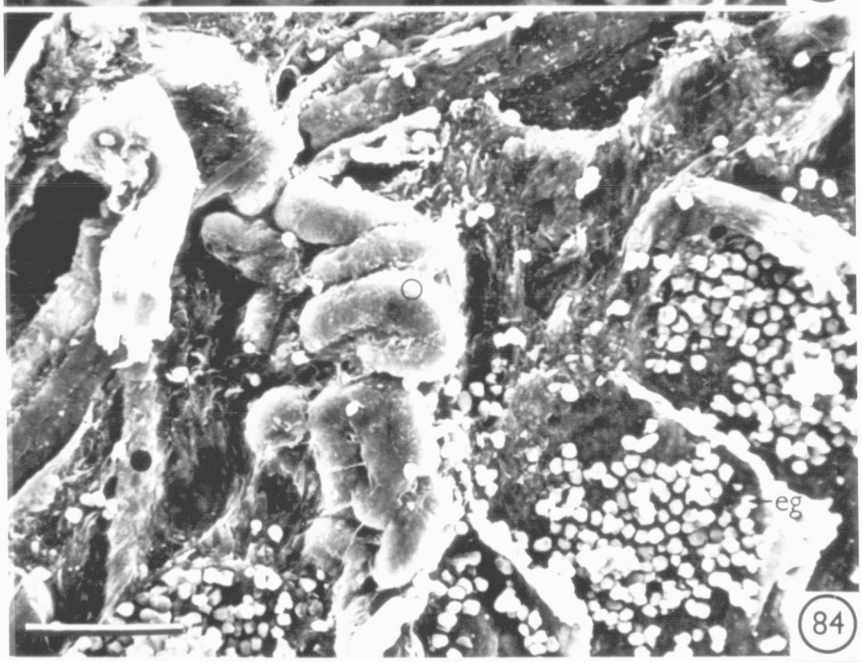
83. Ova (●) are found attached onto strands within each ovary chamber. (Scale = 10 μm)

84. A mature proglottid teased apart to reveal the much-coiled cirrus (○) and the vagina (●). Many eggs (eg), presumed to be fertilized, are found within the lateral branches of the uterus. (Scale = 100 μm)

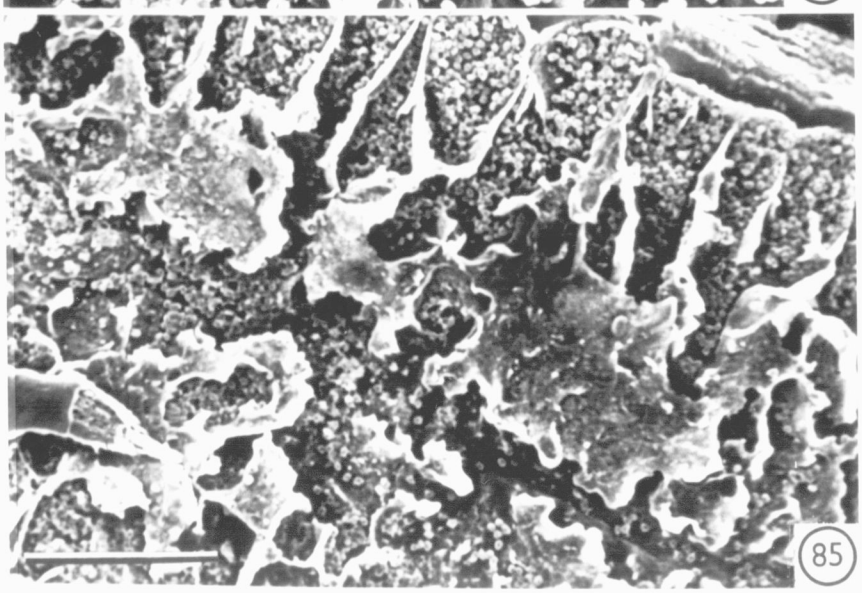
85. In a gravid proglottid, the uterus and its lateral branches occupy the whole segment and are filled with eggs. The rest of the reproductive structures have regressed completely. (Scale = 500 μm)



83



84



85

Plates 86 - 90. Transmission electron micrographs of the tegument on the mature proglottids of adult T. crassiceps.

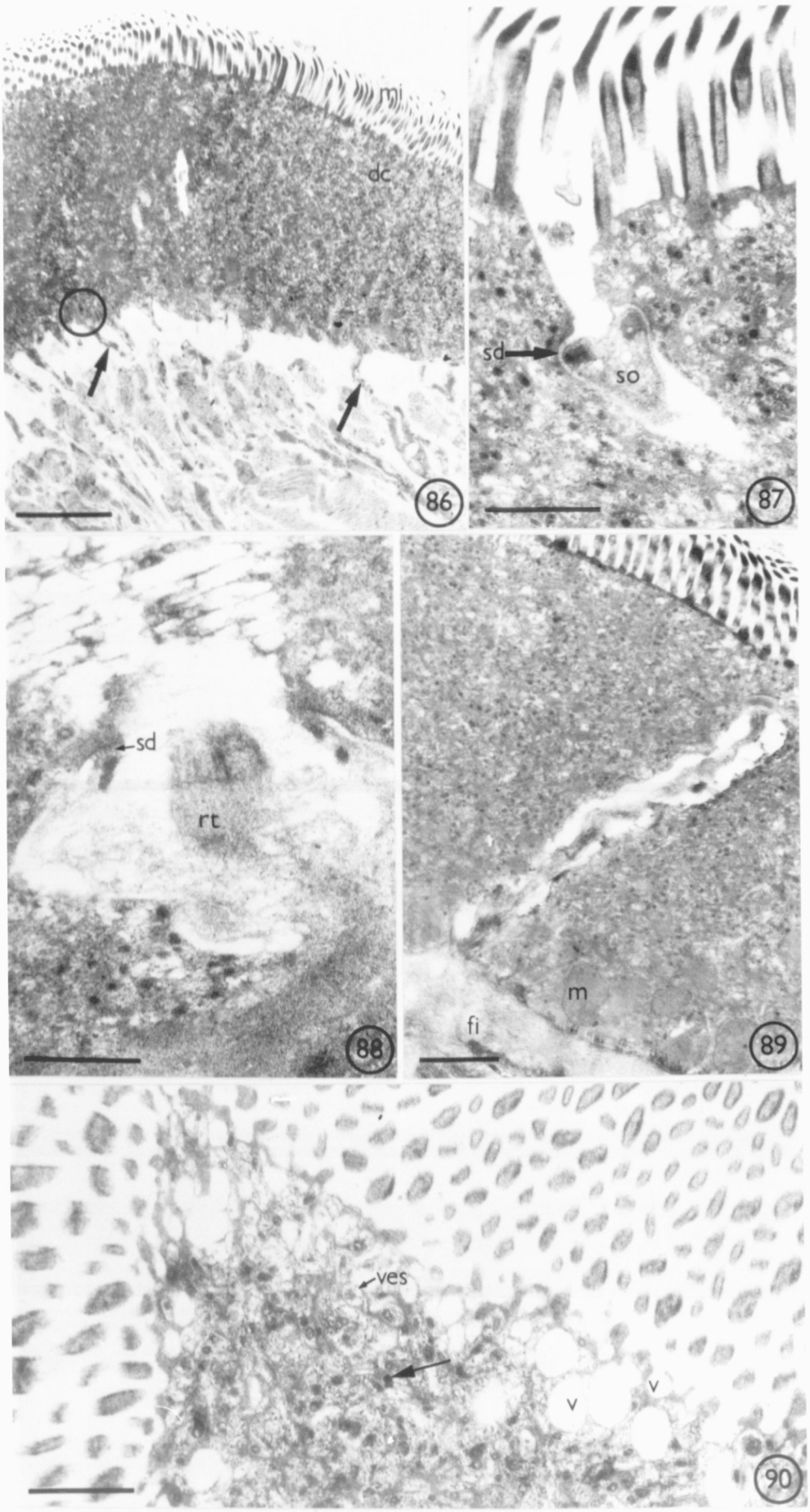
86. A general view of the tegument with the usual microthrix border (mi) projecting from the distal cytoplasm (dc). Numerous cytoplasmic connections (arrow) extend from the perinuclear cytoplasm (not shown here) into the distal cytoplasm. Note invaginations of the inner plasma membrane (circle). (Scale = 2 μm)

87. The terminal process of a sensory organelle with septate desmosomes (sd) at the junction with the distal cytoplasm. Note the relatively long basal portion of the microtriches. (Scale = 0.5 μm)

88. A sensory organelle containing the rootlets (rt) of the sensory cilium (not shown). Note the septate desmosomes (sd) connecting with the distal cytoplasm. (Scale = 1 μm)

89. The rootlets of the sensory cilium is clearly shown here extending right to the fibrous zone (fi). At the basal portion of the distal cytoplasm there are numerous mitochondria with few cristae (m). (Scale = 1 μm)

90. High magnification of the distal cytoplasm showing large vacuoles (v) on the surface, dense bodies (arrow) and granular vesicles (ves). (Scale = 0.5 μm)

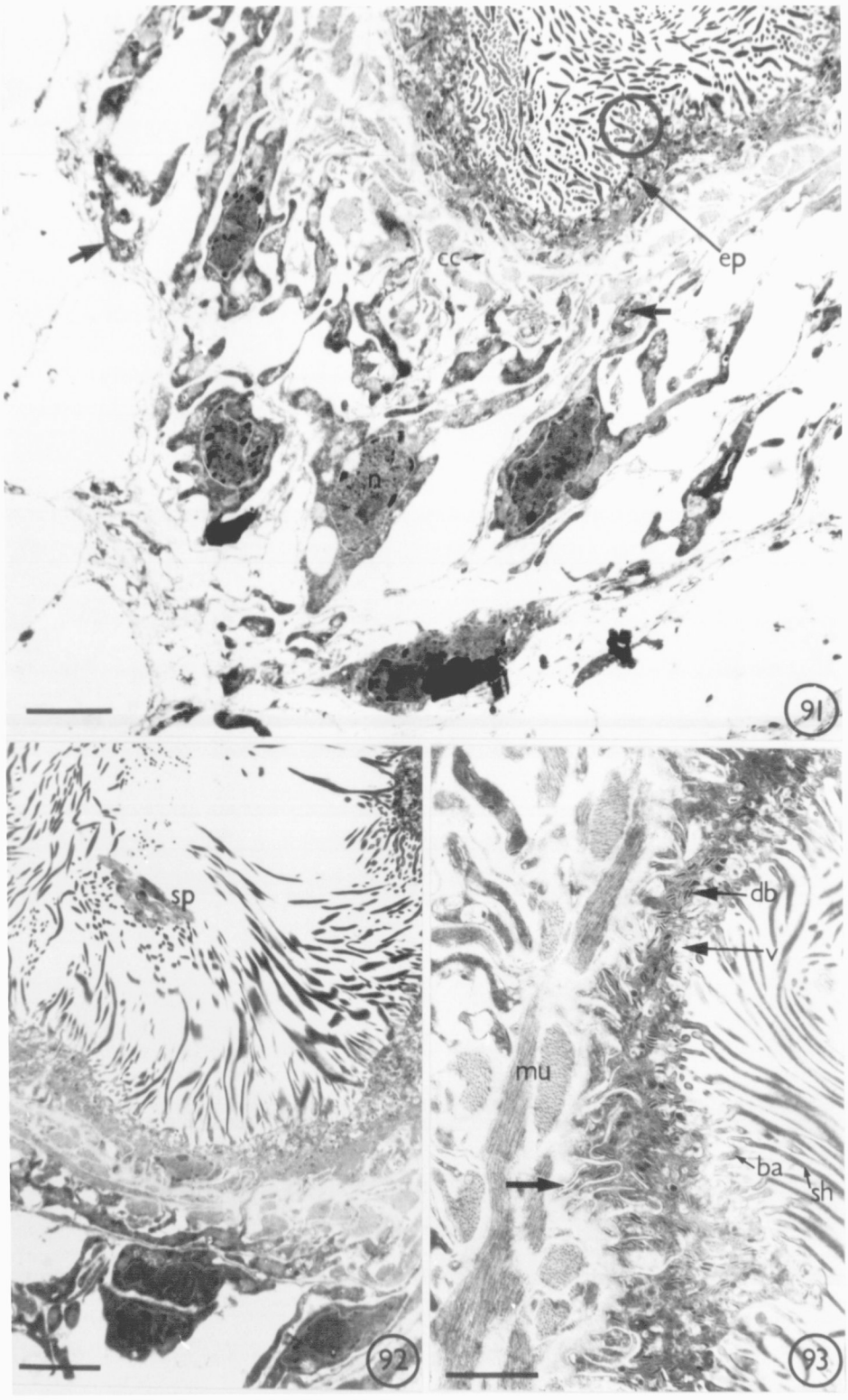

mi
dc
86
sd
so
87
sd
rt
88
m
fi
89
ves
v
v
90

Plates 91 - 93. Transmission electron micrographs of the vaginal canal.

91. The wall of the vagina resembles the tegument in its basic structure. Fine "epithelial projections" (circled) extend into the lumen from the epithelium (ep). Muscle layers are present between the epithelium and the "sub-epithelial cytons" which have large nuclei (n) and cytoplasmic connections (cc) with the epithelium. Disc-shaped bodies (arrows) are present in the cytoplasmic extensions of the cytons. (Scale = 2 μm)

92. Sperms (sp) are occasionally found in the vaginal canal. (Scale = 2 μm)

93. High magnification of the vaginal epithelium and muscle layers (mu). Note the disc-shaped bodies (db) and vacuoles (v) in the epithelium and the involutions of the basement lamina (arrow). The epithelial projections have an electron dense shaft (sh) on a base (ba). (Scale = 1 μm)

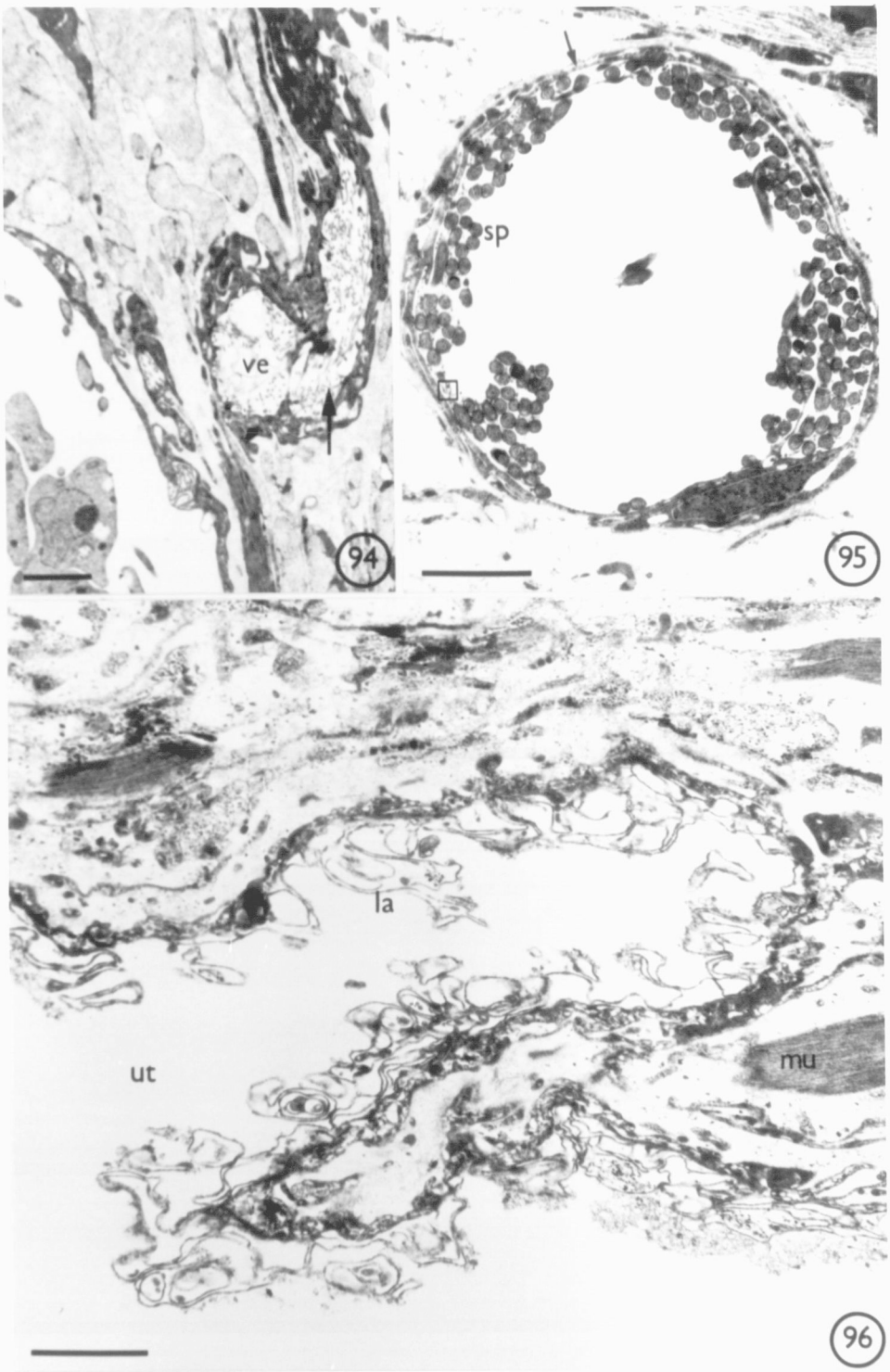

cc
ep
n
91
sp
92
db
v
mu
ba
sh
93

Plates 94 - 96. Transmission electron micrographs of some reproductive structures.

94. The vas efferens (ve) that leads fully formed sperms from the testes (te) has a thick wall that projects fine filamentous microvilli (arrow) into the lumen of the duct. (Scale = 2 μm)

95. The sperm duct or vas deferens which collects sperms (sp) from the vasa efferentia have a thin muscular wall (arrow) Note the fine filamentous microvilli along the wall (square). (Scale = 2 μm)

96. The uterus (ut) has a thin wall from which folds of lamellae extend into the lumen. Muscle fibres (mu) are present beneath the uterus wall. (Scale = 2 μm)


ve
94
sp
95
la
ut
mu
96

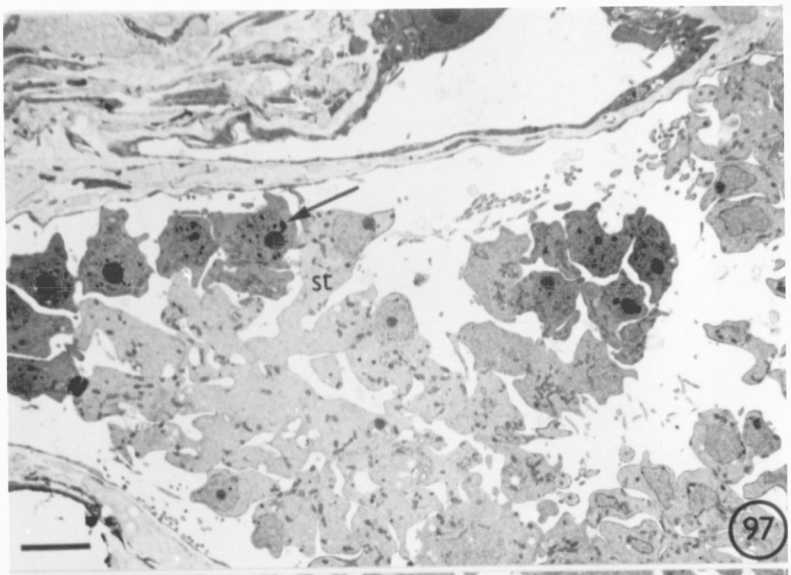
Plates 97 - 100. Transmission electron micrographs of spermatogenesis in T. crassiceps

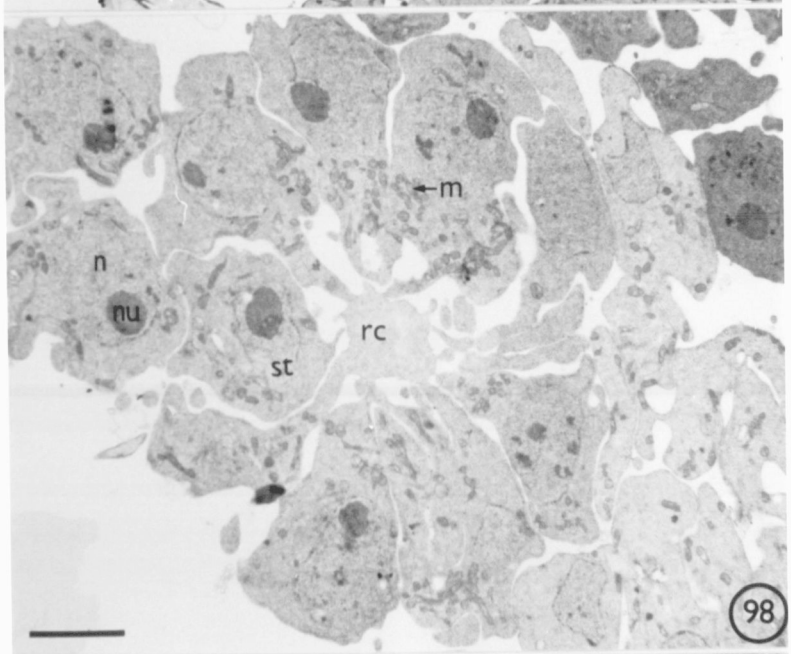
97. A mature testis with spermatids (st), occurring in groups, associated together in cytophores. Note the darker stained spermatids (arrow) with dispersed chromatin material in the nucleus. (Scale = 5 μm)

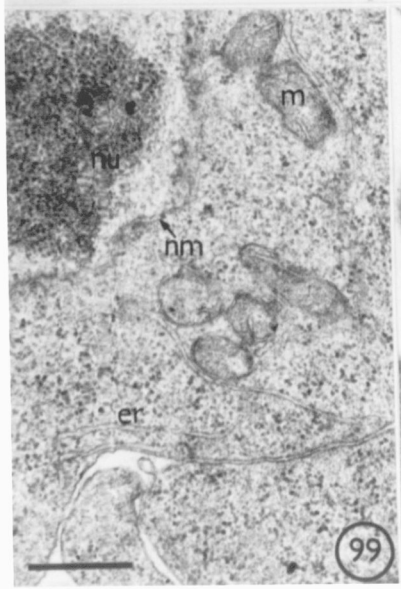
98. A cytophore consisting of spermatids (st) attached to a residual cytoplasm (rc). Note the large nucleus (n) with prominent nucleolus (nu), and the numerous mitochondria (m). (Scale = 3 μm)

99. High magnification of a spermatid. The nucleolus (nu) is prominent within the nucleus. The nuclear membrane (nm) is clearly seen. The cytoplasm contains many granules, probably ribosomal, numerous mitochondria (m) and endoplasmic recticulum (er). (Scale = 0.5 μm)

100. The axial filament (af) originates as a modified extension of the spermatid cytoplasm and is enclosed within a wall of microtubules (mt). Occasionally, a basal body (bb), from which the axial filament probably arises, is observed in the spermatid. (Scale = 1 μm)


st
97


m
n
nu
rc
st
98


m
nu
nm
er
99


bb
mt
af
100

Plates 101 - 105. Transmission electron micrographs of the sperm tails.

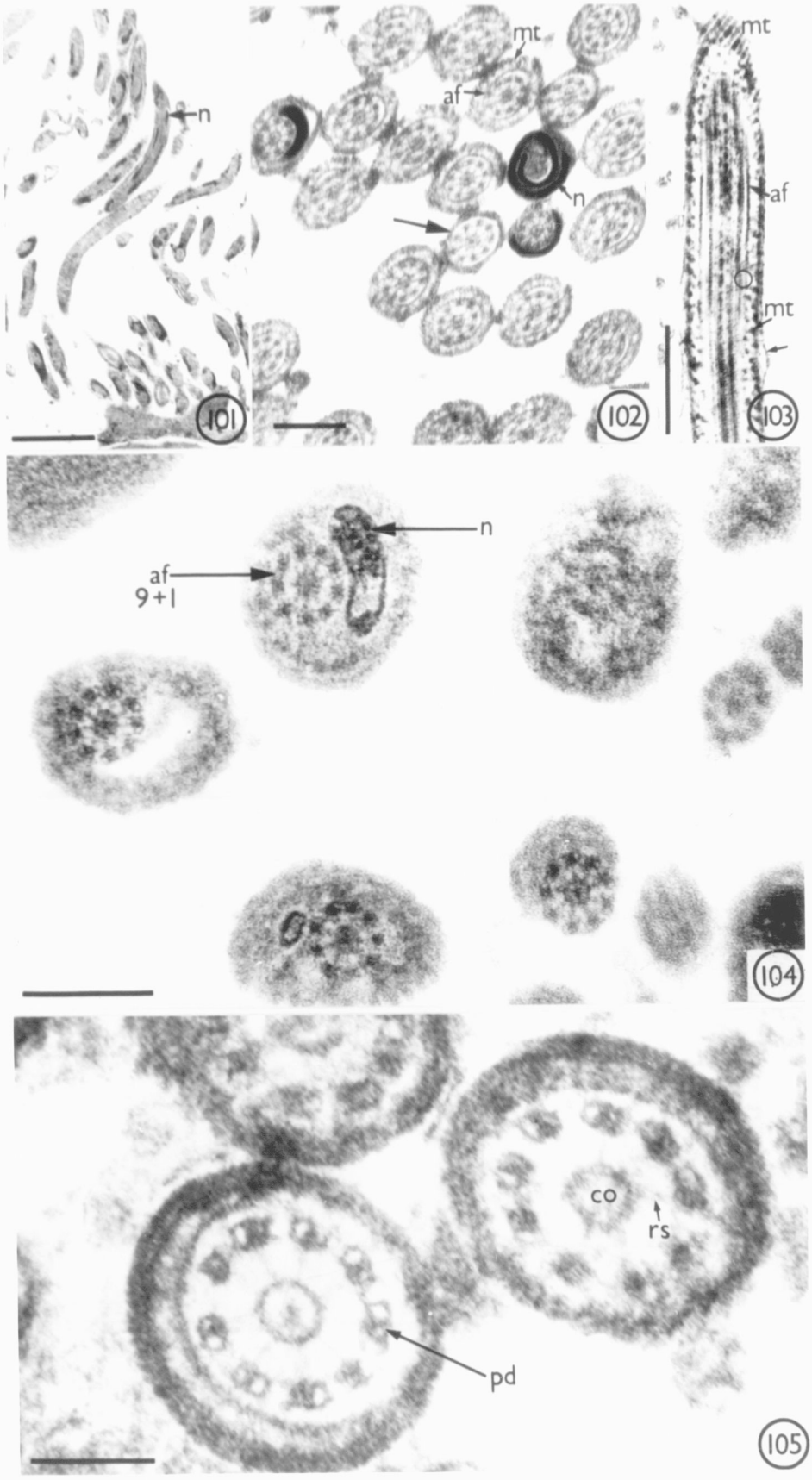
101. The nucleus (n), here revealed as a dense, long chromatin structure, is seen arranged as a spiral around the axial filament in longitudinal sections of sperm tails. (Scale = 2 μm)

102. Sperm tails in transverse section. An outer limiting "sheath" consisting of microtubules (mt) encloses the central axial filament complex (af) in most planes along the sperm tails but occasionally the axial filament is not enclosed (arrow). Note the spiral arrangement of dense nuclear material (n), possibly at the "head" level of the sperm cell. (Scale = 0.25 μm)

103. A longitudinal section of a sperm tail reveals the axial filament (af) as a complex of peripheral tubules enclosed within a striated sheath (circled). The whole complex is again enclosed by an outer "sheath" of "membrane" (arrow) which consists of microtubules (mt) arranged helically along its internal aspect. (Scale = 0.5 μm)

104. The axial filament complex (af) of microtubules with a 9 + 1 arrangement. Note also the nucleus (n) containing chromatin material. (Scale = 0.25 μm)

105. The axial filament complex consists of peripheral microtubules arranged radially as 9 pairs of doublets (pd) connected by radial "spokes" (rs) to the central core (co). (Scale = 0.1 μm)

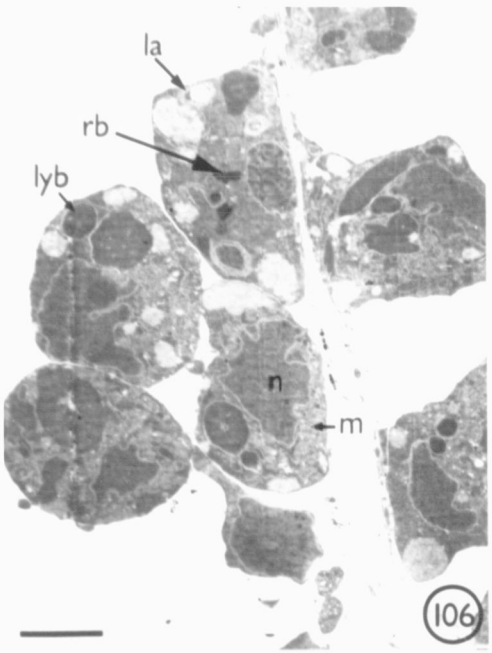

n
101
mt
af
n
102
mt
af
mt
103
n
af
9+1
104
co
rs
pd
105

Plates 106 - 108. Transmission electron micrographs of some reproductive structures.

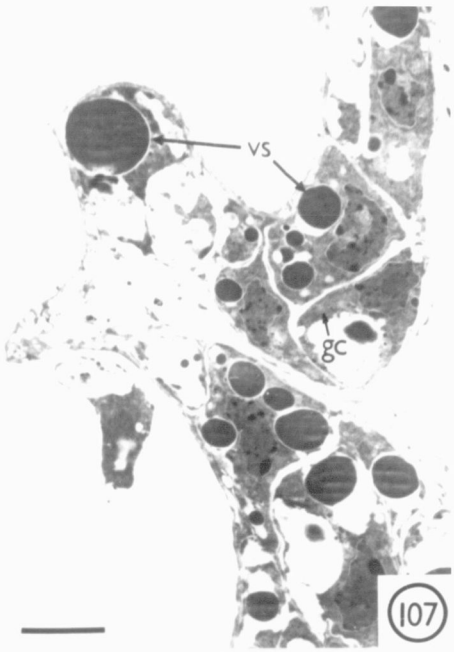
106. These cells, found attached to stalks of cytoplasmic strands within the ovary, are probably degenerate ova. They contain a large nucleus (n), large concentrations of mitochondria (m), and several whorls of lamellae (la). What appears like "lysosomal bodies" (lyb) and "ribosomal bodies" (rb) are also present. (Scale = 3 μm)

107. Cells in the vitellaria containing vitelline secretory bodies (vs) in various sizes. Note the golgi complexes (gc). (Scale = 3 μm)

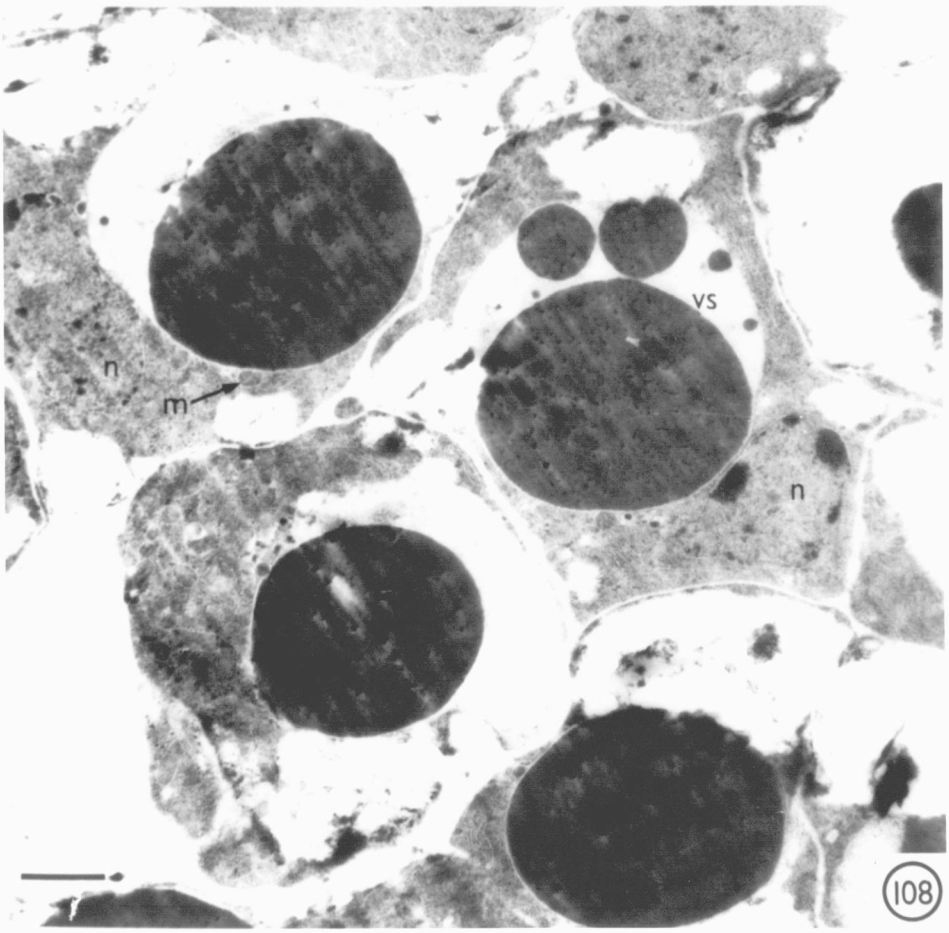
108. Vitelline gland cells containing large electron dense vitelline secretory bodies (vs) within large vacuolated spaces. Note the prominent nucleus (n) and the numerous small mitochondria (m). (Scale = 1 μm)



106



107



108

unsuccessful, presumably due to the fact that the proglottids examined were too mature. Only degenerate ova were found in the ovary (Plate 106). These are usually attached to a stalk. These degenerate ova contain a large nucleus, large concentrations of closely packed mitochondria, and numerous whorls of lamellae. Some unresolved structures include darkly staining bodies, possibly "ribosomal bodies", and what appears to be "lysosomal bodies". The membranes of the ova and the organelles within are either degenerate or not well preserved by the fixatives used. Cells of the vitellaria are easily identified by their large, electron dense secretory bodies (Plates 107,108). These vitelline gland cells have a prominent nucleus displaced peripherally by large vacuolated spaces which contain the vitelline secretory bodies. These secretions appear to increase in size by the accretion of smaller droplets. The cytoplasm is rich in free robosomes, and has numerous mitochondria and extensive golgi complexes.

D. DISCUSSION

The development and morphology of the eggs of cyclophyllidean cestodes have been the subjects of a number of reviews (Smyth & Clegg, 1959; Rybicka, 1966). These reviews of cestode embryogenesis indicated a common pattern in the formation of embryonic envelopes in all species studied. There are 3 basic envelopes surrounding the oncosphere, viz. (1) a capsule, (2) the outer envelope, and (3) the inner envelope. The surface of the outer envelope, in many species, is subsequently covered by a 'shell'. The inner envelope in pseudophyllideans, forms a ciliated embryophore, while in cyclophyllideans, an embryophore of hardened secreted material is formed

within it. Beneath these 3 basic envelopes lies the oncosphere which is surrounded by an oncospheral membrane. With increasing acceptability of the electron microscope there have been several ultrastructural researches on embryonic envelopes in cestodes (Morseth, 1965; Pence, 1967,1970; Collin, 1968; Nieland, 1968; Swiderski, 1968,1973; Rybicka, 1972,1973) resulting in a deeper understanding of cestode egg structure, development, and functional biology. The biology of cyclophyllidean eggs, incorporating light and electron microscopy and histochemistry, has been reviewed recently by Lethbridge (1980).

Taenia crassiceps eggs obtained from gravid proglottids resemble those described in a previous study by Bilqees (1968b) except that the dimensions reported by her (30 x 25 µm) are significantly different from the present study (22.5 x 18.5 µm). This may be due to several factors : Biqees (1968b) measured live eggs with the light microscope while in this study the eggs were measured with a scale at the SEM after they had been subjected to a series of fixation and dehydration; further, she was examining the TOI strain as opposed to the KBS of this study. Under the light microscope the eggs appeared normal, with a fully-formed striated embryophore and the complete set of hexacanth hooks. However, the extremely small number that "activated" when subjected to artificial digestive medium may suggest some abnormality although it is recognized that this is a common problem with taeniid eggs. The medium used was based on that used by Bilqees (1968b). The most likely cause for the non-viability of the majority of eggs is that they were not mature enough as these were from 29-day old worms. Freeman (1962), although using the TOI strain, found eggs in infected dog faeces only 32 days post-infection. The few oncospheres that exhibited motility were observed to have "blebs"

attached on their anterior end. These blebs are thought to be secretory in function and originate from the penetration gland(s) (Heath & Smyth, 1970; Lethbridge & Gijsbers, 1974). The position of these glands were not clearly located with the live material; Bilqees (1968b) used toluidine blue on fixed material to reveal 2 elongated and irregular structures laterally, resembling the model by Lethbridge & Gijsbers (1974) for Hymenolepis diminuta.

The fine structure of T. crassiceps eggs conforms largely to other taeniid species (Morseth, 1965; Nieland, 1968). The capsule and outer envelope are closely associated with lipid droplets which might be remnants of a vitelline cell. The hypothesis that the capsule originates from the vitelline cell (Rybicka, 1966) is supported by the ultrastructural studies of King & Lumsden (1969) who showed that vitelline cells in H. diminuta contain large numbers of secretory granules. The outer envelope cell(s) described in the present study forms the outer envelope. Swiderski (1973) suggested that this cell is a macromere originating from the embryo in Catenotaenia pusilla, but there was insufficient evidence in the present study to suggest this. It is only speculation, but it is more than likely that the vitelline cell forms both the capsule and outer envelope in T. crassiceps. Rybicka (1965) considered 2 possibilities regarding the capsule : either the capsule once formed persists around the embryo as a coat, or the capsule is only a protective membrane for the cleaving embryo, disintegrating at a later stage. Swiderski (1968) was of the opinion that, at least in C. pusilla, the first view is more accurate as the capsule was present throughout the stages of embryonic development. He further suggested that the presence of vital organelles, especially mitochondria, indicates that

the outer envelope is a viable structure and should not be termed "shell" as ascribed by Rybicka (1966) for H. diminuta. In T. crassiceps, the outer envelope appears as an extensive cytoplasmic reticulum with abundance of granules, probably ribosomal, and both this envelope and the capsule contain mitochondria. It would seem likely that while these 2 envelopes are retained throughout egg development as a protective covering for the initial cleavage stage of the embryo, they are also involved in energy-requiring metabolic activities of an unknown nature even after the formation of the thick embryophore. One may hypothesize that these envelopes serve in a "placental" capacity, providing nutrition to and removing waste products from the developing oncosphere and embryophoral cell(s).

The inner envelope originates from the embryophoral cell or cells. This conforms to most studies on cyclophyllidean eggs (Morseth, 1965; Nieland, 1968). The process by which the embryophoral block material is secreted by the embryophoral cell and accrues as spherical blobs at the under-surface of its outer membrane is almost identical to the one reported for T. taeniaeformis (Nieland, 1968). Swiderski (1968) was of the opinion that the embryophore and the inner envelope in C. pusilla arise from 2 different cells but this has been refuted by Rybicka (1972). The role of the embryophoral cell in the synthesis of block material is widely accepted (Rybicka, 1972) and evidence has been provided in this as well as many other studies, e.g. Morseth (1965) and Nieland (1968). The occurrence of mitochondria and ribosomal bodies in abundance is indicative of a highly active metabolism, and Nieland (1968) adds the synthesis of the 'cement' substance as another function of the embryophoral cell. The mitochondria thus may play a unique role in embryophore formation.

While it can be assumed that they mediate energy production, they also appear to contribute to the architecture of the embryophore. The cavities in the embryophore, as observed with the SEM, appear as lacunae under the TEM, and these lacunae often contain a mitochondrion. Similar findings were reported by Nieland (1968) in T. taeniaeformis, and the "circular bodies" in the embryophore of other taeniid eggs (Morseth, 1965) almost certainly refer to these mitochondria. The positive reaction to performic acid-Schiff treatment implies the presence of a keratin or keratin-like protein in the embryophoral blocks. Similar results have been reported in other taeniids (Morseth, 1966) and hymenolepids (Lethbridge, 1971). There is speculation as to whether specific cement substances exist in order to confer host-selectivity properties (Freeman, 1962; Huffman & Jones, 1962). The cement substance reported in this study appears similar in its staining properties to that reported in T. taeniaeformis (Nieland, 1968) but whether their chemical composition is similar remains unknown. If there is evidence for specific cement substances, then the embryophore acts not only as an external protective covering but also to ensure host-specificity. Silverman (1954) demonstrated that the disaggregation of the embryophoral blocks was caused by proteolytic digestive enzymes of the intermediate host, and these enzymes varied between the species studied.

The composition of the oncospheral membrane, the final covering that invests the oncosphere, is unknown, and its origin disputed. In T. crassiceps it would appear that it is formed by the infolding of the outermost membrane, and the whole "oncospheral membrane" consists of 2 laminae containing closely packed vesicles. Nieland (1968) reported a similar structure for the oncospheral membrane of

T. taeniaeformis but attributed its origin to the embryophore cell. This latter view of its origin agrees with that of Rybicka (1973) who reported a delamination of membranes originating from the inner envelope in H. diminuta. It is not known whether the oncospheral membrane in T. crassiceps is derived from the inner envelope earlier on in embryogenesis, but if so, it would seem anomalous to label it as an "oncospheral membrane", especially since it has a structure different from that of a unit membrane. The inner component of this bilaminate oncospheral membrane would seem equivalent to the "peripheral epithelial layer", a term devised by Rybicka (1973). Beneath this is the "peripheral cytoplasm" which invests the whole oncosphere and presumably becomes the future epithelium. Rybicka (1973) used the term "basal epithelial layer" for the equivalent structure in H. diminuta oncospheres. The peripheral cytoplasm has also been described in T. taeniaeformis by Nieland (1968), who first applied the term "sheath" to the modified regions that hold the oncospheral hook blade. From the present study it is not possible to ascertain whether the hook blade lies completely within the sheath, but longitudinal sections of hooks in C. pusilla (Swiderski, 1973) reveal that they remain within the "hook region membrane". It is possible that the folds of cytoplasm from the sheath wall which surround the hooks may act as a form of valve in liberated (activated) oncospheres where the hooks penetrate the oncospheral membrane. The "dense bodies" reported by Nieland (1968) to be concentrated around the sheath are also found in T. crassiceps but more dispersed along the peripheral cytoplasm. While their origin and function remain unresolved in T. crassiceps, Rybicka (1972) showed that in H. diminuta they are synthesized by Golgi and deposited in the "basal epithelial layer"

where they apparently adhere to the oncospheral membrane.

Oncosperal hooks occur intracellularly in hook forming cells or oncoblasts, and presumably are synthesized de novo. Circumstantial evidence for de novo synthesis is provided by the fact that oncoblasts are rich in ribosomal granules and mitochondria, a situation that is associated with active synthesis or metabolism. Similar findings and interpretation were offered by Swiderski (1973) in his studies on C. pusilla. The bipartite structure of the fully formed oncosperal hook and its keratin nature bear close resemblance to the hooks found in other species, e.g. T. taeniaeformis (Nieland, 1968), H. citelli (Collin, 1968), H. diminuta (Pence, 1970), and C. pusilla (Swiderski, 1973). The arrangement of oncospheral muscles was not followed serially in this study. An excellent account of muscular arrangements in H. citelli has been given by Collin (1968) upon whose study is based much of current knowledge. Some muscles in T. crassiceps oncospheres originate at the basal lamina where they are anchored by a unique bulb-like modification inserted into an evaginated portion of the lamina. In T. crassiceps, hook muscles appear to originate from the basal lamina and are attached by desmosomes. They are attached at the "collar" region of the hook, not directly on the hook as reported by Collin (1968), but on the basal lamina that has folded in to the collar. Ogren (1958,1961) reported that hook muscles are attached to the oncoblast surface in H. nana.

While the penetration glands are a major characteristic of cyclophyllidean oncospheres, they were not identified until Sinitsin (1931) noted them in Moniezia expansa. Penetration glands have been described for a number of oncospheres using light microscopy with histochemistry (reviewed by Lethbridge, 1980). Electron microscopy

studies have been reported by Nieland (1968), Collin (1969), Pence (1970), and Lethbridge & Gijsbers (1974). Penetration gland cells in T. crassiceps oncospheres can be easily identified by the large number of dense bodies or granules in the cytoplasm. The dense bodies presumably correspond to the "penetration gland" granules described in taeniids by Silverman & Maneely (1955). Nieland (1968) and Pence (1970) have suggested that liquefaction changes occur in the inclusions as maturity approaches. Lethbridge & Gijsbers (1974), in a series of histochemical and microdensitometric study on H. diminuta, followed the activity of the penetration gland and suggested that secretion is initiated and maintained by the muscular contractions of the oncosphere. Ultrastructural studies extablished that the gland inclusions were progressively exported into the surface epithelium and here accumulated in epithelial "blebs" which were released periodically. A similar secretory mechanism has recently been proposed for H. nana (Furukawa et al, 1977). A similar mechanism is presumed to exist in T. crassiceps but the present set of ultrastructural and histochemical studies was not extended to detect such a mechanism. However, epithelial "blebs" occurred frequently in cultures of oncospheres. The function of the secretory inclusions remains a subject of speculation. Their PAS-positive characteristic (Silverman & Maneely, 1955; Barker, 1970; Heath, 1971) implies that the secretions may be mucoid, and it has been suggested that they (1) facilitate the adhesion of the oncosphere to the intestinal mucosa, (2) lubricate its passage through the tissues, or (3) protect the oncosphere from the digestive enzymes of the intermediate host (Lethbridge, 1980).

The effects of penetration gland secretions on host tissue is a

subject of much controversy. There are contradictory findings with regard to whether of not lytic destruction of host epithelium is necessary for oncosphere penetration (Barker, 1970; Lethbridge, 1971; Heath, 1971). Whatever the means by which the oncosphere penetrates the host epithelium, its presence and continuous secretory activity would present antigenic material to stimulate antibody production. The importance of the immunogenetic properties of oncospheres (Gemmell, 1976; Gemmell & Johnstone, 1977; Clegg & Smith, 1978) has received increasing attention since excretory-secretory antigen originating from T. ovis oncospheres, either during in vitro culture or while maintained in vivo in diffusion chambers, produced significant levels of protection in sheep against oral challenge (Rickard & Bell, 1971, a,b). The contribution of penetration gland secretions to the "functional" antigens involved in conferring protection has been the subject of much investigation (Barker, 1970; Heath, 1971; Anderson & Lethbridge, 1975; Rickard & Adolph , 1977a).

While it is possible to examine the oncosphere and various metacestode stages in T. crassiceps with the present model system, the developmental stages between the oncosphere and the first lacuna present formidable difficulties. Their extremely small size and migratory habits make it impractical to follow such developments in vivo (Bilqees, 1969). Heath & Smyth (1970) and Heath (1973 a) showed the advantages of in vitro cultivation of oncospheres to study post-oncospheral developments. However, shortage of eggs and failure to "activate" oncospheres in the present study precluded this approach, so, detailed study was confined to advanced metacestode stages.

In a population of T. crassiceps metacestodes, the rate of asexual budding determines the reproductive rate of the parasites. The TOI

strain studied had about 6 times as many buds and budders as the KBS strain. This might explain why the TOI larvae reproduce much faster in mice than the KBS strain (routine observations). Other differences included the higher frequency of abnormal evaginated cysticerci, scoleces and hooks. These and other differences were noted in the ORF strain of the parasite (Freeman, 1962; Dorais & Esch, 1969; Fox et al, 1971) and were attributed to an aneuploidy in the nullisomic ORF (Smith et al, 1972a). The TOI strain, previously considered normal, would appear to have become "abnormal" possibly due to continuous passage of the metacestodes, the same observation made by Bilqees (1967) for the ORF abnormalities and attributed to genetic and other factors. Smith et al (1972a) proposed that a somatic mutation in stem cells of polyembryonic larvae like T. crassiceps would favour production of new stocks or clones, especially if the mutant population has a high reproductive rate. Abnormalities in the scoleces and hooks will not be disadvantageous since the larvae are continuously passaged into intermediate hosts and are not required to develop in the intestines of definitive hosts. The mechanisms and kinetics of the population genetics of this phenomenon has been proposed by Smyth & Smyth (1964) for Echinococcus spp. and need little extrapolation to fit the T. crassiceps situation. Other than random mitotic or meiotic mutations, the rate of such mutations could have been increased by injury to the cells during passage possibly resulting in "germinative foci differences" (Schiller, 1973). Kuntz (1943) attributed multicephalic T. taeniaeformis to injury, while Crusz (1948a) confined it to purely genetic mutations. Multicephalic or polyradiate metacestodes have been reported in T. taeniaeformis (Southwell & Krishner, 1937; Crusz, 1947), T. pisiformis

and Dipyllidium caninum (Clapham, 1939).

The structure of the body wall of cestodes as revealed by the electron microscope is recognised as resembling, in many ways, that of the Digenea and the Monogenea, i.e. it consists of a syncytial outer layer (distal sytoplasm) formed from cytoplasmic extensions which arise from an inner nucleated layer containing tegumental cells (perinuclear cytoplasm) (Smyth, 1976). The extensive literature on the tegument and the confusing terminology has been reviewed by Lee (1966, 1972), Smyth (1972) and Lumsden (1975a). The earliest studies on cestodes established the "living" nature of the "tegument", a term applied by Rothman (1959,1960,1963), and its continuity with "sub-tegumental" cells (Threadgold, 1962).

The distal cytoplasm is thrown into projections which were first termed "microtriches" by Rothman (1959). These have earlier been called "villi" or "microvilli" (Read, 1955; Kent, 1957). The microtriches on the tegument of T. crassiceps have a basic structure resembling those of other cestode species, e.g. E. granulosus (Morseth, 1966; Jha & Smyth, 1969), T. hydatigena and T. pisiformis (Morseth, 1966), and Diphyllobothrium erinacei (Yamane, 1968). Baron (1968) and Belton (1977) have studied the microtrichal structure on T. crassiceps and they revealed structures that are generally similar to those elucidated in this study. However, they only described one type of microthrix, and either neglected or overlooked polymorphism among the microtriches. Most of the microtriches have the same pattern of a cylindrical basal portion and an attenuating distal shaft. The blunt-tipped microtriches found in this study are confined only to the inner basal surface of the sucker cup, suggesting special adaptations, possibly to obtain a more close-fitting grip on the gut epithelium.

The microtriches on the rim of the rostellum are long and thin; a possible advantage for this modification would be that the rostellum, being in closest contact with host tissue, will have an increased surface area if microtriches are long and thin rather than short and thick. There are extremely fine microtriches on the rostellar protrusions that are frequently encountered at the rostellar tip. These are probably microtriches on tegument that is in the process of being modified into a "hook organ" or "hypertrophied tegument" (details later). All the other different microtriches encountered in this study are not distinct enough for them to be afforded "modified" status. Most likely, the differences, especially those on the bladder, buds, or old budders, are due to the age of the metacestode/bud, or the artifactual effects of fixation and subsequent processing. TEM studies reveal that microtriches on adult strobilae have a longer basal portion relative to the distal shaft. The significance of this remains uncertain but may be an adaptation for absorption rather than attachment as is the case in scolex microtriches. Similar observations were made by Andersen (1975) and Yamane (1968) who looked at microtriches on the scolex and proglottids in Diphyllobothrium spp. Polymorphic microtriches in the scolex canal of T. crassiceps metacestodes were observed by Prokopic & Hulinska (1979) but their micrographs reveal (what seem to be) artifactual crystals that obscured the microtriches and might have misled their interpretations. Other species of cestodes that show microtrichal polymorphism include some hymenolepids (Berger & Mettrick, 1971) T. hydatigena (Featherston, 1975) and the cysticercoid of Tatria octacantha (Rees, 1973).

The structure of the distal cytoplasm and its inclusions reported

in this study is similar to those of Baron (1968) and Belton (1977). The presence of membrane-bound disc-shaped dense bodies and vesicles, vacuoles, and mitochondria appears almost universal in all the tapeworms studied. However, there has been no unequivocal evidence to attach specific function(s) to the disc-shaped bodies or vesicles. That the distal cytoplasm serves a digestive-absorptive function is virtually universally accepted. Lumsden (1966) and Smyth (1969a) drew the parallel between the microthrix border of tapeworm tegument and the microvilli on gut epithelial cells. Lumsden (1965,1966) provided convincing evidence that structural and enzymatic proteins are synthesized in the perinuclear cytoplasm and transported to the distal cytoplasm. Glycans in the distal cytoplasm have been shown to be continuously and rapidly replaced (Trimble & Lumsden, 1975). Histochemical studies by Lumsden (1966) and Öhman -James (1968) show similarities to results obtained in this study. The microthrix border, distal cytoplasm and perinuclear cytoplasm contain muco-substances such as mucopolysaccharides and mucoproteins. Acid mucopolysaccharides are known to assist with electrolyte transport on cell membranes (Philpott, 1964). They can also bind host protein prior to possible "contact (membrane) digestion" in Hymenolepis and Moniezia (Taylor & Thomas, 1968). In the present study, there is a fairly even distribution of mucosubstances throughout the adult tegument but in the cysticercus they appear to be more prevalent in the anterior regions. In Didentricum, general enzymatic activity, for example that of adenosine triphosphatase, has been shown to increase towards the scolex of adults, but at low levels in the plerocercoids (Öhman-James, 1968). Mucosubstances are probably synthesized and packed in vesicles at the golgi complex in the cell bodies of the perinuclear

cytoplasm. The golgi apparatus has been implicated for proteinaceous secretory products and mucopolysaccharides (Caro & Palade, 1964). Experimental evidence for the establishment of an active, absorptive function of the tegument have been provided by Murrell (1968) who showed that the intake of glucose by T. crassiceps involves an energy requiring mediated process. This was followed by Haynes (1970,1973), also working with T. crassiceps, who demonstrated the absorption of amino-acids is mediated by an active transport mechanism. While the scolex and rostellum of a tapeworm could play an important role in contact (membrane) digestion and, in some cases, stimulation of strobilization (Smyth, 1972), the strobila must play the major role of digestion and absorption as it constitutes the main surface area which comes into contact with host gut contents. It is therefore hardly surprising that there are more mitochondria and ribosomal granules in the tegument of adult T. crassiceps than in the cysticercus. An increase in the number of mitochondria in adult tegument has also been observed in Diphyllobothrium latum (Bråten, 1968) and D. erinacei (Yamane, 1968). The distribution of these mitochondria along the proximal or lower region of the distal cytoplasm has also been observed in other taeniids (Morseth, 1966). Mitochondria found in specialized tissues, such as the tapeworm tegument, where many varied metabolic activities take place, would require special adaptations. The mitochondria observed in the distal cytoplasm of T. crassiceps, and in most other tapeworms, have relatively few cristae. Green & Fleischer (1960) have stated that, in general, the greater the number of ancillary reactions catalyzed by mitochondria the smaller the number of cristae per mitochondrion.

In this study, it was found that the distal cytoplasm of adult

worms contain much more vacuoles or vesicles, especially near the surface, than the larval stage. These vesicles or vacuoles of varying sizes could be circumstantial evidence for pinocytotic activity. It would seem likely that pinocytosis would be frequently encountered in the distal cytoplasm where active secretion, digestion and absorption are going on continuously. Uptake of macromolecules across the tegument membrane by pinocytosis would be expected to complement uptake of small molecules by diffusion, active transport or facilitated transport. Pinocytic vesicles have been described in a number of studies purely on morphological grounds (Threadgold, 1962, 1965; Rothman, 1963; Jha & Smyth, 1969). The uptake of electron-dense particles would be required to demonstrate this phenomenon. Yet, experiments with tapeworms incubated in media with markers have rarely provided unequivocal evidence for pinocytosis (Smyth, 1972). Recently, Hopkins et al (1978) established that pinocytosis occurs in Schistocephalus solidus. The reason for the scarcity of experimental proof may lie in the fact that adult cestodes show a keen selectivity for molecules of specific shape, size and charge (Lumsden, 1970; Threadgold & Arme, 1974). Besides an increased amount of mitochondria and vacuoles or vesicles in the adult tegument of T. crassiceps 2 other changes are observed. One is that the distal cytoplasm is much thicker than in the larva. An increase in adult tegumental thickness has also been observed in E. multilocularis (Sakamoto & Sugimura, 1968), T. hydatigena (Featherston , 1972), Raillietina cesticillus (Blitz & Smyth, 1973) and Diphyllobothrium spp. (Andersen, 1975). This increase in thickness is probably just due to proportionate growth with the maturing proglottid and its increasing physiological demands. The other, more significant, change observed in adult distal cytoplasm is

the near-disappearance of disc-shaped bodies. Similar observations have also been reported in D. erinacei (Yamane, 1968). Smyth (1972) speculates that this may be related to the increase in mitochondria and other vesicles and that these bodies may play a part in the biogenesis of mitochondria - a process which has been shown to accompany larval-adult differentiation in E. granulosus (Jha & Smyth, 1971).

Although microtubule-like invaginations of the internal limiting membrane in the distal cytoplasm of cysticercus bladder wall have been found in T. crassiceps it is difficult to speculate their significance. Microtubular complexes have been reported in H. diminuta (Threadgold & Read, 1970b) but these do not resemble those found in T. crassiceps. Threadgold & Read (1970b) suggested that they may function in water or ion transport, or both. Such a function would be necessary in the bladder of T. crassiceps which consists of a large fluid filled lacuna. Baron (1968) reported dense whorls of phospholipid membranes in the perinuclear cytoplasm and the distal cytoplasm. In this study, they are rarely found, and only in the perinuclear cytoplasm. Their function or significance is unclear. Lamellated bodies have also been reported in D. latum (Bråten, 1968) and their relationship with "tube-like structures" in microtriches has been postulated.

The origin of rostellar hooks has been a subject of much debate. Crusz (1947, 1948b) stated that they originate from the fusion of "cuticular hairs", presumably microtriches, while Mount (1970) was of the opinion that they arise from the enlargement of special microtriches. Baron (1968), Bilqees & Freeman (1969) and Bilqees (1970b) suggested de novo synthesis in specially modified tegumented structures. But

most workers are of the same opinion that after the primary formation of hooklets, whatever the means, secondary thickening is probably due largely to accretion of hook material from specially modified tegument (Crusz, 1948b; Bilqees, 1970b; Mount, 1970). This structure has been described and termed as "hook organ" (Bilqees & Freeman, 1969) and "hypertrophied tegument" (Baron, 1968; Mount, 1970). It is also probably equivalent to the "vacuolated tegument" described for E. granulosus by Smyth et al, 1969. The term "hook organ" is used in the present study. It is morphological and functionally different from the distal cytoplasm and is closely associated with the rostellar hooks. Mount (1970) has described inclusions such as ribosomal aggregates and hook material in this structure. Crusz (1948b) mentioned the presence of tegumental disc-shaped bodies but it is clear from the present study that these bodies are much larger in the hook organ than in the distal cytoplasm. Bilqees & Freeman (1969) and Bilqees (1970b) reported that the hook organ is formed early in the morphogenesis of the rostellum, even before hooks are formed. They related the formation of normal hooks with the hook organ and found that abnormal hooks are found frequently in metacestodes in which hook organ formation is abnormal. The present study revealed that the dense disc-shaped bodies are similar in size and shape with granules that occur in secretory cells of rostellar and sub-rostellar glands. It is possible that the disc-shaped bodies of the hook organ are actually synthesized by these gland cells and transferred to the hook organ. It is also possible that the hook organ, in addition to supplying the material for secondary hook thickening, functions as a "secretory gland" with secretory granules originating from rostellar and sub-rostellar glands. Secretory

globules, β-metachromatic to toluidine blue, have been demonstrated arising from the hook organ. Similar staining reaction occurs in the cells of the rostellar and sub-rostellar gland.

Rostellar gland cells are found frequently in T. crassiceps. The location of these cells above the rostellar pads and within the ring of hooks is comparable with similar findings of "rostellar gland" cells reported in E. granulosus (Smyth, 1964b,1969a; Smyth et al, 1969). However, no nuclear secretions (Smyth et al, 1969) were detected in T. crassiceps. In the cysticercus, sub-rostellar gland cells have been demonstrated and their secretory products may be transported to the hook organ as discussed earlier. The appearance of rostellar gland cell secretion in the adult may provide some clues to its function. Smyth et al (1969) proposed that the secretion may be related in some way, to the control of morphogenesis, or it could serve as a protective layer which forms a barrier between the worm and host tissue thus lowering the antigenic contact between these tissues or acting as an anti-inflammatory substance in some way.

The term "fibrous zone" for the electron-lucid zone between the distal cytoplasm and the underlying muscle layers has been adopted from Morseth (1966). The region has also been called "interstitial material" for the trematode Fasciola hepatica (Threadgold, 1963) and "fibrous interstitium" for E. granulosus (Sakamoto & Sugimura, 1969). Histochemical tests reveal that this zone contains collagenous material. This may provide support and rigidity to the zone which acts as a form of external skeleton to which muscles may be attached. Lumsden (1966) and Sakamoto & Sugimura (1969) reported that there are regular hemidesmosomes on the plasma membrane along this zone. Morseth (1969) observed dark strands that run across the fibrous zone

connecting the distal cytoplasm to the sub-tegumental regions. In T. crassiceps, 2 types of desmosomes or desmosome-like structures are found, both along the fibrous zone that surrounds the muscular sucker. Along the external aspect of the sucker, between the muscles and the distal cytoplasm are what appear to be huge desmosomes that transect the whole zone at frequent intervals. These may function to anchor the distal cytoplasm to the sucker muscles during muscular contractions especially when strong suction is applied to hold on to host tissue. Beneath the fibrous zone, the muscle fibres attach to a limiting lamina by means of desmosomes. These desmosomes occur all round the sucker wherever a muscle terminates at the fibrous zone. The occurrence of desmosomes where muscles terminate at the fibrous zone, together with its collagenous nature, confers upon this structure a "skeletal" function.

The nervous system of T. crassiceps, as revealed by localization of specific cholinesterases, resembles that of several other species of tapeworms studied. Only a few studies have been concerned with the demonstration of esterases in simple whole-mount preparations. Hart (1967) used the cholinesterase technique to demonstrate the nervous system of intact tetrathyridia of Mesocestoides corti; Ramisz (1967) reported on cholinesterase localization in the nervous system of Dilepis undula and Choanotaenia unicornata; Wilson & Schiller (1969) described the nervous system of Hymenolepis spp. through cholinesterase localization; Shield (1969) applied the indoxyl acetate and cholinesterase techniques to D. caninum, E. granulosus and H. taeniaeformis; and Leflore & Smith (1976) described the nervous system of C. fasciolaris using histochemical methods for esterases and cholinesterases. The acetyl- and butyryl- choline iodide method

clearly demonstrates the nervous system of T. crassiceps in whole-mount preparations. The pattern of distribution of the reaction product in the scolex and neck is generally similar to that described for adult C. fasciolaris and Hymenolepis spp. (Shield, 1969; Wilson & Schiller, 1969; Leflore & Smith, 1976). The observation on the nerve supply to the bladder as an intricate nerve-net type of arrangement has only been reported once before in C. fasciolaris (Leflore & Smith, 1976). It has been suggested that the bladder is a specialized structure which plays an important role in nutrition and metabolism as well as a storage reservoir for energy rather than simply as an excretory structure (Baron, 1968; Nieland & Weinbach, 1968; Slais, 1973). It appears likely that such an array of physiological activities would require an extensive nervous innervation. The scolex and rostellum, especially the suckers, are intensely stained for cholinesterase with large nerve cords and ganglia. This extremely high concentration of nerves are presumably vital to the tapeworm for the reception of stimuli as it searches for a suitable hold-fast in the definitive host. Special "stretch receptors" have been implicated in order to maintain the attachment of a scolex to the host mucosa by continuous contraction of the sucker muscles (Rees, 1961). The fine structure of the nervous system has been studied in several helminths, e.g. Cyathocotyle bushiensis (Erasmus, 1962), Schistosoma mansoni (Morris & Threadgold, 1967), Diclidophora merlangi (Halton & Morris, 1969), Raillietina cesticillus (Blitz & Smyth, 1973), and E. granulosus (Jha & Smyth, 1971). Sensory receptors appear in the distal cytoplasm of all these helminths as apical bulb-like structures with an apical "cilium" and connected to a nerve trunk or process. In the present study, the (9 + 2) filament arrangement was

not observed in the sensory "cilium" although microfilamentous structures have been observed within. Lyons (1972) described several types of single and compound receptors in Monogeneans which contained (9 + 2) ciliary receptors. Most sensory organelles T. crassiceps occur as simple, single receptors, but occasionally double receptors were found. No compound receptors have been observed. Double, and triple, receptors have also been reported in Diphyllobothrium spp. by Andersen (1975), but Morseth (1967) found only single receptors in E. granulosus. The relative abundance of sensory organelles in the sucker region observed in this study by SEM and TEM further implicates the importance of the nervous system to the searching and attaching activities of the suckers.

The structure of the calcareous corpuscle, and its relation to glycogen-rich parenchymal cells, resembles that described for E. multilocularis as described by Sakamoto & Sugimura (1969). It has been suggested that they are species specific in terms of composition and crystalization patterns (von Brand, 1965; von Brand et al, 1965). The morphogenesis of these corpuscles was not followed in this study. Swiderski et al (1970) showed that they are synthesized intracellularly in I. madagascariensis. The function of calcareous corpuscles have not been determined. One possible function may be to buffer anaerobically produced acids (von Brand, et al, 1960). It is probable, however, that the role of these corpuscles is more complex and vital. They are found in many species of cestodes and trematodes, especially noticeable in larval forms. They may act as a major reserve centre for vital ions that will be readily available when a large amount of energy is required, say when a cestode larva enters the intestine of the definitive host. A possible confirmation of

this veiw is the fact that the first indication of strobilar development of Echinococcus is a gradual reduction in the numbers of the calcareous corpuscles during the early phases of development (Smyth, 1967).

Flame cells are found throughout the cortical parenchyma of T. crassiceps, including the scolex. Baron (1968) reported that he failed to find flame cells in the scolex of T. crassiceps. The features described for flame cells in this study are virtually identical to typical flame cells found in many biological systems. Their function in parasitic helminths is related to the excretory system which is based on the platyhelminth protonephridial system with flame cells and collecting vessels. The system in T. crassiceps was not the subject of in-depth investigation in this study, but the structure of the flame cell and excretory canals resemble that described for other species of cestodes, e.g. Cotugnia digonopora (Siddiqi, 1961), E. multilocularis (Sakamoto & Sugimura, 1969).

Wardle and McLeod (1952) has described in detail the gross morphology and organ relationships of the reproductive structures in tapeworms. The architecture of the reproductive organs in mature T. crassiceps proglottids conforms to the general pattern for the cyclophyllideans. An interesting result from the present study was the discovery of very similar structures between the vaginal wall and the tegument. It may seem straightforward initially since the vaginal canal is continuous with the tegument. However, it has also been established that the vagina originates from somatic primordial cells in the medullary region of a proglottid after the tegument has long been formed and differentiated (Sulgostowska, 1974). Current knowledge on the embryology of cestodes is scanty. Studies with

D. dendriticum have shown that special germinative cells give rise to the anlagen of the genitalia (Wikgren et al, 1971). Sulgostowska (1974) was of the opinion that in H. diminuta embryonic cells give rise to a single somatic primordium from which germinative cells can be distinguished. From this primordium both male and female genitalia are differentiated. This interpretation reconciles the observations reported in T. crassiceps. The germinative cells that differentiate to form the vagina, though different from tegumental cells, originate as embryonic cells that are retained in the neck region and dispersed into segmenting proglottids. However, this interpretation would require stringent examination before it can be shown to be accurate. Apart from the question of origin, the "epithelium" of the vagina is of interest because of its inclusions which resemble those in the tegumental distal cytoplasm. It is likely that, as in the case of the tegument, sub-epithelial cell-bodies synthesize secretory materials which are then transported to the epithelium and lumen via the cytoplasmic connections. Evidence for this is the observation that the disc-shaped bodies are also found in sub-epithelial cytons. These secretory materials may be important in maintaining suitable conditions along the vaginal canal for the survival and movement of sperms. The function of the microthrix-like epithelial projections, together with the sub-epithelial muscle layers, is likely that of assisting the movement of sperms to the seminal receptaculum and oviduct. The rest of the female reproductive organs consist of the ovary, the vitelline glands (vitellaria), and the uterus. Proglottids containing early stages of oogenesis and uterus formation were not examined. Further studies on sections of proglottids that are more anteriorly located would be required in order to understanding oogenesis and uterus

morphogenesis.

The function of the extensive folds of lamellae on the uterus wall may be that of providing an increased surface area to cope with the physiological processes that would accompany egg maturation. The fact that the uterus is not an open-ended sac, as in the Pseudophyllidea, would mean that metabolic waste disposal, osmoregulation, and other processes are mediated across the uterus wall. There has been practically no published reports on the ultrastructure of the cestode reproductive system, especially the female reproductive structures. A comparative study between T. crassiceps and other species is, consequently, not possible.

The structure of sperms and the stages of spermatogenesis and spermiogenesis have been studied at an ultrastructural level in a number of cestodes, e.g. H. diminuta and H. nana (Rosario, 1964), E. granulosus (Morseth, 1969), and T. hydatigena (Featherston, 1971). The association of spermatids as a rosette-shaped cytophore appears to be common in all the species studied. The abundance of mitochondria and prominent endoplasmic reticulum may be related to the intense metabolic activity involved in cell-division, meiosis, and microtubule synthesis. Featherston (1971) observed that the cytophore is formed by divisions of the primary spermatogonium but there is no complete separation by the cell walls. This produces a multinucleate syncytium which is retained until the spermatozoa are released from the residual cytoplasmic mass. In other platyhelminths species, a fusion process is considered to occur at the spermatocyte stage of development where primary spermatocyte cells fuse to form the rosette cytophore (Rybicka, 1966). This disparity has not been resolved in the present study. The process of spermatozoa formation appears to

be a continuous process. In any section of young testis, mature sperms occur among developing sperms and spermatids. This presumably ensures that a constant source of sperms is available for insemination. Both self-insemination and inter-proglottidal insemination are believed to occur in tapeworms. Although self-insemination has been demonstrated as far as E. granulosus is concerned (Smyth & Smyth, 1969) there has been no conclusive evidence for cross-fertilization. However, continuous production of sperms in mature proglottids, in which the ova are either fertilized or degenerate, may suggest that they are just not meant to be "wasted" but used to inseminate ova in other proglottids, either on the same worm or another. Scanning electron micrographs of the genital pore on mature worms of T. crassiceps revealed sperms emerging from the pore, thereby providing circumstantial evidence that inter-proglottidal insemination involving these sperms might occur.

The fully formed sperm in T. crassiceps, as in most other helminths, consists mainly of a long tail-like structure. The interchangeable usage of sperm or spermtail (Morseth, 1969) is therefore acceptable. The arrangement of the axial filament complex peripheral elements agree with the (9 + 1) pattern widely reported in most platyhelminths. This has been established in several species of turbellarians (Silveira & Porter, 1964), trematodes (Sato et al, 1967; Davies, 1976), and cestodes (Rosario, 1964; Morseth, 1969; Featherston, 1971). In trematodes, axial filament complexes typically appear in pairs while in most cestodes they occur as single units. A notable exception was reported by von Bonsdorff & Tellka (1965) in a pseudophyllidean, D. latum, where the sperm tails possess paired axial filament units. An elongated nucleus along part of the length of the sperm is also a

common feature (Morseth, 1969; Featherston, 1971). The present study reveals that this nucleus is coiled like a spiral around the axial filament complex, along part of the worm. Rosario (1964) illustrated an uncoiled nucleus in his study on Hymenolepis spp. Another difference between the sperms of T. crassiceps and Hymenolepis, as interpreted by Rosario (1964), is that the outermost membrane of the sperms of T. crassiceps have the microtubules arranged on its inner aspect as opposed to the outer aspect in the other species. A significant feature in T. crassiceps sperms is the apparent absence of mitochondria or at least detectable mitochondria. This may be characteristic of cestodes, as mitochondria have not been reported in H. diminuta and H. nana (Rosario, 1964), E. granulosus (Morseth, 1969) and T. hydatigena (Featherston, 1971). On the other hand they have been found in the trematodes Paragonimus miyazakii (Sato et al, 1967) and Microphallus similis (Davies, 1976). The other features of T. crassiceps sperms resemble closely those described by Morseth (1969) in E. granulosus and by Featherston (1971) in T. hydatigena. The general morphology of T. crassiceps sperms contributes to the emergence of a common morphologic pattern of platyhelminth spermatozoa.

E. SUMMARY

Taenia crassiceps eggs contain the basic cyclophyllidean pattern of embryonic envelopes. The capsule and outer membrane appear to originate from an outer envelope cell, probably a vitelline cell. They contain mitochondria and ribosomal granules, possibly indicating not only a protective function but also physiological ones for the developing embryo. The inner envelope consists of an embryophore cell(s) which synthesize keratin-like proteinaceous material which

accrue beneath the outer plasma membrane of the cell to form the embryophore. Mitochondria from the embryophore cell are closely associated with embryophore formation, resulting in characteristic cavities or lacunae in the interstices between the embryophoral blocks. The oncosphere is invested by a bilaminate oncospheral membrane and a layer of peripheral cytoplasm which is modified into sheath-like structures to accommodate the blade portion of oncospheral hooks. Muscles are anchored onto the basal lamina by desmosomes or special bulbous structures and they are involved in hook movements and, possibly, the secretory activity of penetration glands.

Continuous passage of metacestodes may have resulted in the creation of abnormal "strains" of T. crassiceps. The TOI strain that has been maintained in this laboratory might well have become "abnormal" judging from the prevalence of abnormal scoleces and rostellar hooks.

The tegument of T. crassiceps is generally similar in structure to that found in most platyhelminths. Polymorphism among the microtriches at the rostellum rim and inside the cup of the sucker may be related to their respective specialised functions of maximising host-parasite contact for digestion and as an attachment organ. Changes in the tegument in the larval-adult transition include a decrease in the numbers of disc-shaped bodies, an increase in mitochondria, increase in vacuoles or vesicles, a general increase in the thickness of the distal cytoplasm, and the relative increase in length of the basal portion of microtriches. These changes are probably related to the intense digestive-absorptive activities in the adult tegument. The majority of sensory organelles, which occur more frequently along the rim of the suckers, are simple receptors containing a single "ciliary" process.

The scolex and rostellum of T. crassiceps are regions of special interest. Histochemical and ultrastructural studies reveal that "sub-rostellar gland" cells, found in cysticercus and adult, may be involved in maintaining the hook-forming and secretory activities of the hook organ. Rostellar gland cells are found predominantly in the adult worms; their secretions may be important for contact (membrane) digestion. From their β-metachromatic reaction to certain dyes, the secretory material found in both these gland cells are probably acid-mucopolysaccharides.

Many of the other somatic features of T. crassiceps, e.g. the flame-cell, secretory canal and calcareous corpuscle, are similar to most published accounts of flatworm structure. Desmosomes have been found all round the fibrous zone that surrounds the muscles in the suckers. Special dark bands, possibly large desmosomes, are located at intervals across the fibrous zone that separates the sucker muscles from the distal cytoplasm, presumably to serve as anchoring points during strong contractions, especially when the sucker is attached to host mucosa.

In the reproductive system, the wall of the vaginal canal has been shown to resemble, in many ways, the syncytial arrangement found in the tegument. The uterus wall consists of extensive folds of lamellae. Vitelline cells contain prominent globules of vitelline secretions in large vacuolated spaces. Mature ova and ovogenesis were not observed in this study. All the features of spermatogenesis and spermiogenesis that were examined conform to the general platyhelminth pattern. The sperm consists of a single, central axial filament complex with a typical cestode (9 + 1) arrangement of peripheral elements. This central axial filament complex is enclosed

within an outer sheath or membrane which contains microtubules arranged in a helical manner. The elongated nucleus is coiled like a spiral around the central axial filament complex along part of the length of the sperm.

SECTION II. IN VITRO CULTIVATION OF TAENIA CRASSICEPS

A. INTRODUCTION

The use of *in vitro* culture as a tool in parasitology is well established and needs little elaboration. It enables the study of parasite anatomy, physiology, biochemistry and immunology in a controlled system with the parasite isolated from host influences. The advantages of an efficient *in vitro* system are obvious as it eliminates the high cost of maintaining hosts under laboratory conditions with especial regards to safety requirements. The availability of commercially viable and effective vaccines against a host of parasitic diseases of medical and veterinary importance may be realised when these parasites can be maintained *in vitro* through all stages of their life cycles. *In vitro* culture can also be used in post-operative diagnosis of parasitic cysts (Smyth & Barrett, 1980).

1. Review of Literature on Cultivation of Cestodes

The literature on *in vitro* culture of cestodes has been reviewed by Clegg & Smyth (1968), Taylor & Baker (1968), Smyth (1969a,1976) and Silverman & Hansen (1971). Mature tetrathyridia have been cultured from *Mesocestoides* spp. oncospheres by Voge (1967) and Voge & Seidel (1968). Berntzen (1967) has grown mature cysticercoids of *Hymenolepis nana* from hatched oncospheres. Monoxenic cultivation of cysticercoids of *H. diminuta* (Graham & Berntzen, 1970) was successful with rat fibroblast cells in mammalian tissue culture media. *H. nana*, known to develop to the cysticercoid in either the insect or the mammalian host, was grown axenically to the cysticercoid in a "mammalian" medium (Berntzen, 1970). Mueller (1959 a,b,c) and Berntzen & Mueller (1972) have cultured *Spirometra mansonoides* from procercoids to adult

worms, so that almost the entire life cycle of this species can now be grown *in vitro*.

Unlike the pseudophyllidean and hymenolepid cestodes which are relatively easy to culture *in vitro* (Smyth, 1976), the taeniid cestodes have proved to be unusually difficult despite some measure of progress made with *Taenia hydatigena*, *T. ovis*, *T. pisiformis* and *Echinococcus granulosus* and *E. multilocularis* (Heath & Smyth, 1970; Smyth, 1971, 1977; Heath, 1973a; Smyth & Davies, 1974b, 1975). The significance of successful culture systems is obvious. Using the techniques of *in vitro* culture of oncospheres established by Heath & Smyth (1970), Rickard & Bell (1971 b) collected the excretory-secretory antigens from cultured larval *T. ovis* and these crude vaccines produced a high degree of immunity to subsequent challenge with *T. ovis* eggs. Following this, vaccines have been obtained against *T. pisiformis* (Rickard & Outteridge, 1974) and *T. saginata* (Rickard & Adolph, 1976). However, the commercial viability of such vaccines cannot be realised unless the entire life-cycle of the parasite can be grown repeatedly in a continuous culture system. With *T. hydatigena*, Osuna *et al*, (1978) has obtained proglottids with early genitalia. All early attempts to culture *Echinococcus* were unsuccessful until it was discovered that a diphasic medium with a solid or semi-solid substrate was necessary for strobilate differentiation to be induced (Smyth, 1967; Smyth *et al*, 1966, 1967; Smyth & Davies, 1974b). Even then the best system resulted only in sexual maturity in the sheep "strain" of *E. granulosus* but without fertile eggs due to failure of impregnation to take place *in vitro*. The horse strain will simply not grow and differentiate *in vitro*, while *E. multilocularis* develops "monozoic" strobila which may undergo a form of "pseudo-

segmentation" but no true segmentation. The main problems seem to be the dual ones of establishing the optimal biochemical and physical requirements for each species or "strain" to mature sexually, and, when mature, to achieve impregnation and fertilization of ova by spermatozoa.

2. The *in vitro* Cultivation of *Taenia crassiceps*

Attempts to develop *in vitro* culture methods for taeniid cestodes from post-embryonic larval stages to the adult have rarely been made (Silverman, 1965; Silverman & Hansen, 1971). Notable attempts have been reported by Smyth (1967), Smyth *et al* (1966) and Smyth & Davies (1974b) on *E. granulosus*, and by Esch & Smyth (1976) on *T. crassiceps*. Little attempt has been made to culture *T. crassiceps*; the earlier works by Voge (1963) and Taylor (1963) merely aimed at maintaining the parasite in chemically defined media without real progress in culturing the worms to mature adult stages. Esch & Smyth (1976) established a basic protocol for the cultivation of *T. crassiceps* from the cysticercus to the strobilate stage; sexually mature, but not gravid, proglottids were formed. Subsequently, Rickard & Smyth (1977, unpublished work) were able to greatly simplify the procedure and reported results that were comparable in some ways to those of Esch & Smyth (1976). However, there were some aspects of these two studies that were contradictory. Esch & Smyth (1976) reported that a diphasic medium consisting of a complex liquid medium and an underlying base of coagulated newborn calf serum was essential for advanced development of the worms, while Rickard & Smyth (unpublished) found that the diphasic medium was not required for development to the stage of genitalia formation. Since the work of Esch & Smyth (1976) and Rickard & Smyth (unpublished), the liquid medium used by both

groups, Parker's 858, is no longer available commercially. Attempts were thus made in this study to find another suitable medium for culture as well as to determine the optimal requirements, both physical and biochemical, for efficient cultivation of T. crassiceps.

One important aspect of the developmental biology of T. crassiceps that has not been examined hitherto is the changes in the fine structure of the worm as it develops, especially during the larval-adult transition. Jha & Smyth (1971) have shown that in E. granulosus, during the very early stages of strobilization, marked changes occur in the composition of the cytoplasmic constituents; moreover, these changes appear to be correlated with changes in activity of a small group of gland cells at the tip of the scolex forming the so-called rostellar gland (Smyth, 1964b; Smyth et al, 1969). At present, many of the hypotheses put forward concerning the larval -adult transformation of cestodes and the possible relationships between mitochondrial activities, membrane digestion and differentiation need critical examination. T. crassiceps seems to provide a suitable model for such studies especially if its developmental biology can be studied in an in vitro situation.

In this study attempts were made to culture T. crassiceps oncospheres to cysticerci and cysticerci to adult worms.

B. MATERIALS AND METHODS

1. Source of Parasite

Eggs were obtained from gravid segments of adult KBS worms from laboratory infected dogs. These eggs were used for in vitro hatching and activation experiments. Cysticerci of the TOI and KBS strains, routinely maintained in laboratory mice, were used for in vitro

culture to obtain the strobilate adult worms. Ten- and seventeen-day old worms from autopsied dogs were cultured to maturity in vitro. Details of parasite source and maintenance have been given earlier under General Methods and in Section I.

2. Physical Conditions of Culture

a) Culture Vessels : the following vessels were used :-

i) Falcon Flasks (Falcon Plastics)

These are disposable sterile, rectangular, screw-capped plastic flasks, of 30 ml volume.

ii) Tissue Culture Flasks (Sterilin, Costar)

These are disposable, sterile, screw-capped plastic flasks, with a volume capacity of 200 and 50 ml.

iii) Leighton Tubes (Bellco)

These are screw-capped glass tubes with one flat side. The 20 ml capacity size was used.

iv) Milk Dilution (M.D.) bottles (Gallenkamp)

These are screw-capped glass bottles with a capacity up to 250 ml.

b) Gas Phase : the following gas phases were used :-

i) 5% Carbon dioxide, 10% oxygen and 85% nitrogen.

ii) 10% Carbon dioxide, 5% oxygen and 85% nitrogen.

Sterile gas was bubbled through culture vessels of the various media whenever fresh media was used.

c) Agitation : two different types of agitation were used :-

i) Shaking Water Bath (Gallenkamp)

The culture vessels were gently and continuously agitated with a simple harmonic horizontal motion at about 80 revolutions per minute.

ii) Roller Apparatus (TAAB)

The culture vessels were rotated at an acute angle off vertical at about 25 revolutions per hour in an incubator.

c) Temperature

The routine temperature used was 38°- 39° C in both the water bath and the incubator.

d) pH

The pH was maintained at about 7.2 using the colour change of the phenol red, in the basic media and salt solutions, as an indicator. Adverse pH changes were buffered by bicarbonate buffers or HEPES (Research Organics) at 25 mM. Alkalinity was easily corrected by gassing the media or adding acid or alkali as required.

e) Routine Replacement of Media

Media replacement was necessary to remove excessive toxic wastes and to replenish nutrients and vitamins. The frequency of such replacements depended upon the particular protocol involved in each experiment and the amount of parasites cultured in the vessels. Usually, the amount of media used for cysticerci culture was 2 ml/parasite, and the medium was changed once every 3 days. Sterile procedures were always employed for media replacement. All infected cultures were discarded.

3. Culture Media

Culture media were usually made up of commercially available basic media supplemented by serum, glucose, yeast extract, bile or sodium taurocholate and antibiotics.

a) Basic Media : the following were used :-

i) RPMI - 1640 (Flow)

ii) CMRL - 1066 (Flow)

iii) modified "858" (Appendix I)

The method of reconstitution of these media from commercially available powder is according to instructions provided by the manufacturers. Occasionally, all or part of the sodium bicarbonate was substituted by the organic buffer, HEPES, dissolved into the medium to give a final concentration of 25 mM. All media were sterilized by negative vacuum filtration through a 0.2 μm millipore filter (MILLIPORE) into sterile flasks under sterile conditions. After incubation for 48 hours, uninfected media were dispensed into storage bottles and the appropriate amount of antibiotics was added. Media were stored at 4°C.

b) Serum : the following were used :-

i) Foetal Calf Serum, FCS (GIBCO, Flow)

ii) Newborn Calf Serum, NBCS (GIBCO, Flow)

Sera are supplied in sterile 100 ml bottles and are stored at -20°C. After thawing, they may be kept for short periods at 4°C. Sera were inactivated, when necessary, by heating in a water-bath at 56°C for 30 minutes.

In some experiments, a diphasic medium was used. This is made up of a liquid phase of culture media over a semi-solid serum base. The latter was made by pipetting an amount of newborn calf serum into the bottom of the culture vessel and heating it at 76°C for about 1 hour or until the base was firm. In some treatments the surface of the coagulated base was pitted using a sterile Pasteur pipette, or roughened by bubbling the serum before coagulation. Serum coagulated

this way was also used to make a macerate by homogenizing in a macerater, and appropriate amounts transferred into sterile culture vessels. Coagulated serum was incubated with culture medium for 48 hours to ensure sterility and stored at 4°C until required.

c) Glucose

A stock of 30% glucose (ANALAR, BDH) solution was made in triple distilled water (GIBCO). After sterilization by filtration through 0.2 μm mesh filters, the solution was stored at 4°C until required.

d) Yeast Extract

A 5% solution (W/V) of yeast extract powder (OXOID) was made up in CMRL - 1066 or RPMI - 1640. It was filtered through millipore filters of 5 μm. 1.2 μm, 0.45 μm pore size, and then through a 0.2 μm pore size filter to sterilize it. After incubation at 37°C for 48 hours all sterile solutions were dispensed into 100 ml bottles and stored at 4°C until required.

e) Bile and Sodium taurocholate (SIGMA)

Bile, obtained from the gall-bladder of dogs, was diluted with HBSS to give a 5% solution. Commercially produced sodium taurocholate (pure, synthetic) was used in the form of a 0.2% solution in triple distilled water. Both the bile and sodium taurocholate were sterilized by filtration through a 0.2 μm pore size filter and stored at 4°C until required.

f) Antibiotics

Penicillin and Streptomycin (BDH, Flow), available as a mixed solution of concentration 5000 I.U./ml and 5000 μg/ml. were stored at 4°C until required. The antibiotics were routinely added to culture media and other sterile solutions at a level of 2 ml/100 ml giving a final concentration of 100 I.U./ml (Pennicillin) and 100 μg/ml (Streptomycin).

4. Standard Procedure for *in vitro* Culture

a) Sterile Collection of Larvae

Larvae for *in vitro* culture were usually not collected from infections that were less than 3 months old. Infected mice were killed by cervical dislocation and fixed securely in a supine position on a dissecting board that had been previously cleaned with alcohol. The abdomen of the mouse was sterilized with 2 coats of alcoholic iodine, and then opened along its length using sterilized instruments. Metacestodes were removed aseptically from the peritoneal cavity into a sterile beaker of warm HBSS and then washed in several changes of HBSS.

b) Pepsinization and Evagination of Cysticerci

Cysticerci were pepsinized by incubating 50 - 100 larvae for ½-1 hour at 38°C in about 30 ml of 0.2 or 0.5% pepsin made up in HBSS and maintained at pH 2.0 with 5.0 N hydrochloric acid. Following this, the worms were washed repeatedly in 3 or 4 changes of warm sterile HBSS. The evaginating solution (EVS) consisted of sterile HBSS containing the following constituents :-

pancreatin	0.5%
$NaHCO_3$	0.5%
cholesterol	0.01%
sodium taurocholate	0.2%
trypsin	0.015%
antibiotics	2 ml/100 ml

Larvae were incubated in 20 ml of EVS for 3 - 20 hours at 38°C and then washed at least 3 times with warm sterile HBSS.

Incubation for both pepsinization and evagination was carried out in serum bottles placed in a constant-temperature water-bath with

continuous shaking at about 90 oscillations per minute.

c) In vitro Culture

Cultures were carried out in a variety of culture vessels depending on experimental design. Evaginated worms were placed in a petri-dish and examined under a dissecting microscope. Only fully evaginated cysticerci with normal scoleces were selected for in vitro culture. These were transferred by sterile pipettes into culture vessels containing culture medium. A complete culture medium consisted of RPMI - 1640 or CMRL - 1066 supplemented with the following :-

foetal calf serum (FCS)	25%
yeast extract (YE)	0.5%
glucose	0.6%
dog bile (or sodium taurocholate)	0.02%
HEPES buffer	25 mM
antibiotics	2 ml/100 ml

Normally, the foetal calf serum (FCS) was heat-inactivated in a water-bath at 56°C for 30 minutes. The amount of culture medium varied with the type of vessels used and specific experimental protocol. In a number of culture experiments, various physical and chemical factors were altered to determine the role and importance of such factors. The many variables examined included the duration of pepsinization and evagination, the presence or absence of a coagulated serum base in diphasic systems, the type of culture vessels, the crowding effects of cultures, and the type of basic medium used. Also examined were the effects of storage on the viability of larvae for culture, and the importance of medium supplements such as yeast extract.

5. Assessment of Worm Development

Worms in the culture vessels were examined using an inverted microscope placed in a cabinet maintained at 38°C. At times worms were removed and processed for histological examination. Two methods were used mainly, viz. as whole mount squash preparations, and histological sections. At regular intervals, worms were also removed from the culture vessels and processed for ultrastructural studies. Details of light and electron microscopical techniques used have been given earlier in Section I.

6. In vitro Cultivation of the Oncosphere and Adult

Attempts were made to culture the oncospheres of T. crassiceps in vitro (to observed their development to cysticerci). Eggs were removed by teasing from the gravid segments of adult worms placed in a watch-glass with distilled water. They were washed several times in distilled water with antibiotics and stored in distilled water at 4°C. About 2,000 eggs were pipetted into a Leighton tube containing 0.5% pepsin adjusted to pH 2.0. The eggs were pepsinized for 3 hours in an incubator at 38°- 39° C. After pepsin treatment, the eggs were concentrated by centrifugation at 3000 revolutions per minute for 3 minutes, the supernatant drawn off leaving 2 - 3 mm of liquid above the eggs and EVS added. The eggs were kept in EVS up to 24 hours for observations. Owing to lack of "activation" of the oncospheres few attempts only were made to culture the parasite from this stage and these are described in greater detail in the Results section.

Mature strobilae, obtained 10 and 17 days post-infection of pups on autopsy, were washed in warm sterile HBSS with antibiotics several times on a roller tube. These were then cultured in 50 ml

of CMRL - 1066 containing 25% FCS, 0.5% YE, 0.6% glucose, 0.02% dog bile and antibiotics. A diphasic system was used with a coagulated base of NBCS. The worms were cultured in MD bottles at 38°- 39° C in a continuous shaking water bath at about 80 revolutions per minute.

C. RESULTS

1. Hatching and Activation of Oncospheres

About 20 minutes after incubating in EVS, hatching, i.e. removal of the embryophore, of eggs was observed. The keratin embryophoral blocks began to be detached from one another and the embryophore could be seen to disintegrate slowly. Following the disintegration of the blocks, the oncospheres were liberated from the embryophores. The oncospheres, averaging 20.3 x 15.5 μm in size, appeared to be still wrapped in their thin oncospheral membranes. Only a very small number of these oncospheres showed any activity. "Activation", which includes complete liberation of the oncosphere from its enclosing membrane, was not observed in any of the attempts to stimulate it, though in several of the larvae, oncospheral hooks were observed in frenetic activity. Hatching and "activation" experiments were attempted using various concentrations of bile and sodium taurocholate as well as incubating in diphasic medium with coagulated whole and macerated serum. However, none of the experiments resulted in fully "activated" oncospheres. Oncospheres nevertheless increased in size, reaching an average of 45.2 x 26.5 μm after 4 days incubation in CMRL - 1066 supplemented with serum, yeast extract, glucose and bile. There was no further development even in 12-day cultures; most of the oncospheres showed signs of sluggishness and ceased all movement after 4 days incubation in culture media.

2. In vitro Cultivation of Cysticerci

Pretreatment with Pepsin and Evaginating Solution

Larvae placed in a warm solution of HBSS showed gentle, rhythmic undulatory contractions along the whole worm but more obviously and pronounced in the bladder region. A few invaginated cysticerci evaginated after prolonged incubation in HBSS for more than 1 hour.

Upon exposure to a solution of pepsin, larvae did not appear to change in respect to the magnitude of the rhythmic contractions of the bladder wall, nor did the scoleces exhibit behaviour that was different from that of untreated worms. However, treatment with the evaginating solution (EVS) elicited a rapid response (within 3 minutes). There was an increase in frequency and magnitude of contractions followed by the evagination of scoleces. The neck region separating the scolex from the bladder began to contract and extend alternately, this observation concurring with that made by Esch & Smyth (1976) who termed this action "trumpeting". After evagination, the scoleces and rostellum became very active, exhibiting a "searching" behaviour, extending and contracting in different directions. However, the behaviour described by Esch & Smyth (1976) as "hook raising", in which the rostellum evaginated and the anteriorly pointing hook blades in non-stimulated worms were directed posteriorly, was observed only on very rare occasions. However, throughout the duration of the full enzyme-bile salts (PTT) treatment, "trumpeting" and "searching" behaviour persisted. When the PTT treatment was removed and the larvae were transferred to culture vessels, these movements continued but at a less intense pace.

After the PTT treatment, the bladder portion of the larva was usually digested away. In some experiments, almost 100% digestion was

observed leaving the scolex and a small strobilate region. It appears that the full treatment was necessary for complete digestion of the bladder. When pepsin alone was used, there was little or no digestion of the bladder; similarly so when only trypsin and bile salts were used. However, more carefully controlled experiments are required to verify this as the properties of pepsin are affected by the narrow range of pH within which it can act effectively; furthermore, different batches of pepsin from commercial sources are known to show variations in their activities. In experiments where EVS alone was used to stimulate evagination, growth and development did take place at about the same rate as cultures in which full PTT treatment was employed , but there appeared to be less vesiculation of the worms during culture; the details and discussion of such experiments are dealt with below. In several experiments, cultures were set up where the cysticerci were transferred to culture media without prior treatment with pepsin or EVS. In these, the cysticerci failed to exhibit intense and frenetic contractions and "trumpeting" and "searching" behaviour. However, growth and development proceeded to the same stage as worms which had been subjected to the full PPT treatment, although development was slightly slower.

Growth and Development of Worms in vitro

For the purposes of description, it is convenient to divide the in vitro development of the worms into a series of numbered stages (Fig. 7), although differentiation is, of course, continuous. Evaluation of success (or otherwise) of a particular culture system can thus be made by identifying the appearance of these stages and assessing the maximum degree of development achieved at any one time.

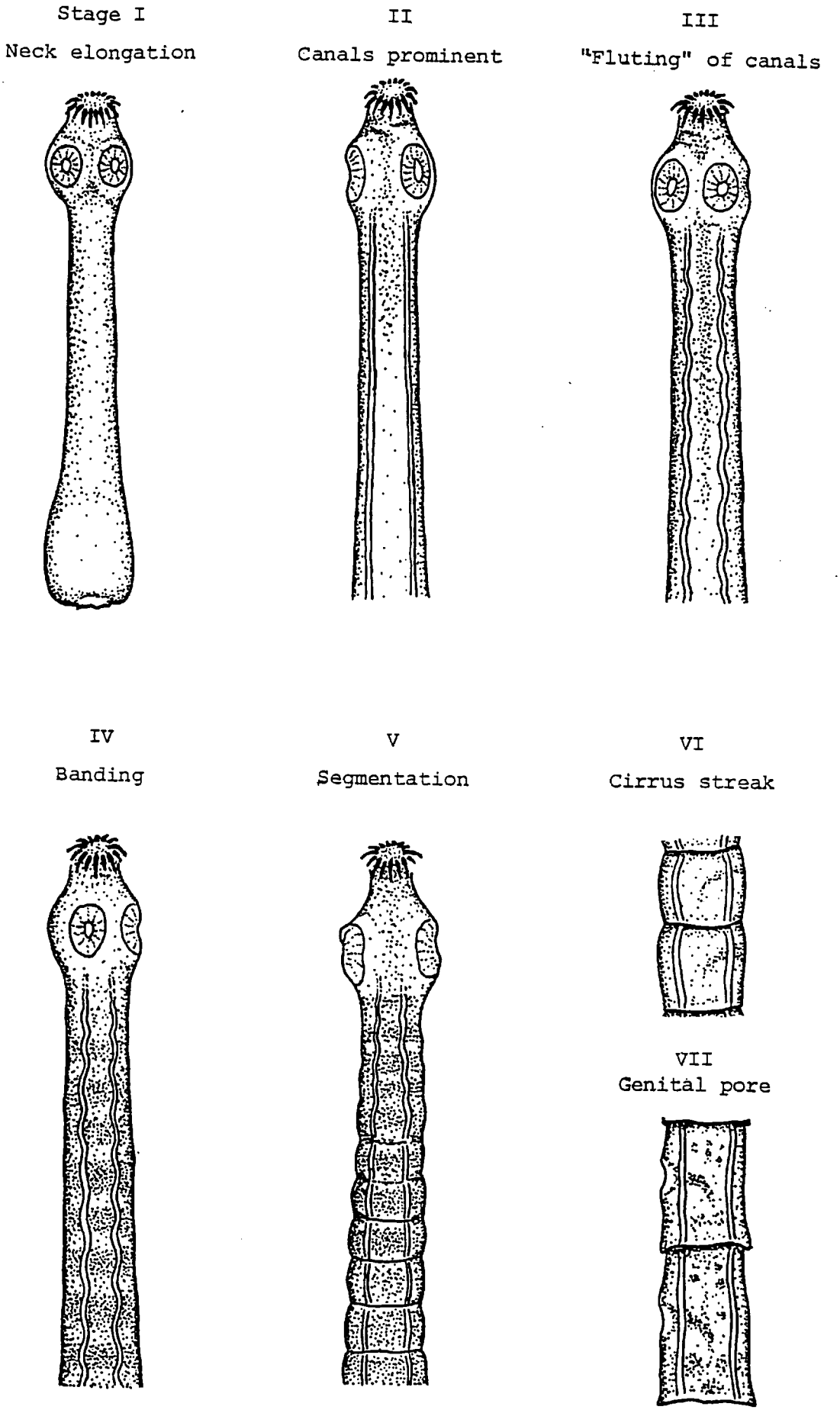


Fig. 7. Stages of Development in the in vitro Cultivation of T. crassiceps

Stage I. Neck Elongation On introduction of activated cysticerci into culture vessels, the worms generally persisted with rhythmic "trumpeting" and "searching" behaviour. Very rarely did larvae penetrate into the coagulated serum base in diphasic culture systems. When holes were punched into the serum base, some of the worms burrowed into the base via the holes and considerable migrations within the base were observed. Within 24 hours of culture, the first signs of development were observed. This involved substantial elongation of the neck, often 2 - 3 times the original length. Whether this lengthening constitutes morphogenesis is uncertain. It could be the result of stretching and relaxation of existing tissue.

Stage II. Appearance of Excretory Canals Following neck elongation, the presence of excretory canals became strikingly apparent, running laterally down the length of the worms. The appearance of the canals seemed to be related to the change in refractiveness of the neck tegument which became more transparent. This could be due to the fact that during this stage the neck region of the worms became more and more flattened laterally.

Stage III. "Fluting" of Canals Within 3 days of development in some experiments, the excretory canals became fluted. The fluting generally began posterior to the scolex and proceeded towards the lower regions as the worm grew in length. The area between the canals became darker and more translucent as fluting and neck elongation continued.

Stage IV. Banding The internal contents of the worms became "consolidated" and segregated into distinct darkened bands between the canals, resulting in the formation of a distinctly banded strobila. This stage was reached as early as Day 6 in some cultures. However,

in other cultures there seemed to be an absence of this stage, i.e. the development of the worms proceeded from Stage III straight to Stage V.

Stage V. Segmentation By Day 8 after introduction into culture vessels, segmentation commenced as indentations of the tegument appeared followed by formation of distinct interproglottid septa. Proglottid formation was more pronounced in areas distal to the scolex. In several cultures this Stage V was reached, but incompletely, with strobila indentations but no distinct septa.

Stage VI. Formation of Cirrus Streak The first reproductive structure to be observed was the cirrus streak, the presence of which was indicated by a denser band of cells extending from the midline laterally in the anterior third of the more posterior proglottids of the strobila. This stage was attained rarely. Most of the worms began to degenerate generally with heavy vesiculation, tegument disintegration and exhibited a tendency to swell up especially in the scolex region. The general appearance of the worms after stages V and VI was dark, and movements were rather sluggish.

Stage VII. Formation of Genital Pore This stage was reached only once in all the culture experiments. A distinct indentation at the tegument where the cirrus streak terminates was observed in the posterior segments. Examination of fixed and stained worms revealed the presence of a lightly stained sperm duct. Neither testes nor ovaries were identified; however, aggregates of darkly stained cells were observed in the expected positions of ovaries, vitellaria and testes.

Rostellar Protrusions

Frequently, scoleces were observed with protruding tongue-like extensions extruding from the tip of the rostellar region. These protrusions appeared either singly or in groups of 2 or 3, and were frequently present on the first 24 hours of culture. Increase in the size of these protrusions *in vitro* would suggest that they are either precipitated secretions of an immunological origin, or they form part of the organ-structures related to the rostellum, perhaps associated with sensory functions. The nature, and possible functions, of these rostellar protrusions are dealt with later when the ultrastructural changes in *T. crassiceps* during *in vitro* culture are considered.

Experiments with Different Culture Systems

The results from experiments in which different factors in the culture system were varied are summarised in Table I.

Experiment 1. In this experiment, 2 variable factors were tested, namely, the use of different evagination times, and culturing in crowded or uncrowded (sparse) conditions. The TOI strain of *T. crassiceps* was used and they were cultured in MD bottles. A monophasic medium consisting of CMRL - 1066 and the usual supplements was used. Thus there were 4 groups of cultures each consisting of 3 vessels :- 2-hour evagination, 30-40 worms; 2-hour evagination, 5-10 worms; 20-hour evaginations, 30-40 worms; and 20-hour evagination, 5-10 worms.

The prolonged period in evaginating solution did not appear to have any physical effect on the larvae. All worms quickly reached Stage I in their development on Day 1. There were no significant differences among the cultures up to Stage IV as the rates of

TABLE I. In vitro Culture of Taenia crassiceps Cysticerci

Exp. No.	Experimental Protocol and Culture System		Number of Days Required for Worms to Reach Described Stage							No. of Days before :-	
	Constant Factors	Variable Factors	I	II	III	IV	V	VI	VII	Degeneration	Termination
1	TOI, CMRL, MD, monophasic	2 hr EVS, crowded	1	4-5	6	8	9-10	-	-	16	21
		2 hr EVS, uncrowded	1	5-6	7	7-8	10-12	-	-	13	21
		20 hr EVS, crowded	1	4	5	7	13	-	-	10	21
		20 hr EVS, uncrowded	1	4	4	7	-	-	-	8	21
2	PTT, CMRL, MD, monophasic	TOI	1	5-6	7	7-8	9-12	-	-	13	21
		KBS	1	3	4-5	6	9-10	-	-	15	21
3	KBS, PTT, CMRL, monophasic	MD, NBCS, uncrowded	1	2	5-6	6	8	-	-	11	22
		MD, NBCS, crowded	1	2	4	6	8	-	-	12	22
		Leighton, NBCS, uncrowded	1	2	3	7	8	11	-	16	25
4	KBS, Leighton, CMRL. diphasic	RPMI	1	3	6	8	9	-	-	13	19
		CMRL	1	2	5	6	8-9	-	-	15	19
5	KBS, Leighton, CMRL, diphasic	3 hr EVS, smooth base	1	3	4	9-10	11	-	-	15	23
		3 hr EVS, holed base	1	4	8	10	14	-	-	16	23
		20 hr EVS, smooth base	1	3	9	10	-	-	-	16	23
		20 hr EVS, holed base	1	3	7-8	11	14	15	-	17	23
6	KBS, MD, CMRL, diphasic	3 hr EVS, smooth base	1	2	7	-	-	-	-	8	11
		3 hr EVS, holed base	1	3	4	7	-	-	-	9	11
		20 hr EVS, smooth base	1	2	7	-	-	-	-	9	11
		20 hr EVS, holed base	1	3	-	-	-	-	-	5	11
7	KBS, MD, CMRL, 20 hr EVS	Rough serum base	2	5	7	8-9	9	-	-	Infected	16
		Smooth serum base	1	3	5	6	8	-	-	Infected	16
8	KBS, CMRL, macerated NBCS	MD	1	4	4	6-7	11	-	-	11	14
		Leighton	1	3	4	5-6	11	14	22	21	36
9	TOI, CMRL, diphasic	Frilled cellulose tubing	1	7	12	-	-	-	-	17	41
10	TOI, COSTAR, RPMI, monophasic	PTT	1	7	13	-	-	-	-	16	25
		EVS only	1	7-8	15	-	-	-	-	16	25
		Pepsin only	4	8	-	-	-	-	-	16	25
		No PTT	4	7-8	12	21	-	-	-	22	25
		Bladders removed surgically	4	7-8	13	21	-	-	-	17	25
11	KBS, MD, "858"	Monophasic, PTT	1	3	5	6	9-10	-	-	16	20
		Monophasic, EVS only	1	3	5	10-11	12	-	-	15	25
		Diphasic, PTT	1	3	5	6-7	10	-	-	15	20
		Diphasic, EVS only	1	4-5	-	-	-	-	-	15	25
12	KBS, COSTAR, RPMI, monophasic, no PTT	Stored for 9 months	-	-	-	-	-	-	-	-	3
		Stored for 7 months	-	-	-	-	-	-	-	-	3
		Stored for 3 months	3	-	-	-	-	-	-	-	5
		Stored for 2 months	5	12	-	-	-	-	-	14	15
		Stored for 3 weeks	2	5	-	-	-	-	-	14	15
13	TOI, COSTAR, RPMI, monophasic	+ YE	1	2	7	13	-	-	-	15	18
		- YE	1	2	8-9	-	-	-	-	14	18

development did not vary between culture groups any more than between individual flasks in a group. However, Stage V was attained by Day 9 in 2-hour evagination groups in the crowded culture, while the 20-hour evagination crowded culture reached this stage after 13 days and the uncrowded cultures failed to do so. The worms in the 20-hour evagination groups began to disintegrate rapidly between Day 8 - 10 while this happened 3 - 8 days later for the 2-hour evagination groups. The results, though not very conclusive, suggest 2 inferences, viz. a crowded situation, as long as it is not excessively so, is more conducive to growth and development; and a prolonged period of incubation in an evagination solution of trypsin/bile salts may be detrimental to subsequent development of the worms in vitro. The experiment was terminated on Day 21.

Experiment 2. This experiment was designed to examine the growth and developmental rate between 2 strains of T. crassiceps, namely the TOI and KBS. Three cultures per group were set up in MD bottles using a monophasic supplemented medium of CMRL - 1066. One obvious difference between the 2 strains is the larger proportion of TOI larvae that are already evaginated upon removal from the abdominal cavity of the mouse. Upon treatment with PTT, many of the TOI metacestodes have their scoleces as well as the bladders digested away. For the purposes of this experiment only larvae with intact scoleces were selected for culture. The KBS strain larvae developed at a slightly quicker rate than the TOI strain. By Day 4, KBS larvae had attained stage III while the TOI larvae took at least 7 days. Neither of the 2 strains developed beyond Stage V and began disintegrating by Day 13 (TOI) and 15 (KBS). The experiment was terminated after 21 days.

Experiment 3. Instead of the normal foetal calf serum used to supplement culture media, new-born calf serum, heat-inactivated at 56°C for 30 minutes, was used. Two types of culture vessels were compared, and another comparison tested between uncrowded and crowded cultures. Four cultures per group were started using KBS strain larvae and CMRL - 1066 with all the normal supplements in a monophasic system. The 3 different groups were :- uncrowded culture in MD bottle; crowded culture in MD bottle; and uncrowded culture in a Leighton tube held on a roller in the oven at ∿38°C. All the worms in this set of cultures exhibited healthy growth rates with Stage V reached in all groups by Day 8. However, the worms cultured in Leighton tubes appeared to develop best, being the only group to reach Stage VI by Day 11, and did not begin to disintegrate on vesiculation until Day 16. The cirrus streaks observed in the worms were faint when examined live, but clusters of cells were observed in H & E stained wax sections.

Experiment 4. Two different culture media were compared in this experiment. Five KBS strain larvae were cultured in each of 8 MD bottles, 4 of which had CMRL - 1066 and the other 4 had RPMI - 1640 with the normal supplements. CMRL - 1066 appeared to be more suitable for development of the worms as worms cultured in it developed faster by between 1 or 2 days at every stage beyond Stage I. Worms in CMRL - 1066 were also alive for a longer period before the tegument became vesiculated and subsequently disintegrated. However, the results were not conclusive as the differences in developmental rate were too small to be significant.

Experiment 5. In this experiment, 2 variable factors were examined, namely, the duration of evagination and the advantage of culture bases

with holes punctured in them. Four groups, each with 3 culture tubes, were set up :- 3-hour evagination and smooth base; 3-hour evagination and holed base; 20-hour evagination and smooth base; and, 20-hour evagination and holed base. All cultures were in Leighton tubes, using a diphasic culture medium of 10 ml CMRL - 1066 with normal supplements, above a solid serum base of coagulated new-born calf serum. Holes were punctured in the bases of 2 groups with a sterile Pasteur pipette. Five KBS larvae were placed in each of the tubes which were gently agitated on a roller in a constant temperature oven. Within 36 hours worms were observed to have burrowed into the holes in the serum base. Beginning with the scolex embedded within the base, there was slow migration inwards into the substrate until after about 10 days when the whole strobilate worm has totally burrowed into the base. In some instances, the worm, as it grew in length, folded upon itself within the holes and reappeared in the liquid phase above the base. Under the highest illumination of the inverted microscope, burrowing worms were observed to be very active, their rostella and suckers exhibiting constant "searching" movements. Stage III was attained first in cultures with 3-hour evagination and smooth base at Day 4 while the others took 7 - 9 days. All cultures reached Stage V with 11 - 14 days except for the one with 20-hour evagination and smooth base where development ceased after Stage IV. Worms that burrowed deep into the base were from cultures with 20-hour evagination and punctured bases. In one culture, Stage VI was obtained on Day 15. However, all the worms began to degenerate badly after Day 17 and the cultures were terminated on Day 23.

Experiment 6. The experiment above was repeated here with the use of MD bottles instead of Leighton tubes. The worms were cultured in

40 ml of liquid medium and 10 ml of coagulated serum base, and incubated in a shaking water bath. For some unknown reason(s), the experiment had to be terminated on Day 11 as the worms showed extremely poor development and had degenerated after 5 - 9 days. Development was rather slow, taking 4 - 7 days to reach Stage III. Only one set of cultures reached Stage IV and one did not go beyond Stage II. The poor result could be due to a poor batch of serum (a common problem in tissue culture work), or some inadvertant (unrecorded) drastic changes of temperature of the cultures, or some other unknown cause(s).

Experiment 7. In this experiment, an attempt was made to simulate the rough surface of the canid intestinal lumen so as to compare the variable "smoothness" of serum base of diphasic medium. Crowded cultures were started by transferring larvae which had been pretreated for 1 hour in pepsin and 20 hours in evaginating solution. The serum base of one group was "roughened" by vigorously shaking the NBCS in the MD bottle before coagulating the serum with its foamy surface. The other group of cultures had a smooth surface of coagulated serum base. There were 3 cultures in each group. During the couse of the experiment it was observed that worms tend to remain in the depressions on the rough surface of the base. These worms showed slower development and a tendency to become cystic and dark. The worms in the "smooth" cultures, however, develop more rapidly and appeared more healthy after Day 8. Worms on a smooth base reached Stages III - V in Days 3,5,6, and 8, whereas worms on a rough base showed delayed development by 2 days. Unfortunately, all the cultures were infected by a fungus after Day 10 and had to be discarded subsequently. Contradictory to expectations, there was poorer development in the rough-surfaced cultures. In

retrospect, it could be due to the fact that the depressions on the surface of the base might have trapped waste-material around the worms that were lodged in them, hence the rapid darkening and cystic tendency of the worms.

Experiment 8. In this experiment, a diphasic medium was used. However, instead of a serum base, the semi-solid phase consisted of coagulated NBCS which had been macerated by a sterile needle and sieved through a 2 mm-mesh. A comparison was made between the use of MD bottles in a shaking water bath and Leighton tubes on a roller. The worms showed almost similar development rates up to Stage V which was reached by Day 11 in both types of culture vessels used. Worms in MD bottles stopped developing after this stage and began to show signs of disintegration soon after. On the other hand, the worms in Leighton tubes continued to develop, and by Day 14 had reached Stage VI. One worm developed a small genital pore on Day 22; this was the only occasion in all the experiments when Stage VII was attained. However, the Stage VII worm was already showing some unhealthy signs of vesiculation, especially along the posterior strobila. All the Leighton tube cultures were kept up till Day 36 with a fair proportion of the worms still alive and fairly active, but there were no further signs of development.

Experiment 9. In this experiment, a roll of cellulose tubing with one side cut into frills was introduced into the serum base in Leighton tubes before coagulation. The liquid phase consisted of CMRL - 1066 with the normal supplements. TOI strain larvae were used. All the worms reached Stage I on Day 1 but thereafter developed very slowly. It was only on Day 7 that some of the worms reached Stage II

and it took 12 days before Stage III was observed. After this, there was no further development; disintegration of worm tegument was observed from Day 17. The cultures were terminated after 41 days when it was obvious that no further development would occur although many of the worms remained fairly healthy looking with intact teguments while some had slightly vesiculated or swollen scoleces.

Experiment 10. In this experiment, different methods used to remove the bladder from the larvae were employed to examine the effects of such removal. The effects of pepsin treatment alone and evaginating solution alone were also observed. There were 3 cultures in each of 5 groups : full PTT treatment; evaginating solution alone; pepsin alone; no pretreatment and, the physical removal of the bladder by cutting with a sharp scalpel blade. Five TOI strain larvae were cultured in a monophasic medium of RPMI - 1640/Hepes in plastic Costar flasks, incubated in a shaking water-bath at ∿38.5°C. Good development was observed in cultures where larvae had their bladders cut away and in those where there was no pretreatment. Stage IV was observed after 21 days of culture. The poorest development was observed in worms that were pretreated with pepsin alone in which there was no development beyond Stage II (8 days). The cultures with PTT and evaginating solution treatment alone attained Stage III by Day 13 - 15 with no further development. Only the untreated worms remained alive beyond 21 days, the rest beginning to degenerate after 16 - 17 days. The cultures were all terminated after 25 days. The poor development and early degeneration of the treated worms could be due to the fact that the enzymes had a severe effect on the TOI larvae, many of which have evaginated scoleces even prior to enzyme-bile-salt treatment. In this particular experiment

no selection was made to culture only those larvae that were active and healthy-looking after the pretreatment.

Experiment 11. In this experiment, two variable factors were examined, viz. monophasic media and the effects of pepsinization. There were 4 groups of cultures each consisting of 3 flasks :- monophasic medium, PTT; monophasic, minus pepsin; diphasic medium, PTT; and, diphasic medium, minus pepsin. The solid base was made of 10 ml of coagulated NBCS and the liquid phase consisted of a modified Parker's 858 made by supplementing CMRL - 1066 with the appropriate amounts of amino-acids obtained from commercial sources. The modified "858" was however incomplete because some of the ingredients were not obtainable at the time of the experiment. Five KBS larvae were cultured in MD bottles, incubated in a shaking water-bath after pretreatment in pepsin (1 hour) and/or evaginating solution (overnight). Larvae that were treated with evaginating solution alone without prior pepsinization retained their bladders and all the buds that were attached at the abscolex end. During the course of the culture, these buds were actively contracting rhythmically. Growth in number and size was observed in these cultures, very much so in the cultures that were in diphasic medium after pretreatment with evaginating solution. Comparing growth and development rate between monophasic and diphasic cultures, there did not appear to be any significant differences. Worms reached Stage V in 9 - 10 days, and began to degenerate in 15 - 16 days; the cultures were terminated after 20 days. Apparently, the modified "858" was not contributing positively to better growth and development. There was a marked difference between larvae cultured in monophasic medium and diphasic medium when no pepsin was used in the pretreatment.

Strobilate growth was not observed in larvae cultured in a diphasic medium, none of the worms developing beyond Stage II. However, worms in monophasic media developed through all the stages up to Stage V in 12 days, just slightly longer than fully pretreated PTT worms. This may indicate that an extensive generation and growth of buds in some way inhibits development of the cysticerci in a strobilate direction. The main factor involved in this inhibition could be nutritional in nature as the rapidly growing buds compete successfully for nutrients directly from the culture medium or/and by drawing nutrients from the main body of the worm. Some growth factors could be present in the coagulated serum which contributed to a medium conducive to bud growth and production since there was very much less growth of buds in larvae culture in monophasic medium. Nevertheless, none of the worms in all 4 groups attained Stage VI in their development but all began to degenerate badly by Day 15 - 16 and cultures had to be discarded after 20 - 25 days.

Experiment 12. Larvae that have been stored for varying periods of time were used for cultures in this experiment. 10 larvae of KBS strain were cultured in Costar flasks using a monophasic medium containing RPMI - 1640. There were 2 or 3 flasks in each of 5 groups of cultures :- 9 months storage; 7 months; 3 months; 2 months; and, 3 weeks. Storage was carried out in MD bottles under a nutrient medium (858 or RPMI - 1640) at $\sim$4°C. Larvae generally took on a more cystic appearance directly proportional to duration of storage. There was no pretreatment with pepsin or evaginating solution; the larvae were washed and directly transferred to culture flasks. There was no growth, nor signs of life, in larvae that were stored for 7 and 9 months. Three-month storage larvae showed slight contractions when

warmed in the culture flasks and attained Stage I after 3 days. Better results were obtained when larvae stored for 3 weeks and 2 months were cultured. The former attained Stages I and II in 2 and 5 days respectively, while the latter took a longer time of 5 and 12 days. However, there were no further signs of development. The worms remained alive but began to rapidly darken and became sluggish by 14 days and had to be discarded after Day 15.

Experiment 13. The importance of supplementing media with yeast extract (YE) was investigated. 10 TOI larvae were cultured in each of 4 Sterilin flasks, divided into 2 groups, one having monophasic medium of RPMI - 1640 with the usual supplements (+YE), and one without yeast extract (-YE). Worms with the benefit of YE developed slowly and reached Stages III and IV by Day 7 and 13 respectively, but -YE worms failed to develop beyond Stage III. After 14 days the worms in all the cultures degenerated rapidly and the experiment was terminated on Day 18.

3. The Ultrastructure of *in vitro* Cultured Cysticerci

In the series of *in vitro* culture experiments described earlier, the best system appears to be that of culturing KBS strain worms in Leighton tubes on a roller using fully supplemented CMRL - 1066 as the liquid phase in a biphasic medium. Worms from this culture system that had been processed for electron microscopy will be described. Only features that show significant differences will be described in detail; those that are similar to *in vivo* worms will be considered only briefly.

After 7 days, worms were generally healthy looking. SEM observations reveal normal tegumental microtriches on the sucker,

scolex and strobila regions of the worms (Plates 109 - 113). The commonly observed rostellar protrusion appeared to be part of the worm and not precipitated material (Plate 109). Microtriches on the suckers and scolex were generally upright with straight shafts, but those on the strobila had shaft tips bent or curved downwards (Plate 113). TEM studies, too, revealed normal internal structures. The tegument was generally similar to in vivo worms with the expected arrangement of microtriches, distal cytoplasm and perinuclear cytoplasm (Plates 114,115). In the sucker region, the fibrous zone between the distal cytoplasm and the muscles appeared normal (Plate 114). Prominent desmosomes were observed where the muscles attach onto the fibrous zone. Numerous aggregates of secretory granules were found in cellular extensions among the muscles. Cells with glycogen granules were also observed. Like the sucker region, the neck region of 7-day cultured worms showed no major differences from in vivo worms (Plate 115). The only difference of note was found in the fibrous material (Plate 116). High magnifications of the tegument revealed normal microtriches on the surface and disc-shaped bodies and vesicles in the distal cytoplasm (Plate 116). Terminal processes and cilia of sensory organelles were also comparable to those in in vivo worms (Plates 116,117). The excretory canals had the usual bleb-like extensions along the canal wall which was covered in a normal plasma membrane (Plates 118,119). One type of cell that has not been described before was observed among the parenchymal cells in the cortical region of the strobila. It had a large nucleus with a prominent nucleolus. This is considered to have a secretory function as numerous prominent granules were present in the granular cytoplasm (Plate 120). The hook organ in the rostellum had the

usual disc-shaped bodies, vesicles, and canals (Plate 121).

On the 12th day of culture "bubbles" were observed on the neck region of some worms (Plates 122,123). Close examination revealed them to be smooth spherical globules, but they could not be identified further because sections through these "bubbles" were not obtained for TEM examination. While the tegument on the scolex appeared to be normal (Plate 122), it was not the case at the segmented region of the strobila. The tegument appeared to be degenerate and vesiculated. The microtriches were thin and short (Plates 124). Bubbles of varying sizes were observed on the surface (Plates 124,125). TEM observations on the rostellar region revealed the usual basic features (Plate 126). On close examination, the microtriches appeared abnormal. In the scolex region, there were electron dense material condensed in the shaft portion of the microtriches (Plate 127). The peripheral wall of the basal portion of some microtriches appeared to be partitioned. This was also observed in microtriches on the neck region (Plate 128). Further, some microtriches in this region were swollen, and numerous vesicles with varying amounts of electron dense granules were present in the distal cytoplasm. Excretory canals were plentiful along the strobila; these were usually found occurring in groups with smaller canals leading into larger ones. The parenchymal cells were lacking in glycogen granules (Plate 129). TEM studies revealed that the rostellar protrusion had an internal structure with an appearance that resembled the hook organ (Plates 130-132). These protrusions were bounded by a plasma membrane with a sparse distribution of microthrix-like projections on the surface. The internal matrix consists of closely packed disc-shaped bodies and vesicles which were similar to those found in the hook organ. Numerous whorls of electron

dense lamellae and vacuolated spaces were also present. Groups of disc-shaped bodies, packed into vesicles, were present in the matrix (Plate 131) as well as in the vacuolated spaces (Plate 132). Flame-cells were seldom observed in 12-day cultured worms; those that were examined were abnormal in that only the "flame" portion was observed (Plate 133). In normal flame-cells the flame would be found within a cylindrical tube made of interdigitating leptotriches.

Most of the worms that were cultured beyond 15 days had degenerated badly. SEM observations revealed that the most "preserved" features were the microtriches on the scolex and anterior non-segmented strobila region (Plates 134-136). Microtriches on the posterior non-segmented region appeared thin and clumped together (Plate 137); this however, could be due to an artifact of processing. The microtriches on the segmented proglottids were long and filamentous (Plate 138). The scolex of 22-day cultured worms and the microtriches found in this region were still normal in appearance (Plates 139,140). The protrusions at the tip of the rostellum were usually disintegrated (Plate 140, inset). TEM observations revealed gross abnormalities in the tegument and sub-tegumental structures. The distal cytoplasm was thrown into large folds (Plate 141). The microtriches were long and thin; the basal portions were relatively thin and extended into filamentous shafts (Plates 142,143). No tube-like structures were observed between the base and shaft portion of the microtriches. Disc-shaped bodies were packed densely into the distal cytoplasm, some of these bodies were arranged in piles or stacks (Plate 142). The muscle layers appeared hypotrophied (Plates 141, 142). Droplets containing lipids, or possibly waste products, were abundant in the distal cytoplasm and sub-tegumental cells (Plates 141,142,144). Excretory

canals were common and appeared normal. However, the rarely observed flame-cells were malformed, without a cylidrical tube surrounding the "flame" (Plate 144). Most of the cells in the parenchyma had little glycogen granules in the cytoplasm.

4. The Ultrastructure of *in vitro* Cultured Adult Worms

Four worms, obtained from a dog 10 days after feeding with KBS cysticerci, were cultured in a diphasic system (details in Materials and Methods). These increased in length rapidly, but by the tenth day of culture, were entangled in knots and subsequently degenerated shortly. In a second attempt at culturing adult worms, 2 worms obtained 17 days post-infection were cultured for a further 14 days. At the end of the experiment, the terminal proglottids were processed for TEM studies.

The tegument resembled that of *in vivo* adults in its basic structure. The distal cytoplasm had an irregular surface, often with vacuoles and pits (Plate 145). Microtriches were generally thin and sparsely distributed. The cytoplasm was highly granular and contained numerous round shaped dense bodies and vesicles containing varying amounts of granular material. As in *in vivo* adults, the distal cytoplasm was connected to the perinuclear cytoplasm by numerous cytoplasmic connections. Below the distal cytoplasm, the circular and longitudinal muscles, and the perinuclear cytoplasm, were normal in appearance. Occasionally, among the glycogen-rich parenchymal cells, there were darkly stained cells with irregular cytoplasmic extensions. The cell has a large nucleus with a prominent nucleolus and a highly granular cytoplasm containing numerous electron-dense granules, probably secretory, and unidentified strands of darkly stained fibres

(Plate 146). Excretory canals were comparable to in vivo ones, with glycogen-rich parenchymal cells beneath the canal wall (Plate 147). Similarly, the vagina appeared normal; the epithelium of the wall, containing electron-dense bodies, had the usual epithelial projections and basal lamina which was thrown into invaginations (Plate 148). No stages of ovogenesis were observed, presumably because the proglottids examined were too mature and the ova had degenerated or fertilized. Sperms that were found in the vas efferens were apparently normal (Plate 149) and fully-formed. However, in the testes, there were signs of abnormality. There was accumulation of lipid droplets in the gonadal cells (Plate 150). Many of the sperms had their outer microtubular sheath fragmented. No rosette cytophores were observed. In testes where all the sperms had been fully differentiated, many of the sperms were abnormal. Some of the axial filament complexes did not possess an outer microtubular sheath (Plate 151). Further, the microtubular arrangement in the outer sheath appeared disrupted in many sperms, and these often did not contain a central axial filament complex. Only normal, fully-formed sperms were found in the seminal receptaculum (Plate 152). Presumably these were functional and could therefore survive and move down the vaginal canal to the receptaculum. The receptaculum had a much vacuolated and intensely stained wall. In the receptaculum, the sperms were in a heterogenous matrix of vacuoles and amorphous material. There were no observable abnormalities in the structure of the uterus. The wall was made up of highly granular cells and extensive folds of lamellae project into the uterus (Plate 153).

In the uterus, a majority of the developing eggs were observed to be abnormal. Most of these were either underdeveloped or malformed.

In most of these abnormal eggs, the outer envelope had not been formed (Plates 154,155,157). The capsule was observed as a much folded lamina. Within many of these capsules, only the vitelline cell was observed; it is impossible to determine if this was the result of the particular plane of sectioning or the lack of a normal, fertilized embryo. Young vitelline cells showed evidence of metabolic activities. There were numerous intensely stained granules, possible vitelline secretions, golgi complexes and large mitochondria (Plate 154). These darkly stained granules were also observed within vacuoles in the highly granular cytoplasm. In presumably older cells, vitelline material appeared aggregated; there were many degenerated vitelline cells which consist predominantly of these darkly stained material (Plate 155). Developing vitelline cells appear to synthesize another type of material that appeared as lightly stained, homogenous substance collected in vesicles (Plates 155,157). Large mitochondria were often present in these vitelline cells. Many abnormal embryos had malformed embryophores (Plate 156). Embryophore block material were observed accumulating on a membrane-like "template" but not surrounding an oncosphere. The embryophoral cell was not identifiable in these abnormal eggs. The outer envelope cell in such eggs was usually not completely differentiated and contained vacuoles with granular material. On occasions, a partially formed embryophore could be observed next to a vitelline cell within a capsule, but the oncosphere and outer envelope were absent (Plate 157). Normal and fully developed eggs were also observed among abnormal ones in the uterus, though in smaller quantities. These normal eggs resembled, in nearly every structural aspect, those examined in mature proglottids of <u>in vivo</u> worms (Plates 158-161). The capsule, outer envelope, embryophore,

Plates 109 - 113. Scanning electron micrographs of *T. crassiceps* after 7 days *in vitro* culture.

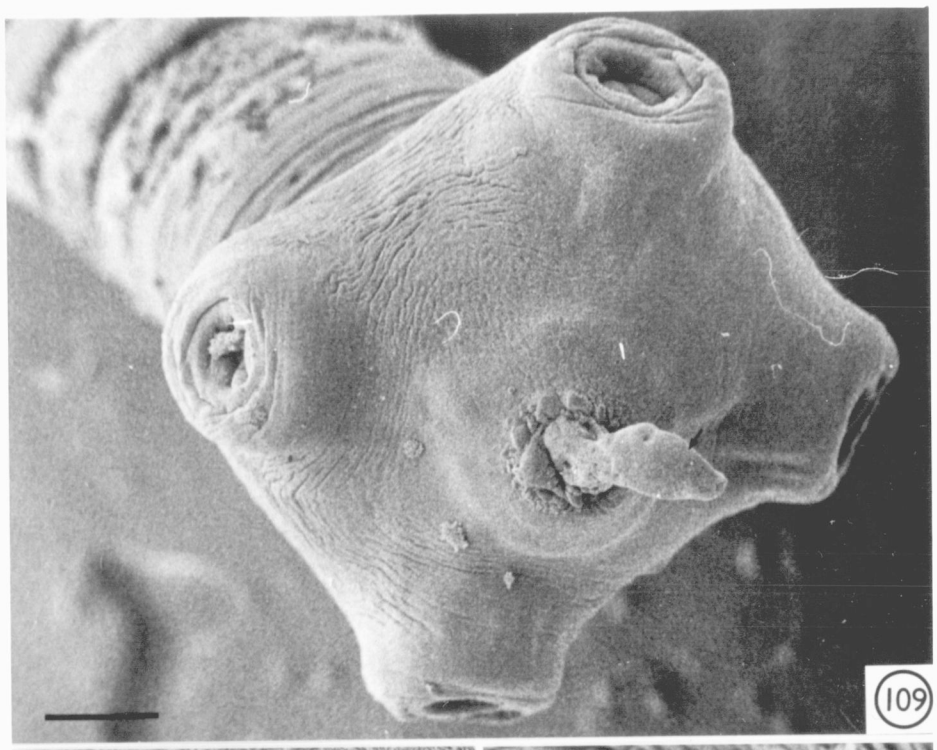
109. The scolex with a non-everted rostellum. Rostellar protrusions (arrow) are often seen protruding at the rostellar tip. (Scale = 100 μm)

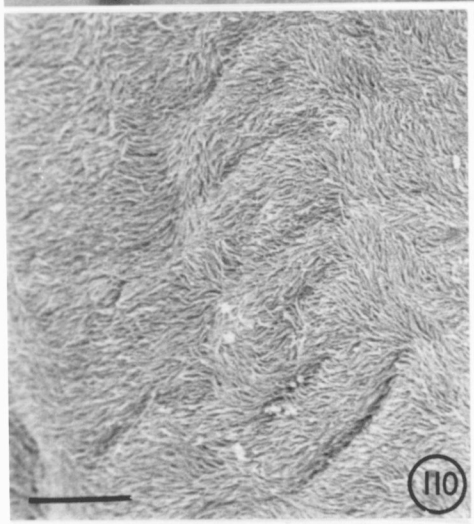
110. Microtriches on the rim of the sucker. (Scale = 10 μm)

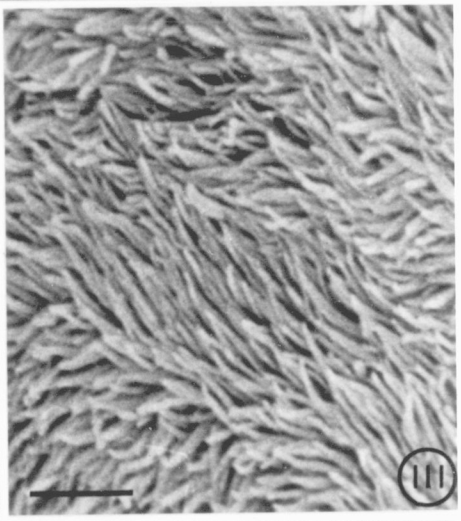
111. Microtriches on the rim of the sucker. (Scale = 2 μm)

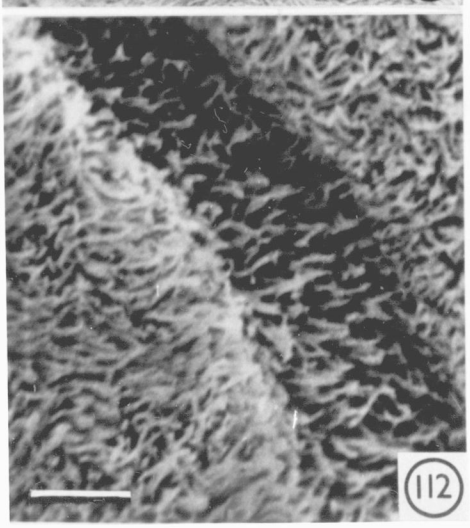
112. Microtriches on the scolex region between the suckers. (Scale = 2 μm)

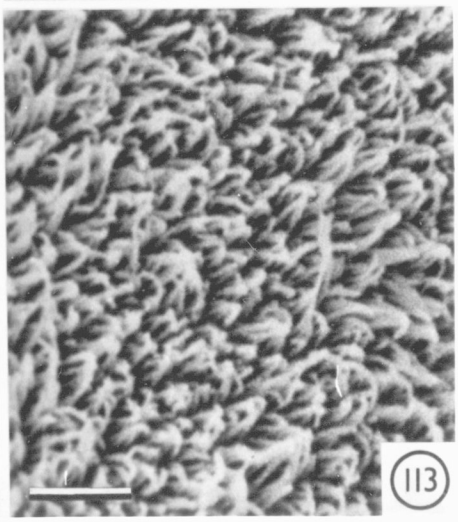
113. Microtriches on the strobila. (Scale = 2 μm)


109


110


111


112


113

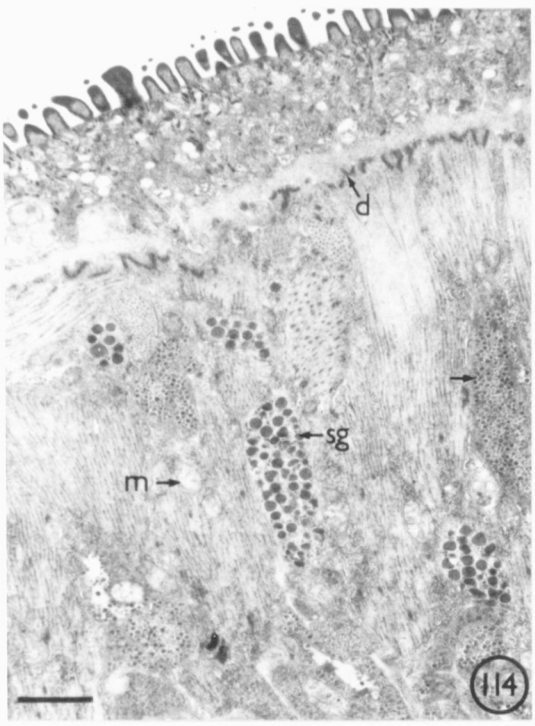
Plates 114 - 117. Transmission electron micrographs of T. crassiceps after 7 days in vitro culture

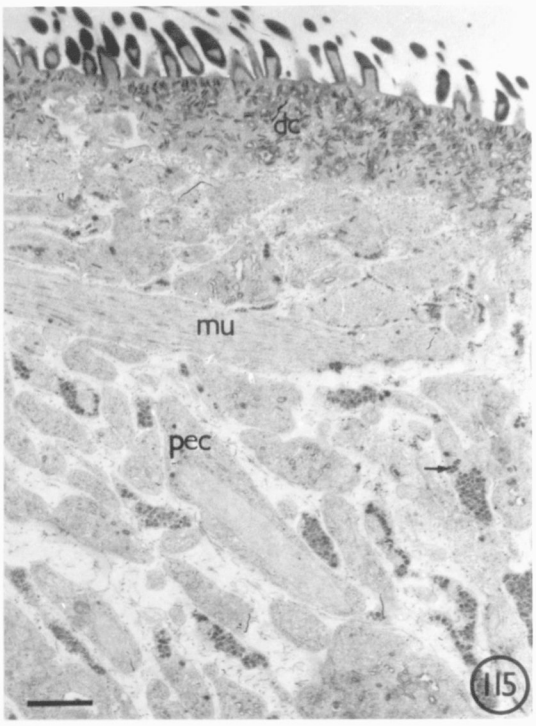
114. Section through the sucker region showing prominent desmosomes (d) where the sucker muscles terminate at the fibrous zone. Aggregates of secretory granules (sg) are found between the muscles. Note the abundance of mitochondria (m) and glycogen-laden cells (arrow). (Scale = 1 μm)

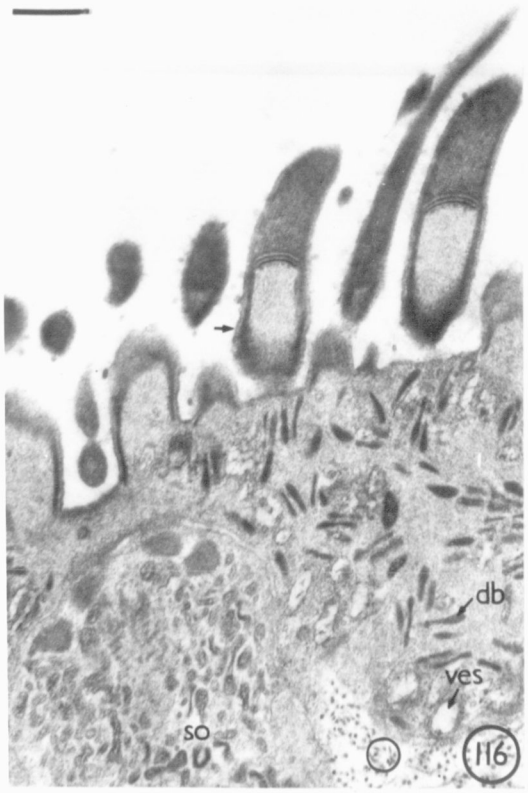
115. Section through the neck (strobila) region showing normal arrangement of distal cytoplasm (dc), muscle layers (mu) and perinuclear cytoplasm (pec). Note glycogen-laden cells among the cytons (arrow). (Scale = 1 μm)

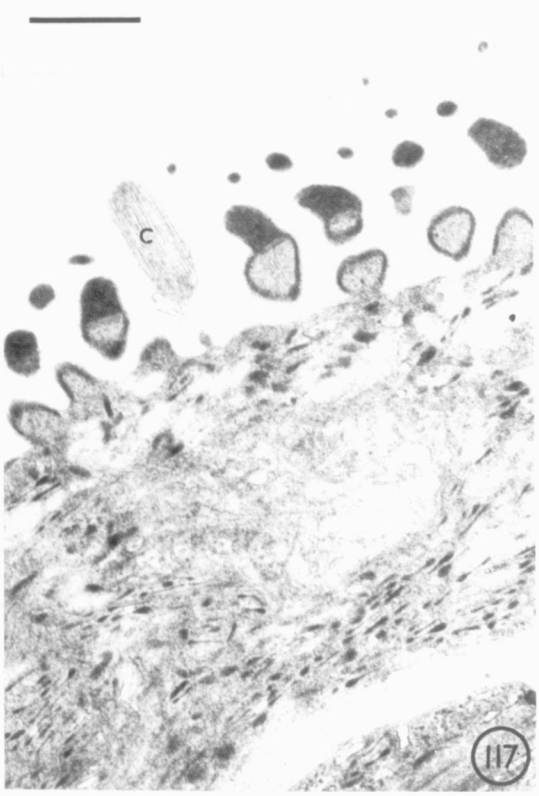
116. High magnification of the distal cytoplasm containing a sensory organelle (so). The microtriches appear normal with a plasma membrane covering (arrow). The distal cytoplasm contains the usual membrane-bound disc-shaped bodies (db) and membrane-bound vesicles (ves). The fibrous zone appears fragmented with strands of condensed material (circled). (Scale = 0.25 μm)

117. The occasional sensory projection or "cilium" (c) is present on the distal cytoplasm. (Scale = 0,5 μm)


d
sg
m
114


dc
mu
pec
115


db
ves
so
116


c
117

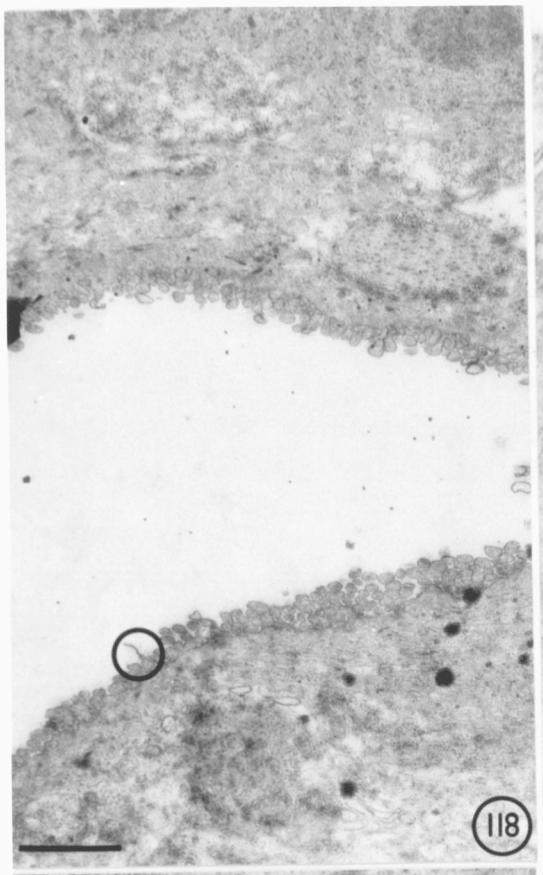
Plates 118 - 121. Transmission electron micrographs of 7-day cultured worms.

118. Section through an excretory canal showing bleb-like projections extending from the wall. Circled region is magnified in Plate 119. (Scale = 1 μm)

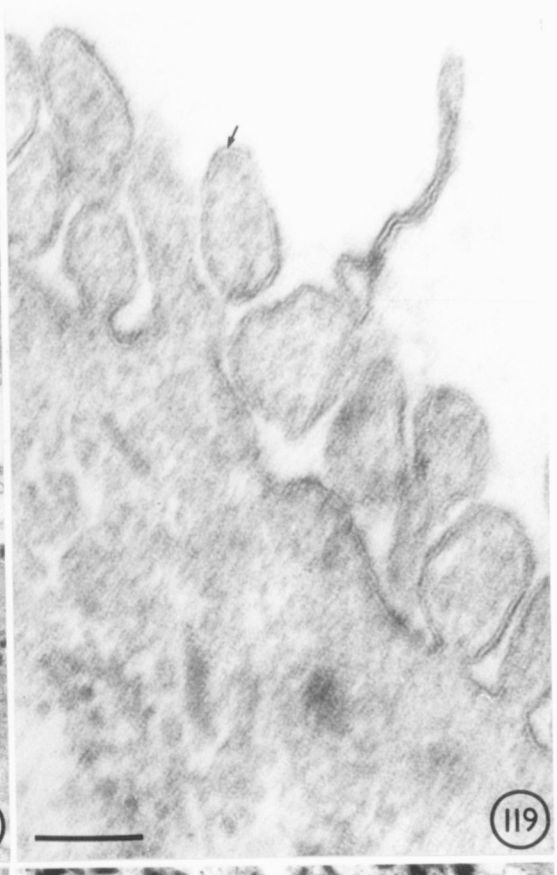
119. The projections from the excretory canal wall are covered by a plasma membrane (arrow). Occasionally these projections end in a filamentous tip. (Scale = 0.25 μm)

120. A secretory cell containing a large nucleus (n) and prominent nucleolus (nu). Note the numerous secretory granules (sg) and mitochondria (m). (Scale = 1 μm)

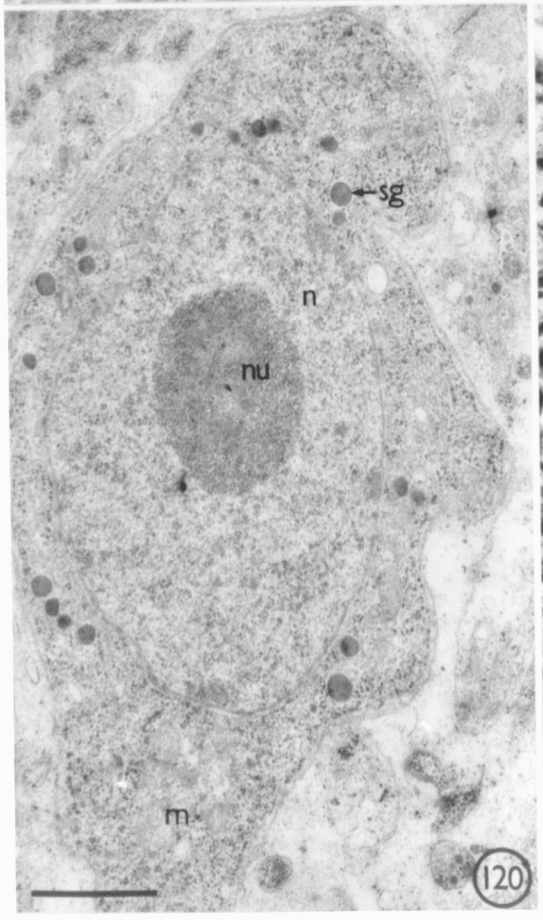
121. Part of the hook organ showing a canal with microthrix-like projections (arrow) and whorls of lamellae (la). Note a pinched-off vesicle containing disc-shaped bodies (▲). (Scale = 1 μm)



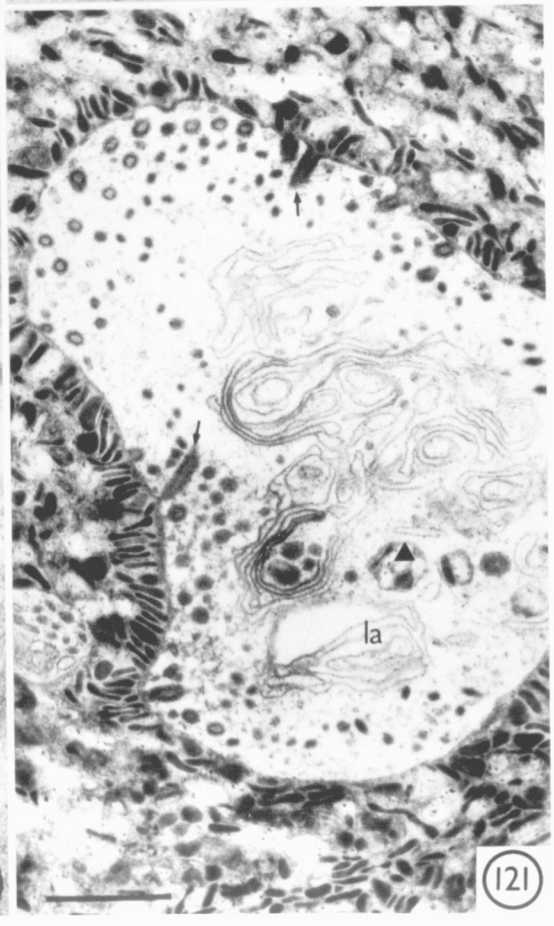
118



119



120



121

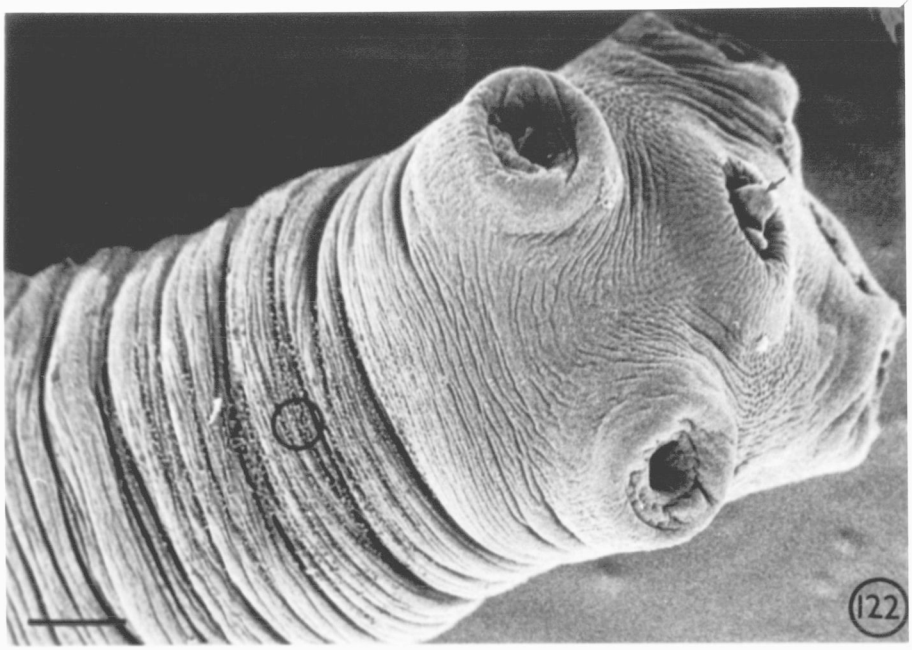
Plates 122 - 125. Scanning electron micrographs of 12-day cultured worms.

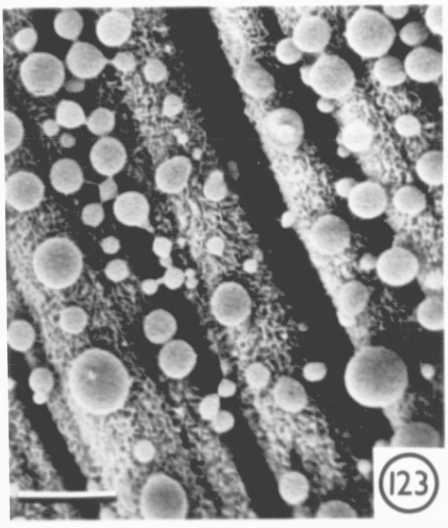
122. The scolex appears normal. The rostellum is non-everted but rostellar projections (arrow) are present. Tegumental bubbles are present on the neck region (circled). (Scale = 100 μm)

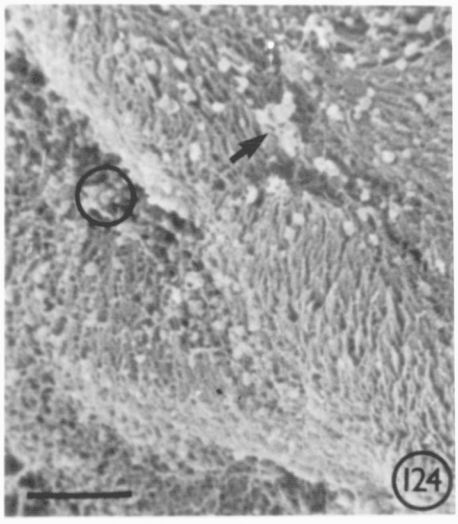
123. High magnification of tegumental bubbles on the neck region. (Scale = 10 μm)

124. The tegument on the segmented strobila. Much of the view is obscured by precipitated material (arrow) and tegumental bubbles (circled). (Scale = 2 μm)

125. A segmented strobila. The tegument appears to be degenerating, and bubbles (arrow) may be seen. (Scale = 100 μm)


122


123


124

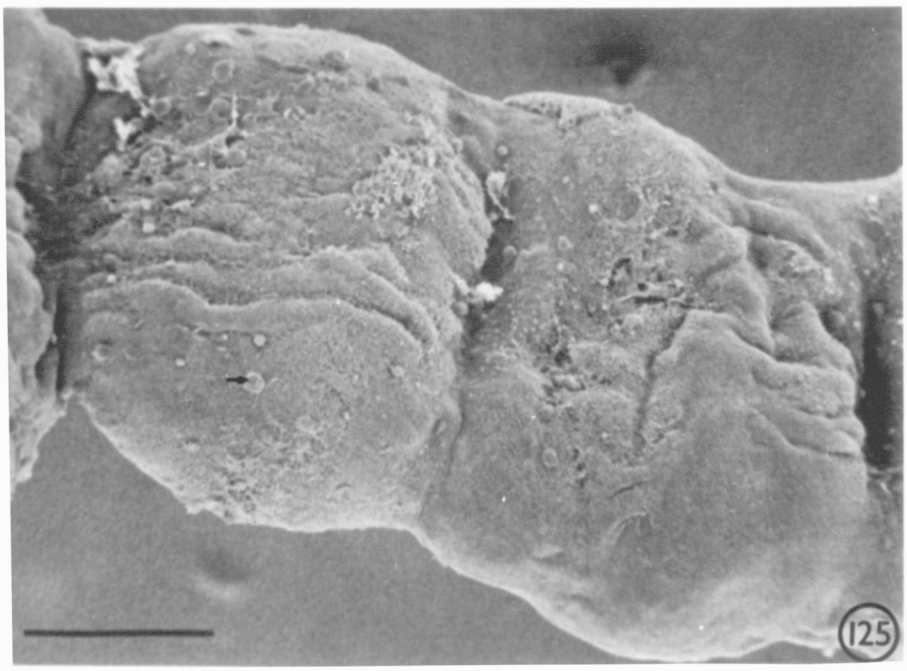

125

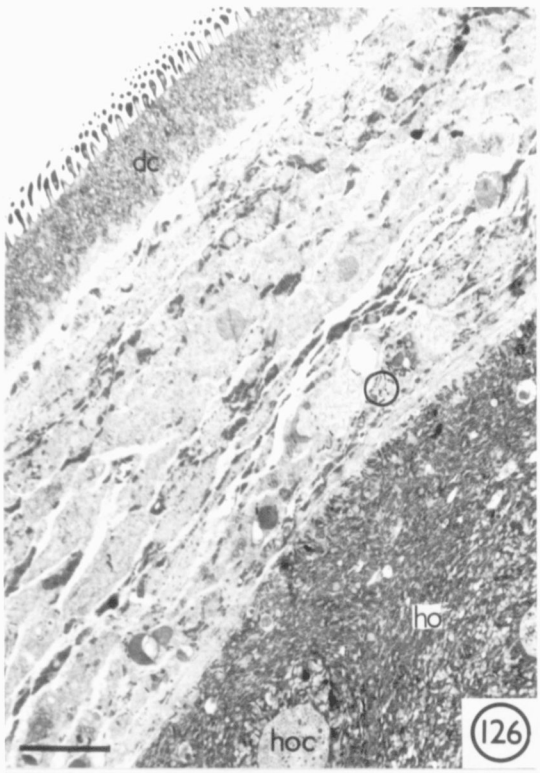
Plate 126 - 129. Transmission electron micrographs of 12-day cultured worms.

126. A transverse view of the rostellum reveals a normal looking distal cytoplasm and part of the hook organ (ho). The hook organ is packed with dense granules and is interupted by canals (hoc). These granules are like the disc-shaped bodies found in the tegument except for their larger size. They are also located in cells bordering the hook organ (circled). (Scale = 3 μm)

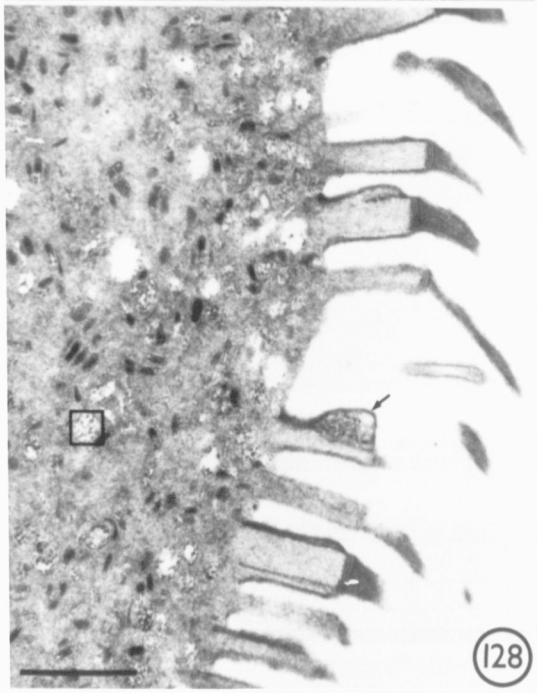
127. At higher magnification, the microtriches appear abnormal. The shaft position appears to contain condensed material of some sort (circled). There appears to be a partition of the peripheral wall in the basal portion of the microtriches (arrow). (Scale = 0.5 μm)

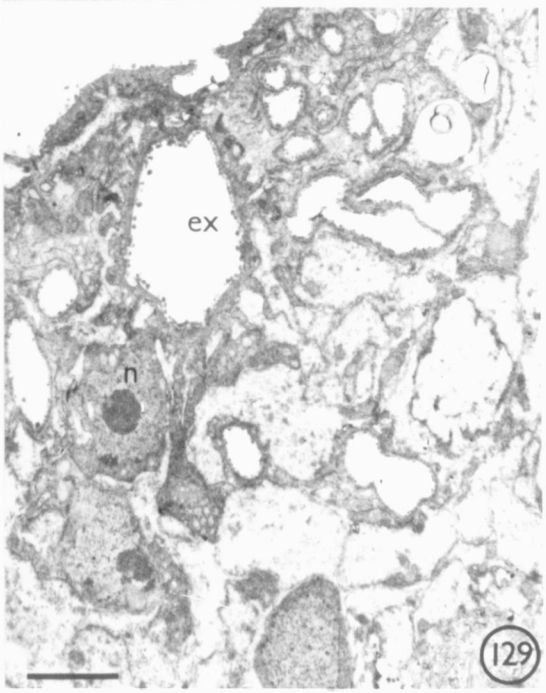
128. The basal portion of some microtriches are swollen (arrow). Note the vacuoles in the distal cytoplasm some of which contain granular material. (Scale = 0.5 μm)

129. A network of excretory canals (ex). Note the cell-body with a large nucleus (n) that forms the canal wall. (Scale = 2 μm)


dc
ho
hoc
126


127


128


ex
n
129

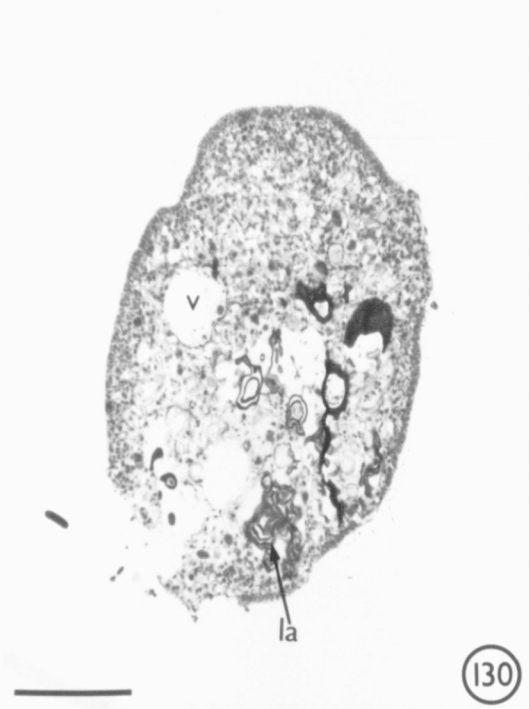
Plates 130 - 133. Transmission electron micrographs of 12-day cultured worms.

130. Section through a rostellar protrusion. It consists largely of dense bodies similar to those found in the hook organ and rostellar gland cells. Also present are large vacuoles (v) and lamellated whorls (la). (Scale = 5 μm)

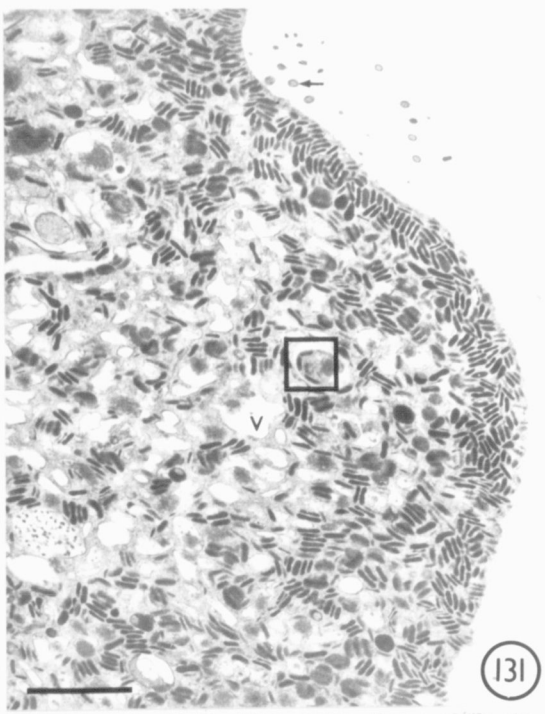
131. High magnification of the rostellar protrusion. There is a sparse distribution of microthrix-like projections on the external membrane (arrow). Note the vacuoles (v) and vesicles containing disc-shaped bodies (square). (Scale = 1 μm)

132. High magnification of a lamellated whorl (la) and a "pinched-off" vesicle containing disc-shaped bodies (arrow) in the vacuolated spaces (v). (Scale = 0.25 μm)

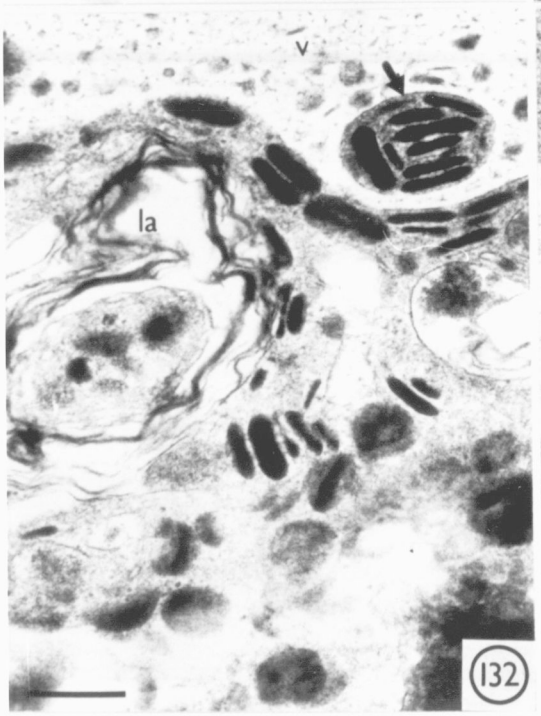
133. The flame of a flame cell with the typical (9 + 2) arrangement of elements in each cilium. (Scale = 0.25 μm)



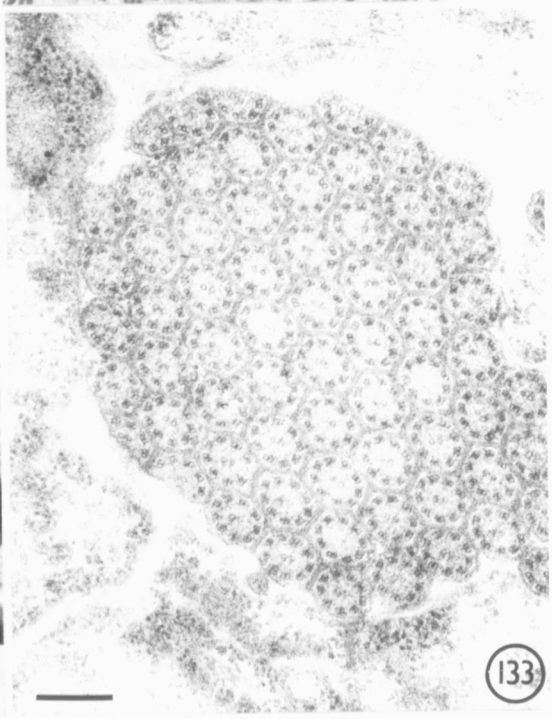
130



131



132



133

Plates 134 - 140. Scanning electron micrographs of worms after 15-days (134 - 138) and 22-days (139 - 140) *in vitro* culture.

134. Microtriches on the sucker rim. (Scale = 2 μm)

135. Microtriches on the scolex between the suckers. (Scale = 2 μm)

136. Microtriches at the anterior strobila region. (Scale = 2 μm)

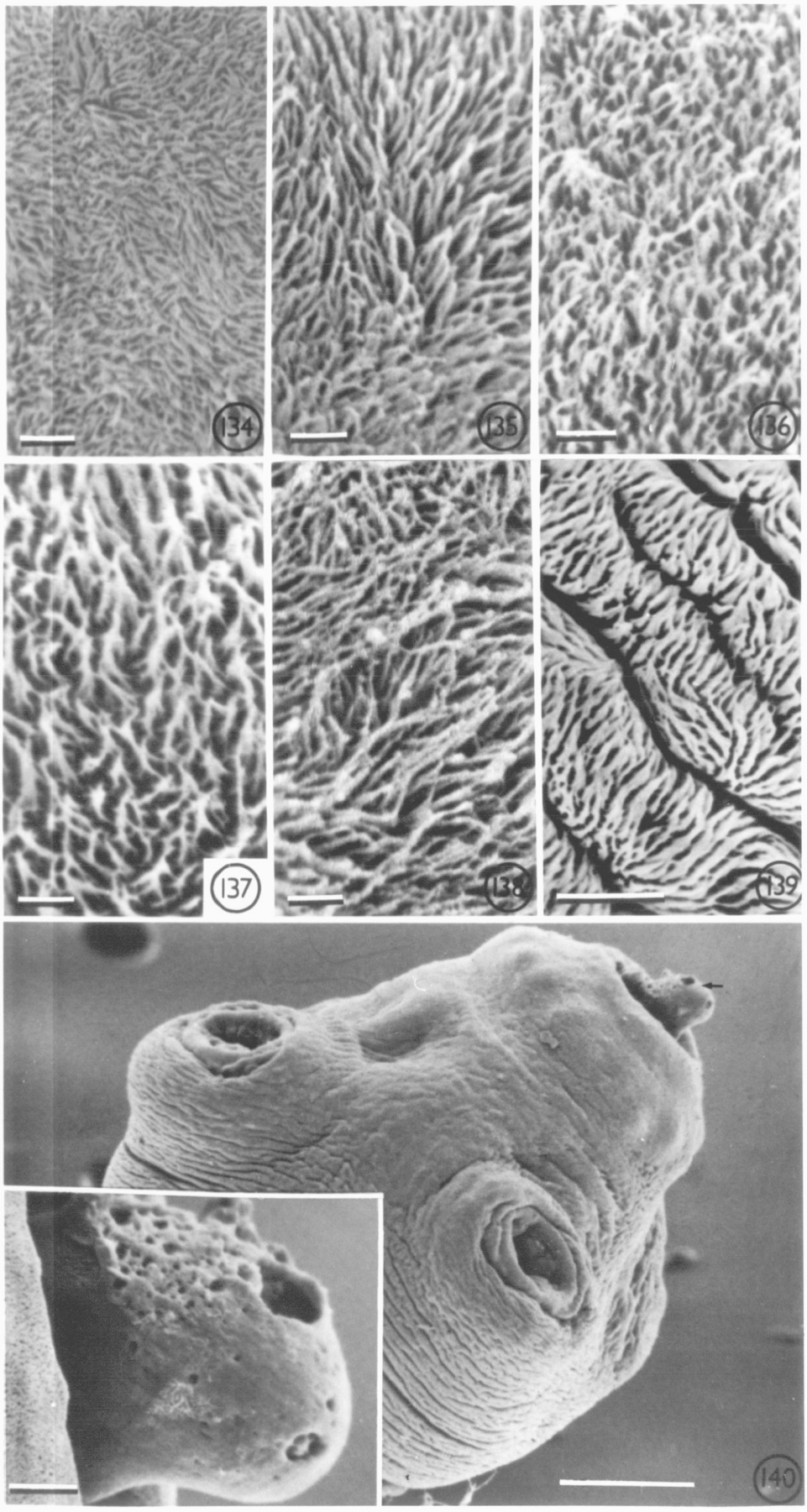
137. Microtriches at a more posterior region of the strobila. (Scale = 2 μm)

138. Microtriches on a segmented proglottid. (Scale = 2 μm)

139. Microtriches on the scolex of a 22-day cultured worm. (Scale = 2 μm)

140. The scolex of a 22-day cultured worm. Note the rostellar protrusion (arrow). (Scale = 100 μm)

140 (inset). A higher magnification of the rostellar protrusion on the rostellar tip of a 22-day cultured worm. (Scale = 10 μm)


134
135
136
137
138
139
140

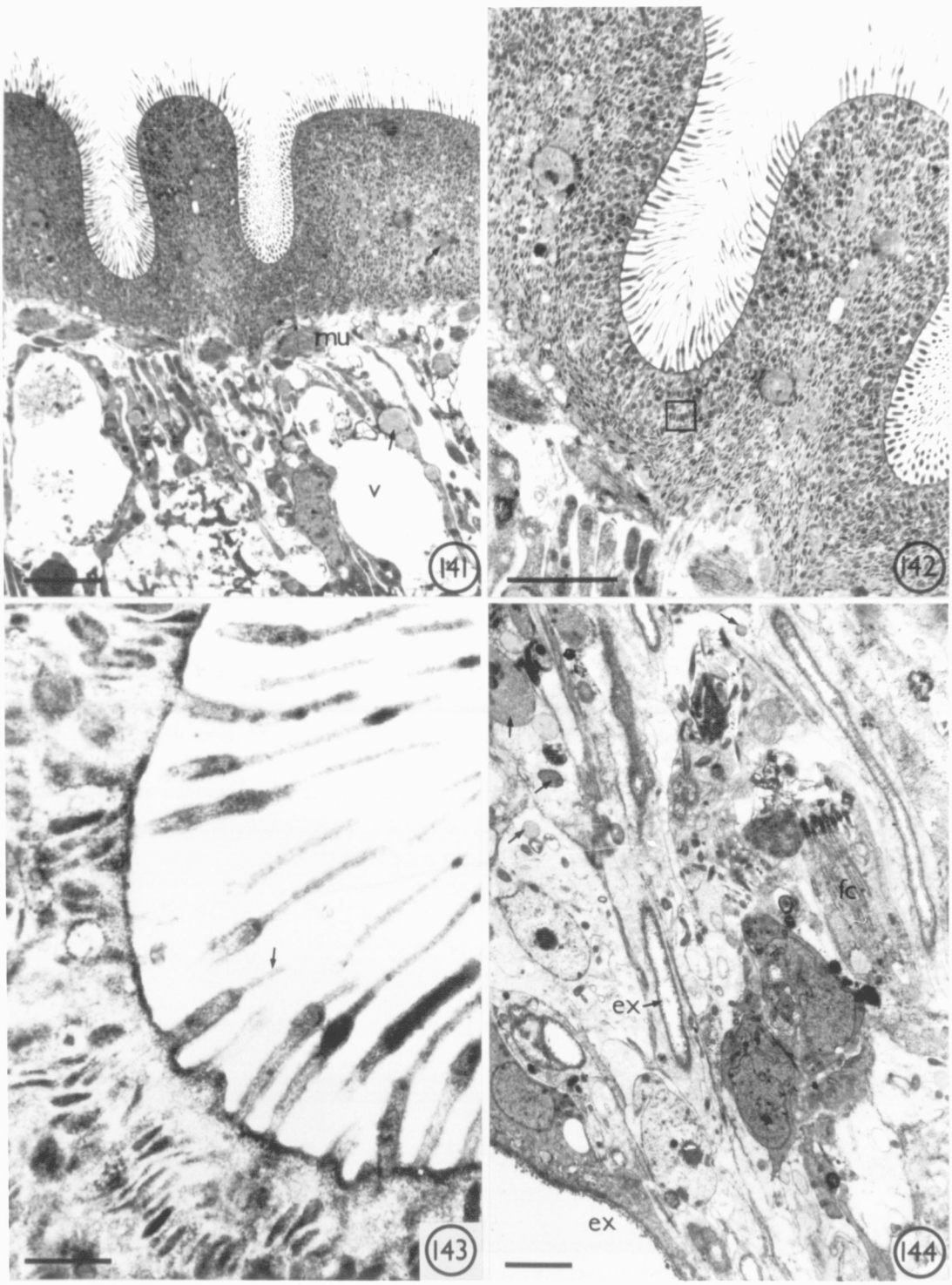
Plates 141 - 144. Transmission electron micrographs of 22-day cultured worms.

141. The distal cytoplasm is thrown into prominent folds. Large vacuolated spaces (v) are found among the peri-nuclear cytoplasm. Droplets which may contain lipids or waste-material are found in the cytons as well as the distal cytoplasm (arrows). Note the hypotrophied muscle fibres (mu). (Scale = 3 µm)

142. The distal cytoplasm with closely-packed disc-shaped bodies, often in stacks (square). (Scale = 2 µm)

143. The microtriches are thin and long, mostly with filamentous shafts (arrow). (Scale = 0,25 µm)

144. Excretory canals (ex) are frequently observed, but the rarely occurring flame cell (fc) appears malformed. Apart from a few darkly stained cells, most of the parenchymal cells have little glycogen granules. Droplets of lipids or waste material are often found in and among cells (arrows). (Scale = 2 µm)


mu
v
141
142
143
fc
ex
ex
144

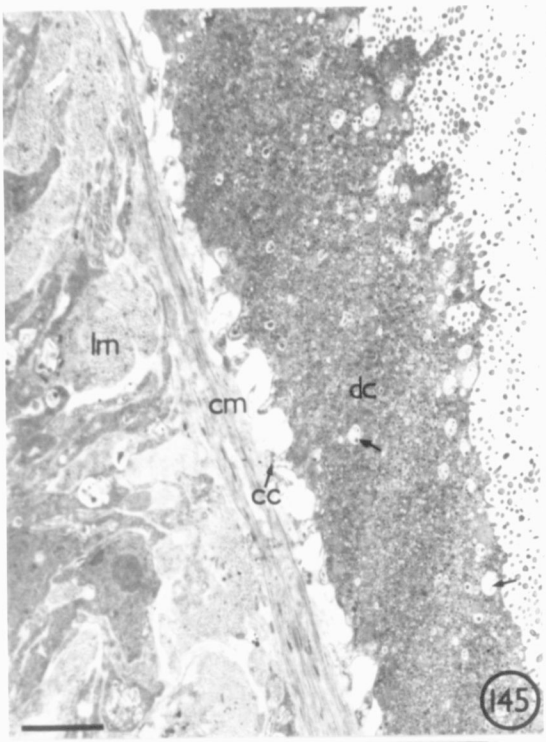
Plates 145 - 161. Transmission electron micrographs of T. crassiceps (KBS) adult worms that were obtained from a dog 17 days post-infection and then cultured in vitro for a further 14 days.

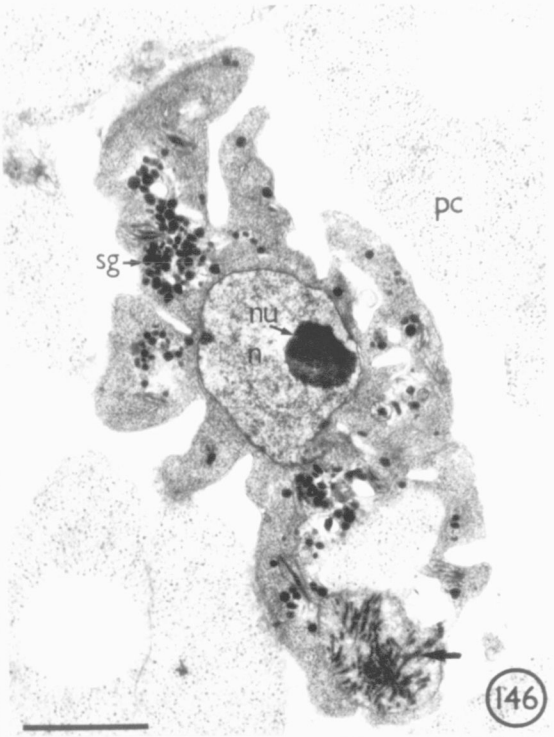
145. The distal cytoplasm (dc) is filled with a granular cytoplasm containing round bodies and vesicles with varying amounts of electron dense material (arrows). The surface of the distal cytoplasm appears irregular, with folds and invaginations. Note the abundance of cytoplasmic connections (cc) and the normal appearance of the circular (cm) and longitudinal muscles (lm). (Scale = 2 μm)

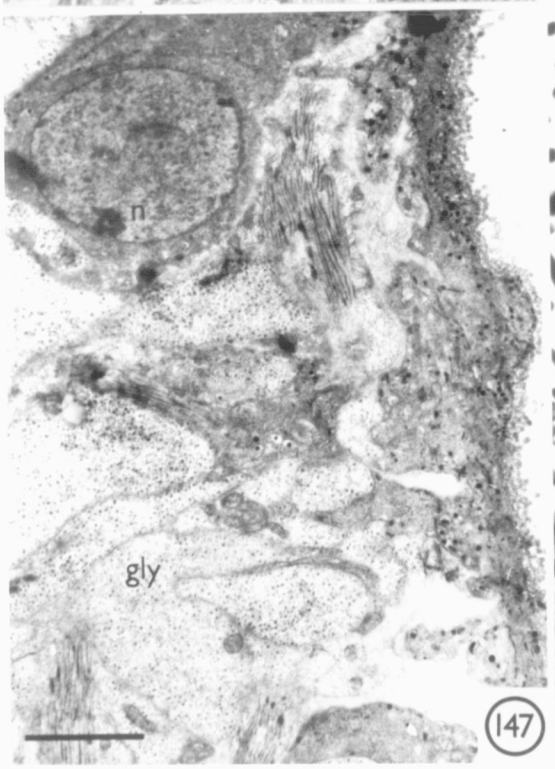
146. A darkly stained cell among the parenchymal cells (pc) of a proglottid. Note abundance of electron dense granules (sg), probably secretory, the large nucleus (n) and nucleolus (nu). Some unidentified strands of dense material are also present in the cytoplasm (arrow). (Scale = 2 μm)

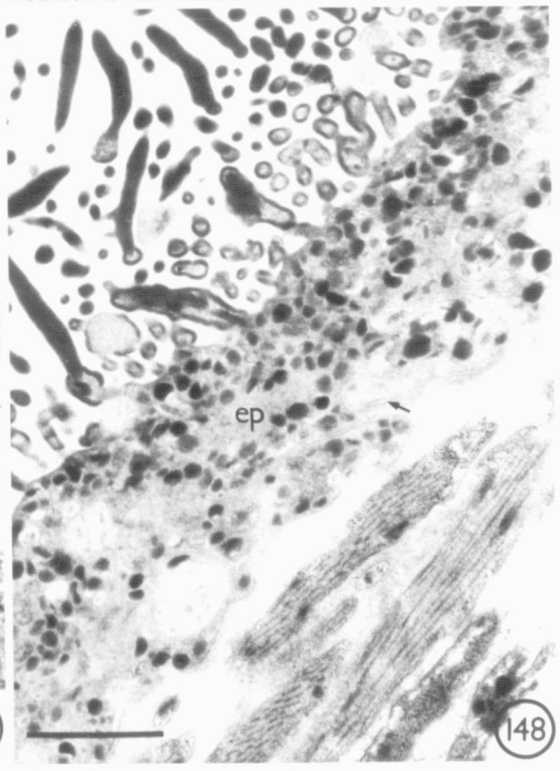
147. An excretory canal with the normal underlying cells. Note the "canal wall cell" with a prominent nucleus (n) and the glycogen-rich parenchymal cells (gly). (Scale = 2 μm)

148. The vaginal wall with a normal epithelium (ep). Note invaginations (arrow) of the basal lamina. (Scale = 1 μm)


lm
cm
dc
cc
145


pc
sg
nu
n
146


n
gly
147


ep
148

149. Normal sperms (sp) in the vas efferens. Note microvillus-like projections lining the vessel wall. (Scale = 0.25 μm)

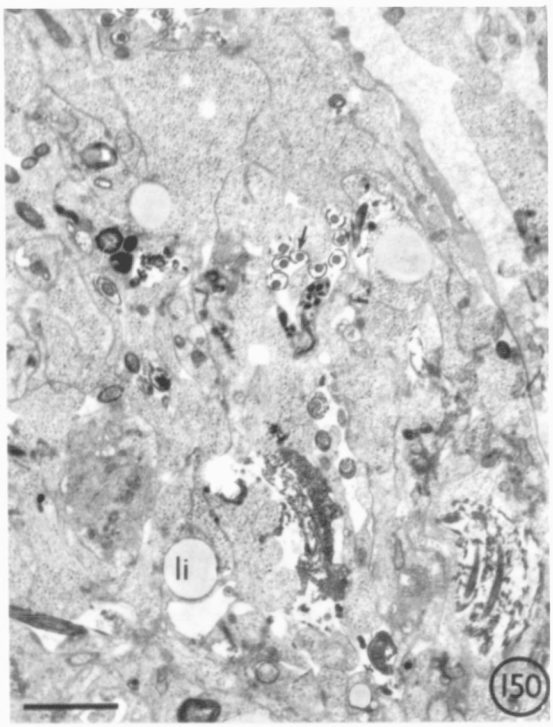
150. Section through a testis showing fully formed sperms among granular cells, presumably spermatocytes. Many of the sperms are abnormal, with fragmented or incomplete outer sheaths (arrow). Note accumulation of lipids in droplets (li). (Scale = 2)m)

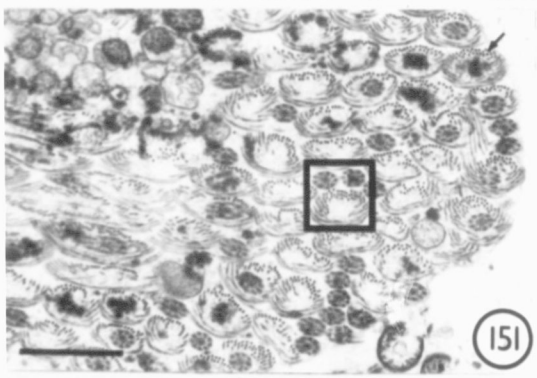
151. Many of the sperms in the testes have an irregular arrangement of microtubules in the outer sheath (arrow). Axial filament complexes are absent in many of the sperm tails, while in others the axial filaments are "naked" (square). (Scale = 1 μm)

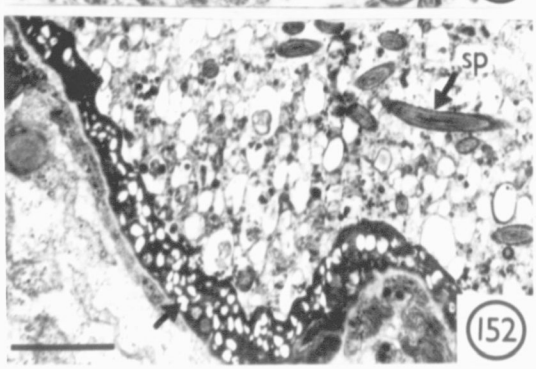
152. Sperms (sp) found in the seminal receptaculum are usually normal. Note the vacuolated epithelial wall of the receptaculum (arrow). (Scale = 2 μm)

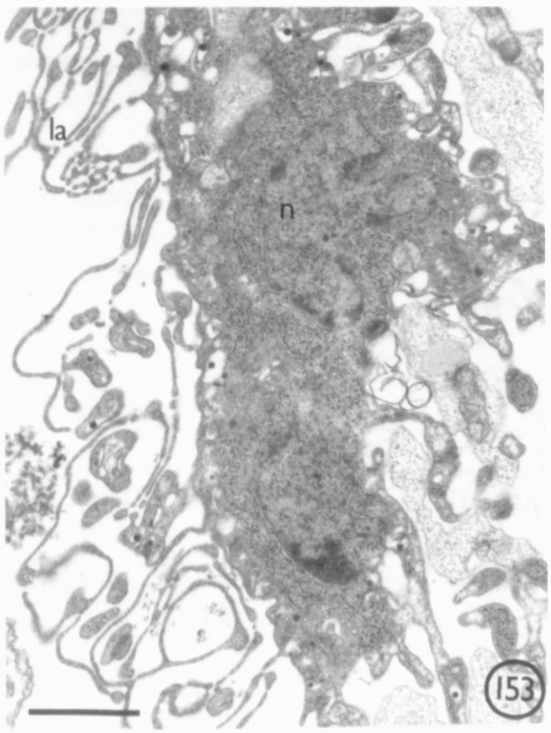
153. Uterus wall with normal lamellated folds (1a). (Scale = 1 μm)


sp
149


li
150


151


sp
152

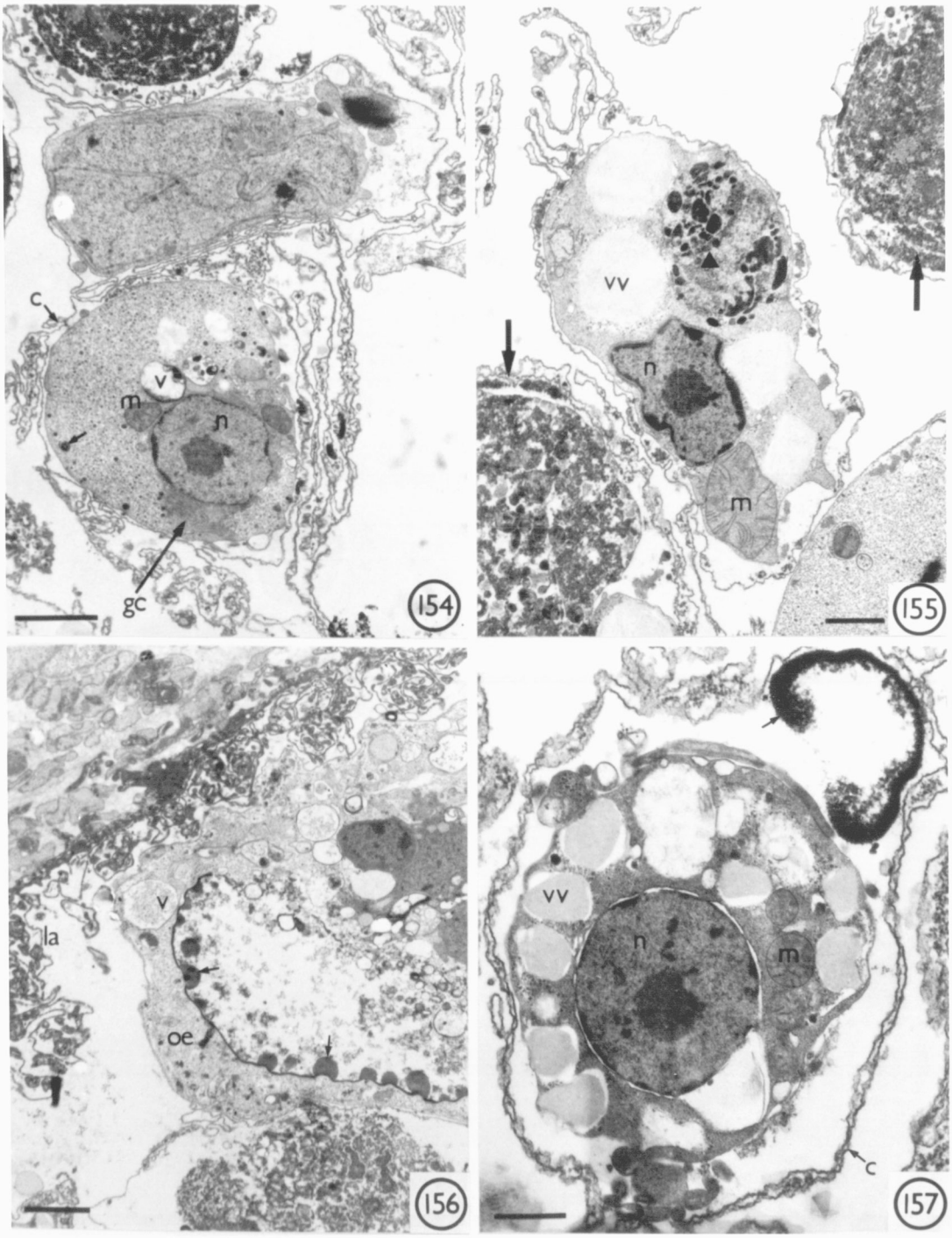

la
n
153

154. A vitelline cell enclosed within the capsule (c). The cell has a large nucleus (n), mitochondria (m), and golgi complex (gc). Vacuoles (v) may contain varying amounts of dense material. Note the dense granules (arrow), possibly droplets of vitelline secretions. (Scale = 2 μm)

155. A vitelline cell within a capsule, among 2 degenerated vitelline cells (arrows). Note the accumulation of dense "yolk" material (▲). Vitelline vesicles (vv) appear to contain a homogenous material, possibly another type of secreted material. Note also the prominent nucleus (n) and large mitochondrion (m). (Scale = 1 μm)

156. An abnormal egg in the process of formation in the uterus. The outer envelope contains vacuoles (v) with granular material. Note the keratin blobs in the process of forming the embryophore which is not surrounding an embryo (arrows). (Scale = 2 μm)

157. An abnormal egg with a partially formed embryophore (arrow) and a vitelline cell within the capsule (c). Note the vitelline vesicles (vv), prominent nucleus (n) and mitochondria (m). The outer envelope is absent. (Scale = 1 μm).


c
v
m
n
gc
154
vv
n
m
155
la
v
oe
156
vv
n
m
c
157

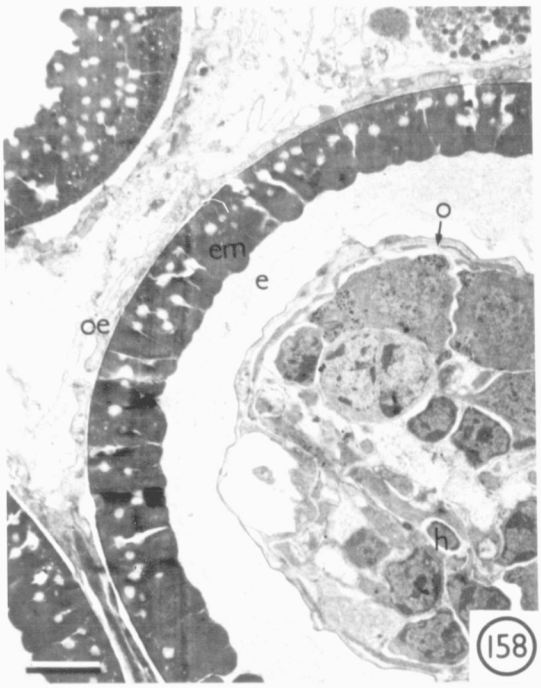
Plates 158-161 are of normal, fully developed eggs.

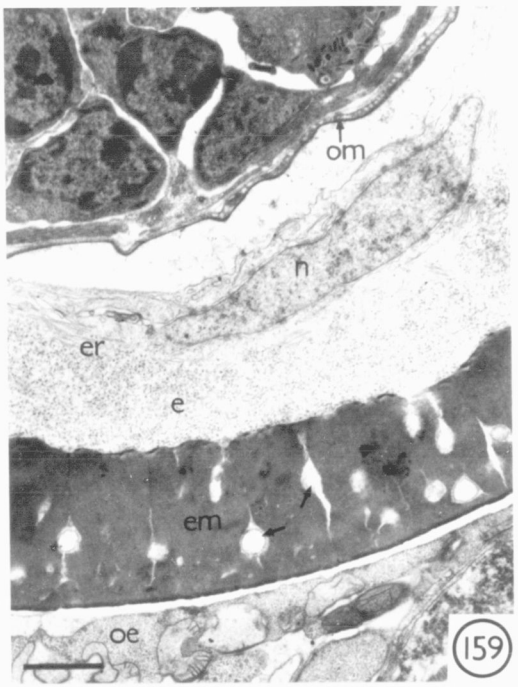
158. A normal, fully formed egg with outer envelope (oe), embryophore (em), embryophore cell (e) and an oncosphere (o) with hooks (h). (Scale = 2 μm)

159. Higher magnification of the egg showing the bilaminate oncospheral membrane (om), the inner envelope consisting of embryophore cell (e) and the embryophore (em), and the outer envelope (oe). Note the extensive endoplasmic reticulum (er) and prominent nucleus (n) in the embryophore cell. Lacunae (arrows) are found in the interstices between embryophoral blocks. (Scale = 1 μm)

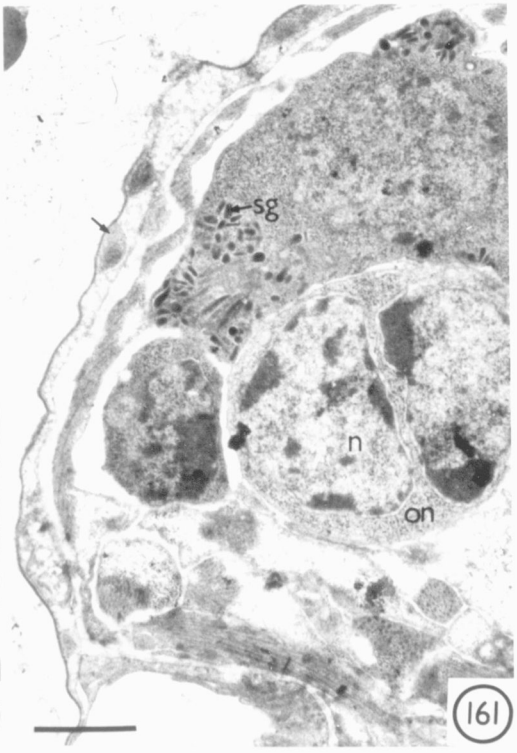
160. High magnification of the embryophore to show lacunae occupied by mitochondria (▲). Note the granular embryophore cell (circled) and the mitochondria (m) in the outer envelope. (Scale = 0.5 μm)

161. An oncosphere with a penetration gland cell containing secretory granules (sg), and an oncoblast (on) with a prominent nucleus (n). Note peculiar dark blobs in the oncospheral membrane (arrow). (Scale = 1 μm)


o
em
e
oe
h
158


om
h
er
e
em
oe
159


m
160


sg
n
on
161

embryophoral cell, and oncosphere appeared similar to *in vivo* formed eggs. Only two differences were noticed. The embryophoral cell, which is the inner component of the inner envelope, had conspicuous endoplasmic reticulum and a prominent nucleus (Plate 159) In comparison, *in vivo* eggs with fully developed structures often had little endoplasmic reticulum and a degenerate nucleus. The other difference that was noticed in *in vitro* formed eggs was that darkly stained "blobs" were occasionally observed in the oncospheral membrane (Plate 161).

D. DISCUSSION

In vivo and *in vitro* studies indicate that hatching of the cyclophyllidean egg involves two processes : (1) the passive digestion of the embryophore, (2) stimulation (= "activation") of the hexacanth embryo to become motile and rupture its enclosing oncospheral membrane (Silverman, 1954). In this report, the term "hatching" is used for the process of embryophoral disaggregation and "activation" for the complete liberation of the oncosphere from its enclosing membrane. For *T. crassiceps* the hatching was observed about 20 minutes after treatment with pepsin and EVS. The exact role of each component enzyme in the treatment is not known. Silverman's (1954) studies on *T. pisiformis* and *T. saginata* demonstrated that the disaggregation of the embryophoric blocks is caused by the proteolytic digestive enzymes of the intermediate host. It is assumed by some workers that disintegration of the embryophore is the result of dissolution of the "cementing" substance holding these blocks together. What appears to be a "cementing" substance has been demonstrated ultrastructurally in this study, and in other species such as *T. taeniaeformis* (Nieland,

1968), while Morseth (1965) reported only a clear space between the blocks in some taeniids. Nevertheless, the fact that hatching is brought about by digestive enzymes does suggest that some cementing material is dissolved. The effect of enzymes on the disintegration of the embryophore varies markedly between species. Pancreatin alone has been reported to bring about hatching in some species, e.g. T. pisiformis (Silverman, 1954). Silverman (1954) showed that the embryophore of T. saginata was unaffected by pancreatin but could be disrupted by shaking after 2 - 3 hours of treatment with pepsin. Freeman (1962) reported that eggs of T. crassiceps hatched when injected directly into the small intestine. This non-obligatory but nevertheless beneficial pretreatment by pepsin has also been observed in T. taeniaeformis (Huffman & Jones, 1962) and E. granulosus (Meymarian, 1961). In the present study, almost all the eggs had the embryophore disintegrated after 20 minutes. In T. taeniaeformis, the rate of in vitro hatching is very variable with 5% of the oncospheres emerging within 60 minutes and 83% escaping over a 3-hour period (Huffman & Jones, 1962). On the other hand, Banerjee & Singh (1969) reported the hatching of C. fasciolaris eggs within 15 minutes in the rat small intestine. Smyth (1969a) has suggested that variable hatching rates may be advantageous in diseminating eggs throughout the intestine, but it is difficult to reconcile this view fully with observations that most oncospheres penetrate the small intestine at definite preferred regions (Banerjee & Singh, 1969; Heath, 1971). The nature of "activation" as a phenomenon is not known, but it is generally believed that it may be related to the surface active properties of bile (Smyth, 1969a). The physiological mechanism through which bile salts stimulate oncosphere activity is unknown and the variable

composition of whole bile in different potential vertebrate intermediate hosts has been stressed by Smyth & Haslewood (1963). It is only possible to speculate why the oncospheres of T. crassiceps did not exhibit "activation" in this study. Firstly, the eggs used for hatching and activation work had been stored in the fridge for 3 to 30 days and this might have resulted in abnormal oncospheres which were unable to participate fully in breaking away the oncospheral mambrane. Secondly, the eggs might have been insufficiently mature since they were obtained from 29-day old worms. Freeman (1962) reported that the earliest time eggs were passed from an infected dog was 32 days post-infection and that a dog killed 29 days after infection harboured immature worms. Although SEM and TEM studies revealed apparently normal eggs, it is possible that these eggs were in fact not fully matured. This seem to be a likely reason since mice were not infected when fed with freshly obtained eggs (see Section III). A third reason may be that the bile that was used in the media for activating oncospheres was actually dog-bile and therefore not specific for the stimulation of T. crassiceps oncospheres. The importance of specific bile has been discussed by Smyth (1969a).

Not many attempts have been made to culture taeniid cestodes from post-embryonic larval stages to the adult. Notable attempts have been reported for T. ovis, T. serialis, T. taeniaeformis, T. hydatigena, T. pisiformis, E. granulosus and E. multilocularis (Heath & Smyth, 1970 ; Heath, 1973a; Smyth, 1967, 1976; Smyth & Barrett, 1979; Osuna et al, 1978). Earlier attempts with T. crassiceps merely aimed at maintaining the parasite in a chemically defined medium (Taylor, 1963). A partially successful effort to culture

T. crassiceps was described by Esch & Smyth (1976). They reported that the best system involved a full enzyme-bile pretreatment followed by culture in Medium V which contained a fully supplemented medium 858 on a coagulated serum base.

In the present study, various combinations of culture vessels, pretreatments, media, media supplements and agitations were assessed to obtain the best system. It is not possible to draw comparative conclusions from all these variables in various combinations because of the sheer impracticality of examining, in the time available, one variable while keeping all the others constant for each of the variable factors. Only broad generalizations can be made with regards to what factors contribute to an "optimal" system. It appears that the best protocol involves a brief enzyme-bile, pretreatment (not more than 3 hours), followed by culturing in a diphasic medium, consisting of fully supplemented CMRL - 1066/HEPES and coagulated NBCS, in a Leighton tube agitated gently on a roller. The worms appeared to develop more quickly and remain alive longer when they were cultured in a crowed vessel.

The developmental rate reported by Esch & Smyth (1976) was better than that obtained in this study. The most obvious reason for this is that they used a medium, 858, which is now not available commercially. Nasset (1962) demonstrated that the relative molar ratios of amino acids in dog intestines are relatively constant. Comparing the 3 media, CMRL - 1066, RPMI - 1640, and 858, it is found that 858 contains amino acid quantities that have the closest resemblance to the molar ratios in the dog. An attempt was made to supplement CMRL - 1066 with amino acids and other constituents, but this was only partially successful because of the difficulties encountered in dissolving

exact amounts of amino acids in solution and the unavailability of certain co-factors. The ratio of amino acids may be important if absorption across the tegument is mediated by carriers. In such a system, an abundance of certain amino acids may exhaust some components of the carrier system such that insufficient amounts of other vital amino acids are absorbed.

Leighton tubes agitated on a roller system appeared to be favourable for culturing. This may be due to the fact that there is a relatively larger volume occupied by media compared to the gas phase; this would result in a more constant and easily maintained gas-liquid equilibrium. It is now more widely accepted that contact (membrane) digestion may be an important phenomenon in the physiology of tapeworms (Smyth, 1972, 1973), although the process has never been unequivocally demonstrated. This may explain why worms developed more favourably in crowded systems, using Leighton tubes and where a coagulated serum base or macerate is present, as these would provide greater membrane-membrane or membrane-substrate contact. Esch & Smyth (1967) reported the necessity of a diphasic medium containing a semi-solid serum base for healthy development of T. crassiceps. This result is contradictory to the later findings of Rickard & Smyth (1977, unpublished work) who demonstrated that a monophasic medium worked equally well. In this study, although a diphasic medium was found to be better for development generally, some worms reached the stage of cirrus streak formation in Experiment 3 where a monophasic medium was used. Esch & Smyth (1976) reported that in monophasic cultures all the worms had vesiculated and died within 13 days before reaching this stage. Smyth (1967) reported that a proteinaceous base was necessary for segmentation and subsequent development by E. granulosus.

Apparently, this is only a partial requirement by T. crassiceps since segmentation will occur when activated cysticerci is grown in a monophasic medium.

In previous studies on taeniid cestodes (Smyth, 1969a; Silverman & Hansen, 1971), there is strong indication that enzyme and bile salt pretreatment is necessary for scolex and/or rostellar evagination, and that this event(s) is important for subsequent strobilization and organogenesis. The present study shows that this may also apply to T. crassiceps, at least in the KBS strain. However, it is difficult to compare this with the results obtained by Esch & Smyth (1976) who showed that pretreatment was not a prerequisite for advanced development because variable factors were involved, e.g. the type of culture vessels, the medium used and the parasite strain. The roles of media supplements in in vitro culture are largely unknown. From this study, the importance of yeast extract was established, but which components of this crude extract actually contributes positively to the system are unknown.

Ultrastructural studies on in vitro cultured worms may provide information on the deficiencies of the system by comparing vital organ structures in cultured worms with in vivo ones. Such an ultrastructural approach has been reported for several trematode species (Kannangara, 1973; Davies, 1976, 1978), but virtually none for taeniid cestodes. T. crassiceps cultured for 7 days were apparently normal in most structural aspects. The only difference was that the fibrous zone was showing signs of abnormality. Instead of being a zone of long fibrous strands (Baron, 1968), it consisted of much fragmented condensed strands. On the 12th day of culture the tegument, particularly at the neck region, showed signs of degenerating. Vesiculation of the tegument

first appeared as bubbles that were clearly identified in SEM. More severe damage to the tegument occurred on the segmented strobila. TEM observations revealed abnormalities in the shaft portion of the microtriches as well as swollen basal portions. The microtriches are believed to be important for the "digestive-absorptive" function of the tegument (Smyth, 1972), and this lack of "structural integrity" found in the microtriches probably resulted in severe impairments to the tegumental functions. After the 12th day of culture microtriches on the segmented strobila continued to degenerate; by the 22nd day all the microtriches on the strobila were thin and filamentous, without the usually distinct base and attenuating distal shaft. The microtriches on the scolex region appeared to retain their normal structure, but this was based only on SEM observations. While excretory canals were frequently encountered and appeared normal, flame-cells were rarely found after 12 days of culture. Those that were examined were malformed in that the "flame" portion, consisting of a collection of cilia, was not enclosed within a tubular structure which, in normal cells, is made of interdigitating leptotriches. The lack of normal flame-cells probably resulted in impairment to excretory and osmoregulatory function. This might be the reason for the accumulation of vesicles, possibly containing waste material, in and among the distal and perinuclear cytoplasm. The amount of disc-shaped bodies was greatly increased in 22-day cultured worms. In comparison, mature <u>in vivo</u> worms showed a decrease in disc-shaped bodies which were possibly replaced by round bodies. There are suggestions that the disappearance of these bodies is related to the increase in mitochondria (Jha & Smyth, 1971; Smyth, 1972). If this is applicable, then it might explain the lack of mitochondria in

cultured worms, a further sign of dysfunction. A general observation of worms after 12 days of culture was that the parenchymal cells began to lose their granular appearance and they contained relatively few glycogen deposits. This indicates that glycogen utilised for energy-requiring metabolic activities was not replaced. This, and the general hypotrophy of muscle fibres, was probably the result of inefficiencies in the digestion and/or absorption of carbohydrates and amino acids from the culture medium.

A general observation in all the cultured worms was that the microtriches on the scolex appeared normal while tegumental "bubbling" and "vesiculation" occurred frequently at the neck region and the segmented strobila. This may be because of the fact that the neck region, being the region of proglottidization, is involved in active synthesis, and the segments are involved in organogenesis. These metabolic activities might have resulted in an accumulation of waste products which were toxic to the cells and were not conducted away efficiently.

The deficiencies of the culture system was not confined to its inability to provide suitable conditions for development of cysticerci to adults but it was also unable, to some extent, to sustain mature 17-day old *in vivo* worms through normal development of sperms and insemination of ova. Many sperms within the testes were abnormal; only normal sperms were observed in the vas efferens and seminal receptaculum. An accumulation of toxic waste products in the testes might be the cause of disruption to normal formation and arrangement of microtubules for the outer sheath. Within the uterus, the majority of eggs were abnormal. There appears to be poor synchrony in the development of the envelopes. In some, the partially formed

embryophore was found but the oncosphere was absent. Nevertheless, several normal, fully-developed eggs were present in the proglottids examined. Since no eggs had been observed in 17-day *in vivo* worms prior to culture, this probably represents a unique instance in which fertilization took place in *Taenia* sp. in an *in vitro* situation, albeit the culture was of an already mature worm.. This suggests that the problems involved in obtaining eggs *in vitro* may be more related to obtaining viable, normal sperms and ova, rather than the physical aspects of attaining insemination. In the cultivation of *Echinococcus* spp. much progress has been made, and spermatozoa are frequently observed but no fertile eggs has been reported (Smyth *et al*, 1967; Smyth & Davies, 1974b; Smyth & Barrett, 1979). While it has been assumed that the absence of fertile eggs is due to failure in obtaining impregnation or insemination, no ultrastructural studies have been carried out to see if the sperms were normal.

The ultrastructural examination of *in vitro* cultured *T. crassiceps* revealed that the "immune precipitate" described by Esch & Smyth (1976) to be extruding from the rostellar region is in fact an intrinsic part of the parasite. This "rostellar protrusion" probably originates as an extension of the hook organ. It contains inclusions that are similar in appearance to those found to pack the hook organ and rostellar gland cells. This suggests that the protrusion may serve a secretory function., when the adult *T. crassiceps* begins to develop in the dog intestine. Special secretory cells were also observed in the strobila of segmented worms that had been cultured for 7 days and in the mature worm cultured after 17 days *in vivo* development. These cells were not observed in cysticerci (Section I), and indicates their secretory role in the adult worm, possibly supplying secretory material to the

tegument for digestion-absorption purposes.

The experiments described in the present study are very much of a preliminary nature. They were designed mainly to obtain a general picture of what combinations of culture conditions may be best suited for the in vitro cultivation of T. crassiceps. More work is required before consistent reproducible results can be obtained. Many of the variable factors involved are practically undefinable. For example, different batches of serum, bile and metacestodes often gave somewhat inconsistent results - a phenomenon well recognized and allowed for in tissue and parasite culture systems (Smyth & Davies, 1974 b). Voge (1973) states: "While the ability to grow any tapeworm is commendable as an initial task, failure to elicit some definite information about the over-all biology, or particularly the biochemical versatility and limitations of the organism grown in vitro, reduces any achievement to a somewhat meaningless 'tour de force', especially when the media or experimental conditions are so complex that definition of essential factors or substances is virtually impossible". The development of the in vitro culture system for T. crassiceps - incomplete as it is - could provide a useful research tool for the study of developmental phenomena in tapeworms. Particularly useful would be controlled experiments to identify totally definable compounds that contribute to optimal growth and development. T. crassiceps could make such an extensive investigation feasible due to its easily maintained metacestode stages and its rapid development.

SUMMARY

Attempts made to culture the oncosphere of T. crassiceps were unsuccessful. While hatching, here taken to mean the disintegration of the embryophore, was obtained in most attempts, none of the

oncospheres were observed to be activated, i.e. liberated from the oncosphoral membrane. The reasons for this failure may be : (1) the eggs used in these experiments had incompletely matured oncospheres, (2) the components of the activating solution were not suitable for this particular species, and (3) the 3 - 30 days storage period had resulted in degeneration of most of the eggs.

Cysticerci were cultured in various combinations of variable factors. The best results were obtained when KBS strain worms, after the full enzyme-bile salts pretreatment, were cultured in gently agitated Leighton tubes, in a diphasic medium containing fully supplemented CMRL - 1066 and coagulated serum macerate. Success in obtaining sexually matured worms were marginal; the most advanced stage obtained showed evidence of gonadal primordia and a genital pore. No fully formed testes, sperms, or ova were obtained. There could be a number of reasons for the lack of in vitro development; (1) the basic media used contained either insufficient or missing vital amino acids or other vital components, or some inhibitory substances, (2) the calf serum used to supplement media was not "compatible" with the parasite whose definitive host is usually a canid, or it lacked vital growth factors, possibly due to heat inactivation, (3) some physical requirements, e.g. a surface for continuous "contact stimulation" of the scolex and rostellum, or particular gas phases, may be lacking or inconsistently maintained, and (4) an inefficient system for waste-removal in the culture systems resulting in an accumulation of toxic substances.

Ultrastructural studies reveal that tegumental structure and function begin to break down between about 7 and 12 days of in vitro culture. Comparing with in vivo worms, abnormalities were observed

in the microtriches, distal cytoplasm, muscles, flame-cells, and parenchymal cells. An accumulation of waste-products might also be the result of abnormal flame-cells which are important in the excretory system. Dysfunction of the tegument as a digestive-absorptive "organ" probably resulted in a depletion of carbohydrates and amino acids.

Sexually mature worms obtained from dogs 17 days post-infection when cultured in vitro produced normal eggs (as observed with TEM). However, many other eggs in the uterus were abnormal. Deficiencies in the culture system also resulted in many abnormal spermatozoa which were probably non-motile since only fully formed sperms were found in the vas efferens and receptaculum seminis.

SECTION III. PATHOGENESIS AND KINETICS OF TAENIA CRASSICEPS INFECTION

A. INTRODUCTION

The Taeniidae are the most important cyclophyllidean family of tapeworms in that many species which are of medical, veterinary and economic importance are found in this group.

Severe pathogenic effects of adult cestodes in their hosts are the exception rather than the rule; in an excellent review, Rees (1967) mentioned that adult cestodes have little effect on the host and are rarely pathogenic. While the adult tapeworm may be relatively harmless to the definitive host, the tissue penetrating and dwelling larval stages in the intermediate host are often very pathogenic. Smyth and Heath (1970) have written a comprehensive review of the pathogenesis of larval cestodes in mammals, and Slais (1973) gave an excellent and detailed account of their functional morphology and its relationship to pathogenicity.

Various facets of larval structure or behaviour may be related to a particular type of pathological effect. Smyth and Heath (1970) summarised these as being (a) morphological, (b) mechanical, (c) nutritional, (d) toxic, (e) immunological, and (f) behavioural. The extent to which these features induce pathological effects is a matter of degree and varies with species or "strain" of both host and parasite and with the host organ invaded.

1. Review of Literature on Pathogenicity of Taeniid Infection

There are 4 main types of larvae in the Taeniidae and in order to place the results with T. crassiceps in context, the pathogenic effects of these are briefly reviewed.

a) The Hydatid Cyst

The general morphology of the hydatid organism and its fine structure are well documented by several workers, e.g. Morseth (1967), Smyth (1969b). Two types of cysts are generally distinguished, viz. the unilocular type caused by Echinococcus granulosus, and the multilocular or alveolar type caused by E. multilocularis. The literature on the pathogenicity of hydatid disease is so extensive that it is beyond the scope of this review to do more than deal briefly with the general principles involved. Excellent reviews on hydatidosis/echinococcosis have been published by Powers & Churchill (1959), Smyth (1964a, 1969 a,b) and Matossian et al (1977). Although it is widely held that 2 species of Echinococcus occur - E. granulosus, with the adult in dogs and the larval stages primarily in herbivores, and E. multilocularis, with the adult in dogs, foxes and cats and with the larval stages primarily in rodents - more recent work has revealed that some "strains" may exist (Smyth & Davies, 1974 a,b; McManus & Smyth, 1978, 1979). The existence of such "strains" might offer some explanation as to the pathogenicity and epidemiology of hydatidosis. Like other larval taeniid infections, the normal route of infection is orally via the alimentary canal. The embryos are carried via the hepatic portal vein to the liver, although they can pass from there to the pulmonary circulation and hence to the other organs. Further accounts of primary hydatidosis due to E. granulosus are those of Webster & Cameron (1961) and Yamashita et al (1961) working with experimentally infected rodents and sheep. The pathology of unilocular hydatidosis in man is adequately dealt with in text-books of parasitology and veterinary science (e.g. Faust & Russell, 1964; Beck & Davies, 1976). According to Jenkins (1949) the detrimental

effects of the hydatid organism are due to pressure, infection, secondary lung complications, anaphylactic shock, rupture, destruction of the bile ducts and death of the cysts. Studying the biochemistry and physiology of hydatidosis, Martikyan & Aslanyan (1962) found disturbances in the bile cholesterol and antitoxic functions of the liver, and impaired glycogen function in severe cases.

One of the early studies on alveolar hydatidosis in rodents conducted by Rausch (1954) revealed in detail the larval development of E. multilocularis in the field vole, Microtus pennsylvanicus. He also described the sequence of events that occur at the primary focus in the liver, namely the migration and infiltration of macrophages, polymorphonuclear neutrophils, fibroblasts and eosinophils, the destruction of liver tissue, dilation of the portal vein, and congestion of the sinusoids. Secondary foci apparently arising by matastasis, have been reported in the spleen and mesentery.

It is more widely accepted that the nature of hydatidosis is often determined by different "strains" of both the host and the parasite. This phenomenon is supported by the work of Smyth & Davies (1974 a,b) which lends weight to observations made by earlier workers, e.g. Lubinsky & Desser (1963) and Yamashita et al (1963).

(b) Cysticercus

This is a general term for the "bladder worm" type of larva whose occurrence is restricted to the Taeniidae. Cysticercosis is of supreme importance due to the scale of the disease and its contribution to human suffering and economic loss in animal husbandry. Much of the knowledge on cysticercosis is based on studies with Cysticercus pisiformis and C. tenuicollis. The mode of cysticercosis infection, as far as is known, is the result of direct ingestion of embryonated

eggs; the possible role played by reverse peristalsis has been considered by several workers and is discussed at length by Webbe (1967). In the majority of cases, the immediate target organ for the hatched and activated oncosphere is the liver (Pullin, 1955; Shepelev, 1958, 1959; Gemmell, 1961). En route to the liver the oncosphere may cause engorgement of blood vessels and considerable haemorrhage, especially in heavy infections (Yoshino, 1933), while the host may exhibit an immunological reaction to the penetration gland secretion (Silverman & Maneely, 1955). The pathological effects of the parasite in the liver and the host tissue reaction have been studied in cysticercosis from C. pisiformis and C. tenuicollis (Sweatman, 1955; Sweatman & Plummer, 1957; Shepelev, 1958, 1959; Liebmann & Boch, 1960 and Schiefer, 1966). Although light infections do not provide any obvious signs of discomfort, clinical symptoms due to heavy infections of C. tenuicollis in pigs and lambs have been reported (Shepelev, 1958, 1959). These may include anorexia, pyrexia, anaemia, jaundice and depression of growth rate. Death could occur after 9 to 13 days post-infection. Acute haemorrhage marks the destruction of the cysticercus capsule as the parasite leaves the liver, resulting in death to the host (Sweatman, 1955; Heath, 1970). From the liver the migrating C. pisiformis larvae proceed to the abdominal cavity of the rabbit where they cause little or no pathogenic effects (Heath, 1970). In heavy infections of C. tenuicollis, migration of larvae from the liver is often associated with trauma such that adhesion of organs occurs as a result of fibrous peritonitis (Pullin, 1955; Sweatman & Plummer, 1957). Encapsulation of cysticerci in established infections is common (Heath, 1970). Other than C. pisiformis and C. tenuicollis, the literature is also extensive for cysticercosis caused by C. cellulosae

and C. bovis. Viljoen (1937) has summarized the earlier literature on their predilection sites. This was followed much later by Silverman (1956), Ginsberg & Grieve (1959), McManus (1960) and Slais (1970). Though there is still some controversy, the general pattern appears to be that in C. cellulosae the cysticerci are found spread throughout the body roughly in proportion to the weight of the organs. In C. bovis distribution depends on the intensity of the infection. In light infections there has been insufficient studies to reveal any pattern, but in heavy infections cysts are evenly distributed throughout the whole musculature (Silverman, 1956). Bellno (1975) concluded that there was no definitive or prefered site for C. bovis in slaughter cattle in Nigeria. Cerebral cysts of C. cellulosae have been reported in the brain of 60% of heavily infected pigs (Viljoen, 1937). Viljoen (1937) quotes Hoefnagel (1923) who found C. bovis in the brain of an ox and Heever & Tustin (1967) found two cysts in the brain of a calf. The literature on human cysticercosis in most cases probably refers to C. cellulosae (Smyth & Heath, 1970) which has received much attention for its pathology, especially when cysticerci are localized in the brain (MacArthur, 1934; Viljoen, 1937; Menon & Veliath, 1940; Dixon & Lipscomb, 1961; Powell, et al, 1966 a,b; Jolly & Pallis, 1971; Tumada & Margono, 1973). Abdussalam (1974) has comprehensively reviewed the prevalence, pathogenesis, prevention and control of cysticercosis throughout the world. Some further recent reviews of cysticercosis have been carried out in the Philippines (Arambulo et al, 1976; Cabrera & Arambulo, 1977), Bali (Simanjuntak et al, 1977), Rwanda (Vanderick & Mbonyingabo, 1972), Korea (Moon, 1976), Mexico (Flisser et al, 1976), Papua New Guinea (Gajdusek, 1978), Chile (Gonzalez & Plaza, 1976) and Zaire (Pandey &

Mbemba, 1976).

(c) Strobilocercus

This larval form is exemplified by C. fasciolaris the larval stage of Taenia taeniaeformis. Much of the literature of C. fasciolaris deals with the biochemistry and immunology of the parasite (Musoke & Williams, 1974; Kwa & Liew, 1975, 1977, 1978a,b). The pathology of C. fasciolaris in rats has been the subject of careful and extensive study especially by early workers interested in its carcinogenic properties (Hutchison, 1958; Dunning & Curtis, 1946). Lewert & Lee (1955) found that the initial stages of infection and early growth increased the solubility of the acellular glycoprotein of the liver; further, there was an inflammatory reaction around the parasite associated with the formulation of a fibrous capsule of host origin. Bullock & Curtis (1924) showed that cells within the host capsule could become malignant after 10 months but usually took 11 to 17 months. Curtis et al (1934 a,b) found direct relationships between the number of tumours and the number of parasite cysts. Non-localized effects of heavy infections have also been reported by Hoeppli & Feng (1933) and Blumberg & Gardner (1940).

(d) Coenurus

Though widely stated in text-books that the coenurid type of cysts found in man and animals are the larvae of 2 species, Multiceps multiceps and M. serialis, studies have revealed that most of the morphological characters used in separating the species may not be valid, and hence many workers contend that immunological or physiological "strains" of only one species might be involved (Nagaty & Ezzat, 1946; Clapham, 1942; Esch & Self, 1965; Larsh et al,

1965; Smyth & Heath, 1970). While coenuriasis of the nervous system in man is rare, it is well documented in domestic animals in which clinical symptoms clearly indicate nervous disorders commonly referred to as "gid" or "staggers" (Fankhauser et al, 1959). The behavioural symptoms in laboratory infected mice have been described in detail by Esch (1964) and these bear resemblance to those occurring in sheep. On the invasion phase of the parasite Esch (1967) speculates that after the initial penetration of the intestine by the oncosphere the larvae remain in blood vessels for quite a while before migrating to other tissues. There is evidence to show that host tissue determines the morphogenesis of the cyst (Esch, 1967). Aspects of the histopathology of the coenurus cyst have been described in a number of hosts including man (Landells, 1949), rabbits (Hamilton, 1950), mice (Larsh et al, 1965) and sheep (Fankhauser et al, 1959).

2. Review of Literature on Pathogenicity of Taenia crassiceps

Probably because of its convenience as a "laboratory organism", T. crassiceps has been extensively studied with regards to its biochemisty and structure. Surprisingly, however, few researchers have taken advantage of this convenient model to study the pathogenicity of such an unusual parasite with its peculiar existence and manner of reproduction.

Larval Stage

Freeman (1962, 1964) initiated interest in T. crassiceps after he established a basic protocol for laboratory maintenance of the parasite, and produced some preliminary results of its pathogenicity. He studied the development of leukocytosis and eosinophilia in the peritoneal fluid and blood of mice following primary infection with the metacestodes. Chernin (1977 a) reported a significant increase

in the number of antigen-antibody complexes with respect to the time, but not the volume, of metacestode infection in mice, and, more curiously, the detection of common antigens in both the parasite and rat hosts (Chernin, 1977 b). A recent study by Siebert et al (1978 a,b) investigated the development of T. crassiceps metacestode populations for the first 2 months following inoculation of primary and challenge doses of the larvae. They found that prior subcutaneous implantation of larvae stimulates immunity to larvae inoculated intraperitoneally and that there are 2 distinct responses by the host to the larvae. The kinetics of parasite population pattern appears to be determined by a humoral reaction from the host followed by a cell-mediated one resembling an allergic inflammatory response. Siebert et al (1978 a) also reported that an increase in larvae burden greatly diminished the allergic inflammatory response, and they suggest that the increased parasite burden resulted in an increase in the amount of immune suppression, a phenomenon reported earlier by Good & Miller (1976).

Laboratory mice are extremely resilient to cysticerci introduced by intraperitoneal inoculation. Freeman (1962) reported a case in which a mouse had lived 553 days after infection, and contained 4412 metacestodes. The abdomens of heavily infected mice may be so distended that all four feet could not touch the floor simultaneously. Death due to the massive numbers of metacestodes and the inferred disruption of feeding and drinking had been observed. The pathological effects of these metacestodes and the burden they inflict on body organs such as the liver, lungs and heart, have not been the subject of much investigation (Chernin, 1973).

Studies by Lubinsky and Baron (1970) suggest a lack of broad host

tolerance in T. crassiceps which does not develop in rabbits, hooded rats or guinea-pigs. Freeman (1962) failed to establish the parasite in guinea-pigs, rabbits, a laboratory rat, a sheep, and some rodents. Blair & Campbell (1976) succeeded in establishing the rat as a laboratory host for T. crassiceps metacestodes. Chernin (1974) reported, what appeared like, a new strain of T. crassiceps from laboratory rats. Anderson & Griffin (1979 a) routinely used the rat as a host in studies on T. crassiceps. Natural infections, though low, have been reported from woodchucks (Marmota monax) in North America (Freeman, 1962; Fleming et al, 1979), and a variety of other rodents in North America and Europe (Sharpilo, 1976; Kazlauskas et al, 1974; Fatzer et al, 1976). The apparent contradiction regarding host tolerance is compounded by, and probably due to, the relative ease with which new "strains" of the parasite evolve.

Feeding experiments with T. crassiceps eggs (Freeman, 1962) revealed that the favoured sites in various wild-caught mammals are subcutaneous regions of the axillae, interscapulae space, underside of neck, in the pleural cavity, abdominal cavity, retroperitoneal behind the kidney, within the pericardial sac and in the lung. Moreover, cysticerci have been reported in the central nervous system of an Alpine marmot (Fatzer et al, 1976); the host showed pathological symptoms of nervous disorder. Metacestodes introduced intraperitoneally largely remain free in the body cavity, although some were attached to various organs particularly the liver, diaphragm, stomach and mesentery. Freeman (1962) also reported that from the infection of metacestodes, living parasites were recovered from 75% of intraperitoneal infections, 55% of subcutaneous inoculations and 15% of intrapleural inoculations. Intraperitoneal injection resulted in minimum host reaction and

maximum rate of parasite reproduction. Subcutaneously inoculated metacestodes were frequently walled off in a thick cyst and produced few progeny, and acute reactions frequently followed intrapleural injections.

Although T. crassiceps is generally regarded as non-pathogenic to humans, a rare case of intraocular T. crassiceps was reported from Canada. In a series of publications, Shea et al (1973), Freeman et al (1973), and Fallis et al (1973) reported the diagnosis and surgical removal of budding cysticerci from the intraretinal space in the eye of a 17 year old Canadian girl who had complained of decreased visual acuity. The cysticerci were positively identified to be those of T. crassiceps and successfully established in laboratory mice. This is the first and only record of a T. crassiceps infection in man.

Adult Stage

The adult worm had been reported in natural infections in arctic foxes in Arctic North America (Rausch, 1952), red foxes in Canada (Freeman, 1962; Smith, 1978), domestic cats in Czechoslovakia (Mituch, 1968) and wild cats in Bulgaria (Yanchev & Genov, 1978). Data on susceptibility of various canids to infection with T. crassiceps and the rate of development of the adults in these hosts is scanty. Freeman (1962) fed metacestodes to red foxes, dogs and coyote pups; and a dog and a fox passed eggs as early as 32 and 42 days post-infection respectively. Though one dog passed proglottids for 9 months before spontaneously losing its infection, the other infections were patent only for a short time. None shed eggs following a second feeding after the initial infections were lost. There are no records of clinical manifestations of adult infestations.

B. MATERIALS AND METHODS

1. Maintenance of Parasite and Infection Regimes

A continuous stock of Taenia crassiceps of both KBS and TOI strains was maintained by serial passaging of the metacestodes intraperitoneally into laboratory mice, as previously described under General Methods.

Standard amounts of metacestodes (either by number or volume) were injected into mice of specified ages. At intervals the mice were autopsied and both total parasite volume and number were recorded.

20 well-developed cysticerci were fed in a gelatine capsule to each of four 8 - week old beagles obtained from an acredited supplier. Three pups were killed by a humane killer 10, 17 and 29 days post-infection and the worms carefully removed from the small intestines under warm saline. The fourth dog was examined for proglottids or eggs in the faeces up till 60 days post-infection. When these were negative, it was re-infected and examined 32 days later.

Eggs were obtained from gravid proglottids at the lower ends of the tapeworms from 29 day old infections. The eggs were washed thoroughly and stored in distilled water at 4°C. 2,000 eggs were fed to mice by stomach tube. At intervals the mice were killed and examined thoroughly for metacestodes.

2. Assessment of Parasite Burden

Direct count was made when assessing the number of adult tapeworms in the infected dogs after autopsy.

In mice, both direct count and volume measurement were employed. In heavy infections all the larvae were washed out of the mice into a 50 ml measuring cylinder, and allowed to settle on standing under

some HBSS. The volume occupied by the parasites was taken to indicate parasite burdens.

3. Pathological Examination

Pathology of host organs was investigated by gross examination as well as by histological examination. Immediately after autopsy organs such as the liver, spleen, ovary, kidney, lung and heart were fixed in Bouin's or formalin and processed in the usual way for wax histology. Sections were stained with Haematoxylin and Eosin, and Casson's Trichrome stain. Organs from mice of similar ages were processed similarly as controls.

Maximow's staining technique was also used to investigate pathogenesis in the infected intestine of the dogs. Segments of intestine with attached tapeworm scoleces were fixed in Zenker's or formal-saline and processed in the usual way for wax sectioning.

Gross and histological examination was also carried out on mice infected with eggs to look for metacestodes and histophathological evidence of migrating oncospheres.

C. RESULTS

1. Larval Stages

(i) Oncospheres

Eight mice were fed by stomach tube with about 2,000 eggs of T. crassiceps from a 29 day old worm. The eggs had been stored for 24 hours at 4 C. One month after infection a mouse was examined for the presence of T. crassiceps. No metacestode stages were found in the peritoneal cavity, thoracic cavity, subcutaneous regions, nervous system and muscles. Gross and histological examination of the liver

revealed no oncospheral tracks nor any other signs of pathogenesis. The same results were obtained on all subsequent examinations of the mice at monthly intervals.

(ii) Metacestodes

Cursory observations on routinely maintained parasite stock suggests that the metacestodes have a progidious ability to propagate in mice. When 2 cysticerci were injected intraperitoneally into mice, rapid growth was evident during the first 8 weeks of infection (Table IIA). The mean number of parasites increased from 2.8 ± 1.3 in the second week to 54.8 ± 8.2 in the eighth week. It would be expected that continued growth of the parasite within the confined area of the mouse peritoneum must come under the constraints of space, nutritional requirements and, perhaps immune suppression. In another experiment, the dose of the inoculate was varied from 2 to 30 cysticerci and the parasite burden was assessed between 6 and 30 weeks after infection. The results are given in Table IIB. This was only an experiment of a preliminary nature; only one mouse was examined for each dosage every 6 weeks. Thus, the statistical significance could not be calculated. However, the results show an interesting feature in the growth patterns of metacestode population. When four metacestodes were injected initially, there was rapid increase in the numbers from 41 after 6 weeks to 2197 after 30 weeks, almost a 54-fold increase. The approximate increase in population between 6 and 30 weeks for the other dosages were : 4 cysticerci - 25-fold; 6 cysticerci - 18-fold; 8 cysticerci - 11-fold; 10 cysticerci - 11-fold; 30 cysticerci - 3-fold. These preliminary results suggest that the growth rate is decreased when the population of metacestodes is high. Most of the mice with very heavy infections were autopsied

TABLE II. Kinetics of Metacestode Population.

(A) 16 TFi mice, divided into 4 equal groups, were each infected intraperitoneally with 2 TOI cysticerci. The mice were examined at fortnightly intervals to assess parasite burden by direct counting. Budders and encapsulated larvae were scored as single parasites.

No. of weeks P.I.	2	4	6	8
No. of metacestodes in each mouse	1,2,4,4	5,7,9,12	25,34,43,47	43,54,56,66
Mean ± S.D.	2.8 ± 1.3	8.3 ± 2.6	37.3 ± 8.5	54.8 ± 8.2

(B) 30 TF1 mice were divided into 6 equal groups. The mice in each group were injected intraperitoneally with 2,4,6,8,10 and 30 TOI cysticerci respectively. A mouse from each group was examined at intervals to assess parasite burden by direct counting. Budders and encapsulated larvae were scored as single parasites.

No. of cysticerci inoculated per mouse	No. of metacestodes recovered in each mouse n weeks post-infection, where n =				
	6	12	18	24	30
2	41(4.9)*	73(5.5)	355(1.4)	884(0.8)	2197(0.4)
4	98(4.1)	230(2.6)	744(1.1)	1841(1.5)	2418(0.7)
6	163(1.8)	402(1.2)	1224(0.5)	2614(0.4)	2922(0.3)
8	281(1.8)	791(1.1)	1734(0.3)	2833(0.5)	3143(0.4)
10+	307(1.6)	964(1.1)	2377(0.4)	3274(0.4)	3440(0.4)
30+	1045(0.4)	1949(0.5)	3045(0.3)	3411(0.4)	3678(0.3)

*Figures in brackets indicate number of encapsulated larvae (% of total)

+These cysticerci were taken from a different sample to that above.

before they became too distended with parasites. It is not possible to say if the parasite population will continue to grow, albeit at a slower rate, until the host dies directly from the infection.

In most of the metacestode populations there were several opaque caseous masses. Gross and histological examination has revealed that these were parasites that have been killed and encapsulated by host reactions (Section I). It is interesting to note that the number of these caseous bodies did not increase proportionately with the parasite population. Rather, the proportion of encapsulated parasites was generally higher in light infections (Table IIB). Most populations with more than 1,000 metacestodes contained less than 0.5% of encapsulated parasites, while in populations with less than 1,000 metacestodes there were usually more then 1% of encapsulated parasites.

(iii) Pathology

Most of the infected mice were extremely resilient to heavy infections. Although an infected mouse may have a grossly distended abdomen (Plate 162) due to the large amount of metacestodes (Plate 163), they do not succumb to the infection, in the sense that very few mice died as a direct result of the parasite burden. The only noticeable effect appears to be a clumsiness in general movements. The presence of such a large amount of metacestodes would be expected to affect the organs in the host, especially in the abdominal cavity where toxic waste-products from the parasite would accumulate. In mice with heavy infections, there was often peritonitis in the abdomen. The loops of the small intestines were often adhered together and there were extensive fibrous deposits around the liver, stomach, gut and spleen. Often, parasites were found adhering to the

small intestines, the mesenteries, and, occasionally, to the liver. Peritoneal exudates were cloudy and contained high concentrations of macrophages and other leukocytes. Examination of host organs revealed size differences only in the heart, liver and spleen (Plate 164). Compared to organs from control animals, the heart and spleen of infected mice always appeared to be slightly smaller in size. Comparison of livers revealed some obvious differences. Liver lobes from infected mice have rounded, lobular edges compared to the attenuating edges in healthy livers. Instead of a dark red coloration, livers from infected mice were often greyish. Histological examination of the organs from infected and non-infected mice were carried out to detect tissue pathology.

Liver Histological examination with the light microscope revealed a conspicuous thick capsule surrounding the liver of infected mice (Plate 165). This was made up of fibrous material and many closely packed cells with highly irregular darkly stained nuclei, probably polymorphonuclear cells. The parenchyma consisted of significant areas where cells appeared atrophic and necrotic. Fibrous tissue heavily infected with lymphocytes were occasionally found in and around the portal tracts. Often the parenchymal cells are separated from the sinusiods by eosinophilic amorphous deposits of amyloid. There were no detectable signs of diapedesis which one might expect in inflammatory reactions. Livers from control mice did not have any of the histopathological changes described above. Very rarely, the parasite was found to have penetrated into the liver. It might have been possible that in a crowded environment, a cysticerci with an evaginated scolex had actually burrowed into the liver. Histological examination revealed that there were no host inflammatory reactions in

the cells neighbouring the parasite (Plate 166). Other than a few necrotic parenchymal cells, the only other histopathological change was a relatively extensive haemorrhage.

Lungs The most obvious histopathological change that was observed in lungs from infected mice was the occurrence of emphysema (Plate 167). The alveolar walls were thin and atrophic, and in places had broken down to form bullae. The alveolar ducts and sacs were enlarged and the lesions were irregularly distributed. This indicated that the emphysema was centrilobular rather than generalised. Oedematous alveoli were seldom detected. There was no proliferation of swollen macrophages with digested haemoglobin which would indicate heart failure. Control lungs had regular alveolar walls and a relatively more uniform pattern of alveolar ducts and sacs.

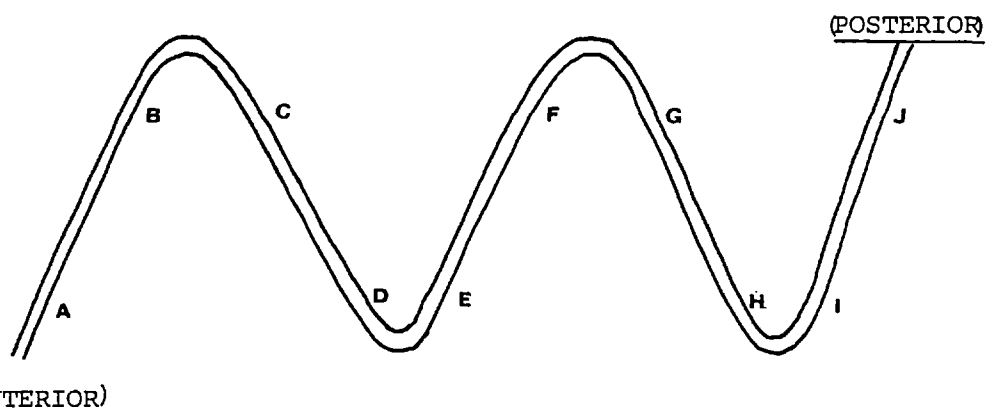
Heart Although the size of hearts from infected mice was usually smaller, the difference was only slight, and there appeared to be little pathological changes. Most of those examined histologically showed little abnormality. The cardiac muscle demonstrated the usual branching and anastomosing muscle fibres, separated by strands of loose connective tissue with a rich capillary supply. There was no obvious evidence of extensive hypertrophy, organically nor histologically, that might be expected from overworked cardiac muscles in a mouse with an extra burden of metacestodes. Closer examination revealed small areas where myocardial infarction might have resulted in necrotic fibres (Plate 168). These fibres were still striated but deeply eosinophilic and with degenerate or no nuclei. There was also a noticeable lack of epicardial fat in hearts from infected mice, but the significance of this must be taken in the light of similar observations in some of the hearts from non-infected mice.

Kidney The kidney of an infected mouse was usually covered by a thick fibrous capsule (Plate 169) consisting of many layers of darkly stained fibres. These fibrous layers had few nuclei and appeared to be largely acellular connective tissue. Within the kidney, there was no evidence of histopathological change. Nephrons were normal with healthy looking glomeruli, richly supplied with blood components, and conducting tubules. The thick fibrous capsule was never observed on any of the kidneys from non-infected animals that were examined.

Other organs The spleen from both infected and non-infected mice showed no detectable differences. There was the usual distribution of haemoglobin break-down product, multi-nucleated giant cells, lymphocytes and other leucocytes. The ovary from infected mice appeared smaller, but contained normal follicles, and no histopathological changes were observed.

2. Adult Worms

Four dogs were each fed with 20 cysticerci of T. crassiceps (KBS). Three were examined after autopsy 10,17 and 29 days post-infection (P.I.). In order to determine the relative position where the worms were found, the whole small intestine was divided into five equal lengths as illustrated below, and the regions designated by the alphabets A to J.



The first dog, examined 10 days P.I., harboured 18 adult worms measuring about 10 - 15 mm. The worms were attached to the mucosa at region 'G'. The second dog, examined 17 days P.I., had 11 worms measuring about 35 - 60 mm, and these were located at region 'H'. Only 3 adult T. crassiceps were found in the third dog examined 29 days P.I. These worms were located in region 'I' and they measured about 50 - 70 mm in length. The fourth dog was kept in order to collect T. crassiceps eggs or proglottids from faecal matter. After 60 days, when no eggs or proglottids were found, the dog was re-infected with 20 cysticerci and examined 32 days P.I., but no adult worms were found.

Since only one dog was available for examination for each infection period, it is not possible to attach much statistical significance to the infection dynamics. The results showed that worm burdens decreased rapidly from 18 to 11 between 10 and 17 days P.I. On the 29th day, only 3 worms were found.

Histological examination revealed that the scolex of the worm was attached shallowly among the tips of intestinal villi (Plate 170). A plug of host gut mucosa was often observed in the suckers. It is difficult to distinguish epithelial damage that was caused directly by the worm from that resulting from processing for histological examination. Generally, there were few signs of pathological changes. Along the villi that were in close contact with the suckers and rostellum the epithelium was flattened and the columnar cells appeared more like pavement type cells (Plate 69). There was also fairly extensive metaplasia of epithelial cells which were transformed into mucus secreting cells (Plate 66). Destruction of epithelial cells by rostellar hooks appeared to be minimal, and any extensive tearing

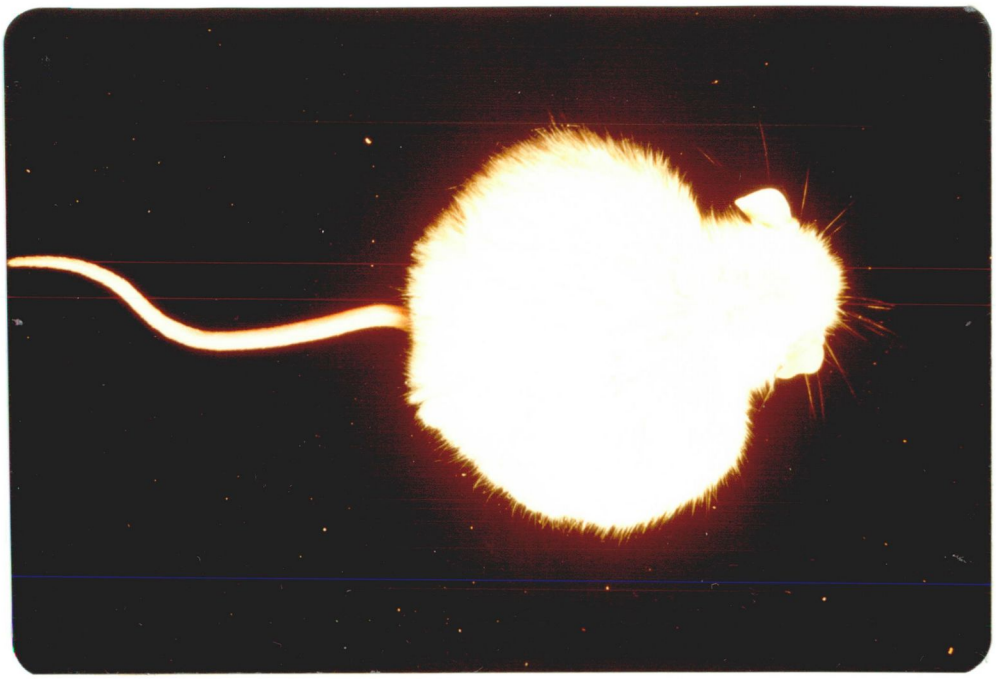


Plate 162. A mouse that has been injected intraperitoneally with about 30 metacestodes of T. crassiceps. This infection was 5 months old. (x 0.75)



Plate 163. A mouse, with a 5-month old infection, dissected to reveal the large volume of metacestodes. (x 0.75)

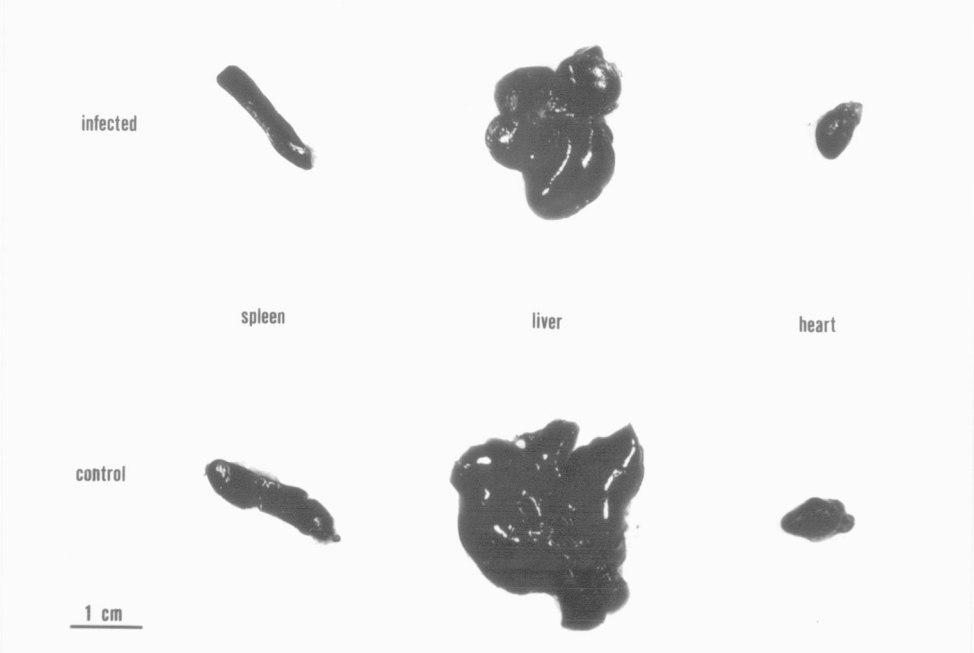


Plate 164. The heart, liver and spleen of an infected and non-infected mouse. The infected mouse was harbouring T. crassiceps metacestodes that occupied a volume of about 20 ml.

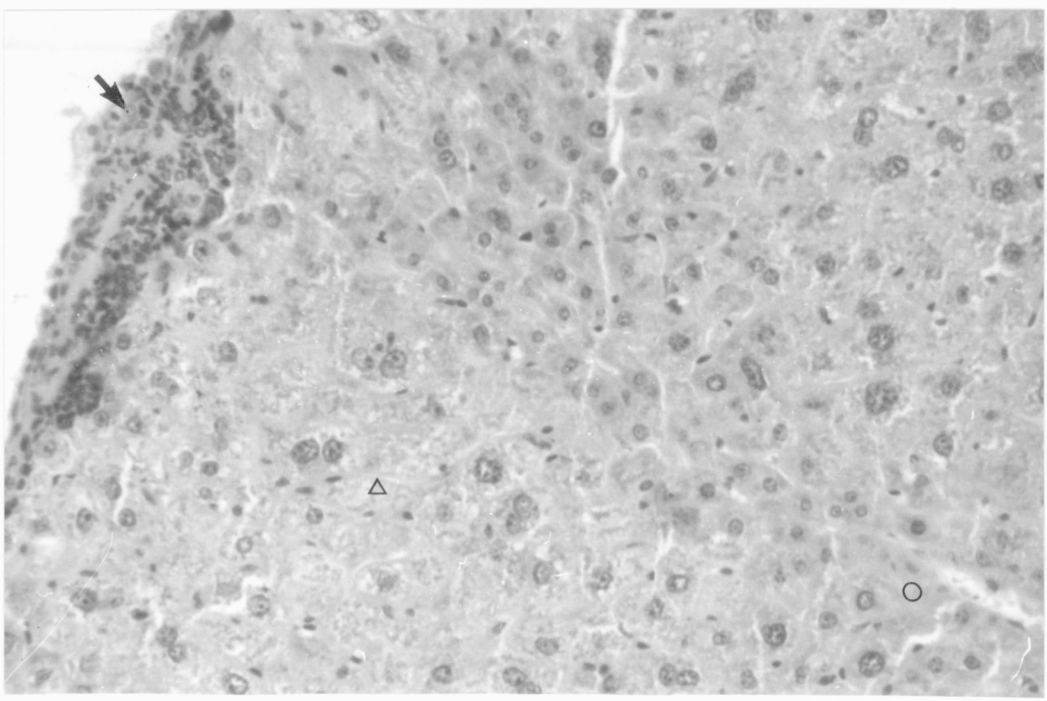


Plate 165. The liver of an infected mouse showing histological abnormalities. The surrounding capsule (arrow) is fibrous and thick containing numerous polymorphonuclear cells. Some of the parenchyma cells are atrophic and necrotic (△) while others are separated from the sinusiods by a dense eosinophilic amorphous deposit of amyloid (○). (Bouin's; H & E) (x 50)

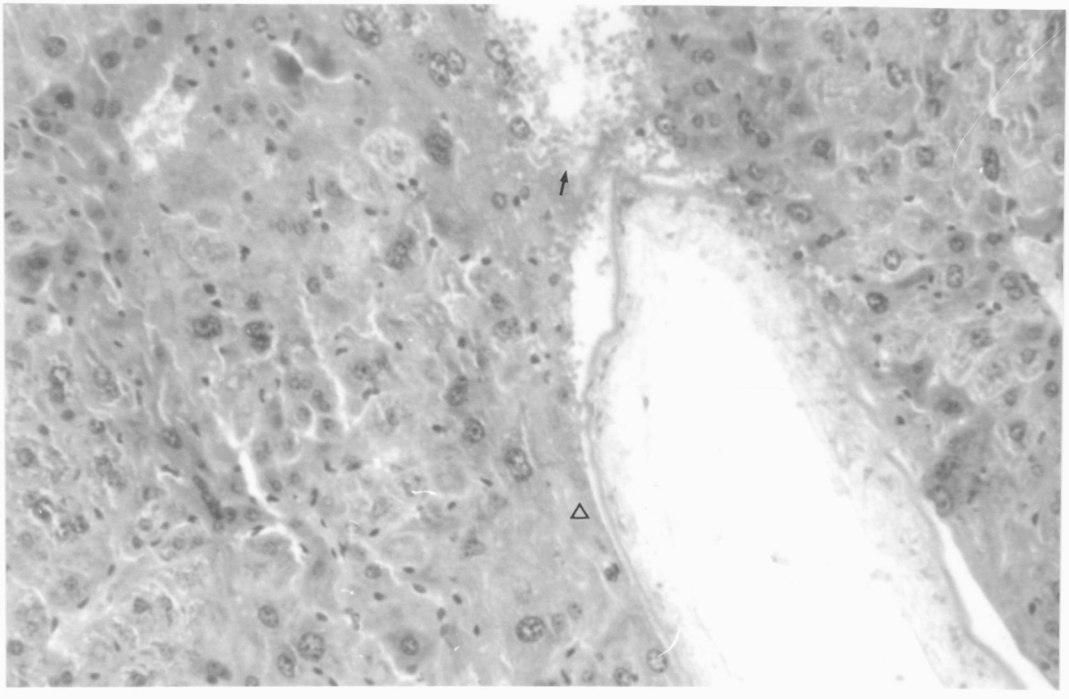


Plate 166. A metacestode observed in the liver. Note the massive haemorrhage (arrow) and the few necrotic cells (Δ). (Bouin's; H & E) (x 80)

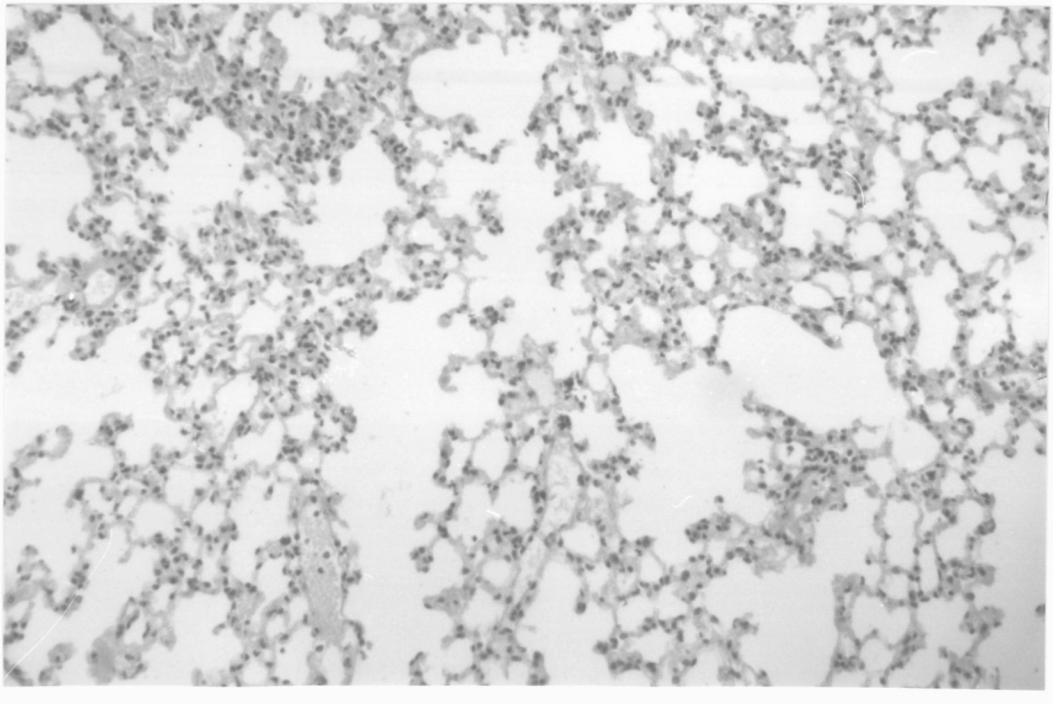


Plate 167. The lung of an infected mouse. There are signs of centrilobular emphysema. The alveolar walls are thin and are broken down in places to form bullae. The enlargement of alveolar ducts and sacs are irregular in distribution and intensity. (Bouin's; H & E) (x 80)

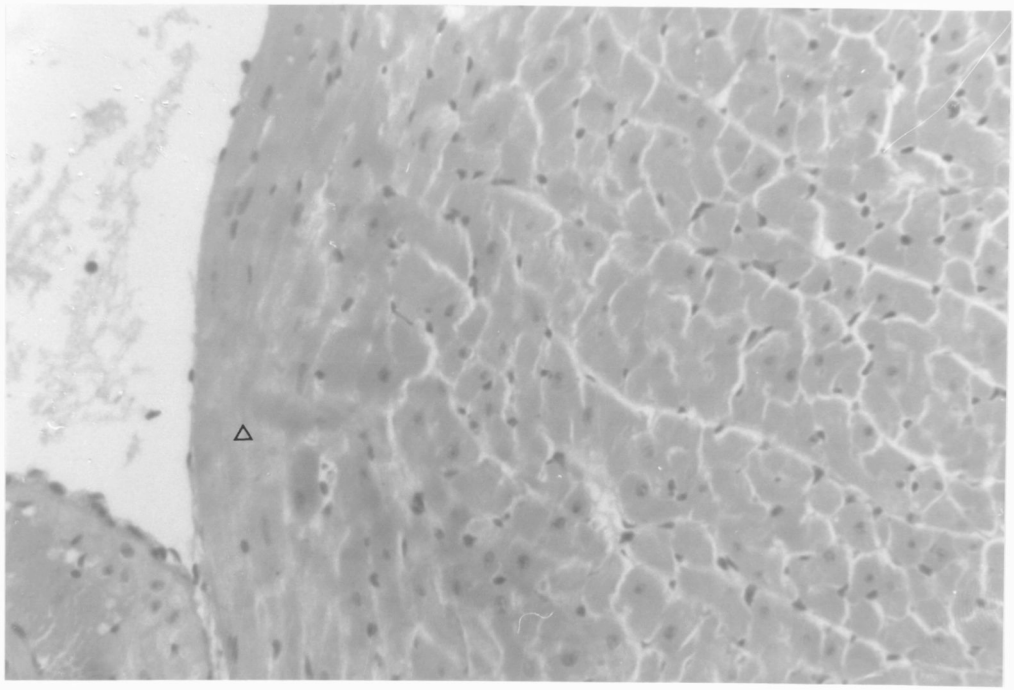


Plate 168. The heart of an infected mouse with only slight infarction of some cardiac muscle (Δ). The rest of the tissues appear normal. (Bouin's; H & E) (x 80)

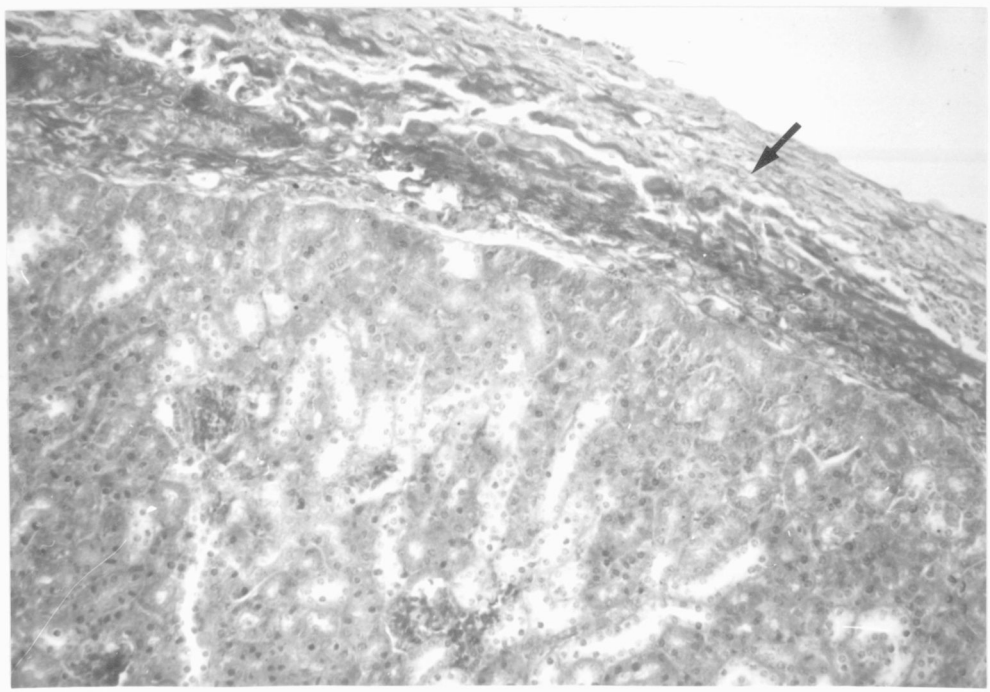


Plate 169. The kidney of an infected mouse. Other than a very thick fibrous layer on the surface (arrow), the rest of the kidney appears unaffected, with normal glomeruli (□) and collecting ducts (○). (Bouin's; Casson's trichrome) (x 60)

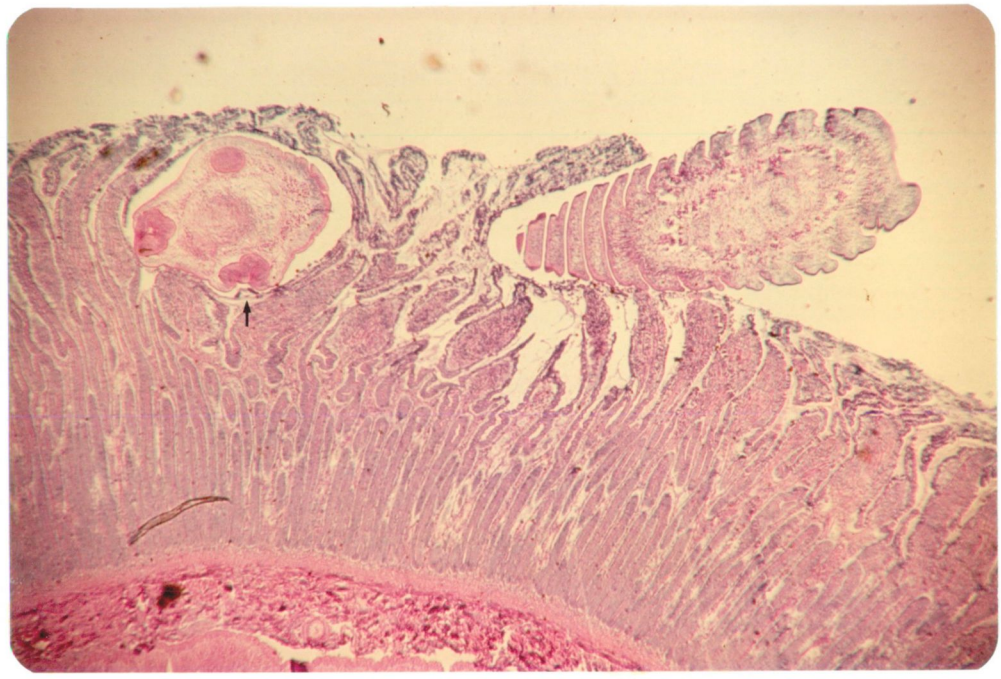


Plate 170. Transverse section through the intestine of a dog showing the attachment of an adult T. crassiceps. A plug of gut tissue is present in one of the suckers (arrow). Note the fairly extensive disruption of tissue around the scolex and strobila. (Zenker's; Maximow) (x 40)

(Plate 67) might have resulted from the sudden withdrawal of the hooks when the infected gut portion was introduced into fixative. The scolex and strobila of the worm might have caused continuous friction on the mucosa at the tips of the villi, provoking inflammatory reactions. However, there was no evidence of extensive invasion of eosinophils or polymorphonuclear cells. Epithelial disruption around the worm (Plate 170) could have been the result of irritation by the worm or an artifact of tissue-processing. There was no haemorrhage or villus hypertrophy to indicate acute inflammation.

Throughout this experiment, a concurrent infection by Toxocara sp. was present in all the dogs examined. The nematodes increased in numbers, size and distribution along the small intestine throughout the duration of the experiment.

D. DISCUSSION

In the present study no metacestodes were recovered from mice infected orally with T. crassiceps eggs. This could be due to a number of reasons. There is the possibility that the eggs used were not fully viable due to lack of maturity. These eggs were from the terminal proglottids of a worm obtained 29 days post-infection (P.I.) Freeman (1962) reported that the earliest time for eggs to be shed from experimentally infected dogs was 32 days and 42 days for foxes. Another possibility for the failure in infecting the mice could be an inadequate dosage of eggs. Bilqees (1967) reported that only 1 to 15 metacestodes were found in mice after oral challenge, but the infective dose was not mentioned. She also noted that the metacestodes were usually surrounded by numerous leukocytes. If only a portion of the 2,000 eggs used in the present study were fully infective, the

insufficient dosage might have resulted in the few oncospheres being overwhelmed by the leukocytes before the mice were examined from the first month P.I. There were no signs of pathogenetic effects of oncospheral migration through liver tissues. Freeman (1962) reported that the main subcutaneous sites where metacestodes were found following an oral infection were the axilla, neck, rib-cage and scapula region. These locations indicate that the migration of oncospheres from the intestines may be mainly via the lymph ducts.

It is interesting to speculate on the mechanisms by which the metacestodes can survive and reproduce so successfully within the "immunologically hostile" environment of the mouse peritoneal cavity. How this is achieved is a matter of great controversy, but parasites appear to be able to do this by avoiding stimulation of the host's immune response or by depressing the level of immune attack. Various mechanisms have been suggested by which parasites could avoid stimulation of the host's immune response. This is achieved by presenting to the host a surface and/or metabolic products which are antigenically very similar to those of the host. This concept of antigen sharing by host and parasite or 'molecular mimicry' (Damian, 1964, 1967) has been shown to be widespread among helminths which do not only share antigens with their hosts but also with other species within their class, or even those from a different class (Capron *et al*, 1968). Smyth (1976) have summarised the possible origins of such 'host-like' antigens : mimicry by natural selection (Sprent, 1962, 1969), mimicry by host "induction" (Capron *et al* 1968), and mimicry by incorporation of host antigens (Smithers *et al*, 1969; Clegg *et al*, 1971; Clegg, 1972). In *T. crassiceps* infection in rats, Chernin (1977a) noted a significant increase in the number of antigen-antibody

precipitins with regard to the duration of the infection and not to the volume of metacestodes. Antibodies produced by rats and mice are apparently not effective against the parasites in so far as they remain largely not destroyed or restricted. Common host antigens between rats and T. crassiceps metacestodes have been detected by Chernin (1977b) but how they might have arisen is unknown. Other than the acquiring of common antigens there is another possibility of how evasion of the host immune response is brought about. Immunological enhancement, a phenomenon which is believed to be involved in survival of tumour allografts (Roitt, 1971), has been implicated in some helminth infections (Varela-Diaz et al, 1972). Here, what are termed 'enhancing antibodies' or 'blocking antibodies' (Rickard, 1974) combine with surface antigens or target cells with the result that these are no longer accessible to the effector arm of the cell-mediated immunity. Immunodepression has been demonstrated in some helminth infections. Kassis & Tanner (1976) have demonstrated anticomplementary actions in the calcareous corpuscles of Echinococcus spp. From the study by Siebert & Good (1979) it was established that complement played an important role in early humoral mediated immunity in T. crassiceps. If anticomplementary function can be attached to the calcareous corpuscles in T. crassiceps (Section I) it may explain why large populations of metacestodes can survive in the mouse. More recently it has been suggested that the rapid replacement of the surface glycocalyx is a mechanism by which helminths may protect themselves against host immunity (Siebert et al, 1978b; Hanna, 1980).

In T. crassiceps, any of the above mechanisms of mimicry, immunological enhancement, immunodepression, or glycocalyx replacement could be in operation either singly or by interaction. In a brief

study described later in Section IV, metacestodes were introduced into an enhanced immune state induced by prior inoculation of parasite antigen. Even in such a situation the parasites could survive and reproduce, although early damage, death and encapsulation was inflicted on some of the larvae. The fact remains that T. crassiceps will continue to exhibit a proliferate reproductive capacity, with an exponential growth rate initially, then slowing down when the population reaches a certain level. It is difficult to determine from the present study whether the slowing down of reproductive rate is due to the lack of space, nutritional requirements, or immunological causes. Freeman (1962) has shown that metacestodes in the abdominal cavity have an increasing rate of reproduction of up to 8 metacestodes per day, while those in the pleural cavity and subcutaneous or tissue sites never exceeded 2 per day, suggesting that propagation may be largely constrained by space.

While it is apparent that the metacestodes can evade the immune response of the mouse to a large extent, it is also interesting that the host is able to tolerate such heavy infections. Very few of the heavily infected mice died as a direct consequence of the parasite. Histopathological examination revealed changes in the lungs and liver of heavily infected mice. Emphysema detected in lungs from infected mice was not generalised, and consequently not very disabling. The cause of emphysema is unknown (Walters, 1979) but it may be associated with chronic bronchitis. It is possible that the presence of the large amounts of metacestodes had lowered general resistance of the mouse, resulting in the bronchitis. The pressure imposed by the metacestodes upon the diaphragm might also have interfered with pulmonary function. There were no signs of heart failure such as

hypertrophy or extensive infarction. The lack of inflammation may be due to the fact that the lungs and heart are isolated from the abdominal cavity, where the metacestodes were located, by the diaphragm. This suggests that any toxic substances excreted by the parasites were localised and not systemic.

The liver is the only organ that suffered from the pathogenic effects of the parasite. Much of the pathology is the result of toxic substances secreted by the parasites. Extensive peritonitis, a sign of toxic imflammation (Walters, 1979), is evident from the fibrous deposits around the mesenteries and abdominal organs. Toxicity might have reached some of the hepatic cells resulting in the observed necrosis. Amyloidosis is believed to be the consequence of pressure atrophy or ischaemia (Curran, 1972); it is possible that the presence of large amounts of metacestodes resulted in generalised external pressure on the liver. No signs of obstructive jaundice was observed in the livers examined. Chernin & Tilleray (1979) reported jaundice in infected mouse liver but noted the lack of symptoms, and suggested that this might be due to the ability of metacestodes to absorb bilirubin. Toxic metabolic products have also been implicated as the cause of liver lesions when H. microstoma was injected into mice intraperitoneally (Simpson & Gleason, 1975). These lesions had degenerating cells and well infiltrated by leukocytes. Lewert & Lee (1955) reported that an initial inflammatory reaction around T. taeniaeformis larvae in the liver was associated with a fibrous capsule but the inflammation disappeared after the initial rapid larval growth had ceased. No inflammatory reaction was noted around the T. crassiceps metacestode found within the liver in this study, although haemorrhage was evident.

No serious histopathological changes were found in the other organs examined. Urinogenital structures appear to be normal; the inflammatory reaction around the kidney did not appear to affect the structures within the cortical or medullary regions of the kidney and the ovary was apparently healthy. Arvy (1950) reported that T. crassiceps often occurred in the region of the genitalia, causing intravaginal cysticercosis in the female and stenosis of the vas deferens and epididymus (resulting in azoospermia) in the male.

To bring T. crassiceps infection into the context of cysticercosis in general, it is interesting to note that T. pisiformis causes little or no pathogenic effects in the abdominal cavity of rabbits, although encapsulation of larvae has been observed in established infections (Heath, 1970, 1971). In C. tenuicollis, adhesion of organs occurs as a result of fibrous peritonitis (Pullin, 1955; Sweatman & Plummer, 1957).

Artificial infection in the dog by adult T. crassiceps did not last more than about a month. No eggs or proglottids were found among faecal matter from the dog after 30 days P.I. The worms were attached four-fifths of the way along the small intestine from the pyloric sphincter in the first dog that was examined but were displaced further posteriorly in the subsequent dogs, until (apparently) totally rejected from the last dog. In this last dog, there was, of course, no evidence that the initial infection had been successful. When the fourth dog failed to pass out proglottids or eggs it was reinfected but there were no worms present at autopsy. Freeman (1962) reported that an infected dog continued to shed eggs for 9 months before being spontaneously rejected from the intestine. He also tried, unsuccessfully, to reinfect another dog that harboured only a short infection. Unfortunately, due to the high cost of dogs and their difficulty of

maintenance (at the London School of Hygiene and Tropical Medicine field station, Winches Farm, St Albans), these dog infections could not be repeated.

There could be a number of reasons for the displacement and the possible rejection of the adult T. crassiceps from the intestine of the infected dogs. The rather superficial attachment site towards the lumen of the intestine might not have provided very strong resistance against normal fluid and peristaltic movements. In comparison, E. granulosus adults are usually deeply attached near the base of the villi with the rostellum embedded in the crypts of Lieberkuhn (Smyth, 1969 a). The importance of morphological, physical and biochemical factors within the gut of the definitive host with respect to adult cestode infections has been discussed in relative detail by Smyth & Smyth (1968). Competition between helminths within a host can be influential in determining rejection rates. One of the earliest reports of interspecific competition between helminths was that of Cross (1934) who observed an inverse relationship between the numbers of Proteocephalus exigus and an acanthocephalan in the gastrointestinal tract of their fish host. Larsh & Campbell (1952) found that the simultaneous exposure of mice to Trichinella spiralis and H. nana produced lower tapeworm yields. Holmes (1961) observed that when H. diminuta and Moniliformis dubius were grown together in rats, the tapeworms showed greater size reductions and were displaced posteriorly in the intestine from positions they occupied in rats with tapeworms only. In the present study the presence of Toxocara sp. which increased in number, size and distribution along the intestine could be a major cause for the displacement of T. crassiceps adults and the subsequent total rejection. The maintenance of dogs free from

Toxocara is almost an impossible task in most animal yards and 'germ-free' conditions are essential to obtain and retain worm-free status. This is a well-recognized problem in dog kennels.

Host immune factors are believed to contribute to the short duration of helminth infections, e.g. H. nana (Schiller, 1959), Moniezia expansa (Hansen et al, 1951), and to serious impairment of growth or damage to the tegument (Hopkins et al, 1972; Gray, 1973; Befus & Threadgold, 1975). While there is the possibility that immunologic interference was involved in the rejection of T. crassiceps and failure to reinfect dogs, no tegumental damage was observed on the worms examined (Section I).

Only minimal damage was caused to the lining of the dog intestine at the attachment site of adult T. crassiceps, any damage observed remains discrete and localized. Metaplasia of the epithelium from columnar to pavement in the villi neighbouring the scolex was also reported by Rees (1958) for Bothriocephalus scorpii. There was no obvious lesions resulting from severe inflammation, invasion by lymphocytes and polymorphonuclear cells and fibrosis as reported for some pathogenic tapeworms, (Williams, 1960; Nath & Pande, 1963; Rees, 1967). Adult T. crassiceps appear to conform to the generally low pathogenicity of most cestodes.

E. SUMMARY

Oral infection of mice with oncospheres from a 29-day worm was unsuccessful; the reasons for this might have been the lack of fully matured and viable eggs.

In heavily infective mice, there was peritonitis with fibrous deposits around the intestine, mesentery and organs. Some histo-

pathological changes were observed in the lungs, where centrilobular emphysema was revealed, and in the liver, where necrosis and amyloidosis were found. The inflammatory changes were attributed to toxic metabolic waste products of the metacestodes which were present in large numbers. Metacestode population growth rate appears to increase exponentially until it became limited by space or nutritional requirements.

Adult worms were found four-fifths along the small intestine from the pyloric sphincter. They appeared to be displaced posteriorly as the infection wore on, and totally rejected from the worm shortly after infection. Only very slight pathogenicity was observed at the attachment site of the scolex.

SECTION IV. PRELIMINARY STUDIES ON THE IMMUNOLOGY OF TAENIA CRASSICEPS

A. INTRODUCTION

1. Development of Parasite Immunology

The rapid development of immunology in the last 20 years has significantly affected all areas of the biological sciences and not least the field of parasitology. The increased interest by immunologists and parasitologists in the immune mechanisms involved in parasitic infections have resulted in considerable progress in the understanding of parasite immunology (Ritts, 1970; Allison, 1972; Capron et al, 1976; Askenase, 1977). This increased interest has also been enhanced by the changing outlook on worldwide diseases; with considerable success in the control of viral and bacterial diseases, more attention is now being focussed on the previously neglected diseases caused by parasitic protozoa and helminths. A greater awareness of these diseases, especially among scientists and sponsors in developed countries now relatively free of major diseases, has resulted in the channelling of much energy and funds into research on parasitic infections which are largely prevalent in tropical developing countries. Finally, the realisation of the limitations of chemotherapy against parasites which can evolve resistance to drugs, have directed research into preventative, rather than purely therapeutic, means of control.

2. Immunology of Cestode Infections.

A measure of contact between a parasite, and its secretory or excretory products, and the tissues of its host is essential if the host is to respond to the presence of the parasite. It may seem logical that where this contact is intimate the response will be more

vigorous and perhaps qualitatively different from that where contact is less close. Thus, it is generally accepted that larval cestodes which develop in host tissues stimulate a higher degree of immunological response than do adult cestodes which reside in the vertebrate gut (Weinmann, 1970). Although contact between the intestinal mucosal surface and the attachment organ of adult cestodes may, in some cases, be very close (Smyth & Smyth, 1968), this is not a necessary prerequisite for the stimulation of antibody response. Recent work with Hymenolepis diminuta and H. microstoma (Harris & Turton, 1973; Befus, 1974; Befus & Threadgold, 1975; Howard, 1976; Hopkins & Zajac, 1976; Hopkins et al, 1977) and Echinococcus granulosus (Williams & Pérez-Esandi, 1971; Herd et al, 1975; Herd, 1976, 1977) have revealed that there is an extensive range of host responses to adult cestode infections. However, there is little conclusive evidence to show the presence of specific immunity, but the responses strongly suggest that the immunological system can operate to limit the success of adult cestodes in the gut lumen.

The importance of larval cestodes as pathogens of man and domestic animals has stimulated much research into the immunological mechanisms operating in the host. The immune response of the mammalian host following an antigenic stimulation by larval cestodes involves the production of an altered state which may (a) have no deleterious effect on either the host or parasite, (b) result in a reaction which is directly beneficial or harmful to the parasite, or (c) result in a state of clinical hypersensitivity which may be harmful to the host and indirectly harmful or beneficial to the parasite (Gemmell & MacNamara, 1972). Within the last decade a number of comprehensive reviews on the immunology of cestode infections

have been published (Gemmell & MacNamara, 1972; Gemmell, 1976; Kagan, 1976; Gemmell & Johnstone, 1977; Williams, 1979). Interest in this area has intensified with the emergence of cysticercosis and hydatidosis as major economic and public health problems in many parts of the world (Schantz & Schwabe, 1969; Williams et al, 1971; Pawlowski & Schultz, 1972). Even in the developed countries, taeniases are still of concern as a result of importation of infected animals, increased international travel, and inexpedient farming practices (Thompson & Smyth, 1975; Abdussalam et al, 1976; Rickard & Adolph, 1977b).

The application of contemporary immunological techniques to experimental parasitology has resulted in many advances in the understanding of host-parasite relationships. Studies on immunity against cestode infections have, until recently, been limited mainly to the humoral aspects of immunity (Gemmell & MacNamara, 1972; Soulsby, 1972). Studies on T. taeniaeformis and Echinococcus spp. have revealed some of the humoral components of the immune response by the hosts (Leid & Williams, 1974; Musoke & Williams, 1975 a,b, 1976; Musoke et al, 1975; Rickard et al, 1977 a,b; Kwa & Liew, 1978a; Lloyd & Soulsby, 1978). The implication of immunoglobulins of the IgA, IgE, IgG and IgM subclasses and their specific roles in the antibody mediated protective mechanism has clarified specific details of the immune response. While studies on humoral immunity have contributed to a deeper understanding of parasite immunology, it is evident from experimental infections that cell-mediated immunity (CMI) responses may be involved in larval cestode infections (Blundell et al, 1969; Nemeth, 1970; Ogilvie & Jones, 1973; Rickard & Outteridge, 1974; Kwa & Liew, 1975; Larsh & Weatherly, 1975; Mitchell et al, 1977;

Anderson & Griffin, 1979 a,b). The likelihood that both humoral and CMI responses could act together or synergistically in the same host was demonstrated by Rickard & Outteridge (1974) and Kwa (1978). Another aspect of the protective mechanism concerns the contribution of complement. Evidence for complement-mediated attack on worms has been presented in work on T. taeniaeformis (Hammerberg et al, 1976; Hammerberg & Williams, 1978a) and Echinococcus spp. (Kassis & Tanner, 1976, 1977; Kassis et al, 1976; Hammerberg et al, 1977).

Several studies have demonstrated antibody mediated responses against early postoncospheral metacestodes (Campbell, 1938 a,b,c; Musoke & Williams, 1975b; Kwa & Liew, 1977; Mitchell et al, 1977). In contrast, mature cysticerci appear to be considerably less susceptible to the protective antibody response (Rickard, 1974; Musoke & Williams, 1976). Once established at the site of predilection, metacestodes survive for extended periods in fully immunocompetent hosts (Ogilvie & Wilson, 1976). The ability of parasites to evade the host immune response and survive for long periods in hosts immune to challenge infection has aroused considerable interest in the past few years (CIBA, 1975). Speculation on how these older cysticerci accomplish this have been made by Campbell (1938c) who, working with Taenia taeniaeformis, was of the opinion that encystment or encapsulation by the host conferred a structural barrier to protect the larvae from immune attack. This was rejected by Musoke & Williams (1975b) whose results suggest that the secretion of an anticomplementary factor is the mechanism by which the older larvae evade the host immune reaction. This view is supported by Kassis & Tanner (1976, 1977) who reported that in vitro studies with E. granulosus and E. multilocularis revealed anticomplementary activities associated

with calcareous corpuscles found in hydatid fluid or in the tissue of the parasite. Rickard (1974) has postulated that host blocking antibodies produced early against T. pisiformis infection may be acquired by the cysticerci and thus interfere with the action of either functional antibodies produced at a later stage or a slowly developing cell mediated immunity. Finally, Kwa & Liew (1978b) appeared to have resolved the problem by suggesting that, for T. taeniaeformis at least, a combination of host blocking antibody and parasite anticomplementary factor(s) may be involved. Further clarification of specific details of the components of protection will contribute to experimental approaches to the evasive mechanisms which worms must employ in order to survive for prolonged periods in immune hosts.

The basic principles and techniques involved in the production of effective vaccines against microbial diseases have been adopted and developed for parasitic diseases caused by cestodes. Artificial immunization schemes against T. hydatigena and T. ovis in sheep (Blundell et al, 1968) and T. saginata in cattle (Wilkerhauser et al, 1974) were developed which involved inoculation with oncospheres. The use of dead vaccines for protection against infection has been evaluated by a number of workers (Clegg & Smith, 1978; Williams, 1979). Campbell (1936) successfully immunized rats against T. taeniaeformis by giving intraperitoneal injections of dried or fresh cyst material. More recent work on vaccination using such "somatic" antigens have revealed that this could be an efficient means of protection. Gallie & Sewell (1976) achieved complete protection by vaccinating calves with a soluble extract of "somatic" antigens, while Kwa & Liew (1977) went one step further by using isolated functional antigenic fractions

from crude soluble somatic antigens extracted from homogenized larvae of T. taeniaeformis to induce sterile immunity. While the use of somatic antigens has been shown to be effective, it is likely that commercially viable vaccines would be more feasible if secreted and/or excreted (ES) antigens can be produced in large quantities from in vitro systems. Using the techniques developed by Heath & Smyth (1970), Rickard & Bell (1971b) vaccinated lambs against T. ovis with antigens collected during in vitro culture. ES antigens have also been used with considerable success to vaccinate cattle against T. saginata (Rickard & Adolph, 1976), rabbits against T. pisiformis (Heath, 1973 b,c, 1976; Rickard & Outteridge, 1974) and rats against T. taeniaeformis (Kwa & Liew, 1977). Isolation of functional antigens from ES products for use as vaccines have yielded encouraging results in T. taeniaeformis and T. pisiformis models (Heath, 1976; Kwa & Liew, 1977).

3. Review of the Literature on the Immunology of T. crassiceps

Taenia crassiceps metacestodes offer many advantages in the study of cestode-host relations and the stimulation of immunity to helminth parasites. Owing to their ability to reproduce asexually by exogenous budding (Freeman, 1962), they can easily be maintained in laboratory mice and rats by regular intraperitoneal passage (Freeman, 1962; Chernin, 1975b; Blair & Campbell, 1976). Direct intraperitoneal inoculation of the larvae avoids the problem of assessment of the immune response to earlier oncospheral stages of the worm and their penetration of host tissues (Gemmell & MacNamara, 1972).

The earliest published studies on antigenic stimulation of murine hosts by larval T. crassiceps are those of Freeman (1964) who

investigated the development of leukocytosis and eosinophilia in the peritoneal fluid and blood of mice following primary infections with the metacestodes. Following these, several aspects of the humoral response of mice and rats to T. crassiceps metacestodes were reported. Chernin (1977a) found that in the mouse host the number of antibody-antigen precipitin lines formed was directly proportional to the duration of the infection and not to the amount of parasites present. He suggested that since they are not effective against these established parasites, the antibodies might have some other functions or that the parasite can hinder the functioning of the antibodies. Using the rat as a host, Chernin (1977b) established the existence of antigens that were common between the host and the parasite, and he postulated that in established infections the parasite might avoid the host's immune system by "disguise" with host antigen(s) - a situation generally recognized to occur with schistosomes (Smithers & Terry, 1976).

From these studies, it was clear that more rigid applications of immunological techniques were required to elicit the exact components of host response as well as the evasive mechanism of the metacestodes. Siebert et al (1978a) reported that in a primary infection of mice with T. crassiceps metacestodes, there was a biphasic response in the host. During the first 4 weeks there was tegumental damage but no detectable cell adherence, while from 4 - 8 weeks the metacestodes became encapsulated with host tissue, resembling an allergic inflammatory response. This biphasic response in T. crassiceps was the first to be reported for larval cestode infections and strongly suggested distinct humoral and cell-mediated immunity responses. In the same study, it was found that there was

a reduction in the host-response associated with increased worm burdens, indicating possible depression of the host immune system first suggested by Good & Miller (1976) who showed that antibody responses to heterologous antigens were depressed in mice infected with T. crassiceps. The process of tegument damage, presumably by antibody, was investigated by electron-microscopy by Siebert et al (1978b). They reported that vacuolation of the tegument began from the first week, and within 2 - 3 weeks the tegument has lost structural integrity, with complete loss of tegument by the fourth week. The initial loss of the glycocalyx, which could be a site for antigenic expression (Lumsden, 1975a), indicated that antibody produced by the host could have attached onto the tegument surface or to vital enzymes in the tegument. The fact that these antibodies could bind complement was supported by the in vitro studies of Siebert & Good (1979) who cultivated metacestodes in various combinations of inactivated and non-inactivated immune and normal sera. They also found that there was no damage to the tegument when metacestodes were incubated in serum from donors with 6-week old infections, indicating the possibility of a temporal production of specific, functional immunoglobulin subclasses by the hosts. Although complement appeared to increase the severity of tegumental damage, neither the presence of heat-labile components of complement nor immune serum was found to be essential for mediation of such damage. As a result of these observations, Siebert & Good (1979) suggested that T. crassiceps metacestodes could produce surface substances capable of activating C3 as well as substances that could activate the alternate complement pathway which is known to occur in other systems (Hammerberg et al, 1976; Hammerberg & Williams, 1978 a,b;

Waksman, 1978). However, it would appear that the predominant mechanism of humoral damage to T. crassiceps larvae in vitro is likely to involve complement dependent specific antibody.

Siebert et al (1979) described the encapsulation process by the host against T. crassiceps metacestodes at the ultrastructural level and discussed its possible mechanism in what appeared to be a CMI response (Siebert et al, 1978a). They found that although the earliest cells to adhere to the metacestode tegument were eosinophils and macrophages there was no sign of tegumental damage to the encapsulated larvae. In fact, the parasites seemed to be secreting toxic substances which caused damage to the adhering host cell membrane. However, when mast cells became prevalent in the later stages of encapsulation, damage to the larval tegument became evident. The study suggested an interaction of eosinophils, mast cells, macrophages and lymphocytes which cooperate to bring about the death of the parasite. More direct evidence of CMI responses were given by Anderson & Griffin (1979 a,b) who, using the rat model, were able to transfer immunity by introducing lymph node cells from immune donors and, to a lesser degree, non-immune ones. In the same series of studies, they discovered that the onset of host resistance to infection with T. crassiceps was coincident with the acquisition of responsiveness to mitogens in the post-natal maturation of rats. These results indicated that functional and mature T-cells were necessary to ensure resistance in a CMI response. These observations led to the suggestion that susceptibility of neonates to infection would be due to immaturity of T-cells, thus providing strong evidence for CMI.

Although Siebert & Good (1979) found tegumental damage in

metacestodes incubated *in vitro* in normal and immune sera, Anderson & Griffin (1979a) failed to establish passive protection by transfer of immunity with immune serum from immune donors. No work has been carried out to investigate whether humoral immunity and CMI responses can act together although Siebert & Good (1979) did suggest that early humoral responses could be due to cell-mediation or cooperation by loosely adhering cells.

In this study, the host response to *T. crassiceps* infection following a primary stimulation of specific immunity was investigated briefly at an ultrastructural level.

B. MATERIALS AND METHODS

1. Source of Parasites

T. crassiceps metacestodes were maintained in laboratory mice by regular passage as described earlier under General Methods and in Section I.

2. Experimental Protocol

T. crassiceps metacestodes of the TOI strain were removed aseptically from heavily infected mice and placed in a beaker of cold normal saline. The metacestodes were allowed to settle and the saline was adjusted to give a total volume of 50 ml. The metacestodes were homogenized in a Waring Blender for 5 minutes. 0.5 ml homogenate was injected subcutaneously in the abdominal region of twelve 6-week old TF1 female mice. Control mice were injected with 0.5 ml normal saline. The mice were then inoculated 2 days later with 10 *T. crassiceps* cysticerci of the TOI strain. Each group of 3 animals were killed by cervical dislocation at 1,3,8 and 12 weeks post-infection. All the parasites in each mouse were collected by rinsing the

peritoneal cavity with warm HBSS. Following 2 washes in HBSS, eosin exclusion tests for cell death (Hanks & Wallace, 1958) were performed as a measure of worm viability (Clegg & Smithers, 1972). Worms which stained pink after exposure to 0.2% eosin for 30 min at room temperature were classified as damaged, and therefore non-viable. Worms that did not take up eosin or were not obviously encapsulated were considered to be viable. After examination, representative larvae were fixed and processed for scanning and transmission electron microscopy as described earlier in Section I.

C. RESULTS

This experiment must be regarded as only preliminary in nature and was designed merely to demonstrate that there is an immune response that could be stimulated by a primary subcutaneous inoculation with parasite homogenate material. Most of the parasites recovered from control mice were normal in appearance under light microscopic examination. The total numbers found in each control group of 3 mice after 1,3,8 and 12 weeks of challenge were 18 (78% viable), 104 (83% viable), 294 (95% viable) and 873 (98% viable) respectively. In experimental mice the numbers were 23 (11% viable), 57 (25% viable), 153 (80% viable) and 402 (90% viable). Of the non-viable larvae, those that were partially or fully encapsulated were found only in experimental animals 8 and 12 weeks post-challenge except for 2 found in a control mouse 12 weeks post-challenge.

It can be seen that in experimental mice there was a considerable level of protection in the first 3 weeks after challenge. However, after 8 and 12 weeks most of the larvae were viable, and the percentage of viable larvae approached that of controls. All viable larvae in

control and experimental mice appeared normal under SEM and TEM.

Most of the metacestodes that were recovered from experimental mice 1 week after challenge were obviously abnormal. From light microscope observations, the metacestodes were irregularly shaped with an abnormally 'patchy' appearance (Plate 171). Many small free buds were found. Several of the metacestodes were budding irregularly with buds developing over wide areas of the budder. No encapsulated larvae were found.

Three weeks after challenge, about 75% of the recovered metacestodes were observed to be grossly abnormal. SEM observations revealed highly irregular budding. The whole surface of the metacestodes consisted of buds of different sizes (Plate 172). The surface of these buds was much folded into deep troughs and ridges (Plates 173,174). Occasionally small aggregates of host cells were observed adhering onto the surface of the metacestodes (Plate 173). TEM observations revealed the much folded tegument thrown into deep invaginations (Plate 175). The distal cytoplasm appeared highly vacuolated, with numerous huge aggregates of vacuoles (Plates 175,176). The vacuoles were approximately 0.15 μm in diameter; many of them were coalesced with each other, forming even larger compound vacuoles. The extensive vacuolation resulted in cytoplasm loss (Plates 177,178). Disc-shaped bodies were still present in the remaining cytoplasm (Plate 178). The perinuclear cytoplasm was darkly stained and the nucleus and other cyton organelles had poor definition (Plate 176). Droplets of electron-lucid material, possibly lipids, were also present in the perinuclear cytoplasm and sub-tegumental parenchyma (Plate 175).

Eight weeks after challenge, about 20% of the recovered parasites were non-viable and had thick layers of host cells adhering on the

surface (Plate 179). The host-cells were closely packed together and had extensions of pseudopodia-like cytoplasmic process (pseudopods) (Plate 180). These processes were observed to be closely entangled with the host microtriches (Plate 181). It is difficult to assign definite identity to these host cells but from their size and surface process they were probably macrophages. The microthrix border was still intact under the host-cells (Plate 182). However, many of the microtriches were extremely long and some appeared fragmented. The predominant cell type along the microthrix border was the macrophage. These had extensive pseudopodia-like cytoplasmic processes that were closely interdigitating with the microtriches (Plates 183 - 185). In some capsules, the microthrix border of the parasite was severely depleted. Strands of fragmented microtriches were often found clumped together (Plate 186). An amorphous, flocculant material was found among the microtriches and host cells. This appeared to consist of immuno-precipitates, and were often found among fragmented microtriches (Plate 187). Normal eosinophils were not observed. Some degranulated ones were observed to have been surrounded by the cytoplasmic processes of macrophages (Plate 182). Many of the macrophages contained vacuoles, probably phagocytic in nature, within which were dense ingested material. Unidentified rods of dense material were also observed in the macrophages (Plate 182). A number of dead and autolysed macrophages were present, particularly in the regions next to the parasite. Lymphocytes were rarely found; these had large nuclei and small amounts of cytoplasm. Fibroblasts, too, were rarely encountered among the host cells. While most of the recovered parasites were not fully covered by host cells, there were a few which were almost entirely encapsulated. These forms were, however, more common among metacestodes recovered

12 weeks after challenge.

About 10% of the parasites from 12-week challenge infections were encapsulated by a thick host-capsule consisting of densely packed cells (Plates 188 - 191). Some of the parasites were only partially encapsulated (Plate 188) while others were almost fully covered. In several instances the encapsulation was incomplete only because of the presence of unencapsulated buds (Plate 190). In "advanced" capsules, the cells appeared to be held together with the help of fibrous material (Plate 191). TEM observations revealed that in capsules in which the larvae were still intact, the microthrix border was severly depleted with only short remnants on the distal cytoplasm. In most of the capsules, however, the parasite had obviously died. It was difficult to recognise the encapsulated larvae. Neither the tegument nor the subtegumental structures were easily identifiable and appeared as a fibrillar mass (Plate 192). Numerous macrophages were observed extending cytoplasmic processes or pseudopods into the dead parasite, presumably phagocytosing the remains of the dead parasite (Plates 192, 193). Macrophages contained unidentified rod-like dense structures and numerous phagocytic vacuoles which contained ingested material (Plates 192 - 194). Macrophages were often observed along the outer surface of the capsules closely associated through interdigitating cytoplasmic processes (Plate 195). Eosinophils were occasionally present but were usually found towards the peripheral regions of the capsule (Plate 197). Macrophages often extended processes to surround other host cells; possibly these might be degenerating cells (Plate 196). Mast cells were rarely seen, and lymphocytes, if present, were not easily recognized because of their resemblance to macrophages. Large amounts of amorphous, fibrous material was present among the host

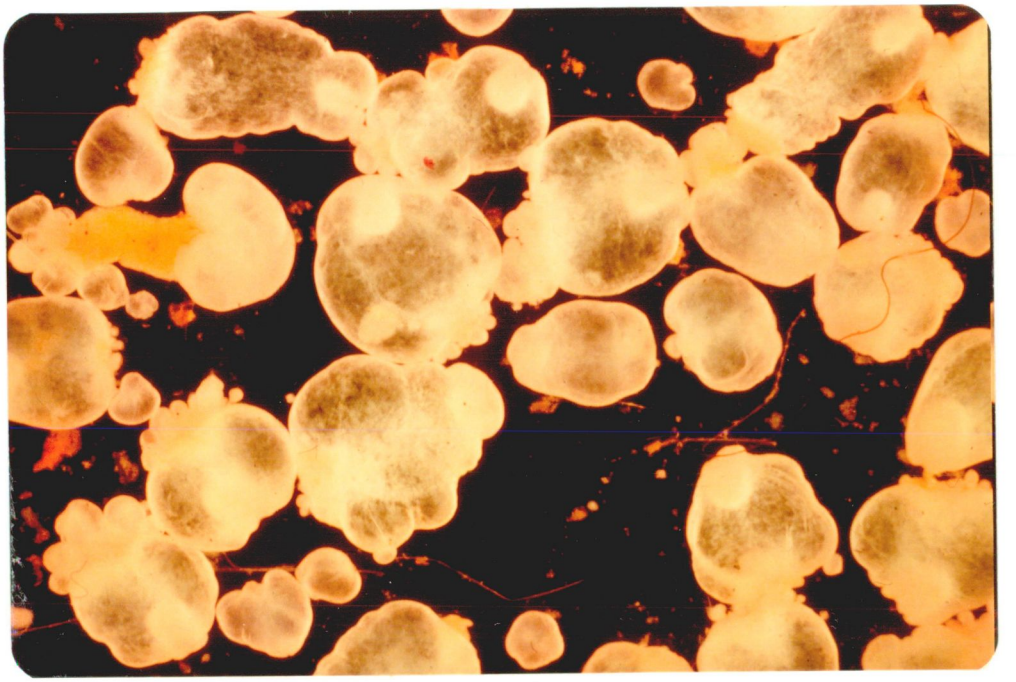


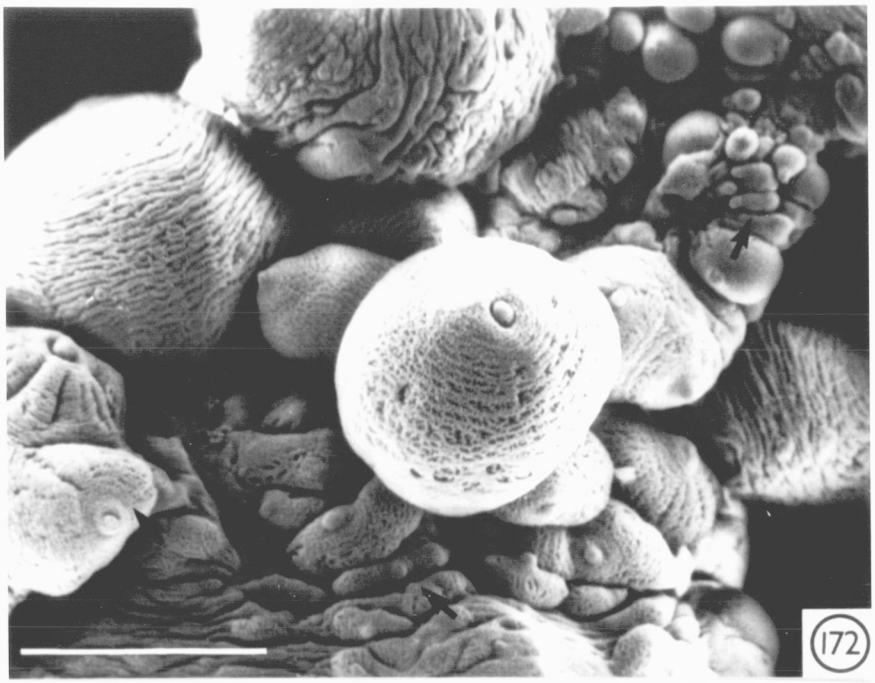
Plate 171. T. crassiceps metacestodes recovered 1 week after challenge infection following a primary inoculum of homogenate. (x 40)

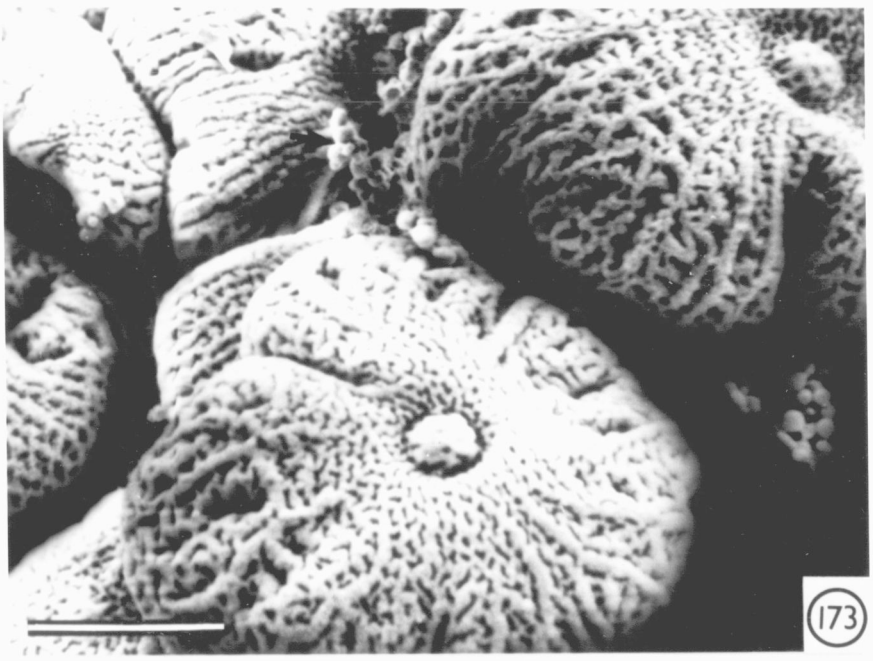
Plates 172 - 173. Scanning electron micrographs of T. crassiceps recovered from a mouse 3 weeks after infection following a primary inoculum of parasite homogenate.

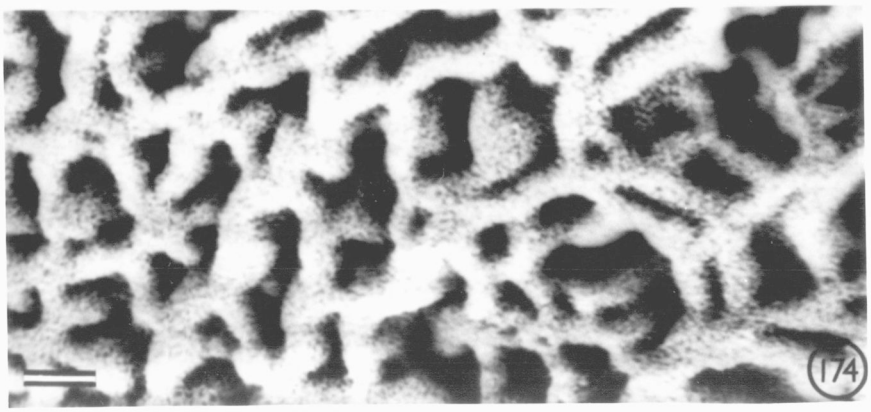
172. The surface of the metacestode is covered by buds of various sizes and shapes (arrows). (Scale = 500 μm)

173. The buds have an irregular, rough surface with the tegument much folded into troughs and ridges. A few aggregates of host cells may be observed between the buds (arrow). (Scale = 100 μm)

174. High magnification of the bud surface to reveal the deep irregular troughs. (Scale = 10 μm)


172


173


174

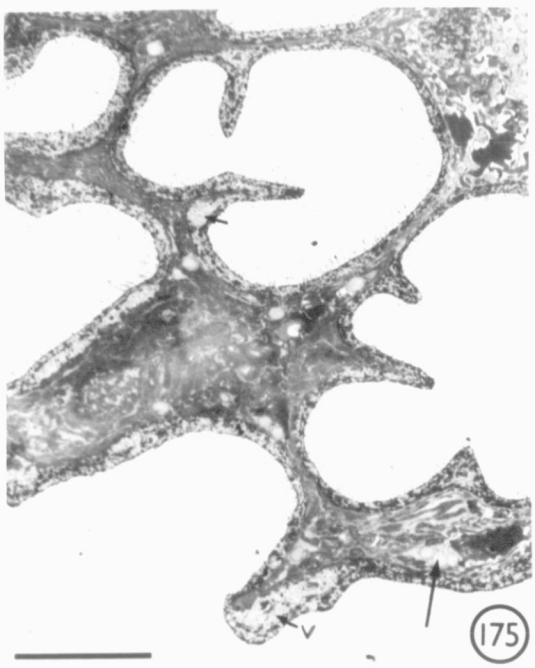
Plates 175 - 178. Transmission electron micrographs of T. crassiceps recovered from a mouse 3 weeks after primary inoculation with parasite homogenate.

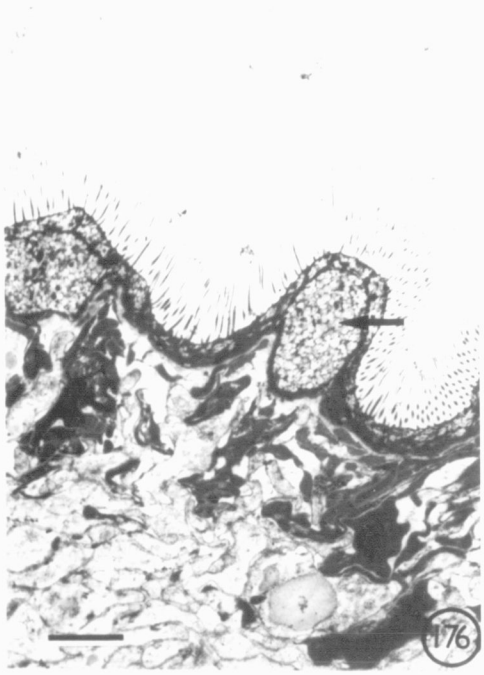
175. The tegument is highly irregular with large folds. The distal cytoplasm is severely vacuolated, with many of the small vacuoles grouped into aggregates (v). Note the large electron-lucid droplets (arrow) possibly containing lipids. (Scale = 10 μm)

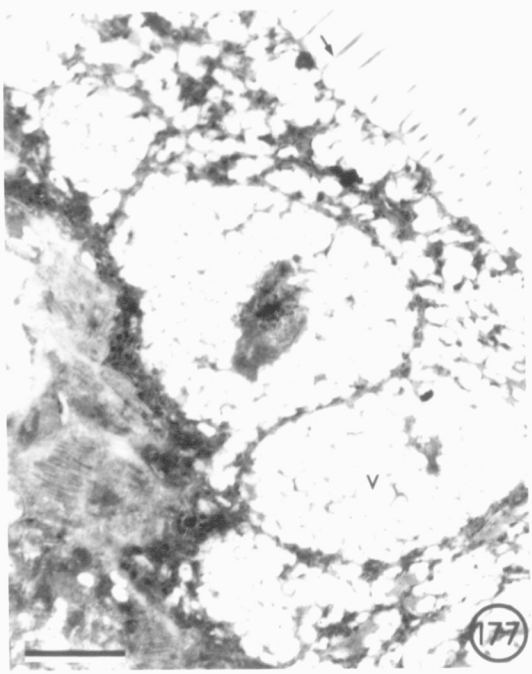
176. High magnification of the tegument to show the aggregates of vacuoles in the distal cytoplasm (arrow). Note the lack of cytoplasmic and nuclear definition in the perinuclear cytoplasm. (Scale = 2 μm)

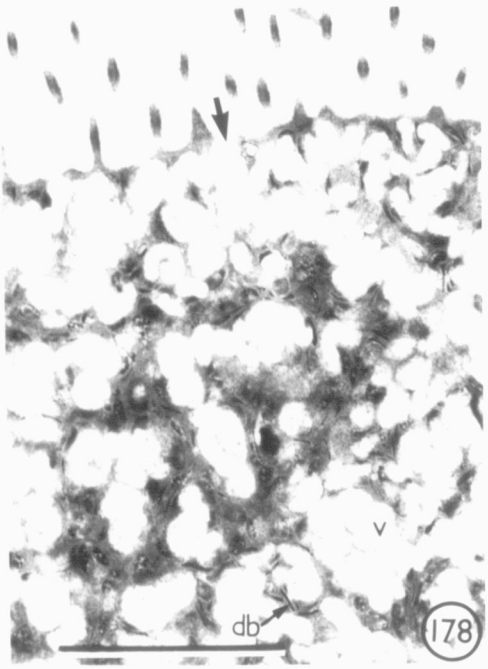
177. A higher magnification of the tegument. Note the thin filamentous microtriches (arrow) and the abundance of vacuoles (v). (Scale = 1 μm)

178. The distal cytoplasm is so packed with vacuoles (v) that little cytoplasm is left. Within the remaining cytoplasm the disc-shaped bodies are still present (db). Note the vacuolated surface (arrow). (Scale = 1 μm)


v
175


176


v
177


v
db
178

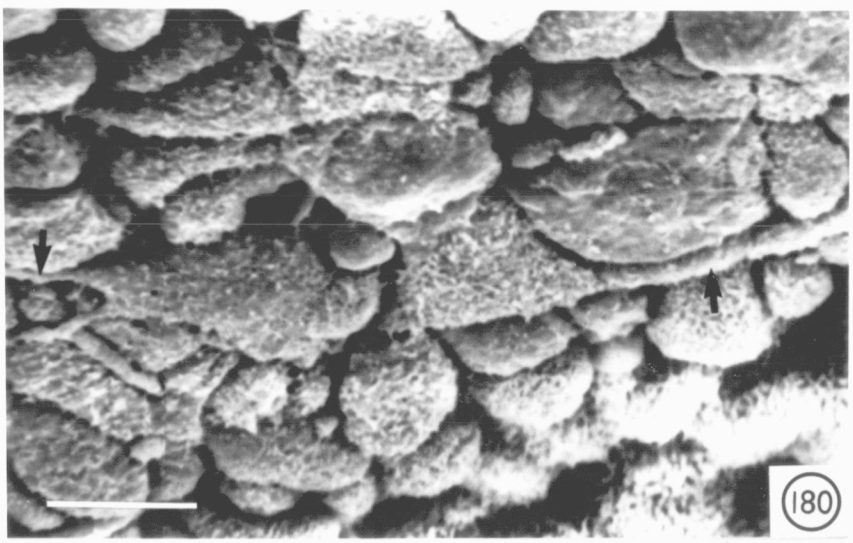
Plates 179 - 181. Scanning electron micrographs of *T. crassiceps* recovered from a mouse 8 weeks after a primary inoculum of parasite homogenate.

179. Large aggregates of host cells (hc) adhering to the surface of the parasite. (Scale = 50 μm)

180. A high magnification of the host cells found on the surface of the parasite. Note the pseudopodia-like cytoplasmic extensions of the macrophages (arrows). (Scale = 10 μm)

181. A high magnification to reveal the close interdigitation (arrow) between the cytoplasmic extensions of host cell (hc) and the microtriches (mi) of the parasite. (Scale = 5 μm)


hc
179


180


hc
mi
181

Plates 182 - 187. Transmission electron micrographs of T. crassiceps recovered from a mouse 8 weeks after a primary inoculum with parasite homogenate.

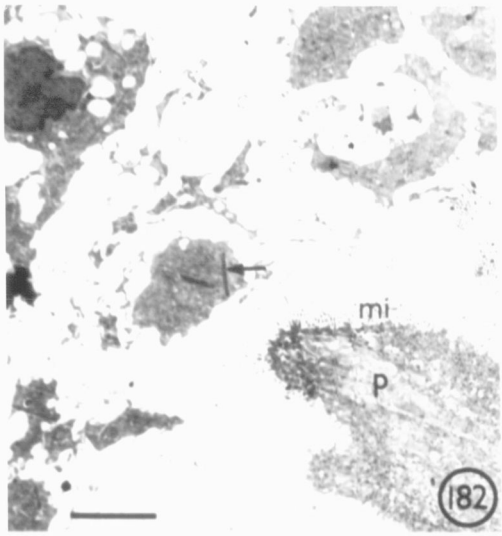
182. This shows the adherance of host cells to the microtriches (mi) of the parasite (p). In one of the host cells, probably a macrophage, there are dense rods (arrow). The macrophages in the top right corner appear to be involved in phagocytosing a degenerate eosinophil. (Scale = 3 μm)

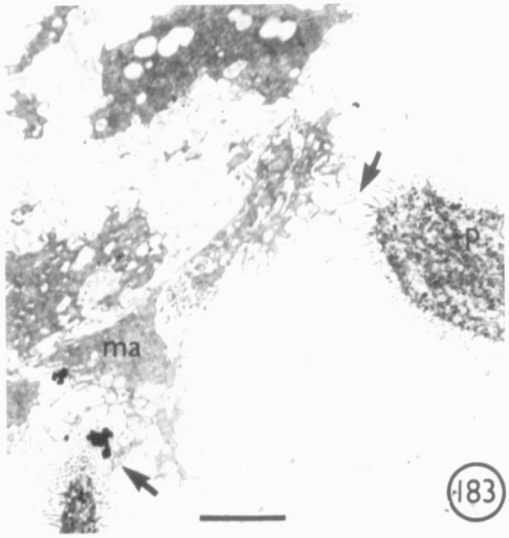
183. This shows a group of macrophages (ma) with extensive pseudopodia-like processes (pseudopods) in close contact with the microtriches of the parasite (p) (arrows). (Scale = 3 μm)

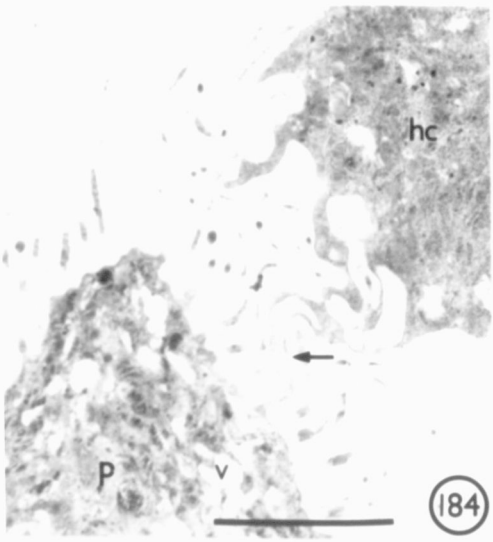
184. Closely interdigitating microtriches and pseudopods (arrow). (Scale = 1 μm)

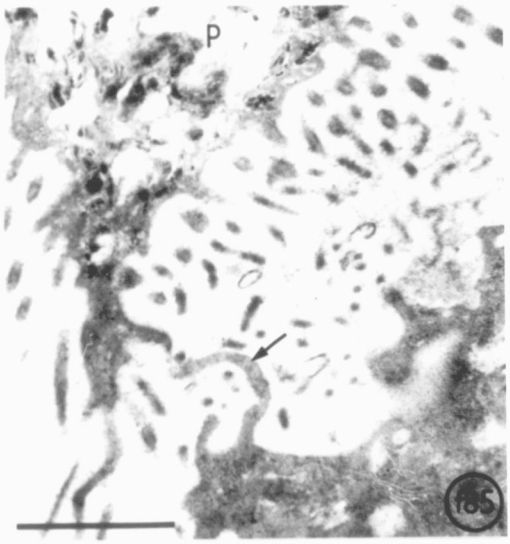
185. Another view to show the pseudopods of the macrophage (arrow) in close association with the parasite (p)'s microtriches. (Scale = 1 μm)

186. In some parasites, encapsulation is more extensive, with an accumulation of amorphous precipitated (probably an immuno-complex) material to form a "ground substance". Note the degenerating microtriches which are often clumped together (arrow). (Scale = 5 μm)


mi
P
182


P
ma
183


hc
P
v
184


P
185


P
186


187

187. Destruction of microtriches appear to be associated with a flocculent material probably an immuno-complex precipitate (arrow). (Scale = 1 μm)

Plates 188 - 191. Scanning electron micrographs of encapsulated T. crassiceps recovered from a mouse 12 weeks after a primary inoculation with parasite homogenate.

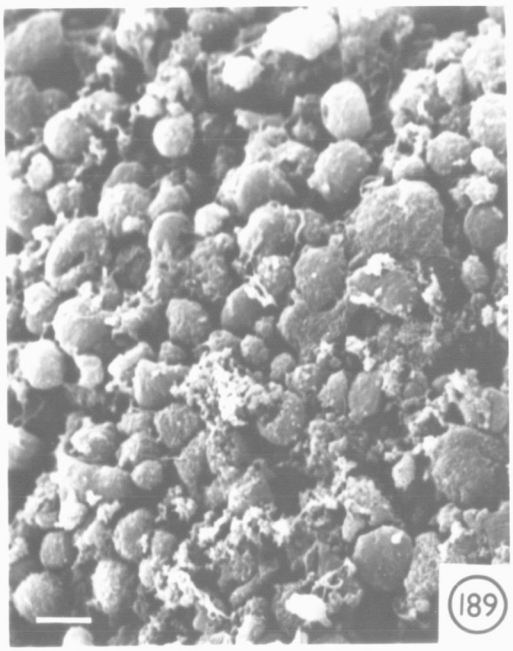
188. A thick layer of host-cells (hc) covering most of the parasite (p). (Scale = 100 μm)

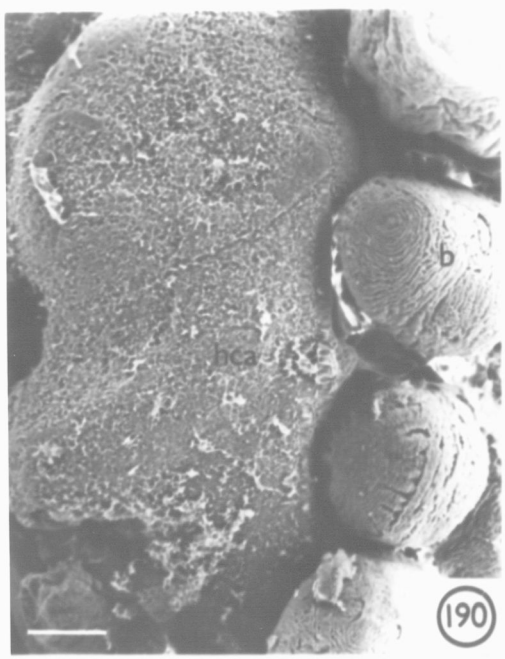
189. A higher magnification to show packed host cells making up the host capsule. (Scale = 10 μm)

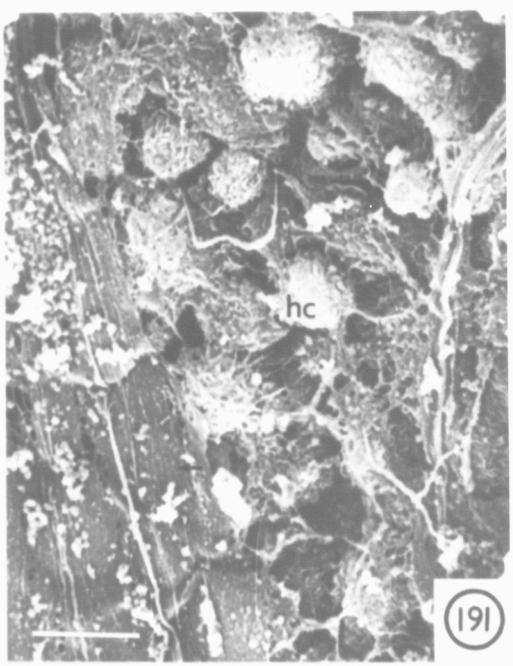
190. The host capsule (hca) encloses most of the parasite, but a number of buds (b) appear to be free of host cells. (Scale = 200 μm)

191. At higher magnification, a fully formed host capsule consists of densely packed host-cells (hc) and fibrous material (arrow). (Scale = 10 μm)


hc
P
188


189


b
hca
190


hc
191

Plates 192 - 197. Transmission electron micrographs of encapsulated T. crassiceps recovered from a mouse 12 weeks after a primary inoculation with parasite homogenate.

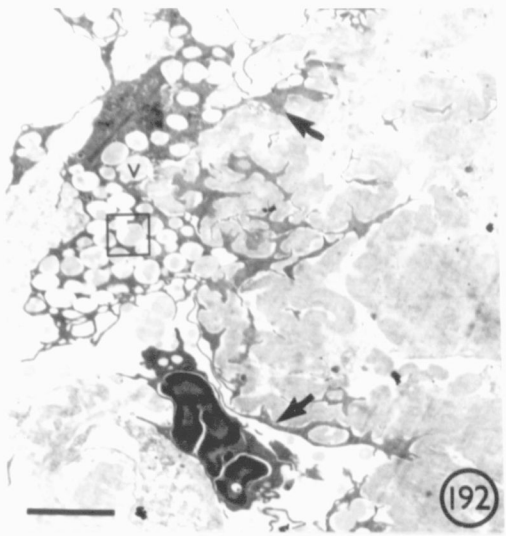
192. The outline of the dead parasite (towards the right) may just be distinguished. Cytoplasmic processes (arrow) from the macrophage penetrate among the dead parasite tissue and phagocytic vacuoles (v) contain ingested material (square). (Scale = 3 μm)

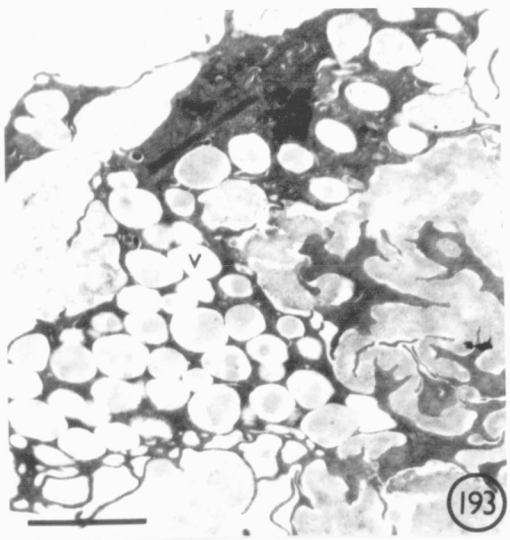
193. A higher magnification of a macrophage with numerous phagocytic vacuoles (v) containing ingested material. (Scale = 2 μm)

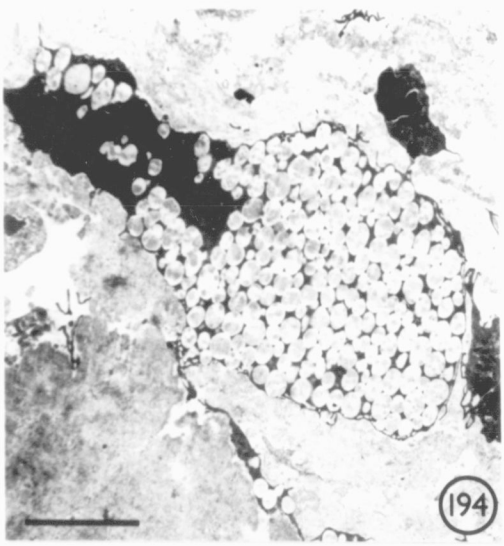
194. A macrophage with an abundance of phagocytic vacuoles. (Scale = 5 μm)

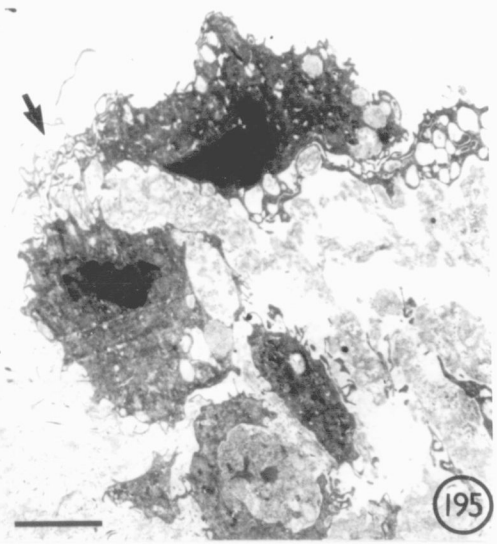
195. Some host cells, probably macrophages, on the external surface of the host capsule. These cells appear to phagocytose dead cells (bottom centre). The macrophages also bind to each other by their pseudopodal extentions (arrow). (Scale = 3 μm)

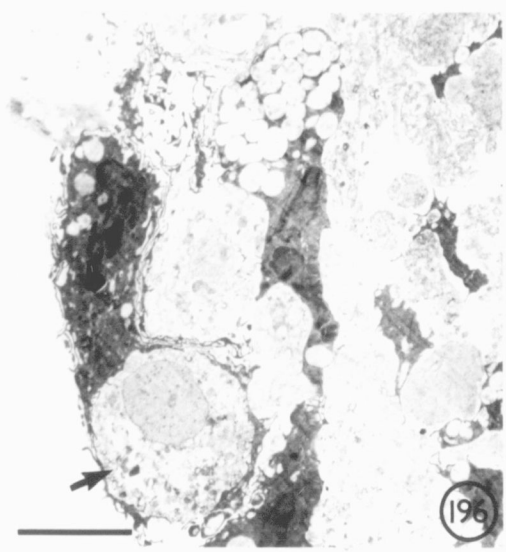
196. Host cells along the outer surface of the host capsule. Cytoplasmic processes are extended around an unidentified degranulated cell (arrow). (Scale = 5 μm)

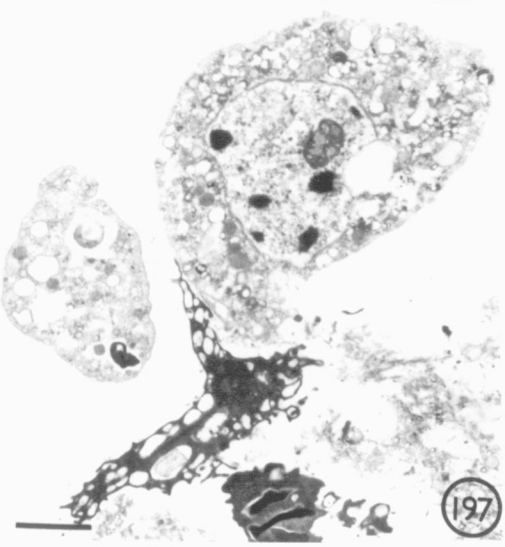

V
192


V
193


194


195


196


197

197. Some unidentified cells, possibly eosinophils, on the surface of the host capsule. (Scale = 2 μm)

cells; these might possibly be collagenous in nature.

D. DISCUSSION

The irregular folding of the tegument of T. crassiceps metacestodes exposed to an early immune response has not been reported before in studies on the same species by Siebert et al, (1978 a,b) and Siebert & Good (1979). However, these workers described a process of vacuolation and microtriches depletion associated with an immune attack which was also observed in the present study. Disruption of the tegument would impair its permeability and absorptive-digestive function resulting in parasite death. Hustead & Williams (1977) reported immunologically mediated effects on the permeability of the tegument to macromolecules in T. crassiceps and T. taeniaeformis. They demonstrated that this disruption of permeability is due to antibody acting in conjunction with complement. Murrell (1971) found that the permeability to sugar and amino-acids in T. taeniaeformis was greatly enhanced in the presence of immune serum.

Encapsulation by host cells was observed on metacestodes that were recovered from mice after 8 weeks challenge. This suggests that the response of mice to infection consists of 2 components. The first component occurs primarily before the first 8 weeks after infection and appears to be humoral mediated, since none of the worms that were examined had obvious host cells adhering on the worm surface. The second component of the response occurred after the 8th week, and appears to be cell-mediated, as suggested by the encapsulation of larvae with host cells. Siebert et al (1978 a) reported a very similar biphasic response using a challenge intraperitoneal dose of 3 metacestodes 3 weeks after a primary subcutaneous inoculum of 3 whole

metacestodes. They further reported that all metacestodes were encapsulated after 8 weeks, but when the challenge dose was increased to 7 worms only 38% became encapsulated and very little encapsulation occurred when 10 or 20 worms were used. In the present study host-capsules were observed only on 20% and 10% of metacestodes recovered 8 and 12 weeks after challenge respectively. This suggests that when more worms are present, the immune response is diminished, and may be related to the ability of the metacestode to depress the host system as suggested by Good & Miller (1976). In infections of E. granulosus and E. multilocularis such an immuno-suppressive mechanism have been shown to be related to apparent anticomplementary activity of the calcareous corpuscles (Kassis & Tanner, 1976).

The present study indicates that metacestodes of T. crassiceps are killed by a combination of humoral and cell-mediated immunity responses. It is likely that digestive-absorptive enzymes and glycoproteins might provide the primary stimulation of host responses to T. crassiceps. In several helminths for which damage due to in vivo immune lysis has been described, the earliest manifestation of the immune attack concerned the digestive-absorptive surfaces (Ogilvie & Hockley, 1968; Hockley & Smithers, 1970; Harness et al, 1973). Ogilvie (1970) stated that enzymes are immunogenic and that host antibodies might inhibit enzyme function. Lumsden (1975 a) suggested that the glycocalyx of platyhelminths is concerned with antigenic expression. Trimble & Lumsden (1975) described the rapid rate of turnover of carbohydrate associated with the glycocalyx of T. crassiceps. Siebert et al (1978 b) provided some evidence that a significant portion of glycocalyx with its acid mucopolysaccharide constituents is lost during a host immune attack. In order to understand the mechanism of

immunity to cestodes, the inter-relation of several factors, e.g. host-immune factors, the cestode glycocalyx, and digestive, enzymes must be determined. Immune factors directed against the glycocalyx would be less effective since the glycocalyx is shed rapidly and complement-binding antibodies directed against the glycocalyx would be shed also, preventing lysis of the tegument membrane. It should be noted that in the trematode F. hepatica there is evidence that glycocalyx turnover serves as a mechanism for protection against host immunity (Hanna, 1980). Antibodies directed against enzymes on the parasite surface (Lumsden, 1975 b) may result in direct lysis of the tegument membrane and/or inactivation of the enzymes could disrupt nutrient absorption and other physiological functions.

Siebert et al (1979) described distinct cellular cooperation among eosinophils, macrophages, mast cells and lymphocytes in a cellular response to T. crassiceps. They noted that in the early stages of encapsulation, eosinophils and macrophages were the primary cell types. Both cell types appeared to be ineffective in causing tegumental damage to the parasite and both cell types showed severe degenerative changes and disruption of cell membranes. Later, when mast cells became common, all of the cell types were normal and damage to the tegument became apparent. Ultimately, interaction of all cell types resulted in death of the encapsulated larvae. In the present study, macrophages were the predominant cell type that was identified. However, there were many unidentified degenerated cells in the capsule, some of which were being phagocytosed by macrophages. These dead cells may be the result of lytic or toxic substances secreted by the parasite as a defensive mechanism to avoid destruction by host cells. A similar reaction has also been suggested for

T. crassiceps by Siebert et al (1979) and other helminths (Ubelaker et al, 1970; Bogitsch & Wikel, 1974). In E. multilocularis infections, the protoscolicidal effector cell has been identified as the adherent monocyte-macrophage cell (Baron & Tanner, 1977). It is believed that the initial adherence of immune cells to the target depends on cytophilic antibody. Apparently, adherence itself does not cause injury but serves to establish the close cellular contact necessary for the subsequent cell destruction (Temple et al, 1973). It is possible that the destruction of T. crassiceps metacestodes in immune hosts could provide a similar phenomenon. On the other hand it is also conceivable that a concurrent interaction between the humoral and cellular arms of immunity could be in operation. One class of immunoglobulins may be involved in early humoral attack on the parasite while another acts in conjunction with activated cells. Such an antibody may play an important role in the encapsulation and phagocytic activities of activated cells by identifying targets on the metacestode tegument to the cells. Baron & Tanner (1977) suggested that antibody is necessary for the attraction of leukocytes capable of the destruction of Echinococcus spp. A similar result has been reported in the in vitro action of peritoneal macrophages on the larvae of Nematospiroides dubius (Ninnemann & Lueker, 1974). Cooperation and interaction between antibody and macrophages have also been demonstrated for S. mansoni (Perez & Terry, 1973; Perez & Smithers, 1977).

The biphasic response that was observed in this study does not necessarily mean that there is a temporal separation between humoral and cell-mediated activity. Both could have been operating at the same time from the onset of the challenge infection, but the parasite had been able to overcome the initial cellular attack by secreting

lytic or toxic substances as described earlier. It is possible that cellular attack had become effective, manifested by encapsulation, consequent to some degree of damage incurred by the parasite as a result of humoral attack.

It can be observed from the present study that in a population of T. crassiceps metacestodes there were some which were able to evade or survive an initial immune response after which they became quite resistant to further attack. A number of reasons could be possible: (1) the size of the challenge infection could have diluted or exhausted the level of immune attack and surviving metacestodes were able to reproduce quickly enough, and these surviving larvae were able to, in some way, depress the host's immune system; (2) larval populations may be heterogenous with respect to susceptibility to humoral damage by immune serum. Such larvae may possess surface antigens qualitatively or quantitatively different from susceptible larvae, and consequently elicit production of antibodies of a different class or activity and which are relatively less harmful to the parasite; (3) related to the second theory, susceptible larvae may produce buds which are antigenically different from the parent budder, the consequences of which have been described earlier. This third possibility may explain the anomalous situation described earlier in which encapsulated larvae had attached buds that were apparently free of any host cells. It is not possible to determine in such a preliminary investigation what exact mechanism(s) are involved in the evasion of host immunity response. It is likely that more than one may be operating concurrently or consecutively.

This study reveals that there is a considerable level of immunity that can be stimulated by a primary subcutaneous inoculation of

T. crassiceps homogenate as antigen. The ease with which T. crassiceps may be maintained in laboratories makes it a suitable model for more studies on the immunology of cestode infections. One area of research that appears attractive is the investigation of the nature of the biphasic response by the 2 components, i.e. humoral and cell-mediated. Although they appear to be discretely separated by time, there is every likelihood that a synergistic response may be in operation, especially during the latter phase. The large numbers of parasites in this model system will make it easier to separate antigenic components that are functional and to characterize them. Another area of research is to determine if the immunity that can be stimulated is a specific response, since heterologous responses can be obtained for related, or even non-related, species (Baron & Tanner, 1977; Hustead & Williams, 1977). Mechanisms by which T. crassiceps larvae suppress or evade the host's immune attack need to be examined, and such studies could prove valuable in relation to the control of some cestode diseases.

E. SUMMARY

Following a primary subcutaneous inoculation of T. crassiceps antigen, in the form of a parasite homogenate, a considerable level of immunity against a secondary challenge of metacestodes was observed. This immune response consisted of 2 temporal phases. Early damage to metacestodes included abnormalities in the structure and, presumably, function of the tegument, and was probably humoral mediated. After 8 weeks of challenge a second cell-mediated phase resulted in a gradual encapsulation of dead larvae by host-cells which consisted predominantly of macrophages. While some metacestodes were susceptible to the host's immune attack, there were others that were able to successfully overcome it, and these went on to reproduce normally.

GENERAL DISCUSSION

Ultrastructural studies on parasites have revealed much additional detailed information on the morphology of cells, tissues and organ systems not shown by light microscopy. Such information has provided a comprehensive picture of function, physiology and developmental biology, and has deepened our understanding of host-parasite relationships. The ultrastructural studies recounted in this thesis reveals that Taenia crassiceps, like all other helminths that have been studied, is remarkably adapted to its mode of life.

The infective hexacanth embryo, or oncosphere, is protected by a thick embryophore made of keratin-like protein blocks, probably held together by a 'cement' substance(s) which dissolves under suitable stimulation, a process generally termed 'hatching'. After liberation from the embryophore the oncosphere needs to be activated by fairly specific enzyme-bile treatments under certain conditions. In this study, hatching was achieved with relative ease but attempts at activating the oncospheres were largely unsuccessful. Both hatching and activation are believed to require specific conditions. In Hymenolepis, the hatching responses of 4 species were found to be different with respect to optimal conditions of pH, CO_2 concentration, amount of sodium bicarbonate and enzymes (Berntzen & Voge, 1965). The role of specific bile salts in the activation of oncospheres may be important in determining intermediate host specificity (Smyth, 1969 a). The oncospheres of T. crassiceps have structures that are common in many other species (Nieland, 1968; Lethbridge, 1980); the hooks, well innervated with complex muscle arrangements, and the extensive penetration glands are generally believed to be used to penetrate the host intestine. From the intestine these larvae migrate to numerous

sites in the host where they become established and begin to reproduce asexually.

The ability of T. crassiceps metacestodes to reproduce by exogenous budding makes it possible to maintain them by serial intraperitoneal passage into convenient laboratory mice. In the peritoneal cavity, the metacestodes propagate vegetatively at a prolific rate, until slowed down by the constraints of space or nutrition. The host shows a remarkable tolerance to the immense parasite burden. There seems to be a balance between host and parasite; while the parasites are able to avoid being harmed by the host's immune system, they also provoke little pathogenic effect on the host as a whole or in the organs, other than a chronic inflammation in the peritoneum.

This ability of the parasite to evade the host's immune system is evident even when the mouse has been primed to induce an enhanced immune status by prior inoculation of antigen. The host response to challenge infection appears to be biphasic, with initial damage to parasite tegument, probably by the humoral mediated arm, followed by a cell-mediated phase which results in cellular adhesion and subsequent encapsulation of the larvae. Ultrastructural examination reveals vesiculation/vacuolation of the tegument and its consequent dysfunction as the principle cause of death during the initial phase. The encapsulation process probably involves many types of cells but macrophages are predominant within the capsule. The exact mechanism by which this cell-mediated response kills the parasites is not known but it is likely to include cell assisted antigen-antibody recognition and phagocytosis. Although an enhanced immunological response can be stimulated against the challenge infection, a significant proportion

of the metacestodes survive and reproduce. Interaction among the host's immunologic factors, the parasite tegument and it's excretory-secretory products largely determine the immunological relationship. The glycocalyx surface on the tegument has been the subject of much recent research with regard to its role in helminth immunity in view of the fact that it forms the immediate interface with the host (Befus, 1977; Hanna, 1980; Hanna & Jura, 1977; Lumsden, 1975 a,b; Smithers et al, 1969).

Other than the role it plays in immunological relationships with the host, the tegument has to serve all other functions involving exchange of material between host and parasite. In T. crassiceps, the tegument, and its associated structures, resembles that described in most other platyhelminths in its basic structure. It is undoubtedly a living cellular structure consisting of a syncytial outer layer (distal cytoplasm) formed from cytoplasmic extensions from an inner nucleated layer (perinuclear cytoplasm). The tegument functions as a digestive, absorptive, secretory and excretory epithelium, as well as a protective barrier. The tegument is also involved with nervous reception since it is innervated by extensions from the intricate network of nerve cords with special sensory receptors terminating at the distal cytoplasm. The area of its interface with host tissue and fluids is increased by the extensive microtriches throughout the parasite.

The scolex of the tapeworm need not function merely as an attachment organ; it is virtually in continuous intimate contact with host tissue, and may also be especially adapted as an organ for digestion/nutrition. This has been suggested for E. granulosus which may, in some way, be capable of directly absorbing materials from the

mucosal surface (Smyth, 1967; Smyth et al, 1966,1967). Although the exact mechanism is not yet known, the presence of glands in the scolex of many cestodes species may be an indication of release of digestive enzymes at the point of attachment although this has never been unequivocally demonstrated. In T. crassiceps, there are numerous secretory gland cells beneath and above the rostellar pad, as well as at the tip of the everted rostellum. These cells contain large deposits of secretory granules, probably mucopolysaccharides or mucoproteins, which are also present in the hook organ. These findings suggest that the scolex and rostellum may be involved in secretory-digestive activities. Further, rostellar protrusions containing similar secretory granules, are found in evaginated cysticerci during in vitro culture; this suggests that some physical and/or physico-chemical factors trigger these protrusions when the parasite undergoes larval-adult transition, during which the previously invaginated scolex becomes functional.

The vertebrate gut is the habitat for the adult tapeworm to arrive at its most important developmental stage with the ultimate biological purpose of producing eggs to disseminate its kind. The success of the tapeworm must be due significantly to their ability to produce large numbers of eggs and/or to produce them rapidly. The massive nutritional requirements for organogenesis, gametogenesis and embryogenesis are met largely by using the tegument of the strobila as a large digestive-absorptive surface. In T. crassiceps, this function is facilitated by adaptations in the microtriches which develop longer basal portions which are better suited for absorption. There is an increase in mitochondria to cope with the energy requirements. Special sub-tegumental cells, not observed in cysticerci, are found laden with

secretory material, presumably for digestion. Vacuoles become more numerous in the distal cytoplasm suggesting some sort of active process in transferring materials across the tegumental surface. A cestode which makes the maximum use of all avenues for obtaining nutrition can thus be envisaged as lying in the gut cavity with its strobila bathed in semi-digested, semi-solid food, from which it is absorbing food materials by diffusion, active transport and possibly pinocytosis (Smyth, 1969 a). It is only recently that convincing evidence for pinocytosis in cestodes has been demonstrated (Hopkins et al, 1978; Threadgold & Hopkins, 1981). Furthermore, the nutritive materials available from the intestine may be supplemented (a) by carbon and oxygen atoms obtained from CO_2 fixation, (b) by materials released at the host-parasite interface, probably under the action of digestive enzymes secreted by the scolex and rostellum, and (c) by soluble constituents released or diffusing from the mucosal cells (Smyth, 1969a).

The form of the egg in each cestode class is closely correlated to the structure of the female reproductive system. The morphology of this system in T. crassiceps is basically similar to other cyclophyllideans. Spermatogenesis and sperm ultrastructure conform to the general pattern established for other cestodes (Rosario, 1964; Morseth, 1969; Featherston, 1971). The physiology of the insemination process is not known; which is not surprising considering the extreme difficulty of studying this process. Both self-impregnation within the same proglottid and between proglottids on the same worm, or impregnation of a proglottid of a different strobila, are believed to occur. The importance of precise physiological conditions for normal spermatogenesis, oogenesis and embryogenesis is emphasized by the fact that in an in vitro system, even an adult T. crassiceps,

presumably with advanced reproductive structures, could only be cultured to produce few normal eggs. Ultrastructural examination reveals most of the sperms to be abnormal; only normal sperms were found in the seminal receptacle. In the uterus most of the eggs were abnormal. This suggests that while the physical attributes of the system are sufficient for impregnation some physiological deficiencies resulted in either abnormal sperms, ova or eggs.

The usefulness of ultrastructural techniques to assess cultivation methods has been established for trematodes by Kannangara (1973) and Davies (1976, 1978). Using these techniques, a fuller picture can be obtained of the effects of *in vitro* culture at a cellular level. In the cultivation of *T. crassiceps* cysticerci, the main defects of cultured worms were concentrated in the strobila regions while the scolex remained relatively normal. Vesiculation of the tegument, hypotrophy of microtriches, breakdown of the fibrous zone, and accumulation of waste products were detected. The inefficient removal of waste-products from the worm is probably the accumulative result of the culture system, and, consequently, the dysfunction of the tegument as an excretory/osmoregulatory surface. The basic problems involved in the *in vitro* cultivation of helminths have been discussed by Smyth (1969a, 1972) and Taylor & Baker (1968). During the present study, one of the problems has been the inconsistencies of results. Variations between different cultures where the same treatment was used (inter-culture variation) and between duplicate treatments within the same culture (intra-culture variation) were occasionally striking - a problem well-recognized with *in vitro* systems. Such variations are generally accepted in culture work due to the extremely complex requirements of the worms, different microniches within each culture

vessel, different factors in serum, and the intrinsic nature of different worms. The extremely complex and often specific requirements for *in vitro* culture of trematodes were clearly shown by Kannangara & Smyth (1974).

The possibility that the KBS strain larvae used for *in vitro* culture might have become abnormal from continuous passage (Freeman, 1962), and thus unable to develop into adults, was discounted when dogs were successfully infected after being fed with the cysticerci. There is as yet no known *in vitro* system that can sustain a developing *Taenia* sp. from the larval stage to a fully mature adult with fertile eggs. The secret may easily be no more than one missing factor, as was discovered for *H. microstoma* which required hemin as a vital growth factor (Seidel, 1971).

The lack of adult worms imposed severe difficulties throughout this study. The high cost of obtaining and maintaining dogs for experimental infection meant that only 4 dogs were available. These dogs were fed with 20 cysticerci each, an arbitrary amount, since it is not known if a larger dosage might have resulted in stunted or abnormal worms due to 'crowding effects' (Read & Simmons, 1963; Roberts, 1966). From the resulting infection, the small numbers of adult worms had to be used for *in vitro* culture, light histology, SEM and TEM studies. This resulted in very little material available, especially for TEM studies. No previous study had been reported on the detailed growth patterns of the adult *T. crassiceps*, and proglottids had to be chosen arbitrarily for ultrastructural studies. Consequently, there were 'gaps' in the attempt to trace organogenesis, especially oogenesis. Another difficulty encountered with regard to the dog infections was that they had to be kept at Wiches Farm (London School

of Hygiene and Tropical Medicine) in St. Albans where suitable facilities were available. The distance made it impractical to monitor proglottid and egg shedding daily. Due to the rapid rejection of worms from the first two dogs, the third was examined on the 29th day, again arbitrarily chosen, in the hope that any worms remaining would have fully matured and viable eggs in quantity. The difficulty of obtaining viable eggs from such limited material was compounded by the possibility that egg maturation may be independent of chronologic order of the proglottids (Silverman, 1954). Eggs obtained from the 29-day old adults failed to infect mice, suggesting that most of the eggs might have been non-viable. It could be for the same reason that activation of oncospheres in vitro was also unsuccessful. Huffman & Jones (1962) reported that in T. taeniaeformis there was variability in hatching of eggs from different proglottids of the same worm. The small quantity of adult T. crassiceps and the consequent lack of eggs would have resulted in a low probability of obtaining sufficient amounts of viable eggs.

There are many avenues open for future research on T. crassiceps. The availability of large quantities of metacestodes and the rapid development to adults make it ideal for in vitro cultivation studies, as well as studies on the developmental biology of tapeworms, especially with the use of ultrastructural techniques. Further ultrastructural studies on the rostellum should reveal more of the special secretory activities of rostellar glands and the hook organ. Since metacestodes at varying stages of development are available, from buds to fully formed cysticerci, they are ideally suited for the study of scolex morphogenesis, especially rostellar and hook formation. The processes of asexual budding and 'strain' formation can be studied at a cellular

level for information on regeneration, somatic-germinative cell biology, and embryogenesis. From an immunological point of view, knowledge of parasite evasion mechanisms and immunodepression, and development of effective vaccines against larval cestode diseases, may be gained from studies on Taenia crassiceps.

SUMMARY

1. The structure of the egg of Taenia crassiceps follows the typical taeniid pattern. There are 3 basic embryonic envelopes surrounding the oncosphere, viz. the capsule, the outer envelope, and the inner envelope which consists of a thick embryophore made of keratin-like protein blocks and an inner cytoplasmic region.

2. The hexacanth embryo, or oncosphere, is enclosed within a fairly thick oncospheral membrane. The oncosphere has typical hexacanth hooks, penetration gland cells, and well-developed musculature; presumably these assist the oncosphere in penetration and migration through host tissues.

3. The tegument is basically similar in structure to that of other cestodes, consisting of a microthrix border, distal cytoplasm, and the nucleated perinuclear cytoplasm. These 3 components make up a syncytium with cytoplasmic connections between the distal and perinuclear cytoplasm. Cytoplasmic inclusions such as disc-shaped bodies, mitochondria, ribosomal granules, and golgi complexes are found in the tegument. A fibrous zone, possibly collagenous, lies between the distal and perinuclear cytoplasm throughout the worm.

4. Nerve processes terminate at the distal cytoplasm with a sensory 'cilium' projecting beyond the surface among the microtriches. These sensory structures are found throughout the larval and adult tegument but are more common along the rostellar rim.

5. Circular, longitudinal, and sometimes transverse, muscles are found between the distal and perinuclear cytoplasm. Among the cell bodies of the perinuclear cytoplasm are glycogen-rich parenchymal cells and cytoplasmic extensions with secretory granules.

6. The sucker region consists largely of muscles rich in mitochondria; secretory granules are abundant among the muscles. Special desmosomes attach the muscles to the fibrous zone, and the whole muscular sucker to the distal cytoplasm.

7. Special secretory cells containing large disc-shaped bodies/granules are found in the rostellar region of the scolex. Similar inclusions are present in the 'hook organ' and the rostellar protrusions that are observed in *in vitro* cultured worms. From their staining reactions, these granules are probably acid mucopolysaccharides.

8. Histochemical techniques demonstrated the following : the keratin-like nature of the rostellar hooks and embryophoral blocks (performic acid - Schiff); the calcareous corpuscles (silver method of von Kossa); the nervous system (Acetylthiocholine iodide/Butyrylthiocholine iodide).

9. Spermatogenesis in adult *T. crassiceps* is basically similar to that of other cestodes. Sperms consist of an outer microtubular sheath arranged in a helical pattern around a central axial filament complex which has a 9 + 1 arrangement of peripheral

elements. The nucleus is coiled in a spiral around the axial filament along part of the sperm.

10. Cysticerci were cultured to the stage of genital primordia formation. No sperms or ova were obtained. The best system involved a Leighton tube as culture vessel, a brief pepsin-evagination treatment, and a biphasic medium consisting of fully supplemented CMRL - 1066 and coagulated serum macerate.

11. Ultrastructural observations revealed severe tegumental damage in the neck and strobila region of cultured worms, probably resulting in loss of excretory, osmoregulatory, secretory, and absorptive functions.

12. A 17-day old adult worm from a dog, cultured for a further 14 days in vitro produced fully formed eggs that appeared normal. However, there were also many abnormally formed eggs and sperms.

13. Four dogs were fed with 20 cysticerci each; three were examined 10,17 and 29 days post-infection and found to harbour 18, 11 and 3 adults respectively. Eggs were obtained from the posterior proglottids of the 29-day old worms. The fourth dog failed to shed eggs up to 60 days P.I. and was reinfected, but no worms were present at autopsy 32 days later.

14. No metacestodes were found in mice that were fed orally with about 2,000 eggs each. Attempts at the in vitro cultivation of oncospheres were unsuccessful as no activation of oncospheres was achieved.

15. The prolific growth of metacestodes in mice had little pathogenic effects on the host. Apart from a general peritonitis, there was only slight emphysema in the lungs, and necrosis and amyloidosis in the liver.

16. In a brief immunological study, mice were injected with metacestode homogenate subcutaneously and challenged 2 days later with 20 metacestodes intraperitoneally. A biphasic response was detected : an early humoral mediated attack on the parasites during the first 8 weeks followed by a cell-mediated one which resulted in cell adherance on the parasite and subsequent encapsulation. However, a majority of the metacestodes were not susceptible to the immune response and continued to reproduce.

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APPENDIX I

Composition of Hanks Basic Salt Saline (HBSS) (Flow)

	g/litre
NaCl	8.00
KCl	0.40
$CaCl_2$	0.14
$MgSO_4.7H_2O$	0.10
$MgCl_2.6H_2O$	0.10
$Na_2HPO_4.2H_2O$	0.06
KH_2PO_4	0.06
Glucose	1.00
Phenol red	0.02
$NaHCO_3$	0.35

Composition of modified "858"

The following amino acids (Sigma) were added to 1 litre of CMRL - 1066. (It is not possible to determine if all the amounts added was completely dissolved)

	mg/litre
Alanine	25.0
Aspartic acid	30.0
Glutamic acid	75.0
Isoleucine	20.0
Leucine	60.0
Methionine	15.0
Phenylalanine	25.0
Serine	22.0
Threonine	30.0
Tryptophan	10.0
Valine	25.0

APPENDIX II

Only modified techniques/procedures are given below. All other staining methods are from Pearse (1960).

a) Casson's trichrome (ref: Stain Technology, 25:225-226)

distilled water	200 ml
phosphotungstic acid	1 g
Orange G	2 g
aniline blue	1 g
acid fuchsin	3 g

1. Dephariffinize and bring to water.
2. Stain for 5 - 15 minutes.
3. Wash in running tap water 5 - 10 seconds.
4. Dehydrate, clear in xylene and mount in DPX or Canada balsam.

b) Maximow's Technique (modified)

Used after fixation in Zenker.

1. Dephariffinize and bring to water, including the usual treatments with mercuric chloride and sodium thiosulphate.
2. Stain in Delafield's haematoxylin solution for ½ - 1 hour
   5 ml stock solution in 100 ml dist-water.
3. Blue in tap water.
4. Wash in dist. water (two changes) for 3 hours.
5. Stain overnight in the following solution :
   5 ml 0.1% eosin in dist. water
   40 ml Sorensen's buffer, pH 6.8
   5 ml 0.1% Azur II (Hopkins & Williams)

added in the above order.

APPENDIX II (contd.)

6. Rinse in dist. water

7. Dehydrate, clear in xylene and mount in neutral medium.

c) Modified Giemsa-Colophonium

1. Deparaffinize and bring to water.

2. Stain for 1 hour or more in the following :

Giemsa stain	10 ml
Acetone	10 ml
Methanol	10 ml
Buffered dist. water, pH 7.2	100 ml

3. Wash briefly in tap water

4. Differentiate in Colophonium/acetone (15 g/100 ml).

5. Wash in acetone:xylene (70 ml : 30 ml).

6. Clear in xylene and mount in green Euparal.

APPENDIX III

Composition of araldite resin for TEM.

Araldite CY212 Resin	24.500 g
HY 964 Araldite hardener (DDSA)	24.500 g
DBT Plasticiser	0.375 g
BDMA Accelerator	0.875 g

APPENDIX IV : Morphological Forms of Metacestodes. Comparison between KBS and TOI strains.

	SAMPLE POPULATION	SCOLEX ABSENT			SCOLEX INVAGINATED			SCOLEX EVAGINATED		
		Buds	Budders	Total	Budders	Cysticerci	Total	Budders	Cysticerci	Total
A (KBS)	387	54	4	58	8	313	321	6	2	8
	455	71	10	81	17	346	363	9	2	11
	572	75	34	109	21	430	451	8	4	12
	480	78	14	92	13	369	382	5	1	6
	707	115	26	141	42	488	530	24	12	36
TOTAL(%)	2601	393(15.1)	88(3.4)	481(18.5)	101(3.9)	1946(74.8)	2047(78.7)	52(2.0)	21(0.8)	73(2.8)
B (TOI)	293	31	160	191	7	19	26	22	54	76
	346	25	218	243	11	11	22	33	48	81
	622	95	338	233	17	27	44	17	128	145
	518	67	219	286	15	37	52	58	122	180
	492	47	216	263	33	51	84	59	86	145
TOTAL(%)	2271	265(11.7)	1151(50.7)	1416(62.4)	83(3.7)	145(6.4)	228(10.0)	189(8.3)	438(19.3)	627(27.6)

Note :- All metacestodes removed from a mouse were placed in a beaker of warm HBSS. During gentle agitation a wide mouthed pipette was used to transfer a random sample of metacestodes onto a petri-dish for counting. On occasions, the metacestodes had been stored for up to 5 days at 4°C before counting.