DEVELOPMENT AND IN VITRO CULTURE OF SOME DIGENEA.

VOLUME 2.

by

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FASCIOLA HEPATICA.

Plates 1 - 4 : Light microscope photographs.

Plates 5 - 35 : Transmission electron micrographs of metacercariae and developing adults.

Plates 36 - 73 : Transmission electron micrographs of cultured flukes.

Plates 74 - 79 : Scanning electron micrographs of cultured flukes.

(Scale bars on Plates denote lum.)

KEY.

Light Microscope Photographs -

A	anterior	os	oral sucker
dc	digestive caeca	P	posterior
ex	explant	vs	ventral sucker
h	hepatocytes		

Scanning Electron Micrographs -

A	anterior	RS	rings of spines
C	cilium	S	spine
DP	"domed" papilla	SP	sensory papilla
EP	excretory pore	T	tegument
OS	oral sucker	VS	ventral sucker
P	posterior	X	artifact

Transmission Electron Micrographs -

a	autophagosome	dm	degenerating macrophage
ag	"adult" granule	dse	degenerating sensory ending
aly	autophagolysosome	ec	embryonic cell
b	bacteria	er	endoplasmic reticulum
bb	basal body	exc	excretory concretion
bi	basal invagination	f	"flame" of flame cell
c	cilium	fc	flame cell
CAD	ciliated ascending duct	ga	area of Golgi activity
cc	caecal contents	gc	Golgi complex
cd	collecting duct	gca	glycocalyx
cm	crystalline material	ger	granular endoplasmic reticulum
cys	cystogenic cell	gly	glycogen
d	dense material/desmosome	gr	genital rudiment
dc	digestive caeca	im	interstitial material

imv interdigitating microvilli

jc junctional complex

jg "juvenile" granule

k keratin scroll

l lipid

la lamellae

le external leptotriches

li internal leptotriches

ly lysosome

m mitochondria

ma macrophage

MDD major descending duct

mu muscle

mv microvilli

n nucleus

nu nucleolus

pc parenchyma cell

PDD primary descending duct

pp parenchymal process

ps protoplasmic strand

r ribosome

sd septate desmosme

sg secretory granule

sp spine

syn tegumental sy ncytium

tb terminal bar

tc tegumental cell body

uc undifferentiated cell

v vacuole

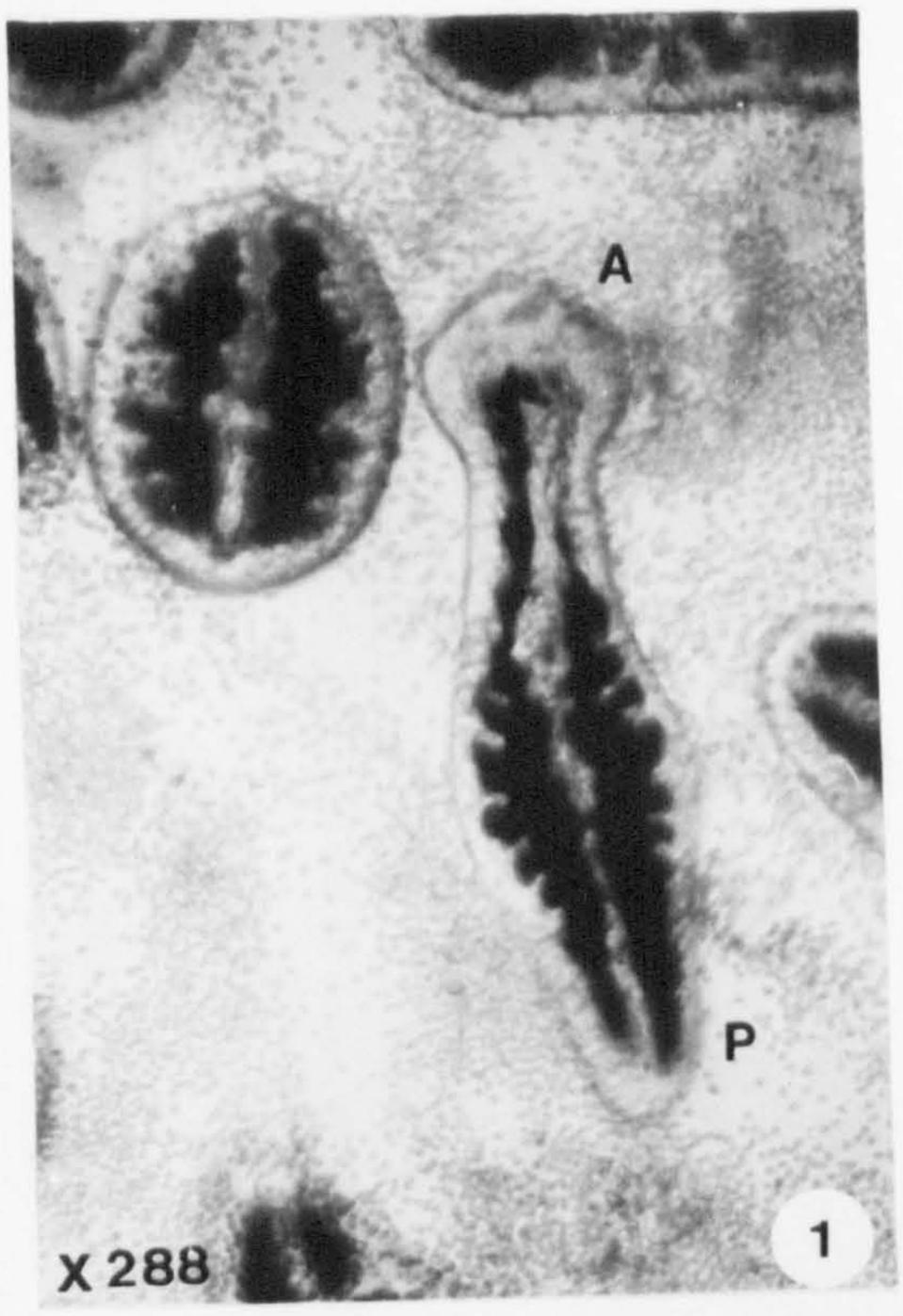
ves vesicle

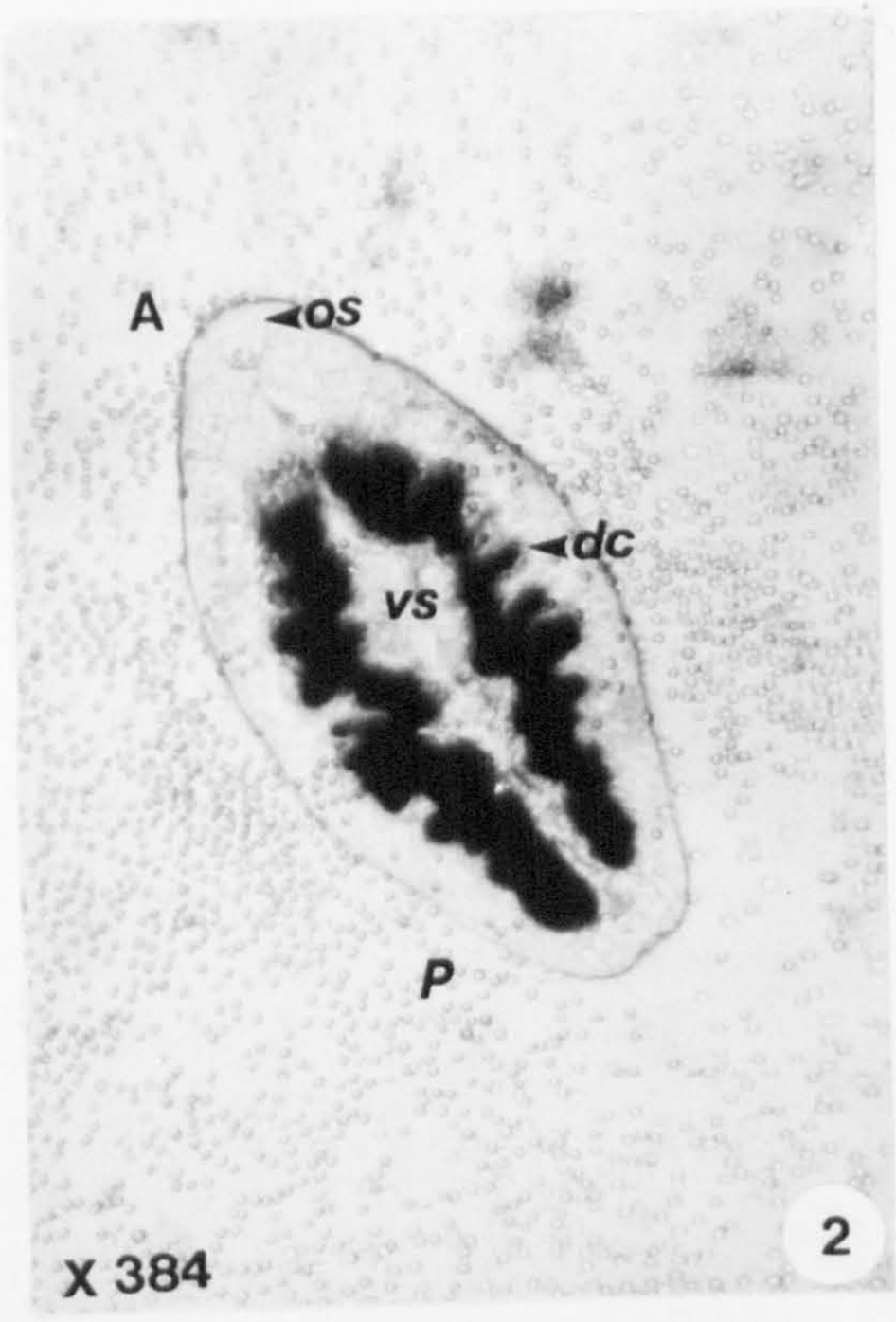
Plates 1 and 2 Flukes after 14 days *in vitro* culture (photographed live).

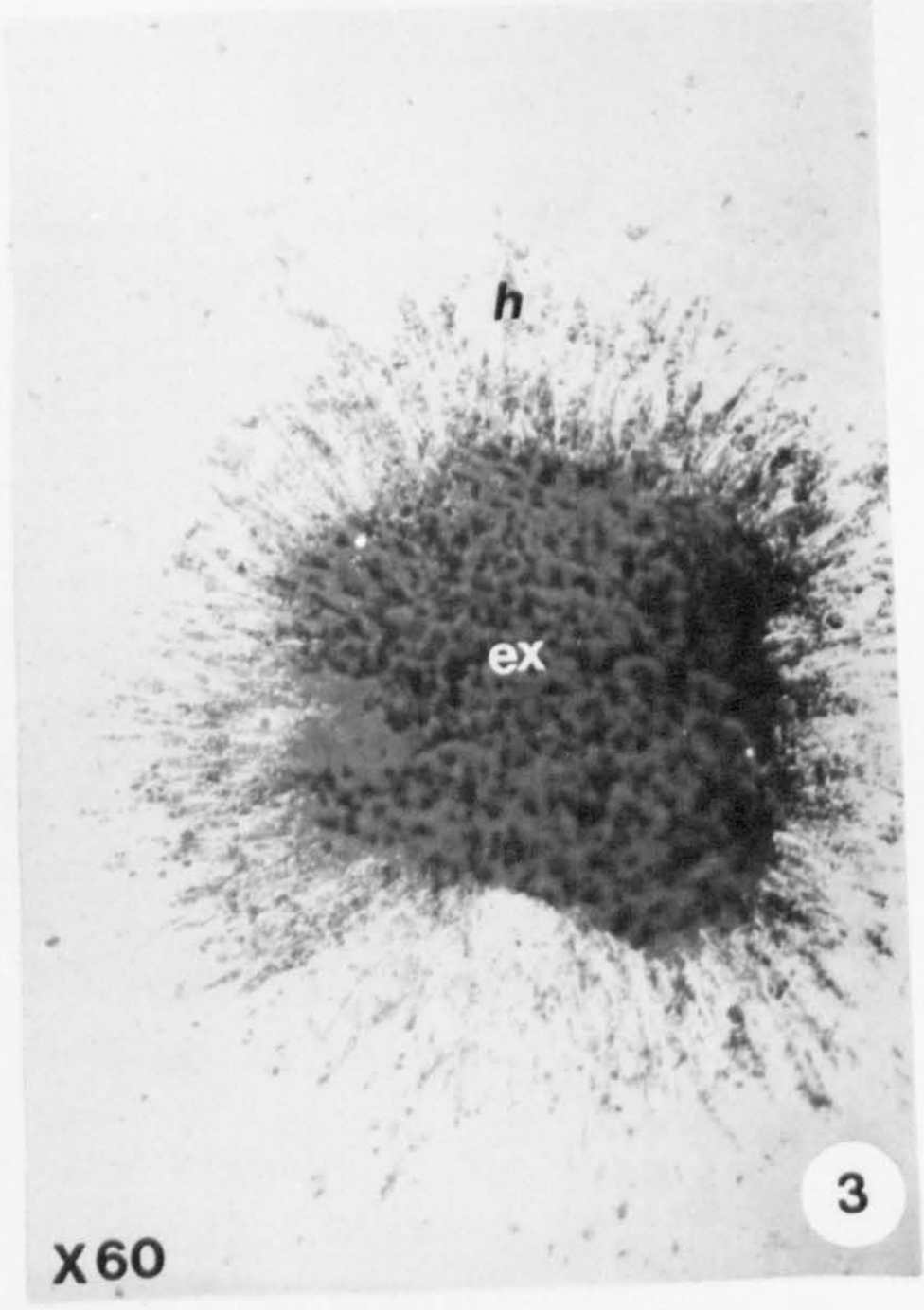
The bilobed digestive caeca are filled with blood and lateral branches have developed.

Plates 3 and 4 Newborn mouse liver explant after 6 days *in vitro* culture (photographed live).

Columns of hepatocytes (h) can be seen migrating out from the original explant (ex).


A
P
X 288
1


A
OS
dc
VS
P
X 384
2


h
ex
X60
3

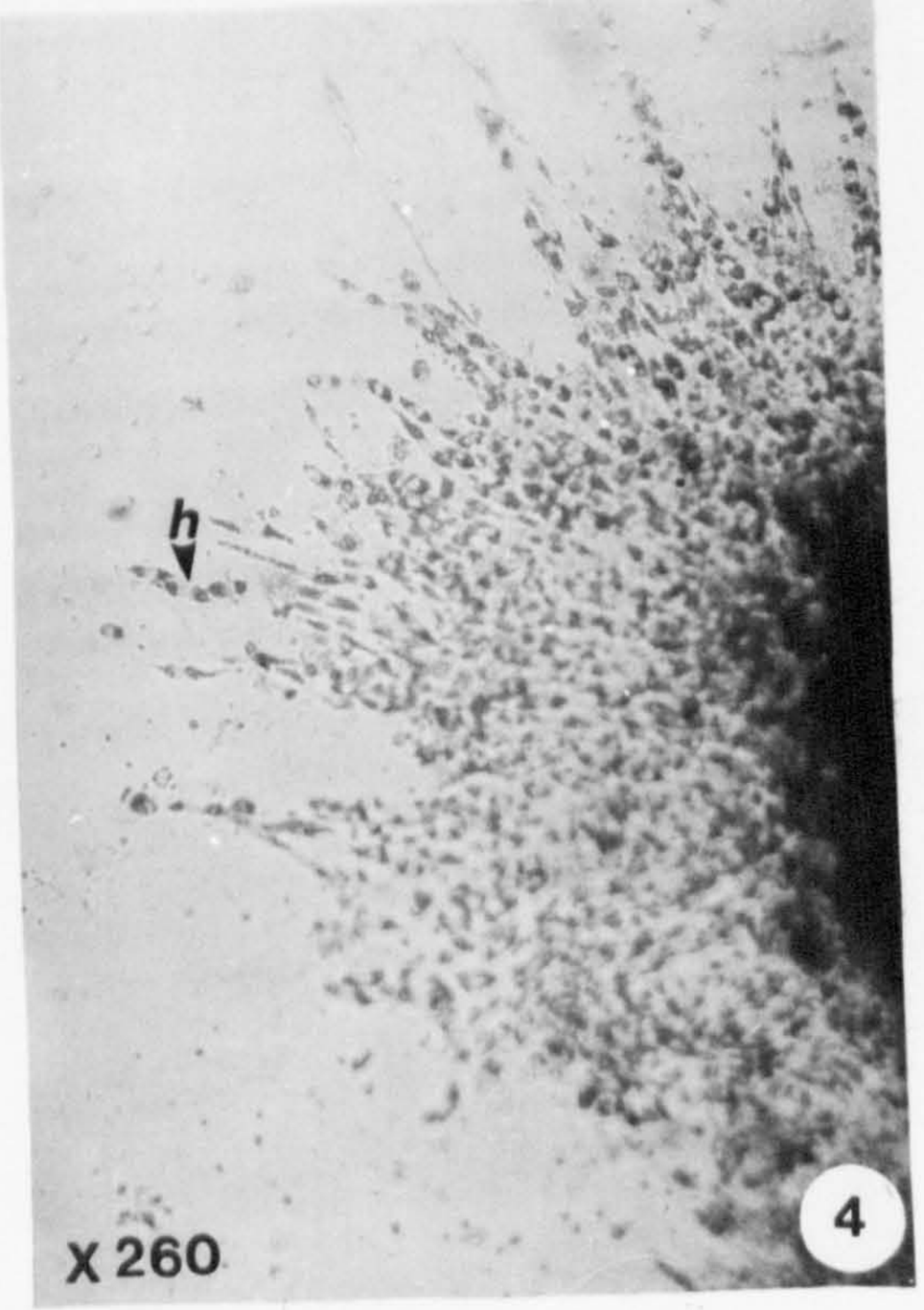
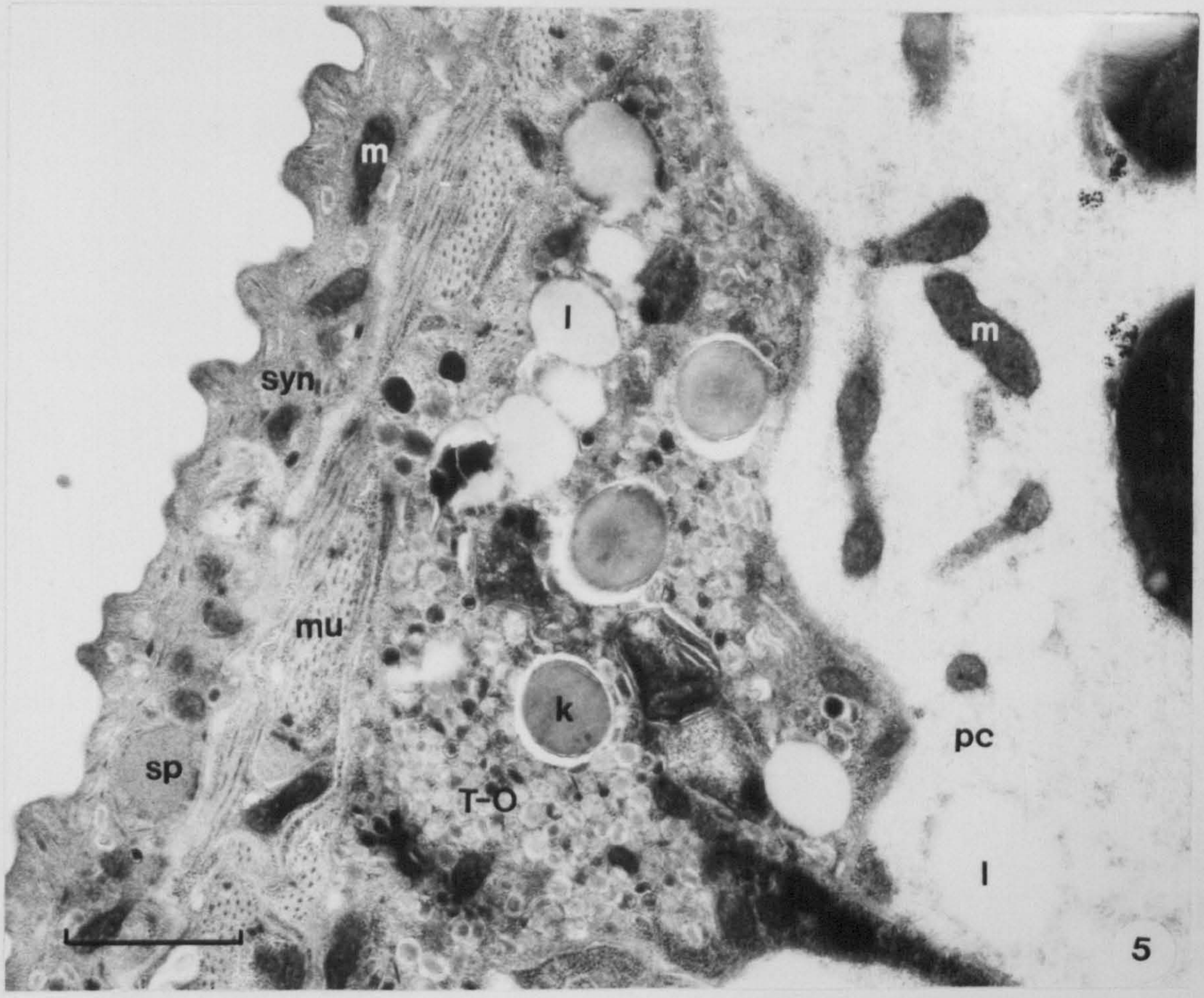

h
X 260
4

Plate 5 Excysted metacercaria.

Spherical (T-O) granules can be seen in both the syncytium and the tegumental cell bodies. Lipid drops (l) and keratin scrolls (k) are also present in the tegumental cells.

Plate 6 Excysted metacercaria.

The remnants of a cystogenic cell (cys) (probably a tanned protein cell, Dixon 1965) lies amongst the tegumental cell bodies. The parenchymal cells (pc) contain numerous lipid drops(l).


m
l
syn
m
mu
k
pc
sp
T-O
l
5

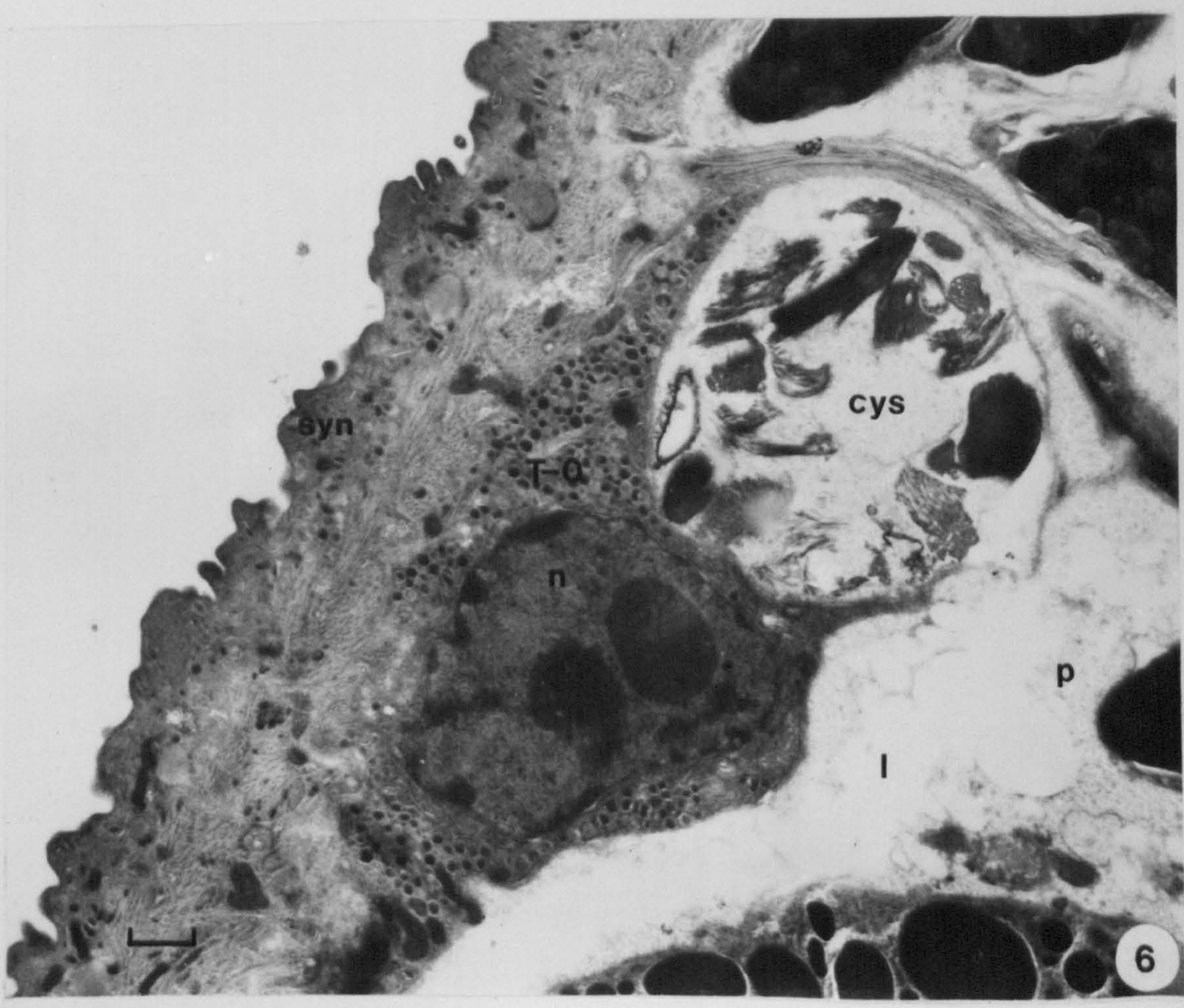
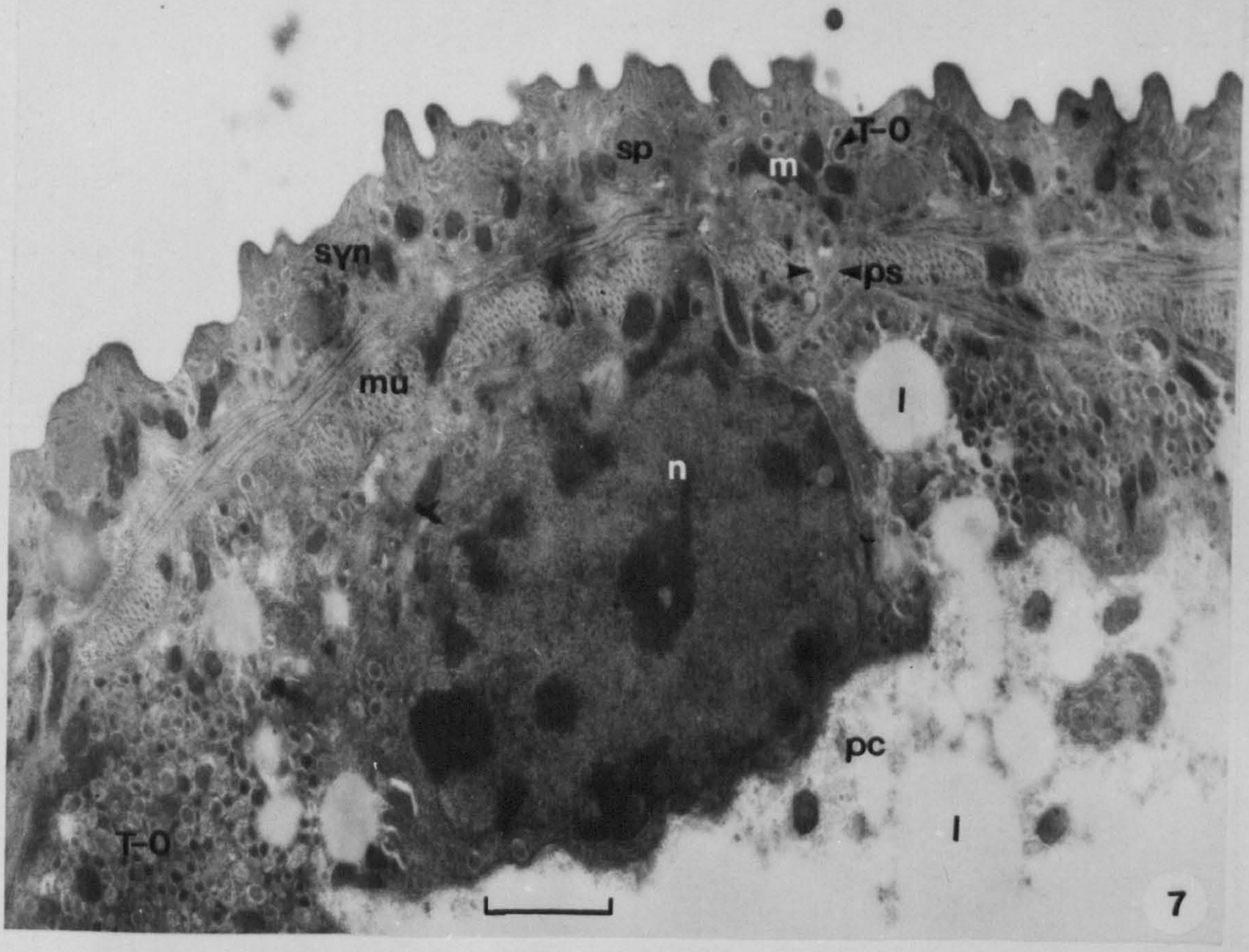

syn
T-O
cys
n
p
l
6

Plate 7 Excysted metacercaria.

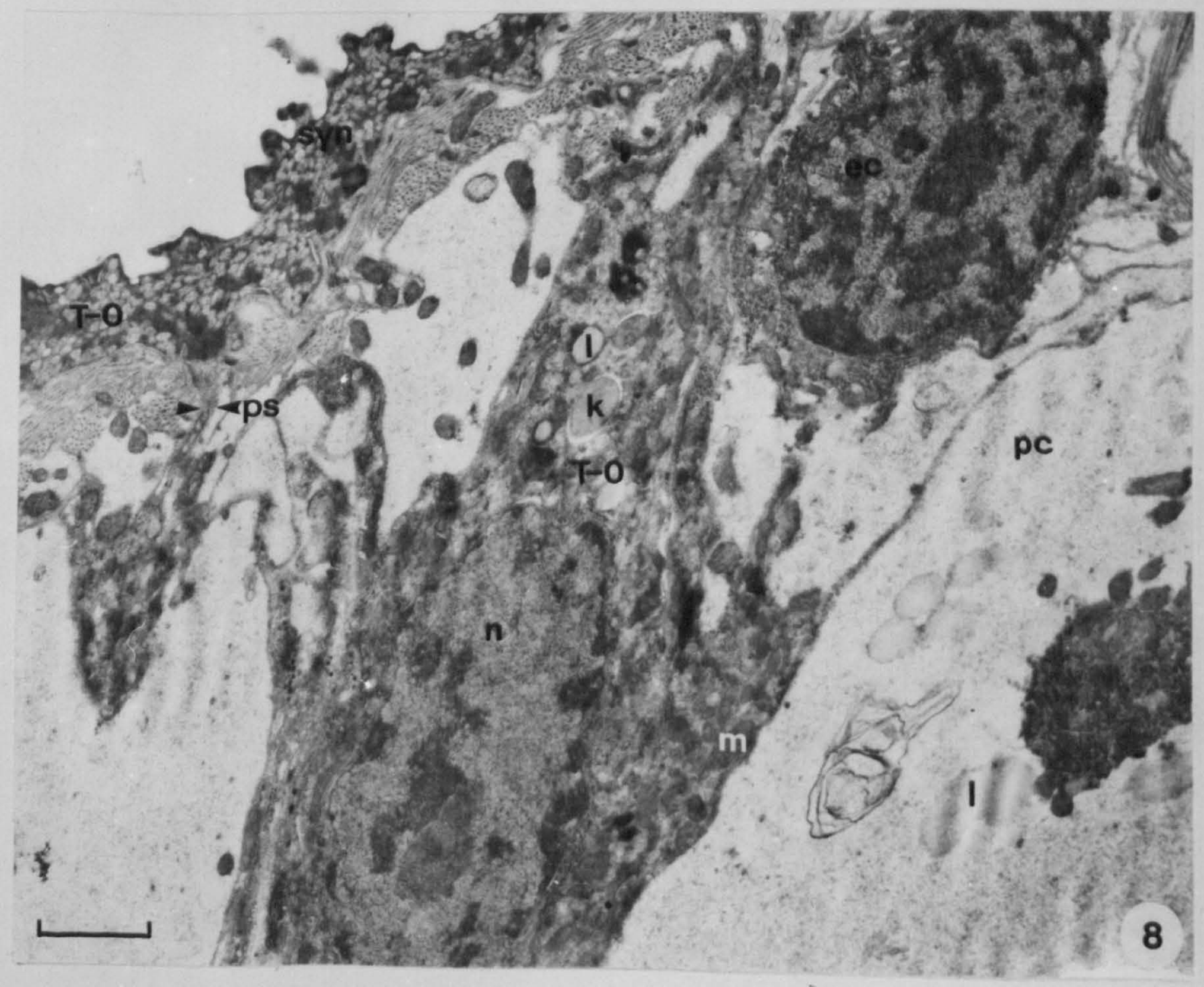
The tegumental syncytium (syn) contains dense mitochondria (m), spines (sp) and spherical (T-O) secretory granules. A protoplasmic strand (ps) can be seen connecting the syncytium with the tegumental cell body which lies below the peripheral musculature (mu). The tegumental cell contains numerous spherical secretory granules (T-O) and some lipid drops (l); the nucleus (n) contains patches of heterochromatin.

Plate 8 Day 3 p.i. - location, abdomen.

The ground substance of the tegumental syncytium (syn) has increased in density and is packed with spherical granules (T-O). A protoplasmic strand (ps) can be seen connecting the syncytium with a tegumental cell body. Keratin scrolls (k) are still occasionally seen in the tegumental cell bodies. An undeveloped"embryonic"cell (ec) can be seen in close proximity to the tegumental cell, it contains no secretory granules.



7



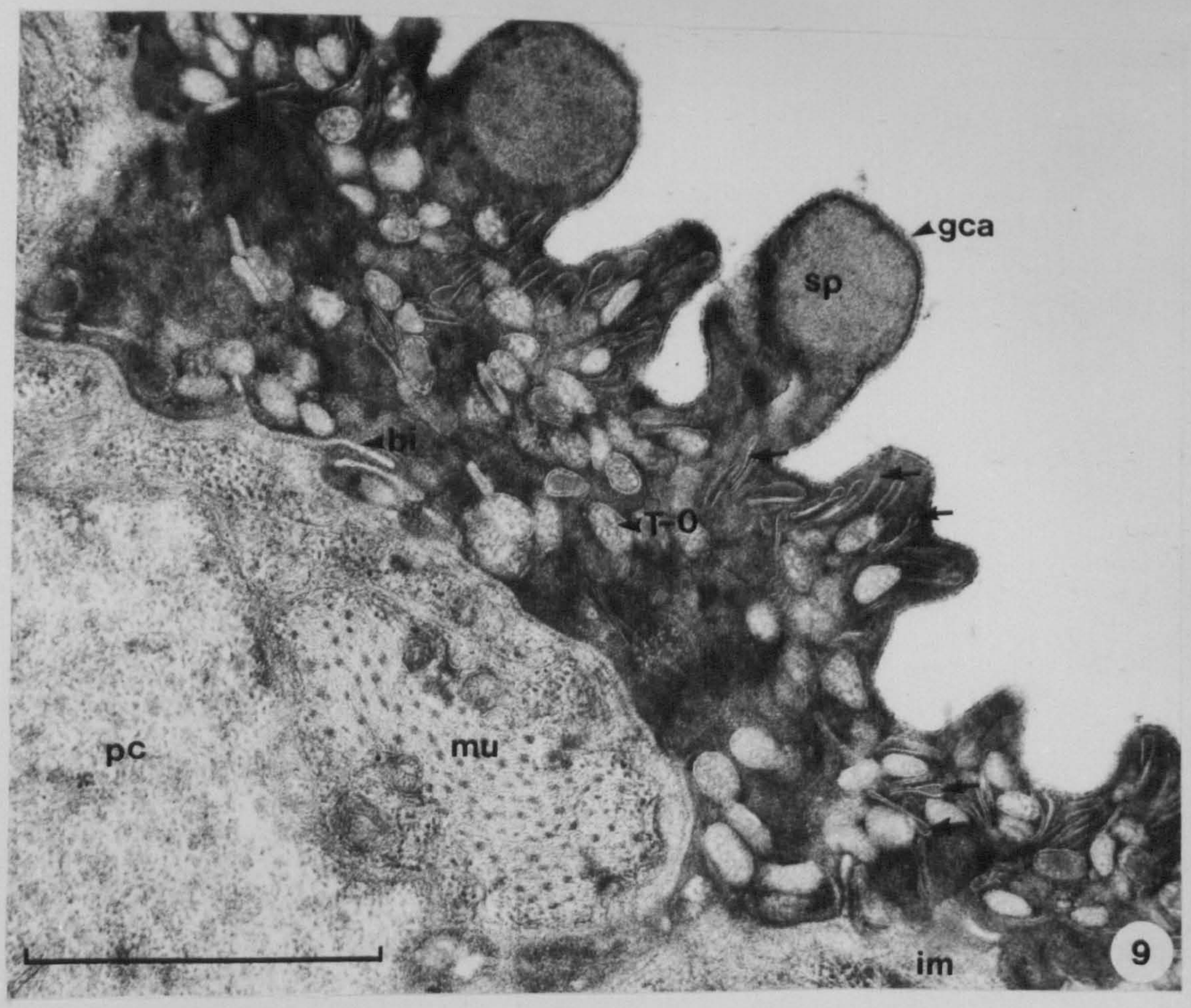
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Plate 9 Day 3 p.i. - location, abdomen.

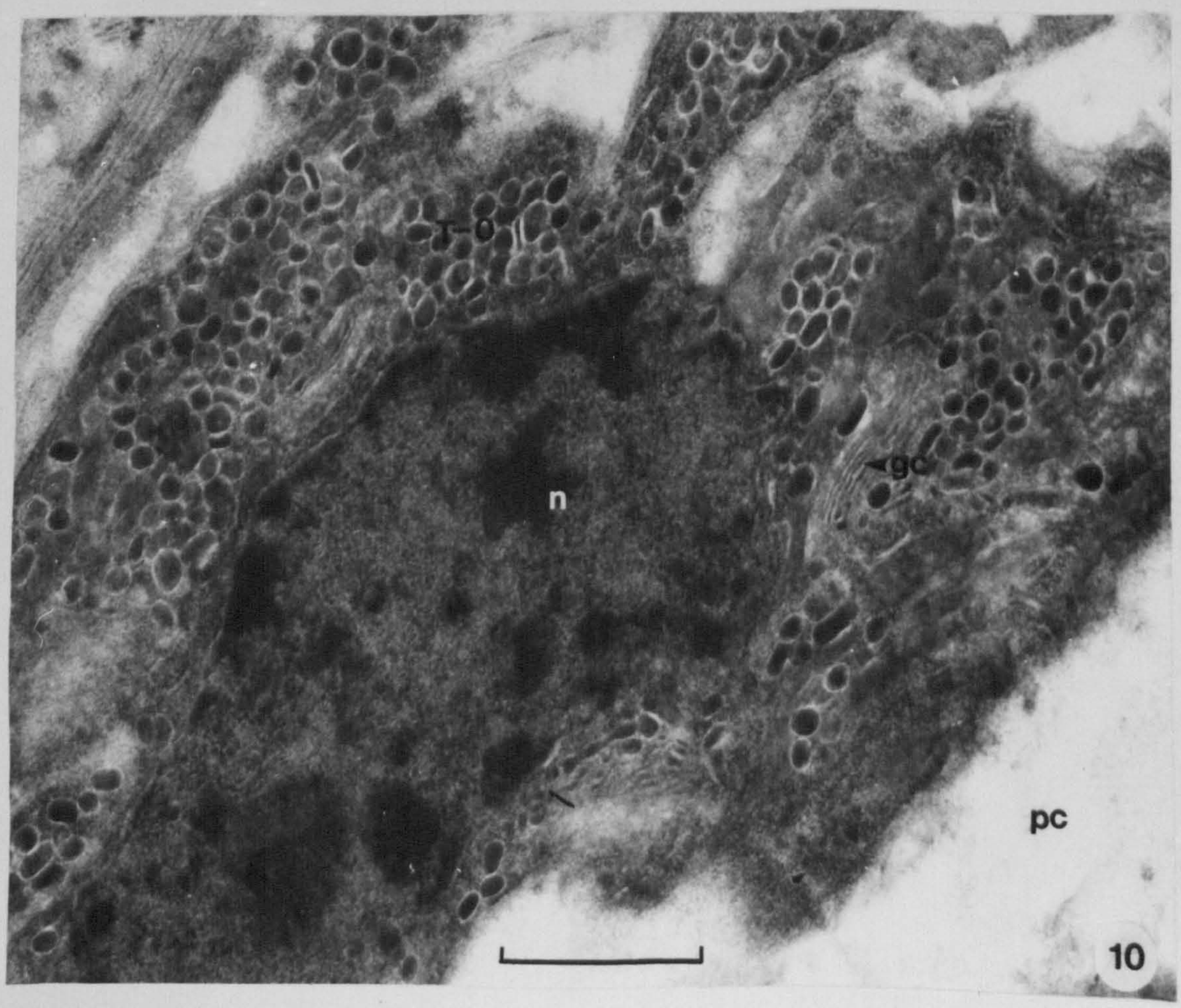
The ground substance of the syncytium is now moderately dense. The spherical secretory bodies (T-O) in the syncytium have granular, electron lucent contents. Biconcave granules (arrowed) are present and are particularly frequent near the surface membrane to which they are orientated at right angles. The surface membrane is coated by a thin, fuzzy glycocalyx (gca). Some invagin--ations of the basal plasmalemma (bi) are present. Between the syncytium and the underlying muscle cells (mu) is a thin layer of interstitial material (im). The parenchymal cells (pc) contain abundant glycogen.

Plate 10 Day 3 p.i. - location, abdomen.

The tegumental cell bodies are packed with numerous spherical granules (T-O) with dense contents. Golgi complexes (gc) are present.



9



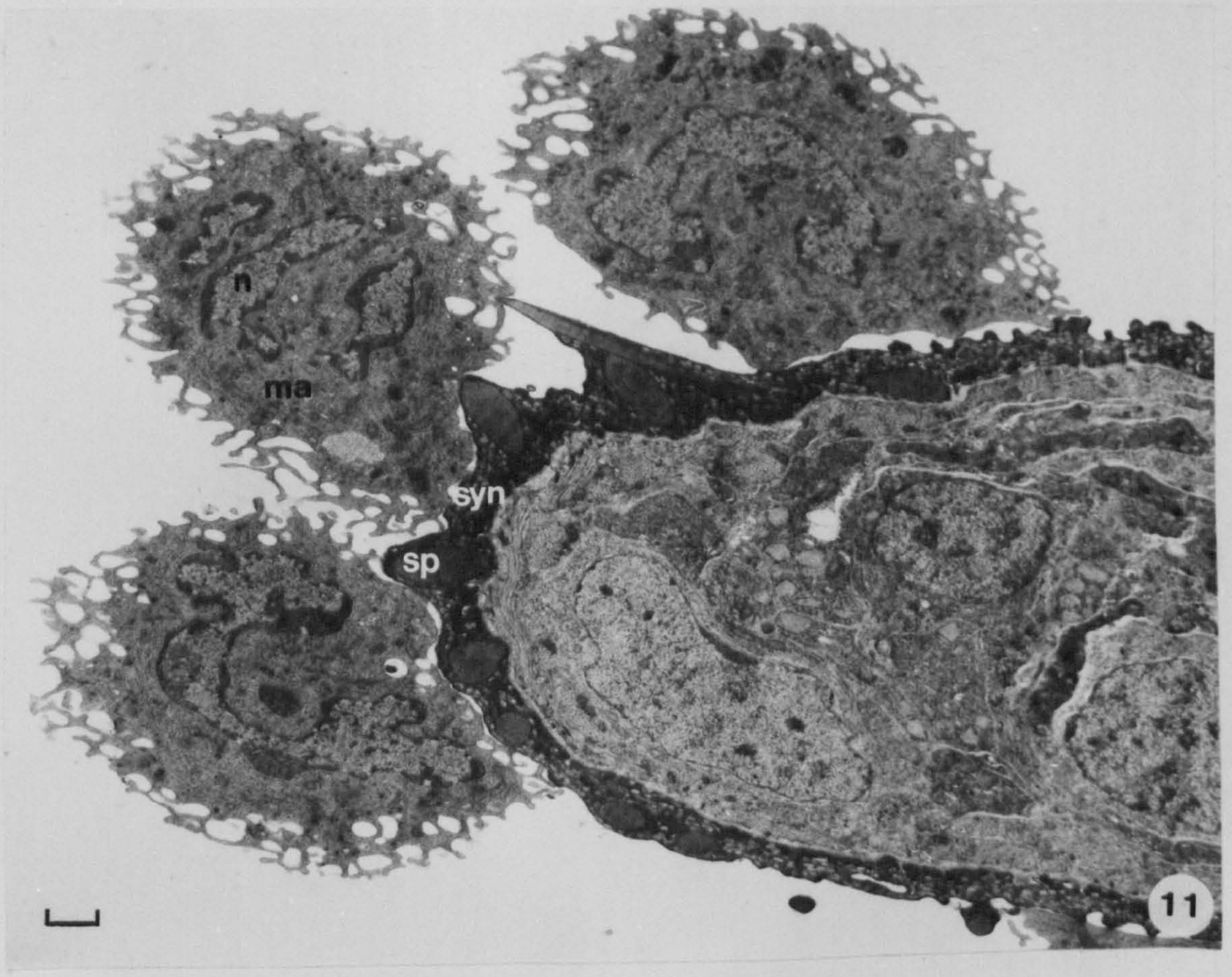
10

Plate 11 Day 5 p.i. - location, abdomen.

Macrophages (ma) from the mouse abdominal cavity adhere to the tegumental syncytium (syn) of the young fluke. The nuclei (n) of the macrophages are very irregular in shape. The surface of the macrophage consists of a reticulum of cytoplasmic processes.

Plate 12 Day 5 p.i. - location, abdomen.

The macrophage (ma) is separated from the tegumental syncytium (syn) by the fluke's surface glycocalyx (arrows); there appears to be no direct contact.


n
ma
syn
sp
11

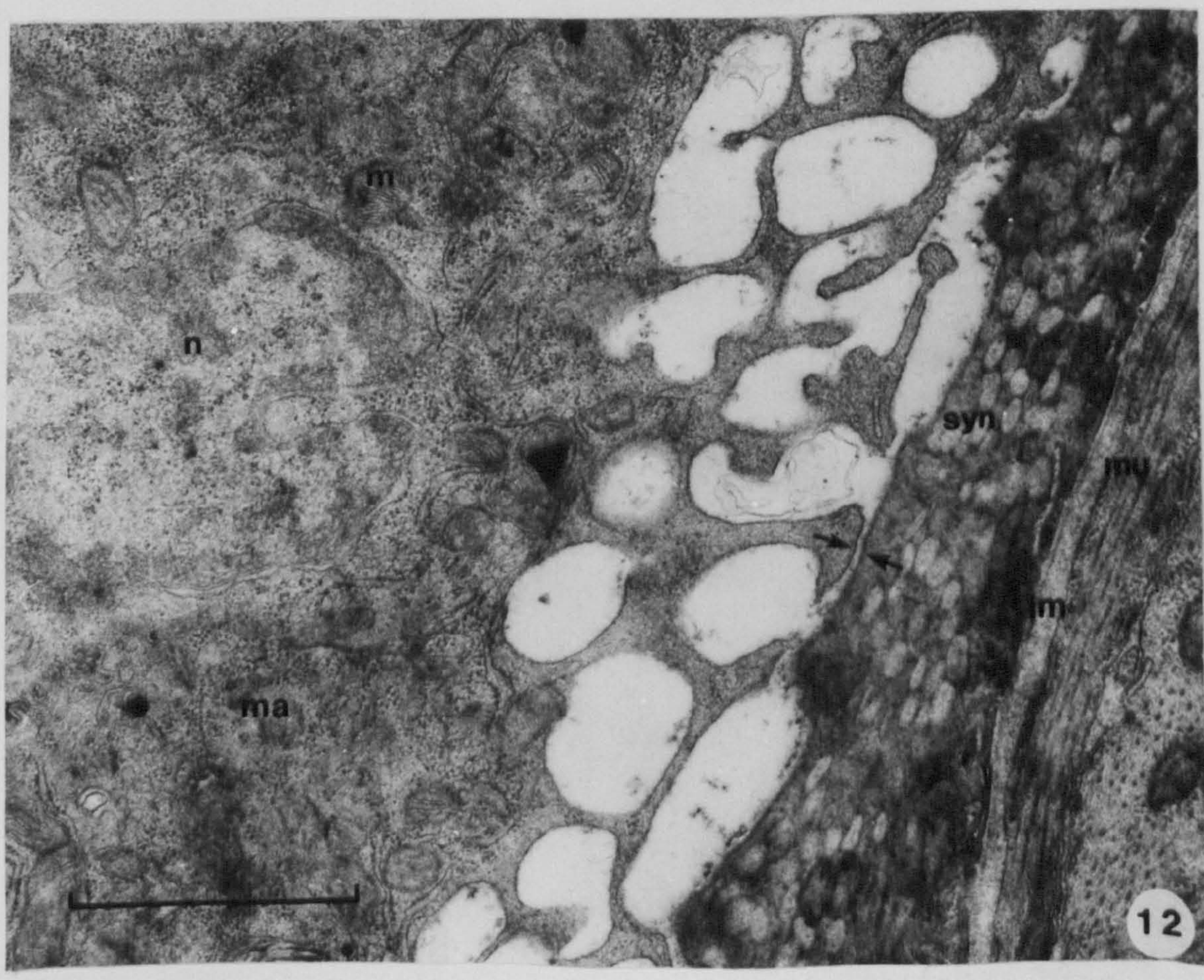
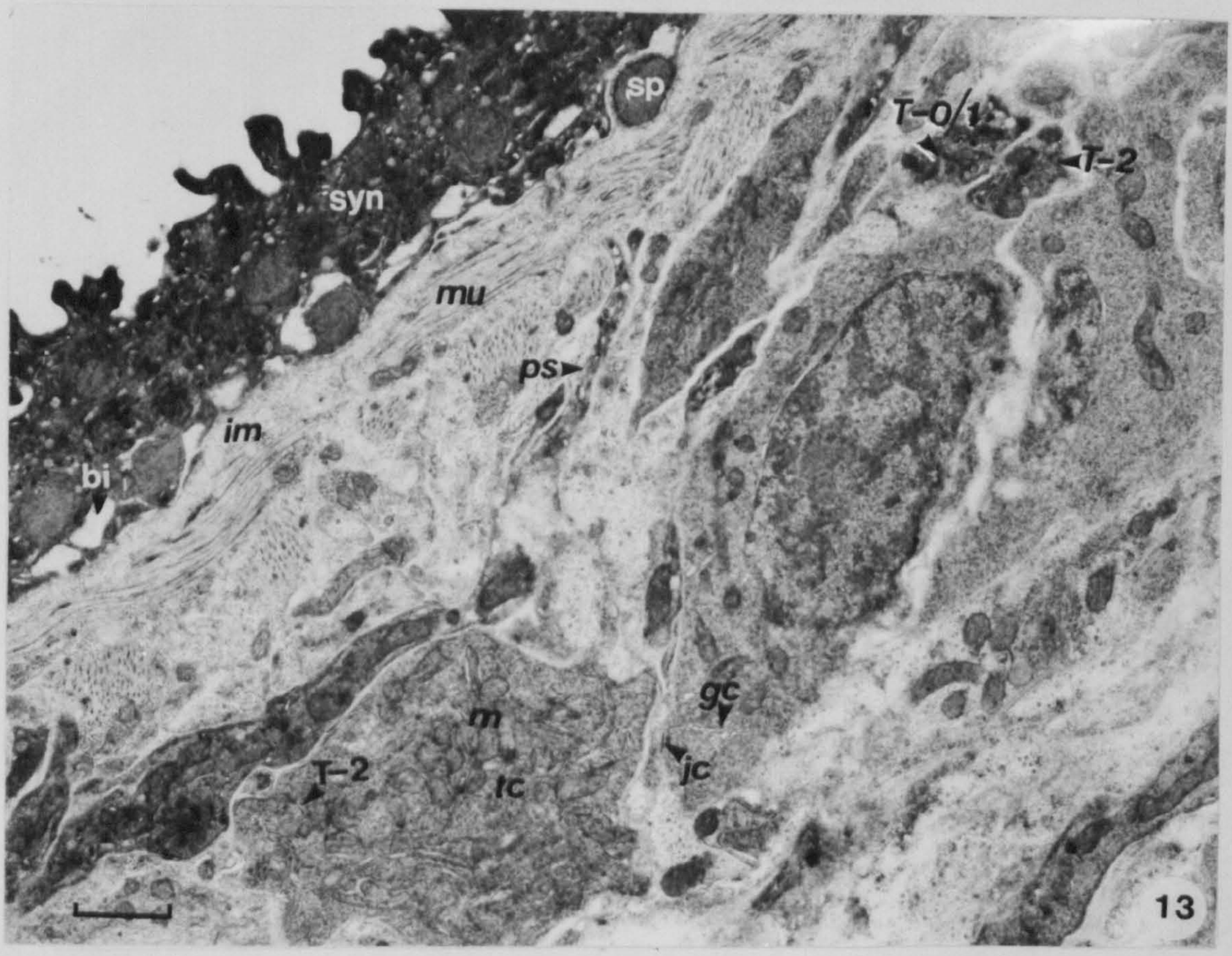

m
n
ma
syn
mu
m
12

Plate 13 Day 9 p.i. - location, liver.

Two types of tegumental cell bodies are now present (tc): Type 2 tegumental cells contain dense, rod-like secretory bodies (T-2) whilst the original Type 0 tegumental cells contain approximately equal numbers of spherical (T-0) and oval (T-1) secretory bodies. Junctional complexes (jc) exist between the tegumental cell bodies and adjacent parenchymal cells.

Plate 14 Day 11 p.i. - location, liver

The tegumental syncytium contains two types of granules - the spherical (T-0) granules often appear granular and relatively electron lucent, the oval (T-2) granules have a dense heterogeneous content. The T-0 granules often appear biconcave near the surface membrane. The surface of the fluke is covered by a thin, fuzzy glycocalyx (gca). There are numerous invaginations of the basal plasmalemma (bi) some of which appear slightly swollen.



13



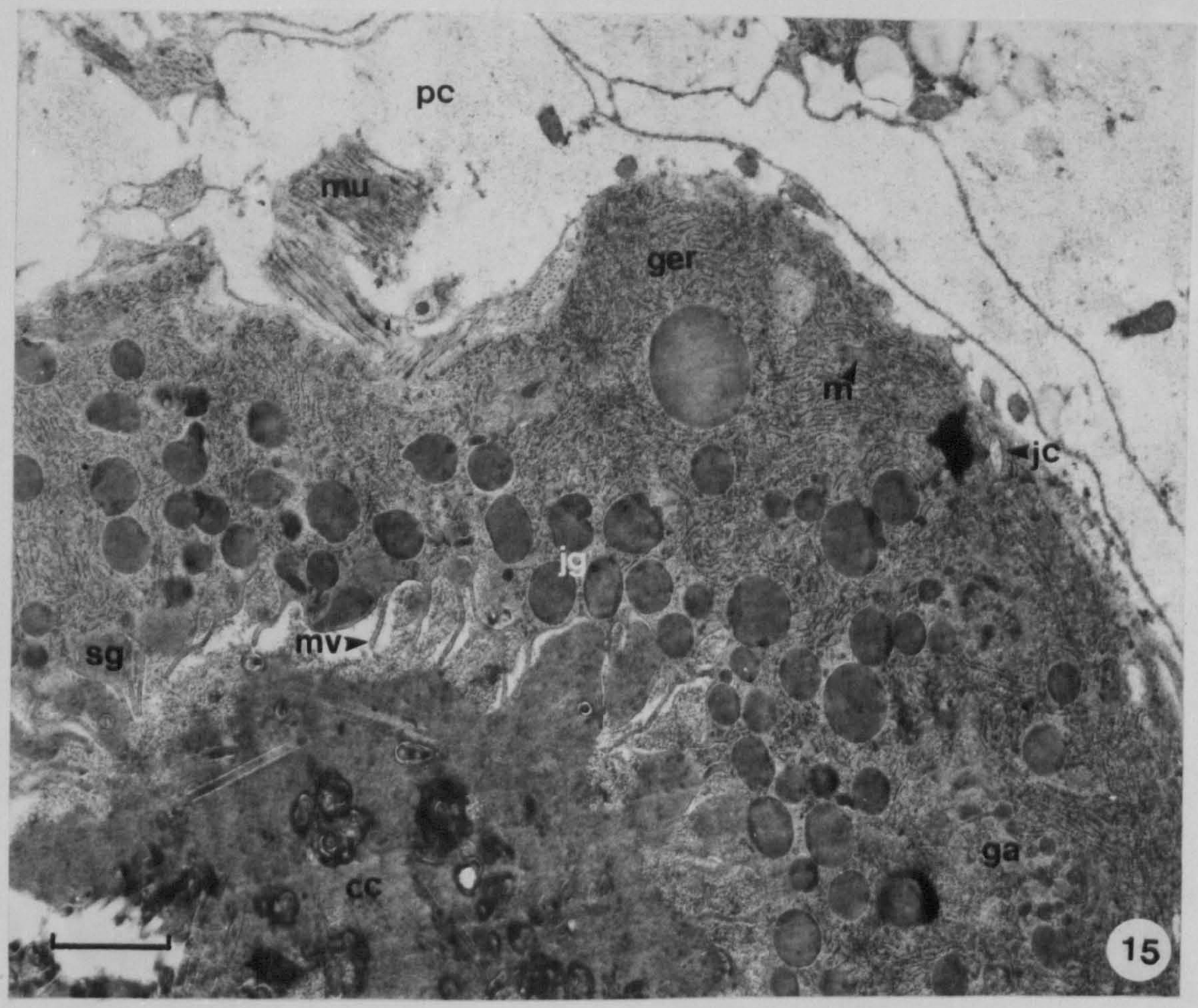
14

Plate 15 Day 3 p.i. - location, abdomen

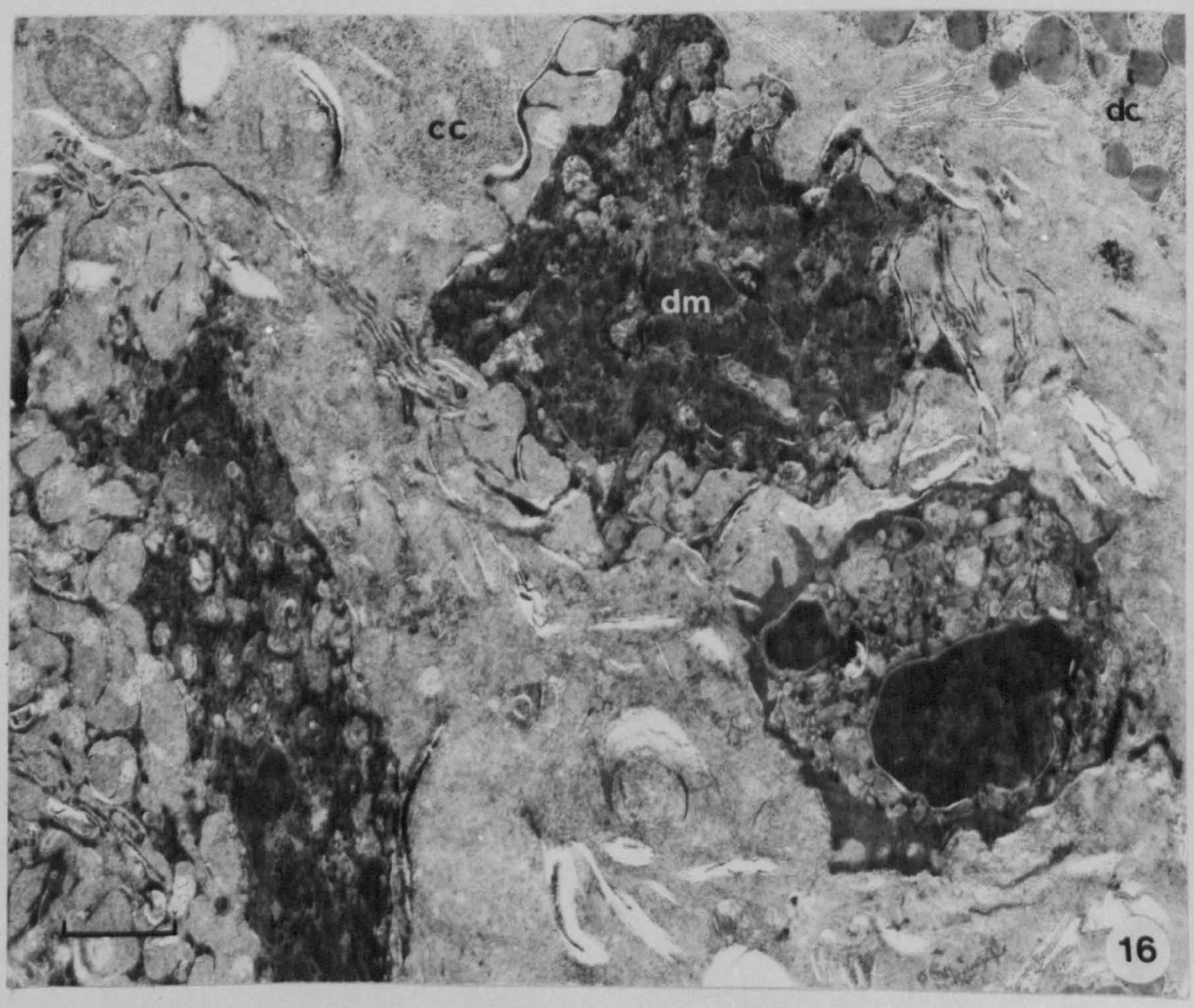
The caecal epithelium contains short strands of GER (ger), mitochondria (m), some areas of possible Golgi activity (ga) and "juvenile" type secretory granules (jg). The luminal surface is thrown up into short microvilli (mv). Junctional complexes (jc) between the caecal cells and parenchymal cell (pc) processes are now present. The lumen of the digestive caeca contains a dense material (cc) of a similar density to the secretory granules; granules can often be seen in the process of secretion (sg) into the lumen.

Plate 16 Day 6 p.i. - location, abdomen.

Degenerating host macrophages (dm) can be seen in the lumen of the digestive caeca of flukes recovered from the abdomen.



15



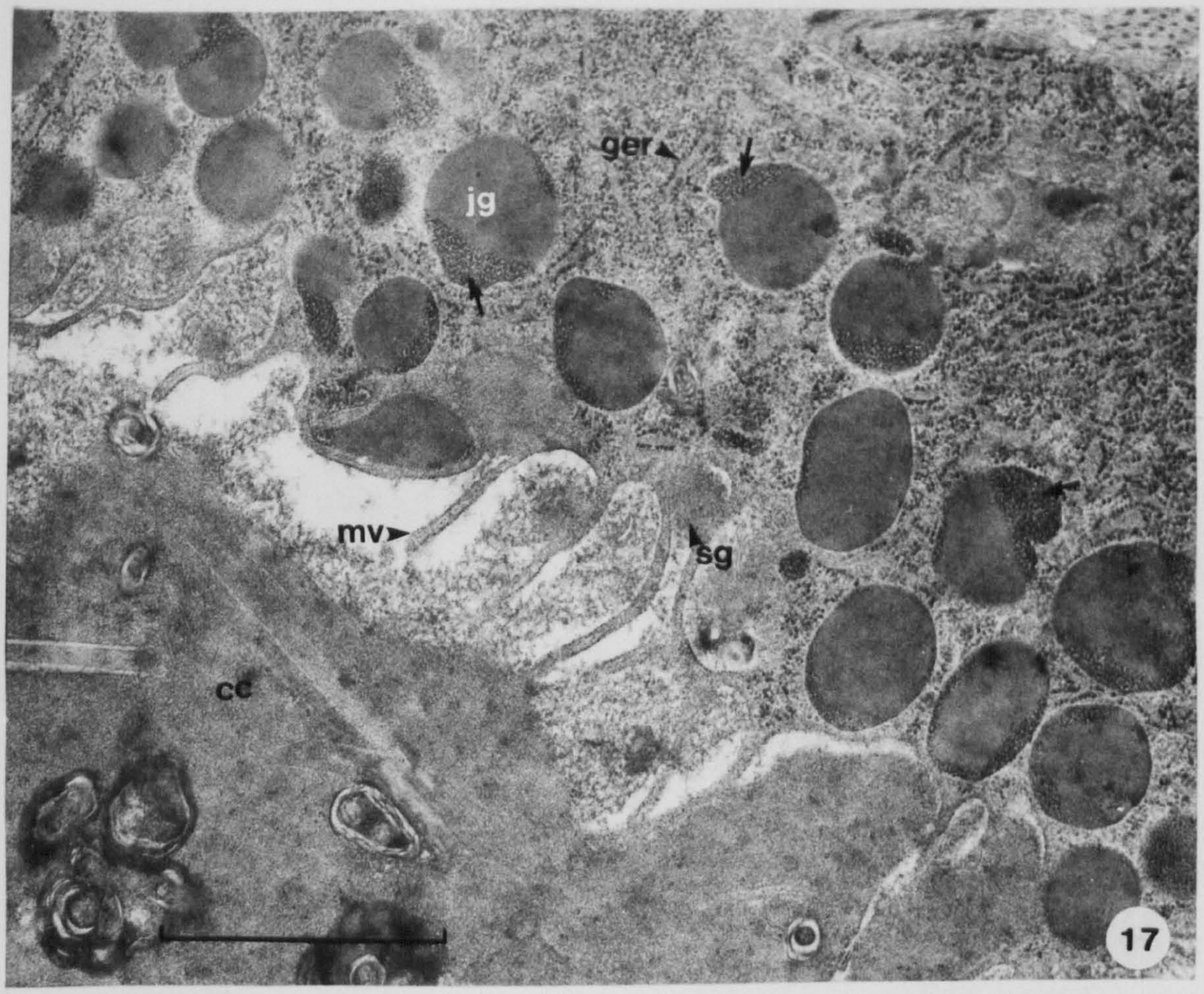
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Plate 17 Day 3 p.i. - location, abdomen.

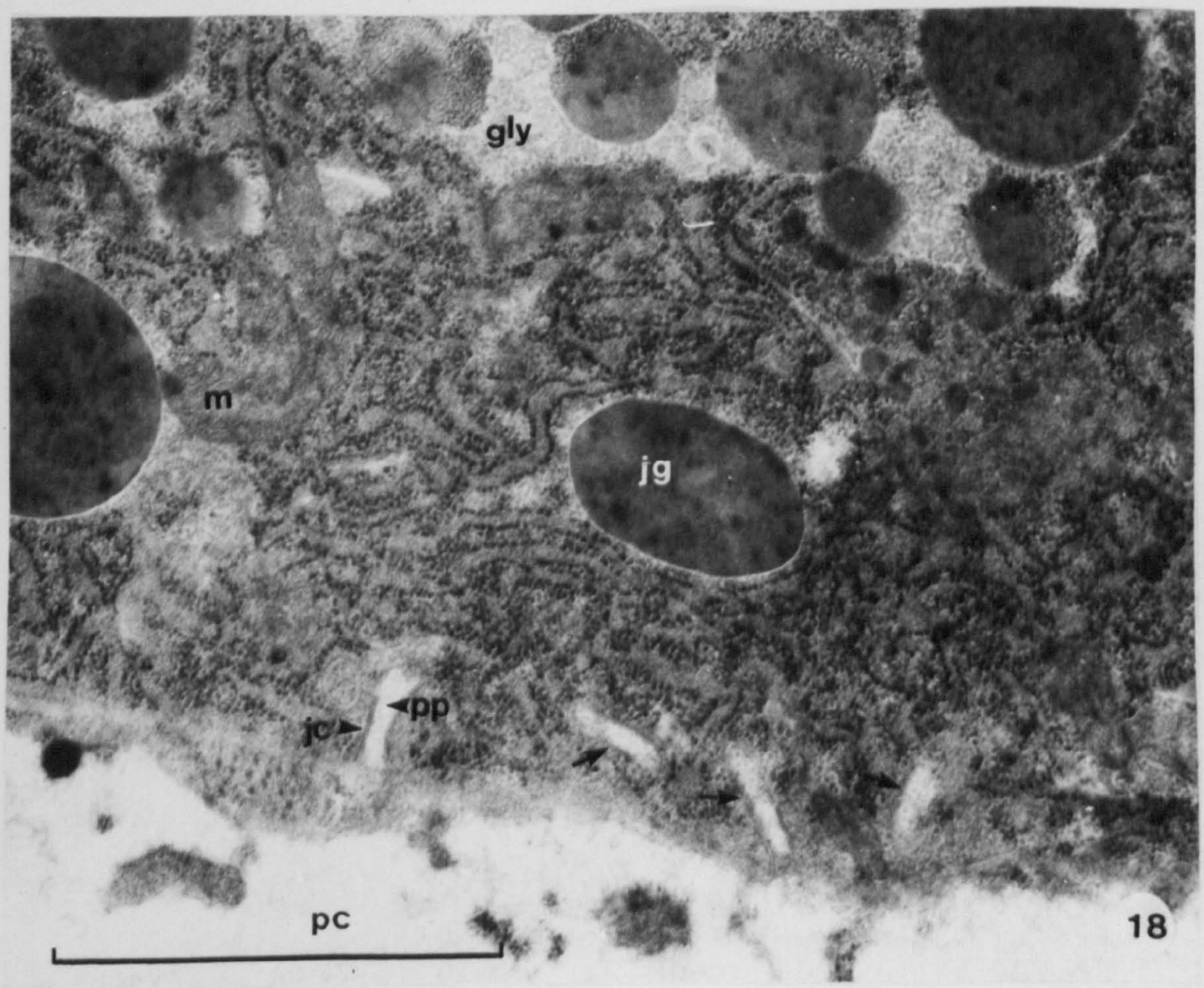
The "juvenile"type secretory bodies in the caecal epithelium of young flukes (jg) often appear granular in certain areas (arrows) particularly around the periphery of the granules. Granules are often seen in the process of secretion (sg) into the lumen of the caeca which is filled with a material of similar density.

Plate 18 Day 3 p.i. - location, abdomen.

Junctional complexes (jc, arrows) between the cells of the caecal epithelium and processes (pp) of the surrounding parenchymal cells (pc) begin to develop at this time. Areas of glycogen are occasionally seen in the caecal cells (gly).



17



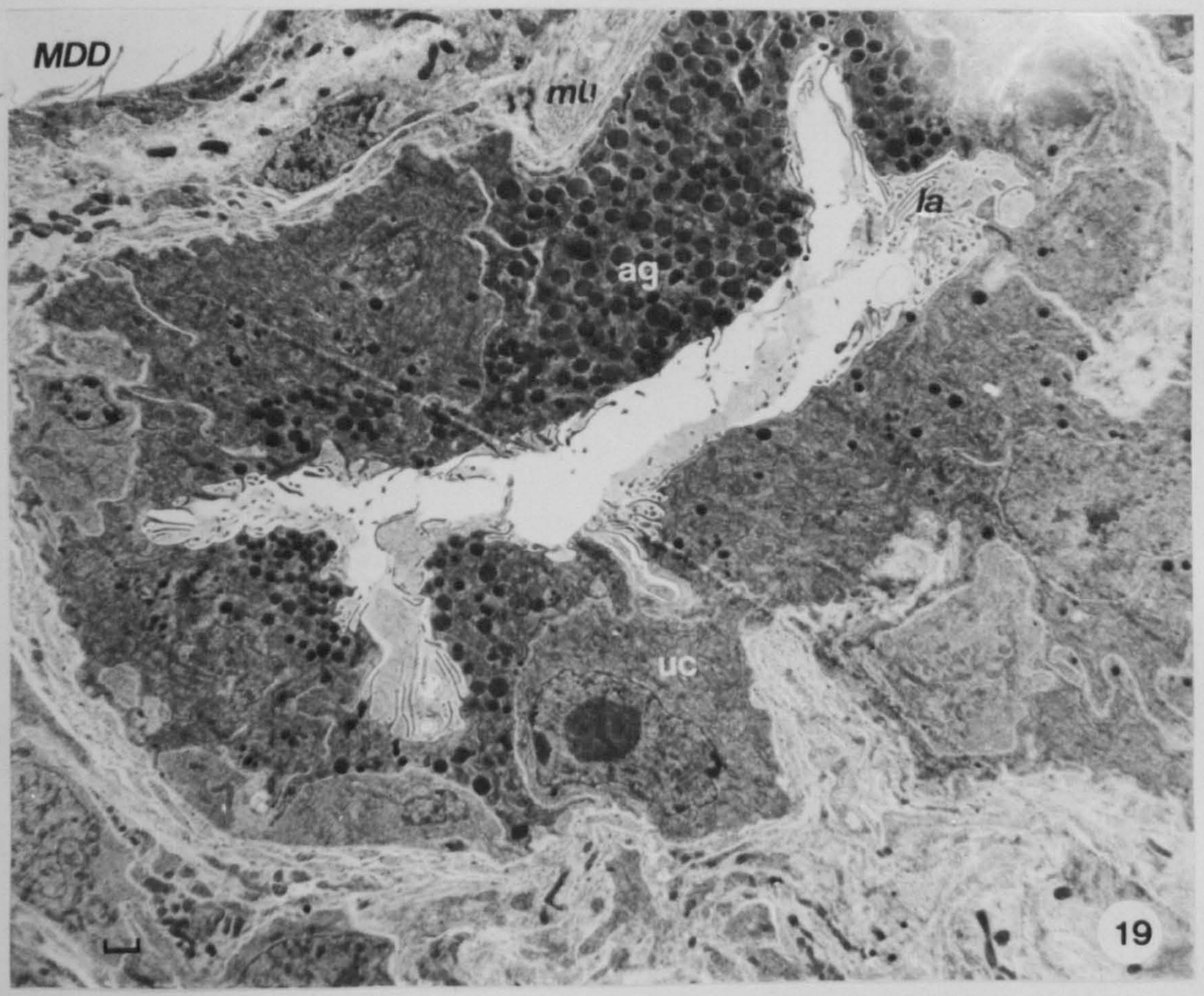
18

Plate 19 Day 5 p.i. - location, liver.

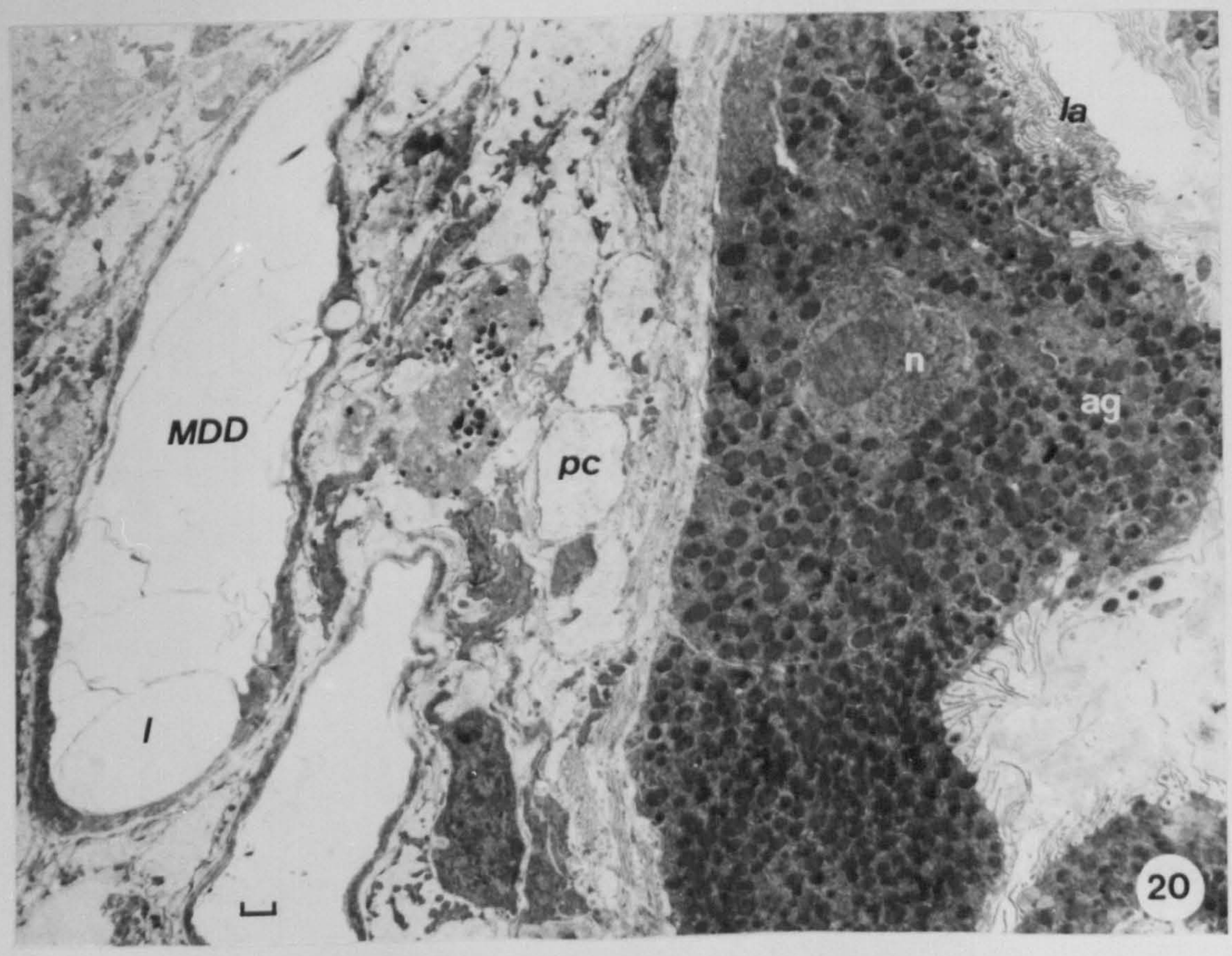
A section through the digestive caeca shows cells containing numerous dense, "adult" type secretory granules (ag). Undeveloped cells (uc) which contain no secretory granules are commonly seen and probably contribute to the growth of the caeca.

Plate 20 Day 9 p.i. - location, liver.

The caecal cells are filled with "adult" type granules (ag). The surface lamellae (la) are now longer and more numerous. The caecal epithelium is increasing in thickness.



19



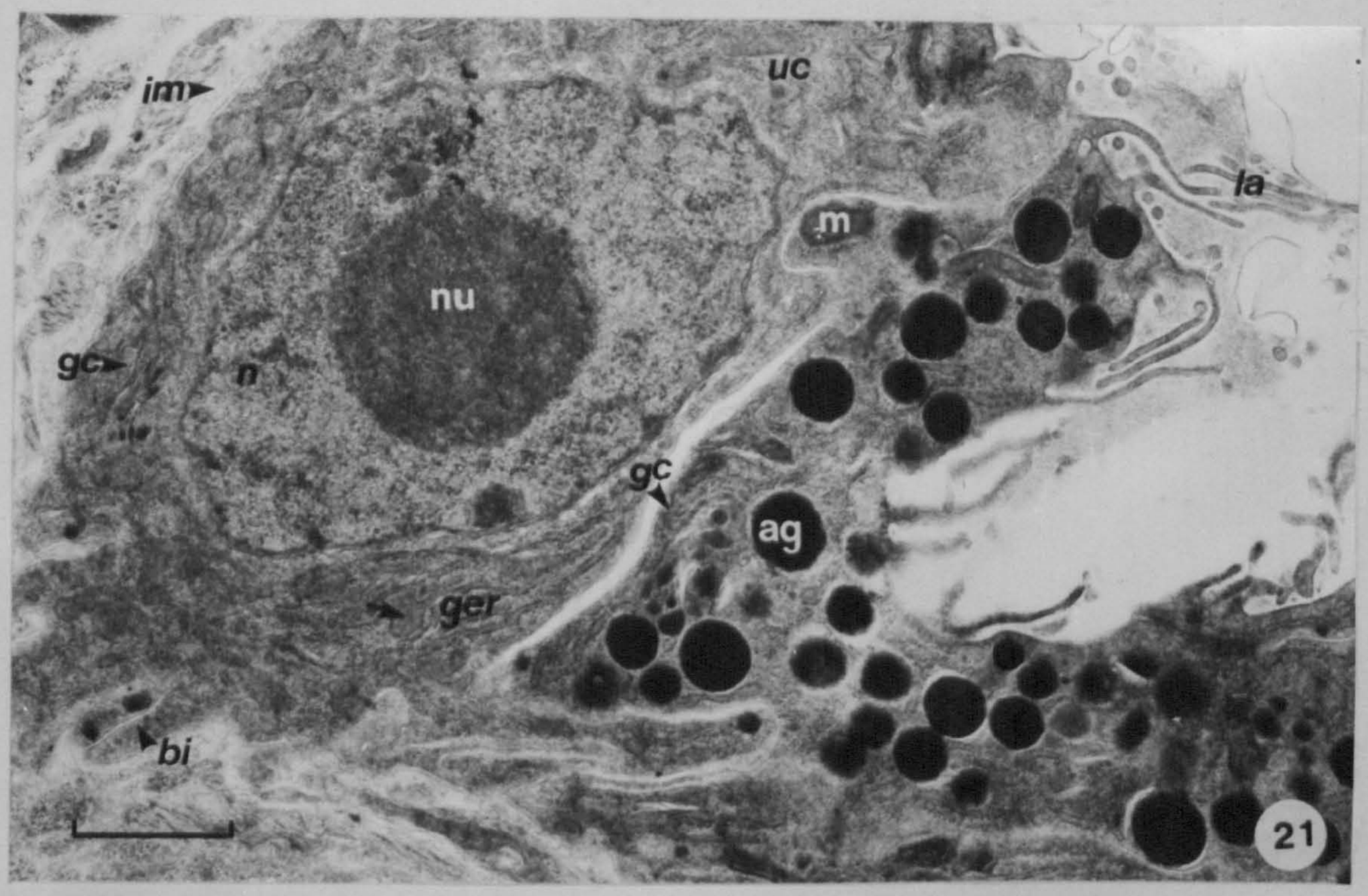
20

Plate 21 Day 9 p.i. - location, liver.

An undeveloped cell (uc) can be seen lying next to an actively secreting cell in the caecal epithelium. The undeveloped cell contains a nucleus (n) with a prominent nucleolus (nu), Golgi complexes (gc) which appear to have dense contents, and short lengths of GER (ger) which are frequently seen to be swollen at the ends (arrow). The adjacent cell contains numerous "adult" type secretory granules (ag), Golgi complexes filled with a dense material (gc), and GER.

Plate 22 Day 5 p.i. - location, liver.

This section shows the basal part of the caecal epithelium which includes an area of stored glycogen (gly), some "adult" type granules (ag), abundant ribosomes and short lengths of GER (ger) which are often swollen with a moderately dense material (arrows).


im
uc
la
m
nu
gc
n
gc
ag
ger
bi
21


n
ag
ger
gly
22

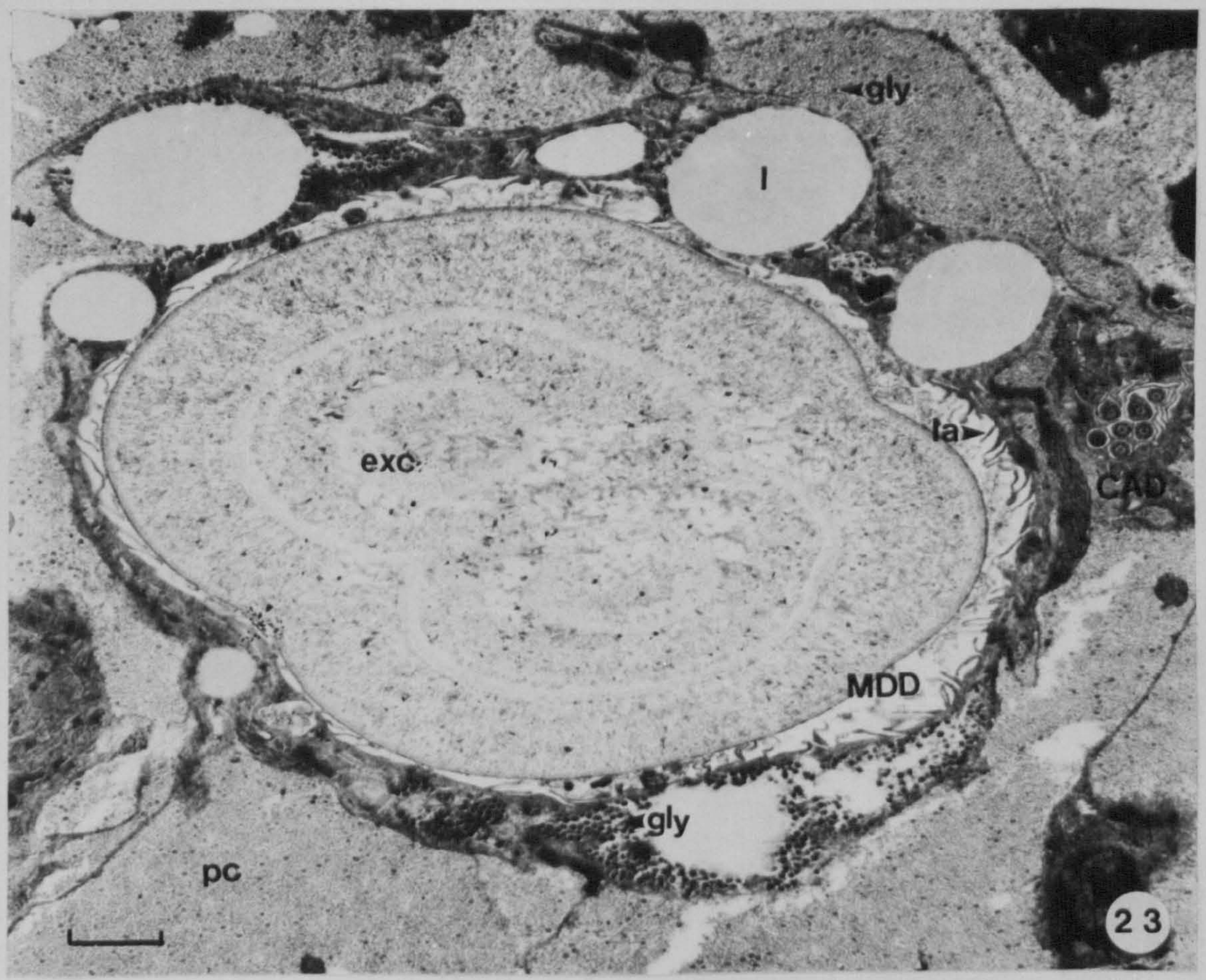
Plate 23 Excysted metacercaria.

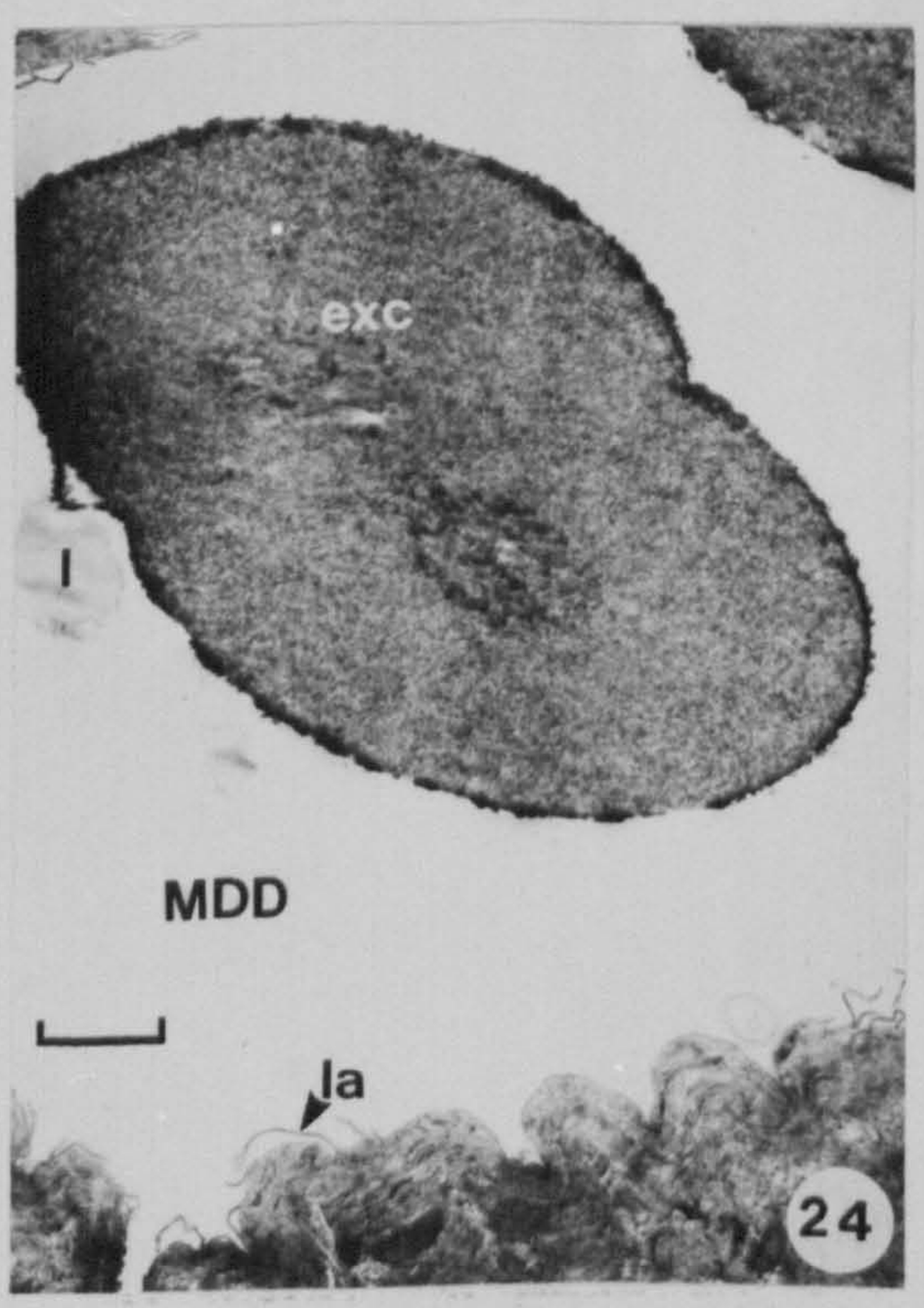
An excretory concretion (exc) fills the major descending duct (MDD) of the excretory sytem. The cytoplasm lining the MDD is thrown up into short lamellae (la) and contains deposits of glycogen (gly) and some lipid drops (l). An adjacent ciliated ascending duct (CAD) can be seen. The surrounding parenchyma cells (pc) are packed with stored glycogen.

The excretory concretion (exc) is formed of concentric layers of material.

Plates 24 and 25 Excysted metacercaria.

These sections show a different type of excretory concretion (exc) which is apparently made up of needle-like crystals (arrows). Around the periphery of these concretions is a layer of granular material (double arrows). Dense material can be seen adhering to the surface of the concretion.


gly
l
exc
la
CAD
MDD
gly
pc
23


exc
l
MDD
la
24

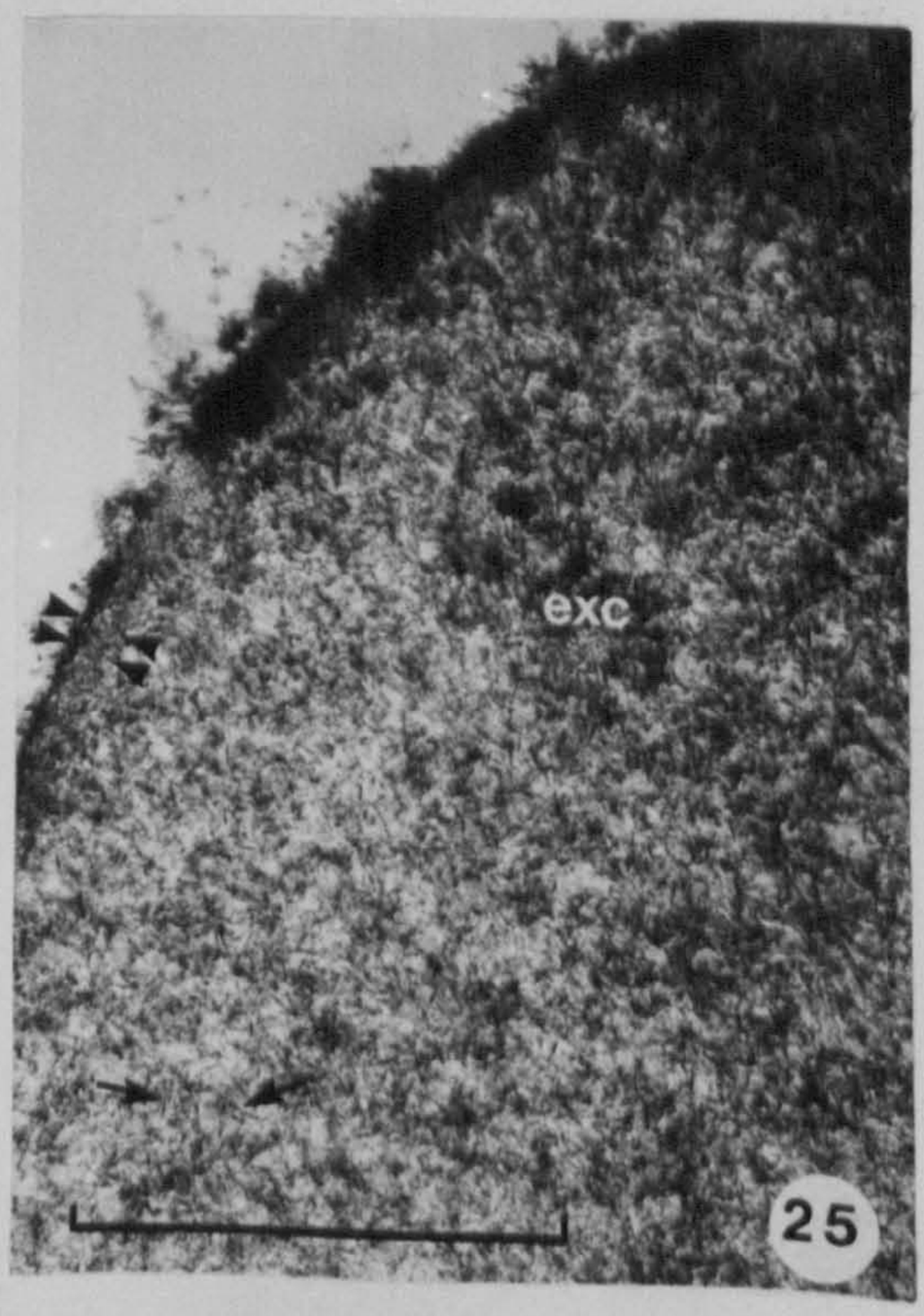

exc
25

Plate 26 Day 2 p.i. - location, abdomen.

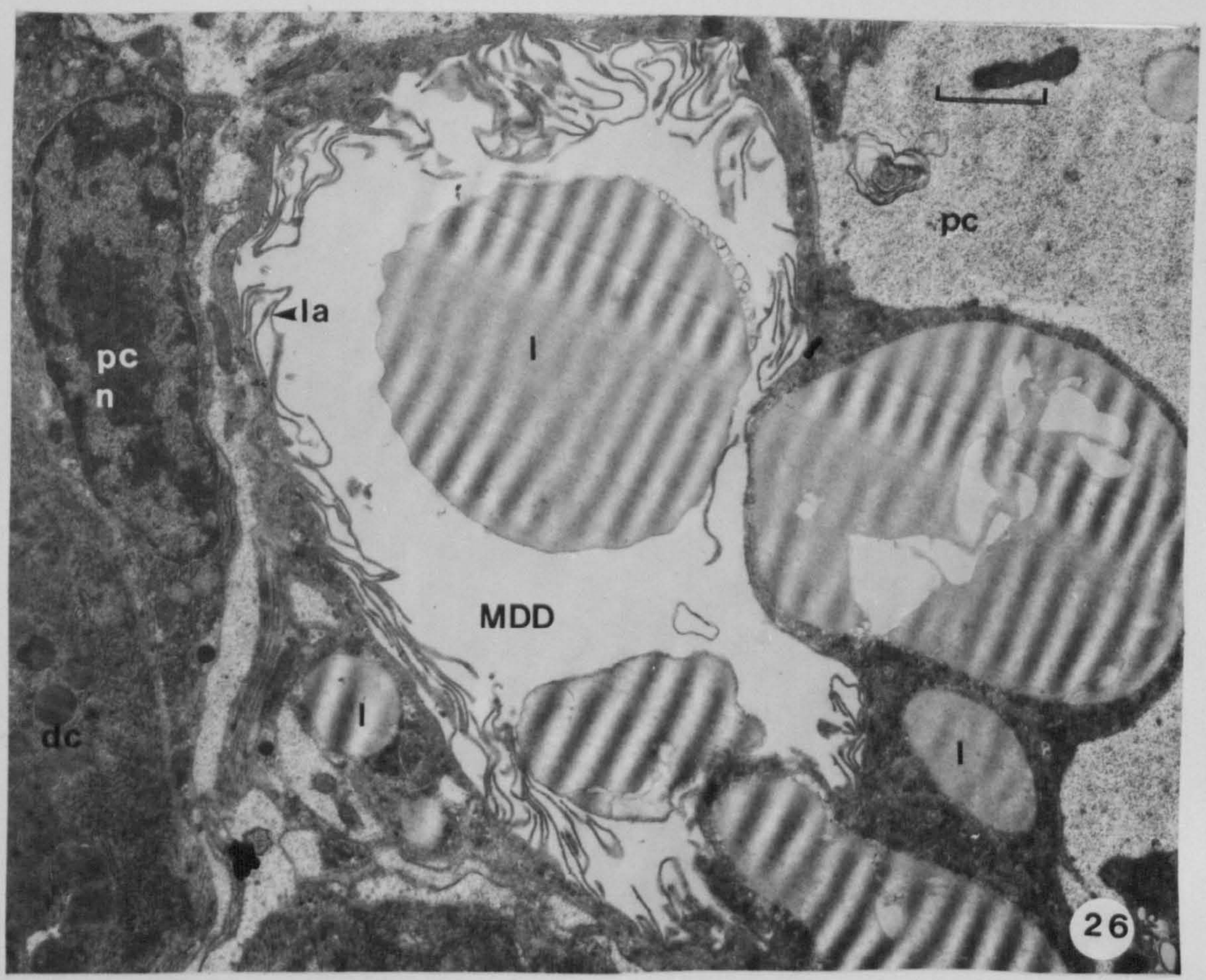
The major descending duct (MDD) contains large lipid drops (l), some of which are free in the lumen, others are embedded in the cytoplasm lining the duct. Glycogen is still present in the cytoplasm lining the MDD and the surface lamellae (la) are longer.

Plate 27 Day 9 p.i. - location, liver.

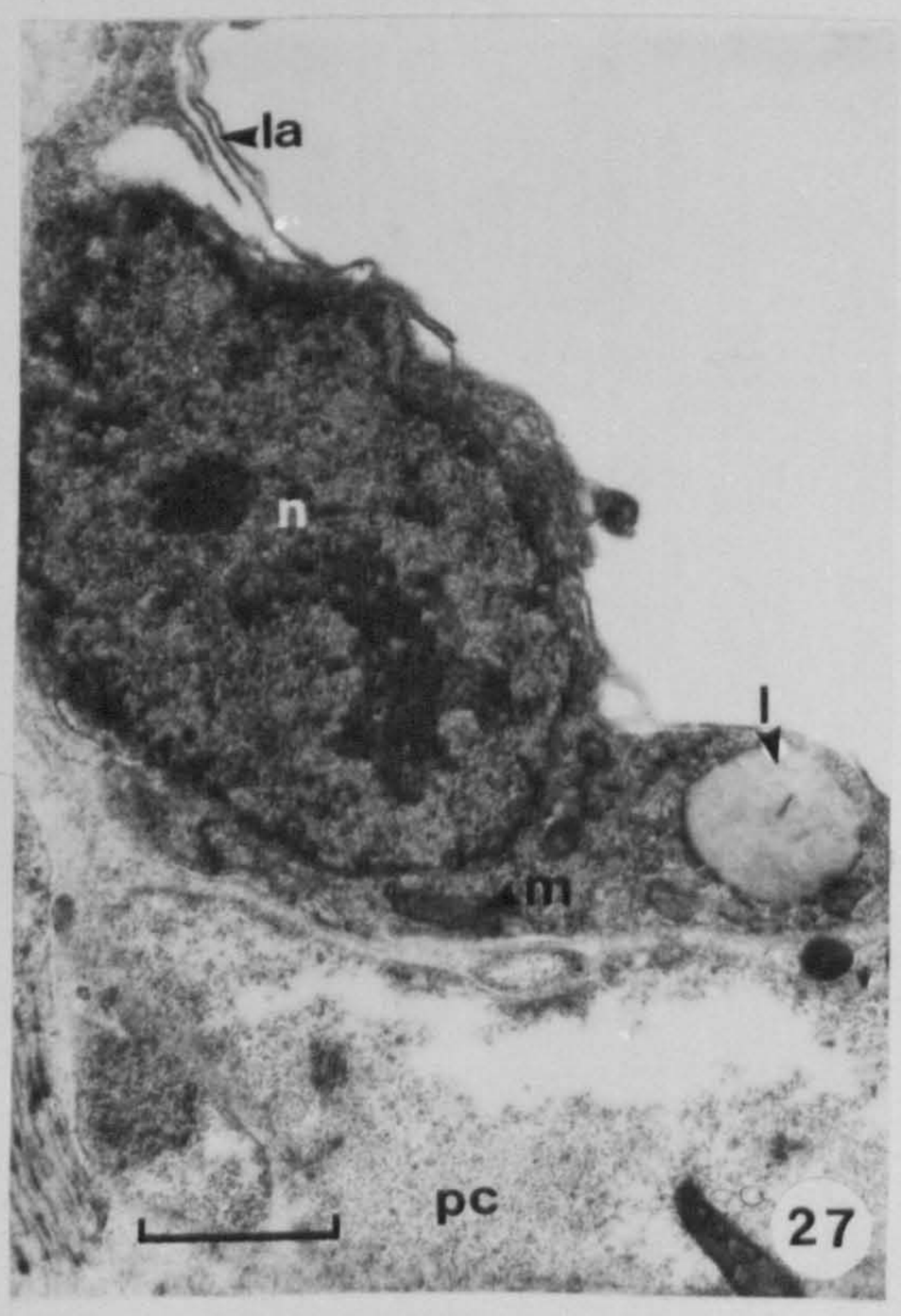
The nuclei (n) of the MDD often project into the lumen since the cytoplasm lining the duct is usually very thin.

Plate 28 Day 9 p.i. - location, liver.

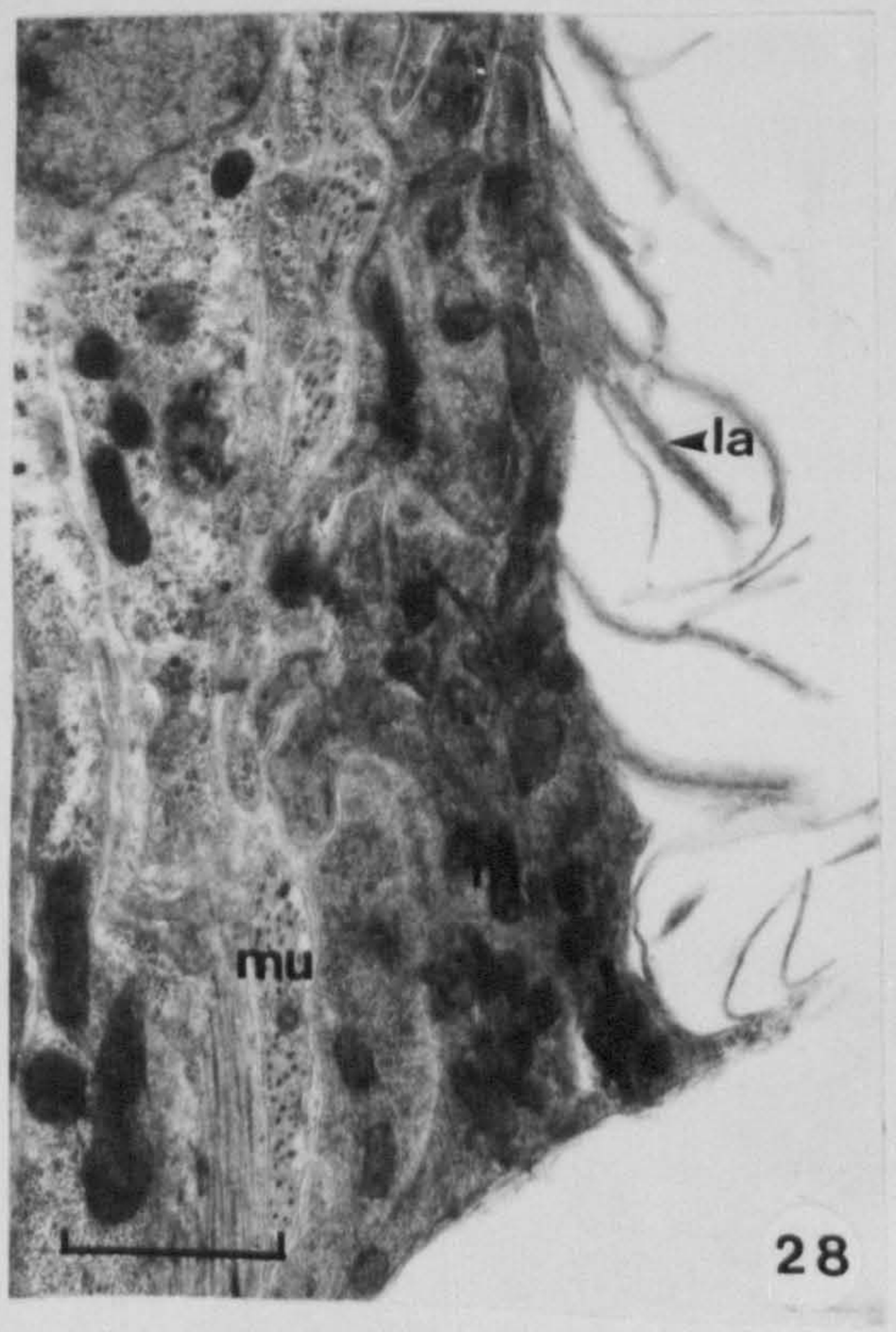
The thin cytoplasm lining the MDD contains small, dense mitochondria (m) and free ribosomes. Muscle cells (mu) surround the MDD but they do not form a continuous band.



26



27



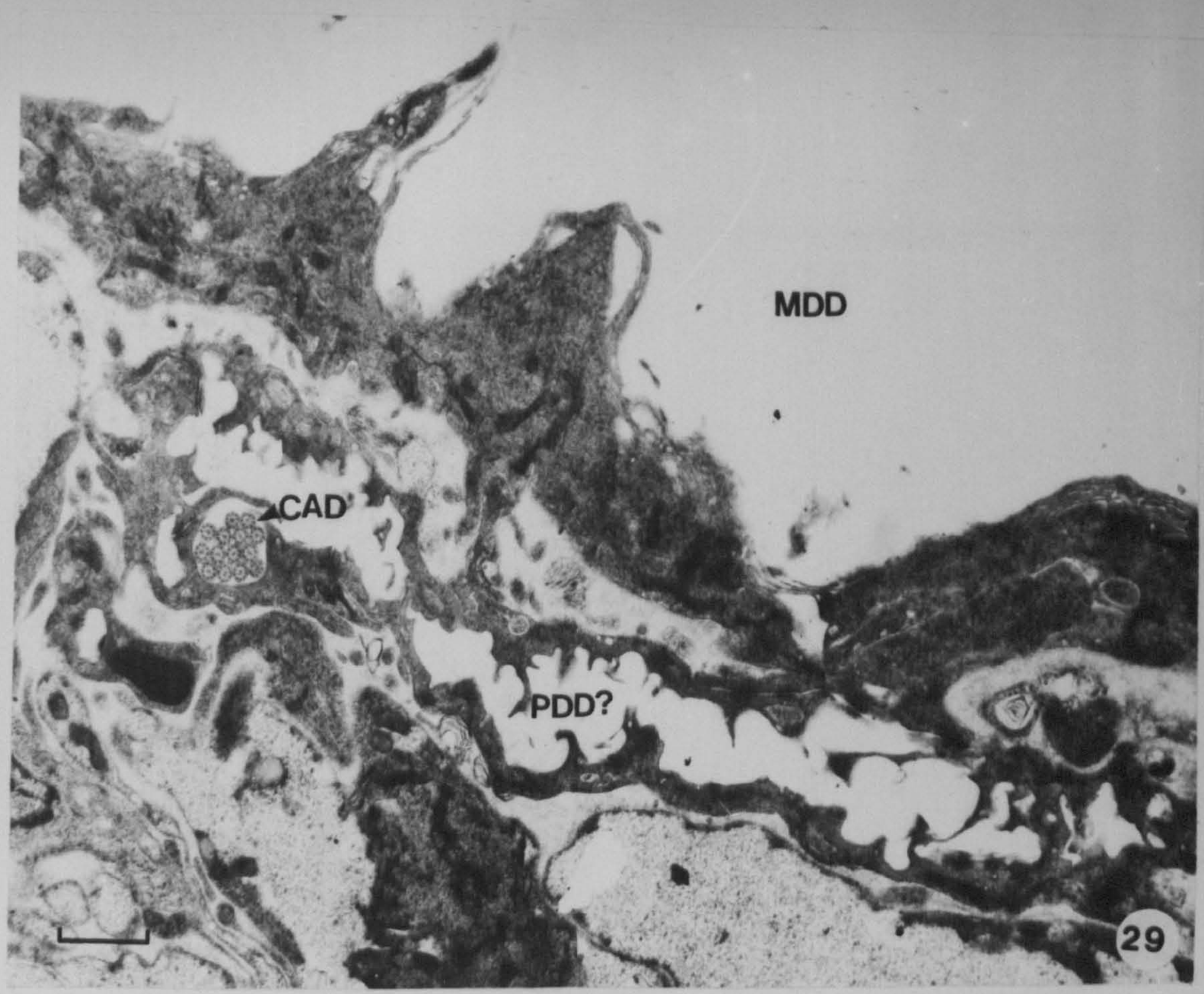
28

Plate 29 Day 7 p.i. - location, liver.

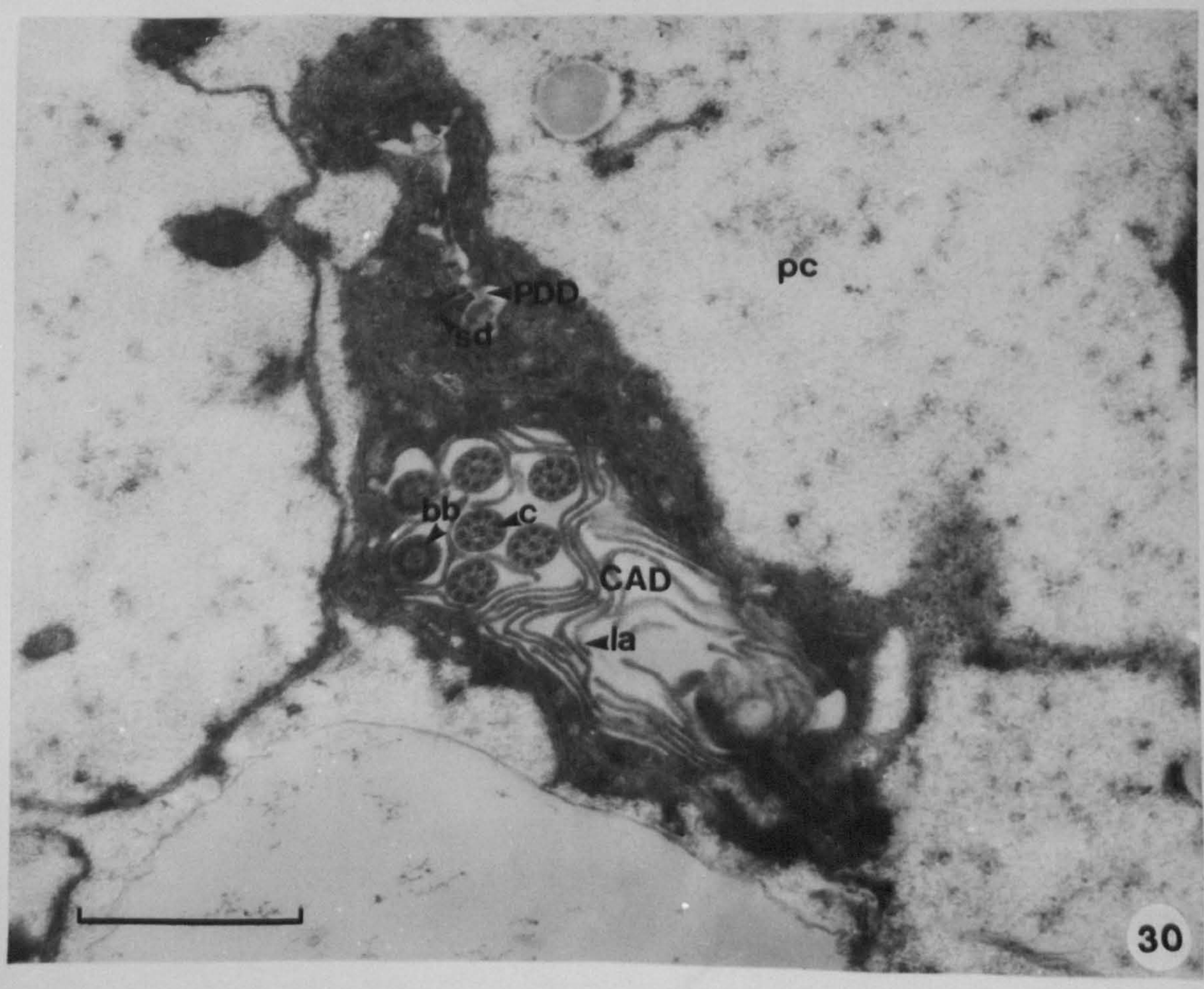
This section shows the major descending duct (MDD), a ciliated ascending duct (CAD) and a longitudinal section through what is probably a primary descending duct (PDD?).

Plate 30 Day 3 p.i. - location, abdomen.

A section through a ciliated ascending duct (CAD) which contains cilia (c) and lamellae (la) in its lumen and several ciliary basal bodies (bb) in the surrounding cytoplasm. Note the typical proximity of the primary descending duct (PDD) and the septate desmosome (sd) which links adjacent cells



29



30

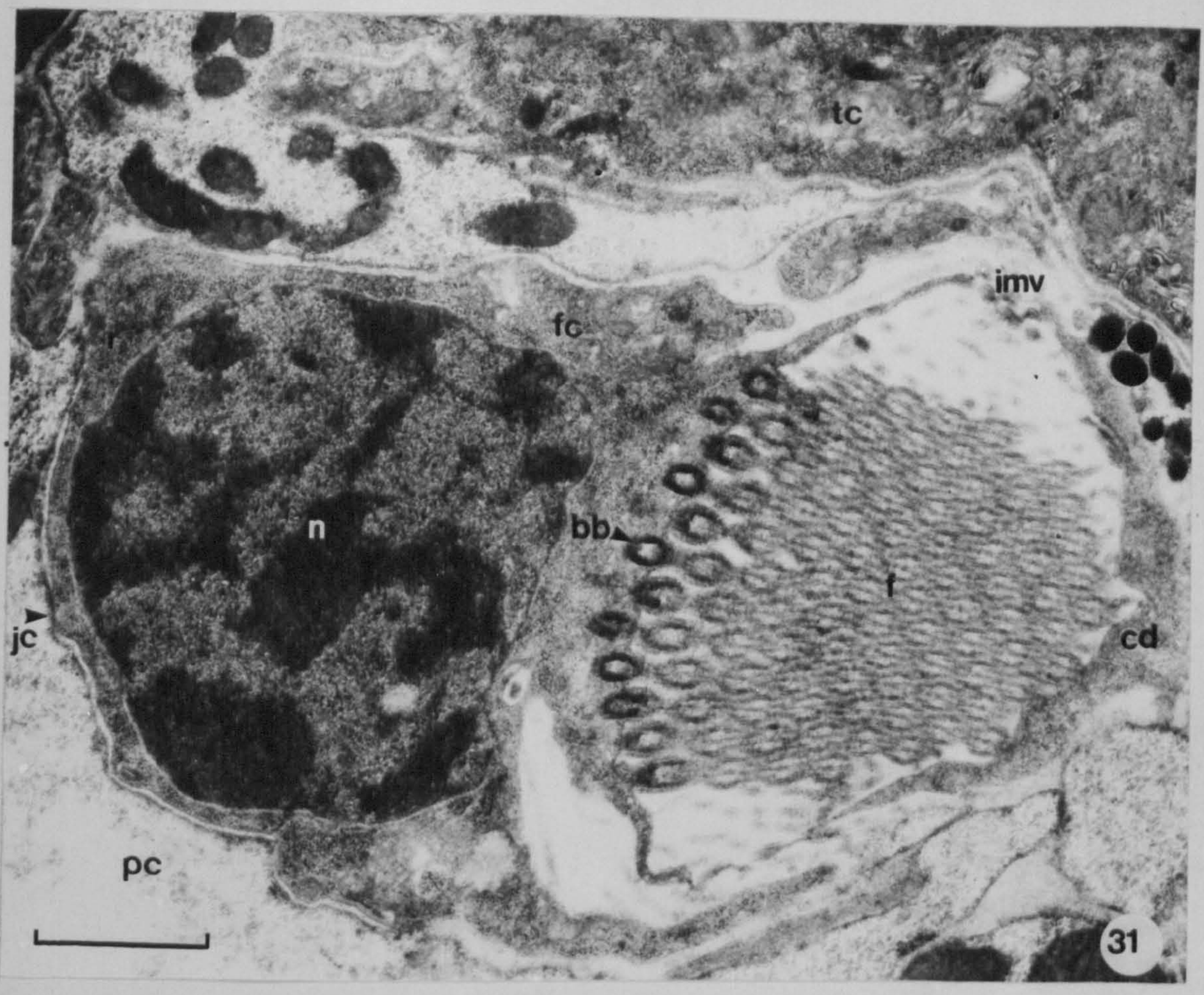
Plate 31 Day 3 p.i. - location, abdomen.

A longitudinal section through a flame cell to show the flame cell body (fc), the "flame" (f), and a cell of the collecting duct (cd).

The flame cell body contains a large nucleus (n) with dense patches of heterochromatin, numerous free ribosomes (r), and the ciliary basal bodies (bb). The flame cell and the cell of the collecting duct are joined by a series of interdigitating microvilli (imv). The flame cell body is connected to an adjacent parenchyma cell (pc) by a junctional complex (jc).

Plate 32 Day 3 p.i. - location, abdomen.

A transverse section through the barrel of the flame cell showing the central mass of cilia which form the "flame" (f), the internal (li) and the external (le) leptotriches and the interdigitating microvilli (imv) separated by a band of dense material (d) which connects the flame cell body to the cell of the conjoined tubule. The barrel of the flame cell is surrounded by a layer of interstitial material (im).


tc
imv
fc
n
bb
f
jc
cd
pc
31

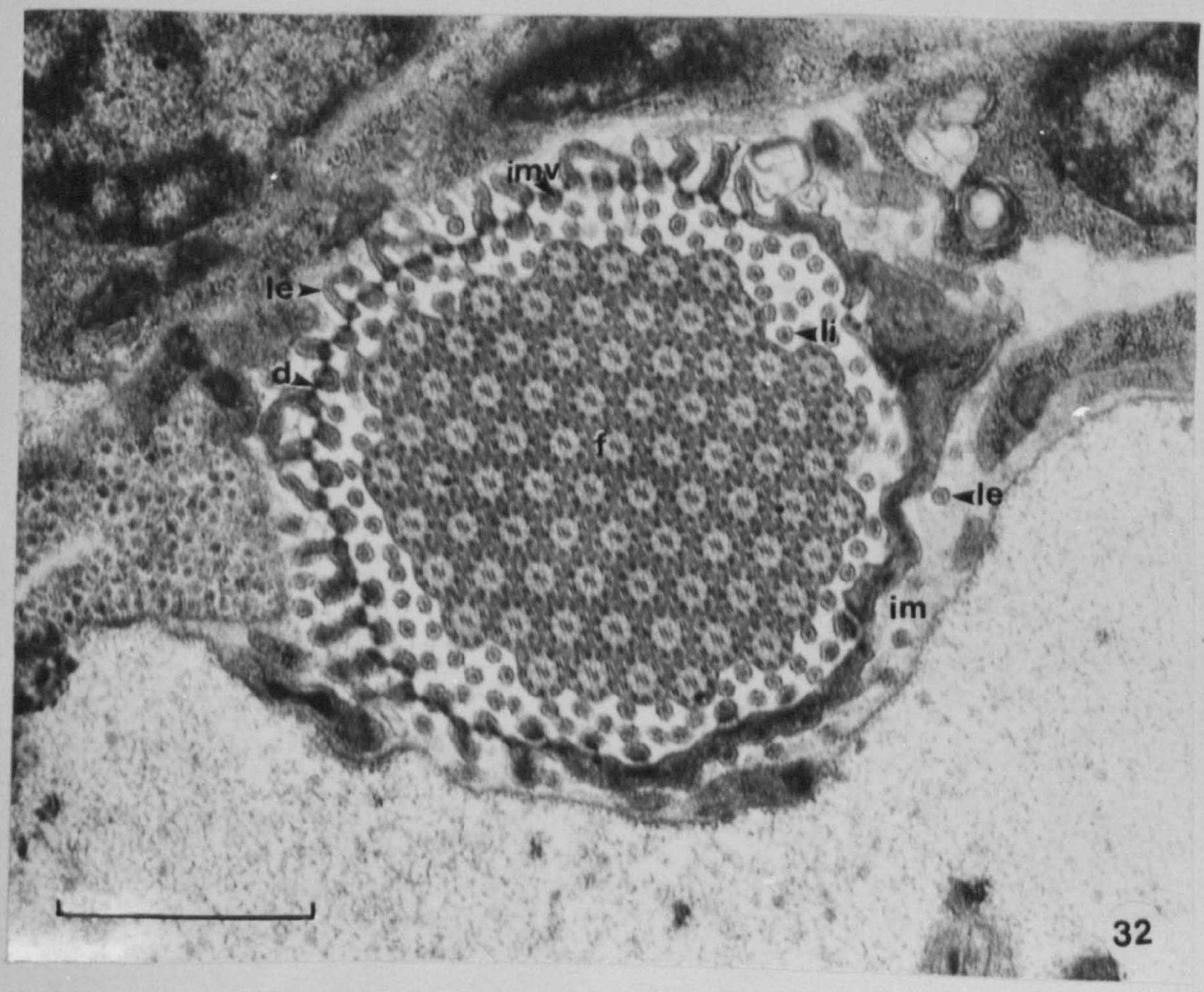

imv
le
li
d
f
le
im
32

Plate 33 Excysted metacercaria.

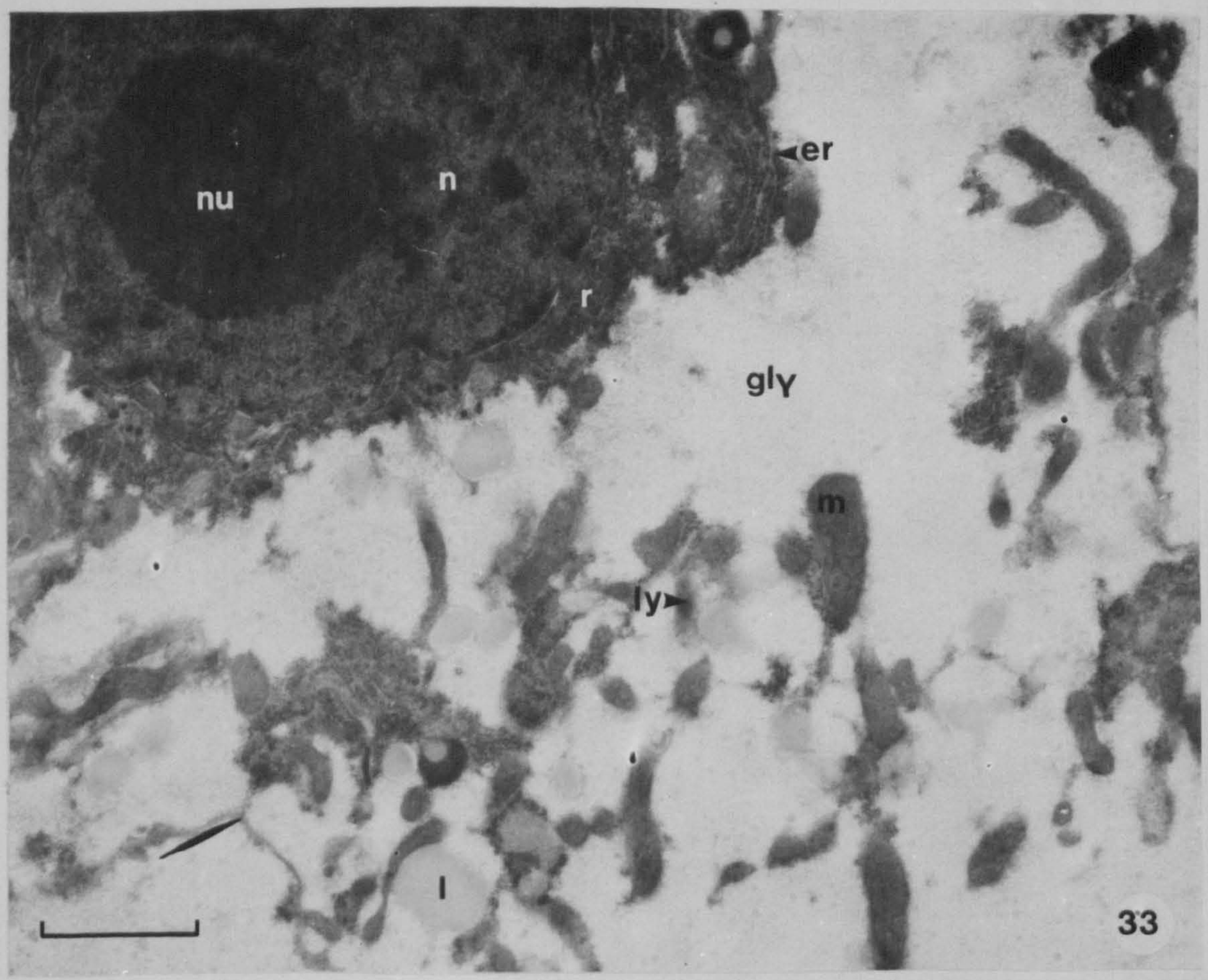
The nuclei of the parenchyma cells (n) are often situated in areas of dense cytoplasm which consists of free ribosomes (r) and GER (er). The rest of the cytoplasm of the cell contains smaller areas of ribosomes and GER, small lipid drops (l), glycogen (gly), mitochondria (m) and some primary lysosomes (ly) or δ bodies.

Plate 34 Day 3 p.i. - location, abdomen.

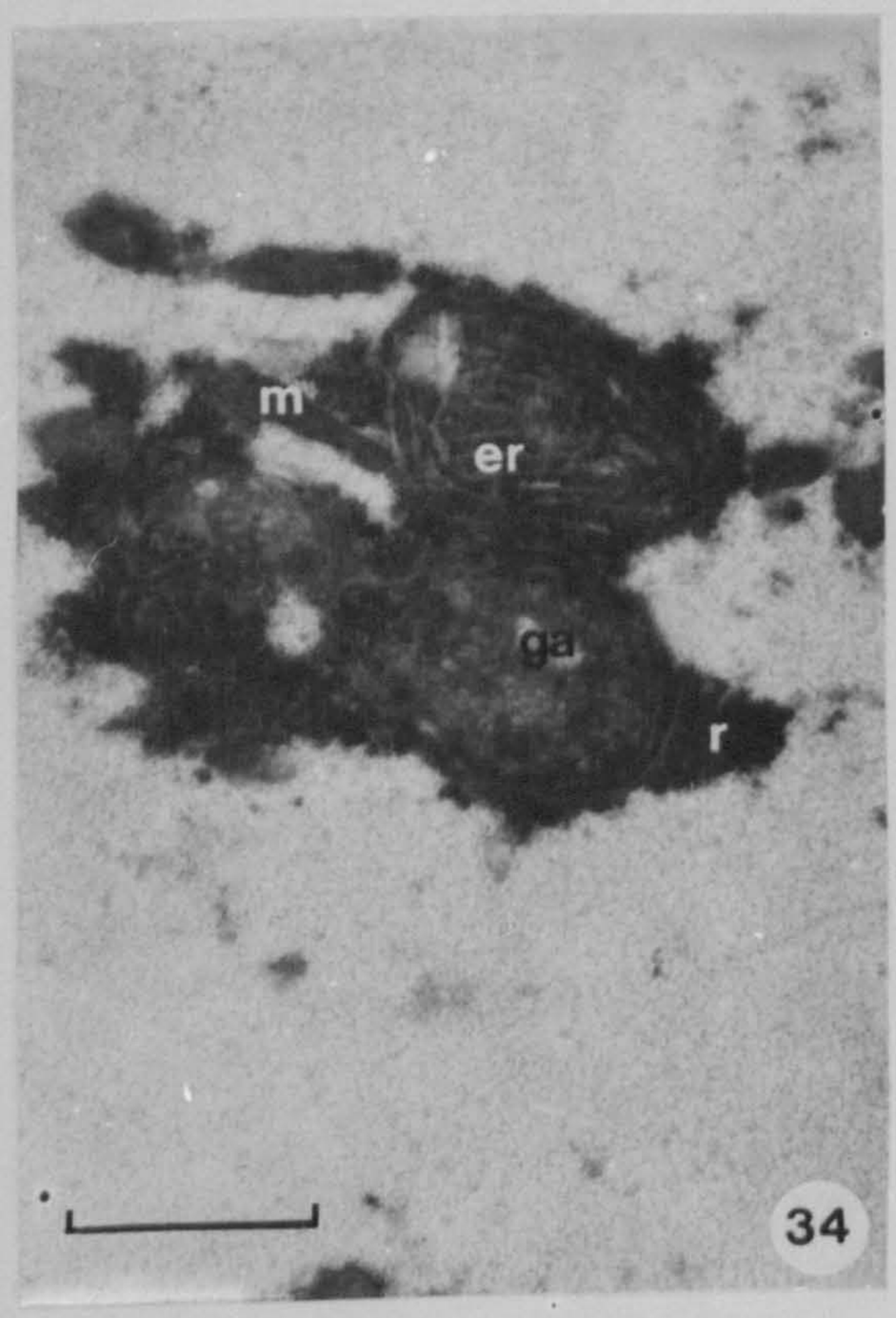
This section shows a dense area of cytoplasm in a parenchyma cell containing mitochondria (m), free ribosomes (r), GER (er) and an area of possible Golgi activity (ga).

Plate 35 Day 3 p.i. - location, abdomen.

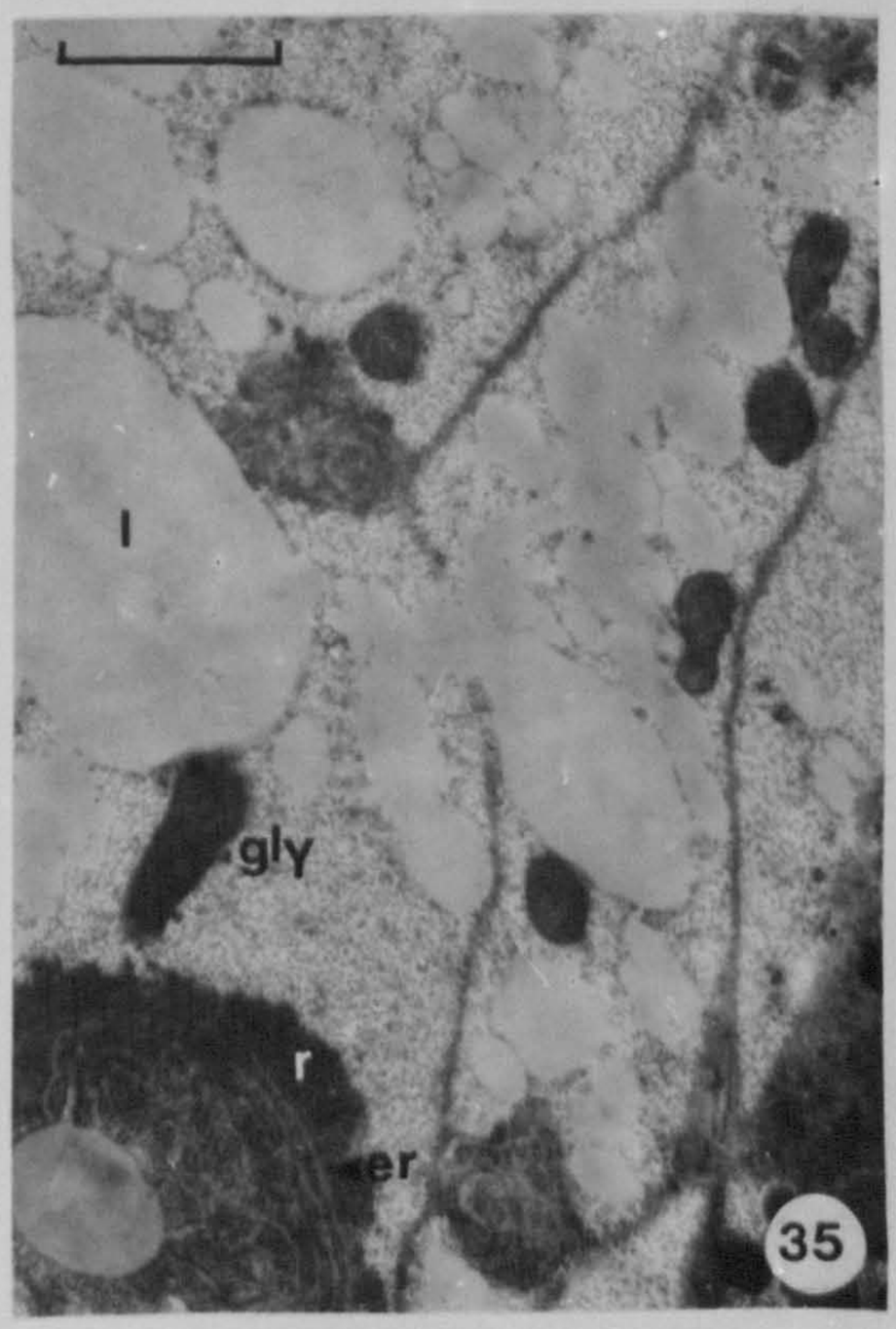
The ground substance of the cytoplasm of the parenchyma cell contains stored glycogen (gly) and numerous lipid drops (l) of various sizes.



33



34



35

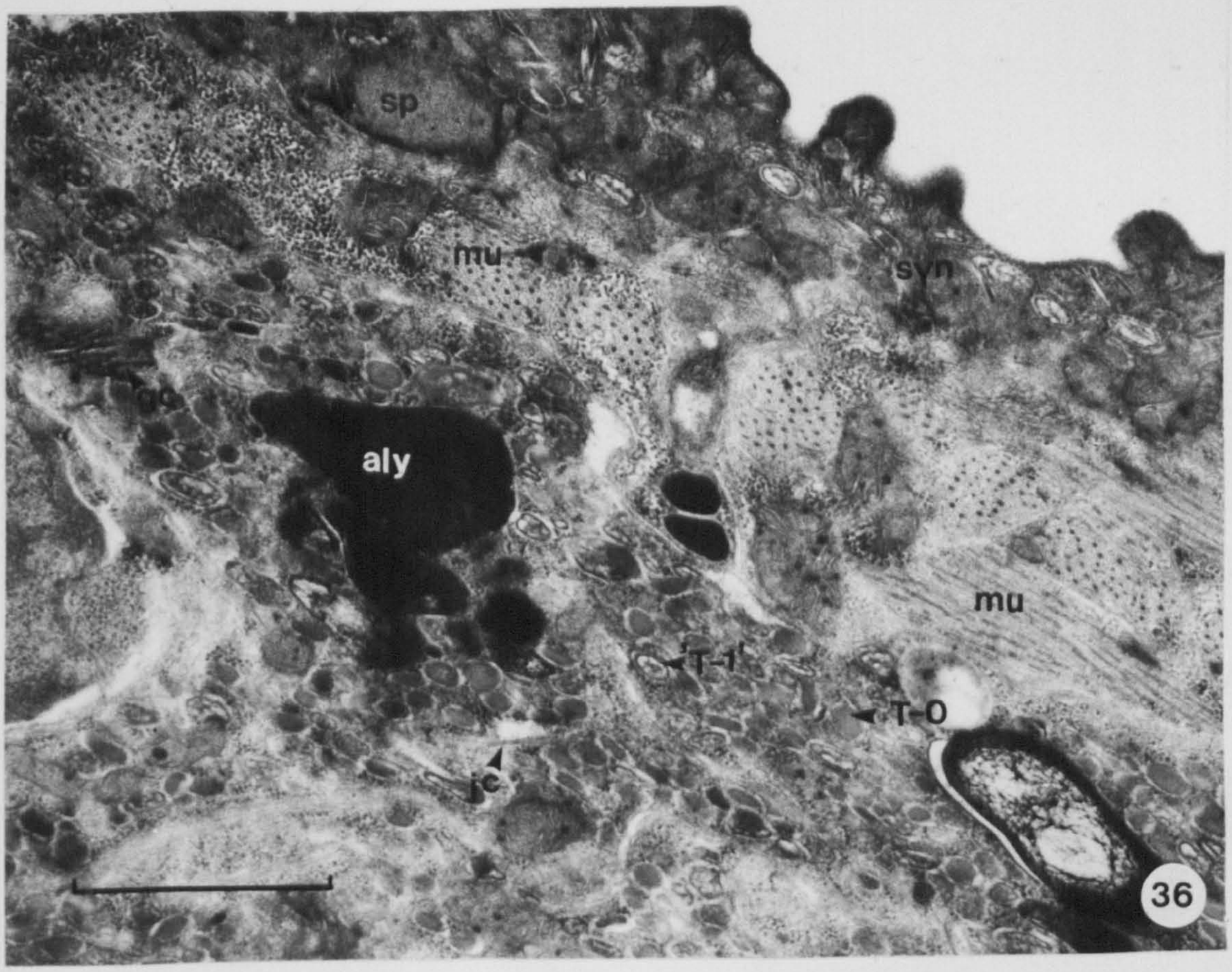
Plate 36 12 hours in vitro culture.

The tegumental syncytium contains typical spherical (T-O) granules. The tegumental cell bodies contain moderately dense, spherical (T-O) granules and some whose contents have concentrated in the centre of the granule to give a "cartwheel-like" appearance ("T-1"); the latter are similar to the oval (T-1) type of granule seen in flukes recovered from the livers of mice. Autophago-lysosomes are also present in the tegumental cell bodies(aly).

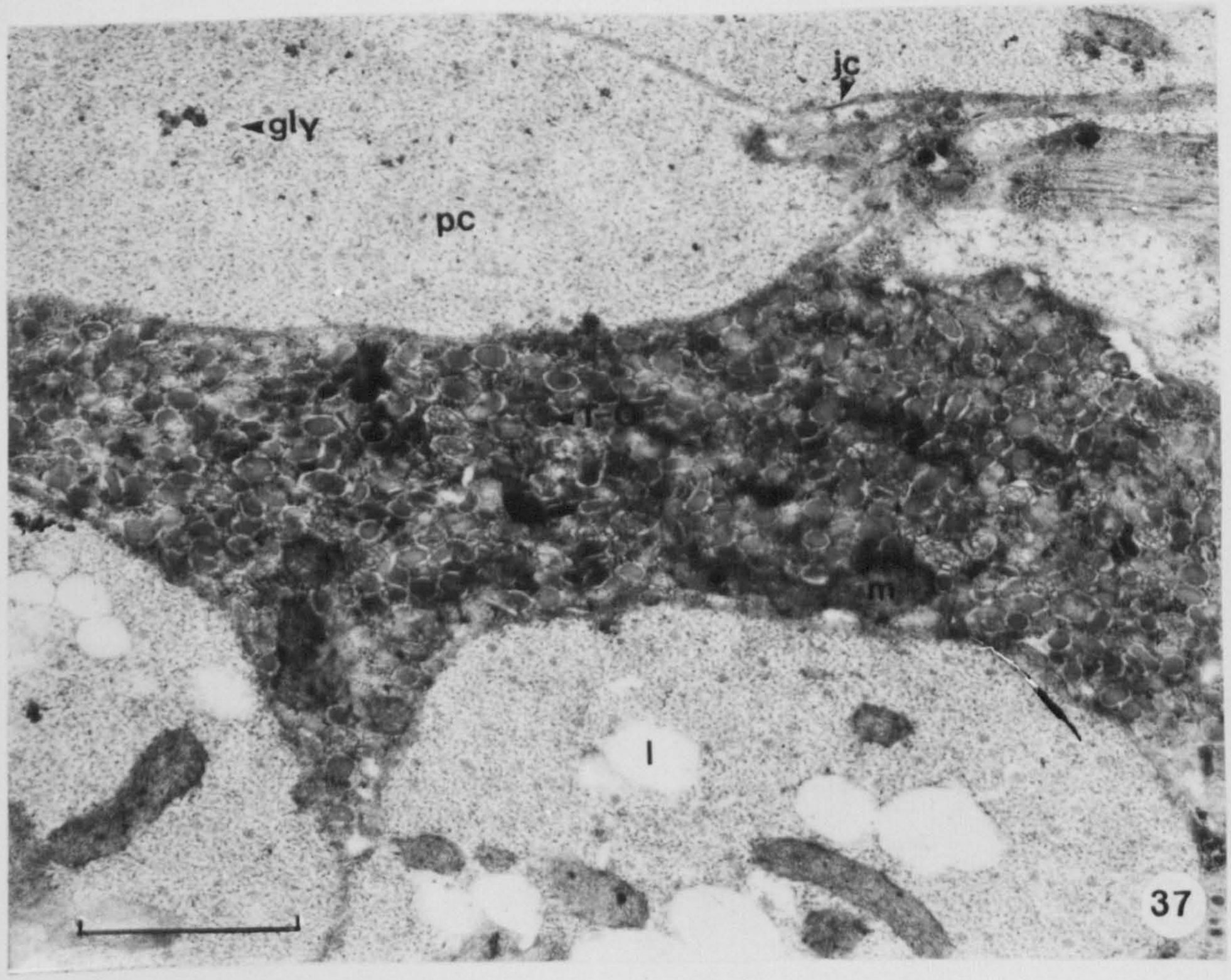
Plate 37 12 hours in vitro culture.

This section shows a tegumental cell packed with spherical (T-O) granules, some of which are homogeneous and dense, whilst others appear granular and more electron lucent.

The parenchyma cells (pc) contain glycogen (gly) and lipid drops (l); adjacent cells are connected in places by junctional complexes (jc).



36



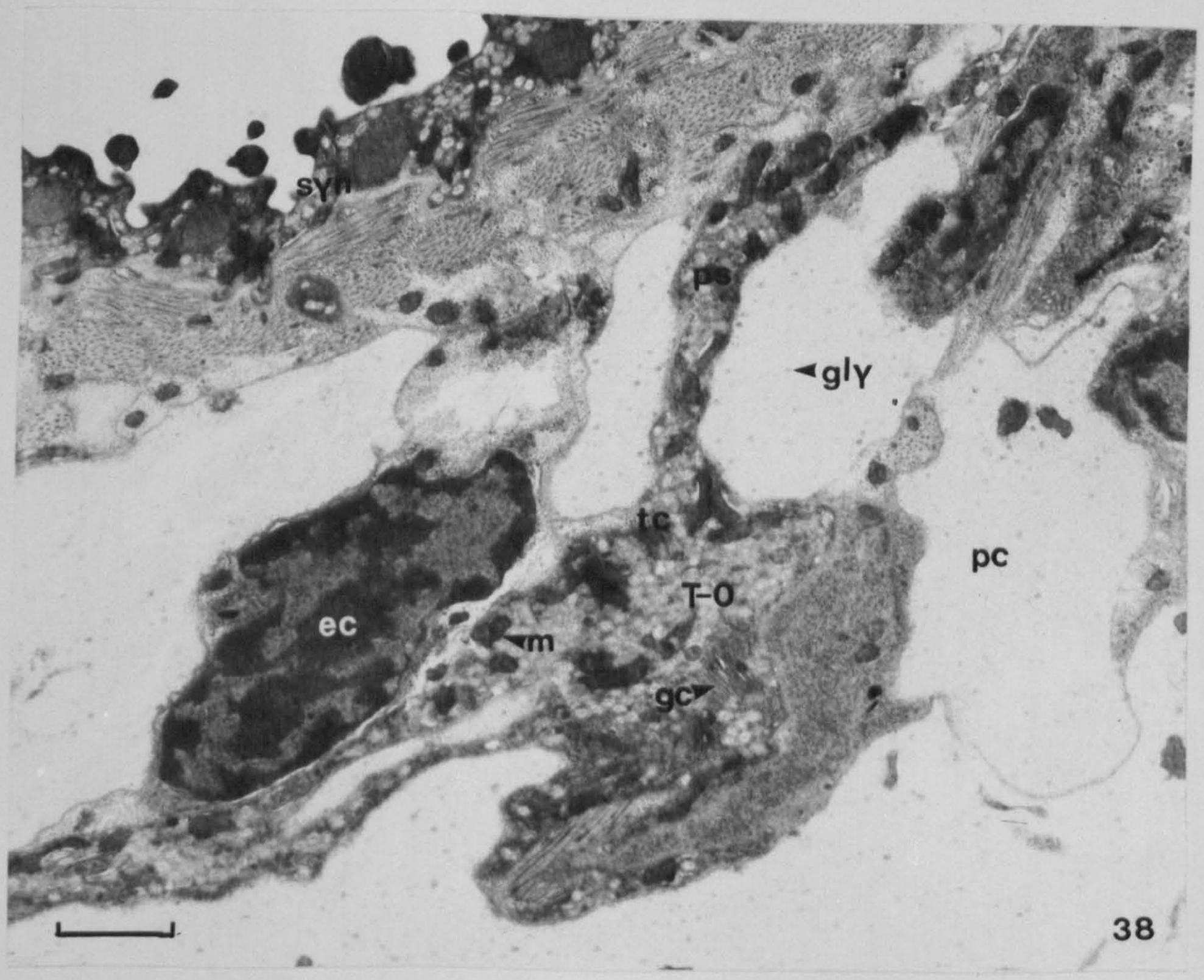
37

Plate 38 2 days *in vitro* culture.

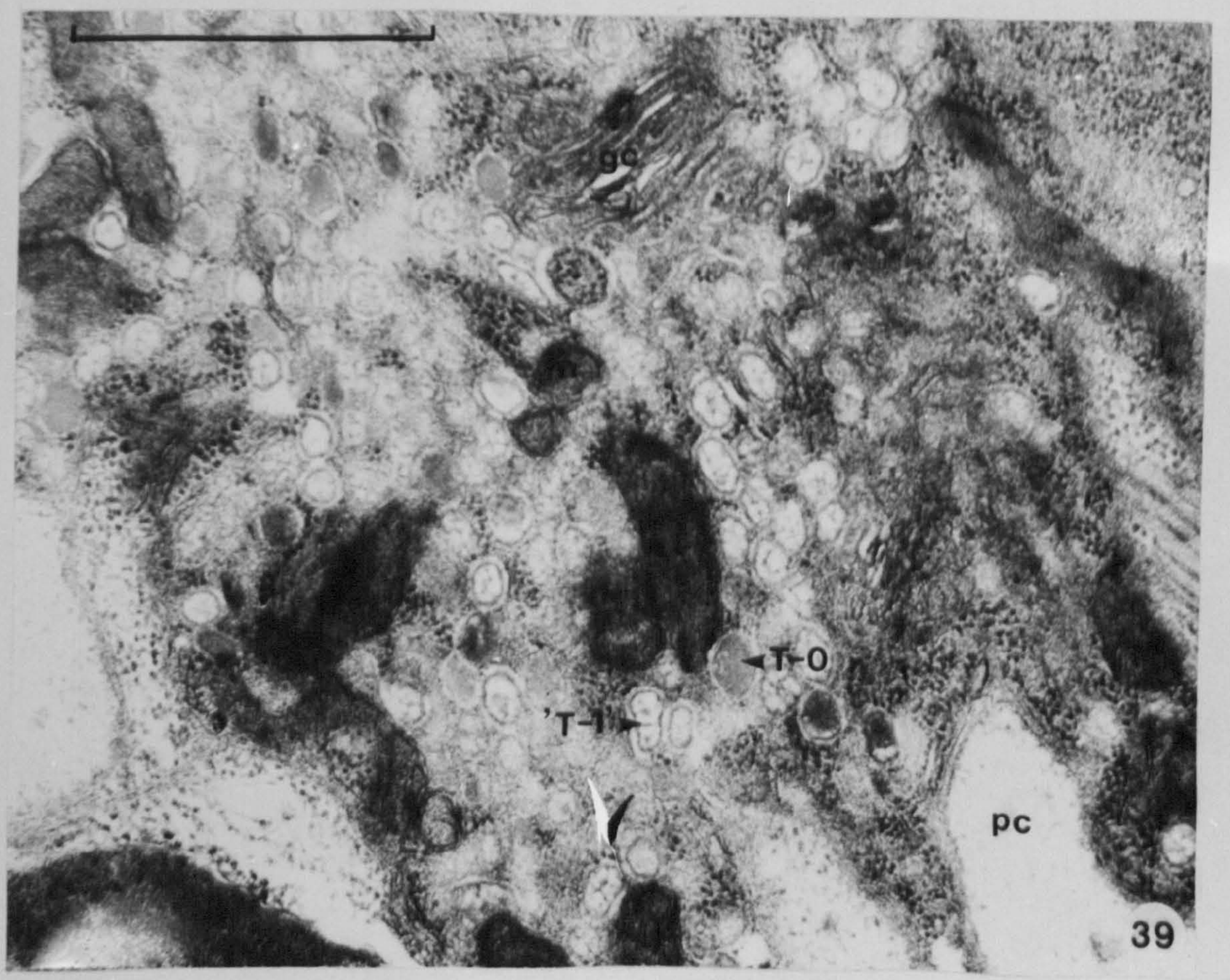
The spherical (T-0) secretory bodies in the tegumental cells (tc) appear to have lost some of their contents and are granular and electron lucent; the Golgi complexes (gc) and mitochondria (m) in these cells are prominent. The surrounding parenchyma cells (pc) contain deposits of glycogen (gly). An undifferentiated, "embryonic" cell (ec) can be seen next to the tegumental cell.

Plate 39 2 days *in vitro* culture

Some of the spherical (T-0) granules in the tegumental cell bodies appear normal (ie. they have a moderately dense, homogeneous content) but the majority have a granular content which is often condensed in the centre to give a "cartwheel-like" appearance which makes them appear similar to the T-1 granules seen in developing adults *in vivo* ("T-1"). The Golgi complexes are prominent (gc).



38



39

Plate 40 1 week *in vitro* culture

The tegumental syncytium contains only the spherical (T-O) type of secretory granule and they appear rather electron lucent. The mitochondria (m) and ground substance of the syncytium are comparatively dense. The fibrous interstitial material (im) between the syncytium and the underlying peripheral musculature is quite thick.

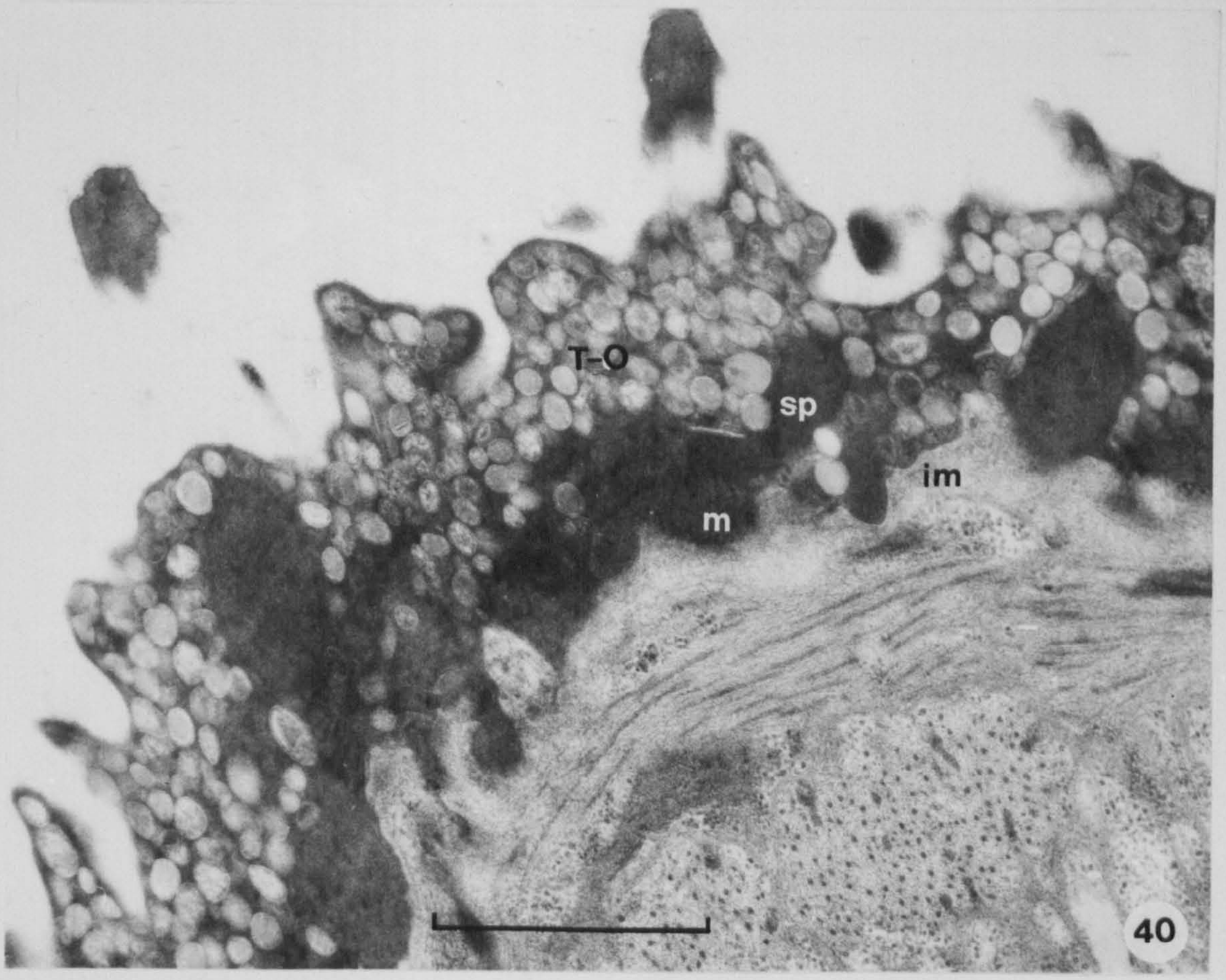
Plate 41 1 week *in vitro* culture.

This section through the tegumental syncytium shows an unusual type of ciliated sensory ending; the cilium (c) is enclosed by fingers of syncytium.

Plate 42 1 week *in vitro* culture

The tegumental spines (sp) often appear to be "sunk" into the underlying tissues although they are invariably surrounded by the sub-syncytial layer of interstitial material.





40



41



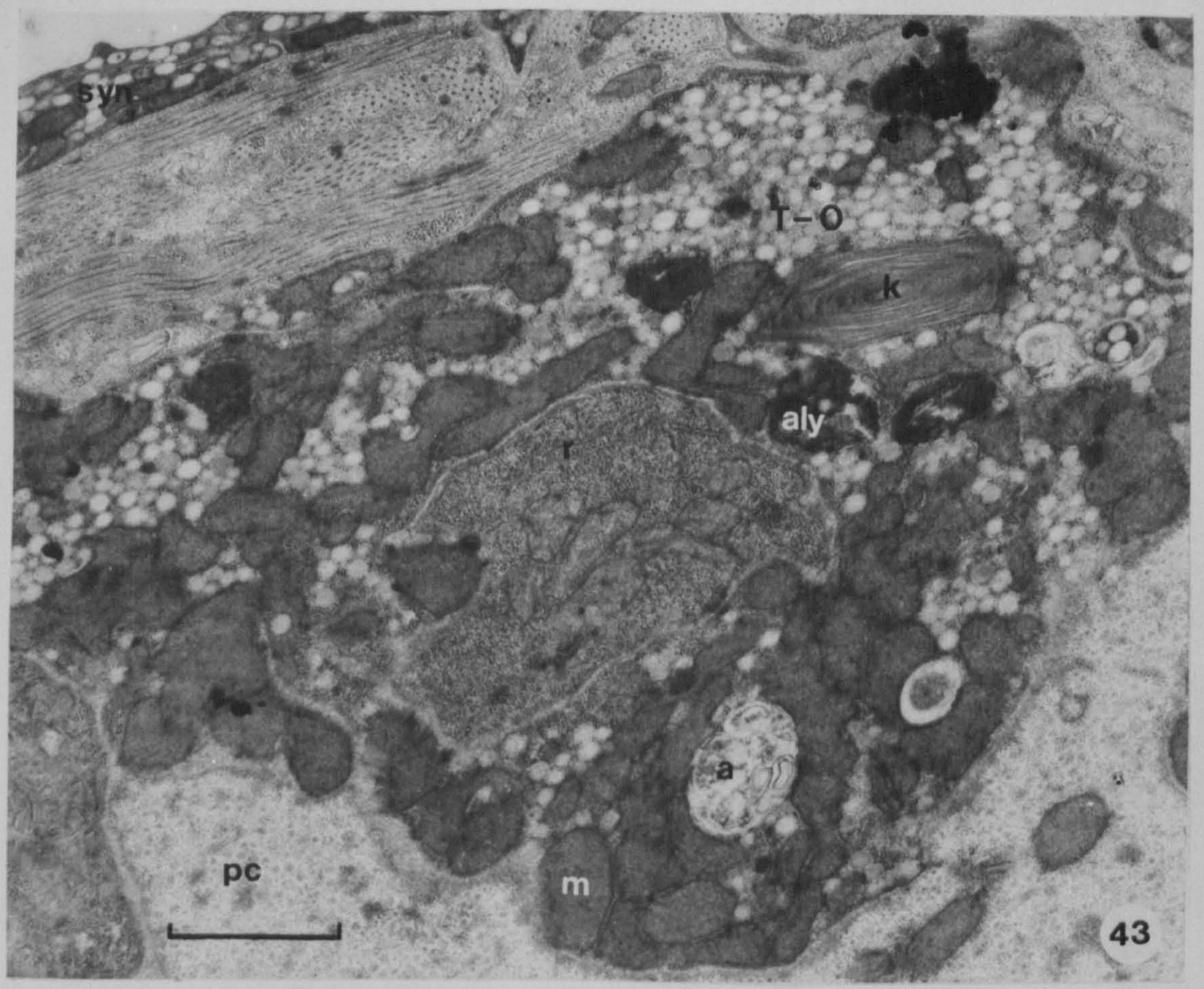
42

Plate 43 2 weeks *in vitro* culture.

The tegumental cell bodies contain spherical (T-O) granules which appear electron lucent. Numerous large mitochondria (m) and free ribosomes (r) are present in the cell bodies which also contain autophagosomes (a), autophagolysosomes (aly) and degenerating keratin scrolls (k). The adjacent parenchyma cells contain stored glycogen.

Plate 44 2 weeks *in vitro* culture.

The spherical (T-O) granules in the tegumental cell bodies show a wide variety of contents - some have a "cartwheel-like" appearance ("T-1") and are similar to the T-1 granules of developing adults *in vivo*. The Golgi complexes (gc) are prominent and consist of 8-10 stacked cisternae; there are numerous large mitochondria (m) and free ribosomes.



43



44

Plate 45 2 weeks *in vitro* culture.

The syncytium in the region of the ventral sucker is abnormal; it is vacuolated (v) and contains autophago-lysosomes (aly), and deposits of glycogen (gly).

Plates 46 and 47 2 weeks *in vitro* culture

These two sections show degenerating sensory endings (dse) in the tegumental syncytium in the ventral sucker region; there is considerable vacuolation of the sensory bulb and/or the surrounding syncytium (v).



45



46



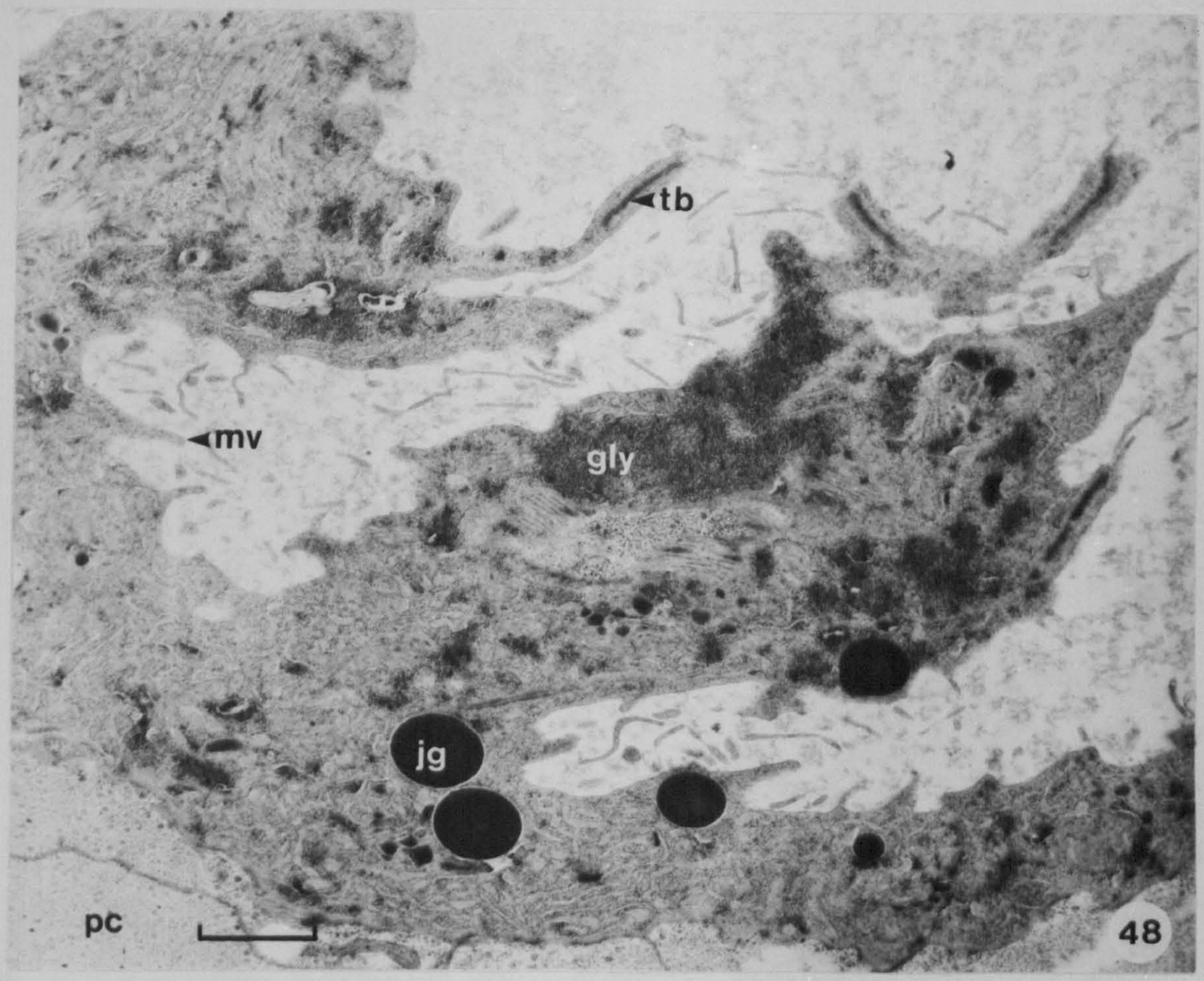
47

Plate 48 12 hours in vitro culture.

The caecal epithelium contains some large, dense, "juvenile" type secretory granules (jg) and areas of glycogen (gly). The terminal bars (tb) or desmosomes which connect adjacent cells, project into the lumen. Small microvilli (mv) are present on the luminal surface of the caecal cells.

Plate 49 12 hours in vitro culture.

Areas of possible Golgi activity (ga) occur in the caecal epithelium and they are often associated with groups of small vesicles (ves). The GER (ger) is fragmented and there are numerous free ribosomes.


tb
mv
gly
jg
pc
48

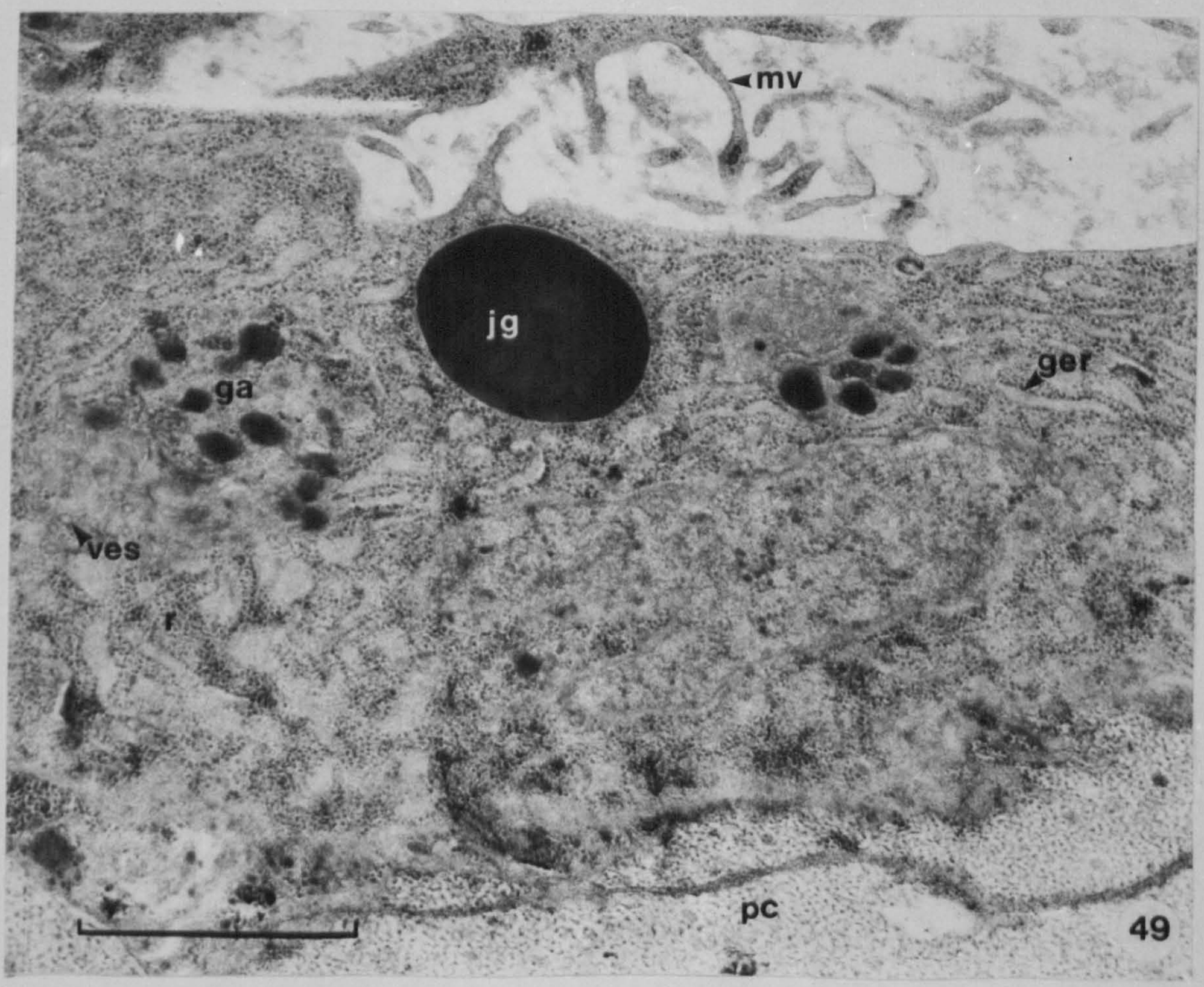

mv
jg
ga
ger
ves
pc
49

Plate 50 2 days *in vitro* culture.

This section is typical of the more abnormal areas of caecal epithelium in cultured flukes. The GER (ger) is fragmented and often swollen and there are numerous free ribosomes; secretory granules are absent or infrequent. The epithelium is thin and the surface lamellae (or microvilli) are relatively short and infrequent.

Plate 51 2 weeks *in vitro* culture.

In some areas of caecal epithelium there are numerous "adult" type secretory bodies (ag). However, their contents are often less dense than usual and they may appear granular around the periphery. The GER (ger) is fragmented and is not orientated, free ribosomes are numerous. Junctional complexes (jc) have developed between the caecal cells and processes arising from adjacent parenchyma cells.

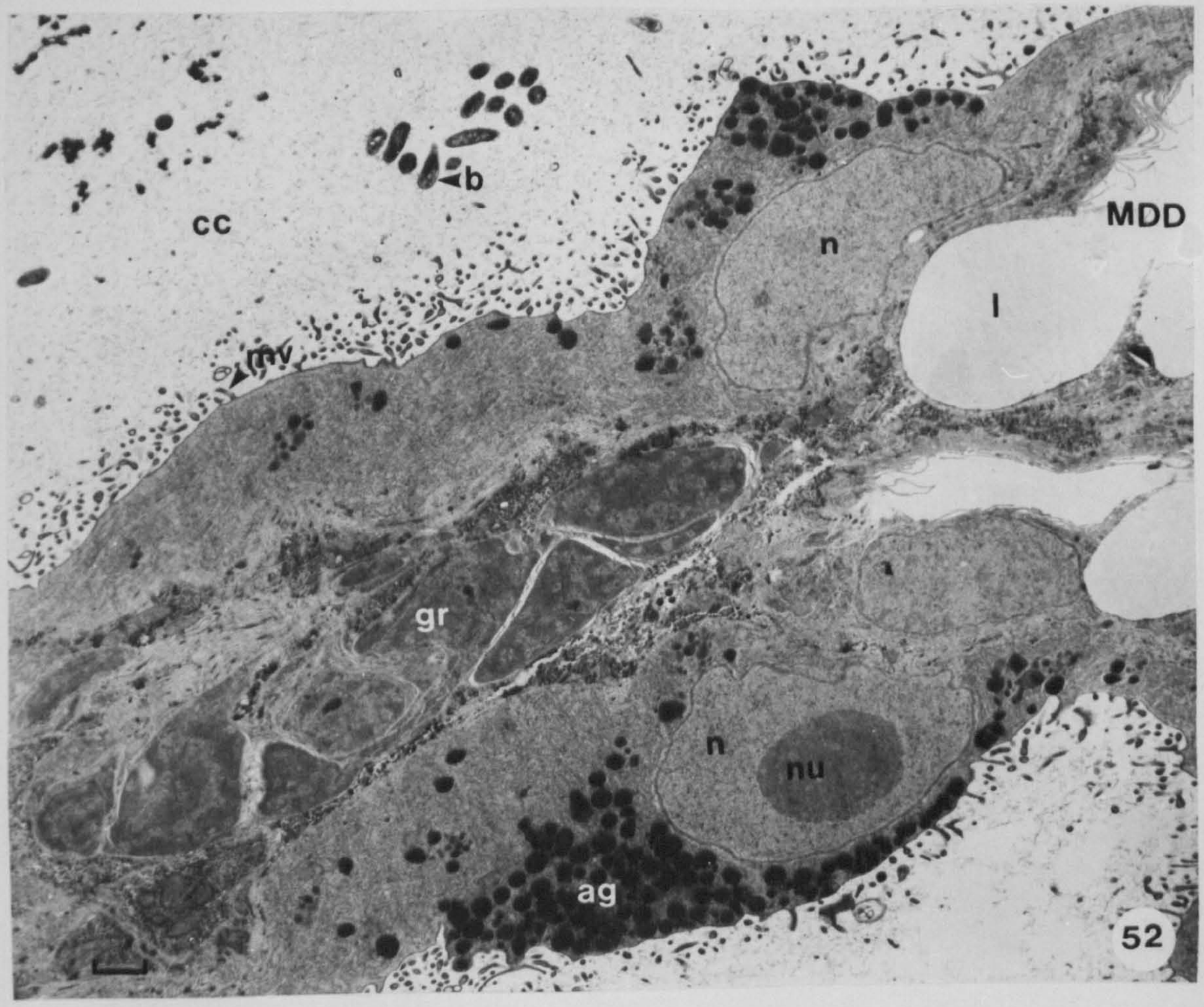
m
mu
bi
m
gly
ger
pc
50
nu
jc
n
bi
ja
ger
ag
im
51

Plate 52 2 weeks *in vitro* culture.

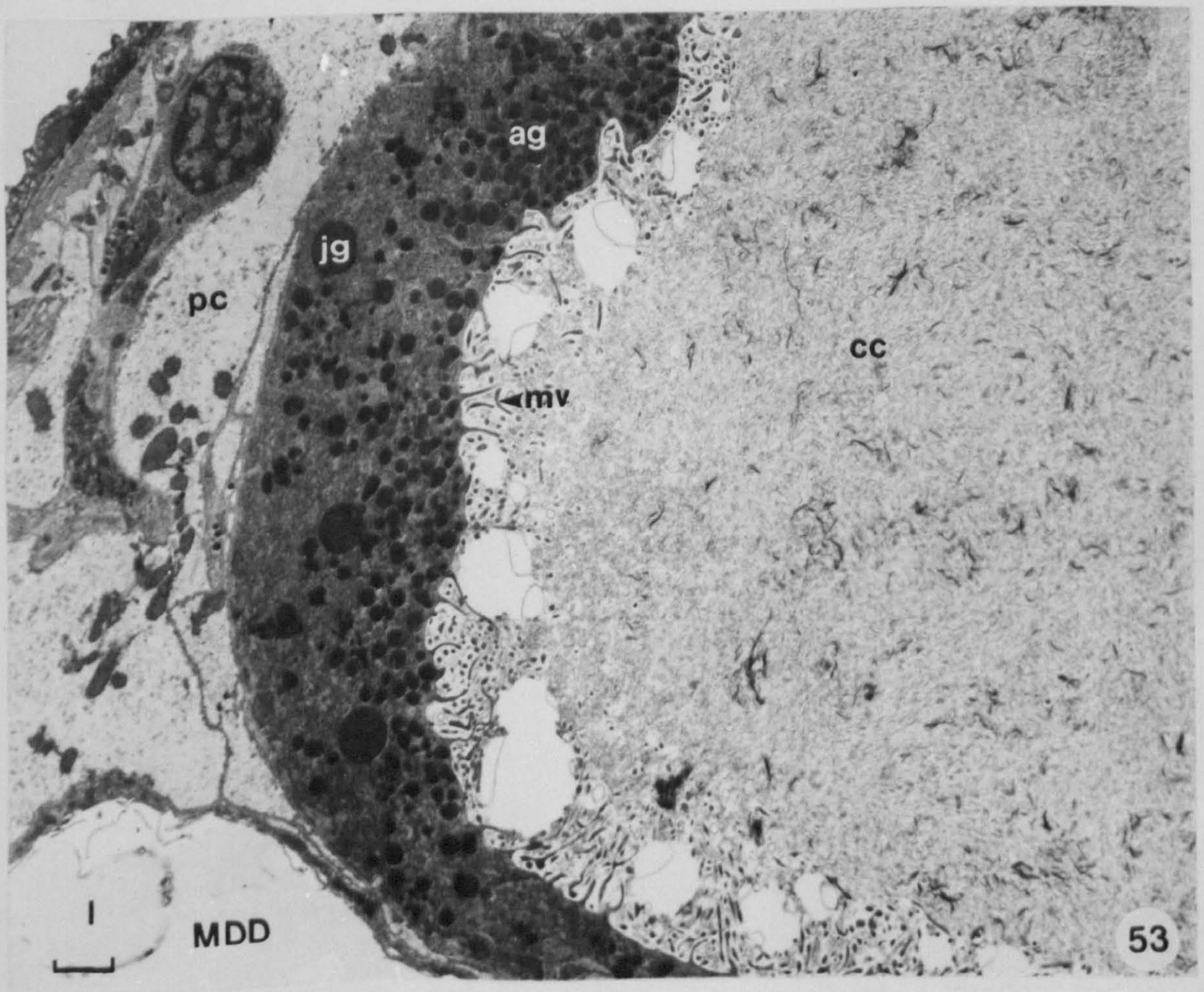
Areas of apparently normal "adult" type secretory granules (ag) are often associated with the nuclei (n) in the caecal epithelium. The surface microvilli (mv) have not increased in numbers significantly since the metacercarial stage.

Plate 53 2 weeks *in vitro* culture.

In this section "adult" (ag) and "juvenile" type (jg) secretory granules can be seen together in the caecal epithelium. The fibrous caecal contents (cc) are typical and probably represent the black solids which accumulate in the gut during *in vitro* culture.



52



53

Plate 54 2 weeks *in vitro* culture.

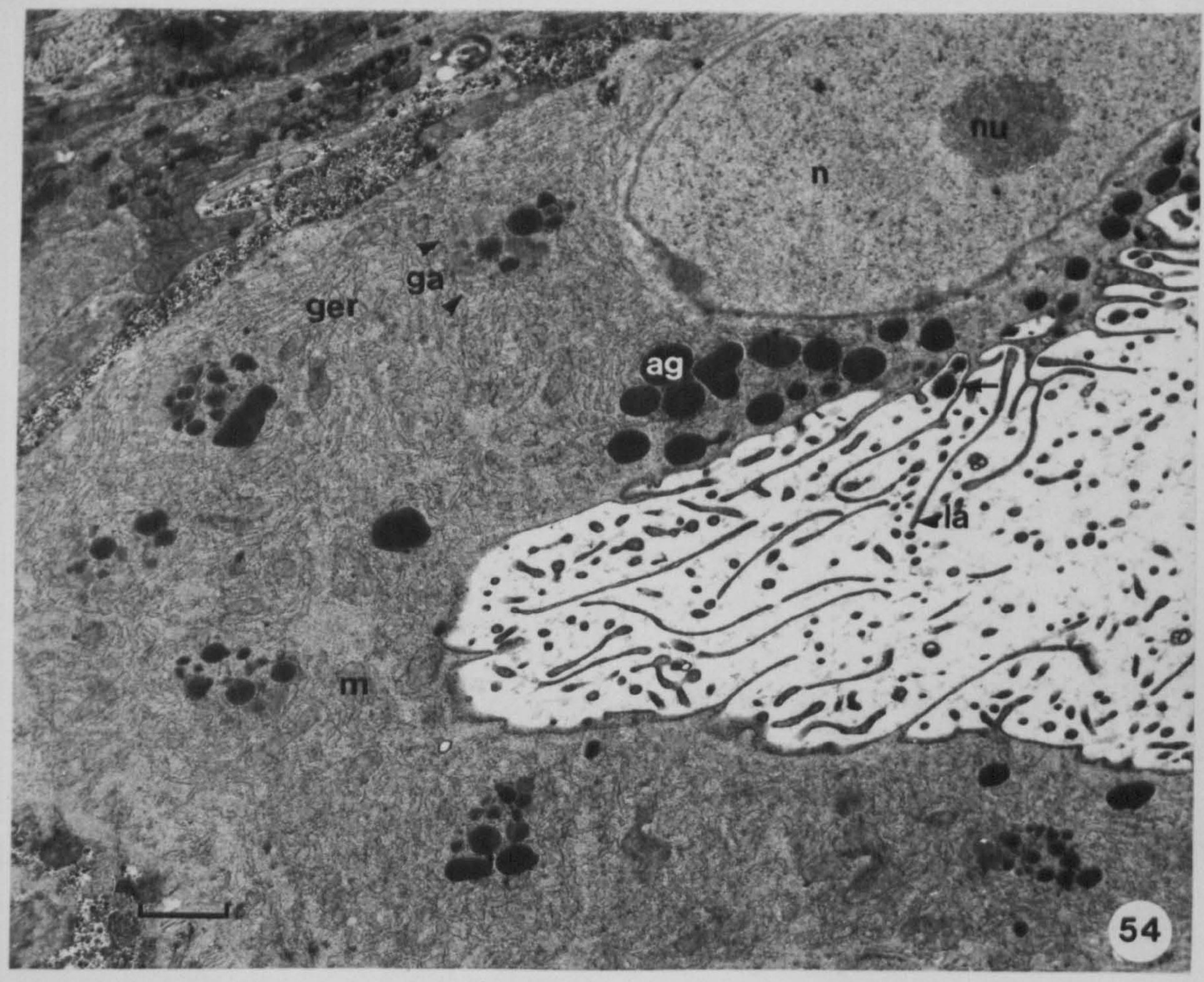
Areas of possible Golgi activity (ga) are present in the caecal epithelium and are probably responsible for producing the "adult" type granules (ag). Some dense material (arrowed) can be seen between the surface lamellae/microvilli (la) and this may represent secreted material.

Plate 55 2 weeks *in vitro* culture.

Junctional complexes (jc) between the caecal epithelium and processes (pp) of the parenchymal cells (pc) are present.

Plate 56 2 weeks *in vitro* culture.

Numerous invaginations (bi) of the basal plasmalemma of the caecal epithelium are present.



54



55



56

Plate 57 12 hours in vitro culture.

An excretory concretion (exc) can be seen in the major descending duct (MDD) of the excretory system. The cytoplasm lining the duct contains glycogen (gly) and is connected to adjacent parenchyma cells (pc) by junctional complexes (jc).

The lumen of the ciliated ascending duct (CAD) contains typical "9+2" cilia (c) and lamellae. The cells of the CAD are connected to each other by a septate desmosome (sd) and to the surrounding parenchyma cells (pc) by a junctional complex (jc).

Plate 58 1 week in vitro culture.

The cytoplasm lining the excretory duct contains lipid drops (l) and some stored glycogen (gly). Large lipid drops can be seen in the process of extrusion into the lumen. The surface lamellae (la) have increased in length.

jc

MDD

jc

pc

la

exo

CAD

sd

57

la

n

l

l

glγ

pc

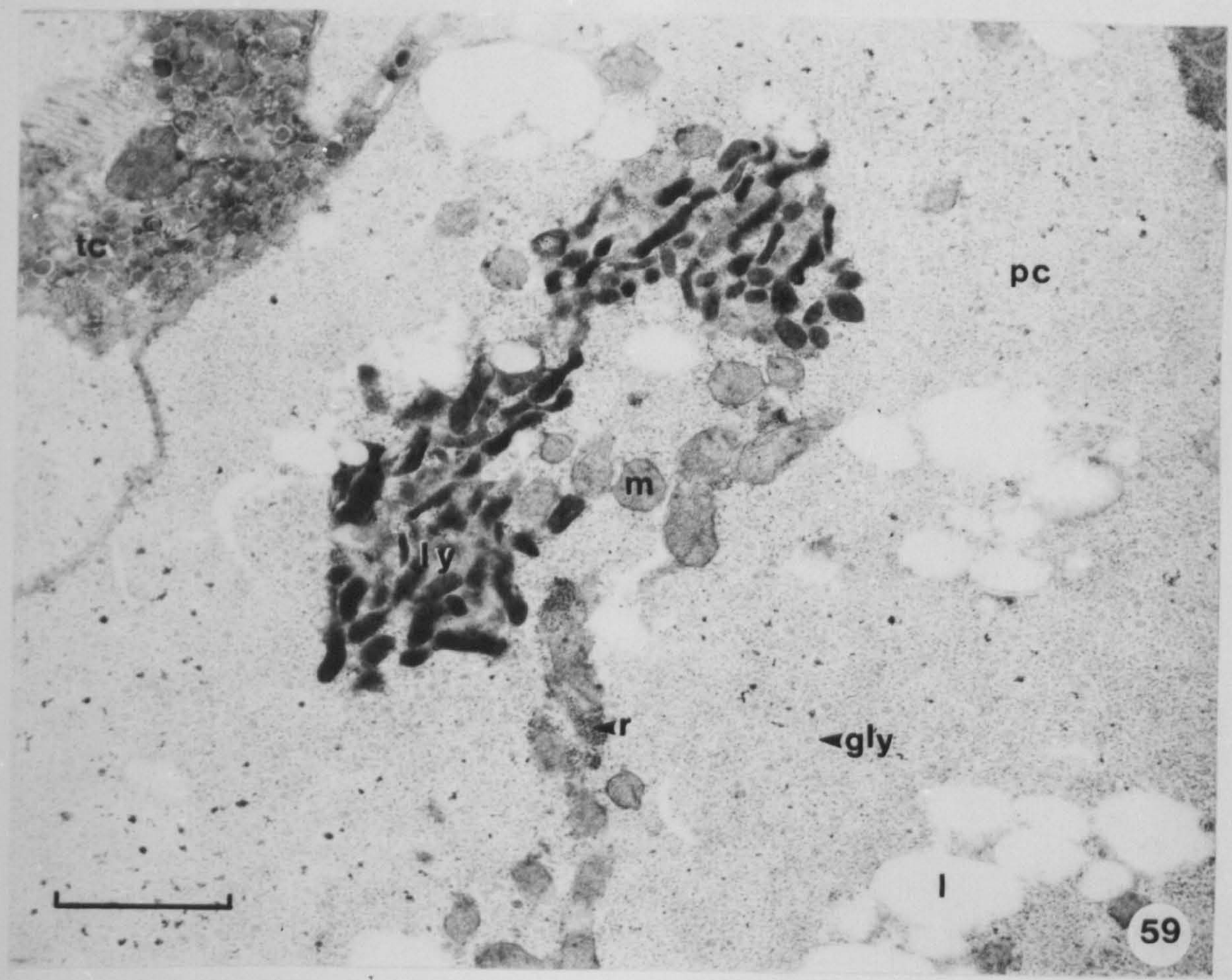
58

Plate 59 12 hours *in vitro* culture.

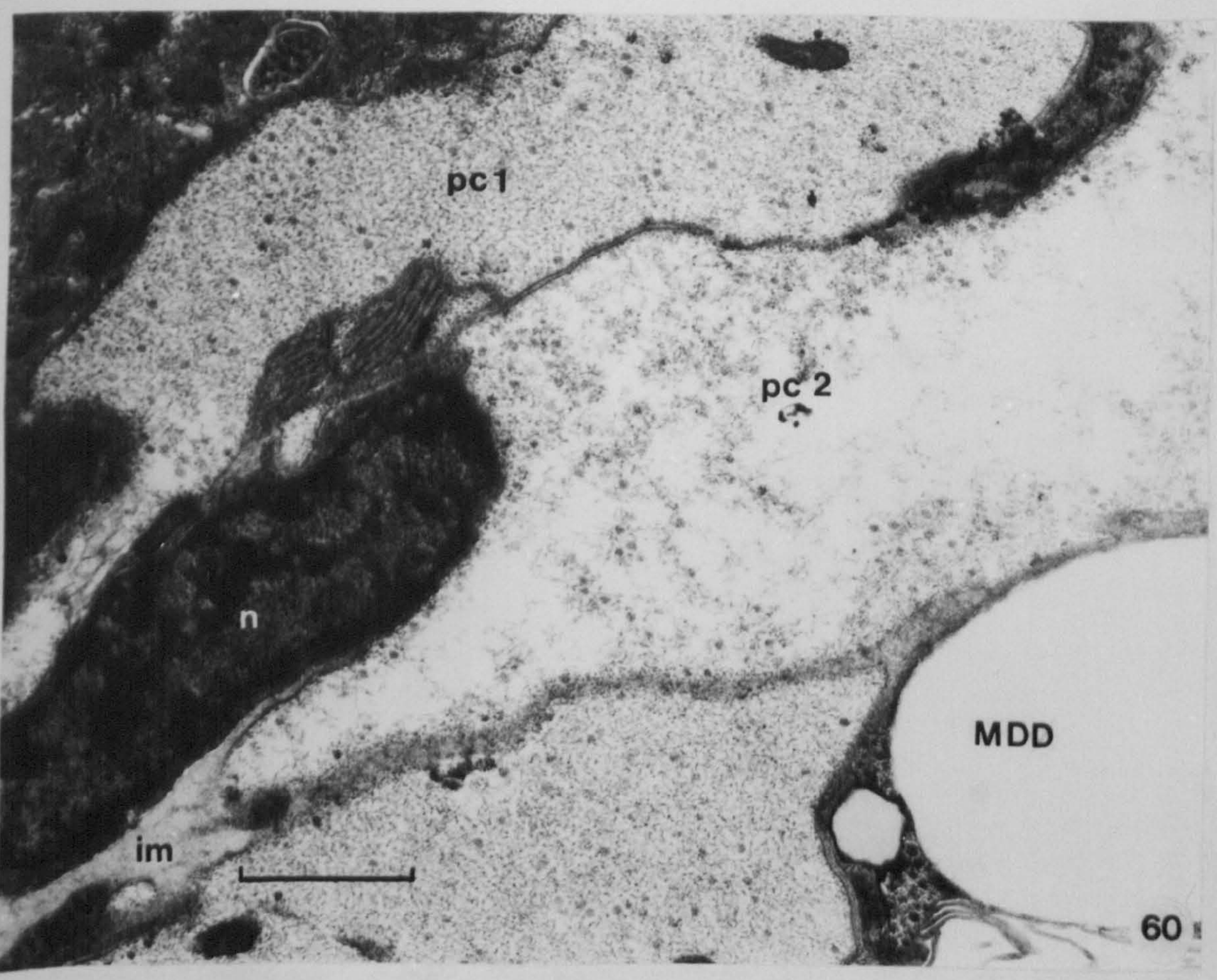
The parenchyma cells (pc) contain glycogen (gly), lipid drops (l), free ribosomes (r), mitochondria (m) and primary lysosomes (ly) or δ bodies.

Plate 60 3 days *in vitro* culture.

Adjacent parenchyma cells (pc) vary in their contents - one of them (pc 2) appears rather "leached out" in comparison to the other (pc 1).



59



60

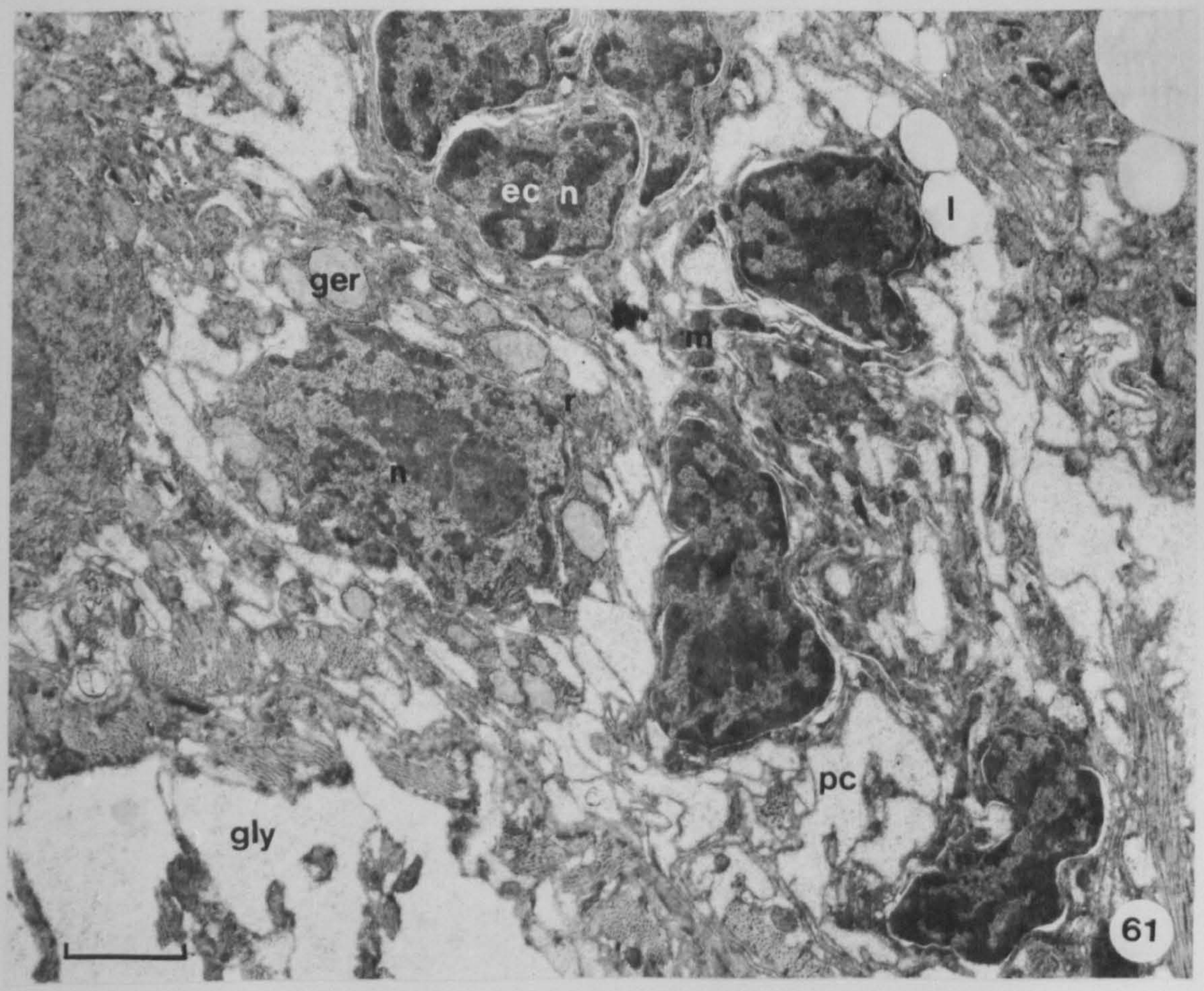
Plate 61 2 weeks in vitro culture.

The parenchymal cells (pc) frequently appear tortuous and convoluted. They contain lipid drops (l), glycogen (gly), nuclei (n), free ribosomes (r), mitochondria (m) and short lengths of GER (ger) which often appears swollen. The nuclei of some "embryonic" cells (ecn)- probably those of the genital rudiment - can be seen.

Plate 62 2 weeks in vitro culture.

The parenchyma cells contain glycogen (gly), mitochondria (m), primary lysosomes (ly) or δ bodies, autophagosomes (a) or γ bodies and autophagolysosomes (aly).

Adjacent cells are separated by a thin layer of interstitial material (im) and connected via junctional complexes (jc).



61



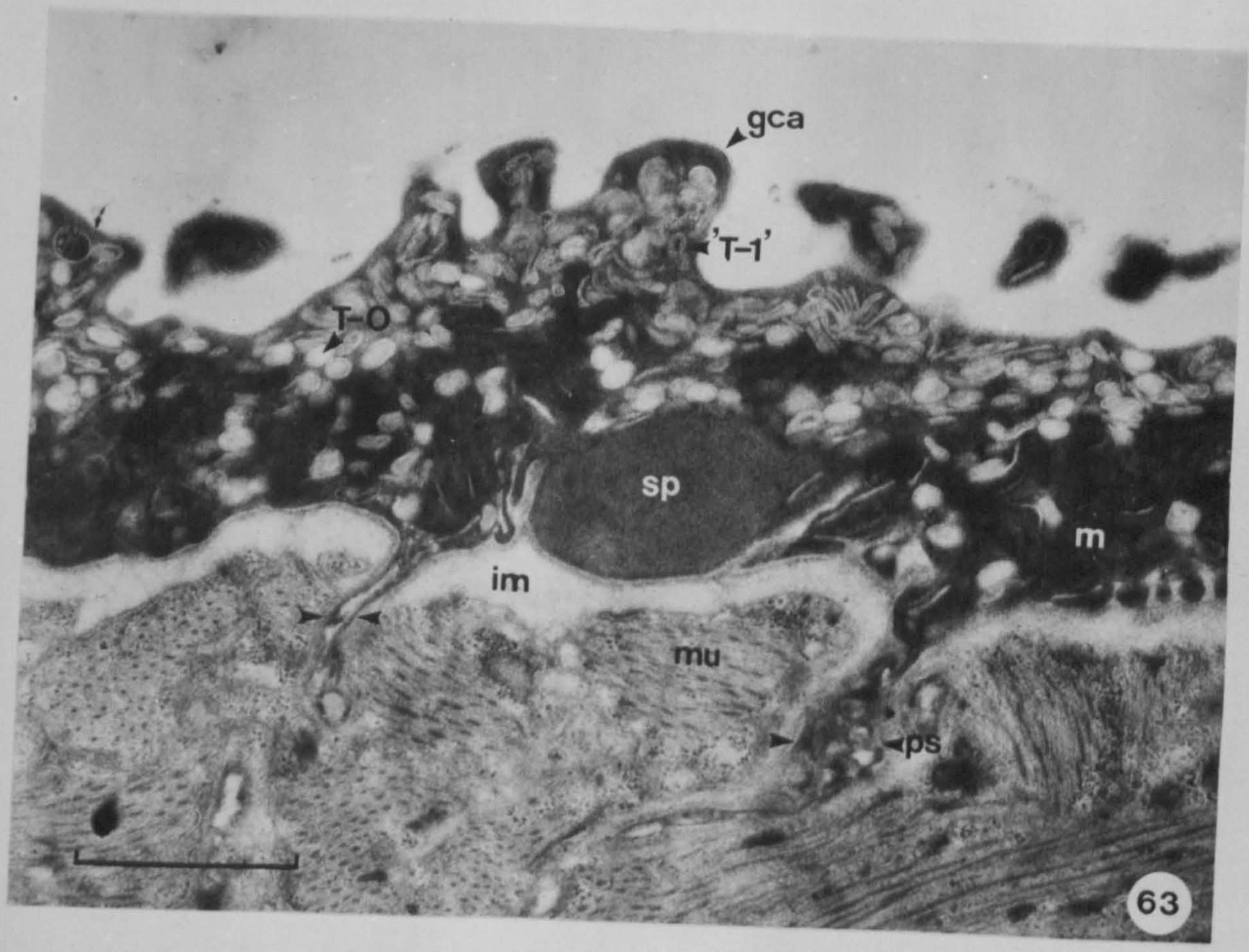
62

Plate 63 12 days *in vitro* culture (Culture 43).

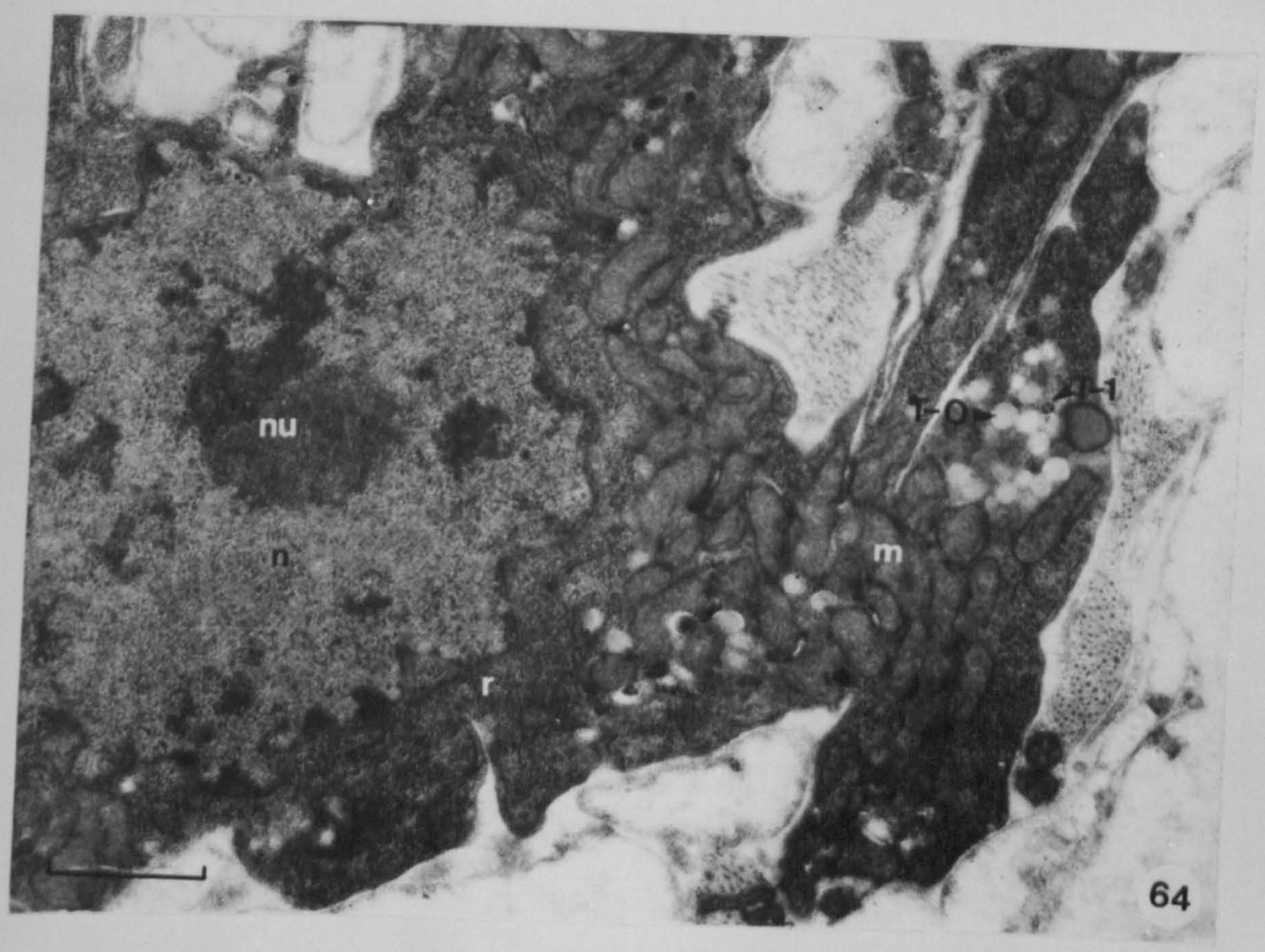
The ground substance of the tegumental syncytium is dense and contains electron lucent spherical(T-O) granules and some whose contents have condensed to give a "cartwheel-like" appearance; the latter resemble the T-1 granules of normal developing adults. Protoplasmic strands (ps),which connect the syncytium and the tegumental cell bodies, can be seen penetrating the peripheral musculature (mu). The layer of interstitial material (im) between the syncytium and the underlying peripheral musculature is thin.

Plate 64 12 days *in vitro* culture (Culture 43).

The tegumental cell bodies contain numerous free ribosomes (r) and mitochondria (m), some electron lucent spherical (T-O) granules and some oval granules which are probably normal T-1 granules.



63



64

Plate 65 12 days *in vitro* culture (Culture 43).

The caecal epithelium contains "adult" type granules (ag), mitochondria and GER (ger) some of which has a parallel orientation. Adjacent cells are connected at their luminal surface by terminal bars (tb). The surface lamellae (l) have increased in length from those of the metacercaria although they often possess swollen or bulbous tips (arrowed). Undifferentiated cells (uc) containing ribosomes, mitochondria and a nucleus but no secretory granules or GER can be seen embedded in the caecal epithelium.

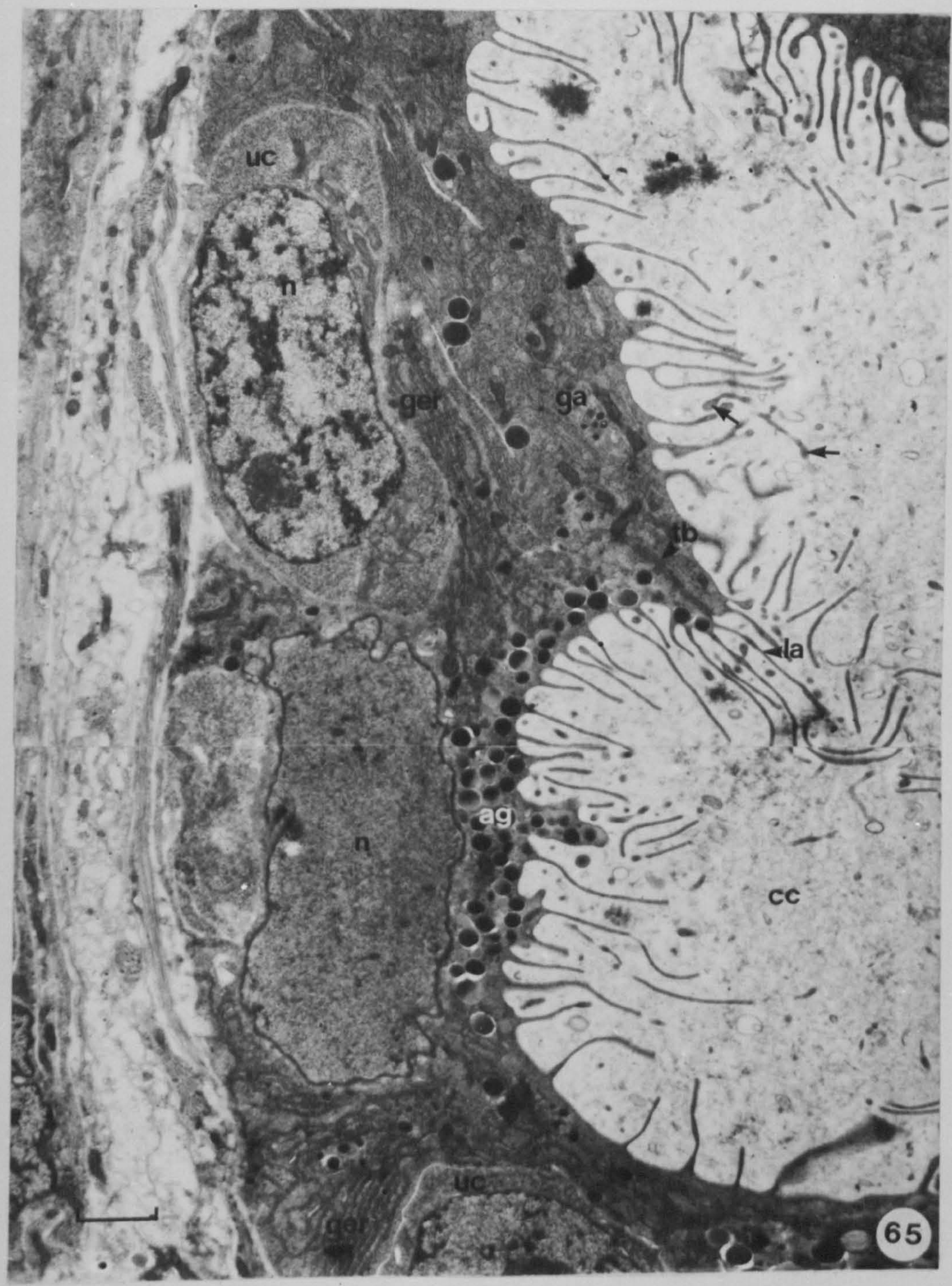
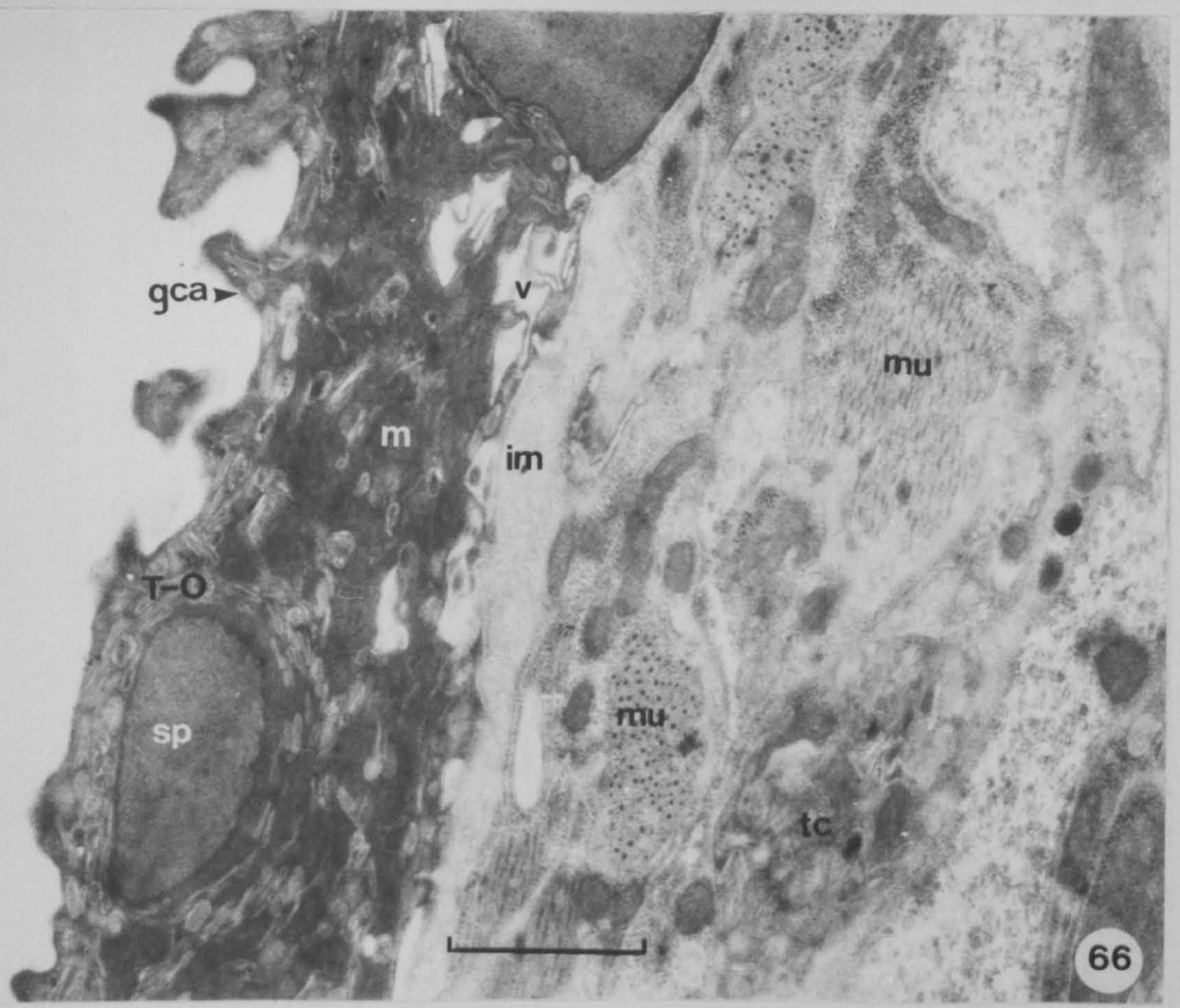

uc
n
ger
ga
tb
la
ag
n
cc
uc
ger
65

Plate 66 Fluke removed from mouse 7 days p.i. and cultured for 7 days (Culture 37).

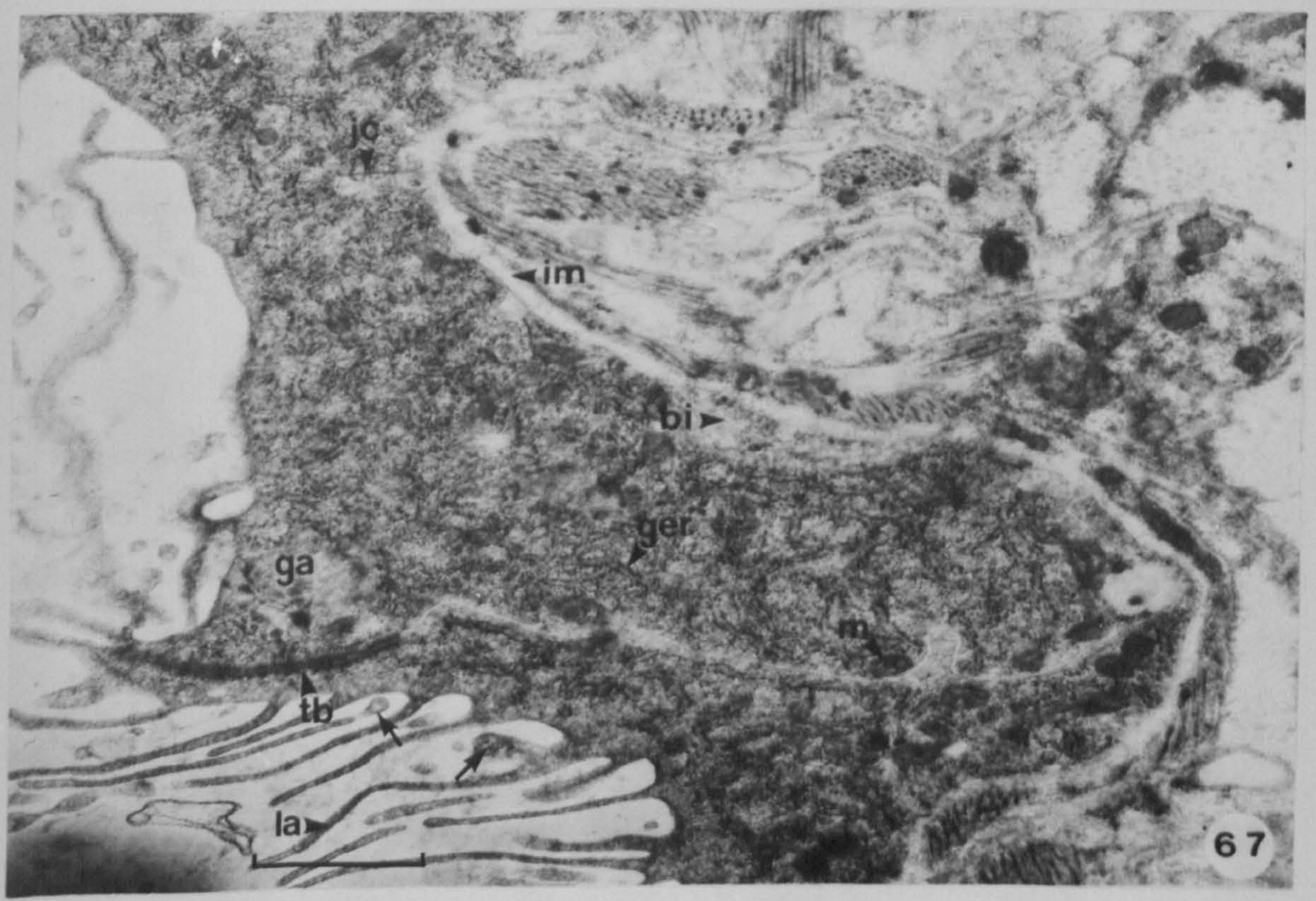
The tegumental syncytium is dense and contains spherical (T-O) secretory bodies. The base of the syncytium often appears vacuolated (v). The layer of interstitial material (im) between the syncytium and the underlying musculature (mu) is thick. Tegumental cell bodies (tc) are infrequent.

Plate 67 Fluke removed from mouse 7 days p.i. and cultured for 7 days (Culture 37).

The caecal epithelium appears highly abnormal. The cells contain fragmented GER (ger), mitochondria (m) and areas of possible Golgi activity (ga); secretory granules are absent or infrequent. Invaginations of the basal plasmalemma (bi) and junctional complexes (jc) with parenchymal cell processes are present. The surface lamellae (la) often have swollen or bulbous endings (arrowed).



66



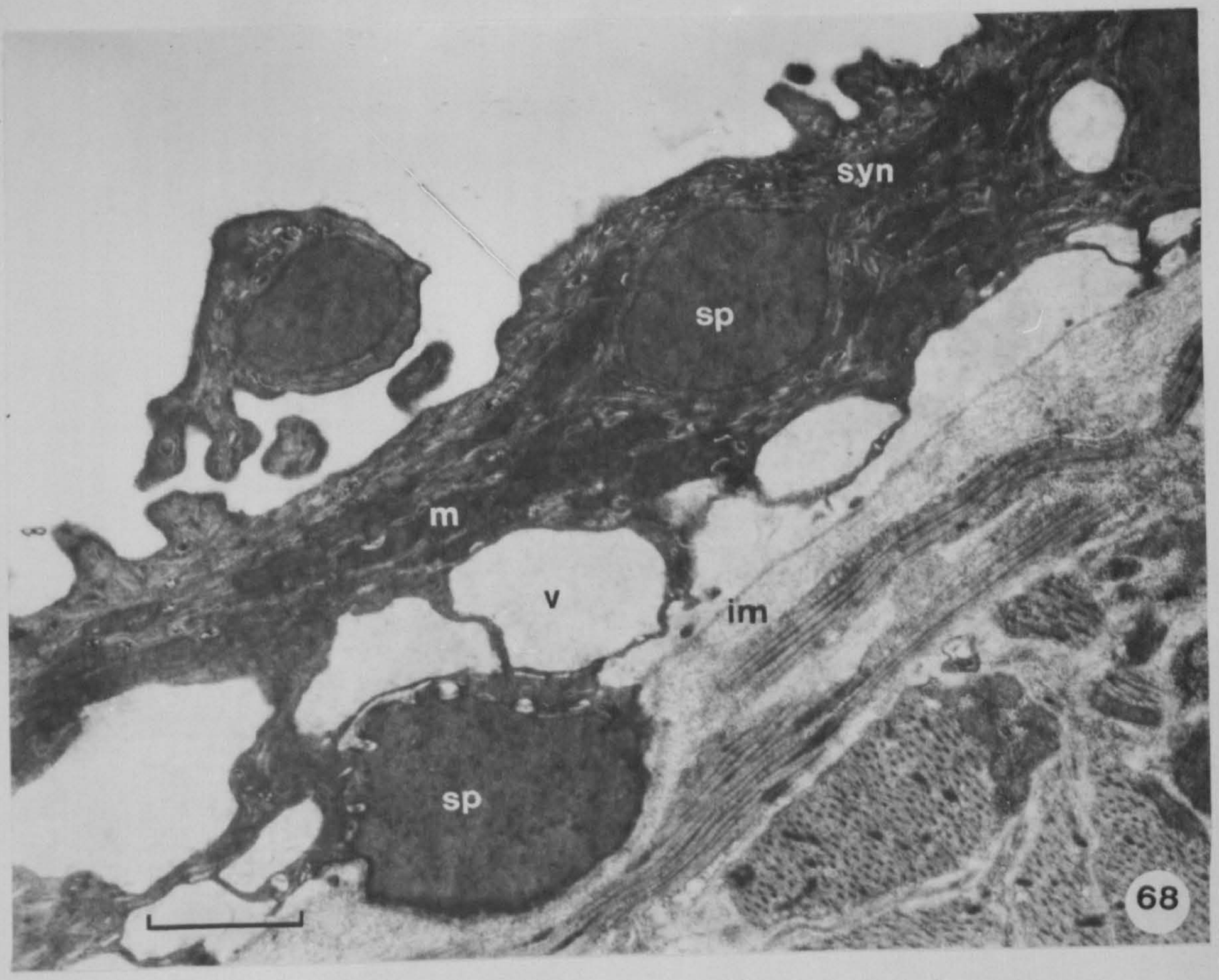
67

Plate 68 Fluke removed from mouse 14 days p.i. and cultured for 7 days (Culture 38).

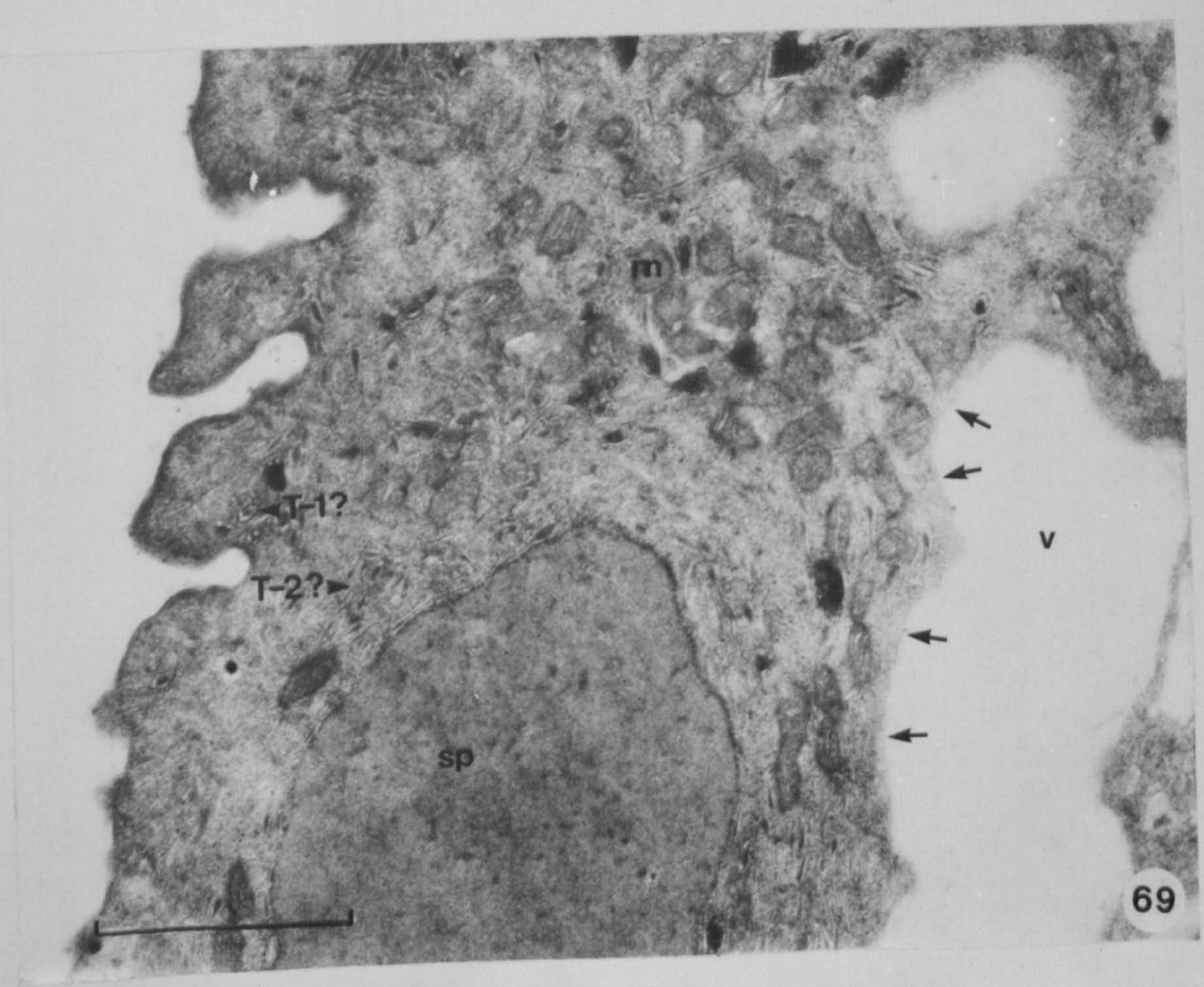
The basal area of the tegumental syncytium (syn) is highly vacuolated (v). The underlying layer of interstitial material (im) is thick and spines are frequently seen embedded in it.

Plate 69 Fluke removed from mouse 14 days p.i. and cultured for 7 days (Culture 38).

The vacuoles (v) in the basal area of the tegumental syncytium are apparently not membrane-bound (arrowed). The granules in the syncytium are probably of the oval (T-1) and rodlike (T-2) types.



68



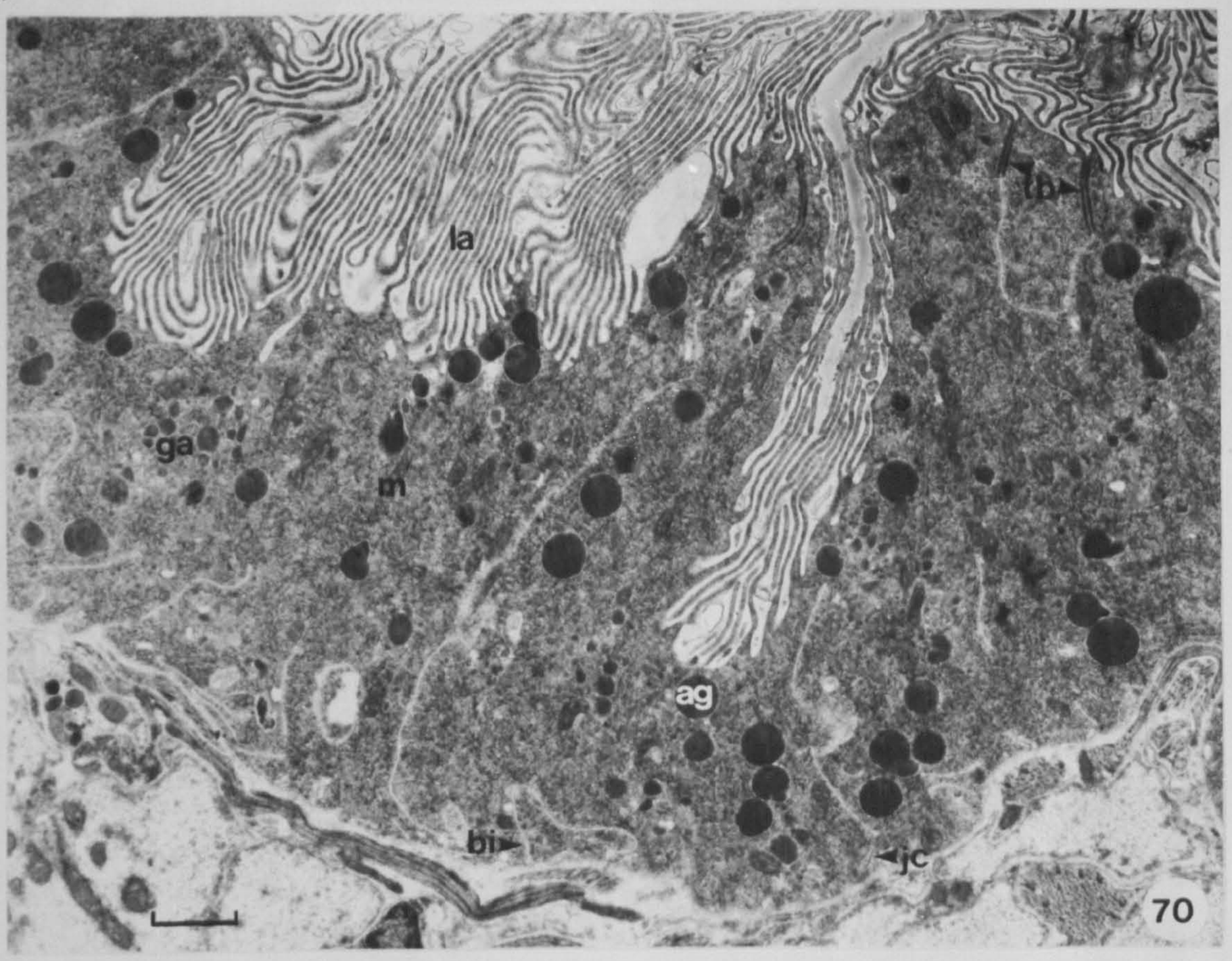
69

Plate 70 Fluke removed from mouse 14 days p.i. and cultured for 7 days (Culture 38).

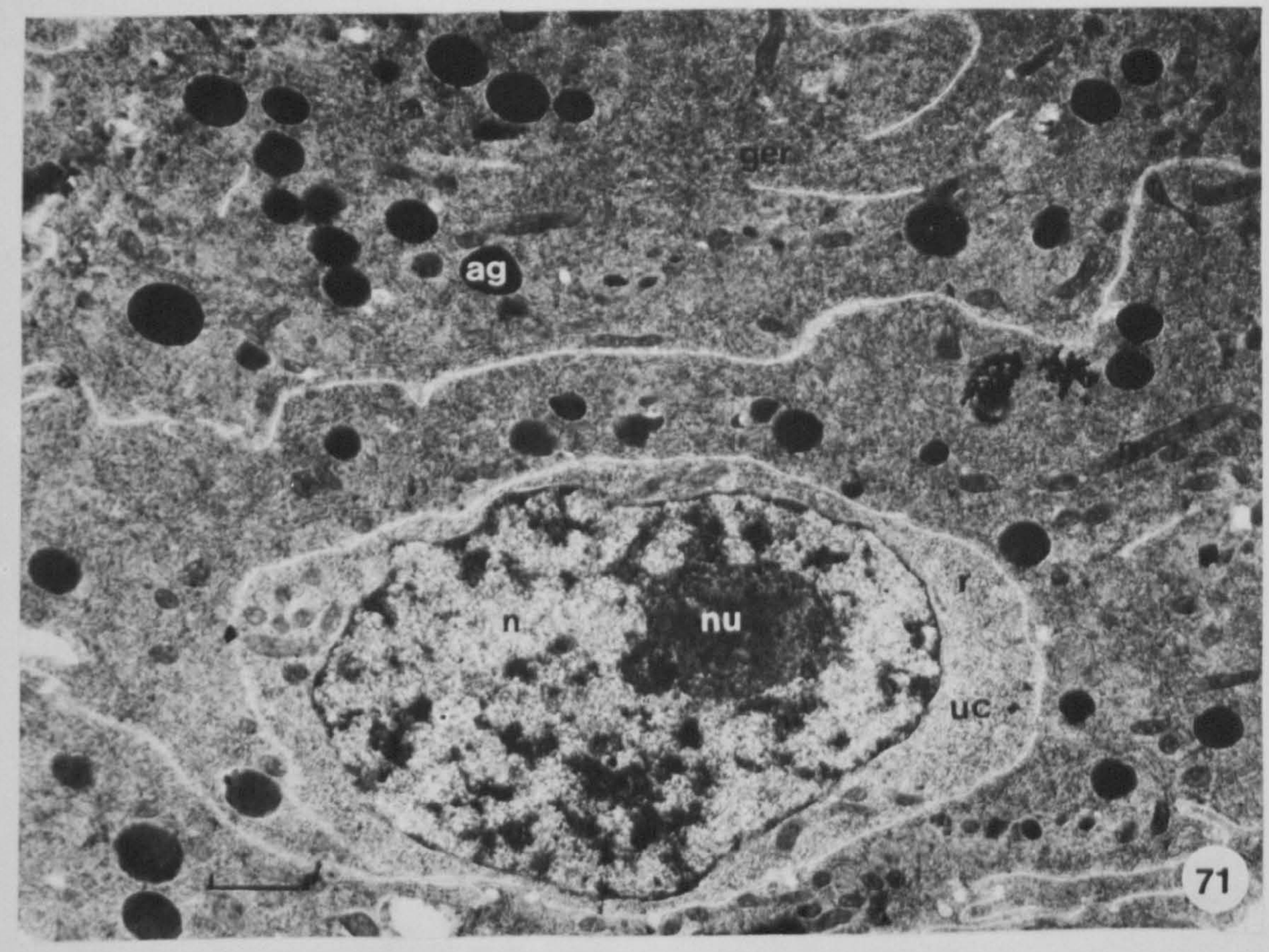
The epithelium lining the main branch of the digestive caeca contains numerous "adult" type granules (ag), areas of Golgi activity (ga) and mitochondria (m). The GER is fragmented and is not orientated. The surface lamellae (la) are long and numerous. Invaginations of the basal plasmalemma (bi) and junctional complexes (jc) with parenchymal cell processes are present.

Plate 71 Fluke removed from mouse 14 days p.i. and cultured for 7 days (Culture 38).

The caecal epithelium includes undeveloped cells (uc) which contain a nucleus (n), ribosomes (r) and mitochondria but no GER or secretory granules.



70



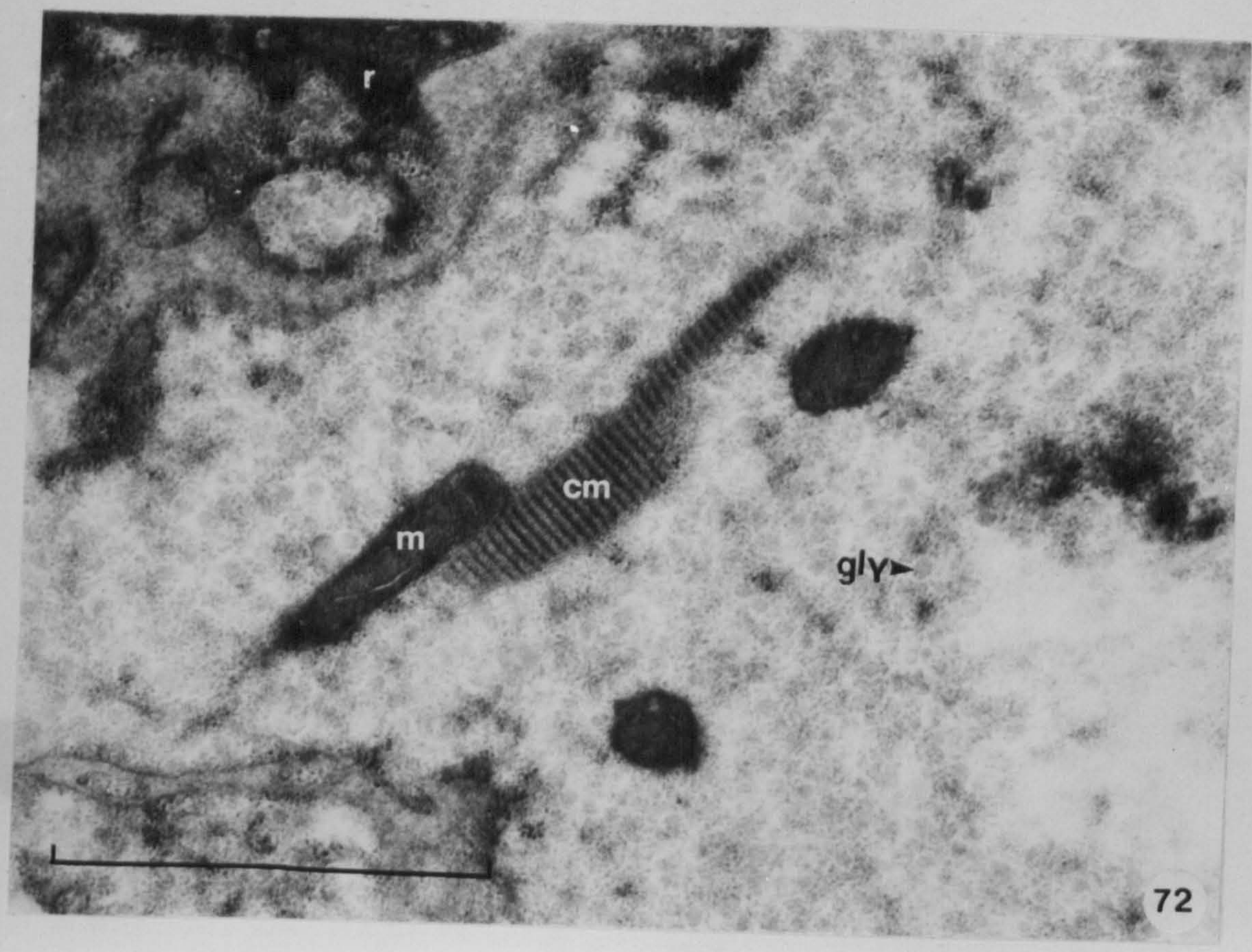
71

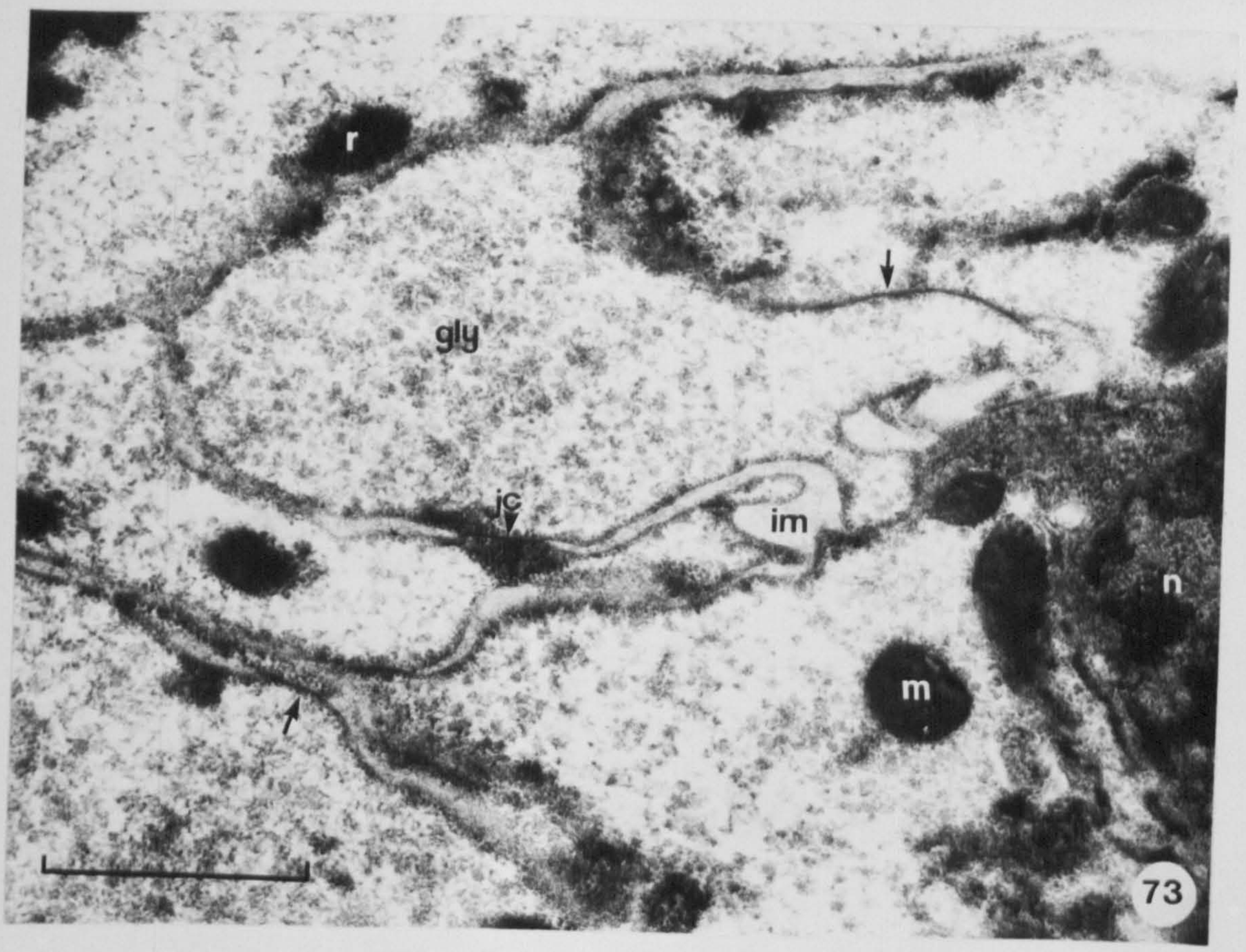
Plate 72 12 days *in vitro* culture (Culture 43).

This section shows a parenchyma cell containing an unusual body of crystalline appearance (cm).

Plate 73 Fluke removed from mouse 14 days p.i. and cultured for 7 days (Culture 38).

Adjacent parenchyma cells are separated by a relatively thick layer of interstitial material (im) and they are connected via frequent junctional complexes (jc - arrows). The cells contain glycogen (gly), areas of free ribosomes (r), dense mitochondria (m) and nuclei (n).


r
cm
m
gly
72


r
gly
ic
im
n
m
73

Plates 74 - 76 7 days in vitro culture.

Plate 74 (X - artifacts)

A cultured fluke showing the oral sucker (OS), ventral sucker (VS) and excretory pore (EP).

Plate 75 (X - artifacts)

The oral sucker (OS) region showing the rings (RS) of spines (S), ciliated sensory papilla (SP), a ring of domed papilla (DP) around the oral sucker (small arrows) and two domed papillae (large arrows) inside the lip of the sucker.

Plate 76

The ring of domed sensory papillae (DP) around the ventral sucker (VS) are in the process of dividing (arrows). The lips of the sucker are spine-free.

Plates 77 - 79 28 days in vitro culture

Plate 77

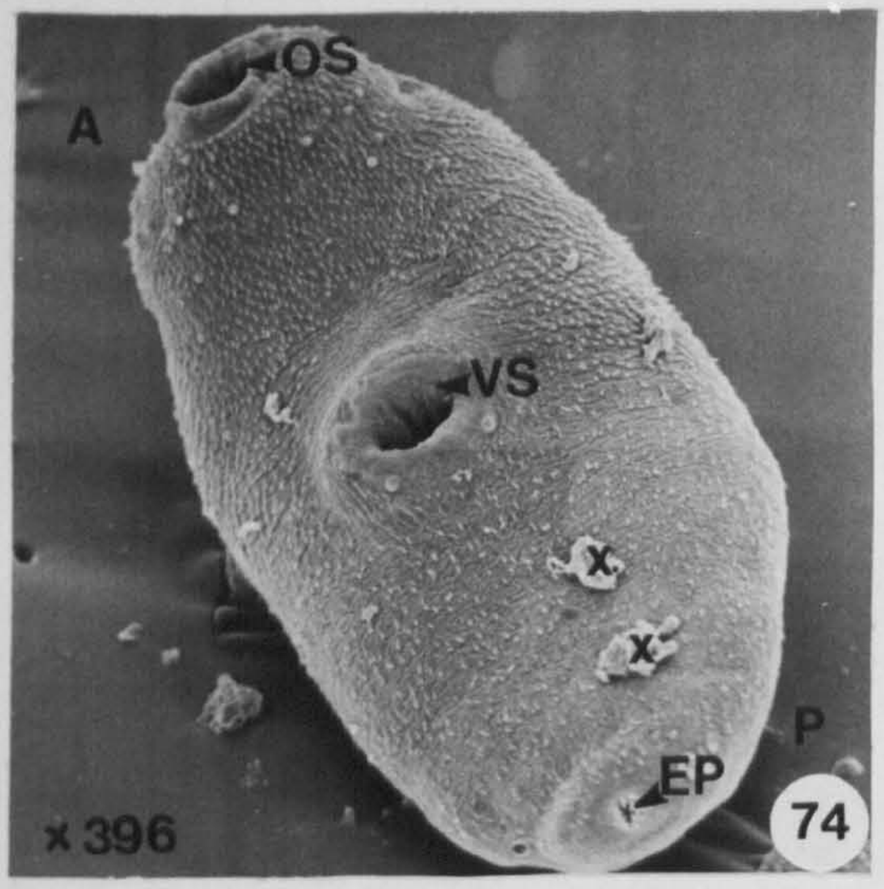
In this view the anterior portion of the fluke is curled round towards the ventral sucker (VS). The domed sensory papillae (DP) around the ventral sucker are in the process of division (arrows) but are not completely separate.

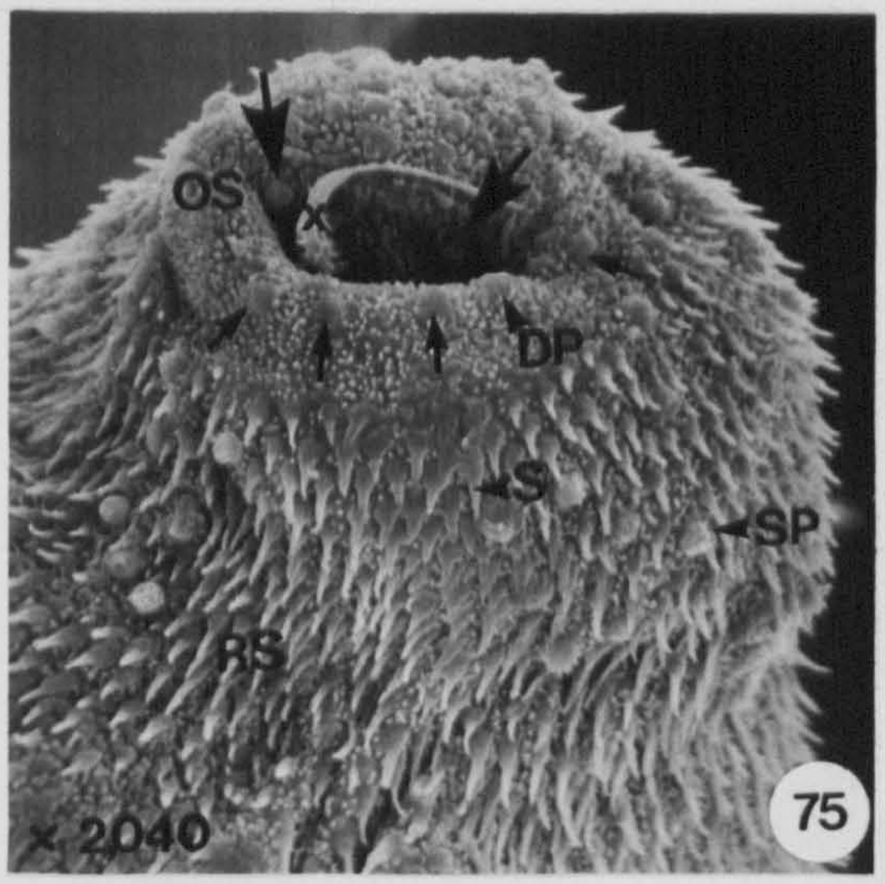
Plate 78

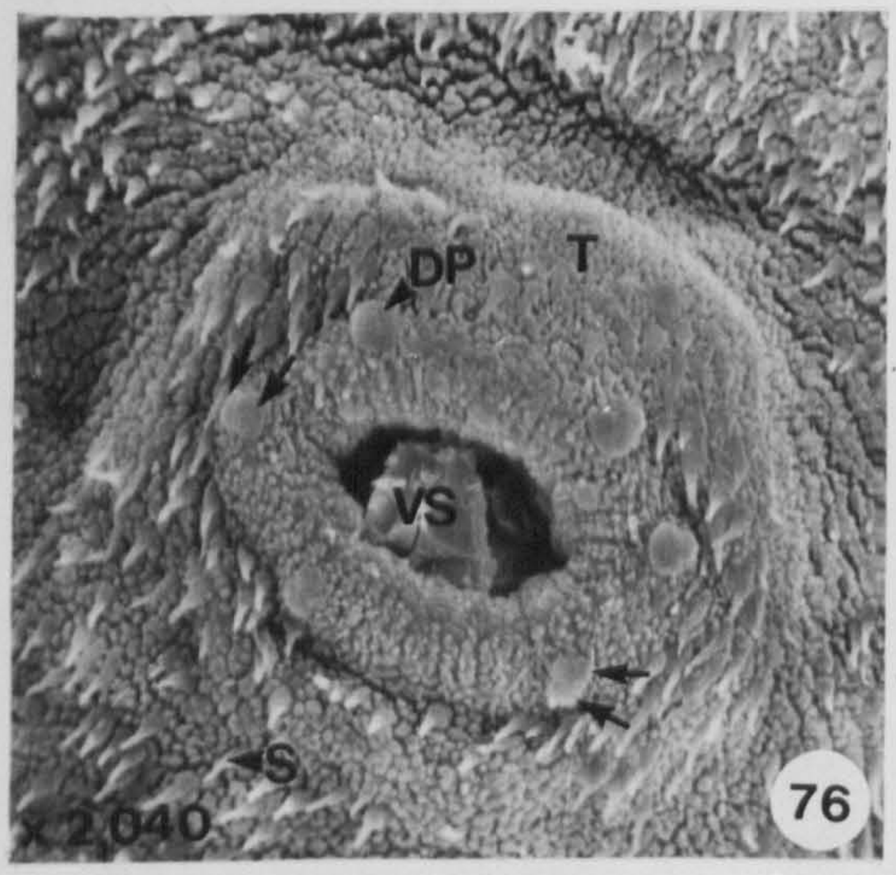
On the anterior surface of the fluke the spines are arranged in rows which ring the fluke (RS). The cilia (C) of the ciliated sensory papillae project an unusual distance from the bulb.

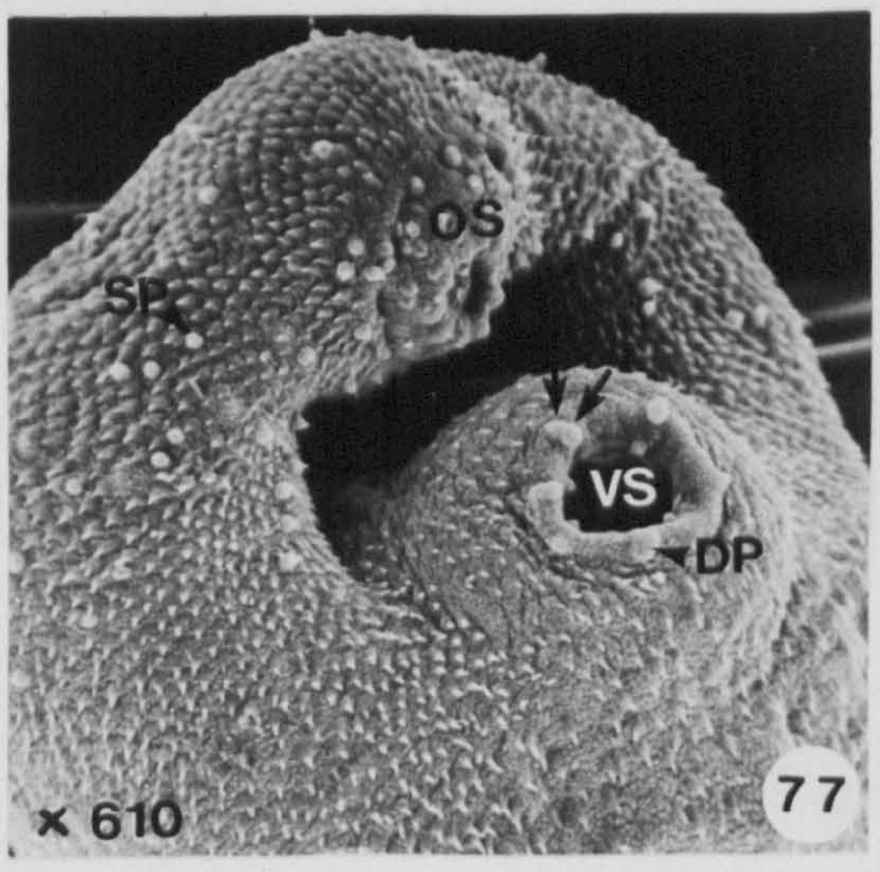
Plate 79

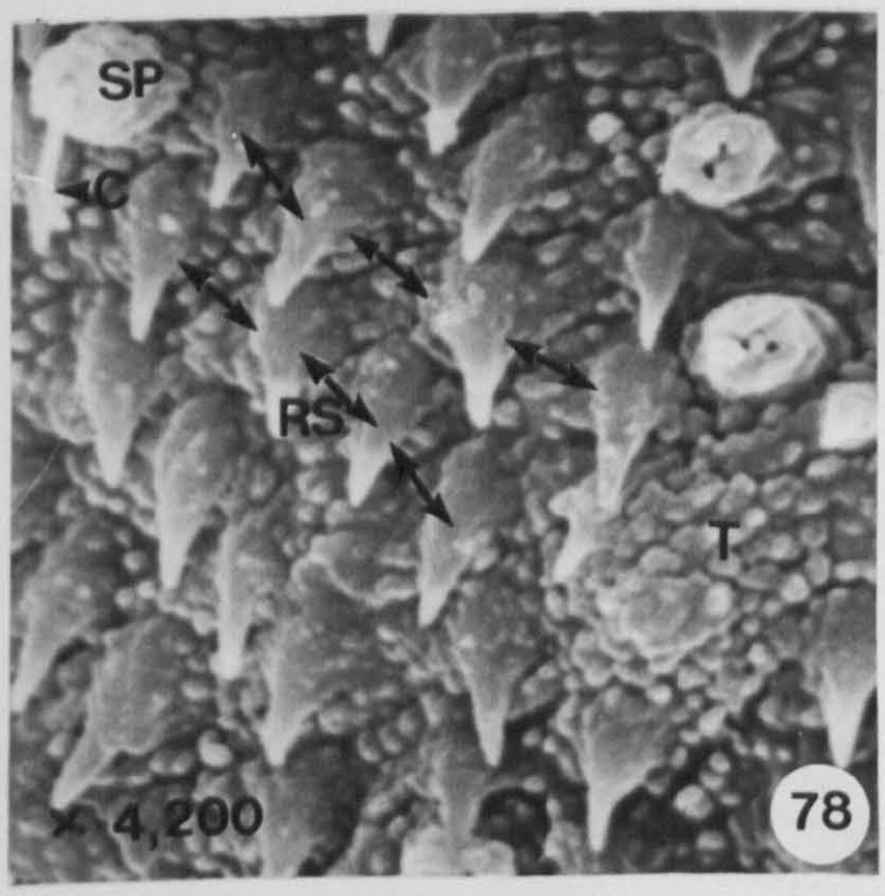
The spines (S) in the posterior region are arranged at random. The surface (T) of the fluke is thrown up into small microvilli.

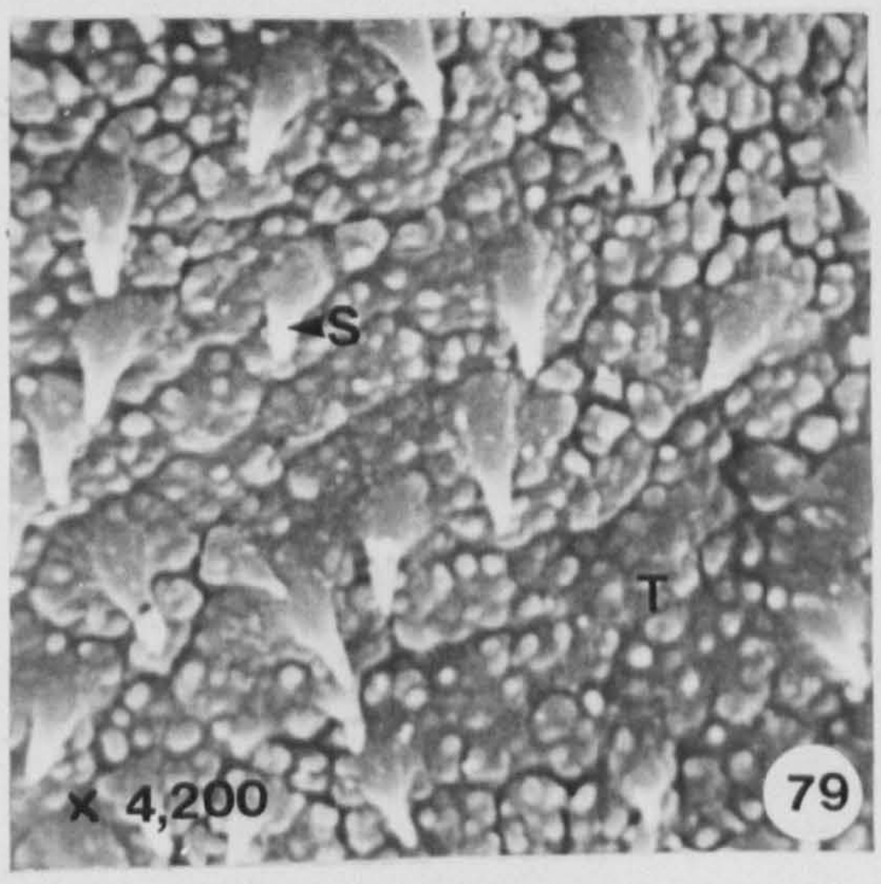

A
OS
VS
X
X
X
EP
P
x 396
74


OS
DP
S
SP
RS
x 2040
75


DP
T
VS
S
x 2,040
76


OS
SP
VS
DP
x 610
77


SP
C
RS
T
x 4,200
78


S
T
x 4,200
79

MEIOGYMNOPHALLUS MINUTUS.

Plates 80 - 82 : Light microscope photographs.

Plates 83 - 107 : Transmission electron micrographs of metacercariae and adults.

Plates 108 - 110 : Scanning electron micrographs of adults.

Plates 111 - 122 : Transmission electron micrographs of cultured flukes.

Plates 123 - 125 : Scanning electron micrographs of cultured flukes.

(Scale bars on Plates denote lum.)

KEY.

Light microscope photographs -

eb	excretory bladder	hyp	hyperparasitized tissue
gx	glycocalyx	meta	metacercaria
hp	plug of host tissue	pl	pallial line tissue

Scanning Electron Micrographs -

A	anterior	S	spine
C	cilium	SP	sensory papilla
CR	"crown" on spine	T	tegument
EP	excretory pore	TB	tegumental bubbles
OS	oral sucker	VS	ventral sucker
P	posterior		

Transmission Electron Micrographs -

af	axial filamentt	eg	excretory granules
bb	basal body	f	"flame" of flame cell
bi	basal invagination	FC	flame cell
bl	basal lamina	ga	area of Golgi activity
cb	"centriole-like body"	ger	granular endoplasmic reticulum
cc	caeeal contents	gc	Golgi complex
cg	cortical granules	glc	gland cell
cn	condensed nucleus	gls	gland cell secretions
cp	central process	go	gonad
cr	"crown" on spine	gx	glycocalyx
CT	conjoined tubule	ht	host tissue
d	dense material/ desmosome	im	interstitial material
EB	excretory bladder	imv	interdigitating microvilli
ED	excretory duct	l	lipid

la	lamellae	sd	septate desmosome
li	internal leptotriches	se	sensory endings
ly	lysosome	sg	secretory granules
m	mitochondria	sp	spine
mt	microtubules	spo	sporonts
mu	muscle	sr	spore
n	nucleus	st	spermatids
nc	nurse cell	syn	(tegumental) syncytium
ne	nerve	tc	tegumental cell body
nu	nucleolus	te	testes
oe	oesophagus	tg	tegumental granules
ov	ovary	ut	uterus
pc	parenchyma cell	v	vacuoles
pgs	prostate gland secretion		
pp	pars prostatica	ve	vesicles
r	ribosomes	VS	ventral sucker
rt	root	vs	vitelline secretions
sb	sporoblast	w	membraneous whorls

Plate 80 Metacercariae *in situ* in cockle tissue (thick section prepared for TEM and stained in toluidine blue).

Normal (meta) and hyperparasitized (hyp) metacercariae can be seen enclosed by the cockle pallial line epithelium (pl). The hyperparasitized metacercaria (hyp) is packed with microsporidian spores. The other metacercariae have a thick surface glycocalyx (gx) and a prominent, voluminous excretory bladder (eb).

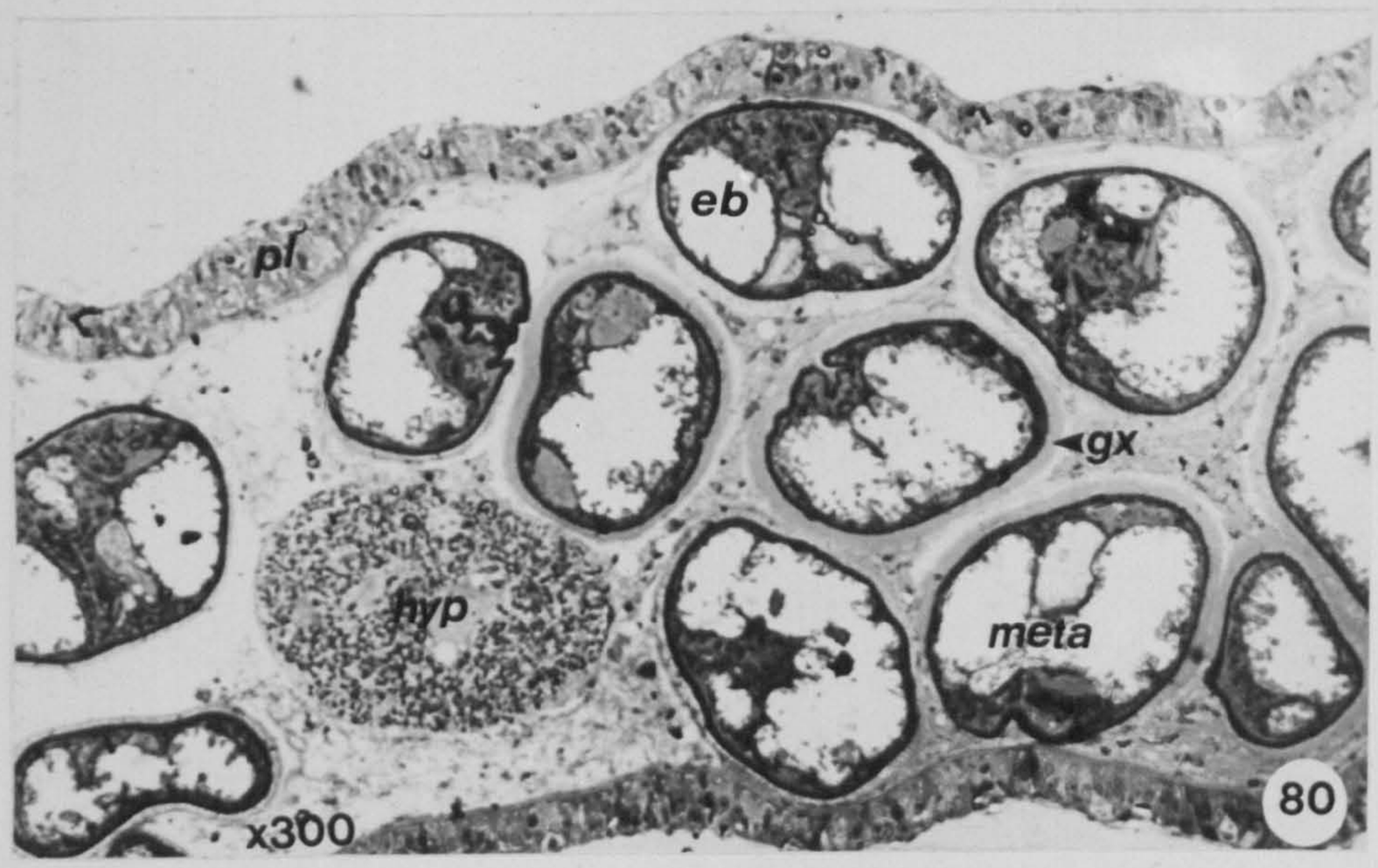
Plates 81 and 82 Adults *in situ* in oystercatcher gut (stained in Haematoxylin & Eosin).

Plate 81

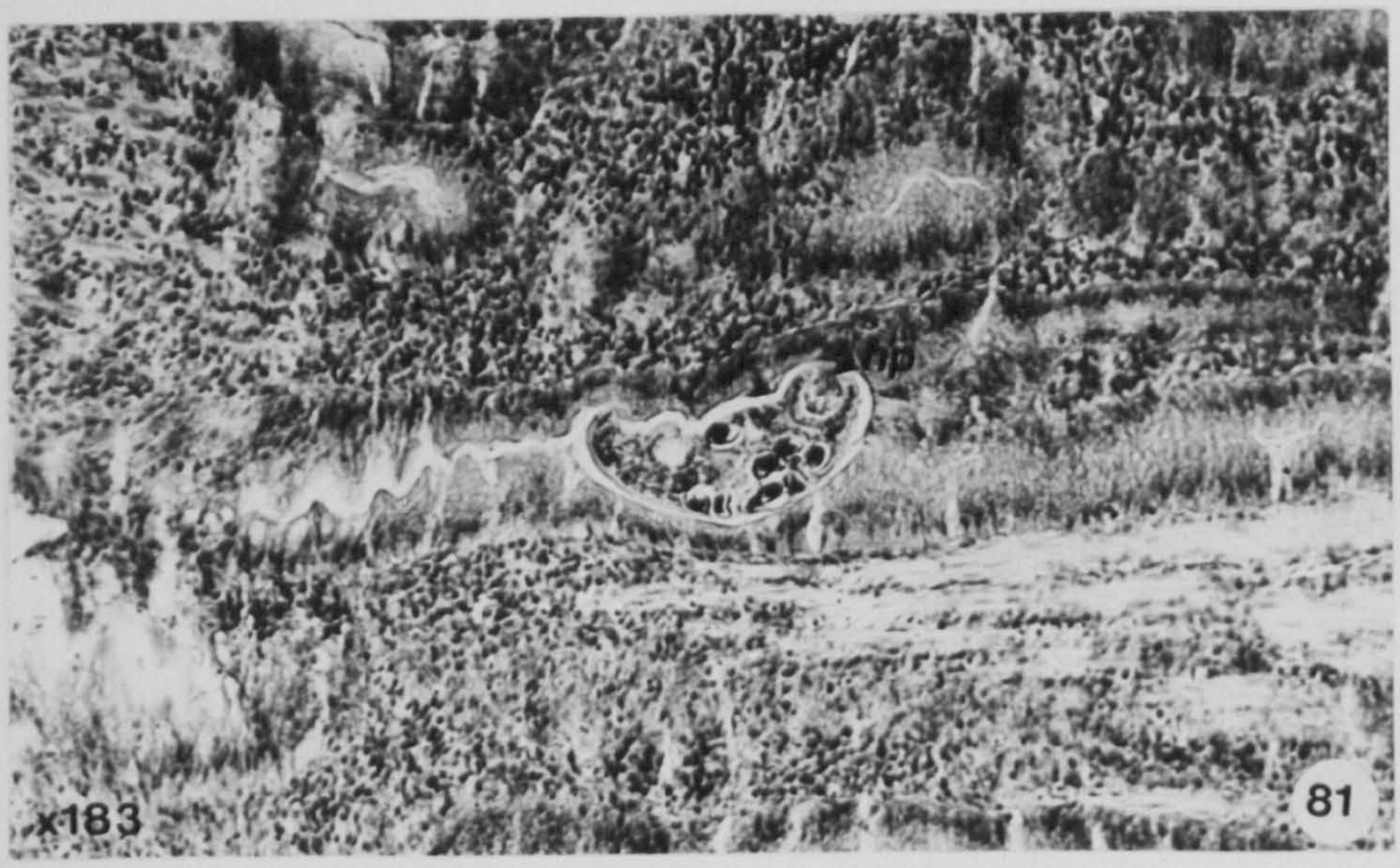
A longitudinal section showing a plug of host tissue (hp) in the fluke's oral sucker.

Plate 82

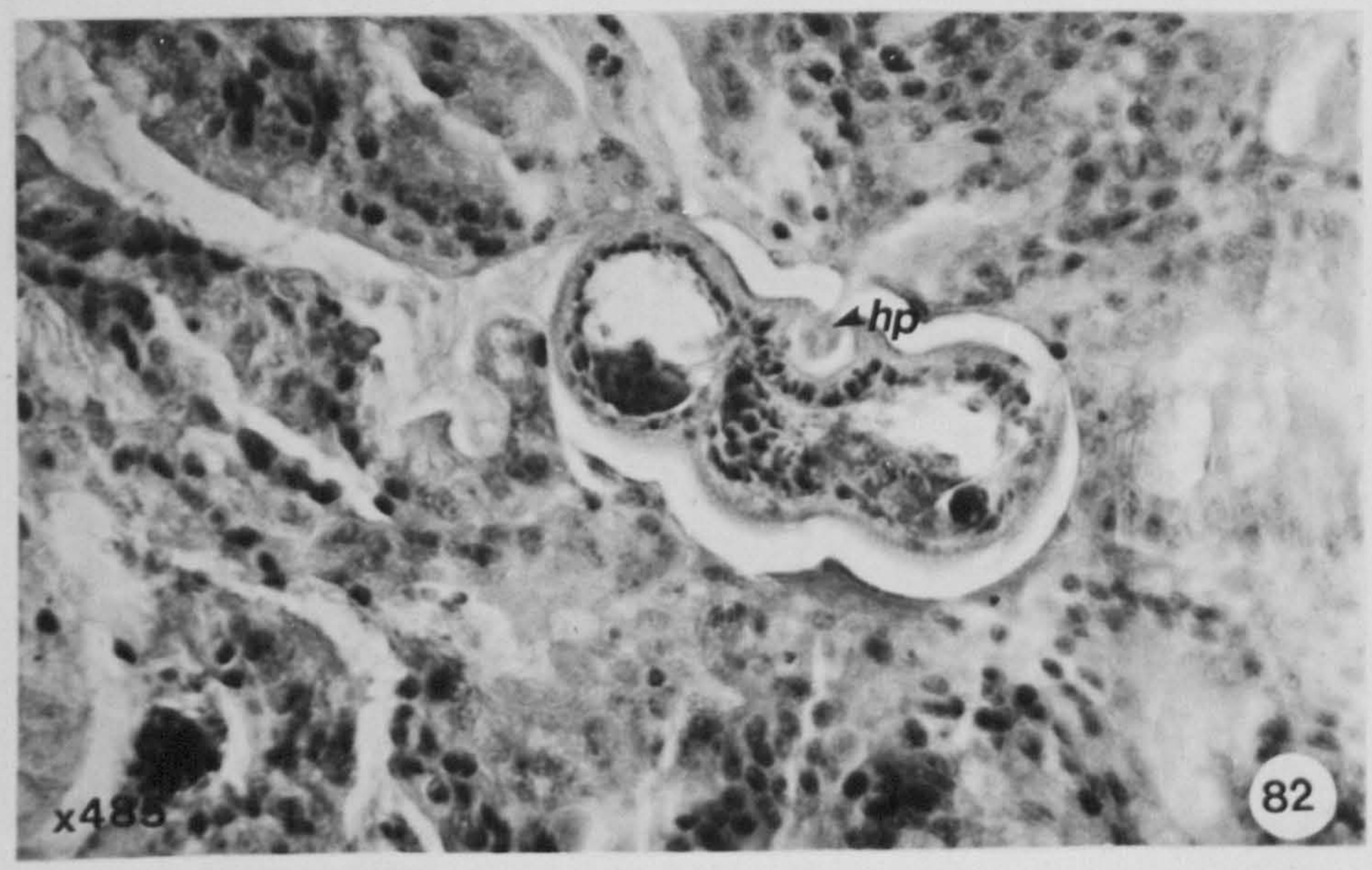
A transverse section showing a plug of host tissue (hp) in the fluke's ventral sucker.



80



81



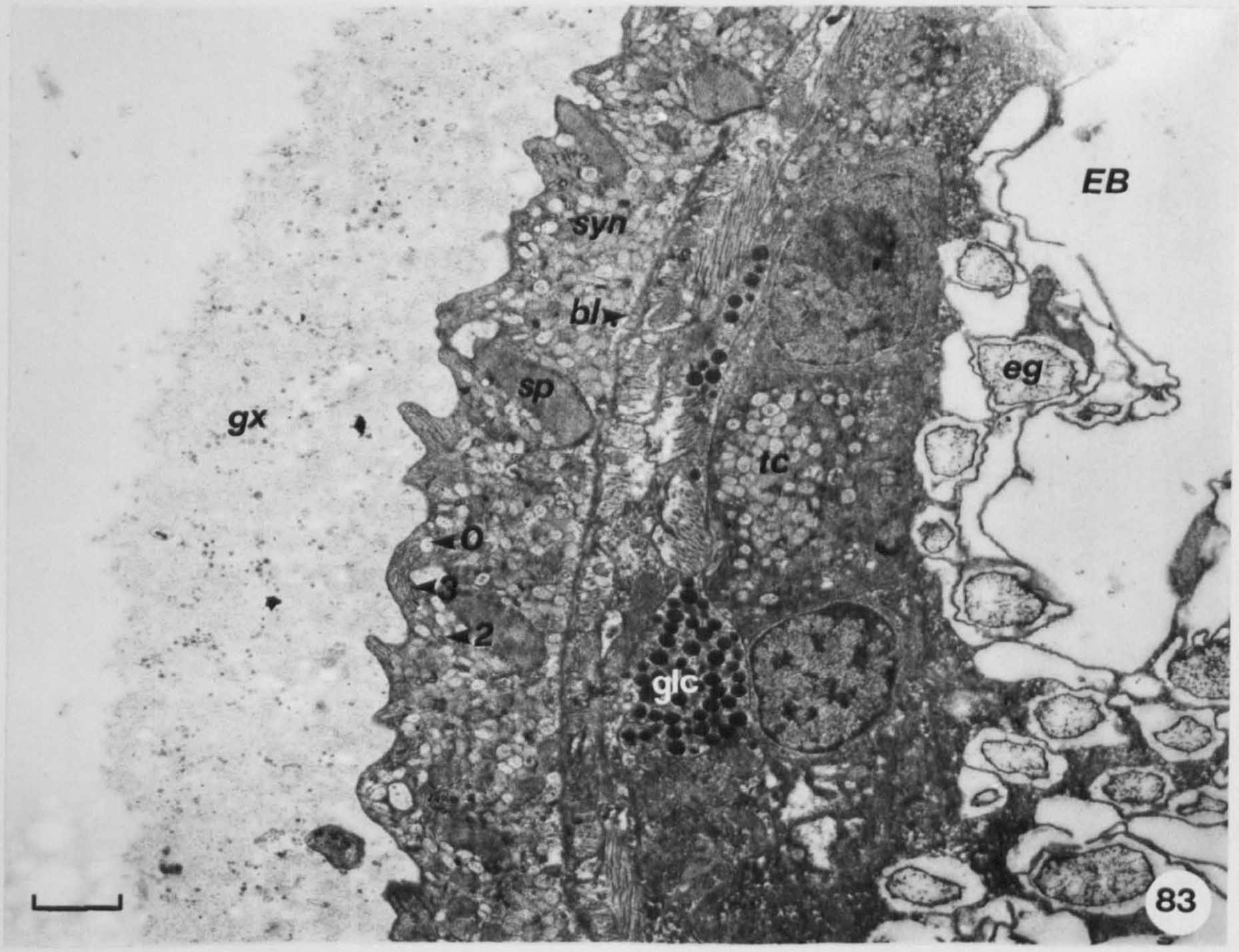
82

Plate 83 Metacercaria.

Covering the tegument of the metacercaria is a thick surface glycocalyx (gx). Three types of granules are present in the syncytium - spherical and moderately electron lucent (Type 0), oval and granular (Type 2), and biconcave and dense (Type 3). Spherical, electron lucent granules are also present in the tegumental cell bodies (tc). The cell body of one of the unicellular gland cells (glc), which are associated with the tegument, is also present. The excretory bladder (EB) contains many excretory granules (eg).

Plate 84 Metacercaria.

A section through the gonad (testis or ovary?) shows germ cells with relatively electron lucent nuclei (n) with prominent nucleoli (nu). The semicircular nuclei of possible nurse cells (nc) can be seen around the periphery of the gonad. An area of cells with denser cytoplasm and nuclei is also present near the periphery


EB
syn
bl
sp
gx
eg
tc
o
3
2
glc
83

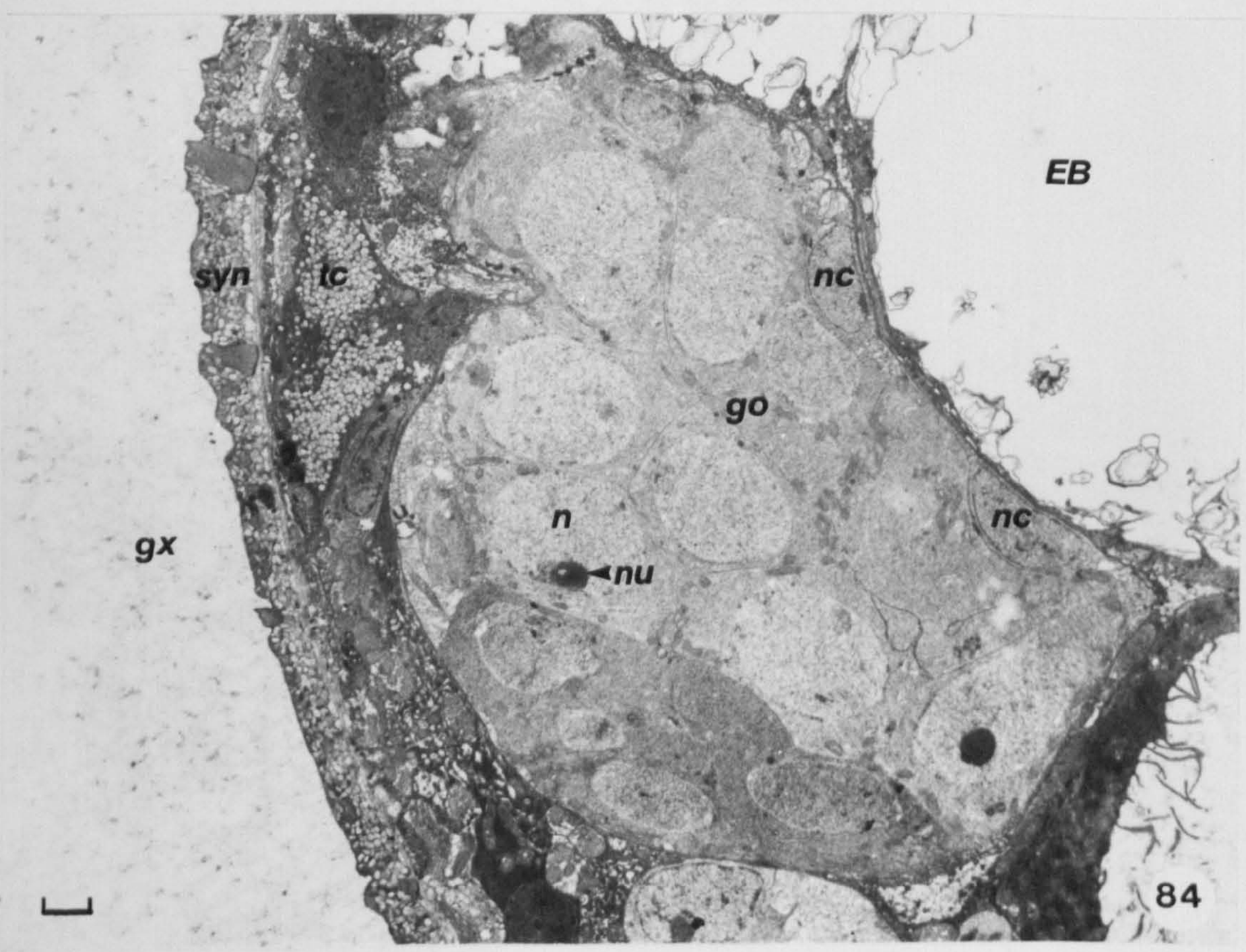

EB
syn
tc
nc
go
n
nu
gx
nc
84

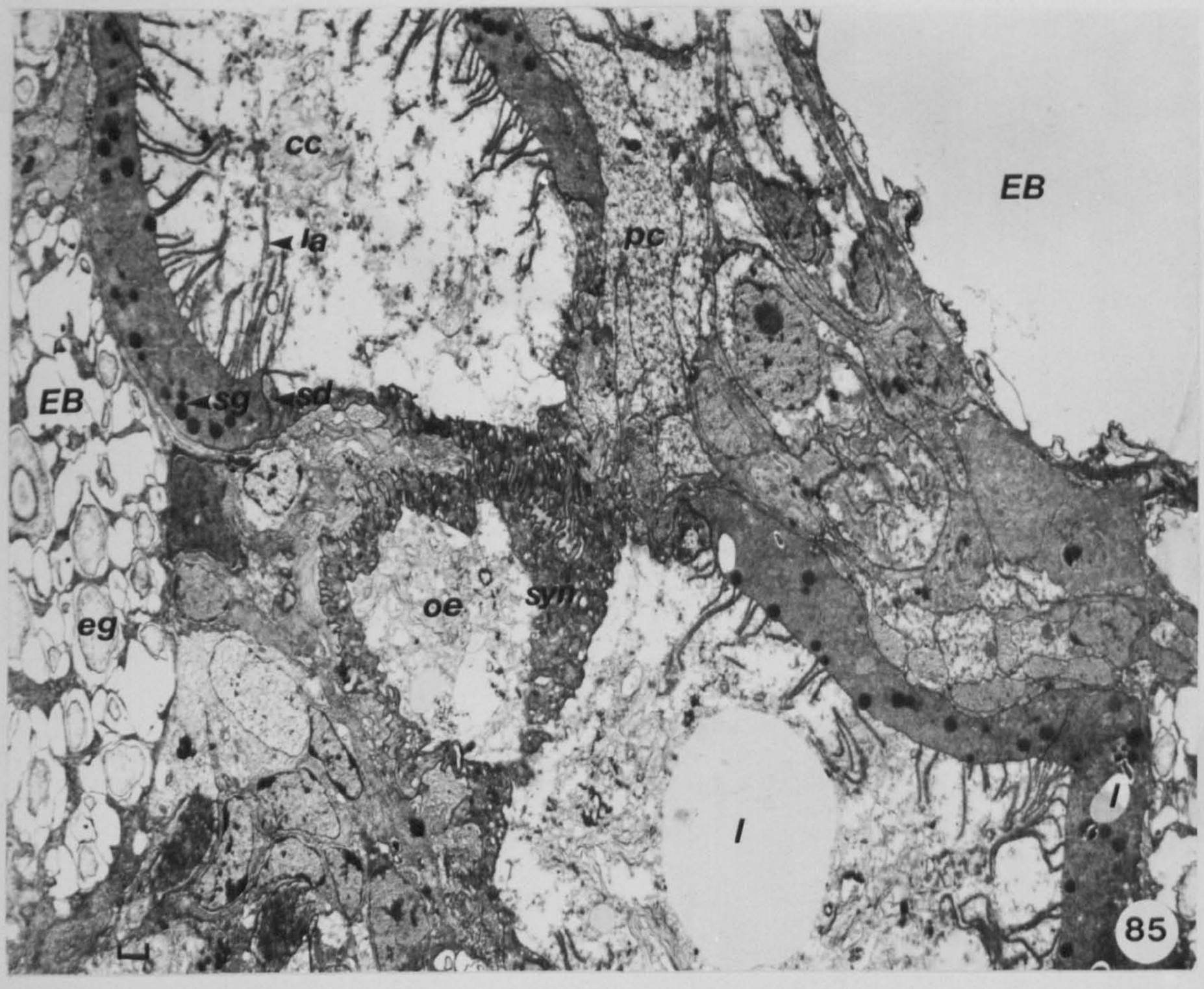
Plate 85 Metacercaria.

This section shows the two branches of the digestive caeca with the oesophagus between (oe). The oesophagus is lined with a syncytium and is connected to the cells of the caecal epithelium by a septate desmosome (sd). The caecal cells bear surface lamellae (la) and contain dense spherical or oval secretory granules (sg) and small lipid droplets (l). The lumen of the caeca contains large lipid drops and varying quantities of debris (cc).

The two branches of the excretory bladder (EB) are also present and parenchymal cells (pc) can be seen which exhibit a variety of contents.

Plate 86 Metacercaria.

Adjacent caecal cells are connected by desmosomes (sd); they contain areas of GER (ger), areas of mitochondria (m), lipid drops of various sizes (l) - some of which are in the process of extrusion into the lumen - and secretory granules (sg). Nuclei are infrequently seen.


cc
la
pc
EB
EB
sg
sd
eg
oe
syn
l
l
85

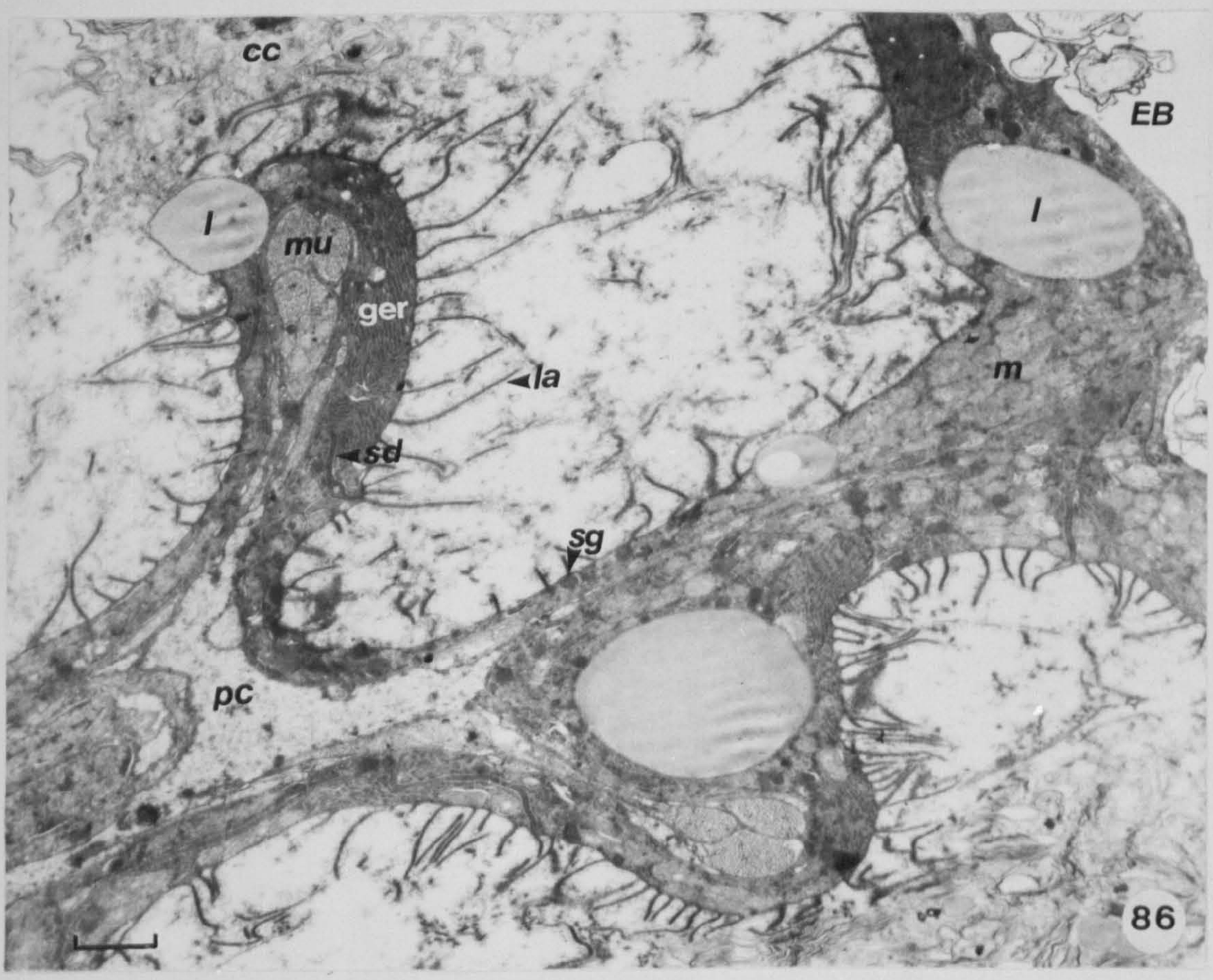
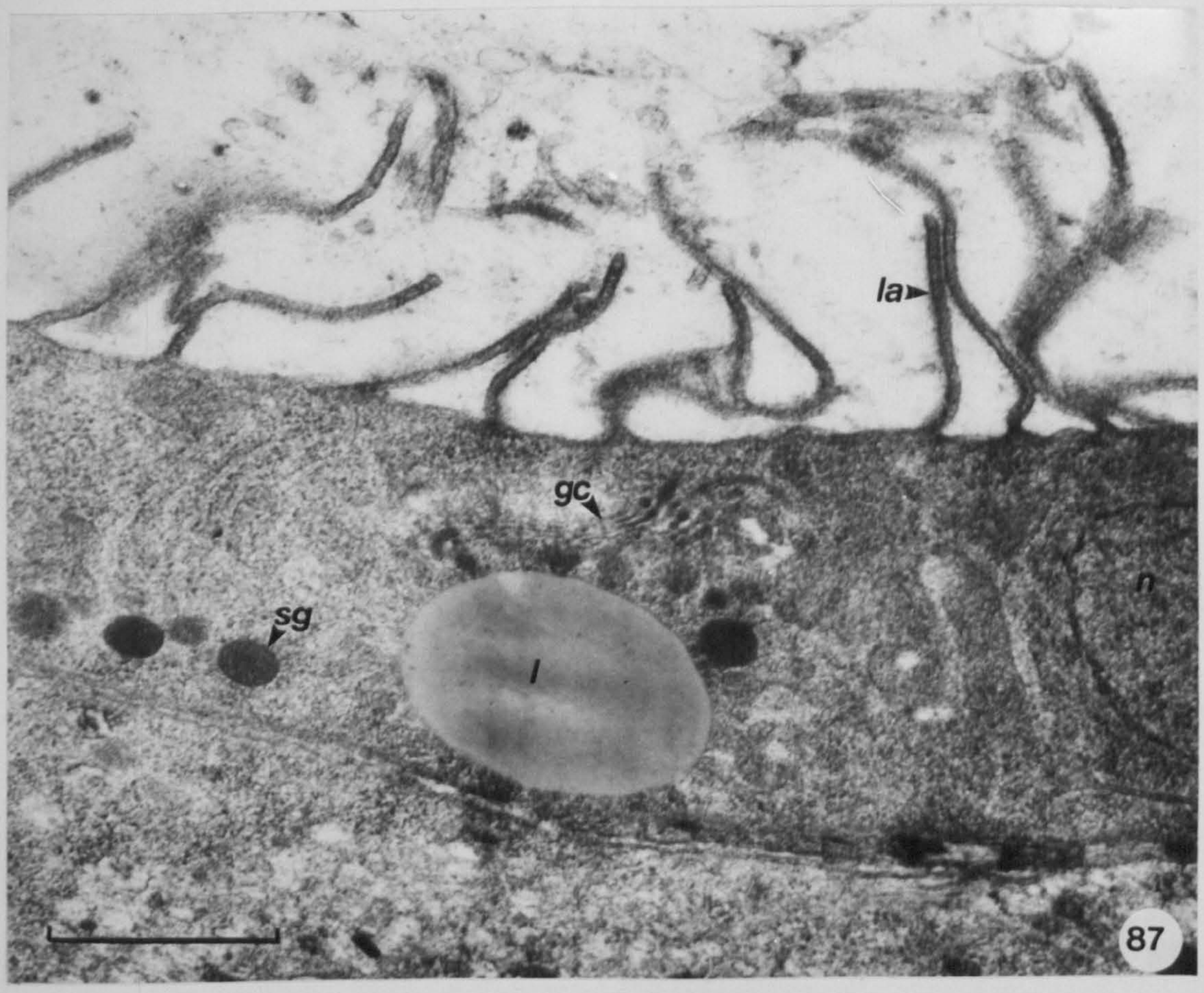

cc
EB
l
l
mu
ger
m
la
sd
sg
pc
86

Plate 87 Metacercaria.

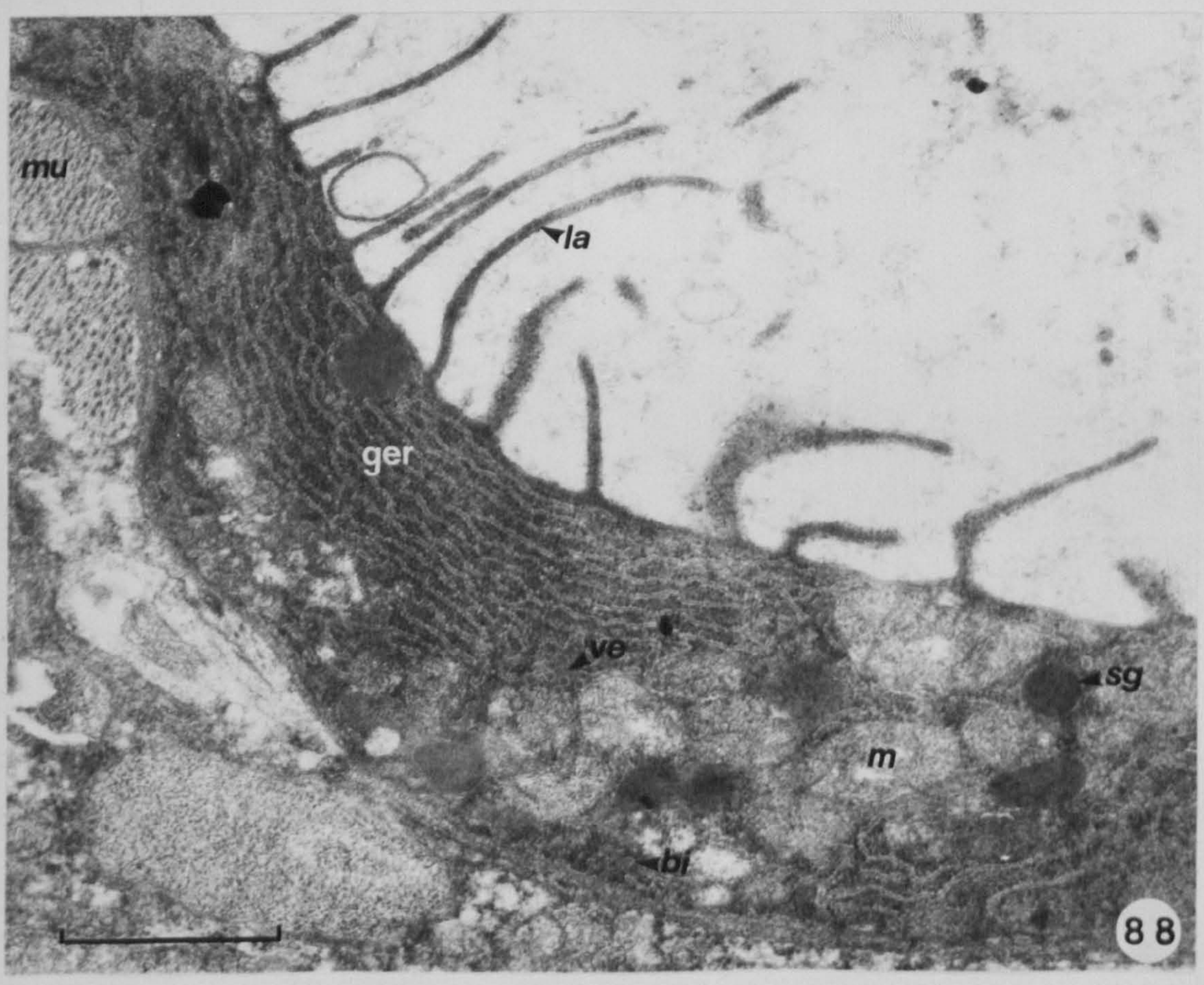
This section through the caecal epithelium shows a Golgi complex (gc) filled with dense material of a similar densityto the secretory granules (sg).

Plate 88 Metacercaria.

This section through the caecal epithelium shows an area of GER (ger) and associated with it several small vesicles (ve) which are filled with a moderately dense material. An invagination of the basal plasmalemma (bi) can also be seen.



87



88

Plate 89 Metacercaria.

The excretory bladder (EB) contains numerous excretory granules (eg) and several small lipid drops (l) which are both closely associated with the long, tortuous surface lamellae (la). In one area (arrowed), the bladder appears to be lined with tegumental syncytium which contains typical tegumental secretory granules. A flame cell (f) is cut in cross section.

Plate 90 Metacercaria.

A high power micrograph showing the structure of that part of the excretory granule (eg) which remains after decalcification.


eg
l
EB
la
89

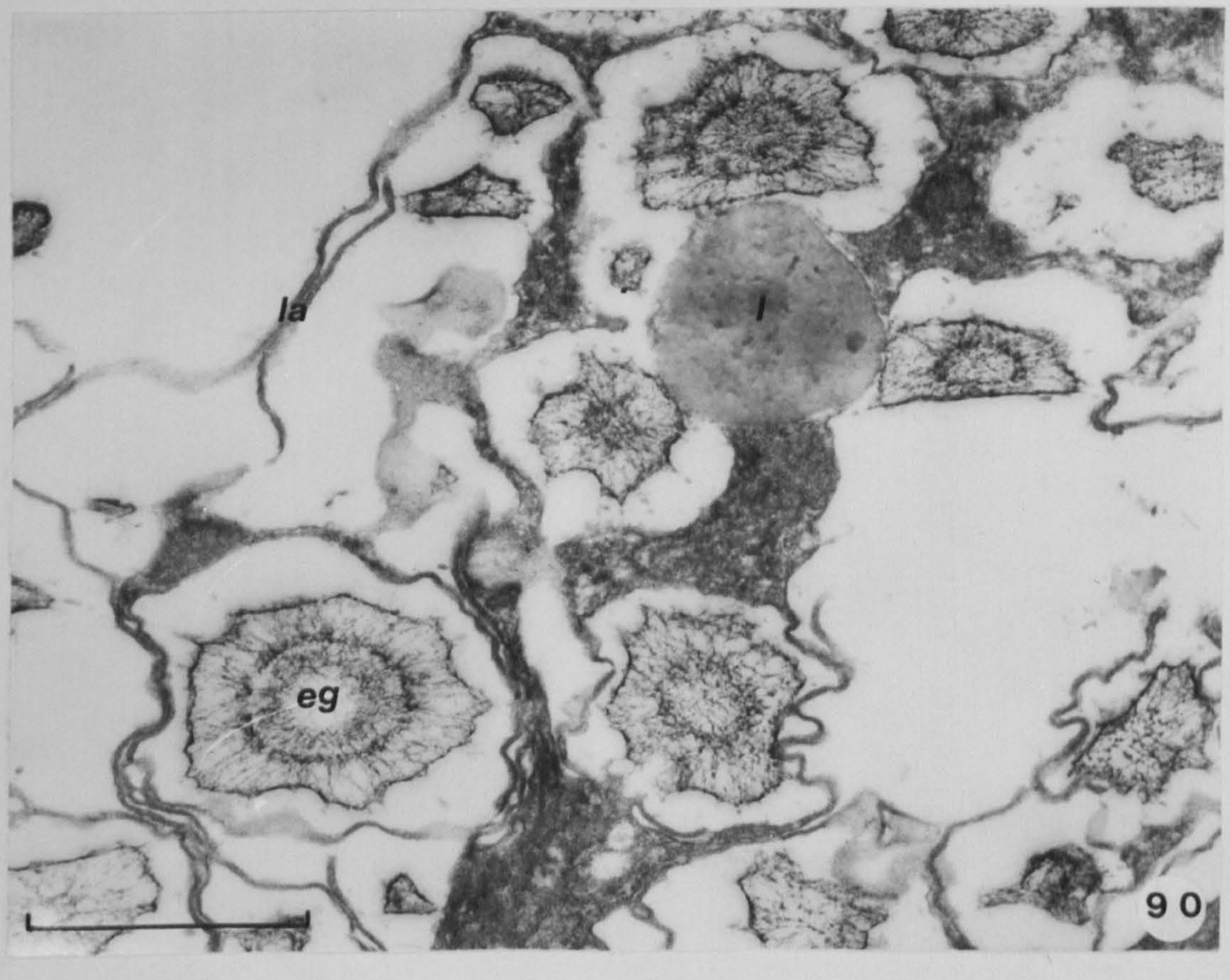

la
l
eg
90

Plate 91 Metacercaria.

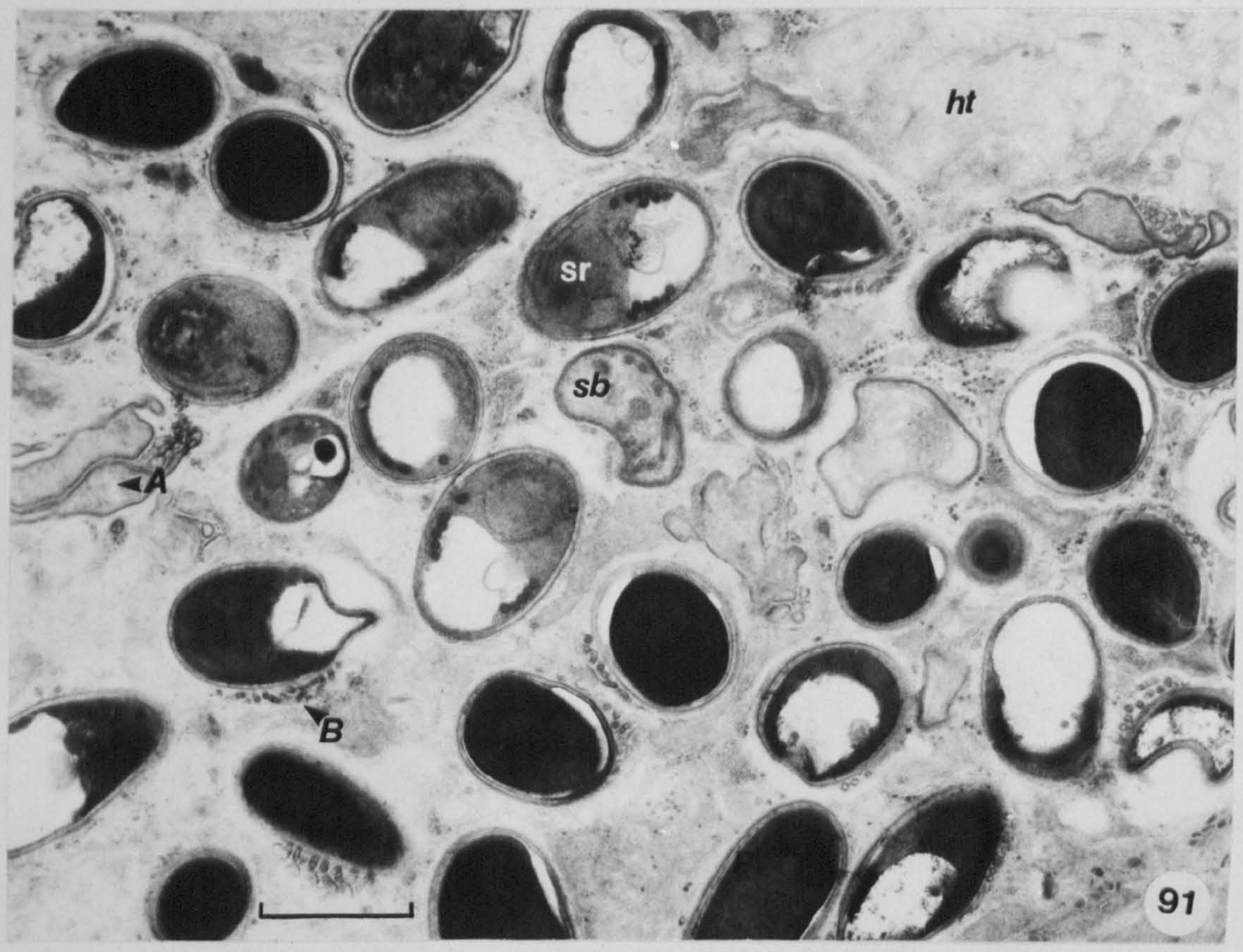
Spores (sr) and sporoblasts (sb) of the microsporidian Unikaryon legeri showing the two types of structures which are often associated with them. One of these structures is membraneous and sac-like (A) and is located in the host tissue (ht) in the vicinity of the spores. The other (B) is more closely associated with the spores.

Plate 92 Metacercaria.

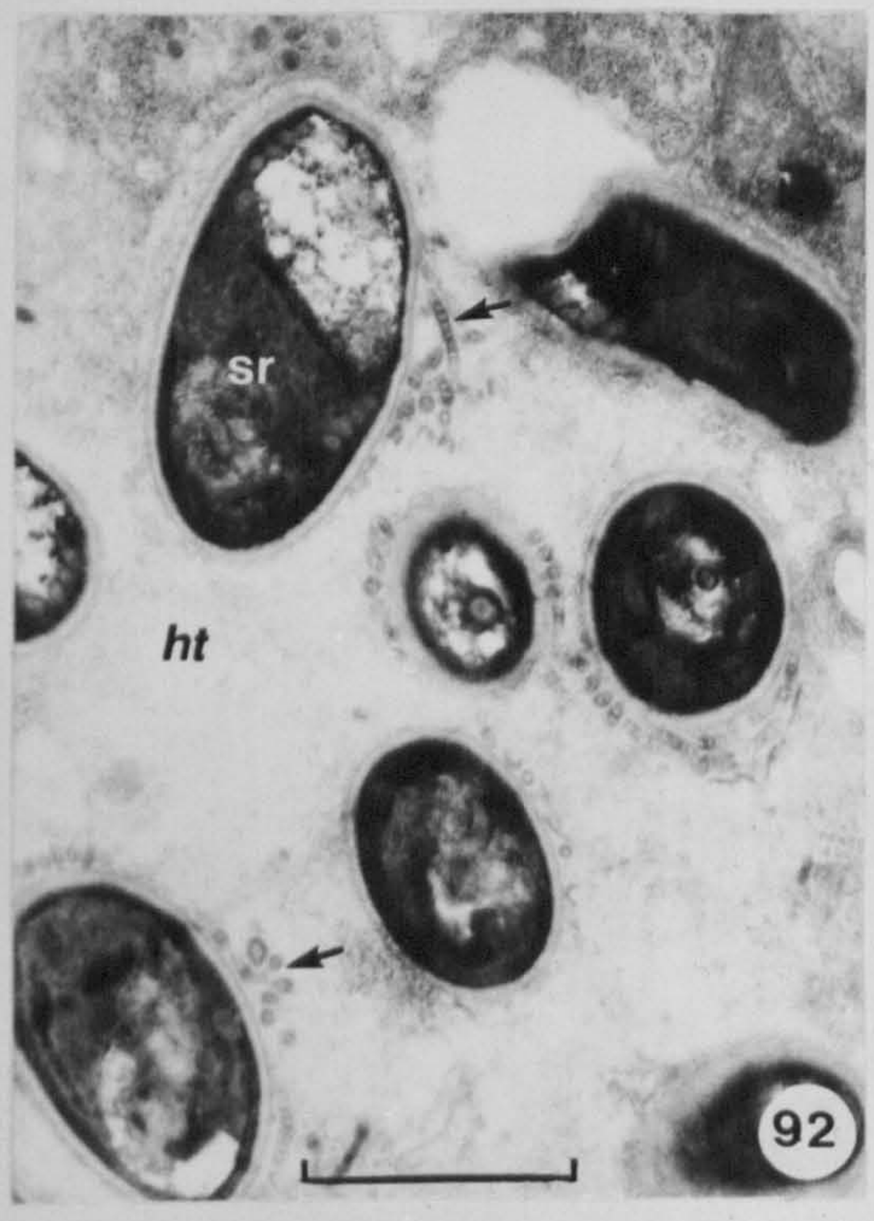
This section shows the second type (Type B) of associated structure (arrowed) more clearly. It is "ladder-like" in longitudinal section and consists of one or two concentric spheres in cross-section.

Plate 93 Metacercaria.

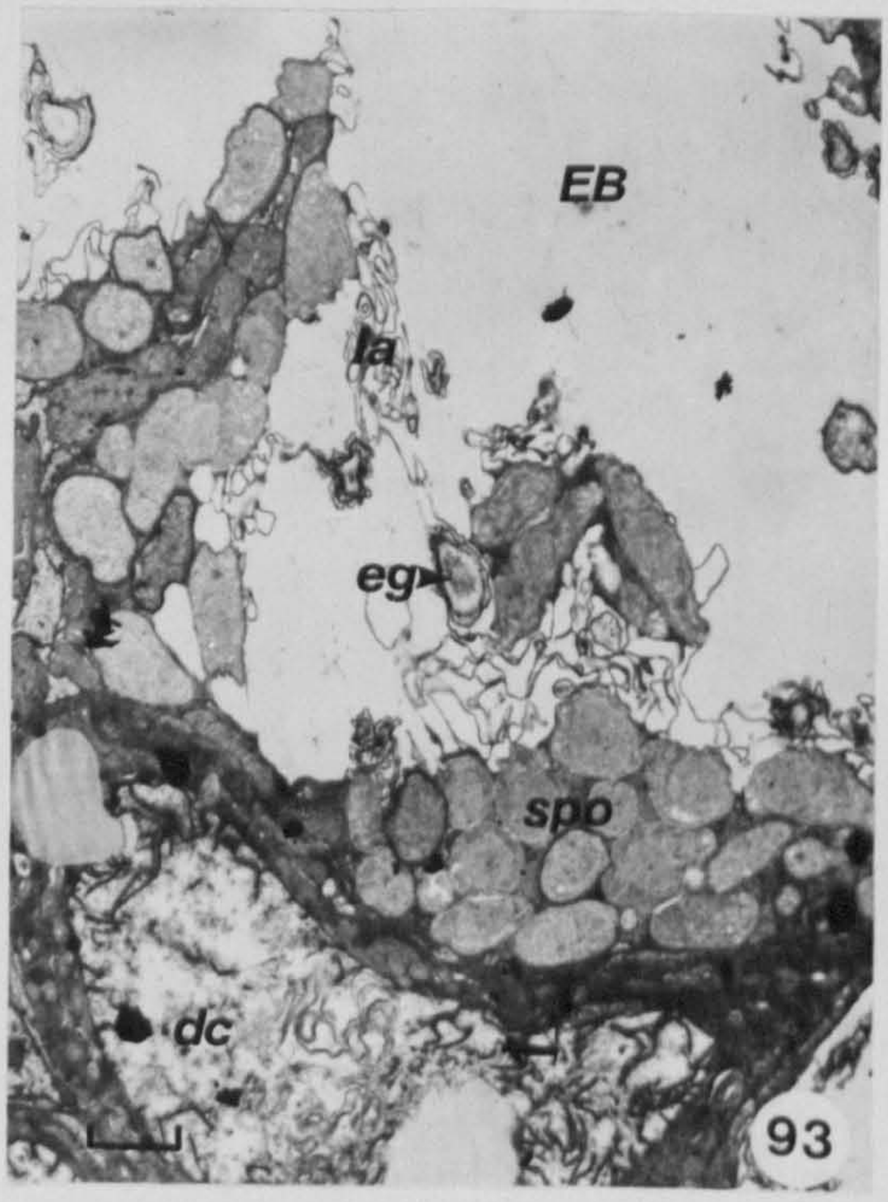
Sporonts (spo) of U. legeri embedded in the cytoplasm lining the excretory bladder (EB).



91



92



93

Plate 94 Adult from oystercarcher.

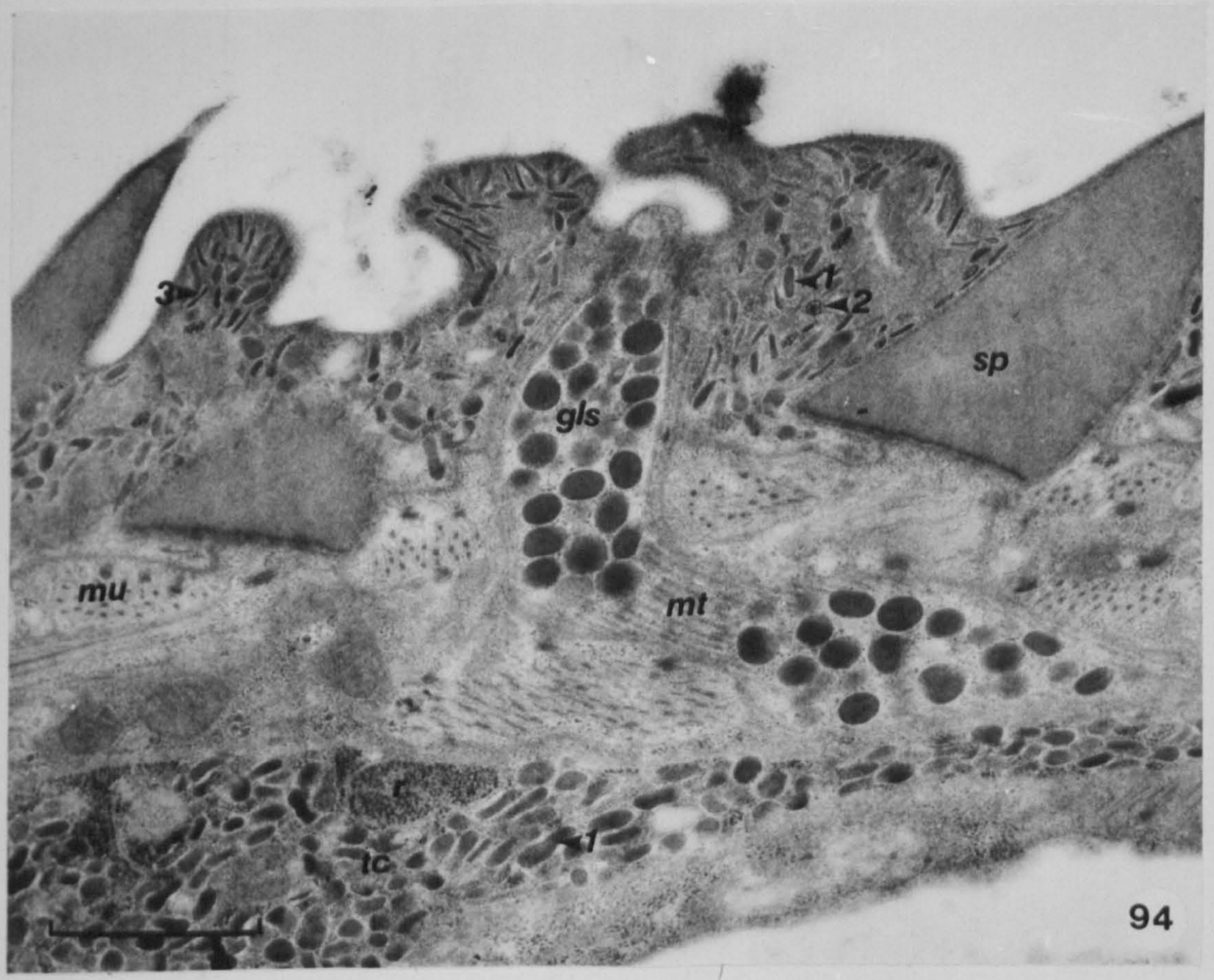
Three types of granules are present in the syncytium - spherical and dense (Type 1), oval and granular (Type 2) and biconcave and dense (Type 3). The tegumental cell body (tc) contains spherical secretory bodies (Type 1) and numerous free ribosomes (r). A duct of a unicellular gland cell can be seen penetrating the syncytium. It contains dense secretory bodies (gls) and is lined with microtubules (mt).

Plate 95 Adult from oystercatcher.

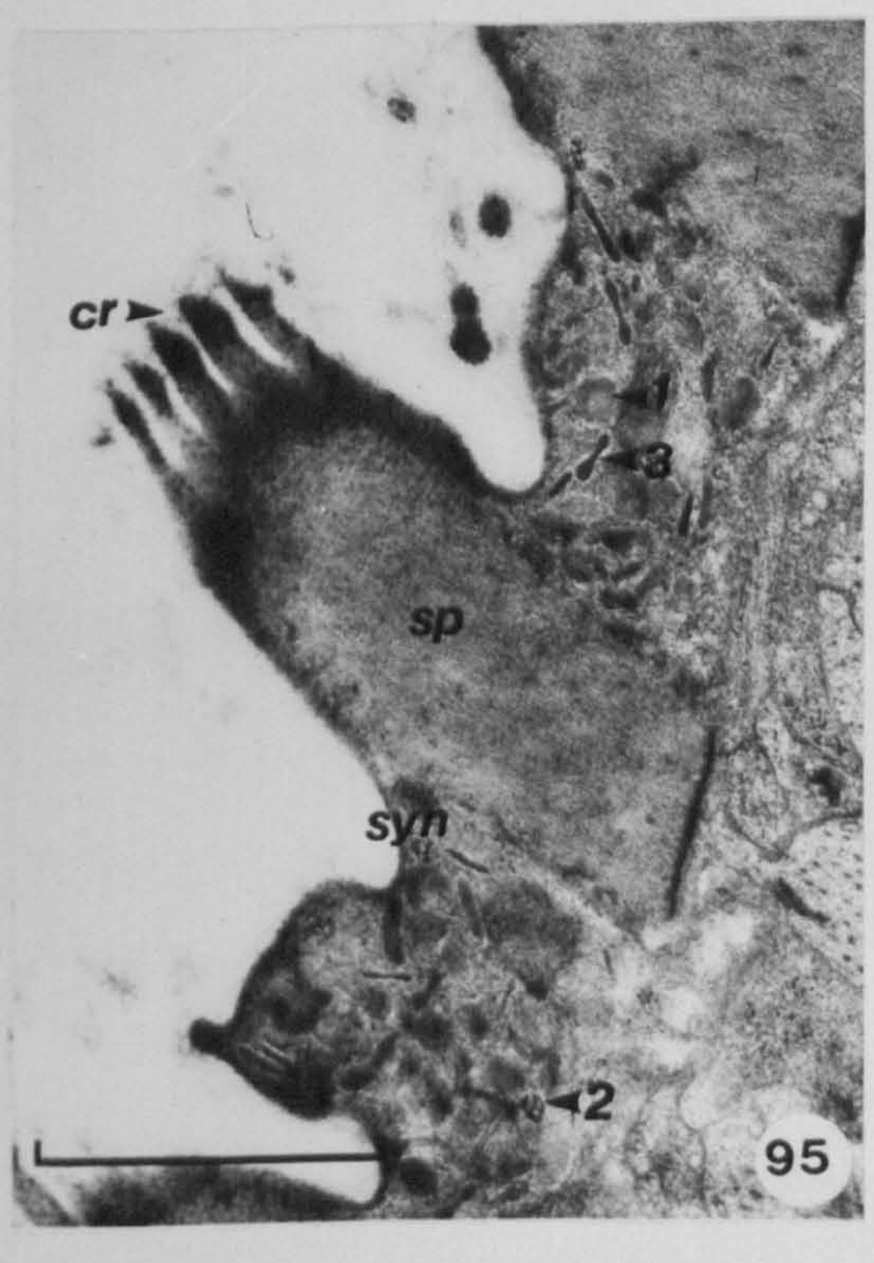
A longitudinal section through a tegumental spine (sp) to show the "toothed" tip (cr).

Plate 96 Adult from oystercatcher.

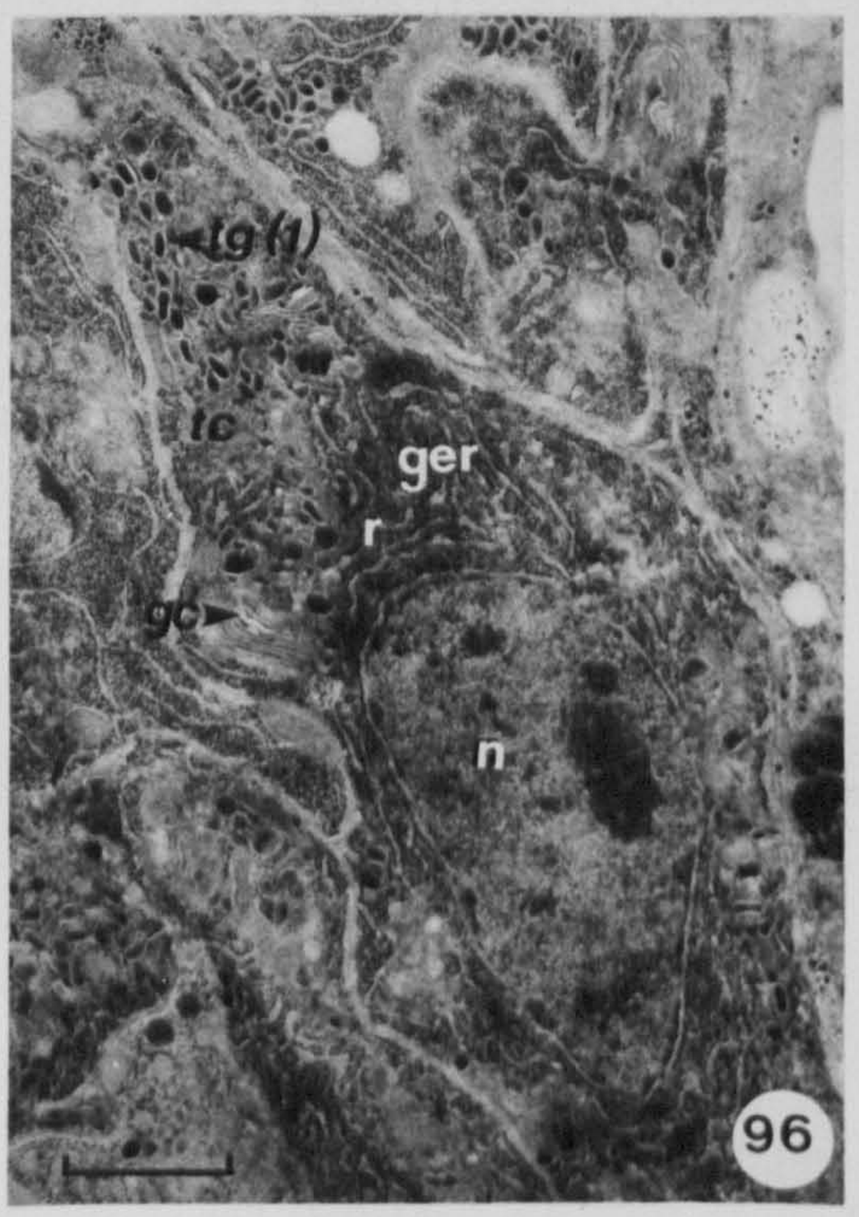
In addition to free ribosomes (r), there is some GER (ger) in the tegumental cell bodies (tc) particularly near the nucleus (n). Golgi complexes (gc) and tegumental granules (tg) are also present.



94



95



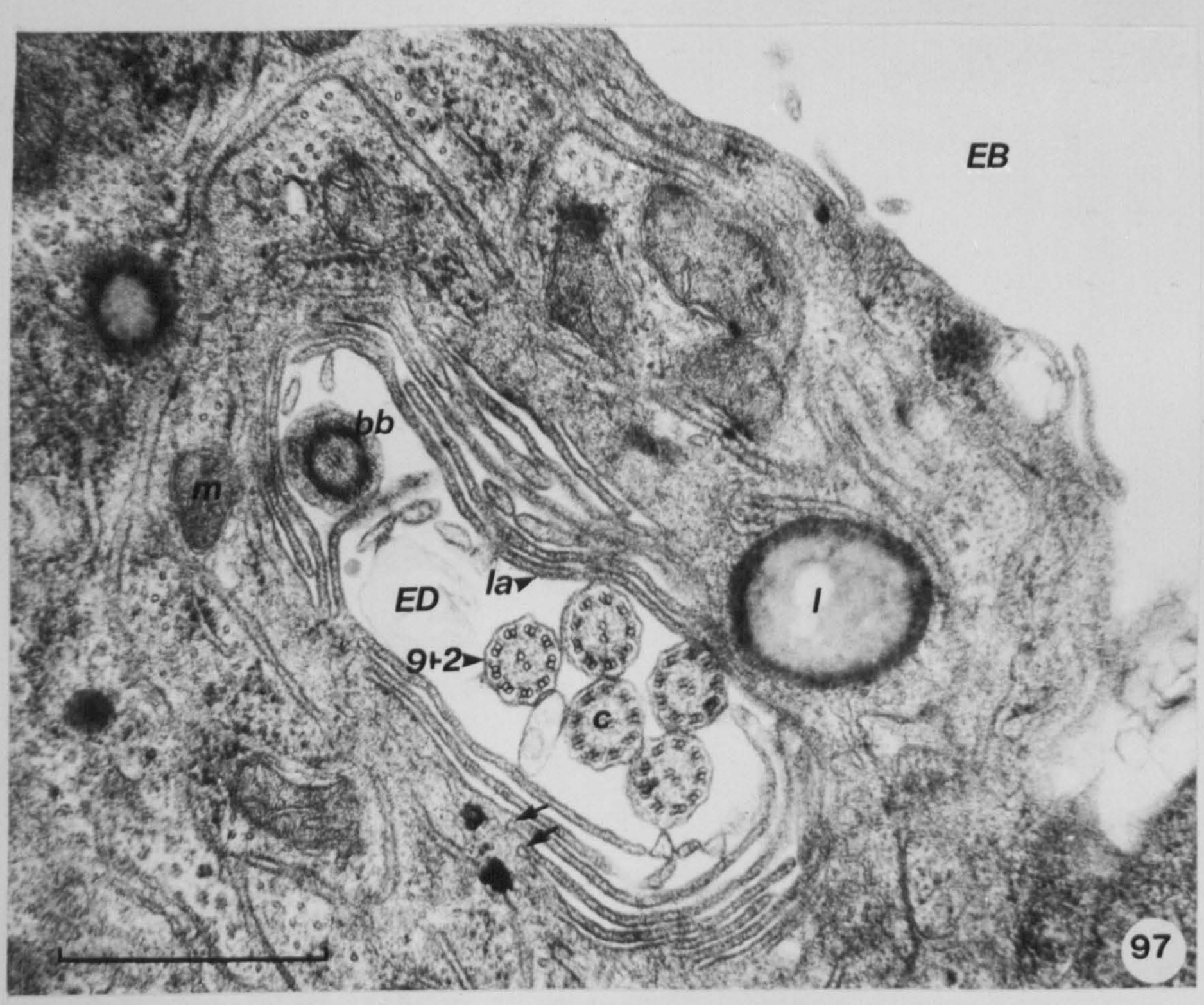
96

Plate 97 Adult from oystercatcher.

A section through a collecting duct of the excretory system (ED) shows that it bears both surface lamellae (la) and cilia (c) with a typical "9+2" arrangement of microtubules. The cytoplasm lining the duct contains mitochondria (m), lipid drops (l) and possible micropinocytotic vesicles which appear to originate at the base of the lamellae (arrowed).

Plate 98 Adult from oystercatcher.

An oblique section through a flame cell shows both the flame cell body (FC) and the cell of the conjoined tubule (CT) which are connected by a series of inter-digitating microvilli (imv) with a small area of dense (d) material at points of contact. The cilia of the flame (f) have a typical "9+2" configuration and typical basal bodies (bb) which are embedded in the cytoplasm of the flame cell body (FC). Internal leptotriches (li) are present and the flame cell is surrounded by interstitial material (im).


EB
bb
m
ED
la
9+2
c
l
97

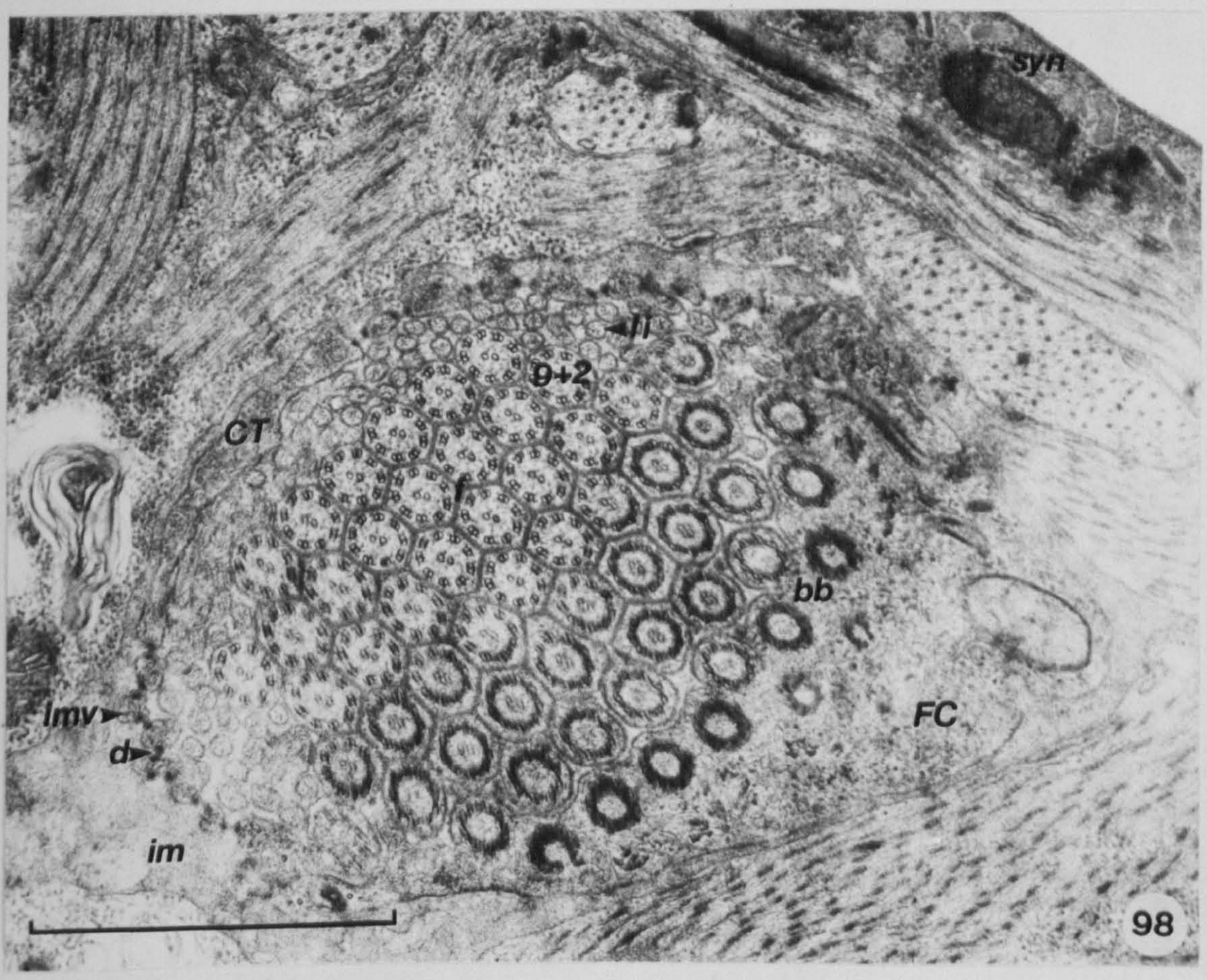

syn
ll
9+2
CT
f
bb
lmv
d
im
FC
98

Plate 99 Adult from Oystercatcher.

This section through the vesicula seminalis shows sperm cut in various planes of section. The sperm typically contain two axial filaments (af) with a "9+1" substructure, a nucleus (n), a mitochondrion (m) and peripheral microtubules (mt).

Plates 100 - 103 Developing adult - Day 1 p.i. from the duckling.

Plate 100

The basal bodies which produce the axial filaments of the spermatozoon in the spermatid stage, are embedded in the central process (cp) of the spermatid which is lined by microtubules (mt). The nucleus of an adjacent spermatid with its condensed chromatin (cn) can be seen.

Plate 101

The lateral and central processes of several spermatids have been cut in cross section; the lateral processes contain the axial filaments (af) and the central(cp) processes are lined by microtubules.. The filament producing apparatus of an adjacent spermatid can also be seen and consists of the "centriole-like" body (cb) and the two basal bodies (bb).

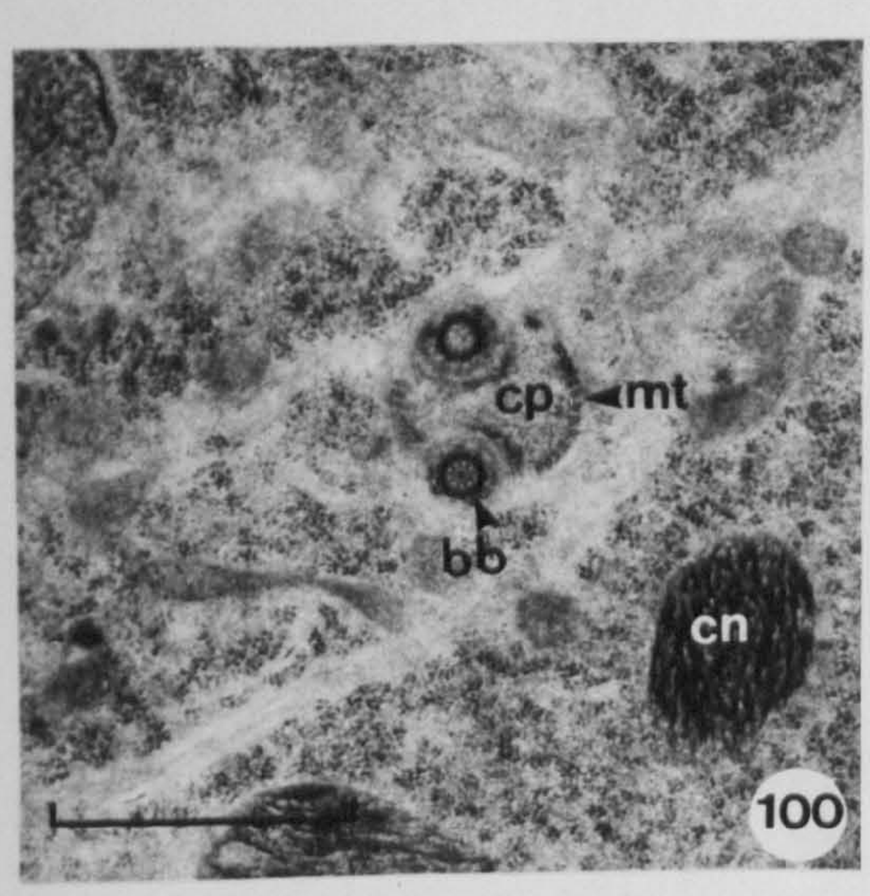
Plate 102

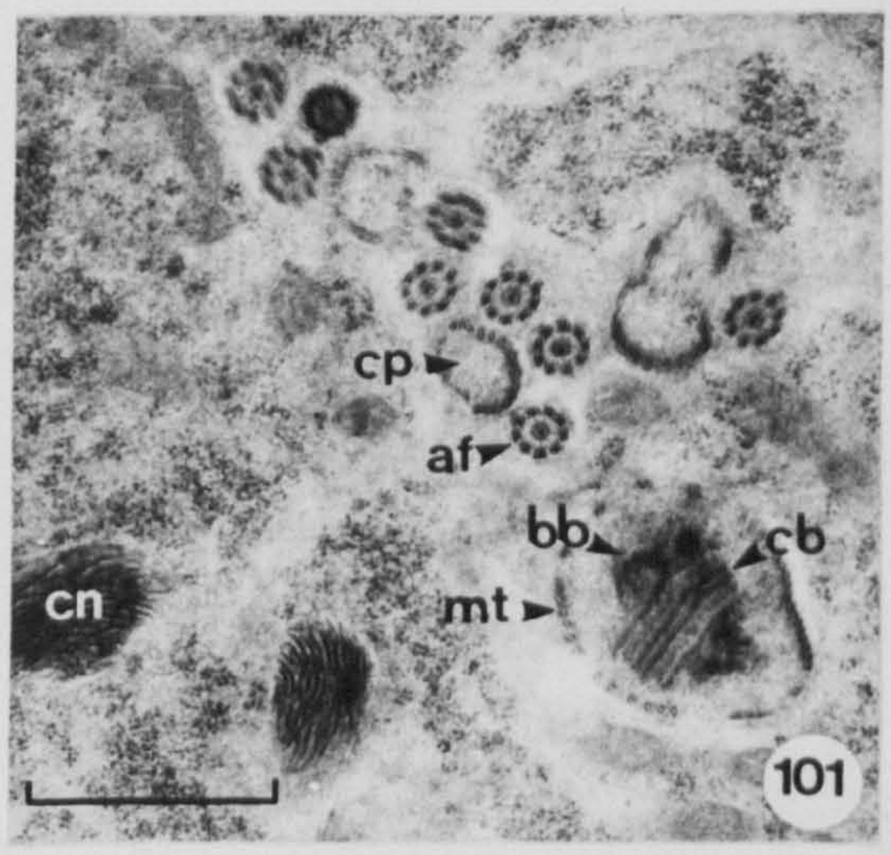
A longitudinal section through a spermatid showing the nucleus (n), peripheral microtubules (mt) and basal body (bb). The nucleus (n), at this stage, does not as yet possess condensed chromatin.

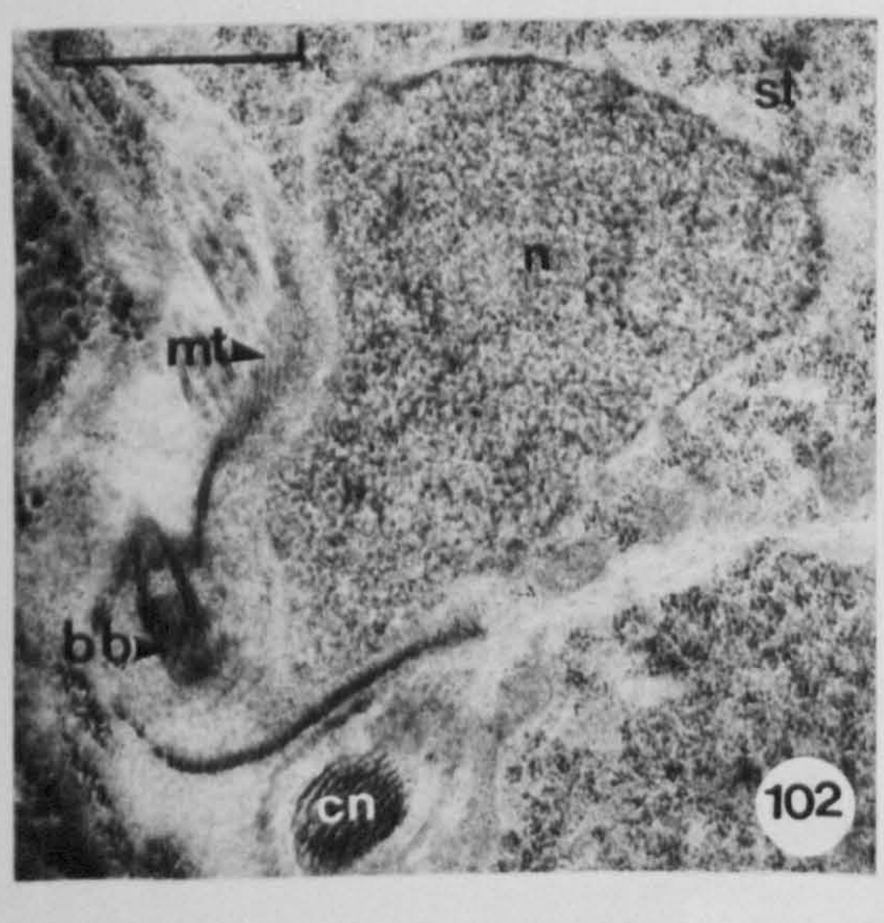
Plate 103

A longitudinal section through a spermatid showing the "centriole-like" body (cb), basal bodies (bb) and attached striated rootlet (rt). The nucleus (n) still bears a nucleolus (nu).


pgs
mt
m
af
9+1
99


cp
mt
bb
cn
100


cp
af
bb
cb
cn
mt
101


st
n
mt
bb
cn
102

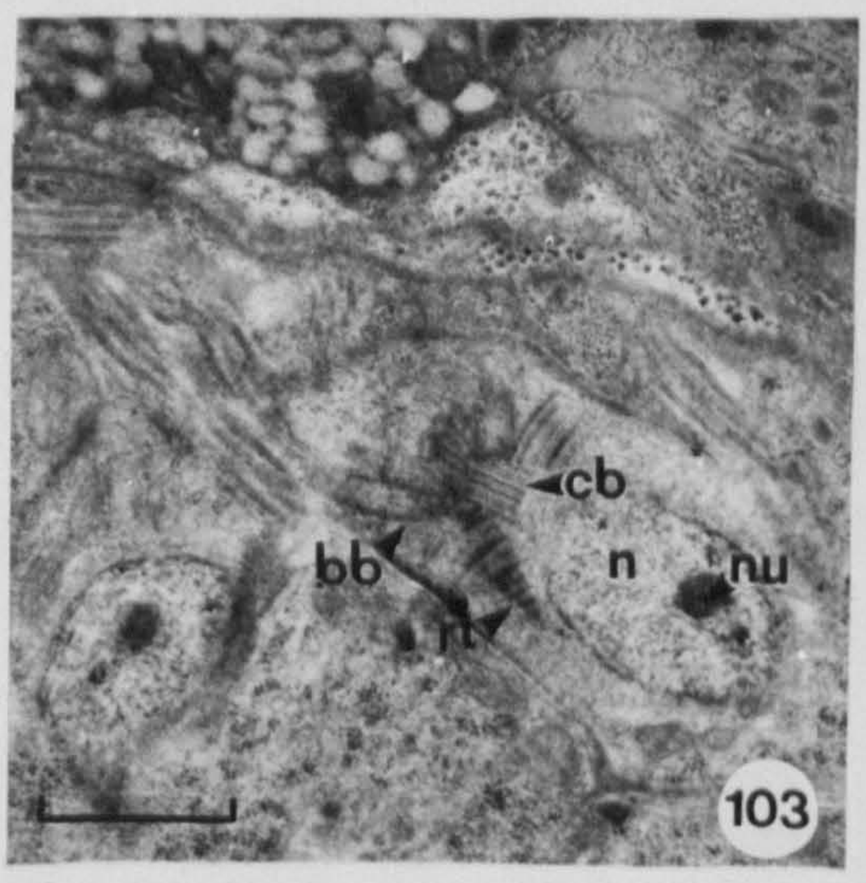
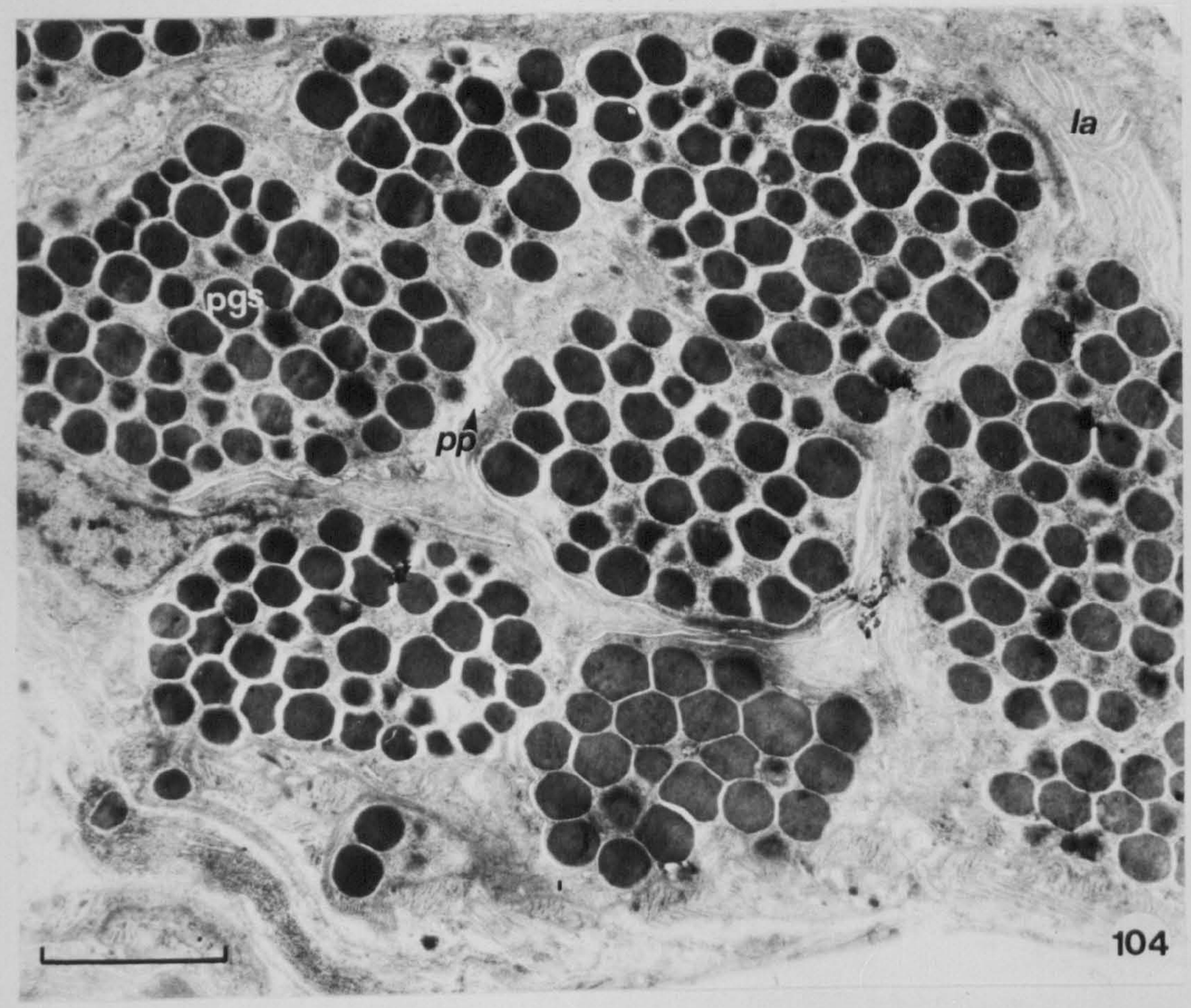

cb
bb
n
nu
mt
103

Plate 104 Adult from oystercatcher.

A section through the prostate gland showing the individual cells which are packed with dense secretory droplets (pgs) and which protrude into the lumen of the gland, or pars prostatica (pp), until it is virtually occluded. Lamellae (la) arise from the surface of the prostate gland cells.

Plate 105 Adult from oystercatcher.

Adjacent cells in the prostate gland are connected by a short septate desmosome (sd) at their luminal surface. The cytoplasm of the cells is filled with dense secretory bodies (pgs) of various shapes and sizes.


la
pgs
pp
104

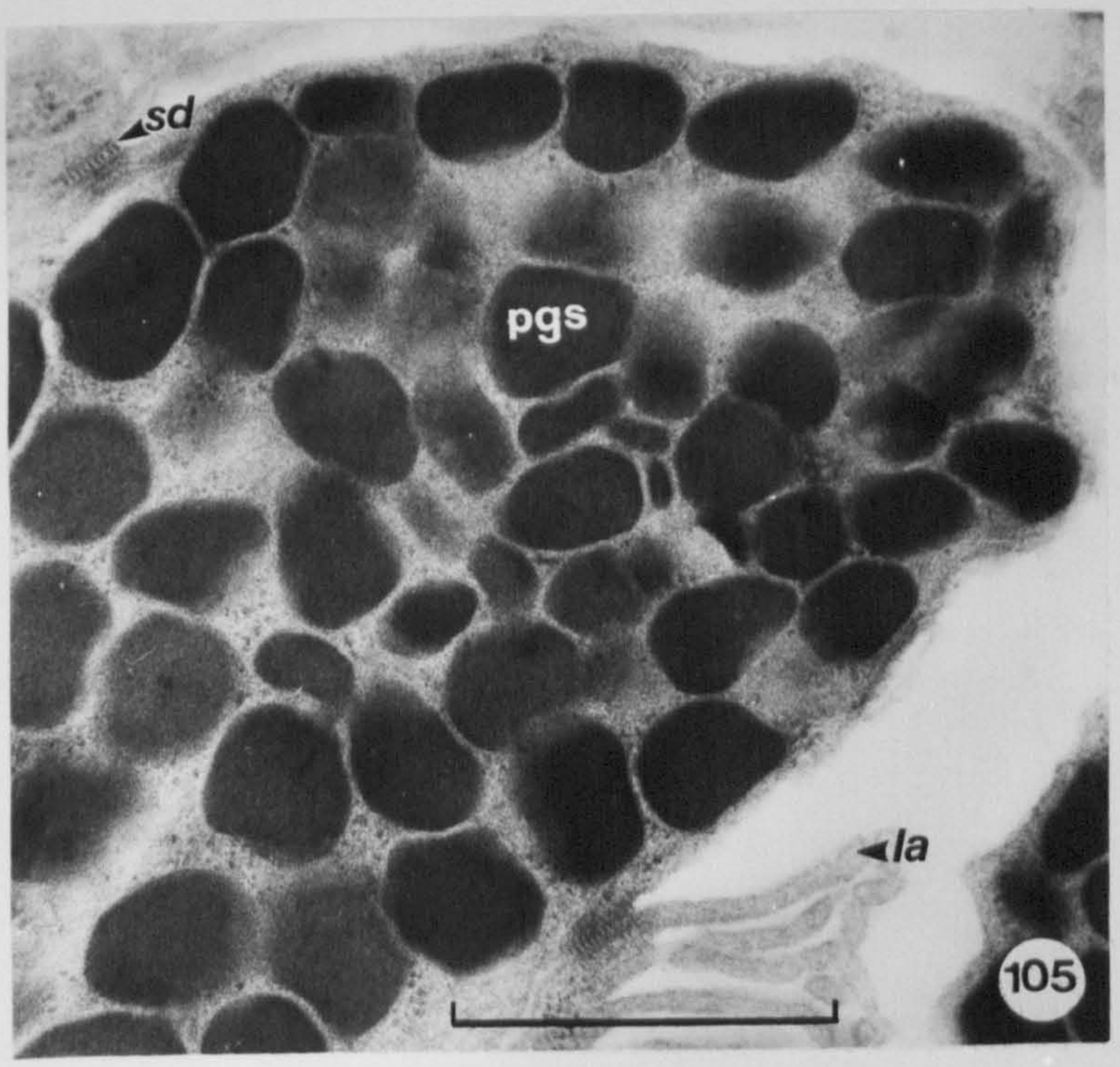

sd
pgs
la
105

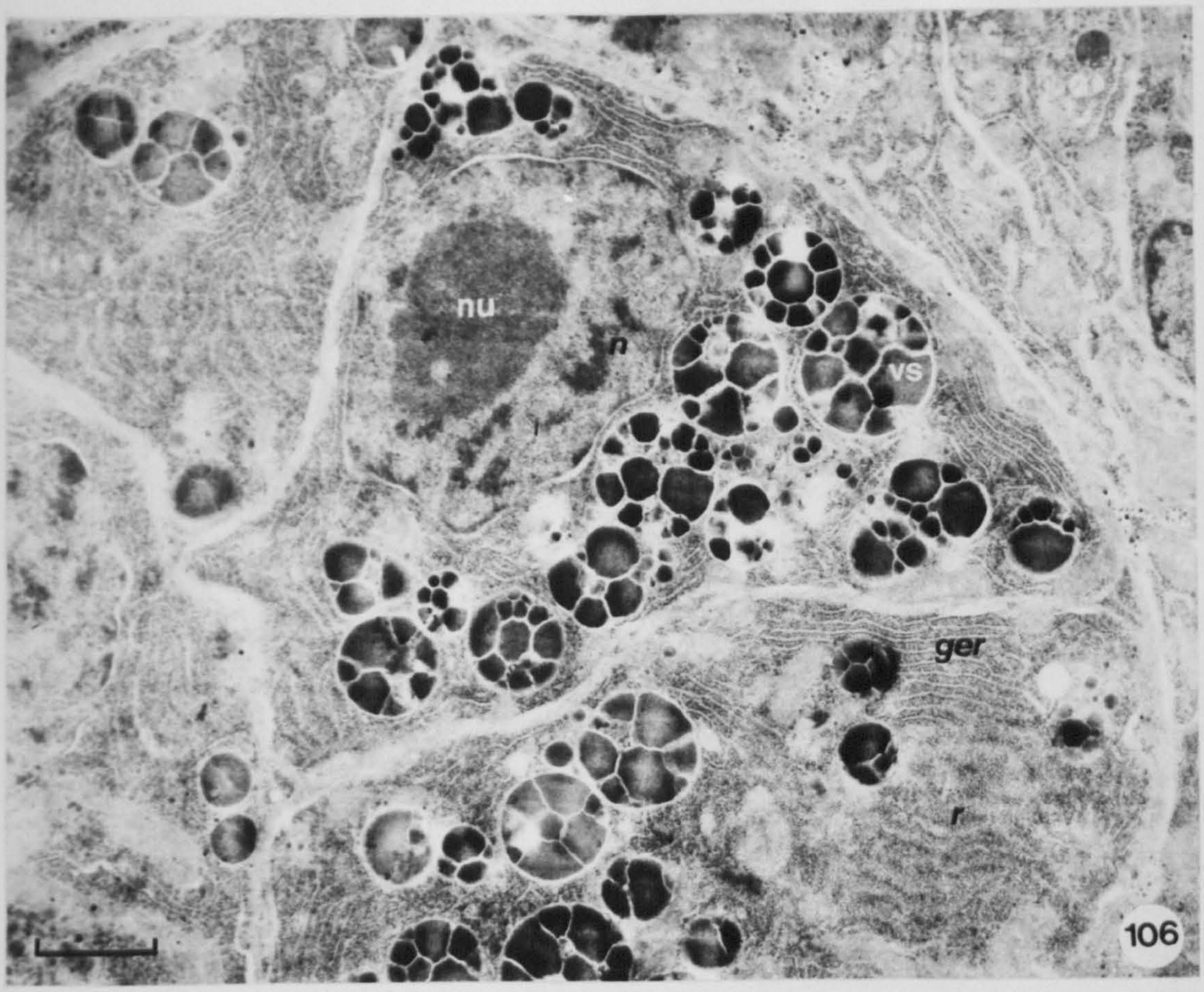
Plate 106 Adult from oystercatcher.

The vitelline cells contain abundant GER (ger) and free ribosomes and the typical vitelline secretory bodies (vs) which are clustered together to form spherical globules.

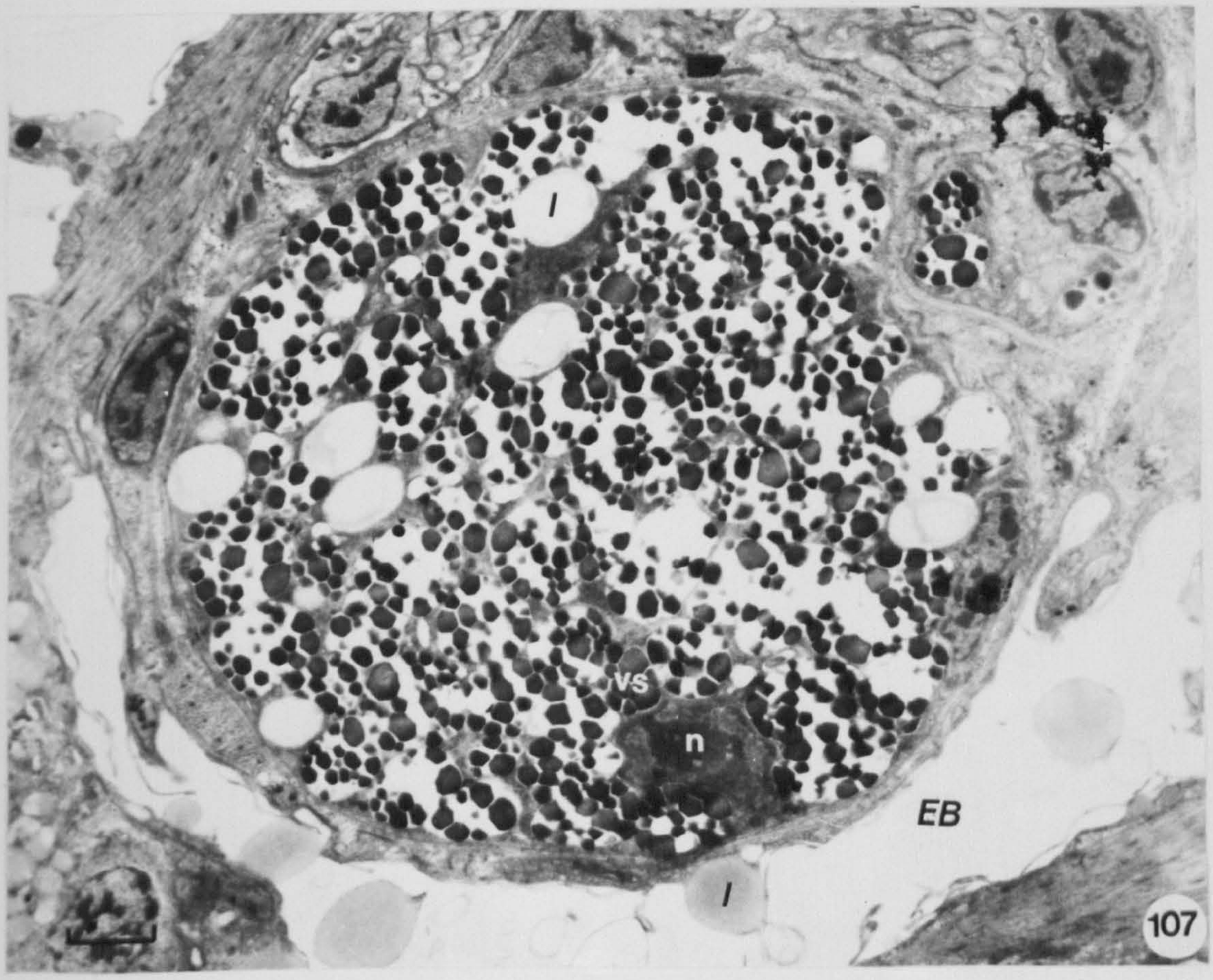
Plate 107 Adult from oystercatcher.

The mature vitelline cell is packed with vitelline secretory bodies (vs) and lipid drops (l). Very little of the cytoplasm is left and, in this particular cell, the only recognisable organelle is the nucleus (n).

The excretory bladder (EB) contains numerous lipid droplets (l) some of which are in the process of extrusion into the lumen.



106



107

Plate 108 Adult from oystercatcher.

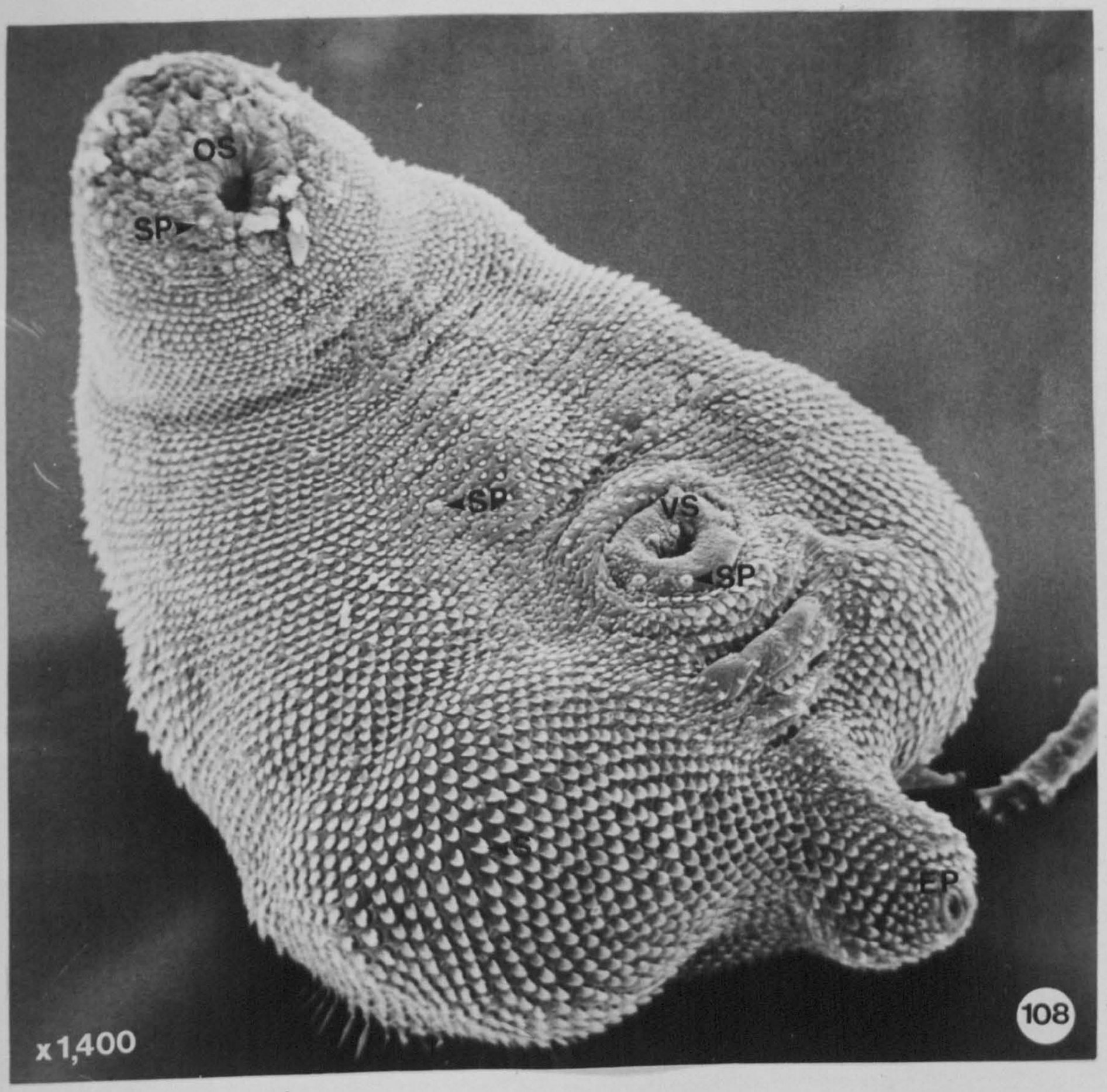
This view shows the typical shape of the adult. The body is covered with rings of spines (S). The oral sucker (OS) is at the anterior end and the ventral sucker (VS) is in the posterior part of the body, just posterior to it is a spine-free ridge. The excretory pore (EP) region often protrudes slightly.

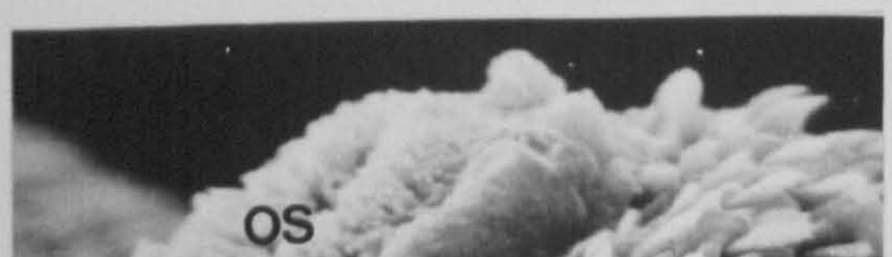
Plate 109 Adult from oystercatcher.

Ciliated (C) sensory papillae (SP) surround the oral sucker (OS) the lips of which are spine free. In this view the "toothed" (CR) tips of the spines (S) can be seen.

Plate 110 Adult from oystercatcher.

The surface of the ventral sucker (VS) is spine-free and it bears several ciliated sensory papillae (SP). Adjacent to the sucker the spines appear to be sunk into pits in the tegument (arrowed).


OS
SP
SP
VS
SP
EP
S
x1,400
108


OS
C
SP
CR
S
x4,800
109

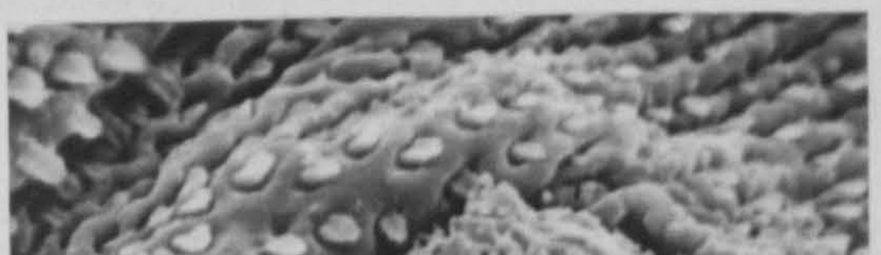
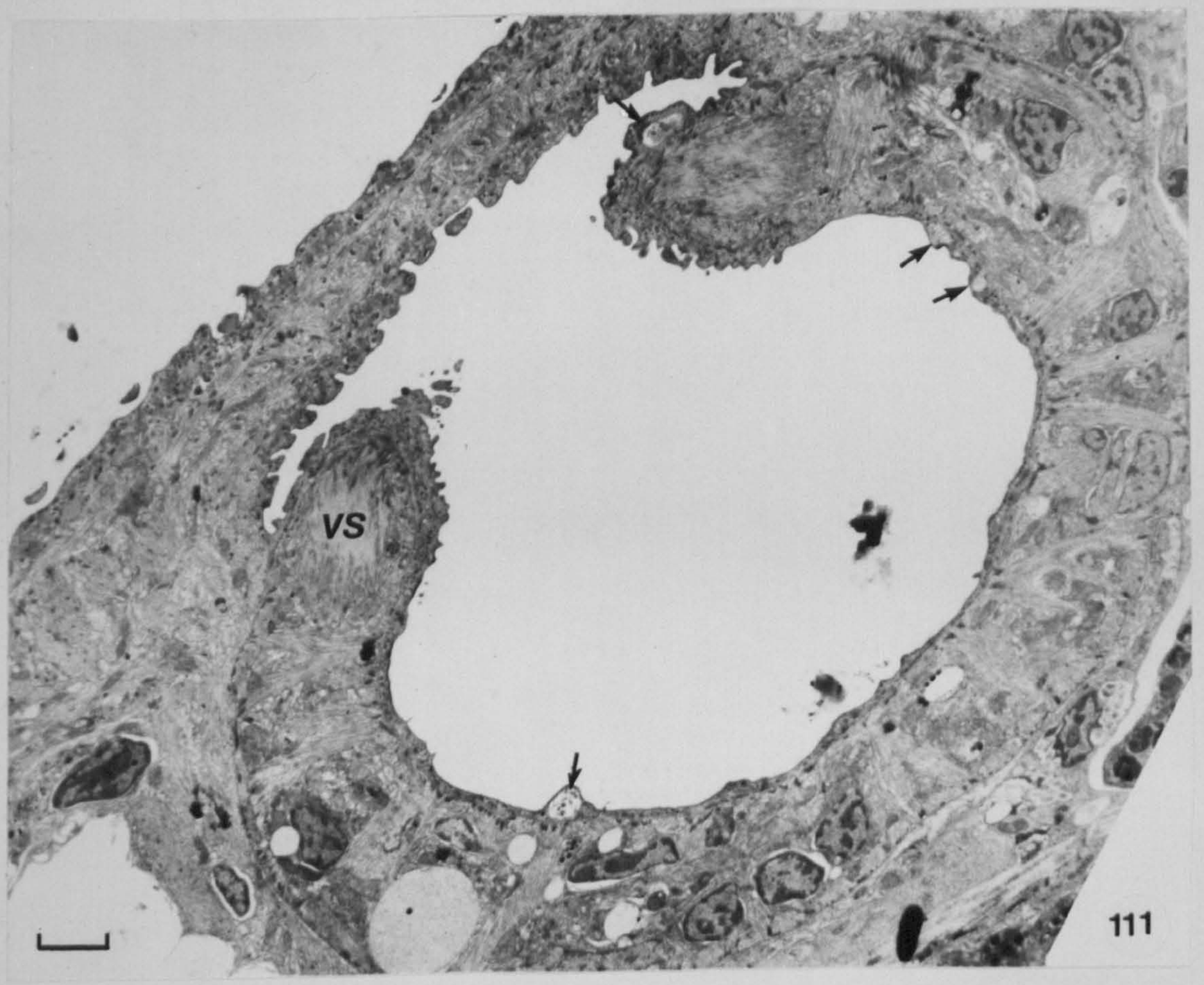

VS
SP
S
C
x4,000
110

Plate 111 4 days *in vitro* culture.

An oblique section through the ventral sucker (VS) showing abnormal "bubbling" of the syncytium (arrowed).

Plate 112 4 days *in vitro* culture.

The tegumental syncytium contains electron lucent, spherical granules (Type 0) and granular, oval (Type 2) granules. Both the oval and the spherical granules are present in the tegumental cell bodies. The tegumental cells also contain large lipid drops (l), Golgi complexes (gc), mitochondria (m) and free ribosomes (r). The layer of interstitial material (im) between the syncytium and the peripheral muscles is thicker than in the metacercaria or the adult.


VS
111

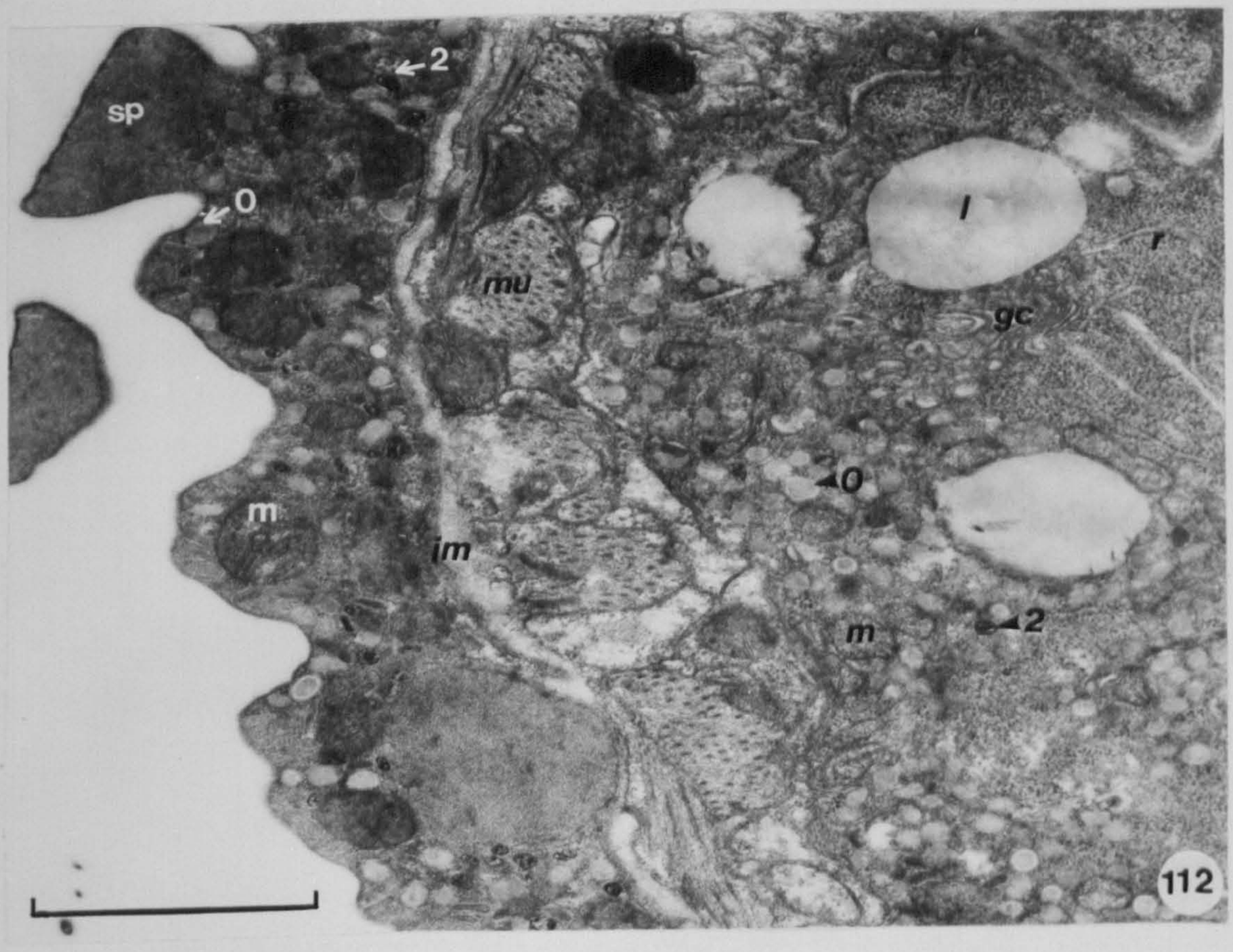
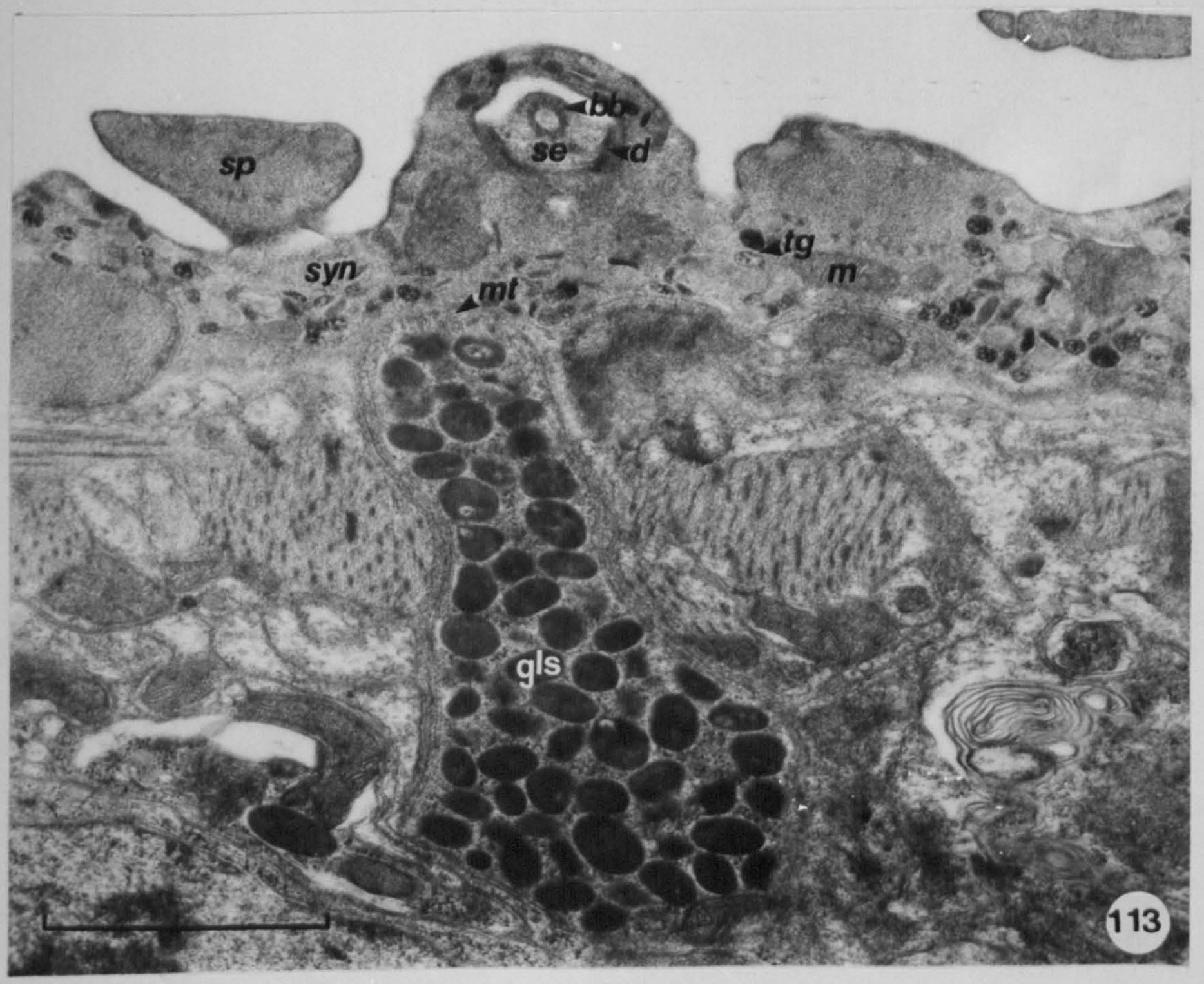

sp
2
0
mu
l
r
gc
m
im
2
112

Plate 113 4 days in vitro culture.

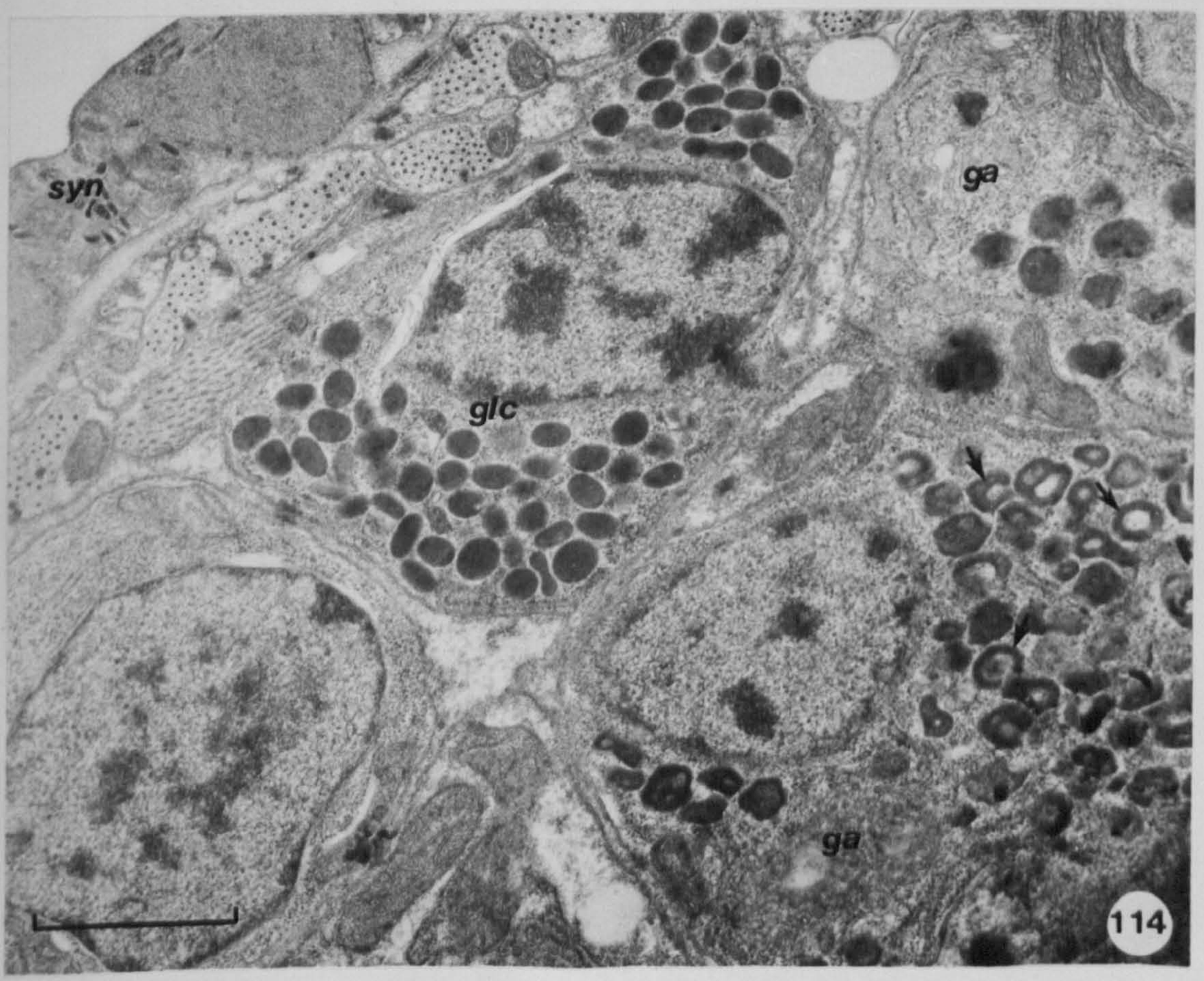
This section shows the duct of an apparently normal unicellular gland cell with typical secretory bodies (gls) and microtubules (mt). A sensory ending (se) is cut in cross section - the basal body (bb) of the cilium and the desmosome (d) connecting it with the syncytium (syn) can be seen.

Plate 114 4 days in vitro culture.

This section shows the cell bodies of three unicellular gland cells (glc) one of which appears normal whilst the other two contain rather abnormal secretory bodies (arrowed). Areas of possible Golgi activity (ga) can be seen in these cells.



113



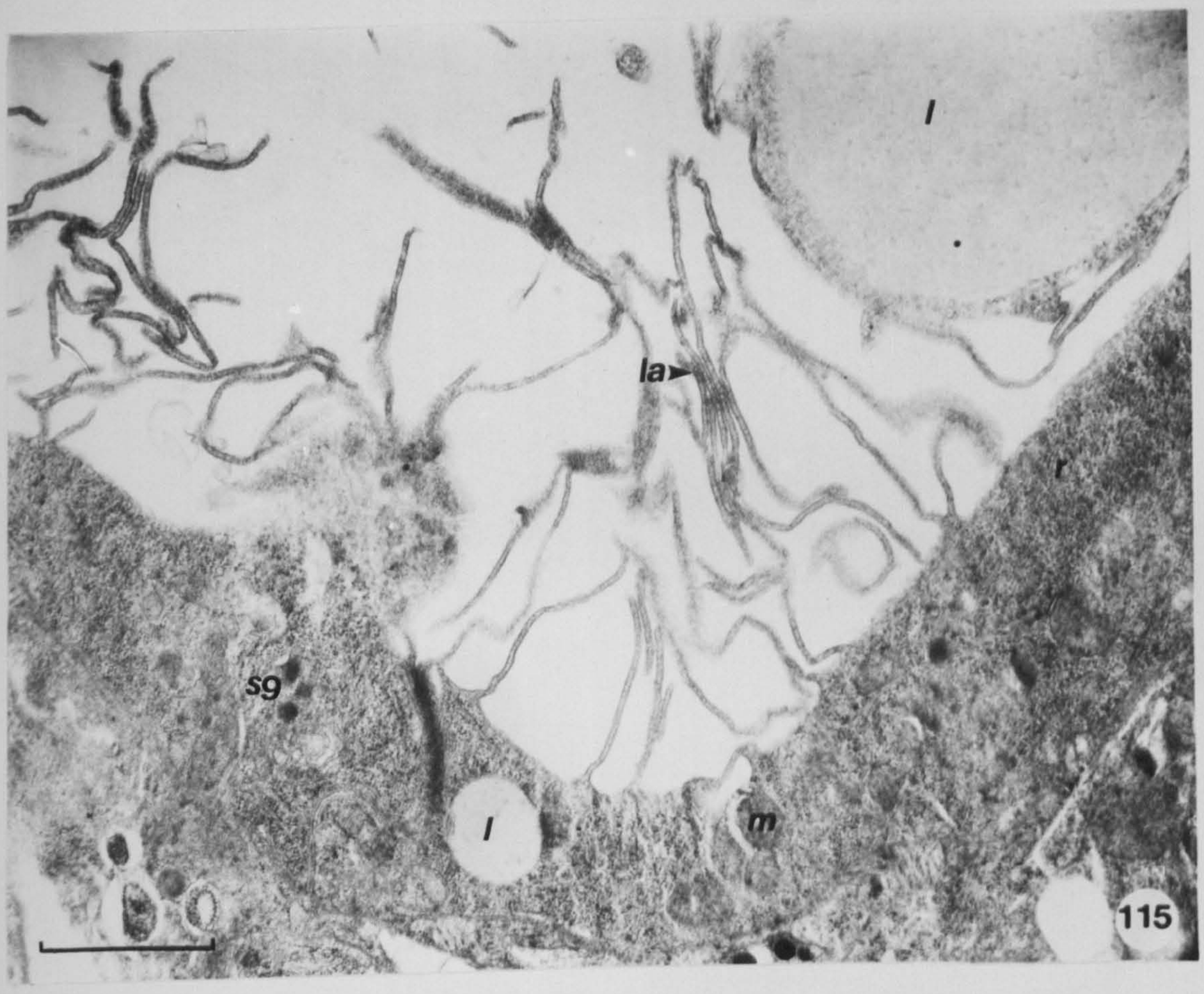
114

Plate 115 4 days in vitro culture.

The caecal epithelium appears highly disorganised; it contains mitochondria (m), small numbers of lipid drops (l), numerous free ribosomes (r) and occasional secretory granules(sg). The surface lamellae (la) are long and tenuous and large lipid drops are present in the lumen (l).

Plate 116 4 days in vitro culture.

Although many of the sperm in the vesicula seminalis of cultured flukes are apparently normal (1&2), abnormal sperm (3&4) with four axial filaments (af), one or two mitochondria (m) and one nucleus (n) are frequently seen.



115



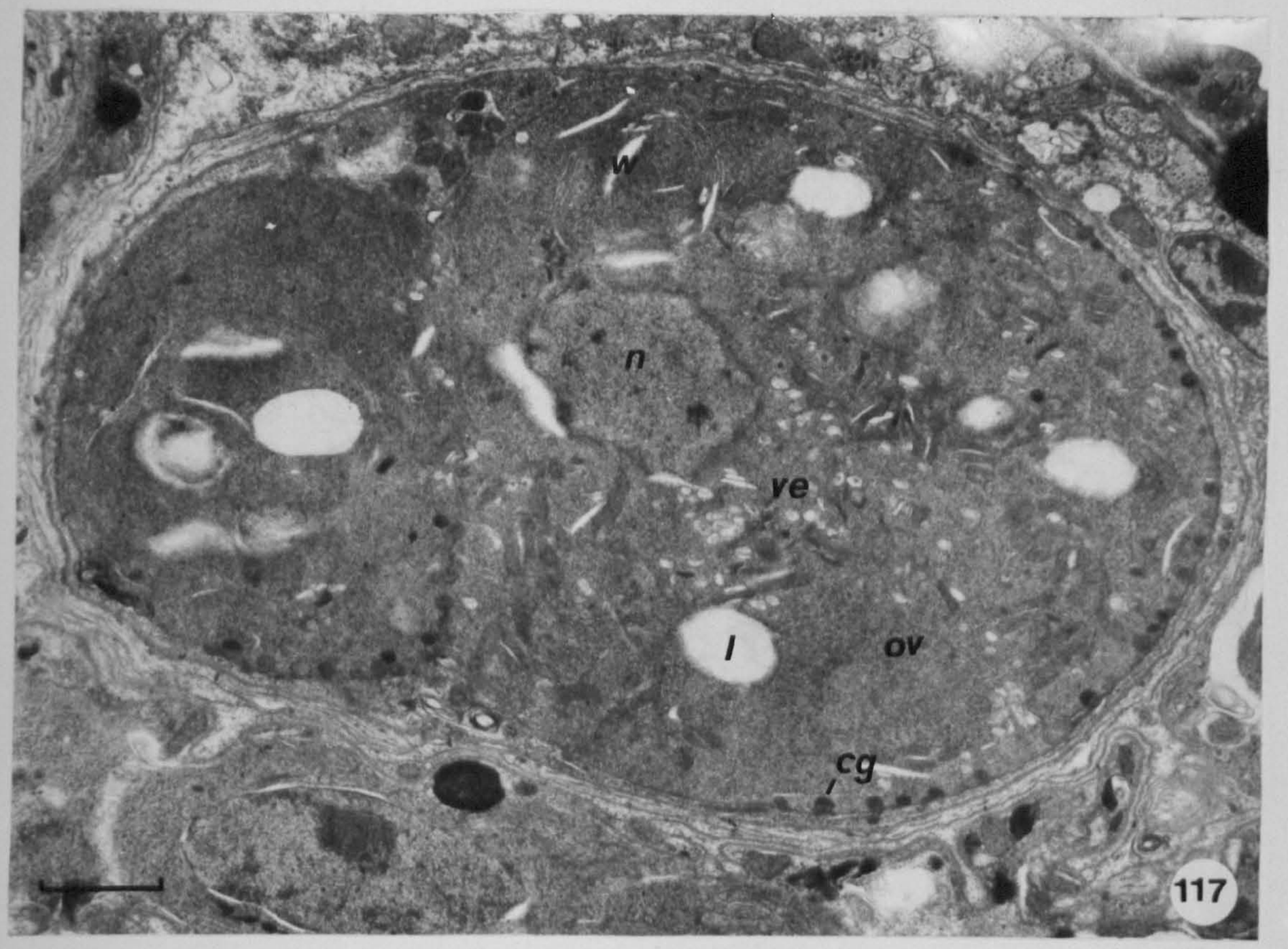
116

Plate 117 4 days *in vitro* culture.

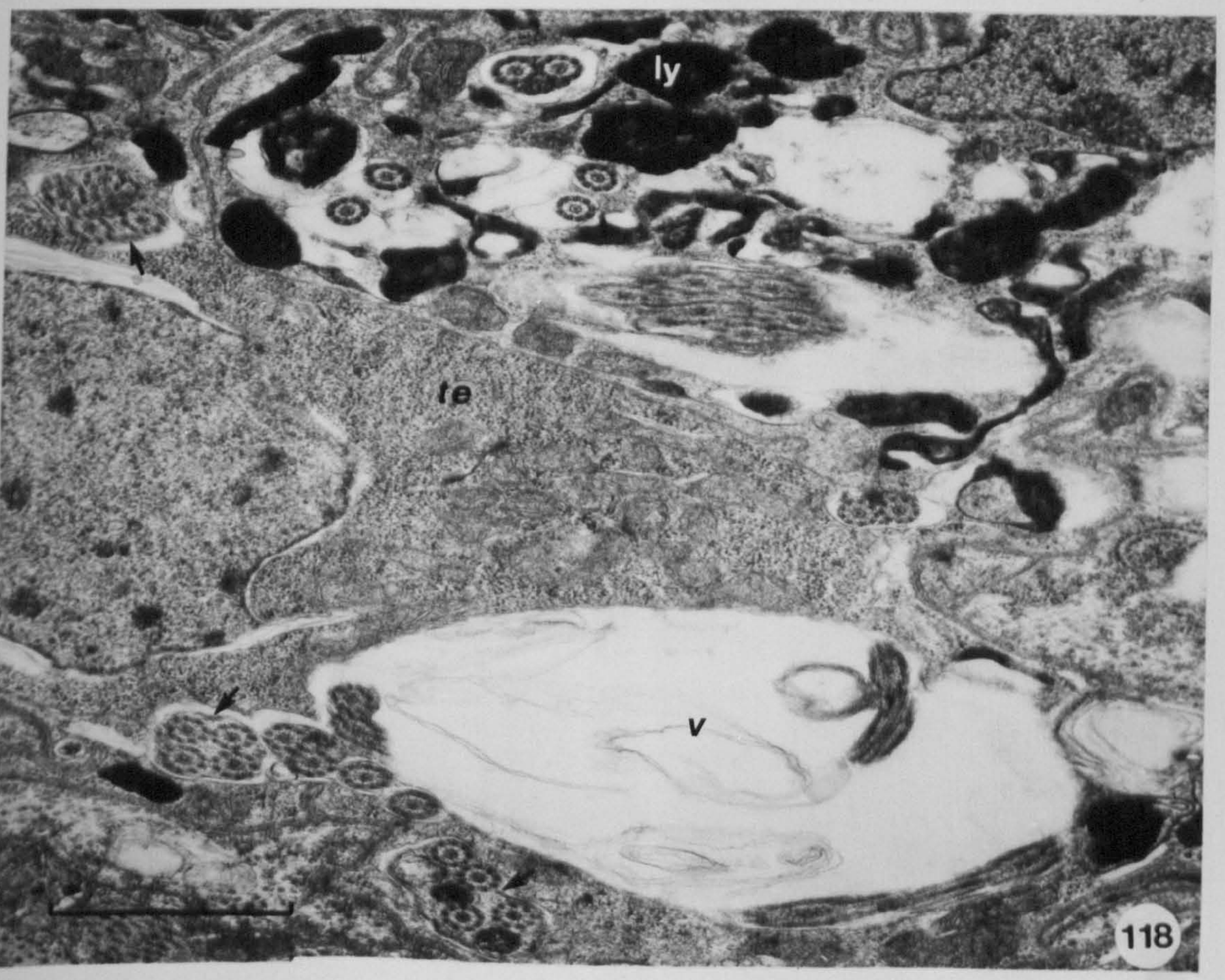
The ovary of cultured flukes appears grossly abnormal. Cell boundaries are difficult to discern and, in addition to cortical granules (cg) and mitochondria, the cells also contain lipid drops (l), vesicles (ve) and membraneous whorls (w).

Plate 118 4 days *in vitro* culture.

The testes (te) of cultured flukes appear abnormal, they contain areas of vacuolation (v) and dense lysosomal bodies (ly). Abnormal sperm with four rather than two axial filaments are present (arrows).



117



118

Plate 119 4 days *in vitro* culture.

The vitellaria contain numerous vitelline secretory bodies (vs), ribosomes (r) and areas of GER (ger). The vitelline droplets occasionally appear rather "ragged" in outline.

Plate 120 4 days *in vitro* culture.

The cytoplasm lining the uterus (ut) contains mitochondria (m), ribosomes (r) and GER (ger); it is thin and bears lamellae (la) or microvilli on its luminal surface. The lumen of the uterus is filled with an amorphous material.


ger
vs
r
l
EB
r
D
119

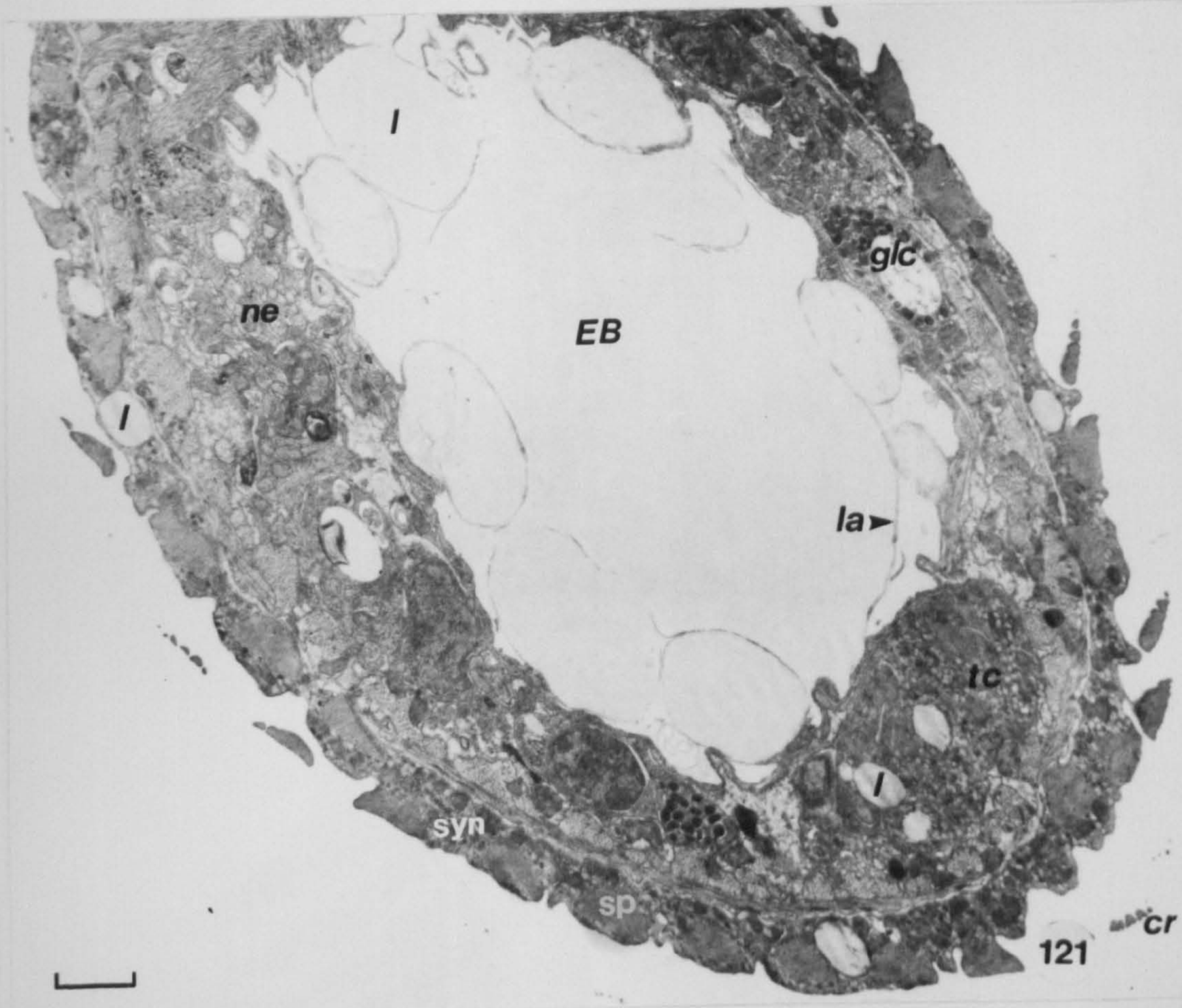

ger
ut
r
m
la
120

Plate 121 4 days *in vitro* culture.

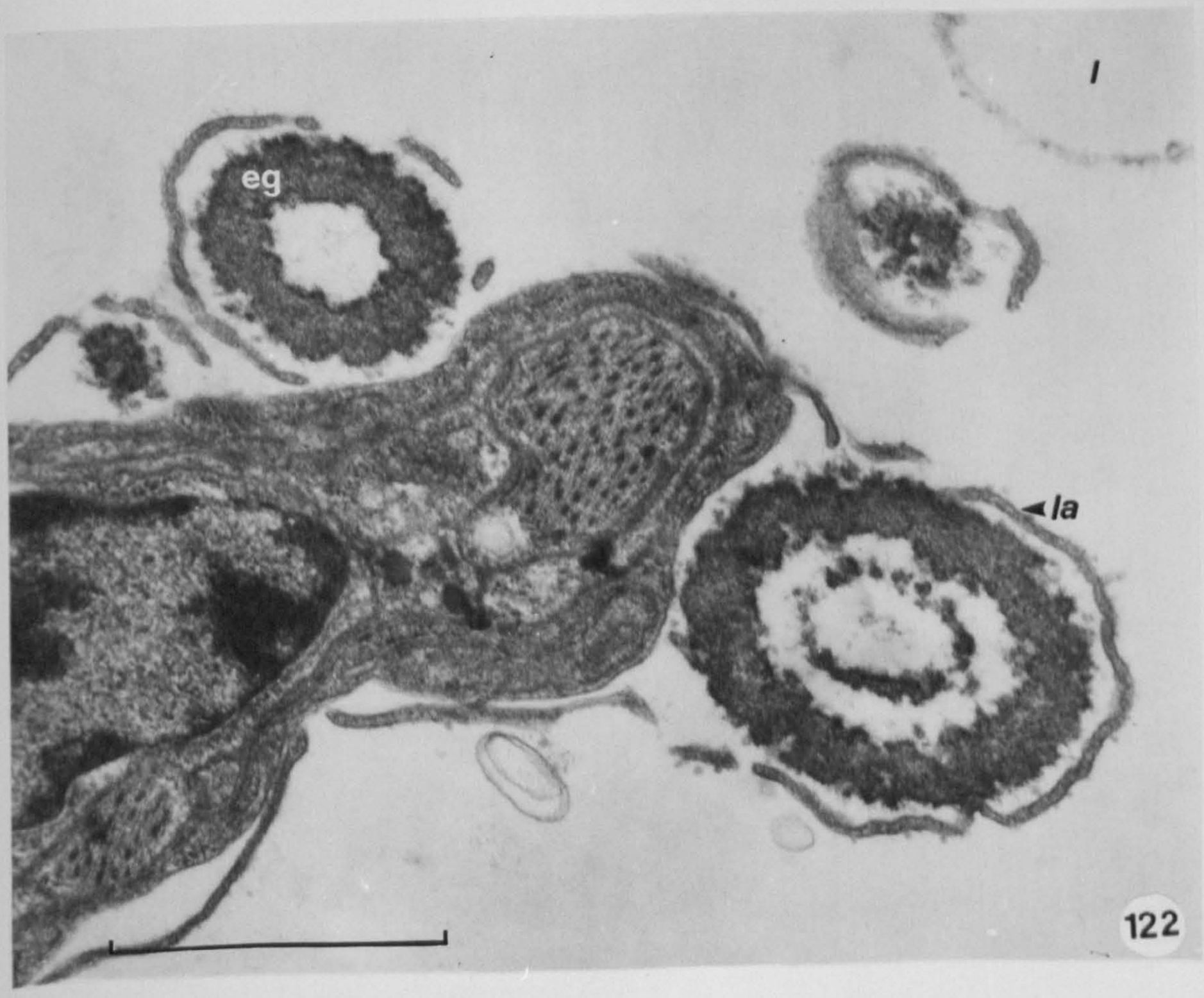
The excretory bladder (EB) contains numerous large lipid drops (l). Smaller lipid drops are also present in the tegumental cells (tc) and in the syncytium (syn). The cell body of a gland cell (glc) can be seen which apparently contains a large area of vacuolation.

Plate 122 4 days *in vitro* culture.

Occasionally some granules (eg) remain in the excretory bladder of cultured flukes for some time. These granules have not been decalcified.



121



122

Plate 123 4 days in vitro culture.

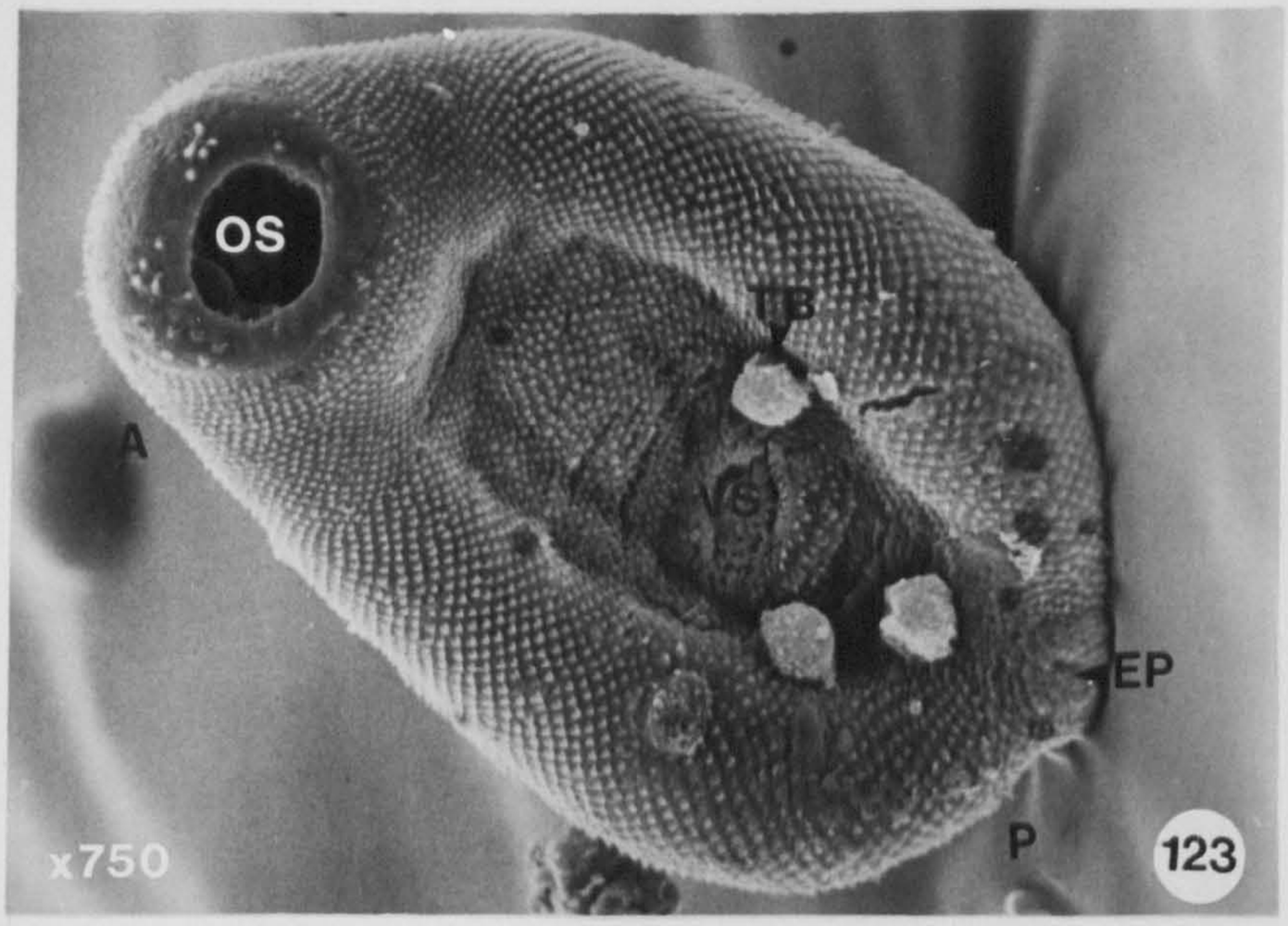
This view shows the typical shape assumed by cultured flukes with the lateral edges curled ventrally. Large, irregular tegumental "bubbles" (TB) are present on the ventral surface.

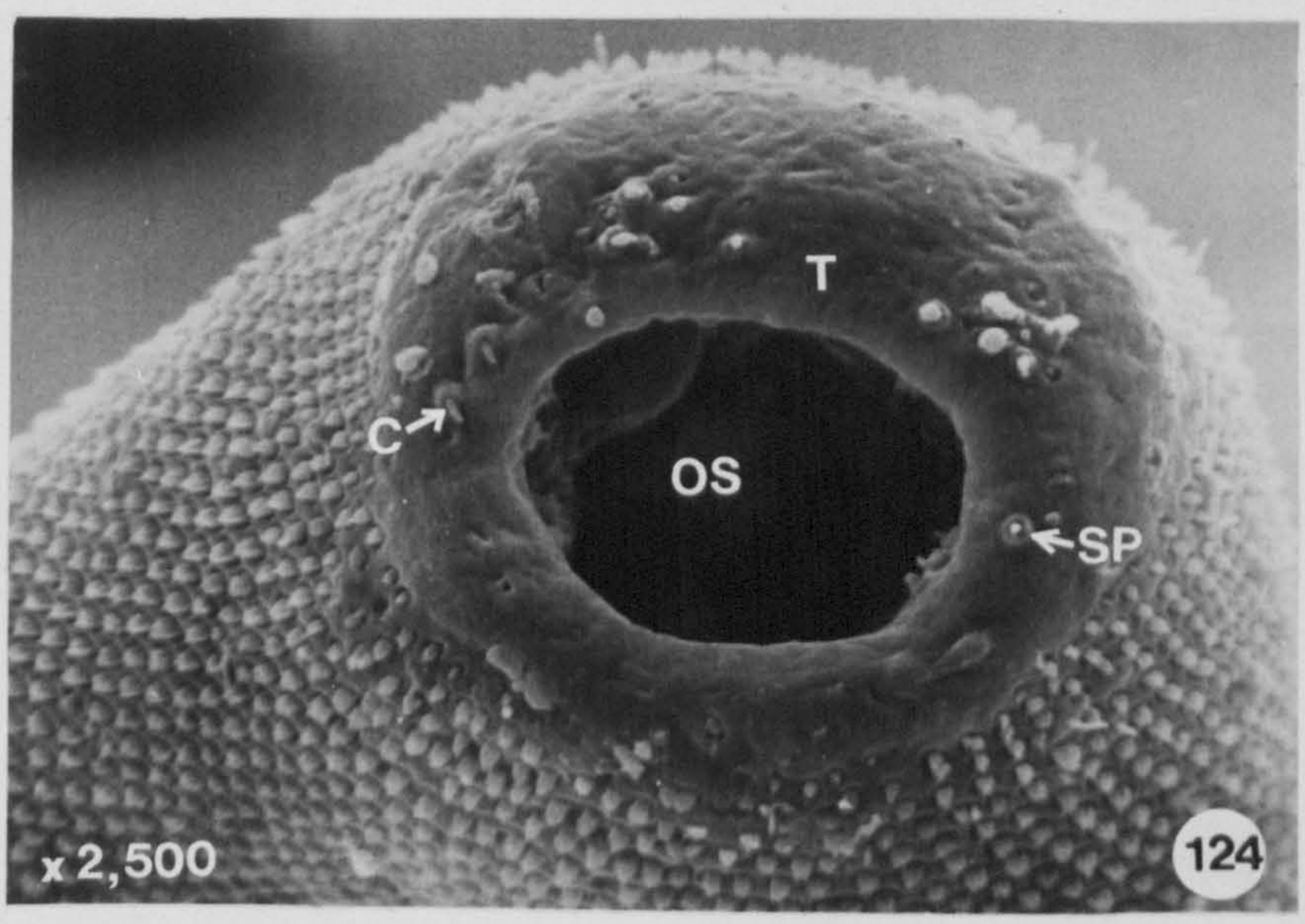
Plate 124 4 days in vitro culture.

The oral sucker (OS) is prominent, bare of spines and gaping. The cilia (C) of the sensory papillae (SP) project an unusual distance.

Plate 125 4 days in vitro culture.

The ventral sucker (VS) is not prominent, the spines anterior to the sucker appear to be sunken into pits in the tegument (arrowed).


OS
A
TP
VS
EP
P
x750
123


T
C
OS
SP
x 2,500
124


VS
SP
S
x 2,000
125

MICROPHALLUS SIMILIS.

Plates 126 - 133 : Light microscope photographs.

Plates 134 - 163 : Transmission electron micrographs of metacercariae and adults.

Plates 164 - 167 : Scanning electron micrographs of metacercariae.

Plates 168 - 194 : Transmission electron micrographs of cultured flukes.

Plates 195 - 197 : Scanning electron micrographs of cultured flukes.

(Scale bars on Plates denote lum.)

KEY.

Light Microscope Photographs -

A	anterior	ov	ovary
ab	abnormal eggs	P	posterior
dc	digestive caeca	t	testis
de	ductus ejaculatorius	tv	tanned vitelline secretions
e	egg	ut	uterus
eb	excretory bladder	v	vitellaria
glc	gland cell	VS	ventral sucker
mc	"mitochondria" cells	ves	vesicula seminalis
mp	male papilla	vr	vitelline resevoir
oo	ootype		

Scanning Electron Micrographs -

A	anterior	P	posterior
C	cilium	RS	ring of spines
CR	"crown" on spine	S	spine
DP	"domed" papilla	SP	sensory papilla
EP	excretory pore	T	tegument
GP	genital pore	TB	tegumental bubbles
OS	oral sucker	VS	ventral sucker

Transmission Electron Micrographs -

abs	abnormal sperm	ch	chromosome
af	axial filament	ce	centriole
bb	basal body	cn	condensed nucleus
bi	basal invagination	cp	central process
c	cilium	co	central core
cb	"centriole-like body"	cr	"crown" of spine
cc	caecal contents	cs	connecting strand

CT conjoined tubule

de ductus ejaculatorius

e egg

EB excretory bladder

ED excretory duct

f "flame" of flame cell

FC flame cell

ga area of Golgi activity

ger granular endoplasmic reticulum

gc Golgi complex

glc gland cell

gls gland cell secretions

glm gland cell material

gly glycogen

im interstitial material

imv interdigitating microvilli

ivs immature vitelline cell

jc junctional complex

l lipid

la lamellae

li external leptotriches

m mitochondrion

mc "mitochondria cell"

mp male papilla

ms membraneous secretory bodies

mt microtubules

mu muscle

mvc mature vitelline cells

n nucleus

nc nurse cell

np nuclear pore

nu nucleolus

oc oocyte

ov ovum

pc parenchyma cell

pd peripheral doublets

pgs prostate gland secretions

pp parenchymal process

ps protoplasmic strand

r ribosomes

rb "ribosomal body"

rc residual cytoplasm

rs radial spokes

rt root

s sperm

sc spermatocytes

sd septate desmosome

sg secretory granule

sp spine

spm spine-like material

st spermatids

syn tegumental syncytium

tc tegumental cell body

tg tegumental granules

tvs tanned vitelline secretions

ut uterus

ves vesicula seminalis

vc vitelline cell

vs vitelline secretions

Plates 126 - 129 Thick sections of flukes prepared for TEM and stained in toluidine blue.

Plate 126 Excysted metacercaria.

A longitudinal section showing the unicellular gland cells (glc) in the forebody, the digestive caeca (dc), and, posterior to the ventral sucker (vs), the testes (t), uterus (ut) and the vitellaria (v).

Plate 127 Adult - 4 days p.i. from the mouse.

A longitudinal section showing the vesicula seminalis (ves) filled with sperm, the digestive caeca (dc), ovary (ov), male papilla (mp), the two testes (t), the ciliated ootype (oo) and numerous shelled eggs (e).

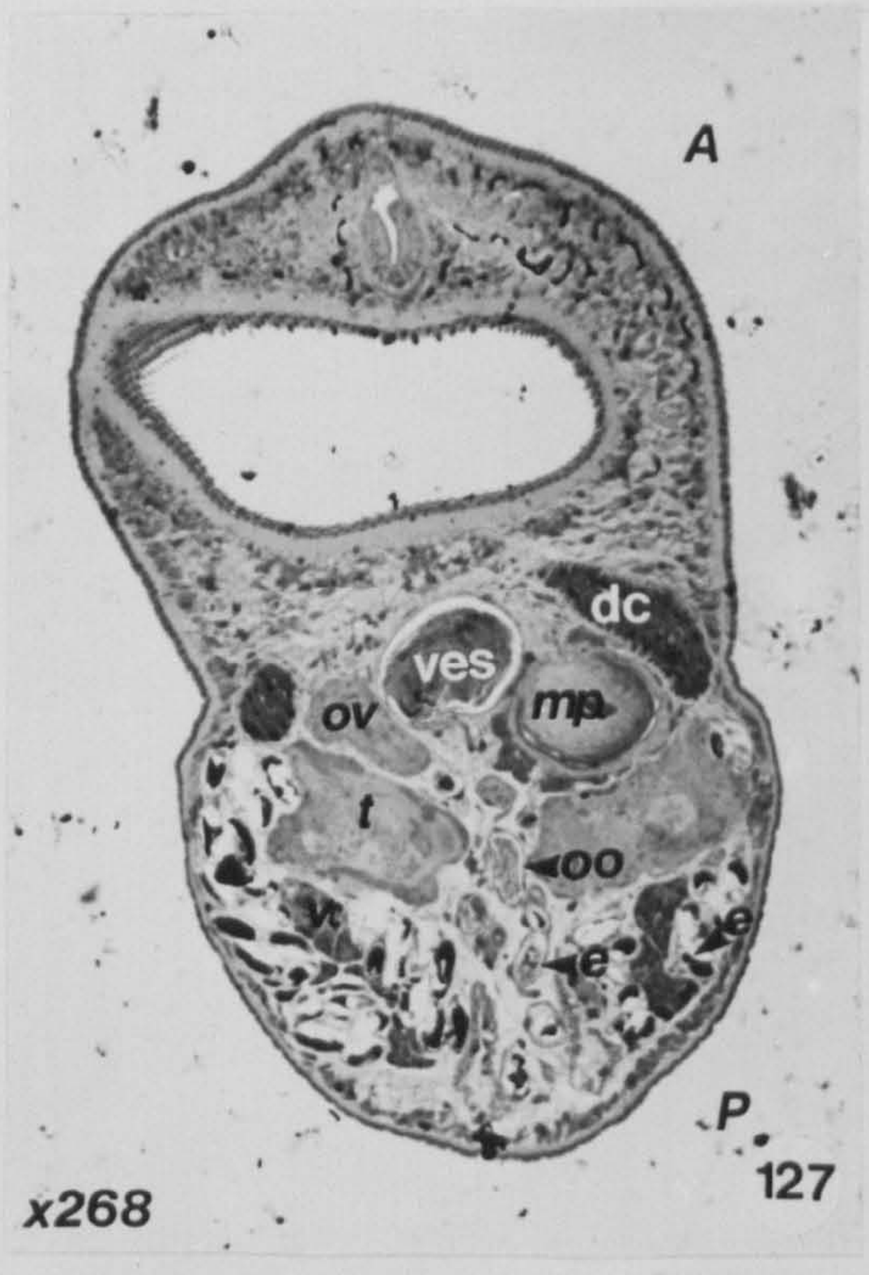
Plate 128 4 days *in vitro* culture.

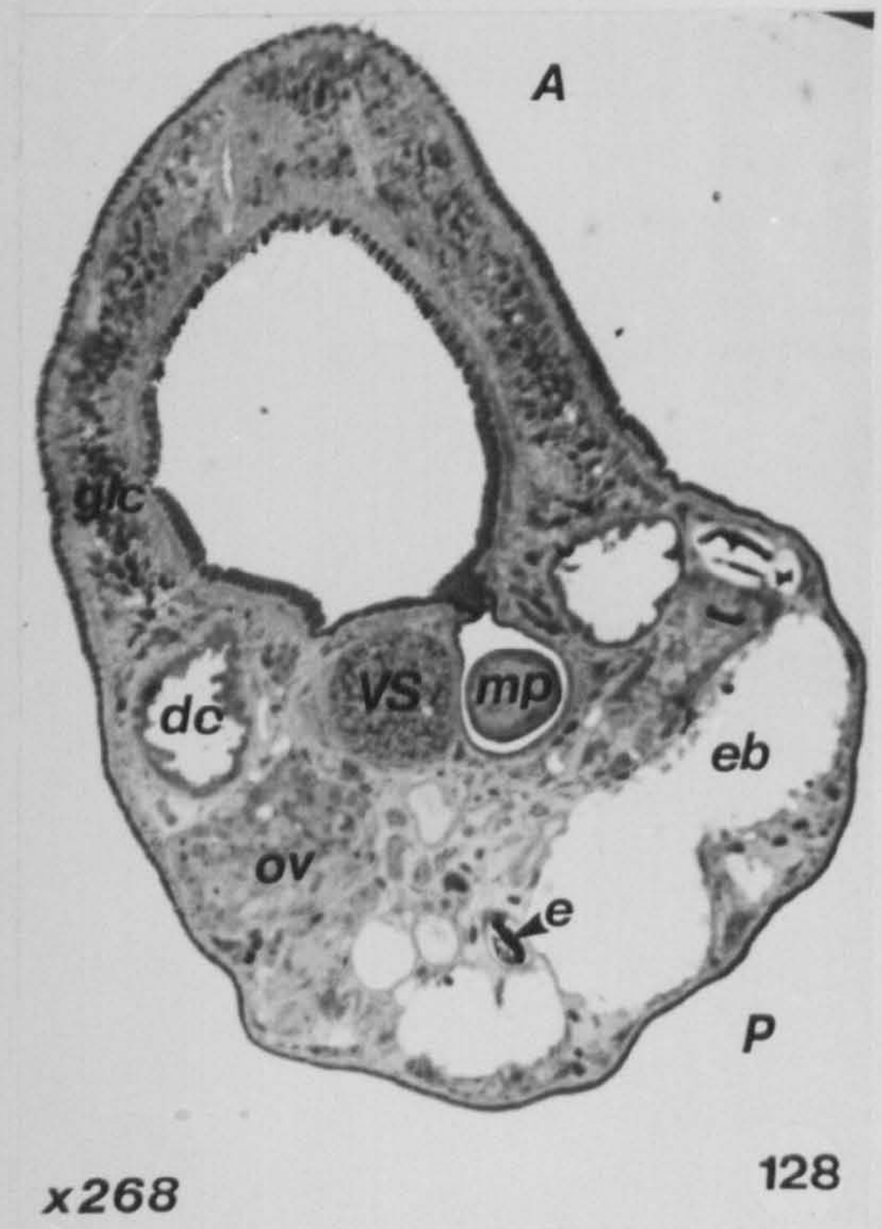
A longitudinal section showing the digestive caeca (dc) with distended lumens, the ventral sucker (vs) and adjacent male papilla (mp), the ovary (ov), the excretory bladder (eb) and small numbers of shelled eggs (e).

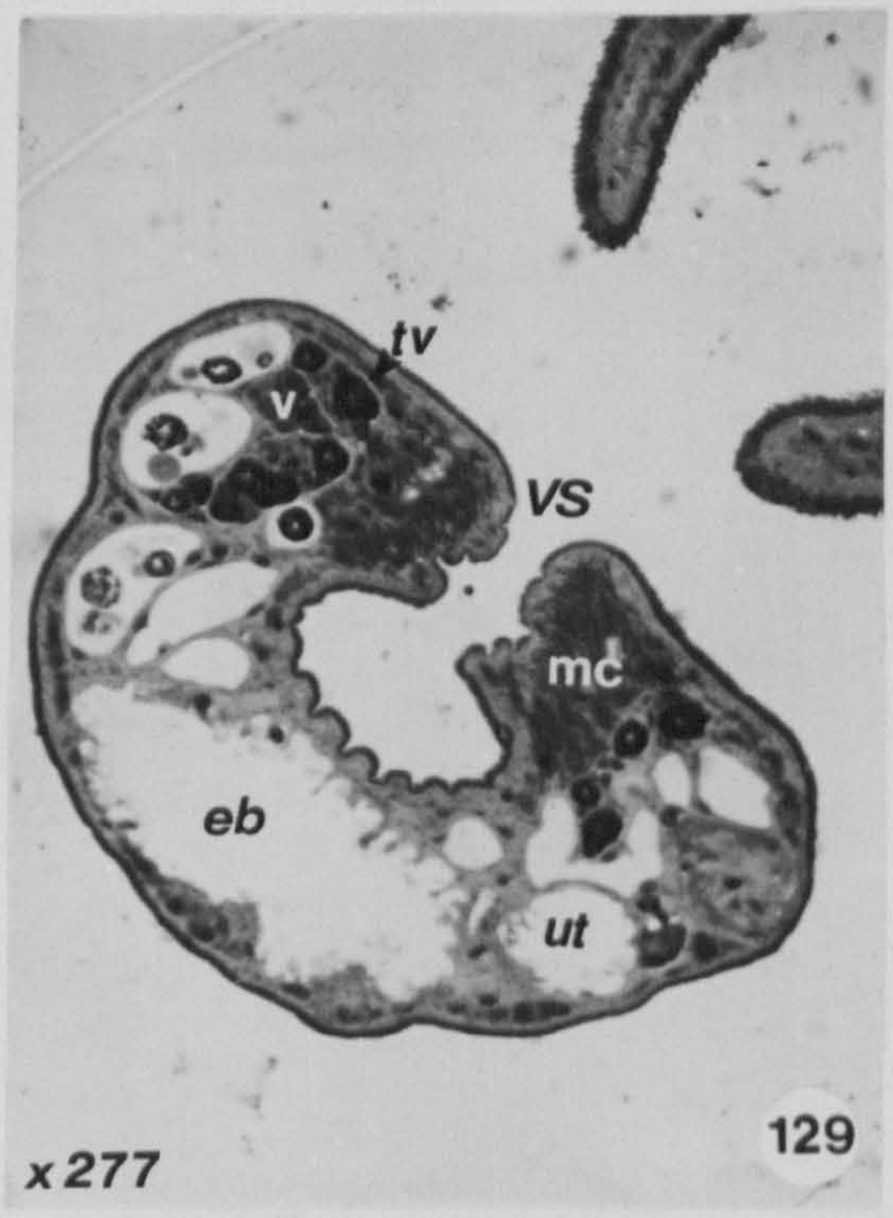
Plate 129 4 days *in vitro* culture.

A transverse section just below the level of the ventral sucker showing the two groups of "mitochondria cells" (mc) and the prematurely tanned vitelline secretions (tv) in the vitellaria (v) and the uterus (ut).


P
v
ut
t
VS
dc
glc
A
126
x264


A
dc
ves
ov
mp
t
oo
e
e
P
127
x268


A
glc
dc
VS
mp
eb
ov
e
P
128
x268


tv
v
VS
mc
eb
ut
129
x277

Plates 130 - 131 Whole mounts stained in Gowers carmine.

Plate 130 Excysted metacercaria.

In the hind body the ovary (ov), two testes (t) and undeveloped vitelline cells (v) can be seen.

Plate 131 Immature adult - 1 day p.i. from the mouse.

The vitellaria have increased in size considerably.

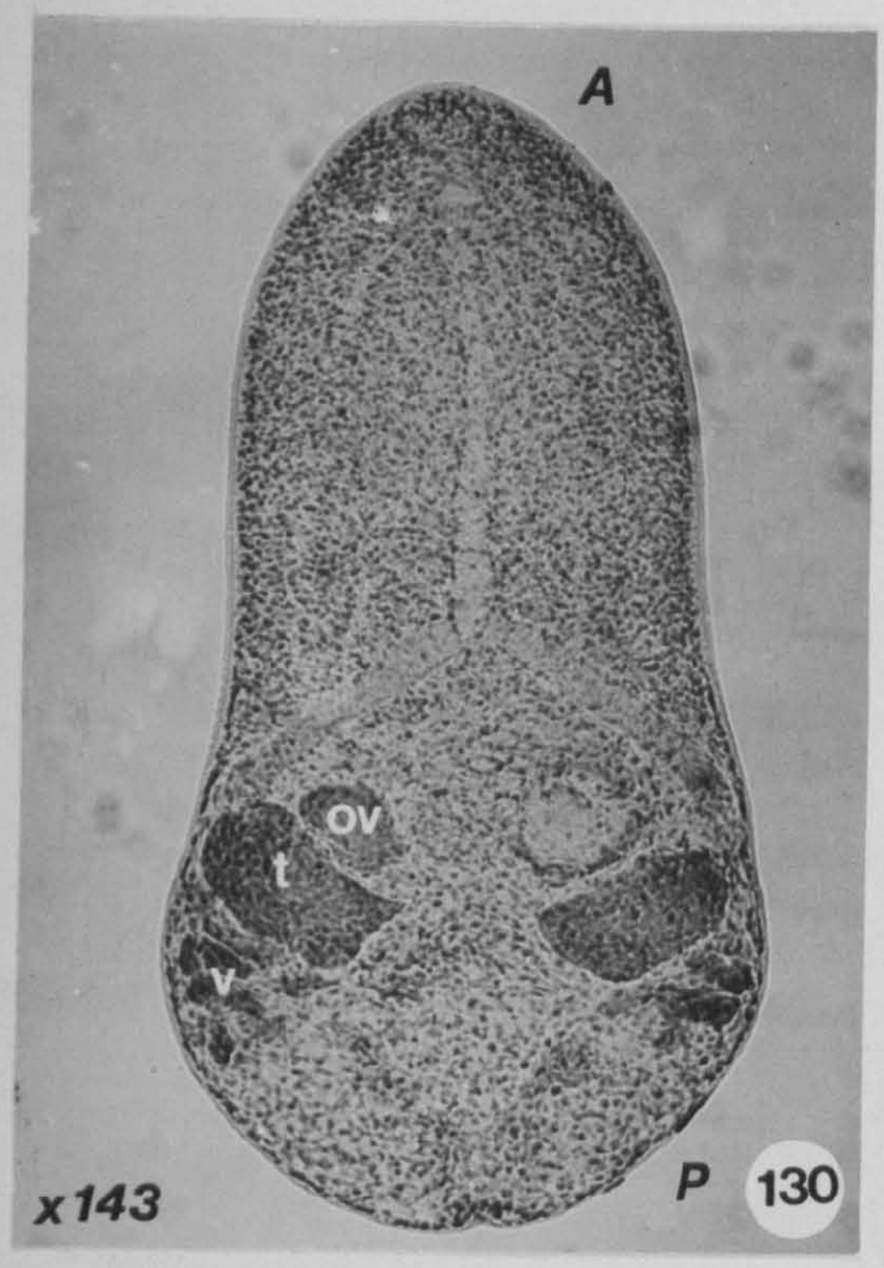
Plates 132 - 133 Unstained live flukes, slightly flattened.

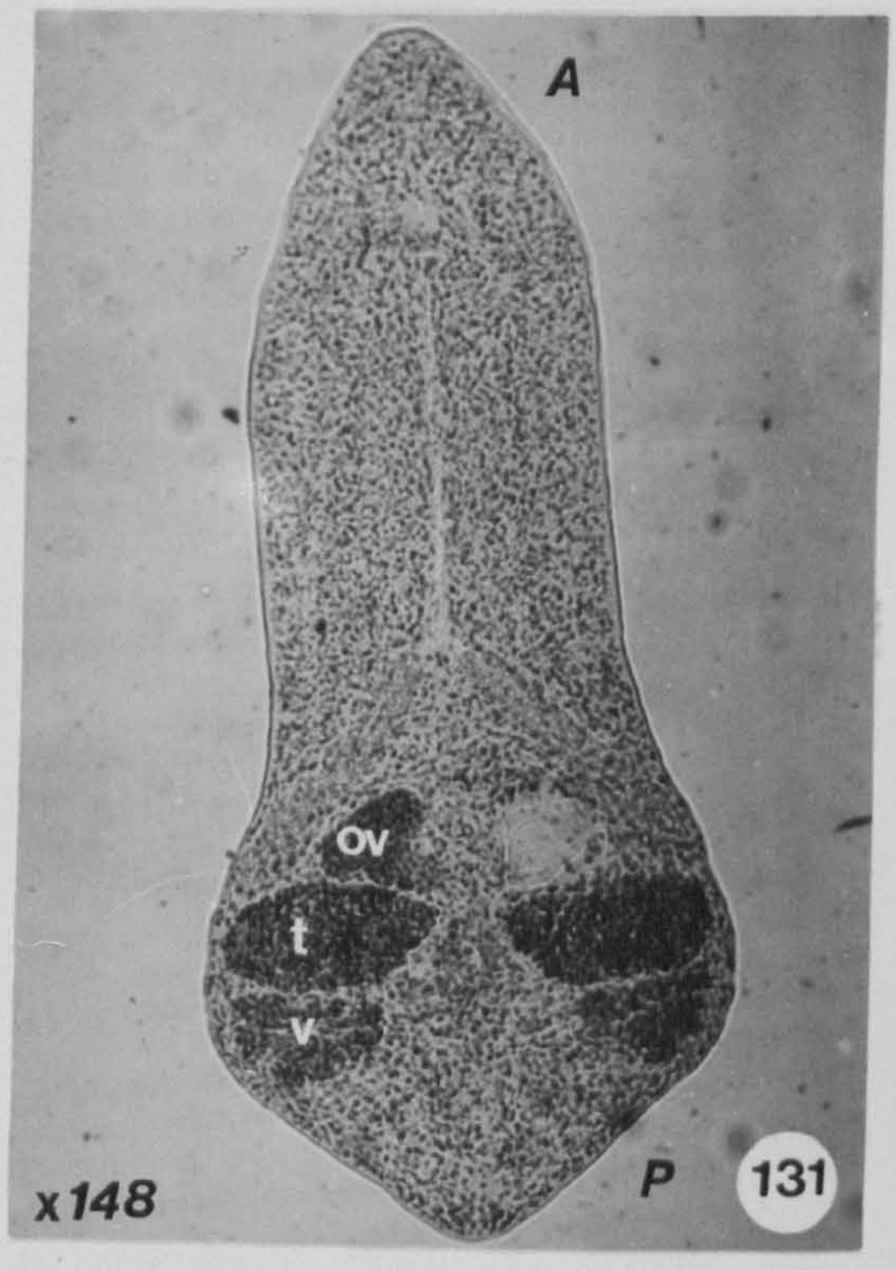
Plate 132 Adult - 4 days p.i. from the mouse.

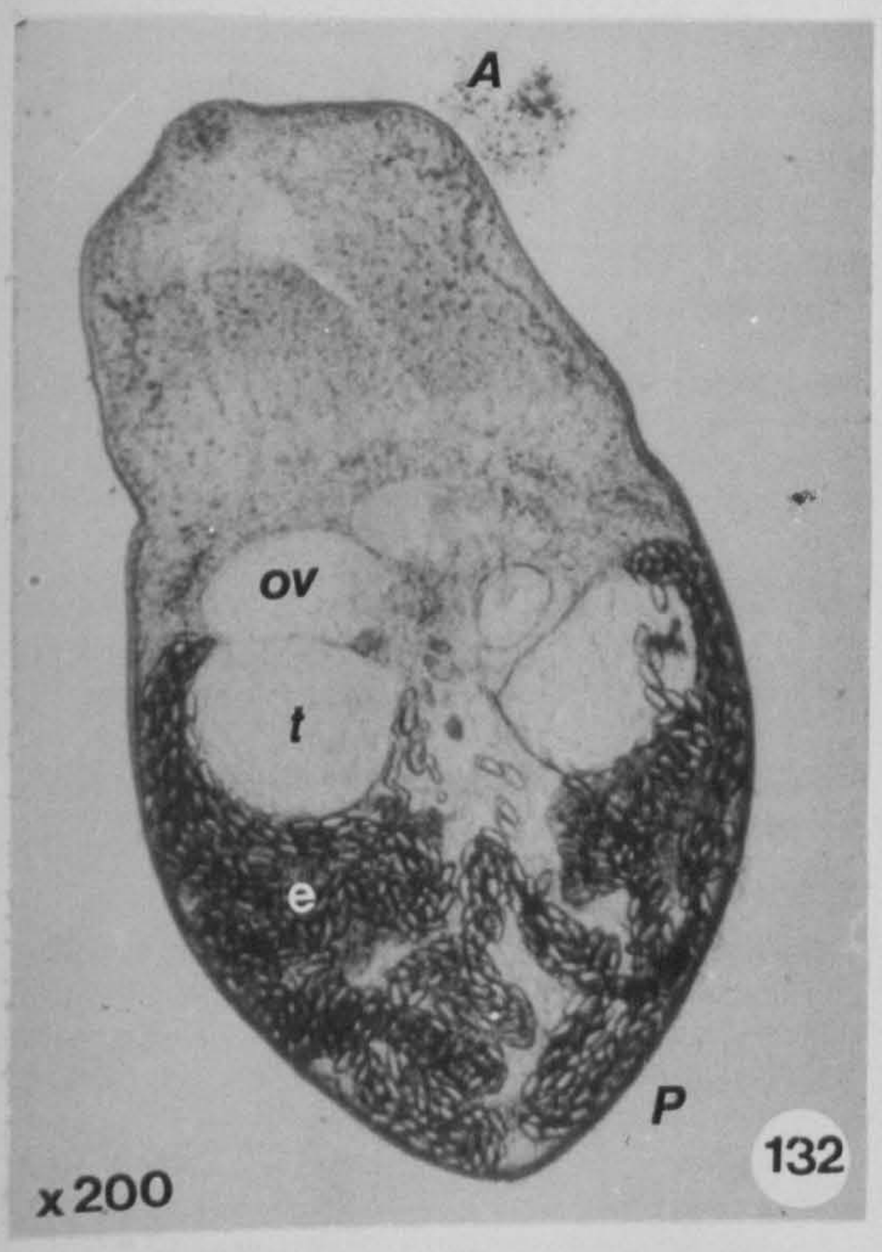
The gonads (t,ov) and the cup-shaped mass of(e) eggs fill the hind body of the adult.

Plate 133 4 days *in vitro* culture.

The hind body of a cultured fluke showing the vesicula seminalis (ves) and ductus ejaculatoris (de) packed with sperm, the vitelline resevoir (vr) containing vitelline secretions, normal shelled eggs (e) with an ovum and vitelline cells and abnormal eggs (ab) which do not contain an ovum.


A
ov
t
v
x143
P
130


A
ov
t
v
x148
P
131


A
ov
t
e
P
x200
132

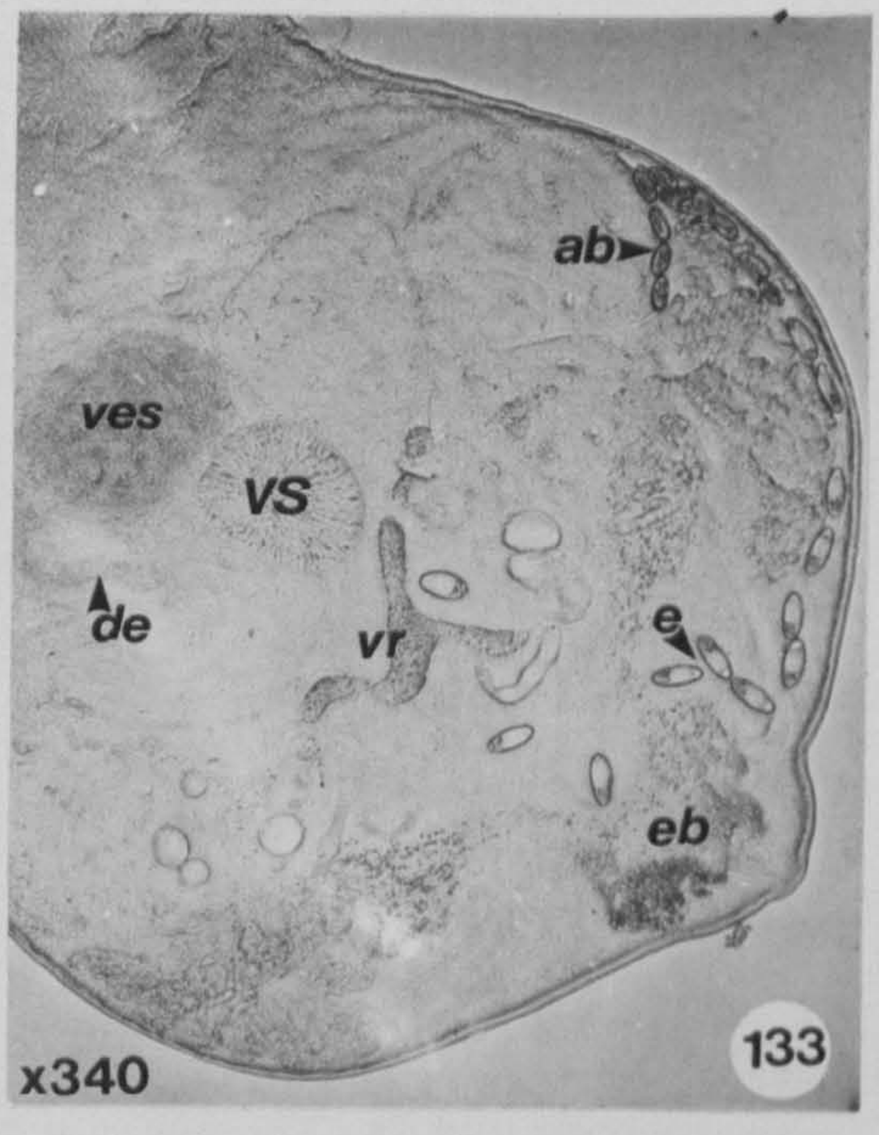
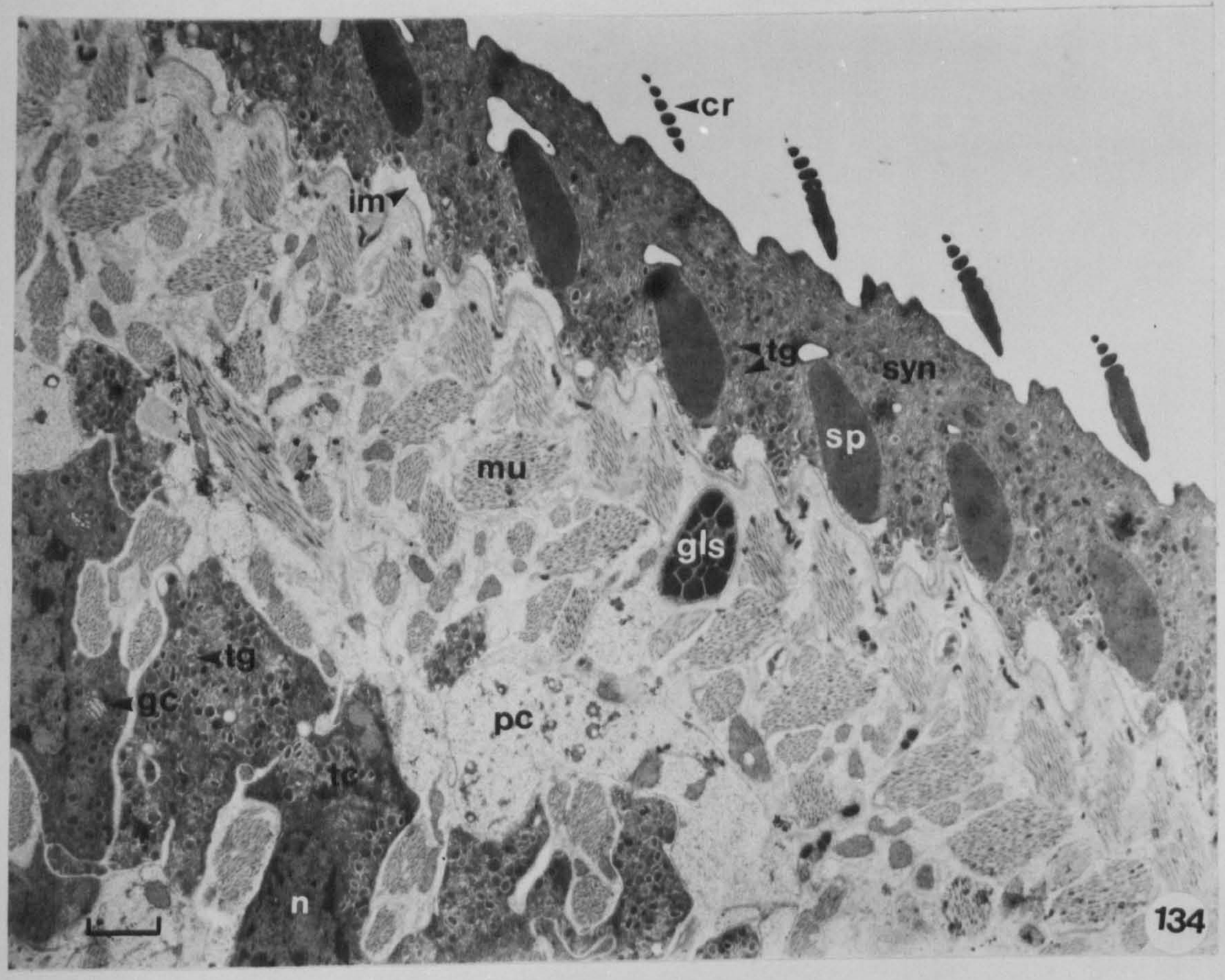

ab
ves
VS
de
vr
e
eb
x340
133

Plate 134 Excysted metacercaria.

A section through the tegument in the forebody showing the "crowned" spines (cr,sp) and granules (tg) within the syncytium (syn). Beneath the peripheral musculature (mu) are the tegumental cell bodies (tc) containing granules (tg) and Golgi complexes (gc). A cross section through the duct of a tegumental unicellular gland cell can be seen (gls).

Plate 135 Excysted metacercaria.

A section through the tegument just posterior to the ventral sucker showing the unusual "mitochondria cells" (mc) and the much folded syncytium (syn). A protoplasmic strand (ps) leading up from the "mitochondria cells" can be seen although it is not clear whether it is continuous with the cytoplasm of the syncytium. A tegumental cell (tc) and a duct of a unicellular gland cell (gls) can also be seen.



134



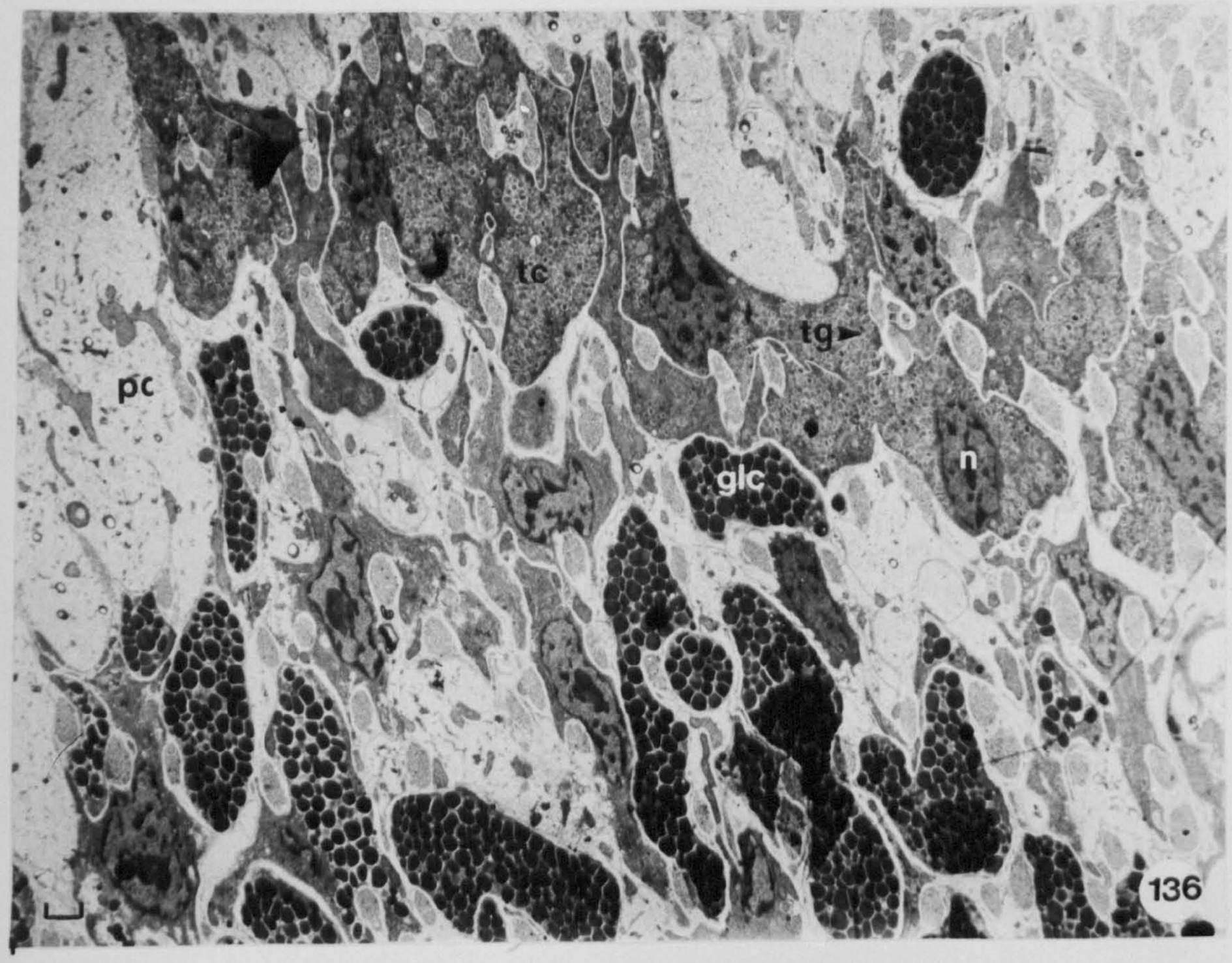
135

Plate 136 Excysted metacercaria.

This section through the forebody shows several tegumental cell bodies (tc) and, beneath them, the cell bodies of the unicellular gland cells (glc) which are virtually filled with dense secretory bodies.

Plate 137 Excysted metacercaria.

The cytoplasm of the tegumental cell body contains a variety of oval or spherical secretory granules (tg), numerous Golgi complexes with distended cisternae (gc), mitochondria (m), a nucleus (n) and free ribosomes (r) although it lacks a GER network.


tc
tg
pc
glc
n
136

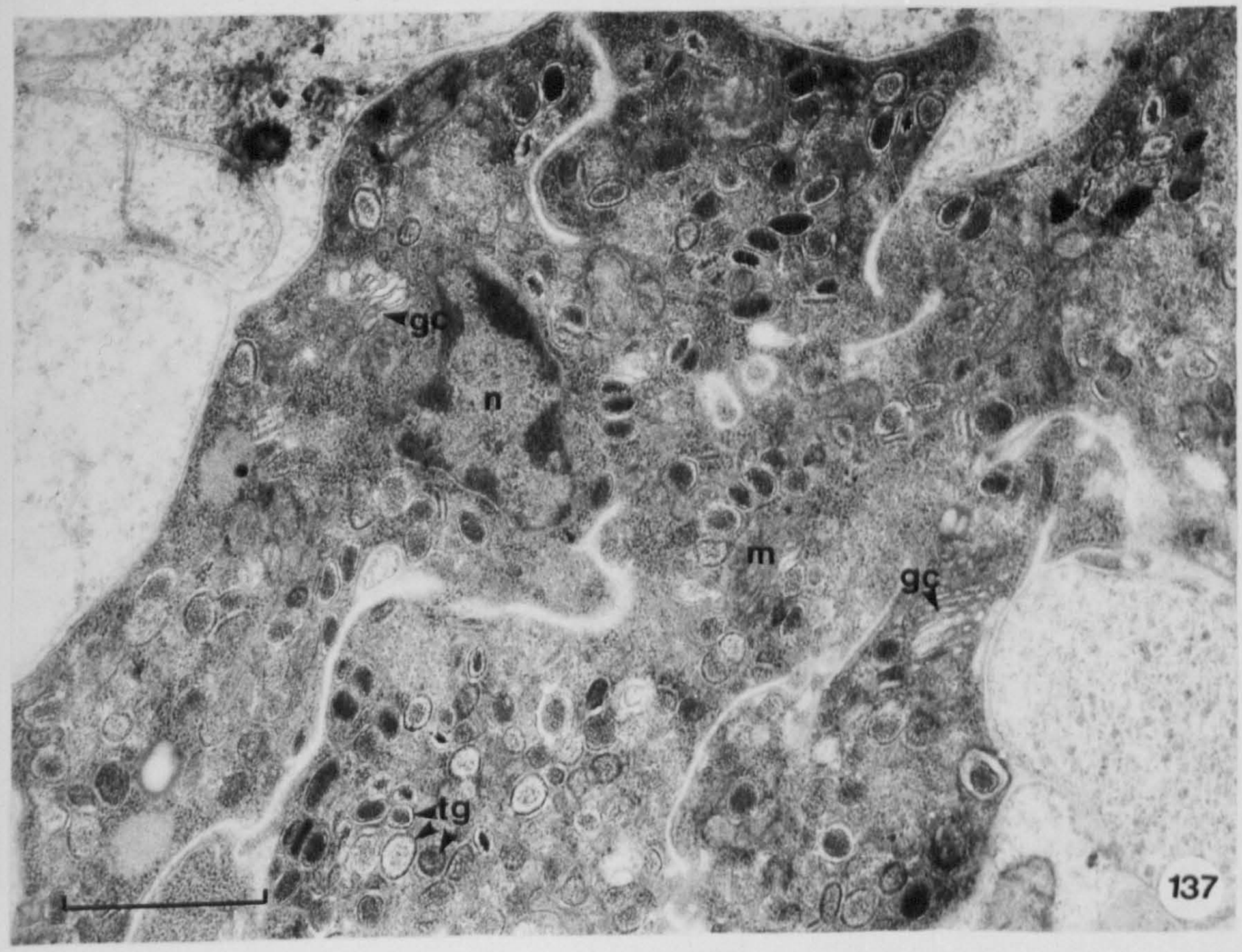
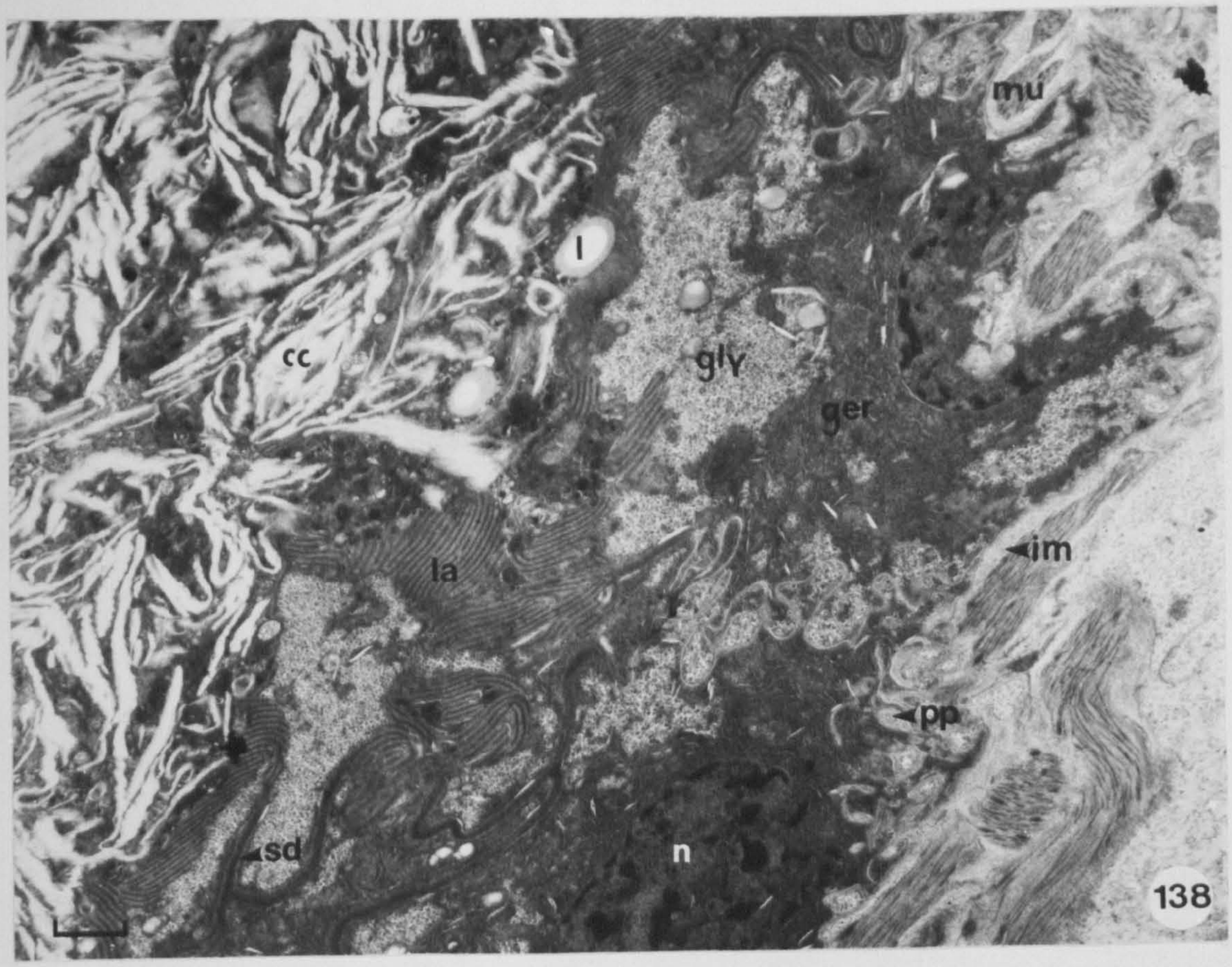

gc
n
m
gc
tg
137

Plate 138 Excysted metacercaria.

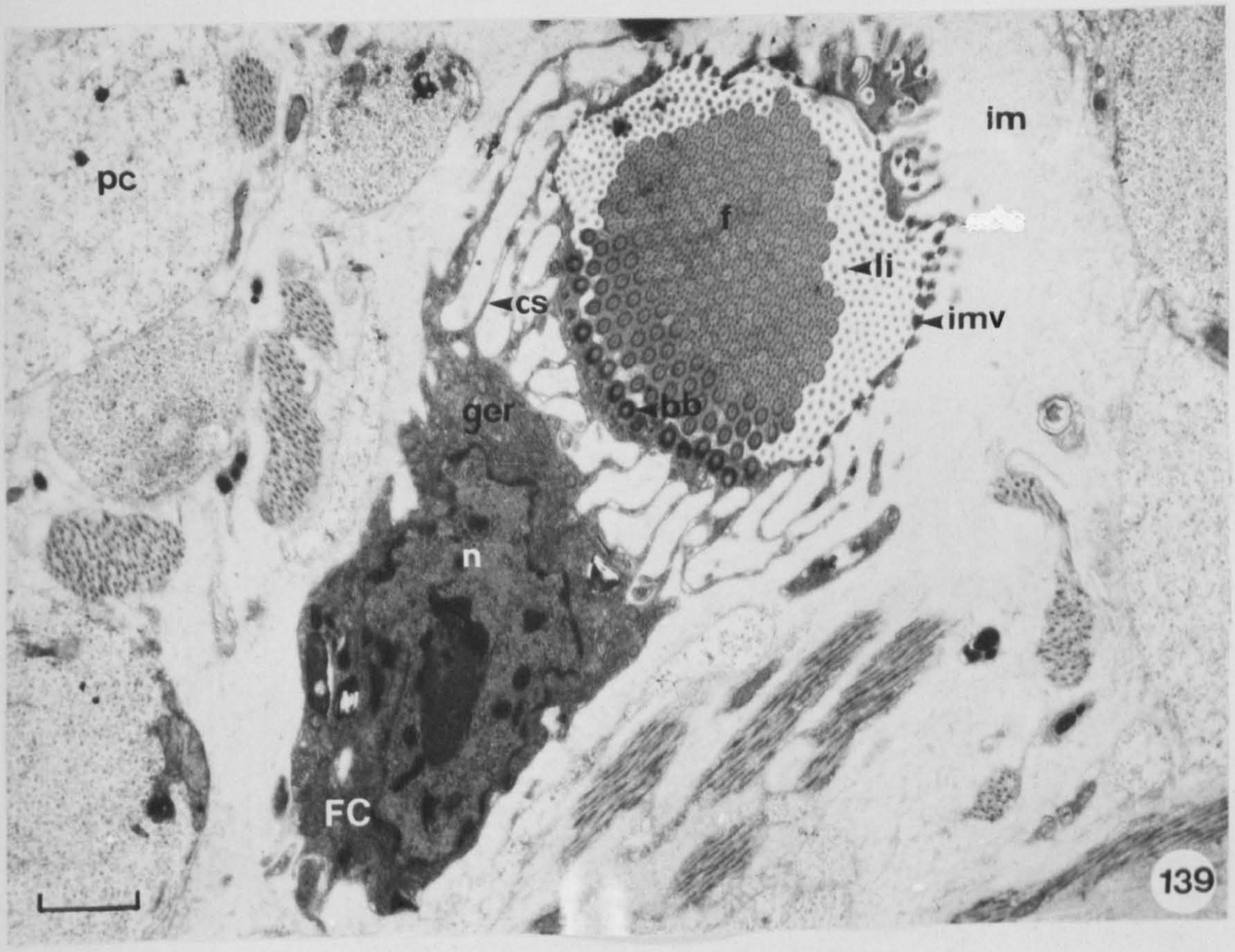
The cells of the caecal epithelium contain areas of glycogen (gly) intersperced with denser areas which contain GER (ger) and the nuclei (n). The plasma membranes of adjacent cells are undulating and the cells are connected at their luminal surface by long septate desmosomes (sd). Processes of parenchymal cells (pp) penetrate the base of the caecal cells although there are no junctional complexes between the two cells. Small lipid drops (l) are seen in the cytoplasm of the cells and in the lumen. The apical surface of the caecal cells is thrown up into short closely packed lamellae (la). The lumen of the caeca is filled with an irregular dense material (cc).

Plate 139 Excysted metacercaria.

The flame cell (FC) body consists of two parts - one part contains the nucleus and various other cytoplasmic organelles such as mitochondria and GER (ger), and the other part contains the basal bodies (bb) of the cilia which comprise the "flame" (f). The two parts of the cell are connected by strands of cytoplasm (cs). There are many internal leptotriches (li) and the junction between the flame cell body and the cell of the conjoined tubule consists of a series of interdigitating microvilli (inv). The flame cell is surrounded by a thick layer of interstitial material (im).



138



139

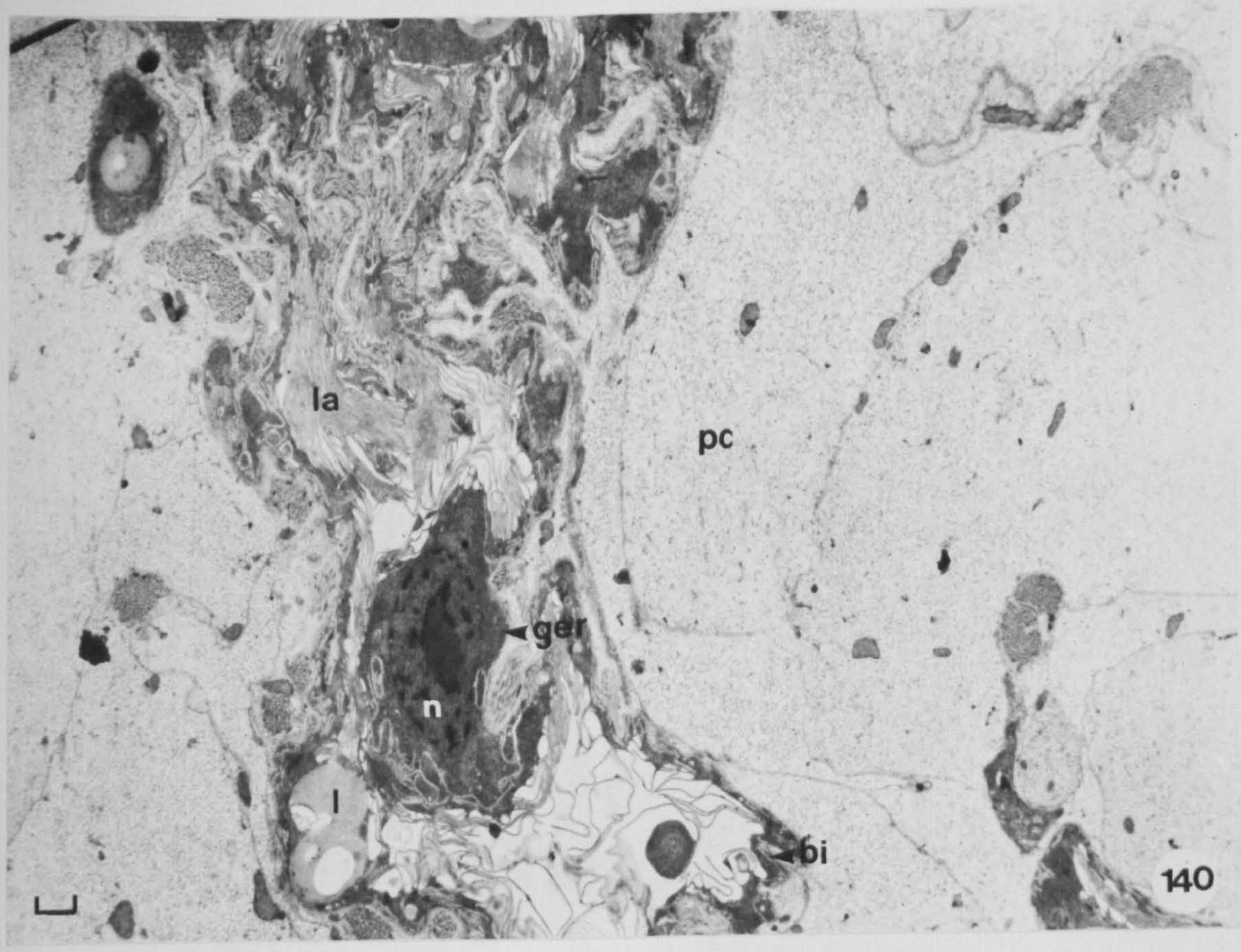
Plate 140 Excysted metacercaria.

The cytoplasm of the excretory bladder contains nuclei (n) with dense heterochromatin, abundant GER (ger), lipid drops (l) and long surface lamellae (la); the basal lamina is much invaginated (bi).

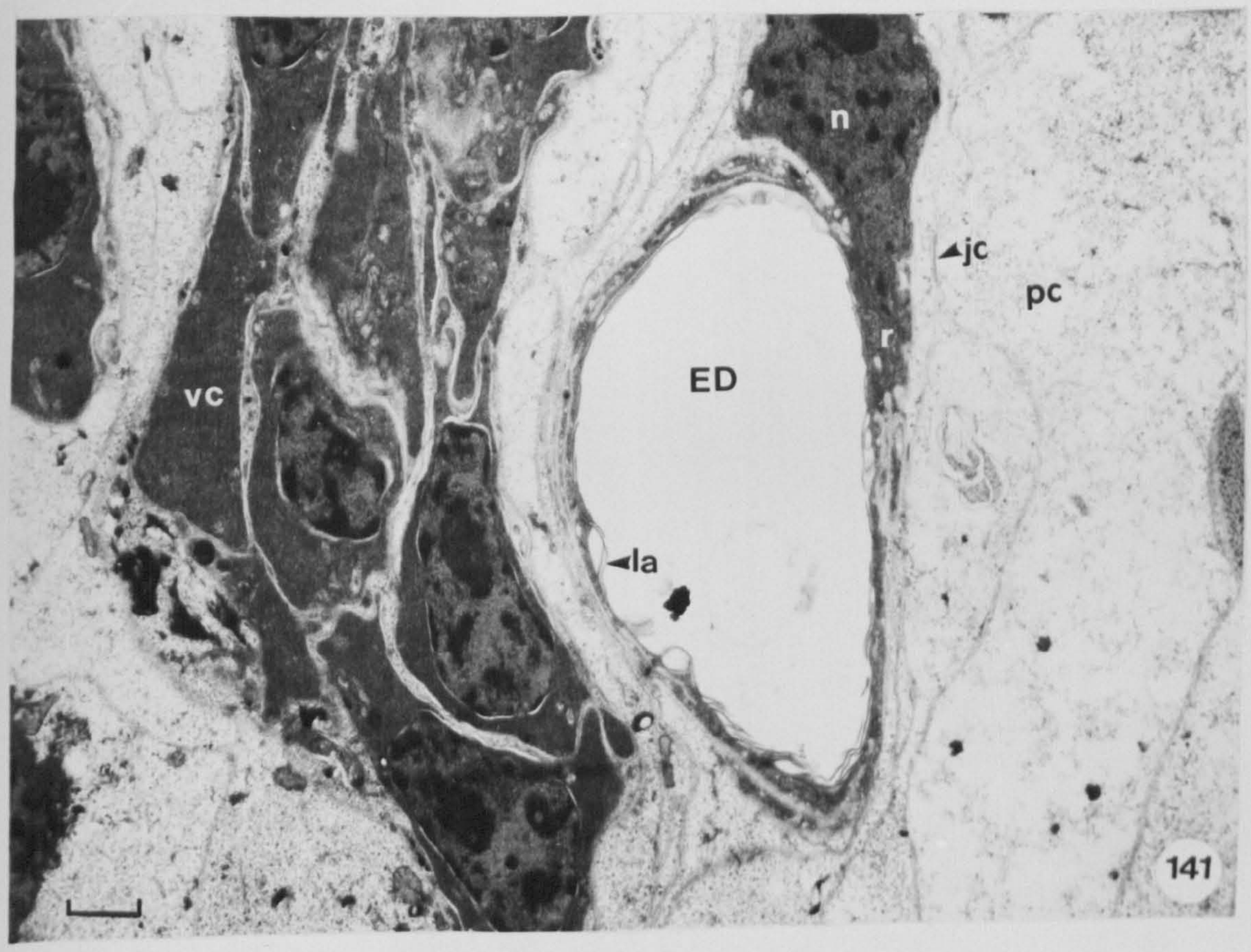
Plate 141 Excysted metacercaria.

The cytoplasm of the excretory duct (ED) - which presumably connects the flame cell to the excretory bladder - is thin and contains ribosomes (r) and some GER. The basal plasmalemma is much infolded and the luminal surface bears lamellae. The nucleus (n) does not protrude into the lumen but is often sunk between the parenchyma cells.

Immature vitelline cells (vc) and a junctional complex (jc) between adjacent parenchyma cells (pc) can also be seen.



140



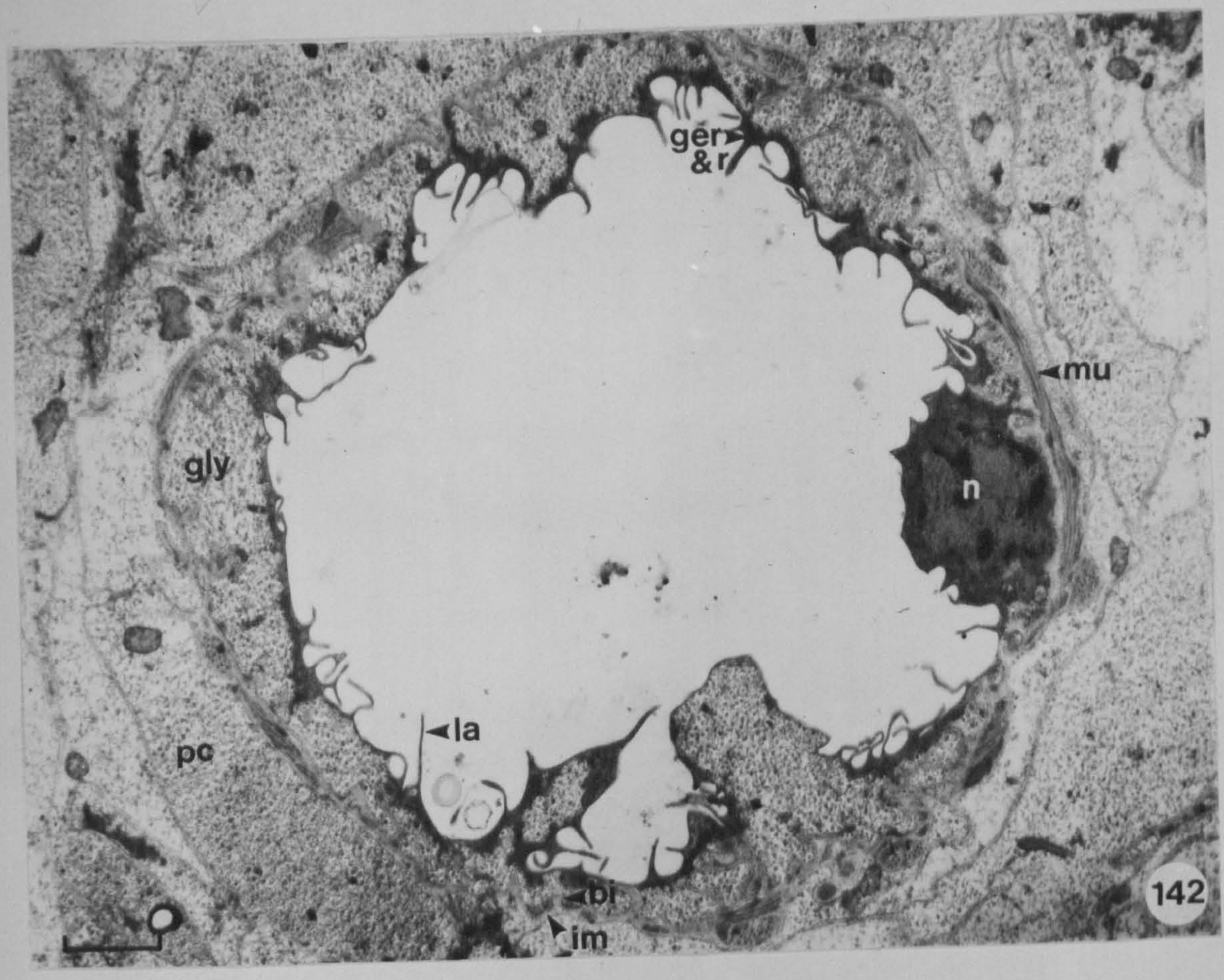
141

Plate 142 Excysted metacercaria.

The cells lining the uterus contain abundant glycogen(gly) and, nearer the lumen, there are denser areas containing ribosomes (r), GER (ger) and sometimes the nuclei (n). The apical surface is thrown up into finger-like projections and lamellae (la) and the basal plasmalemma is much invaginated (bi).

Plate 143 Excysted metacercaria.

The ultrastructure of the ootype is similar to that of the uterus (Plate 142) except that there is less stored glycogen (gly), more basal invaginations (bi) and cilia (c) with typical basal bodies (bb) project into the lumen. There is a virtually continuous layer of muscle (mu) around the ootype.


gér
&r
mu
gly
n
la
pc
bi
im
142

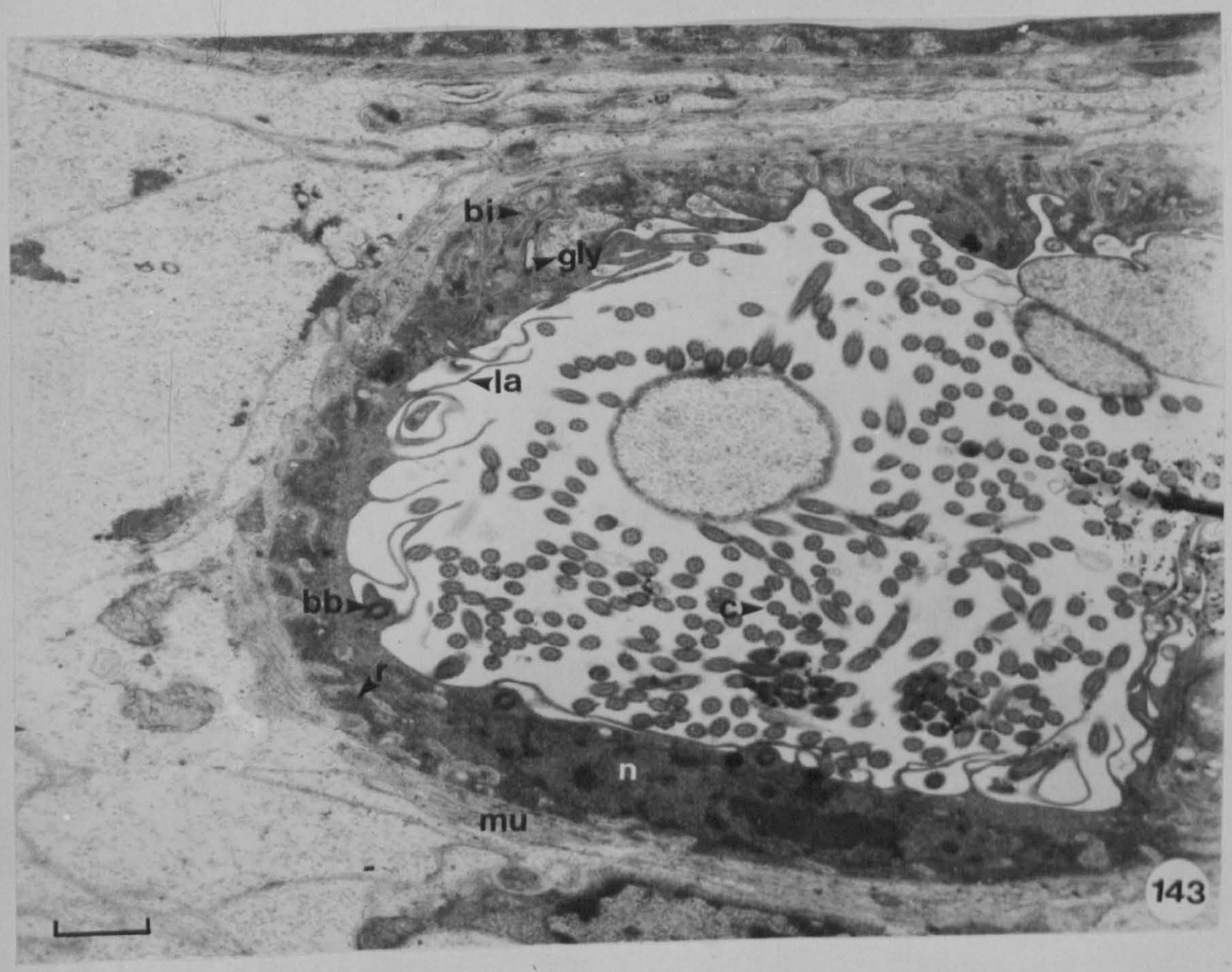
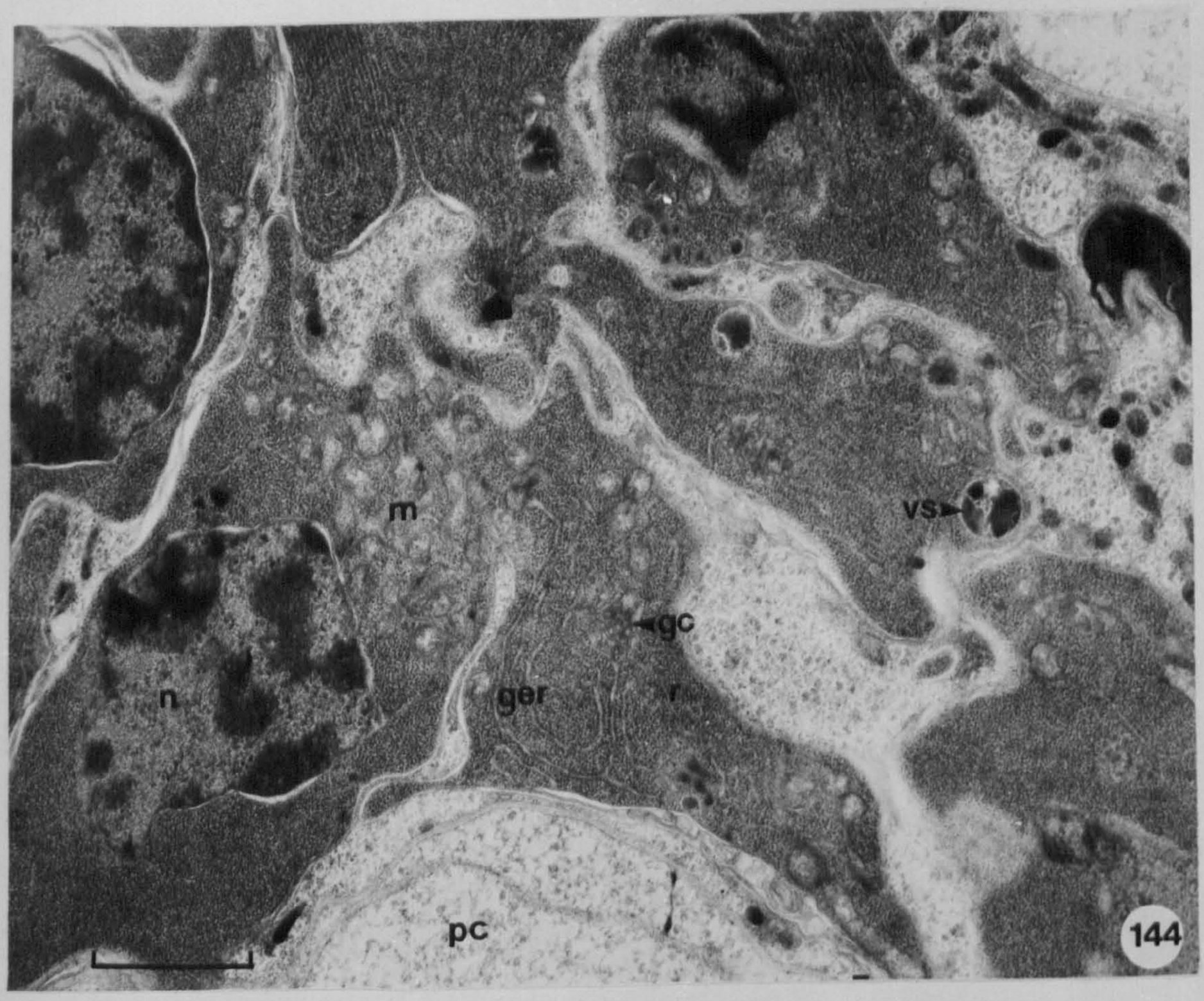

bi
gly
la
bb
c
n
mu
143

Plate 144 Excysted metacercaria.

The immature vitelline cells contain GER (ger), free ribosomes (r), Golgi complexes (gc), mitochondria (m) and occasional secretory droplets (vs).

Plate 145 Excysted metacercaria.

The germ cells in the ovary have an irregular outline and prominent nuclei; they contain abundant free ribosomes (r), mitochondria (m) and Golgi complexes (gc). Between the germ cells are processes from the nurse cells (nc) which contain mitochondria and some GER.


m
VS
gc
n
ger
r
pc
144

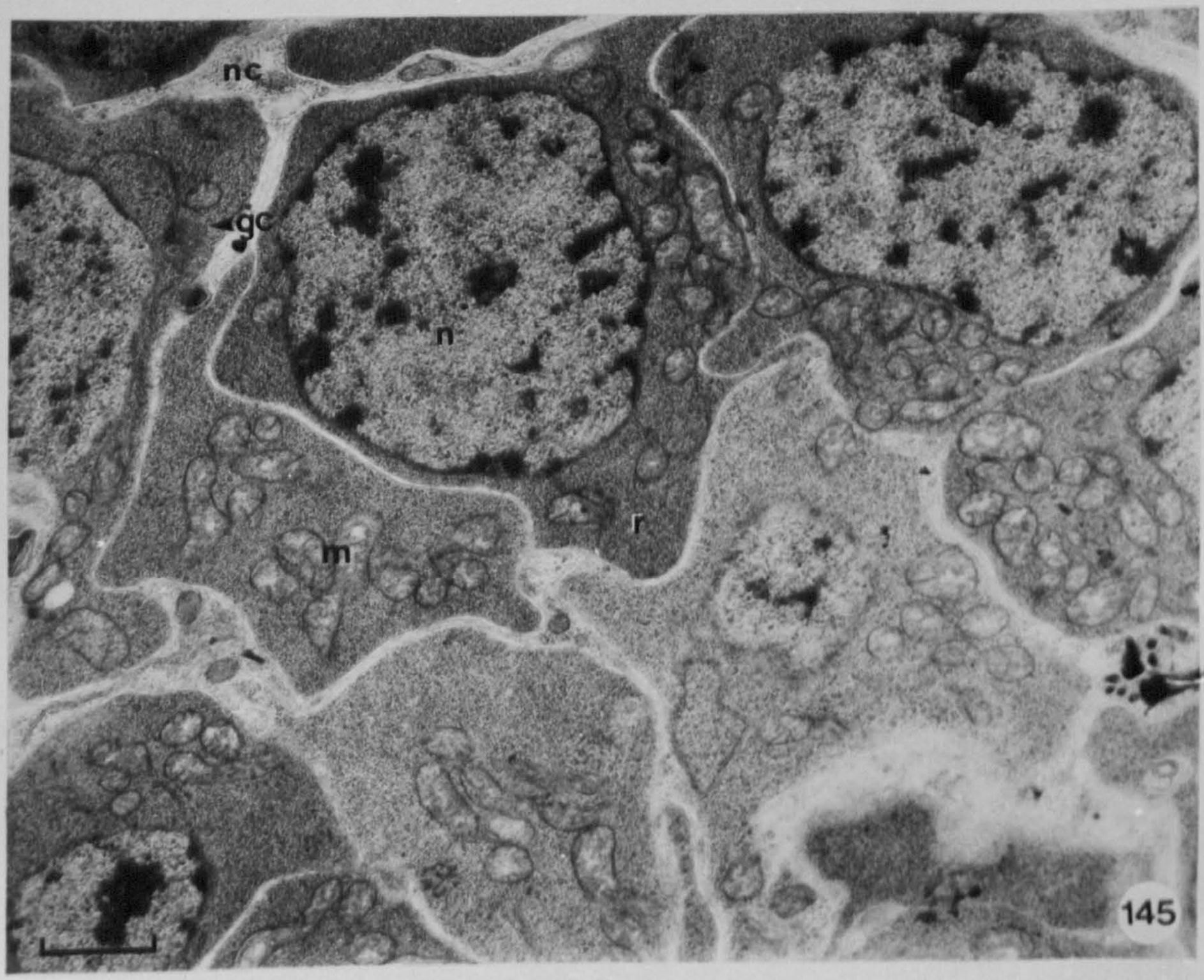
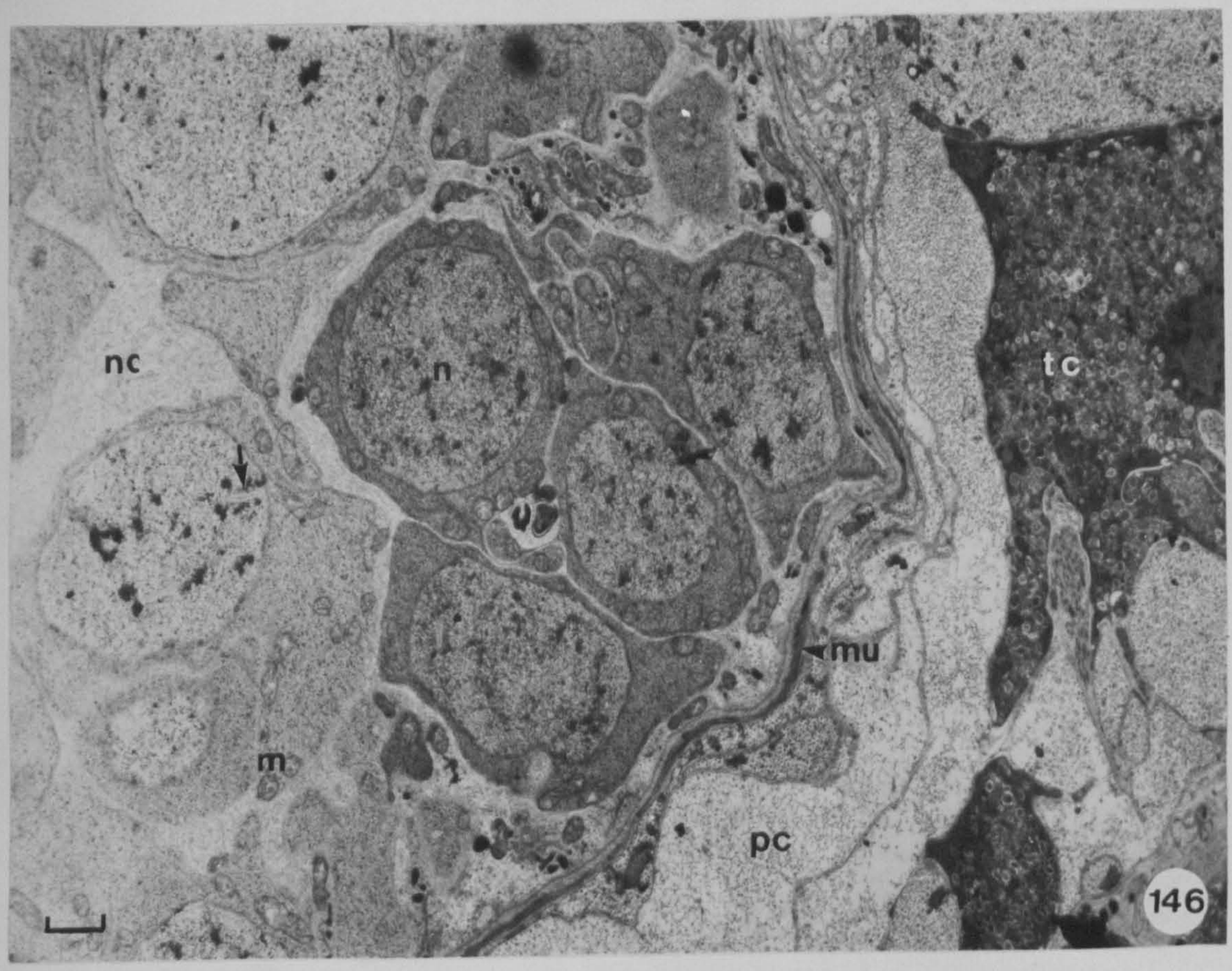

nc
gc
n
r
m
145

Plate 146 & 147 Excysted metacercaria.

The testis is surrounded by a thin but virtually continuous layer of muscle (mu). Small dense cells are often present around the periphery of the testes whereas the central regions contain large irregular cells. The nuclei of the germ cells are prominent and they often possess synaptonemal complexes (arrowed). The cytoplasm of the cells contains numerous free ribosomes , infrequent GER, mitochondria (m) and Golgi complexes (gc). Between the germ cells the cytoplasm of the nurse cells (nc) can be seen - the nuclei of the nurse cells are not seen here although mitochondria are present in the cytoplasm.

In Plate 147 a nucleus is cut obliquely and the pores (np) in the nuclear membrane are visible.


nc
n
tc
mu
m
pc
146

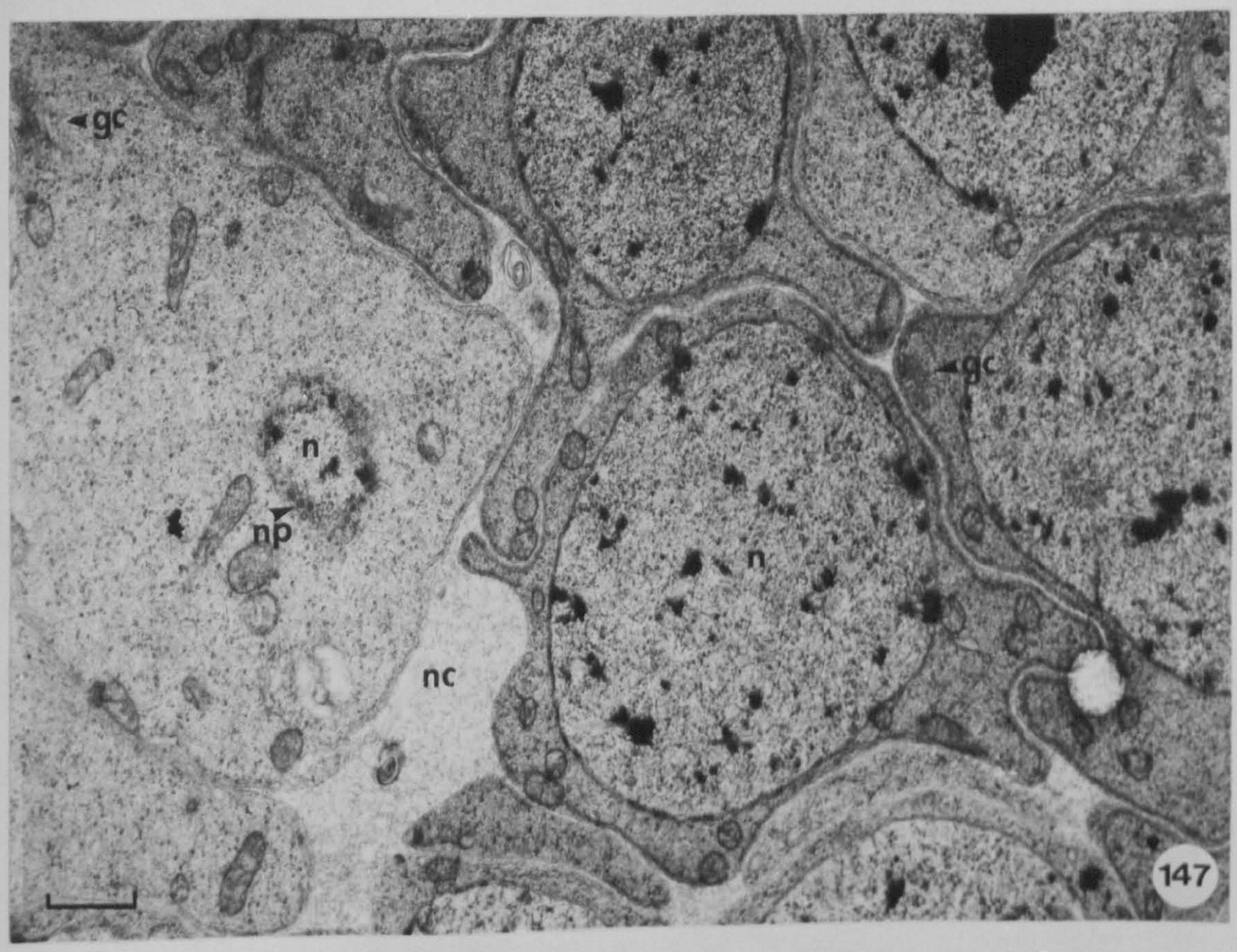

gc
n
np
nc
gc
n
147

Plate 148 Adult - 4 days p.i. from the mouse.

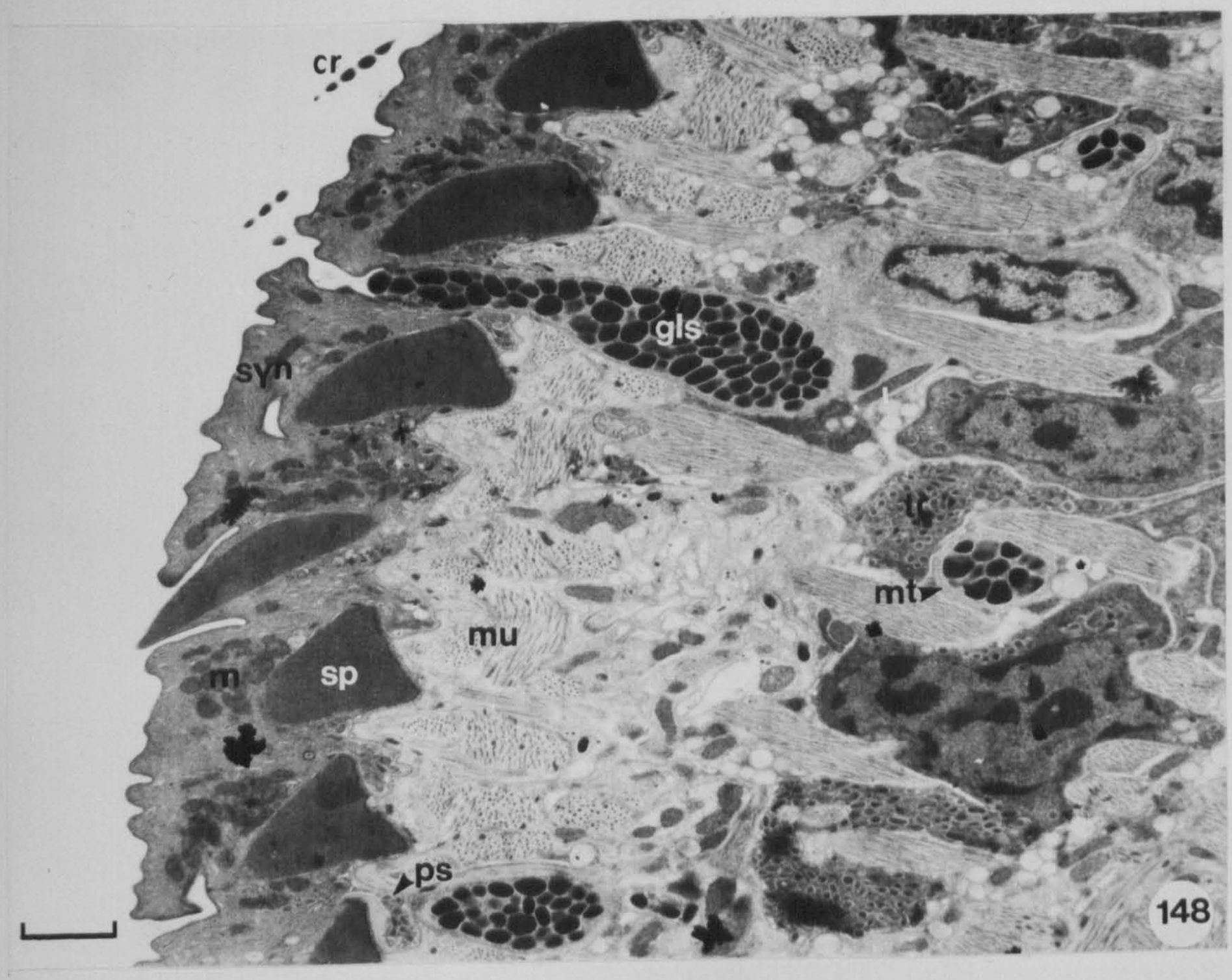
The tegument in the anterior part of the adult consists of a syncytium (syn) - bearing "crowned" spines (sp,cr) and containing tegumental granules and mitochondria (m) - and the tegumental cell bodies (tc) which underly the peripheral musculature (mu). A duct of a unicellular gland cell containing numerous secretory bodies (gls) can be seen penetrating the syncytium. In cross section the duct is lined by a ring of microtubules (mt).

Plate 149 Adult - 4 days p.i. from the mouse.

A slightly oblique section showing the hexagonal arrangement of the spines (sp). The syncytium contains relatively few secretory granules (tg) and the small mitochondria are dense. The syncytium is separated from the underlying peripheral muscles by a layer of fibrous interstitial material (im).

Plate 150 Adult - 4 days p.i. from the mouse.

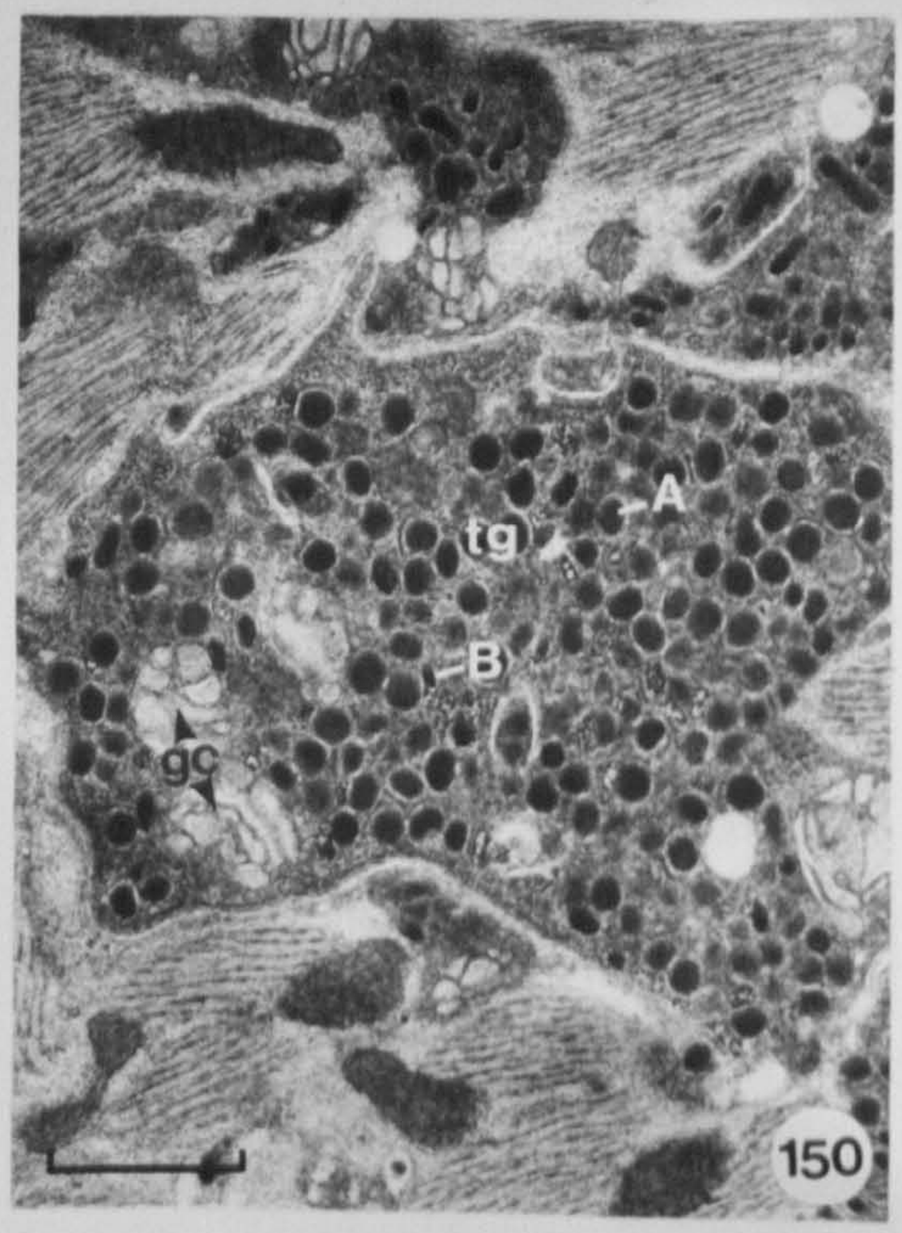
The tegumental cell body contains two types of secretory granules (tg) - a spherical granule with homogeneous dense contents (A) and an oval granule with granular contents (B). Also present in the cytoplasm are free ribosomes and Golgi complexes (gc) with dilated cisternae.



148



149



150

Plate 151 Adult - 4 days p.i. from the mouse.

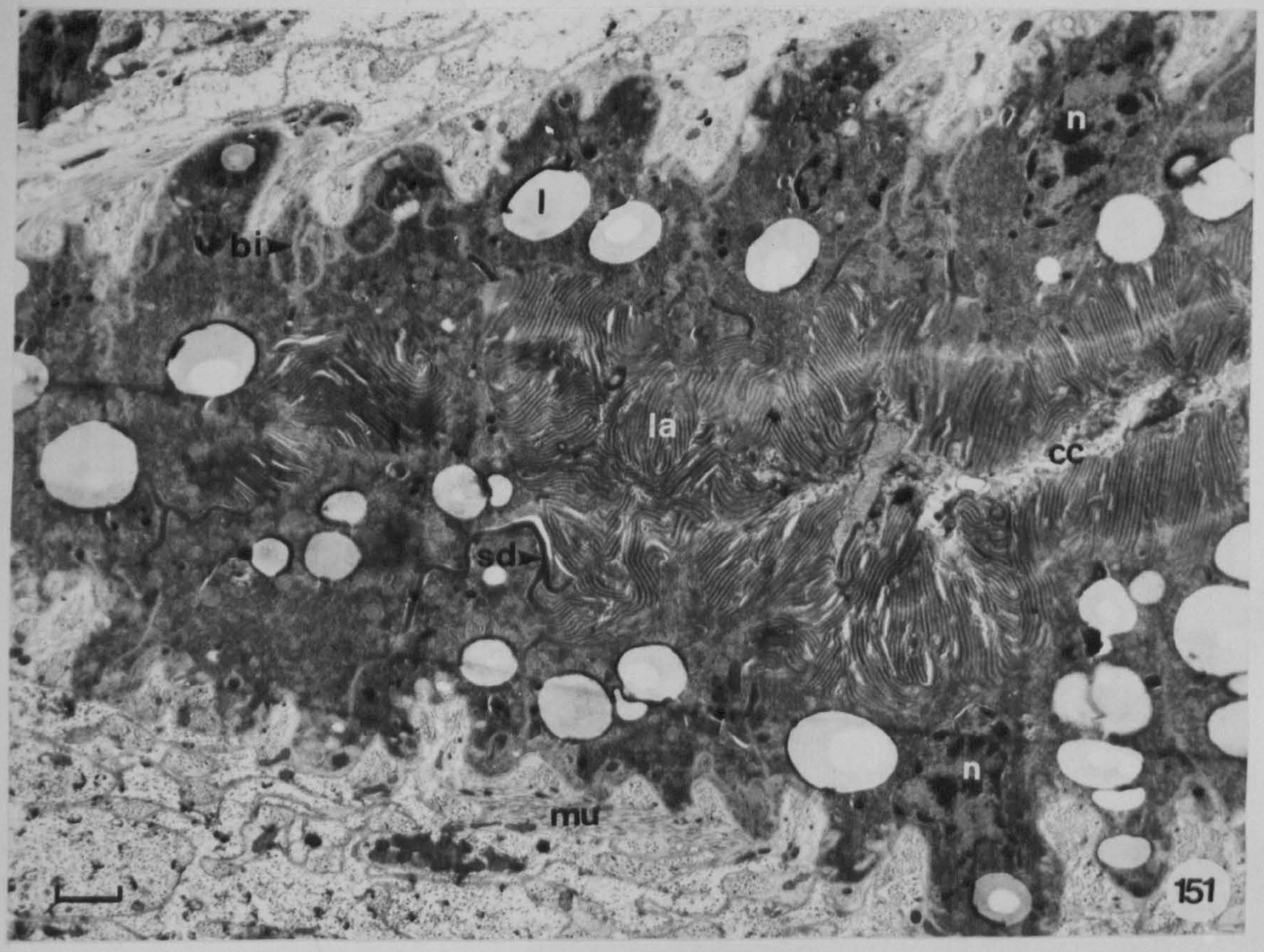
This section is a general view of an arm of the digestive caeca showing numerous lipid drops (l) and closely packed surface lamellae (la). The nuclei (n) are mainly basal and adjacent cells are connected by septate desmosomes (sd).

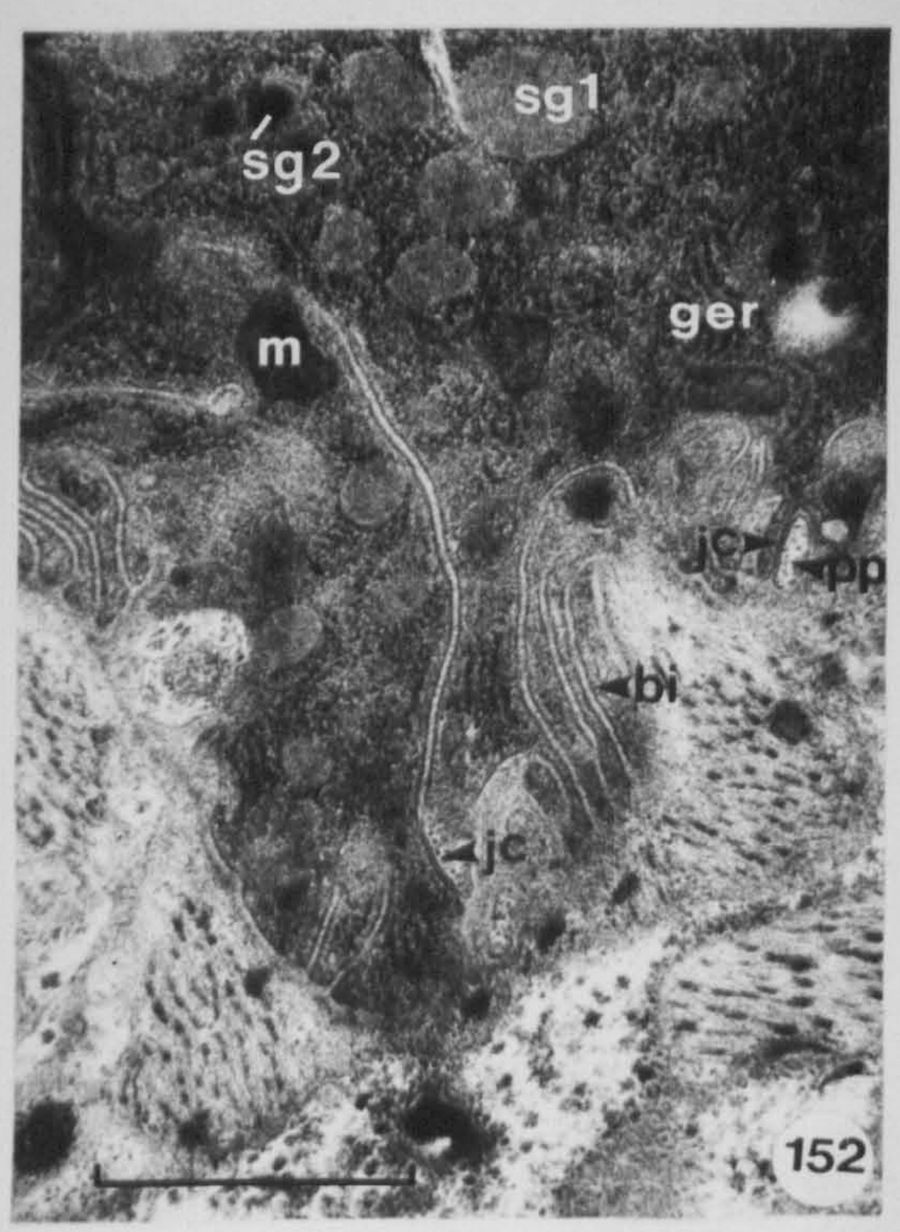
Plate 152 Adult - 4 days p.i. from the mouse.

The basal lamina of the caecal cells is much invaginated (bi) and junctional complexes (jc) are present between the caecal cells and the processes of the parenchymal cells (pp). Two types of secretory body are present - a small dense body and a larger less dense body. The cytoplasm of the cells contains GER (ger) dense mitochondria (m) and lipid drops (Plate 153-1).

Plate 153 Adult - 4 days p.i. from the mouse.

Between the closely packed surface lamellae (la) of the caecal epithelium is a thin layer of electron dense material (arrowed).


n
l
bi
la
cc
sd
n
mu
151


sg1
sg2
ger
m
jc
pp
bi
jc
152

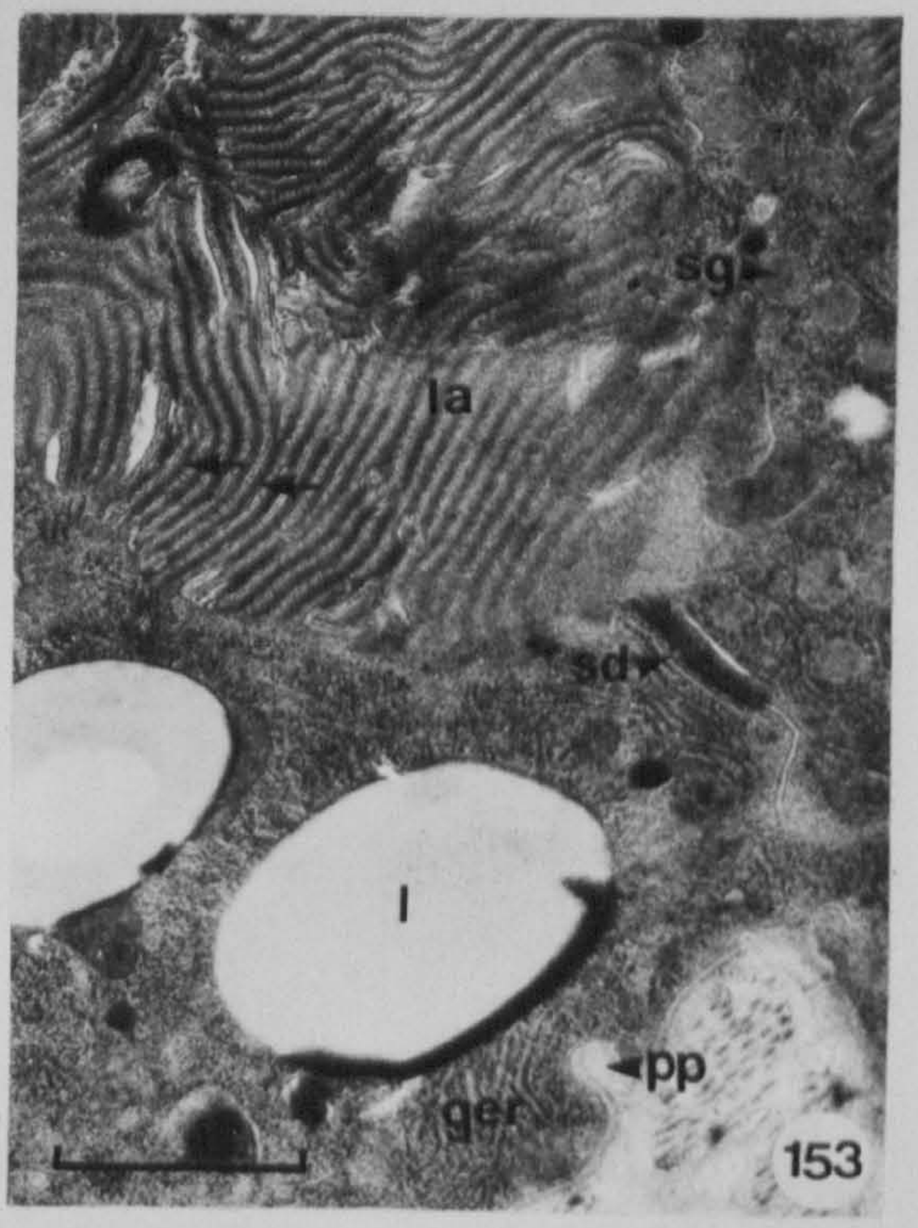
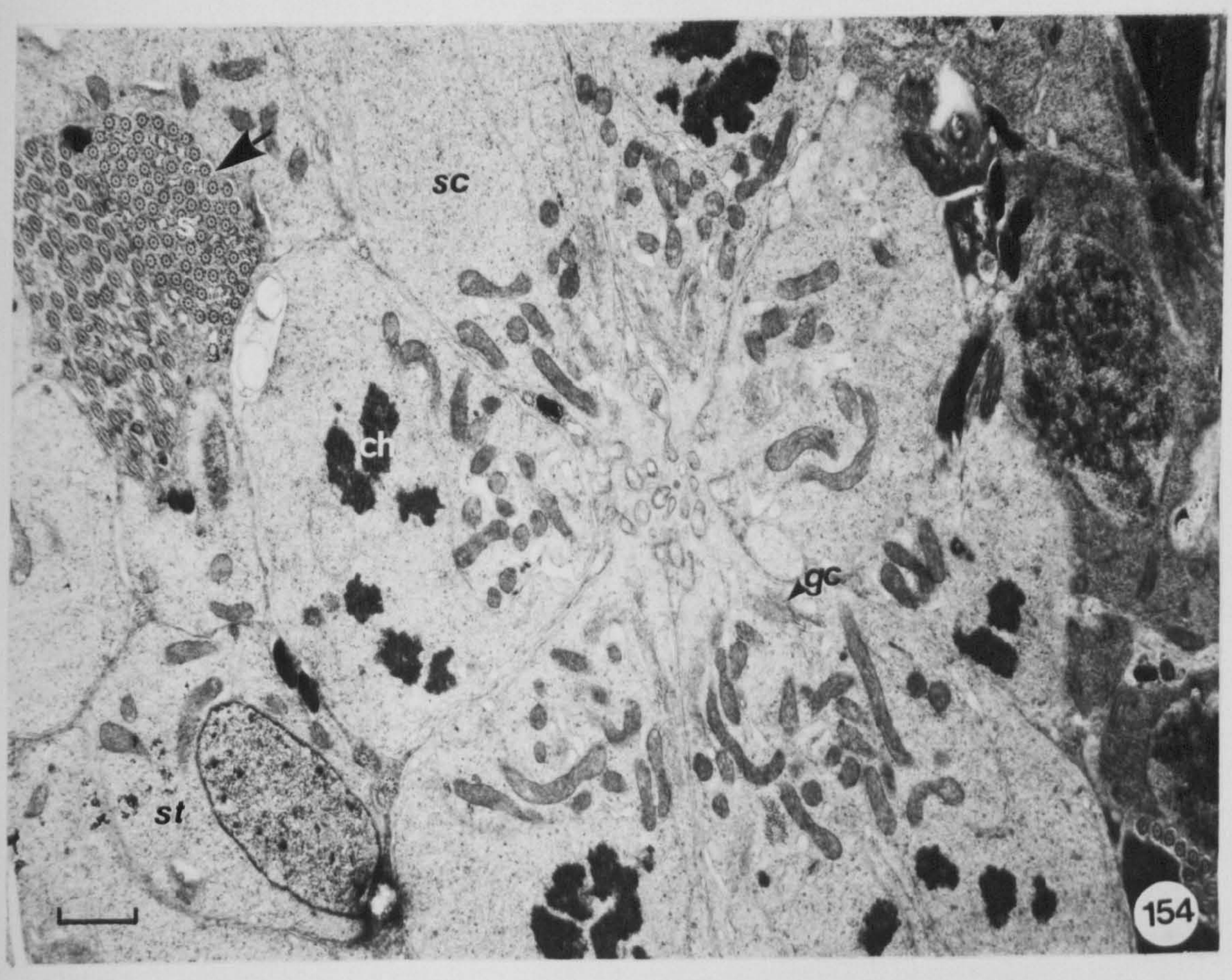

sg
la
sd
l
pp
ger
153

Plate 154 Adult - 4 days p.i. from the mouse.

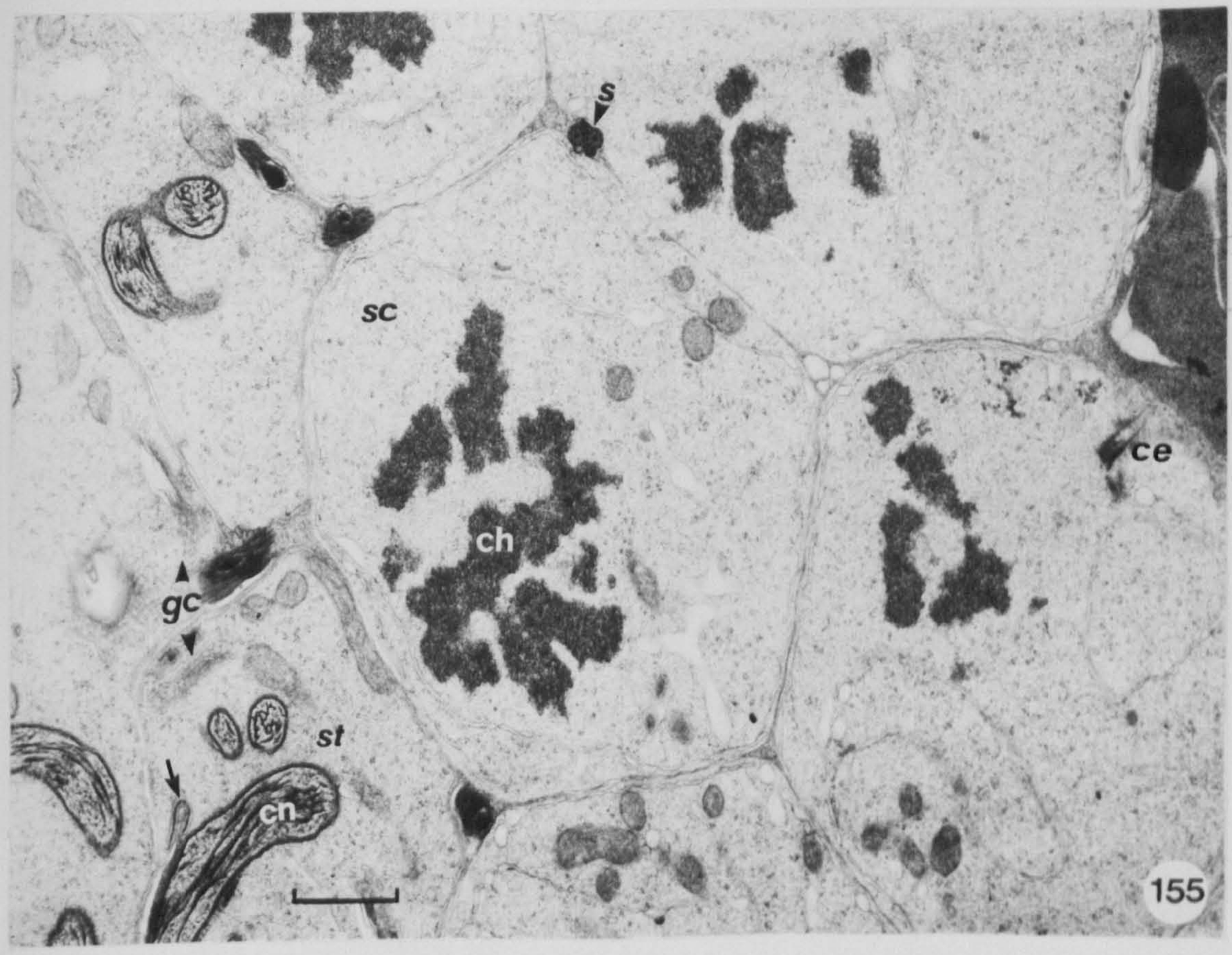
Various stages of spermatogenesis can be seen in the testes. This section shows dividing spermatocytes (sc) associated together in a cytophore, a spermatid (st) and developing sperm (arrowed - see Plate 156).

Plate 155 Adult - 4 days p.i. from the mouse.

This section through the testes shows spermatocytes (sc), spermatids(st) and mature sperm (s). The spermatocytes are in the process of division and chromosomes (ch) and a centriole (ce) can be seen. The nuclear material of the spermatid is condensed (cn) into threads and the elongated nucleus is cut several times in cross section. The cytoplasm of the spermatid contains Golgi complexes (gc) ribosomes and mitochondria. The furrow (arrowed) which forms during the production of the lateral and central processes can be seen.



154



155

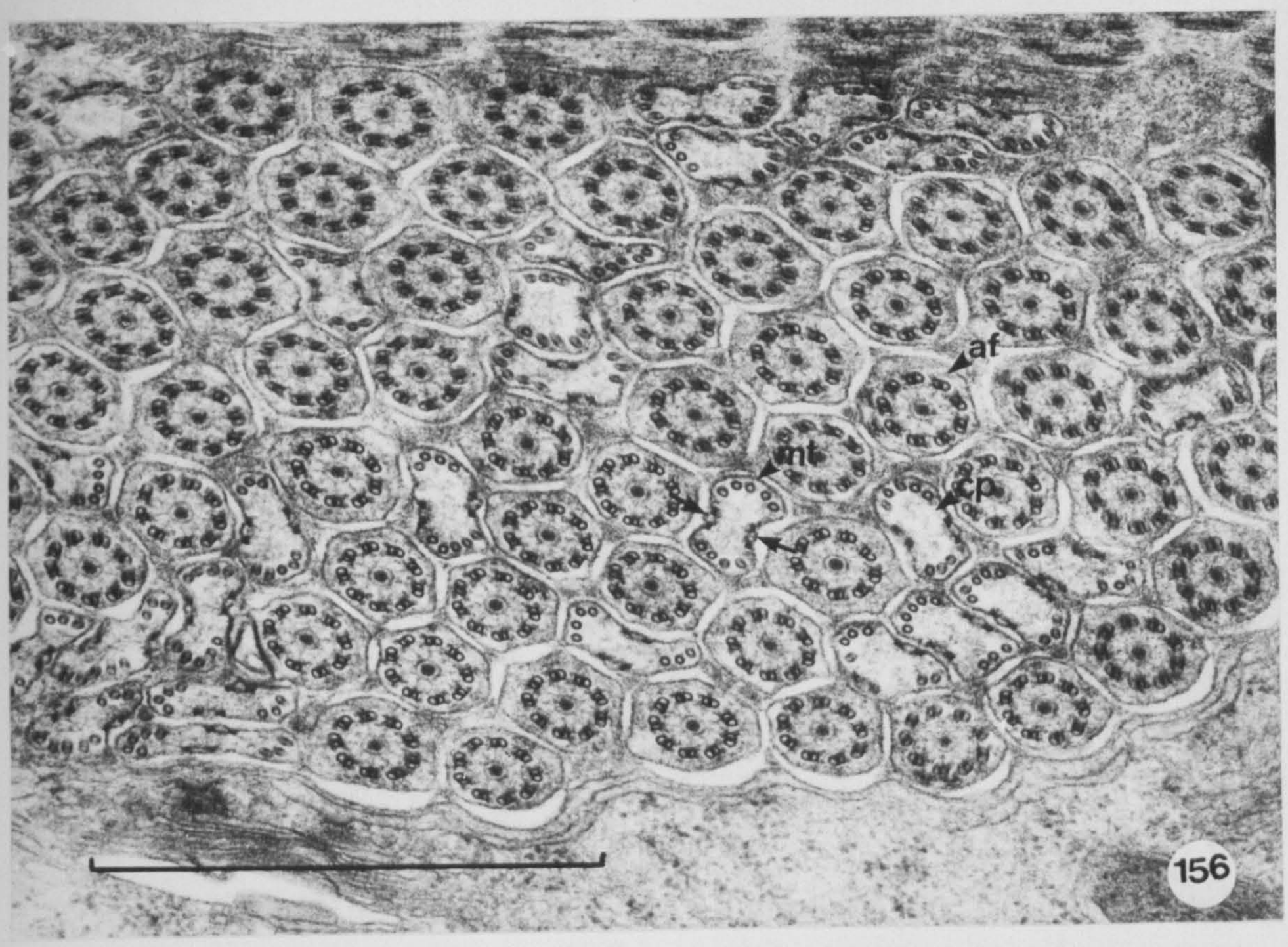
Plate 156 Adult - 4 days p.i. from the mouse.

This section shows the still separate lateral and central processes of adjacent spermatids. The lateral processes contain the "9+1" axial filaments (af). The (cp) central processes contain two groups of microtubules (mt) and, between them, two pairs of dense rectangular plaques (arrowed).

Plate 157 Adult - 4 days p.i. from the mouse.

This section cuts through a spermatid in the region where the lateral processes arise. The axial filament (af), "centriole-like body" (cb), sperm mitochondrion (m), condensed nucleus(cn) and the peripheral microtubules can all be seen.

Note the two dense areas (arrowed) on opposite sides of the nucleus in an adjacent spermatid.

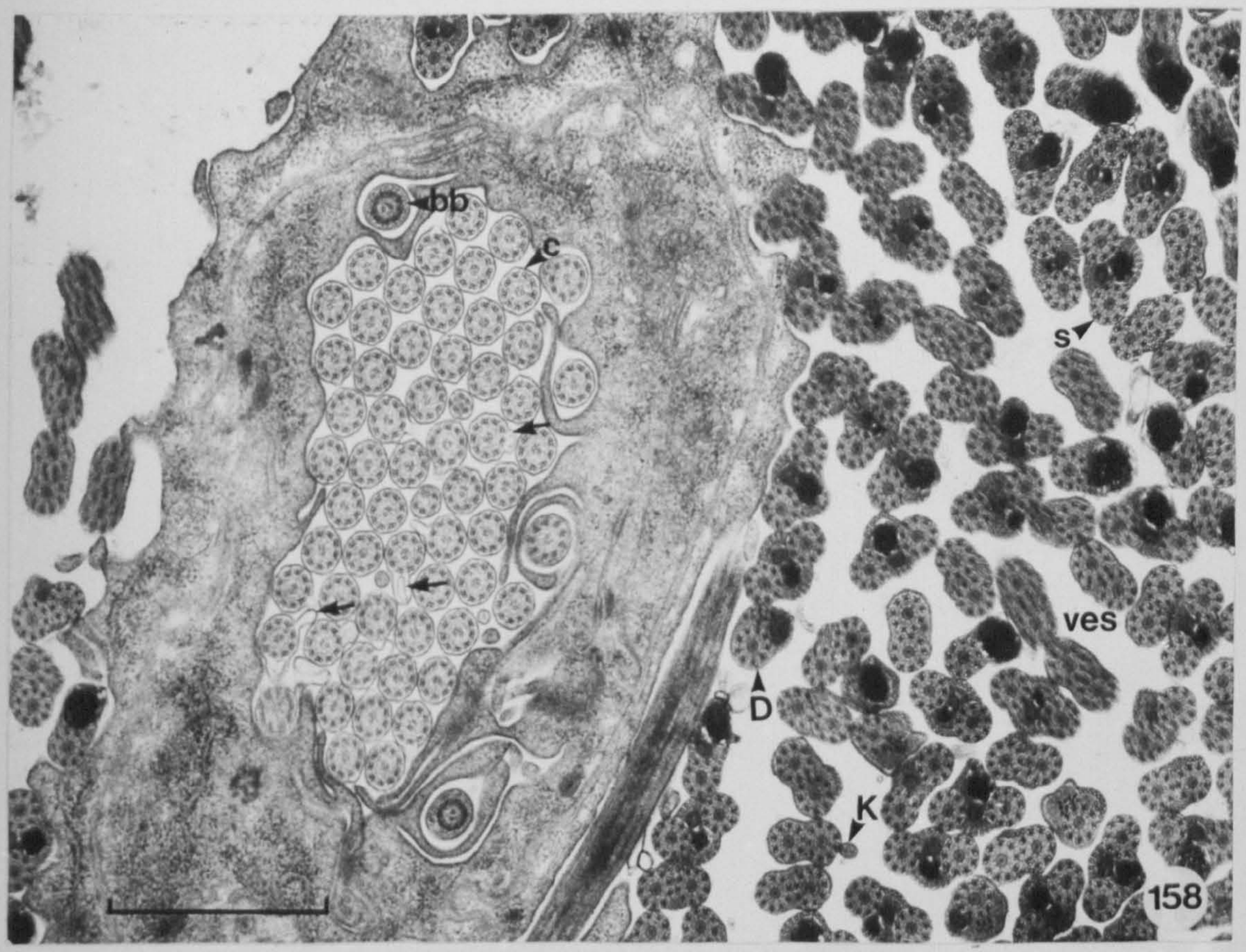

af
mt
cp
156


cb
m
cm
af
mt
m
157

Plate 158 & 159 Adult - 4 days p.i. from the mouse.

These two sections show mature sperm in the vesicula seminalis. Various different types of morphology can be seen in cross section (A-K) and these are described in detail in Volume I, page 331 . Cross section E is typical of the sperm described for other trematodes and consists of a nucleus with condensed chromatin (cn), two "9+1" axial filaments (af), a mitochondrion (m) and two sets of peripheral microtubules (mt).

The role of the ciliated duct next to the vesicula seminalis is unknown. The cilia (c) have typical basal bodies (bb) and a "9+2" arrangement of microtubules; the surface of the cilia is often irregular (arrows).


bb
c
S
ves
D
K
158

H
C
G(b)
F
E
G(a)
J
I
mt
af
9+1
m
cn
A
B
159

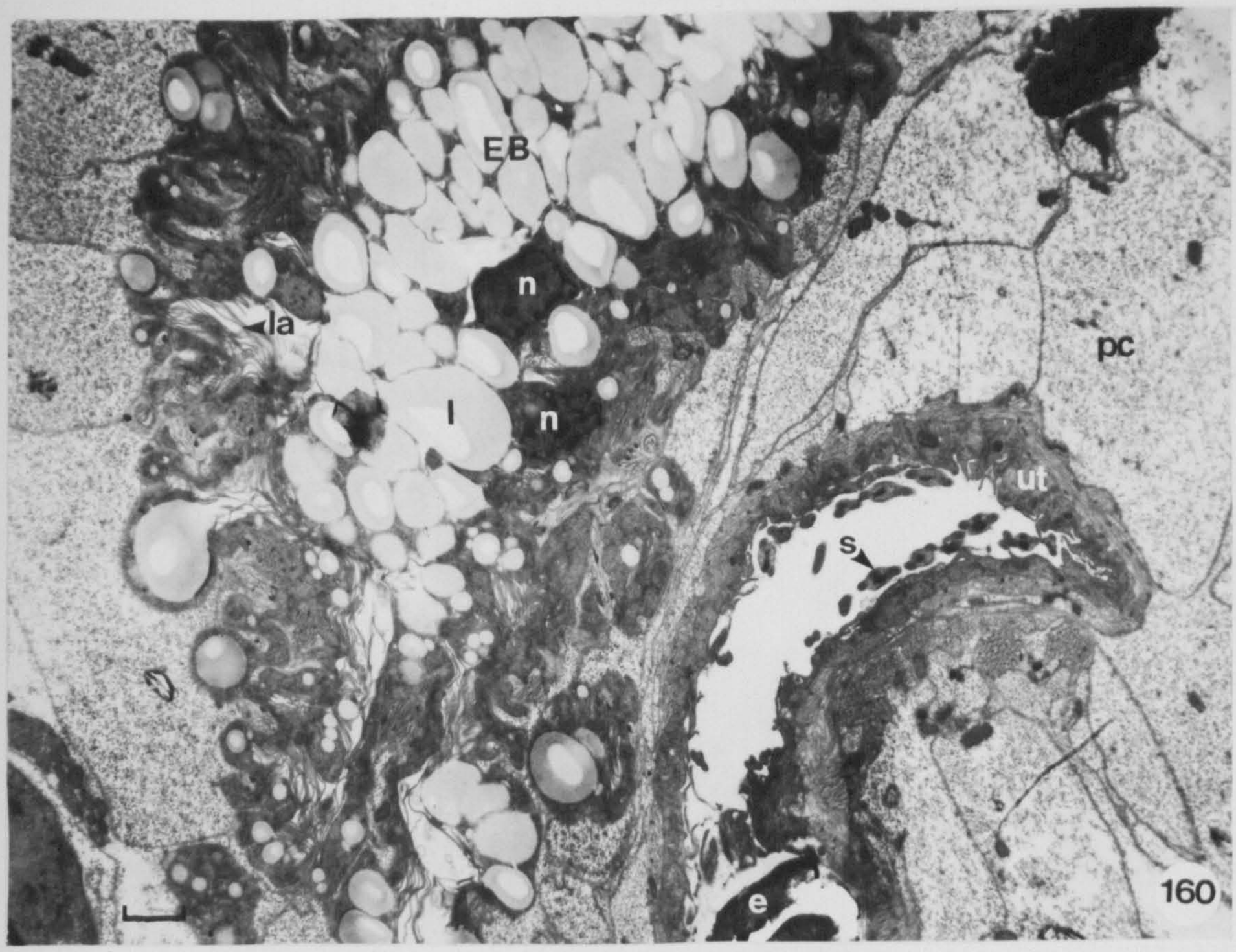
Plate 160 Adult - 4 days p.i. from the mouse.

The cytoplasm and the lumen of the excretory bladder (EB) of the adult contains numerous lipid droplets (l). The uterus (ut) is also seen in this section, it contains sperm (sp) and a tanned egg which is poorly preserved (e).

Plate 161 Adult - 4 days p.i. from the mouse.

The cells of Mehlis' gland contain sac-like secretory bodies (ms), Golgi complexes (gc), GER (ger), mitochondria (m) and nuclei (n).

Note: There may be other cell types in Mehlis' gland.


EB
n
la
l
n
pc
ut
s
e
160

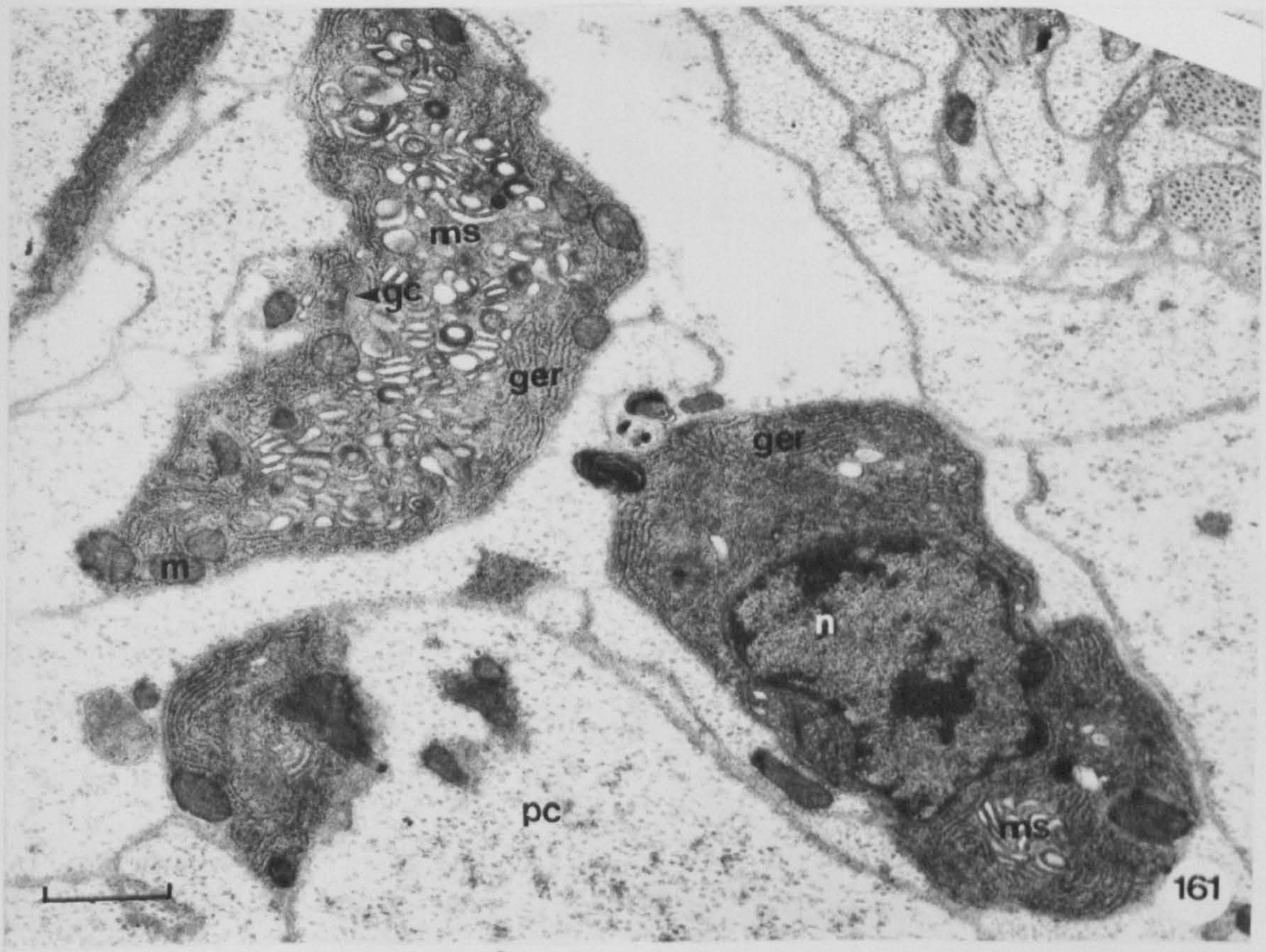
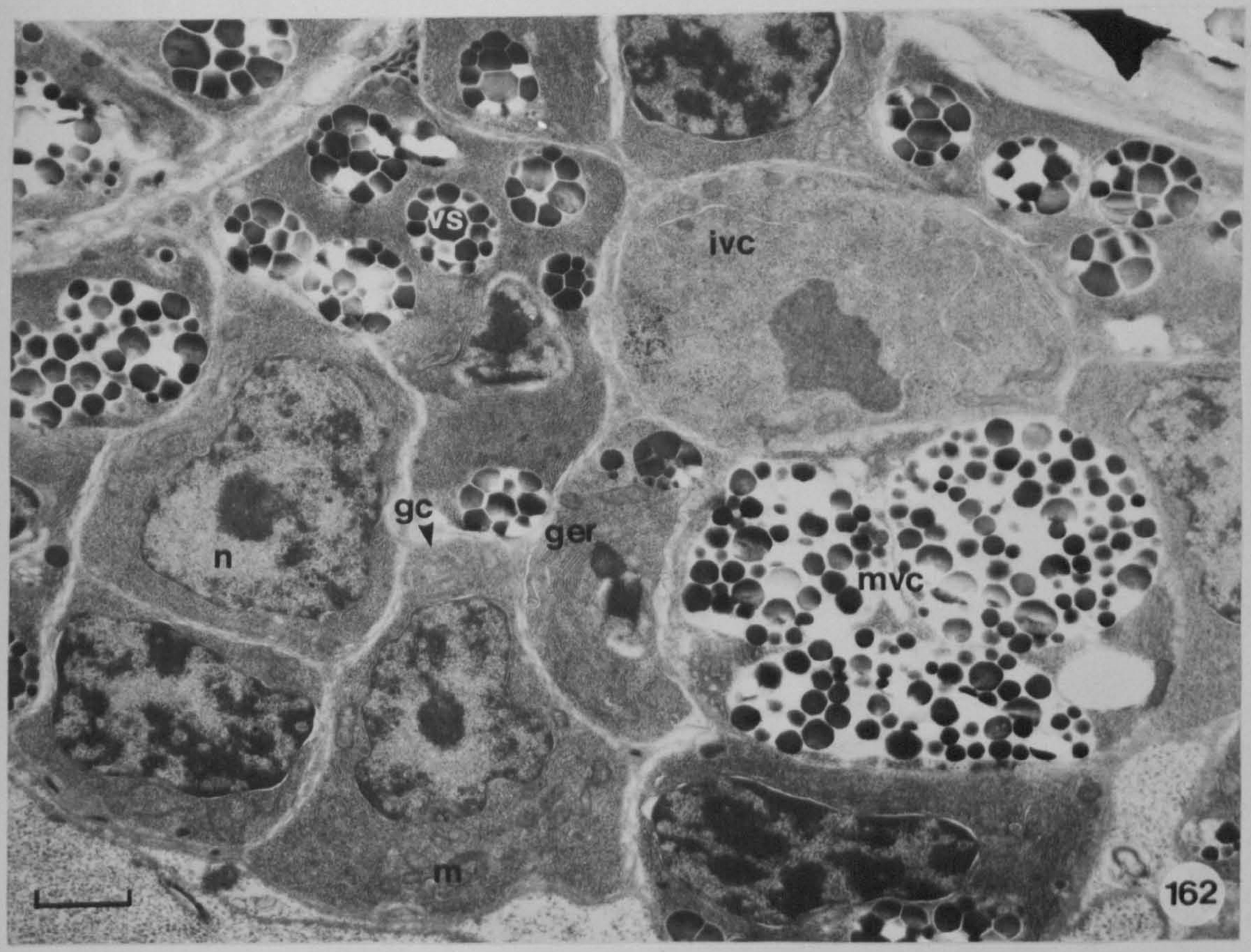

ms
gc
ger
m
ger
n
pc
ms
161

Plate 162 Adult - 4 days p.i. from the mouse.

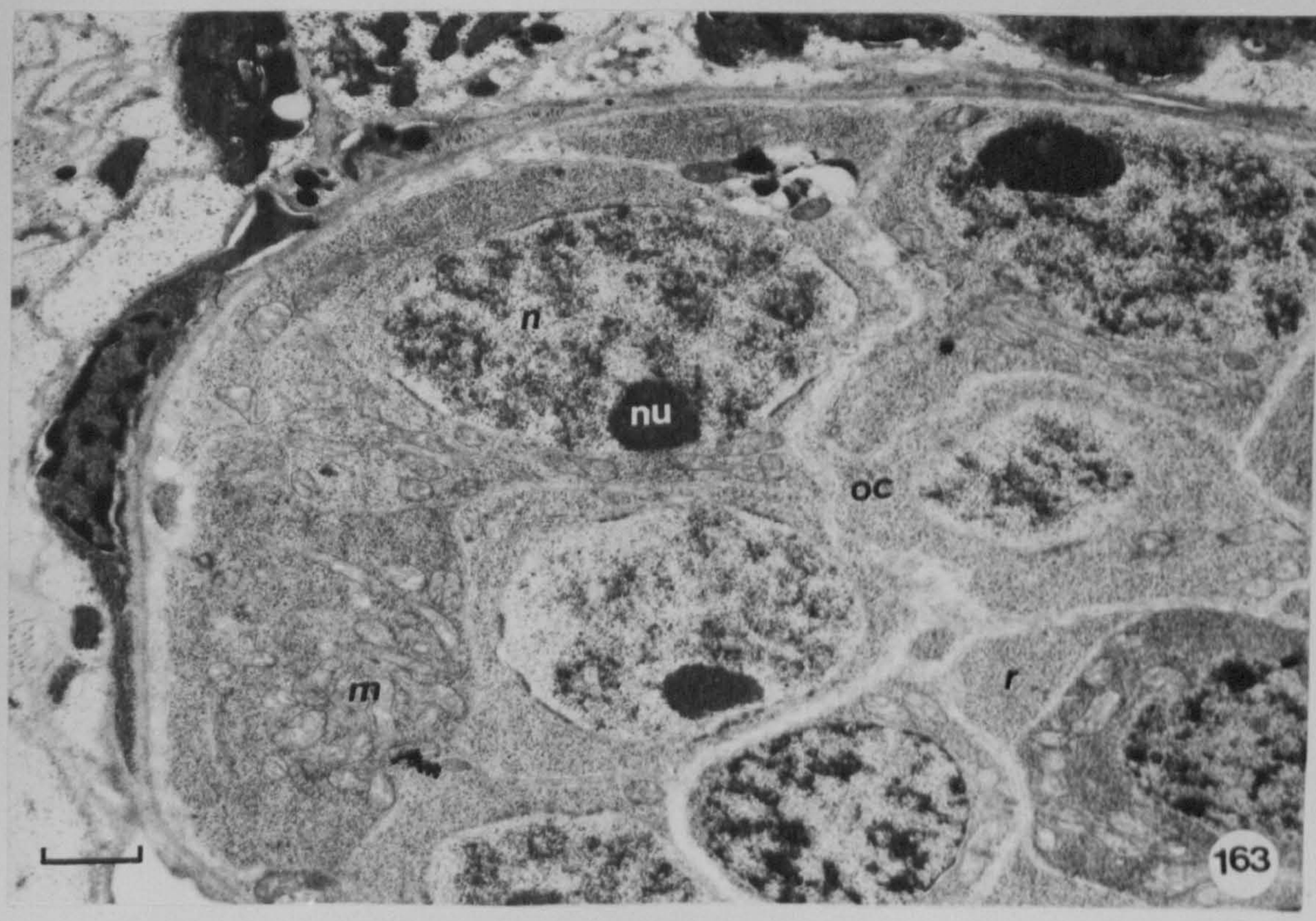
Immature, developing and mature vitelline cells are all present in this section through the vitellaria. The immature cells (imv) contain free ribosomes and some mitochondria. In the developing cells there are clusters of secretory droplets (vs), extensive GER (ger), mitochondria (m) and Golgi complexes (gc). In the mature cells (mvc) only small areas of cytoplasm are left and the vitelline secretions almost fill the cell.

Plate 163 Adult - 4 days p.i. from the mouse.

This section through the ovary of the adult contains cells (oocytes? - oc) which posses nuclei (n) with prominent nucleoli (nu) and numerous dense patches of heterochromatin. The ground substance of the cells contains ribosomes and mitochondria (m) with an electron lucent matrix. Golgi complexes, cortical granules and "ribosomal bodies" appear to be absent.



162



163

Plates 164 - 169 Excysted metacercaria.

Plate 164

This view shows the general shape and surface features of the metacercaria (A-anterior, P-posterior) including the oral sucker (OS), ventral sucker (VS) and excretory pore (EP).

Plate 165

The lateral edges of the fluke just posterior to the ventral sucker are spine-free and have deep circumferential and longitudinal folds (arrowed)in addition to the usual surface corrugations

Plate 166

The ventral sucker (VS) is shallow and just posterior to it is a groove which extends round to one side of it; this probably represents the genital pore (GP). On the ventral surface posterior to the ventral sucker the surface of the fluke bears deep longitudinal folds (arrowed). Sensory papillae (SP) are present on the lips of the sucker.

Plate 167

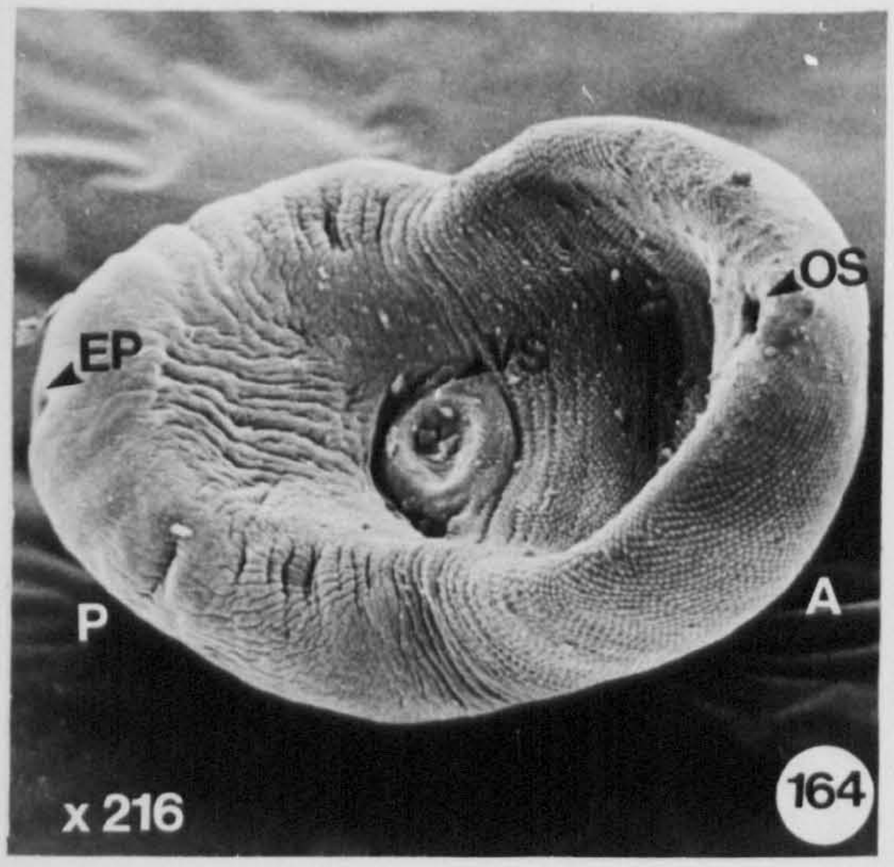
The oral sucker has a bilaterally symmetrical (dotted line) arrangement of ciliated (3P) and non-ciliated (DP) sensory papillae.

Plate 168

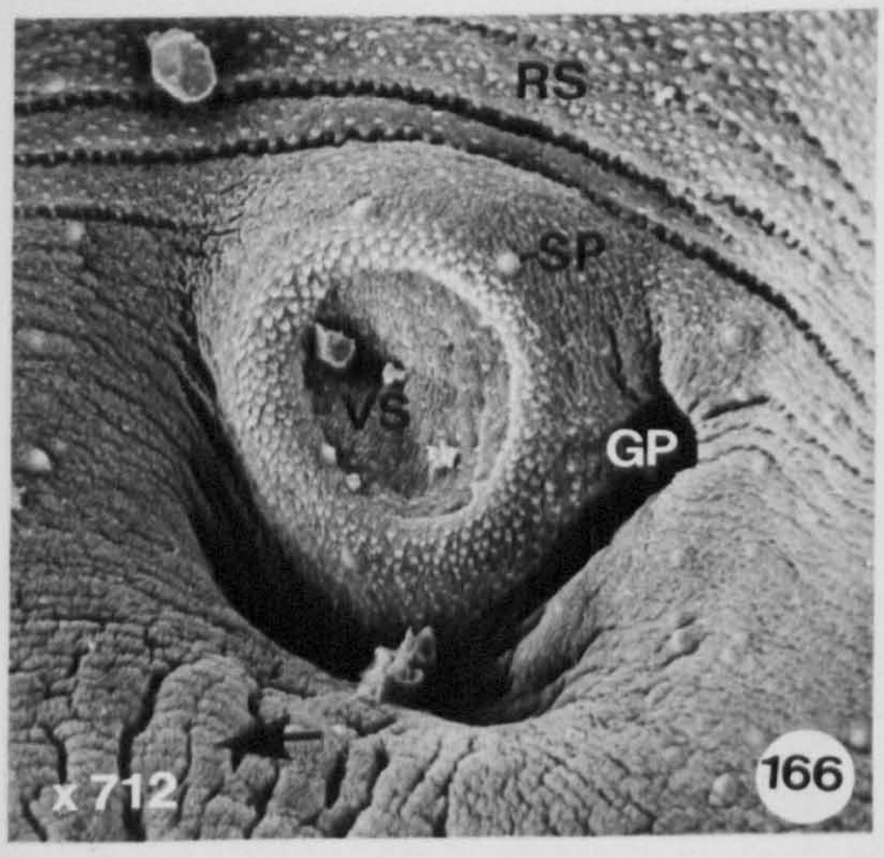
The hindbody is covered with rings of spines (RS) which have a crown (CR) of up to 14 finger-like projections or "teeth".

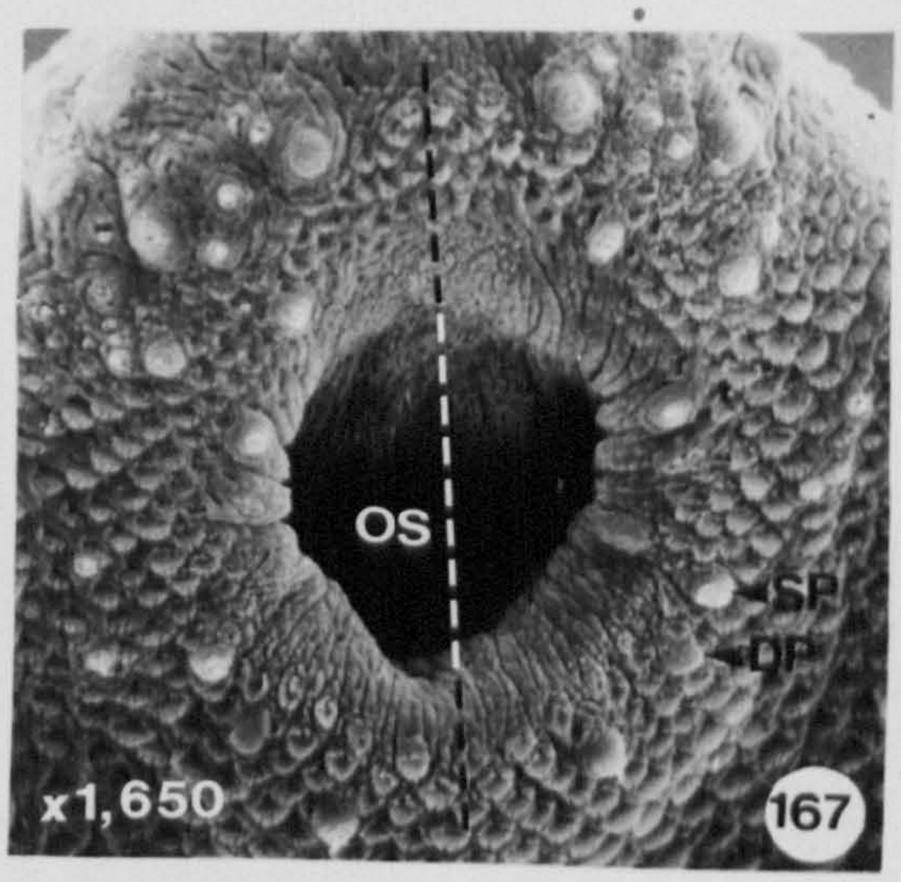
Plate 169

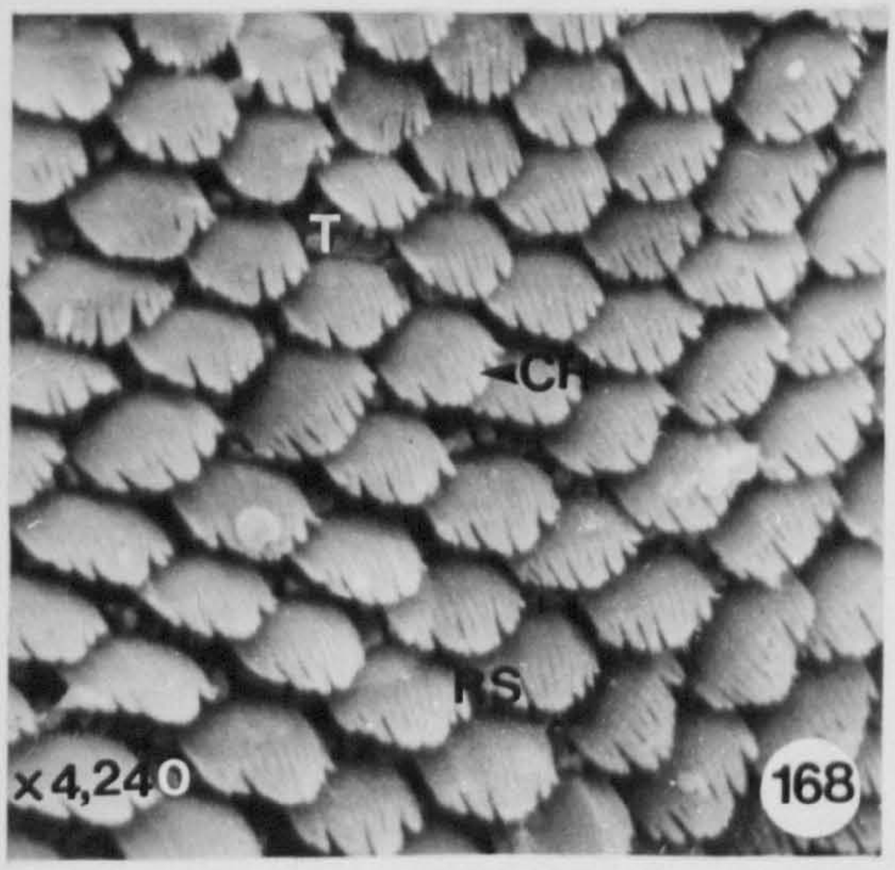
The hindbody bears irregularly placed peg-like spines (S). In the region of the spines the tegument is furrowed (arrows).


EP
VS
OS
P
A
x 216
164


T
x 2,200
165


RS
SP
VS
GP
x 712
166


OS
SP
DP
x1,650
167


T
CP
MS
x4,240
168

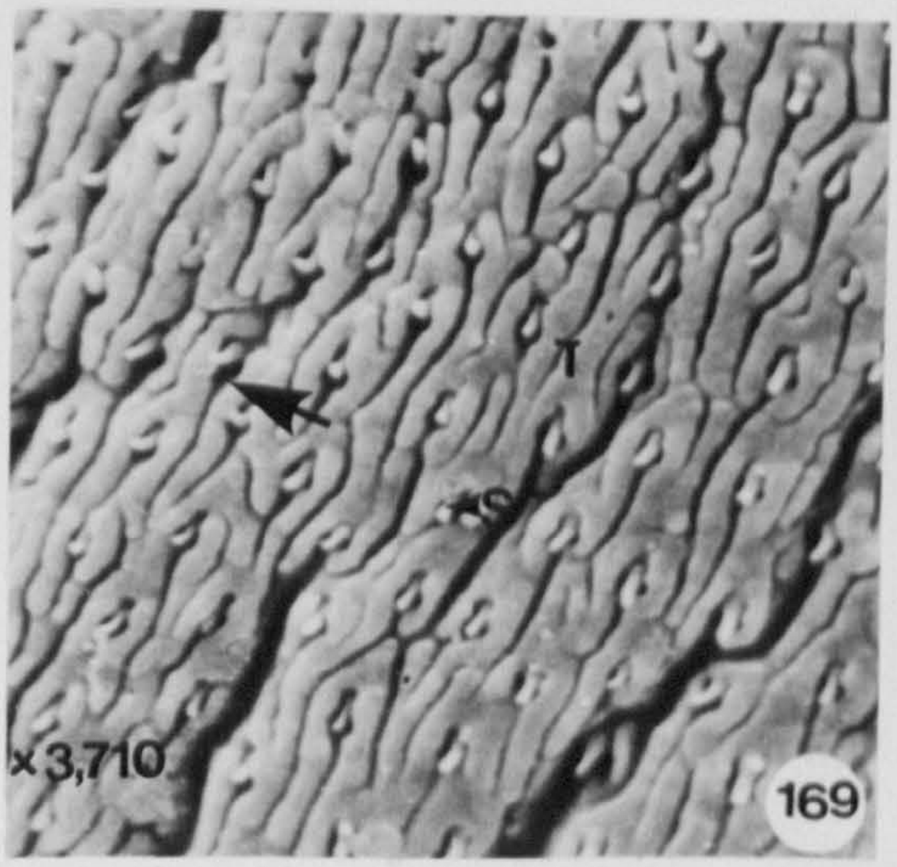
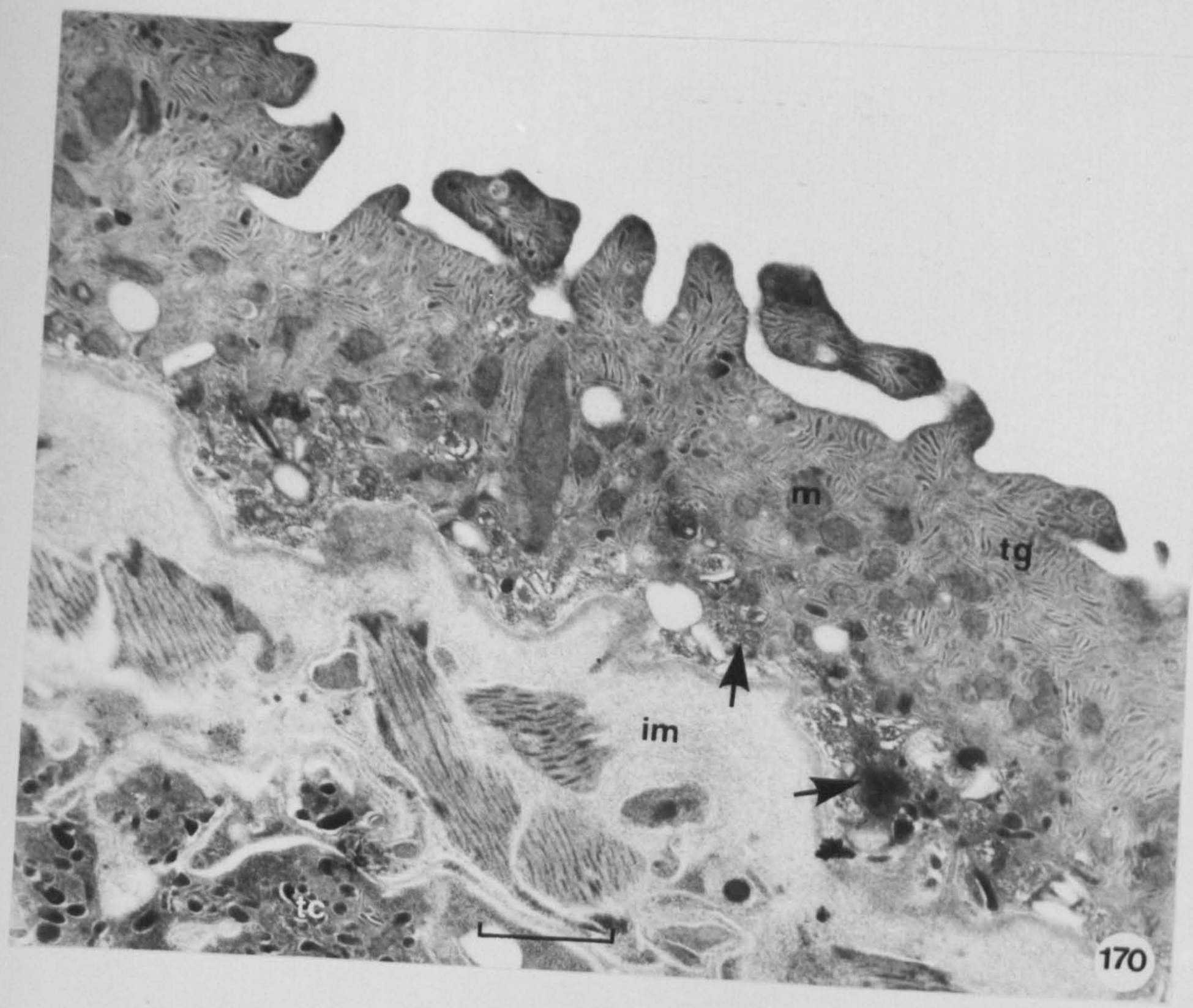

T
MS
x3,710
169

Plate 170 4 days in vitro culture.

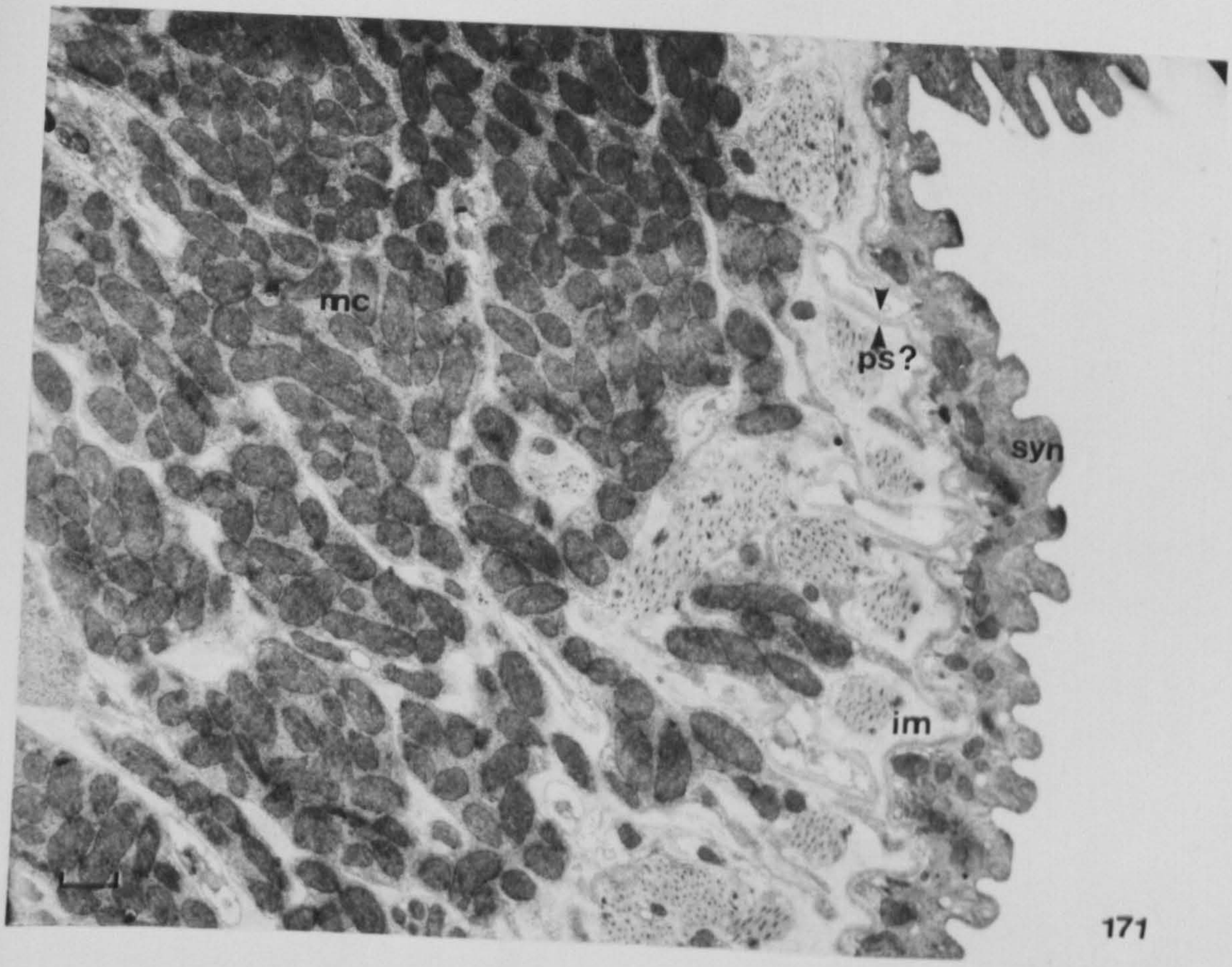
The te gumental syncytium immediately above the basal lamina appears abnormal (arrows), it is granular and contains degenerating secretory bodies and vesicles. The basal lamina and underlying layer of interstitial material (im) are thicker than in the metacercaria or the adult. The apical part of the syncytium contains apparently normal spherical and biconcave granules (tg).

Plate 171 4 days in vitro culture.

"Mitochondria cells " are present in cultured flukes and are similar to those found in the metacercaria.and the adult. They are located in two groups beneath the peripheral musculature on the lateral surface of the fluke just posterior to the ventral sucker.



170



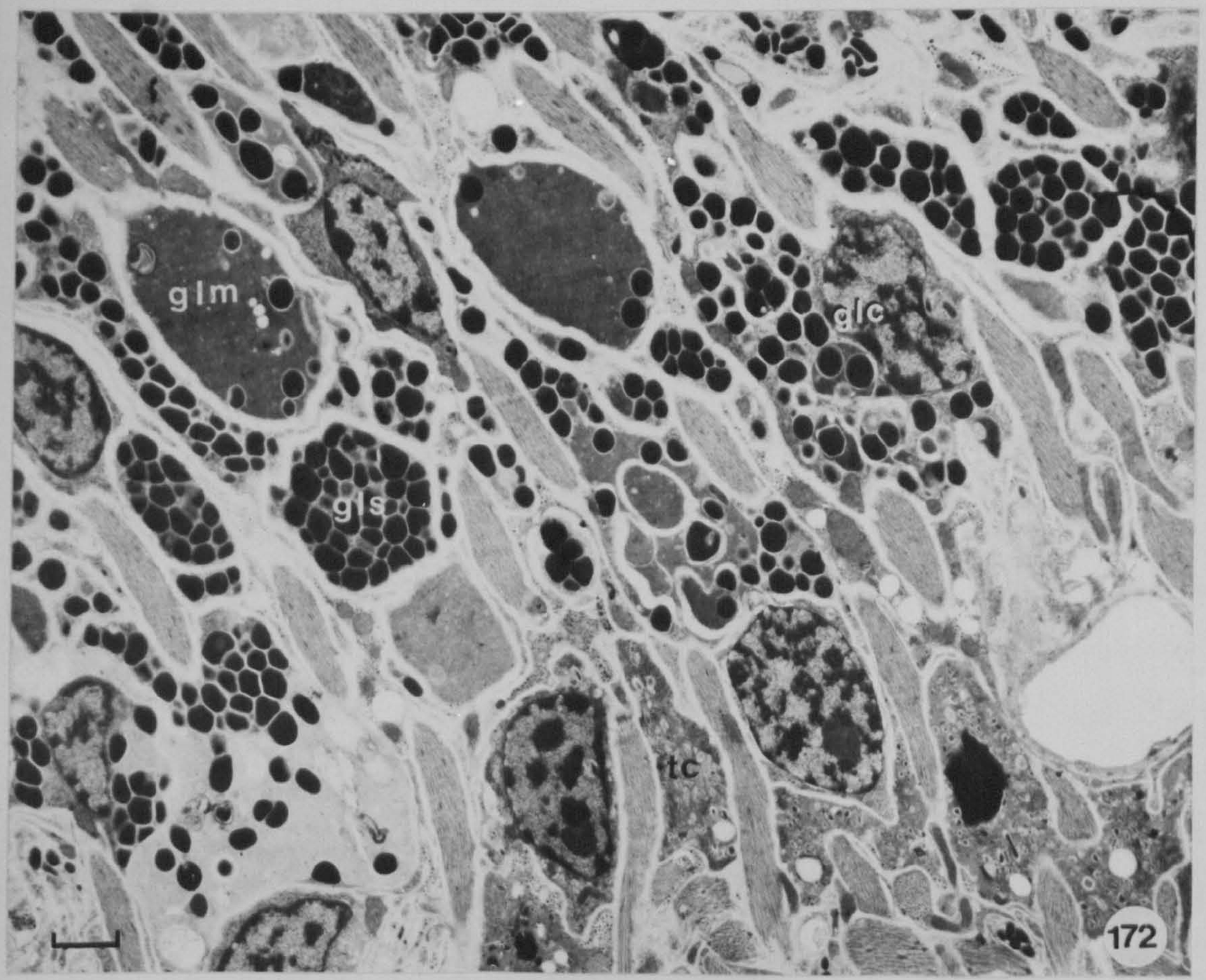
171

Plate 172 4 days *in vitro* culture.

This section shows the tegumental cell bodies (tc) and the underlying cell bodies of the unicellular gland cells (glc). The gland cells contain typical secretory droplets (gls) and, in addition, large irregular masses of moderately dense material (glm).

Plate 173 4 days *in vitro* culture.

The large masses of material (glm) in the gland cell bodies are finely granular and are not membrane-bound. Normal secretory bodies (gls) are also present although they often seem to be in the process of disintegration (arrows) and they appear to be contributing to the mass of granular material which occupies a large part of the cell.


glm
glc
gls
ftc
172

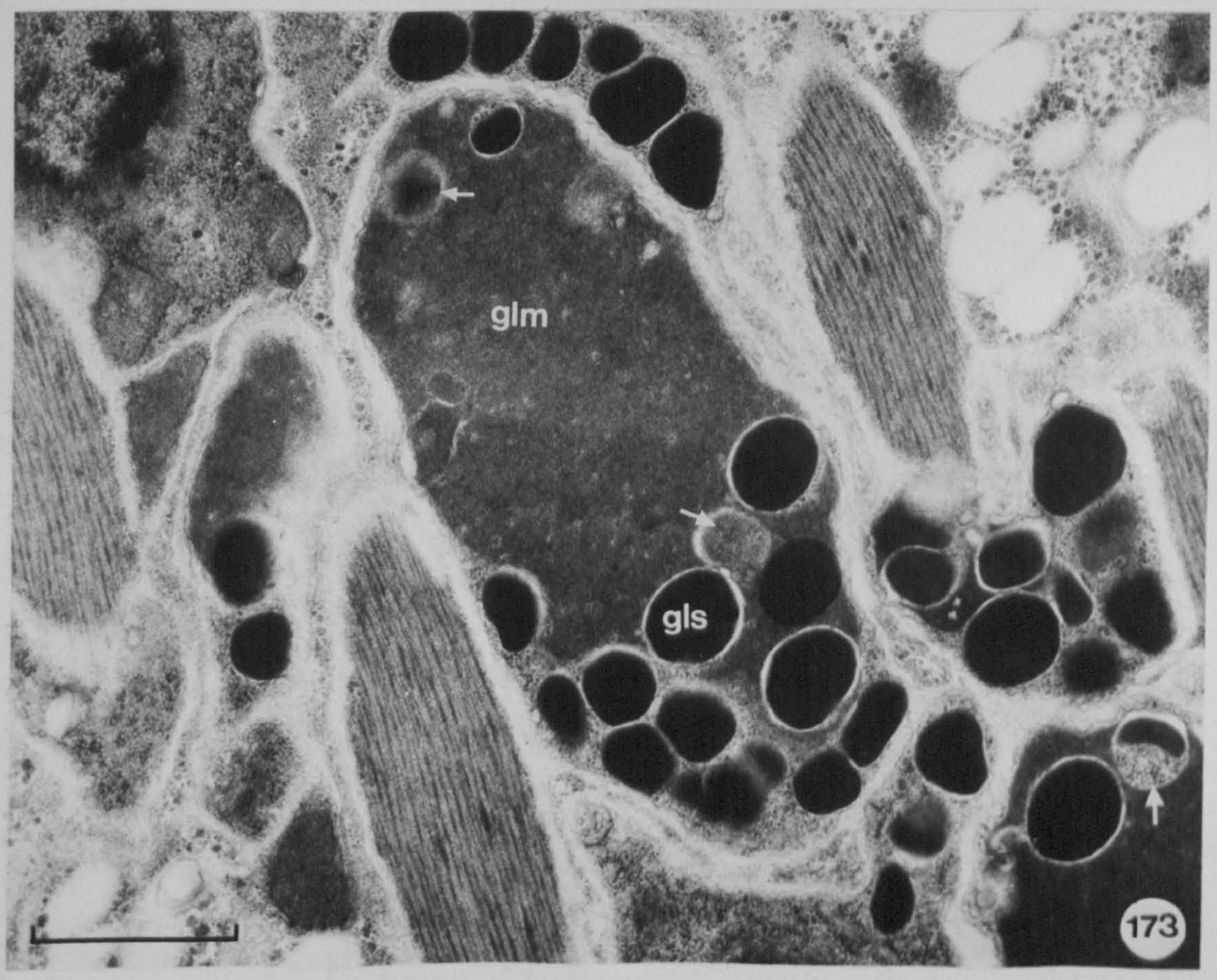
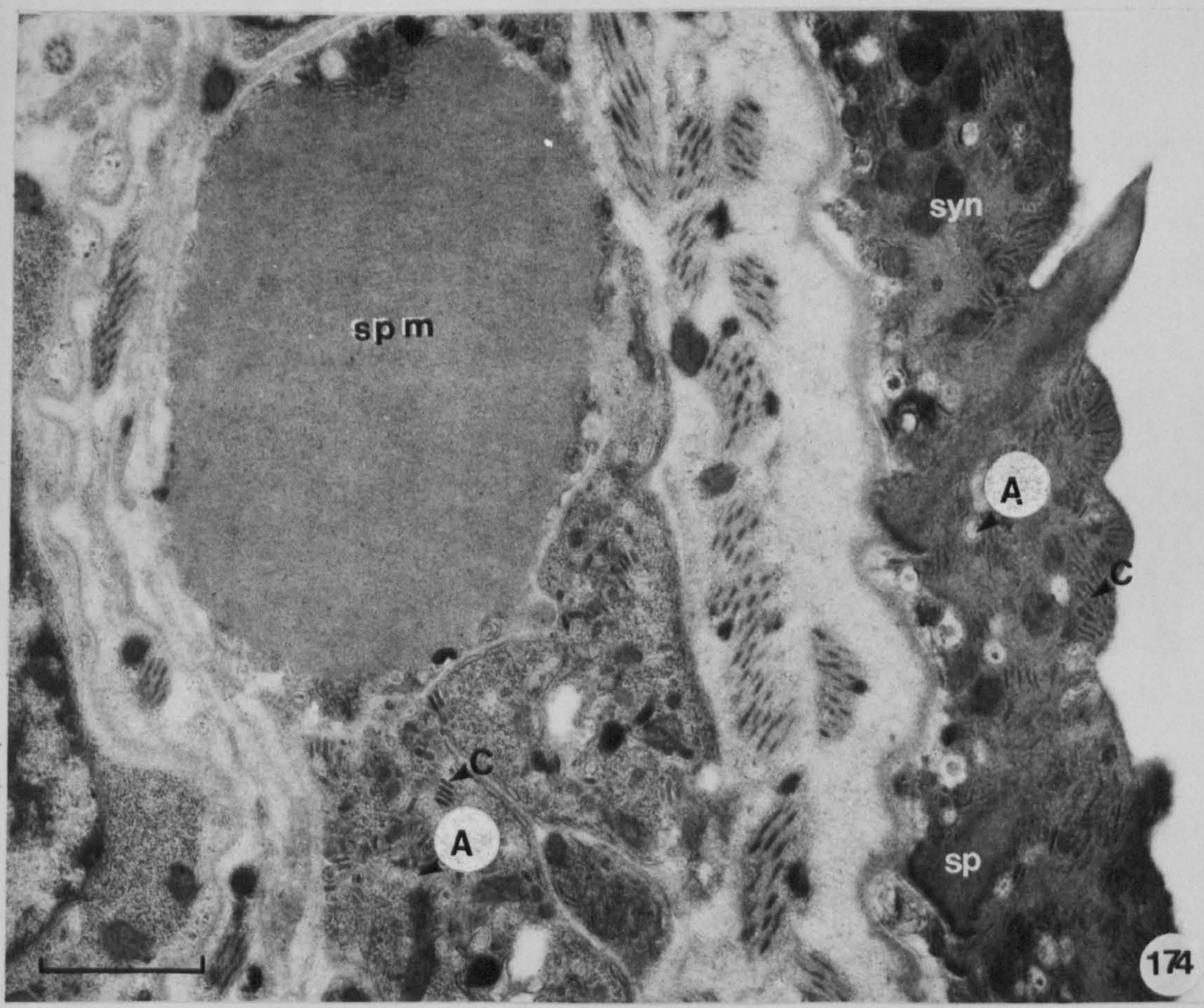

glm
gls
173

Plate 174 4 days *in vitro* culture.

The tegumental cell bodies of cultured flukes contain spherical (A) and rod-like or biconcave (C) secretory granules. In addition they also contain large, often oval, crystalline bodies (spm) which are very similar in composition to the spines (sp) in the syncytium.

Plate 175 4 days *in vitro* culture.

The rod-like secretory granules (C) are often associated with the large crystalline masses(spm) present in the tegumental cell bodies (arrowed). The Golgi complexes (ga) have very dilated cisternae and they are often associated with groups of small, sac-like vesicles (v). The tegumental cell bodies also contain abundant ribosomes (r), mitochondria and some dense areas which are probably lysosomes (ly).


spm
syn
A
C
A
C
sp
174

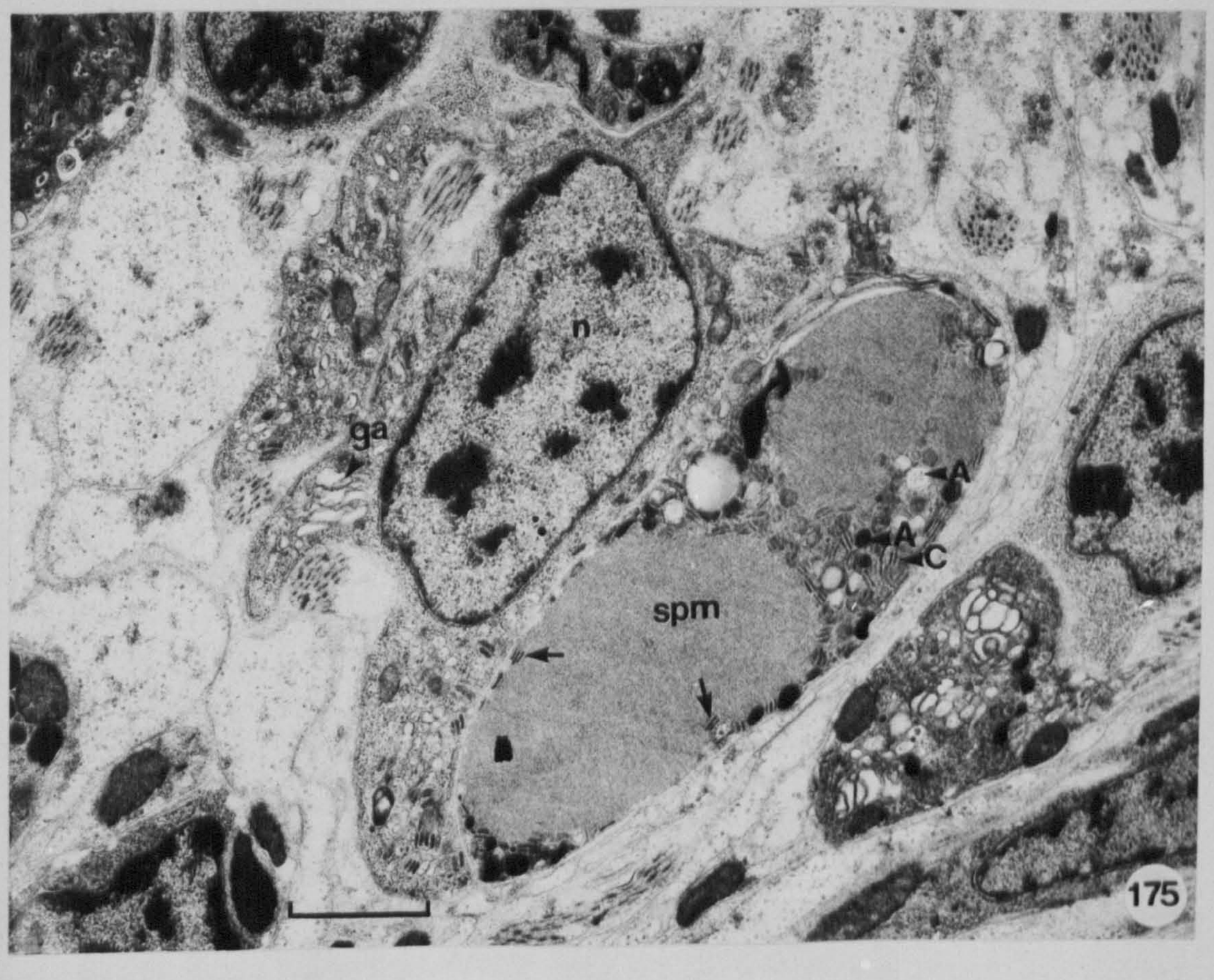

n
ga
A
A
C
spm
175

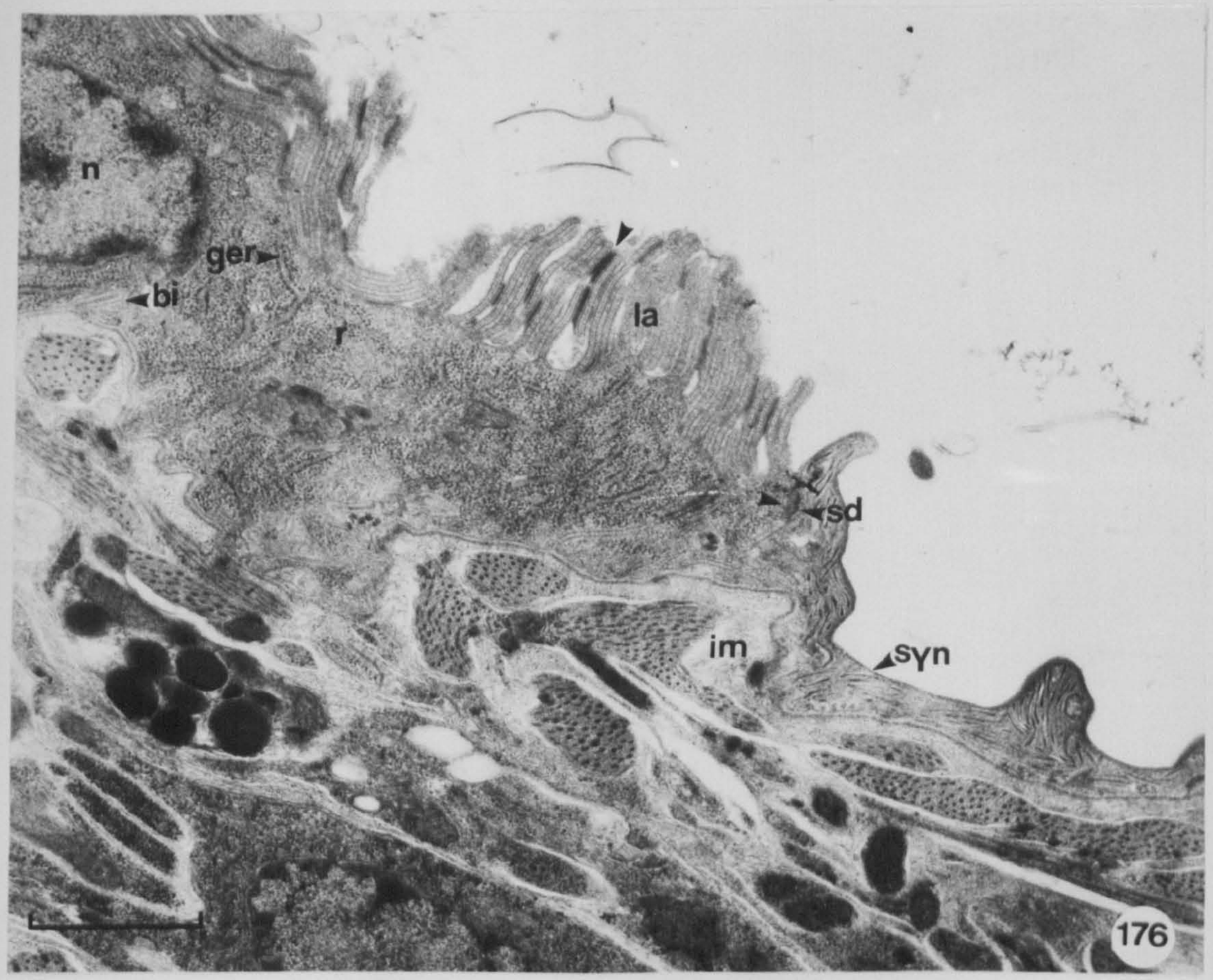
Plate 176 18 hours in vitro culture.

This section shows the caecal/oesophageal junction; the syncytium (syn) lining the oesophagus is connected to the cells of caecal epithelium by a septate desmosome (sd). The cytoplasm of the caecal cells contains short lengths of GER (ger), numerous free ribosomes (r) and mitochondria. The surface lamellae are short and closely packed, clumps of dense material are often present between them (arrowed). Junctional complexes with the parenchyma cells are absent although there are many invaginations of the basal plasmalemma (bi); the underlying layer of interstitial material is thick (im).

Plates 177 & 178 4 days in vitro culture.

The masses of dense material (asterisks) associated with surface lamellae of the gut epithelial cells consist of stacks of long, thin, dense elements similar to those normally seen between the lamellae.

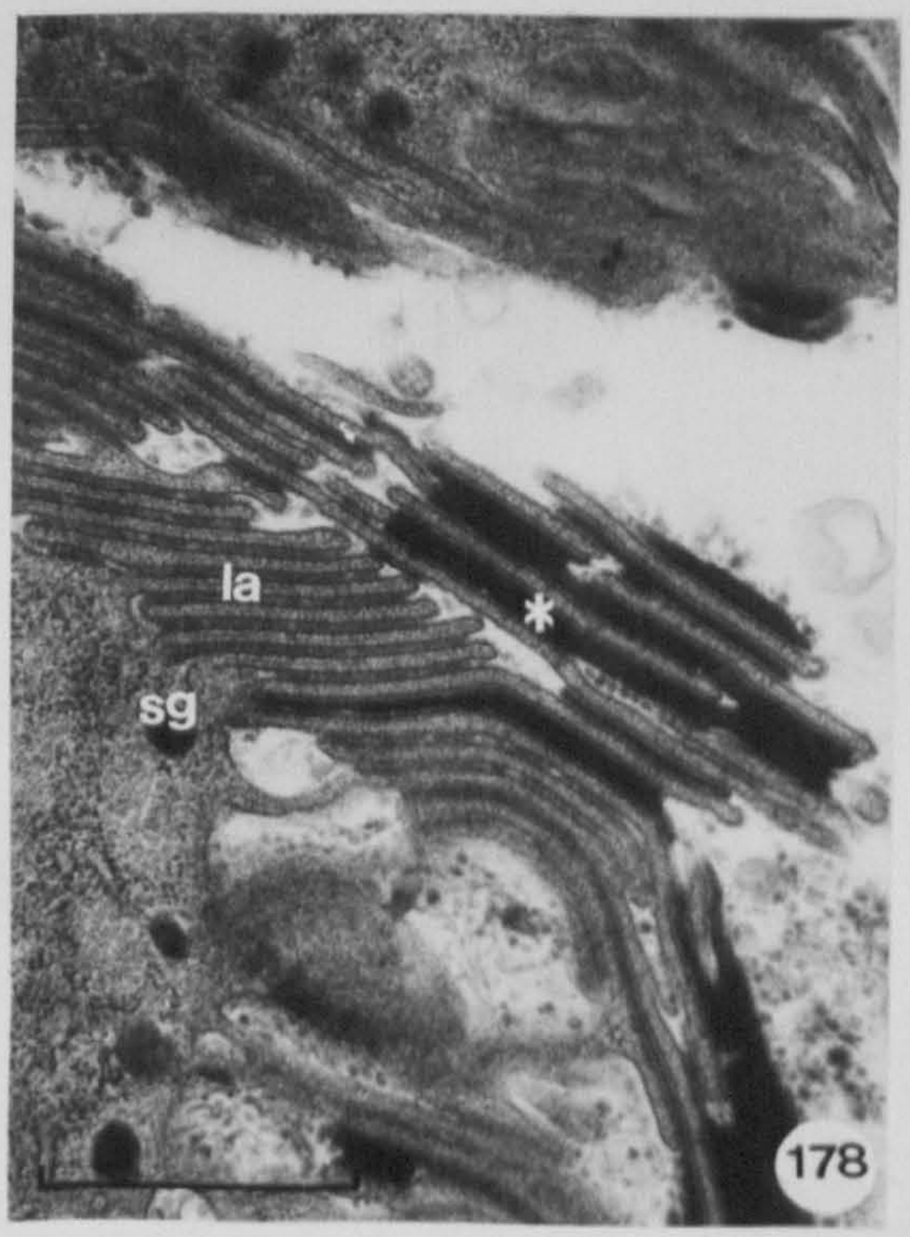
Small, dense secretory granules (sg) are present in the caecal cells especially near the luminal surface.



176



177



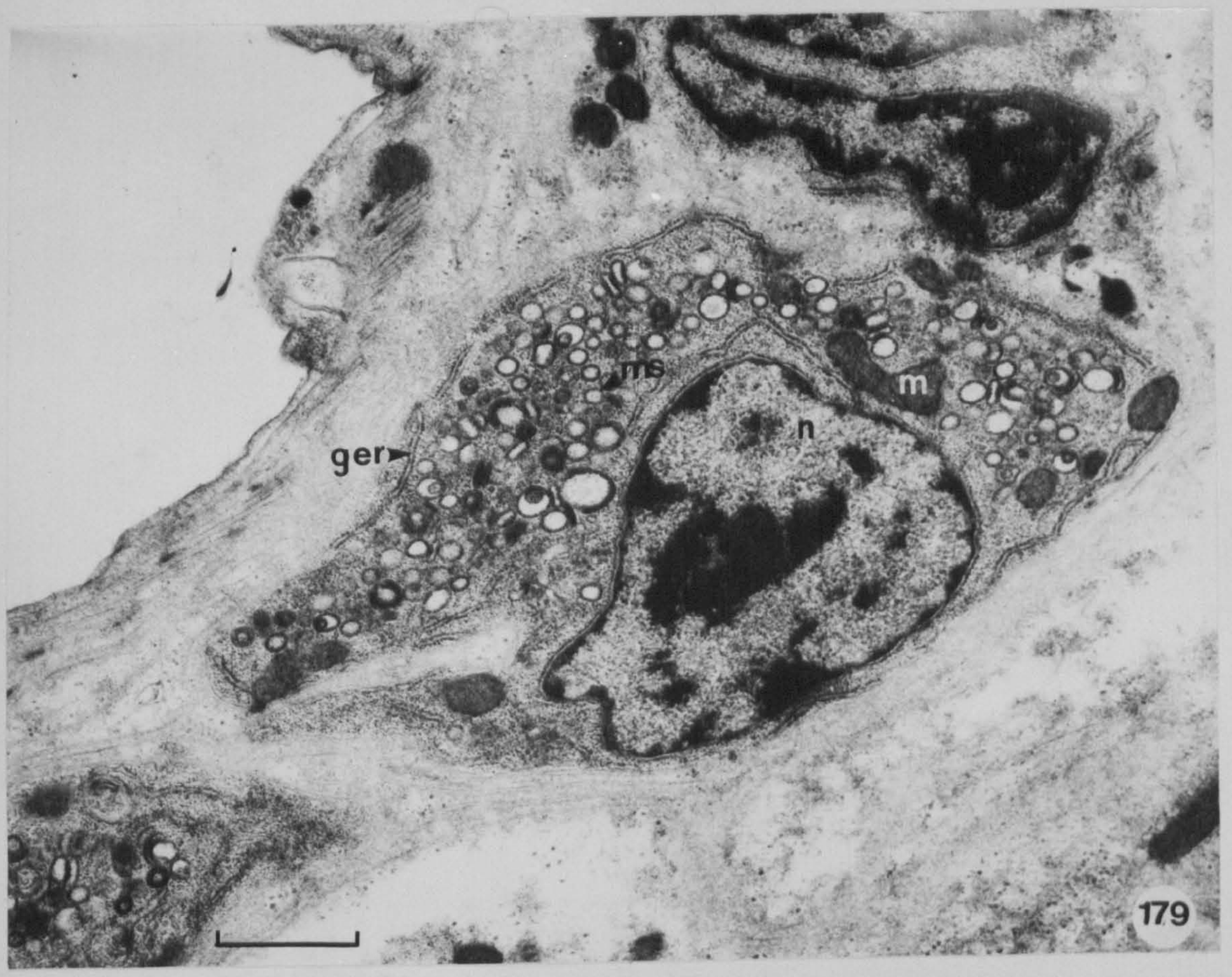
178

Plate 179 18 hours *in vitro* culture.

The cells of Mehlis' gland contain typical sac-like secretory bodies (ms), mitochondria (m), strands of GER (ger) and free ribosomes.

Plate 180 4 days *in vitro* culture.

The cells in the ovary of cultured flukes (oocytes? - oc?) contain nuclei (n) with prominent nucleoli (nu), clusters of mitochondria (m) and dense granular bodies which may correspond to the "ribosomal bodies" (rb) described for the oocytes of *F. hepatica*. No Golgi complexes were observed.


ms
m
n
ger
179

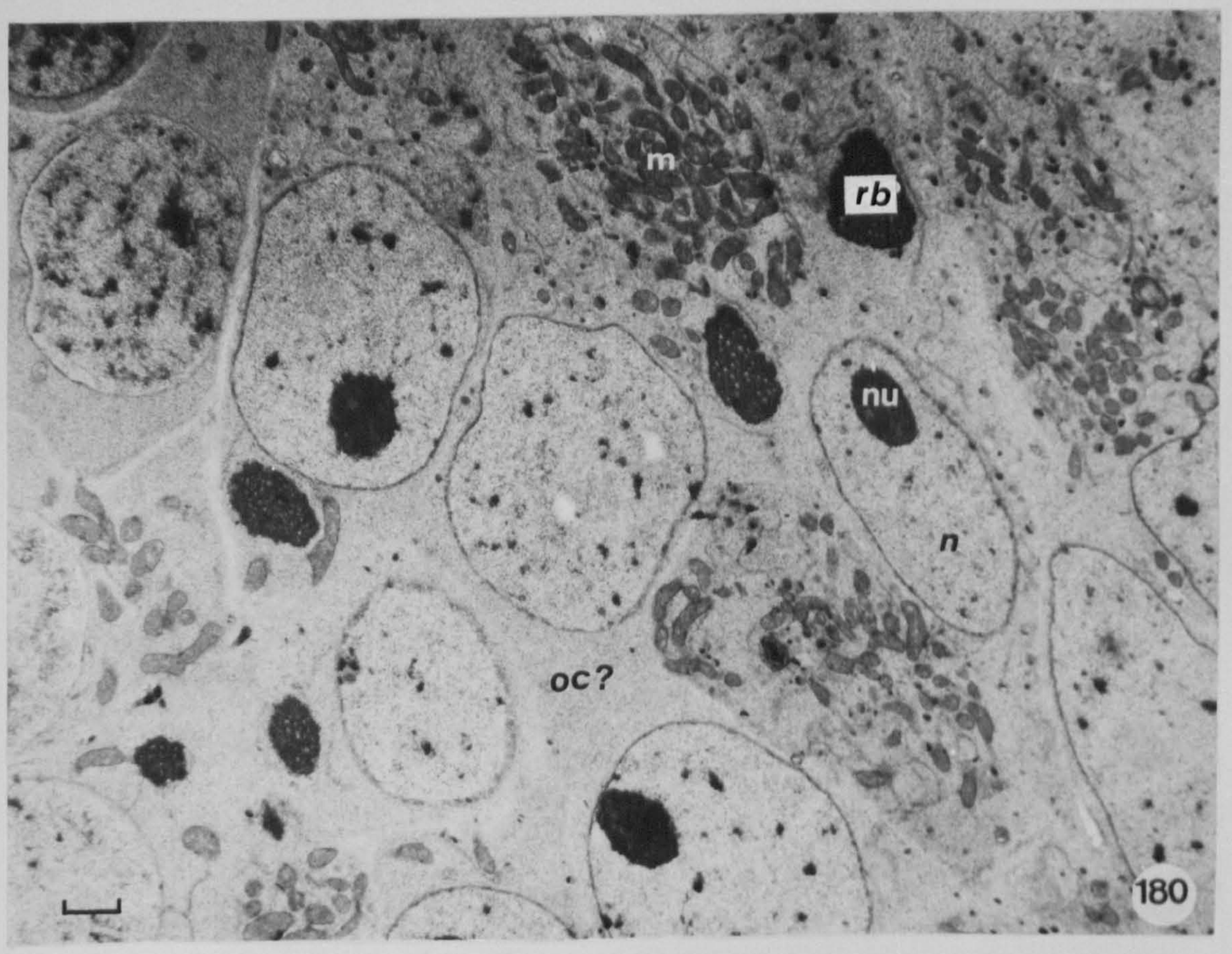
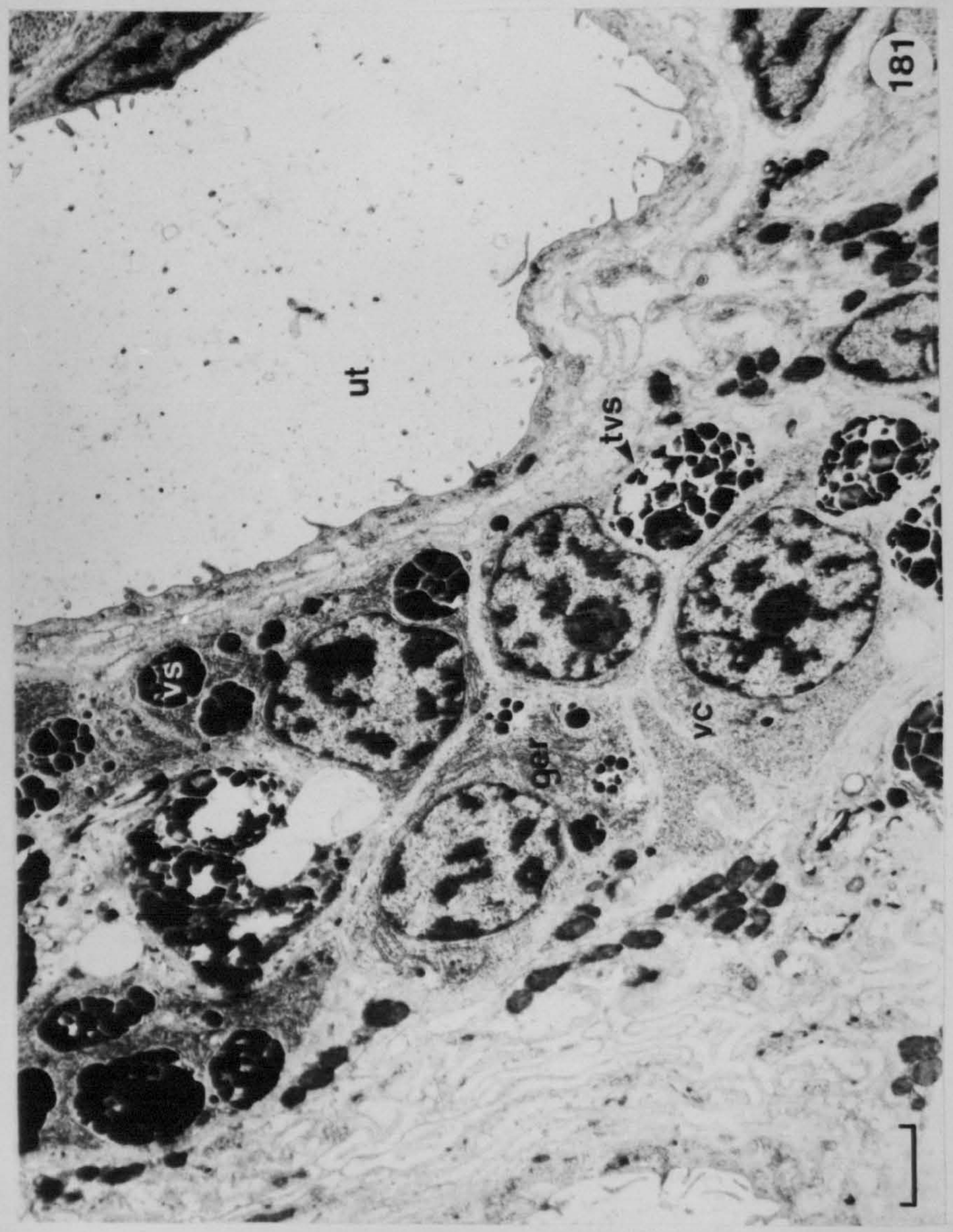

m
rb
nu
n
oc?
180

Plate 181 18 hours *in vitro* culture.

The vitelline cells contain apparently normal secretory droplets (vs) and some whose outline appears "ragged" (tvs); the latter may correspond with the prematurely tanned vitelline secretions seen in the vitellaria of cultured flukes.

Plate 182 4 days *in vitro* culture.

In this section two unshelled ova can be seen in the uterus - one of them is associated with a vitelline cell which contains a nucleus (n), some GER (ger) and numerous dense secretory bodies (tvs). The ovum and associated vitelline cell may correspond to the abnormal eggs seen *in utero* in cultured flukes.


181
ut
tvs
vs
ger
vc


182
ut
ut
ov
ov
tvs
ger
n

Plates 183 - 191 Stages of spermatogenesis within the testes.

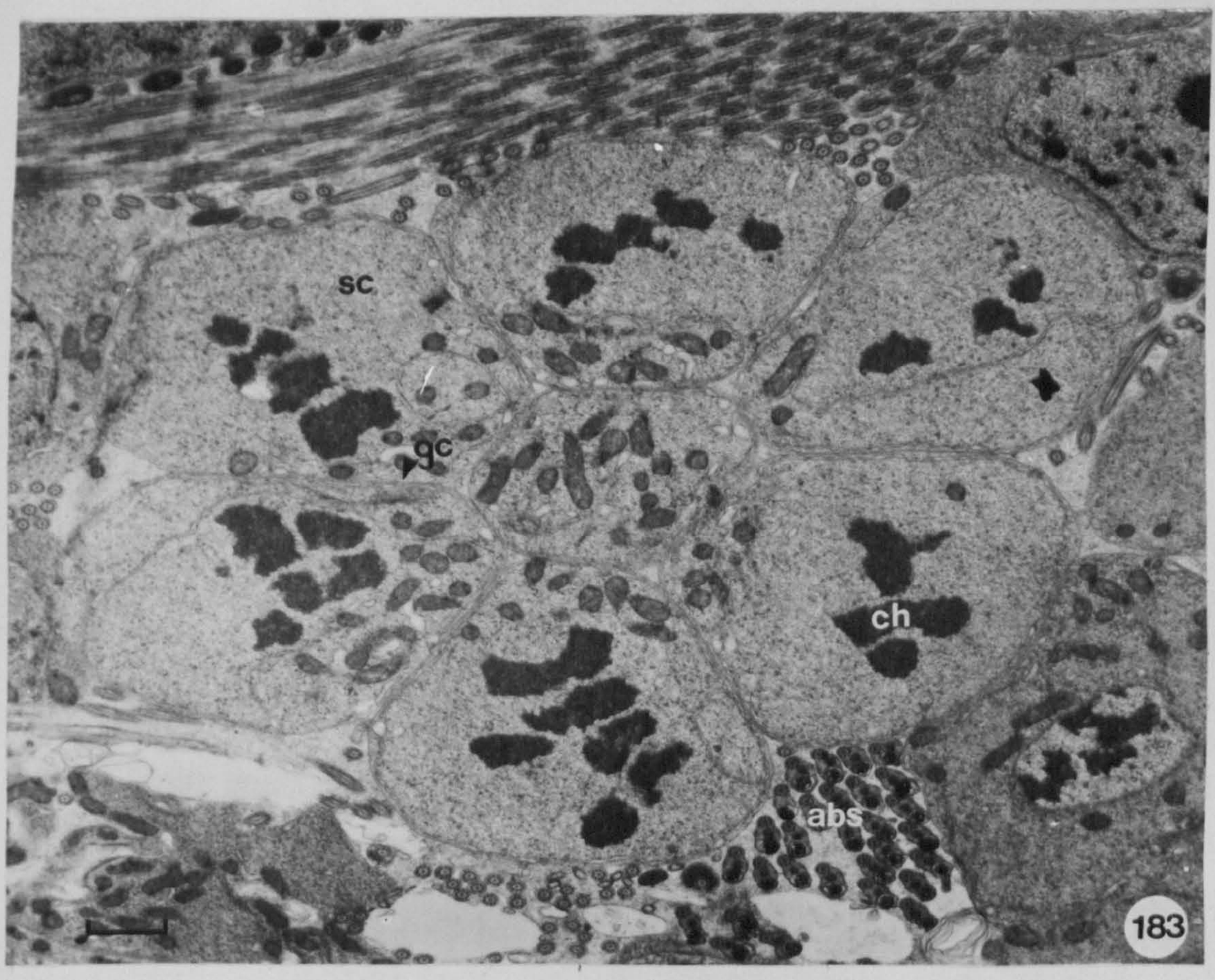
Plate 183 4 days *in vitro* culture.

This section shows a cluster of spermatocytes (sc), associated together in a cytophore, which are in the process of division. The nuclear membrane is not present and dense chromosomes (ch) are clearly visible. The spermatocytes contain ribosomes, Golgi complexes (gc) and dense mitochondria.

Plate 184 4 days *in vitro* culture.

This section shows a group of spermatids (st) associated together in a cytophore. The individual cells are connected to the central mass of residual cytoplasm (rc) by a slender stalk. The nuclear material (n) of the spermatids has begun to condense into dense strands and the "centriole-like bodies" (cb), striated rootlets (rt) and basal bodies (bb) associated with the product ion of the axial filaments are present.

A synaptonemal complex (arrowed) can be seen in the nucleus of an adjacent spermatocyte.


sc
gc
ch
abs
183

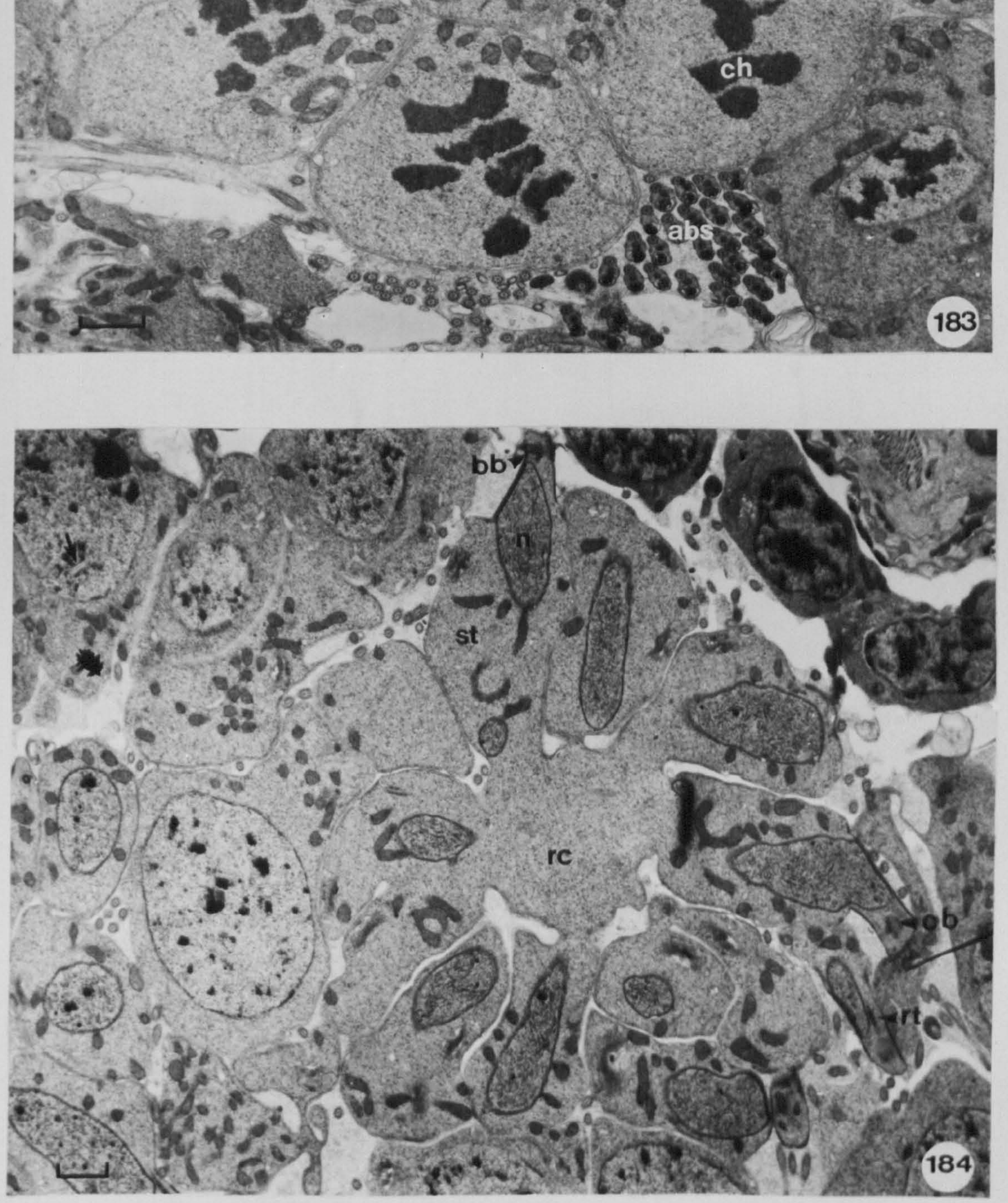

bb
n
st
rc
ob
rt
184

Plate 185 4 days *in vitro* culture.

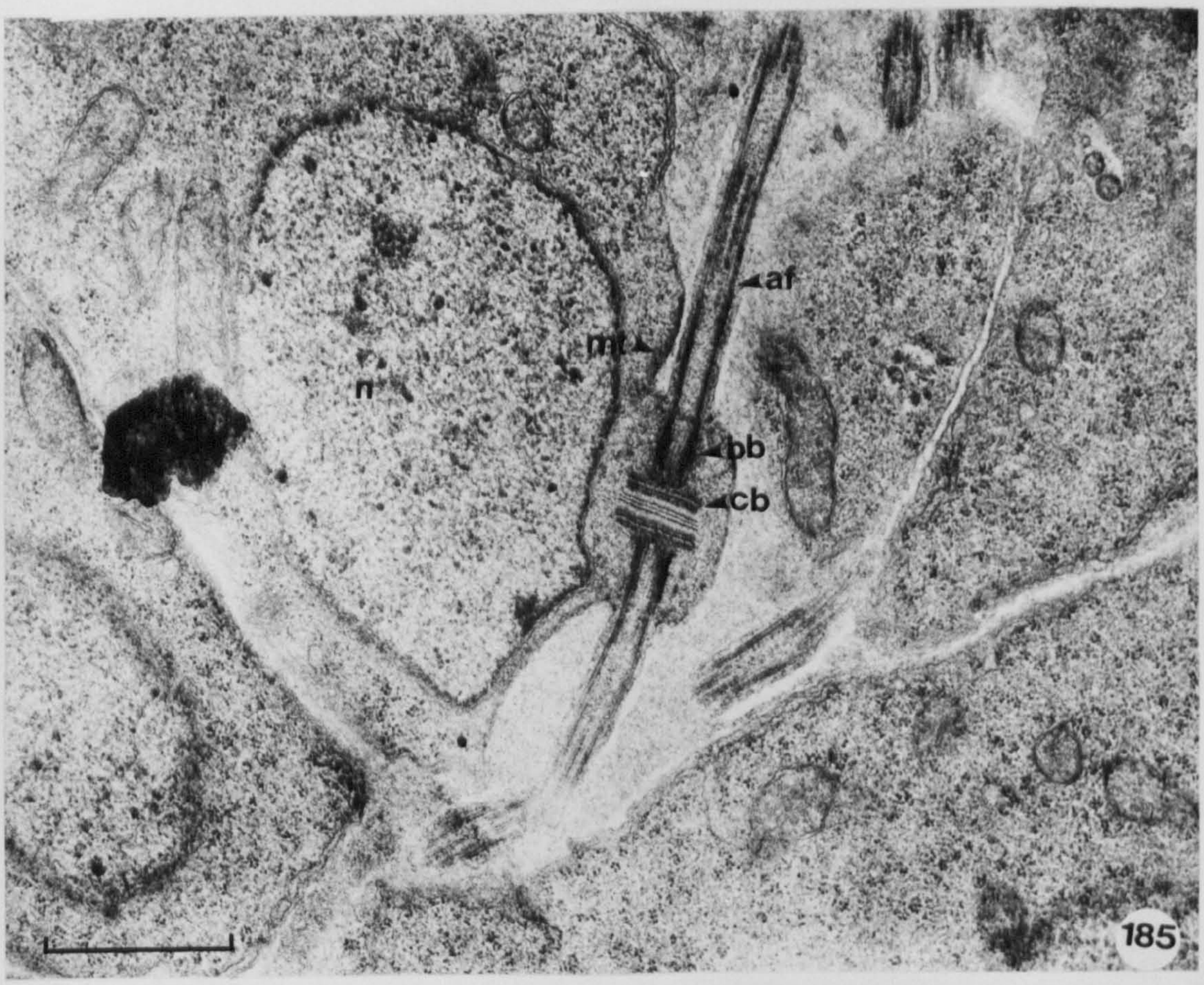
This section shows an early stage of spermateleosis. The apparatus connected with the production of the two axial filaments (af) is clearly visible; it consists of a lamellate "centriole-like body" (cb) and two basal bodies which lie at right angles to it (bb). This apparatus lies in a bulge of cytoplasm, which is lined with microtubules (mt), in the vicinity of the spermatid nucleus (n).

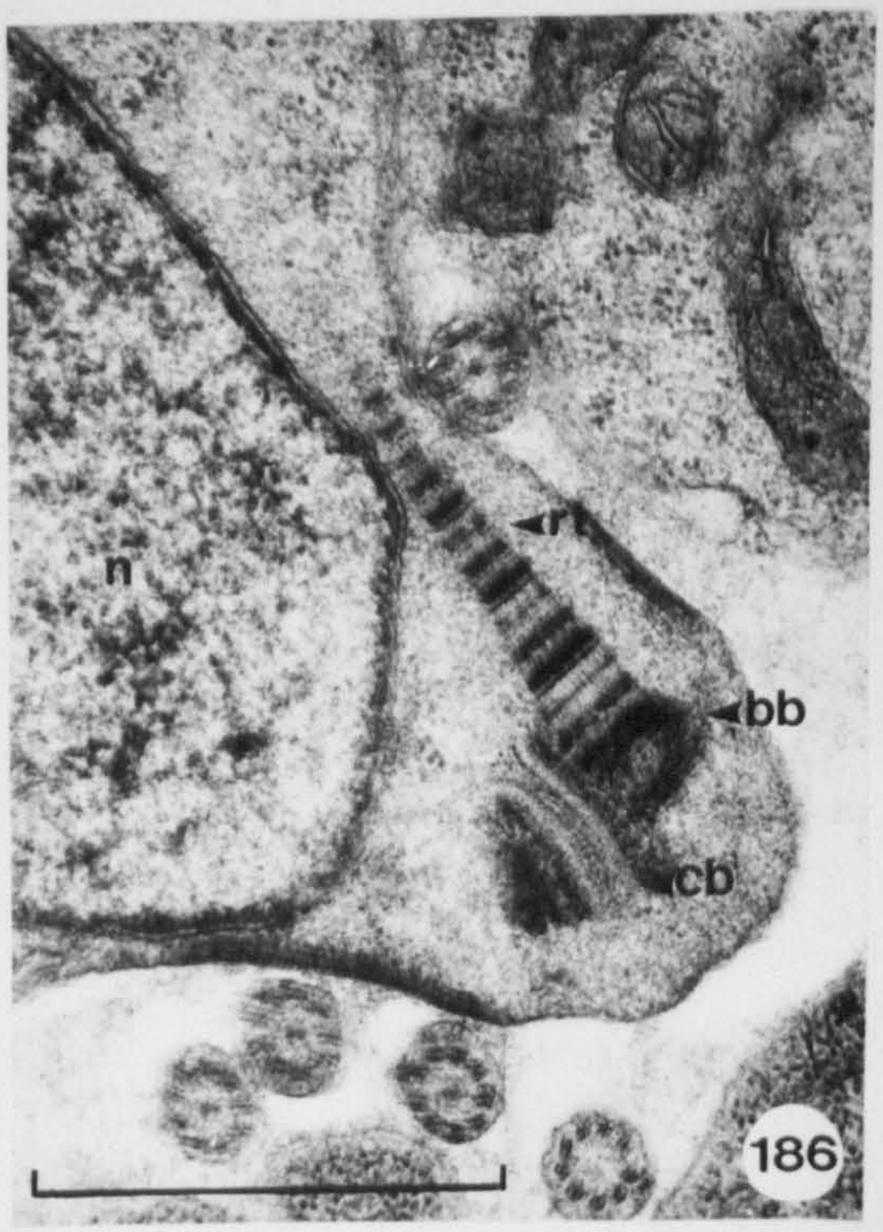
Plate 186 4 days *in vitro* culture.

Attached to the basal body (bb), a tapering striated rootlet (rt) passes backwards into the cytoplasm of the spermatid.

Plate 187 4 days *in vitro* culture.

In cross section, the basal body (bb) has a typical substructure of nine sets of triplet tubules tilted slightly with respect to one another with no central tubules or core.


af
mt
n
bb
cb
185


n
bb
cb
186

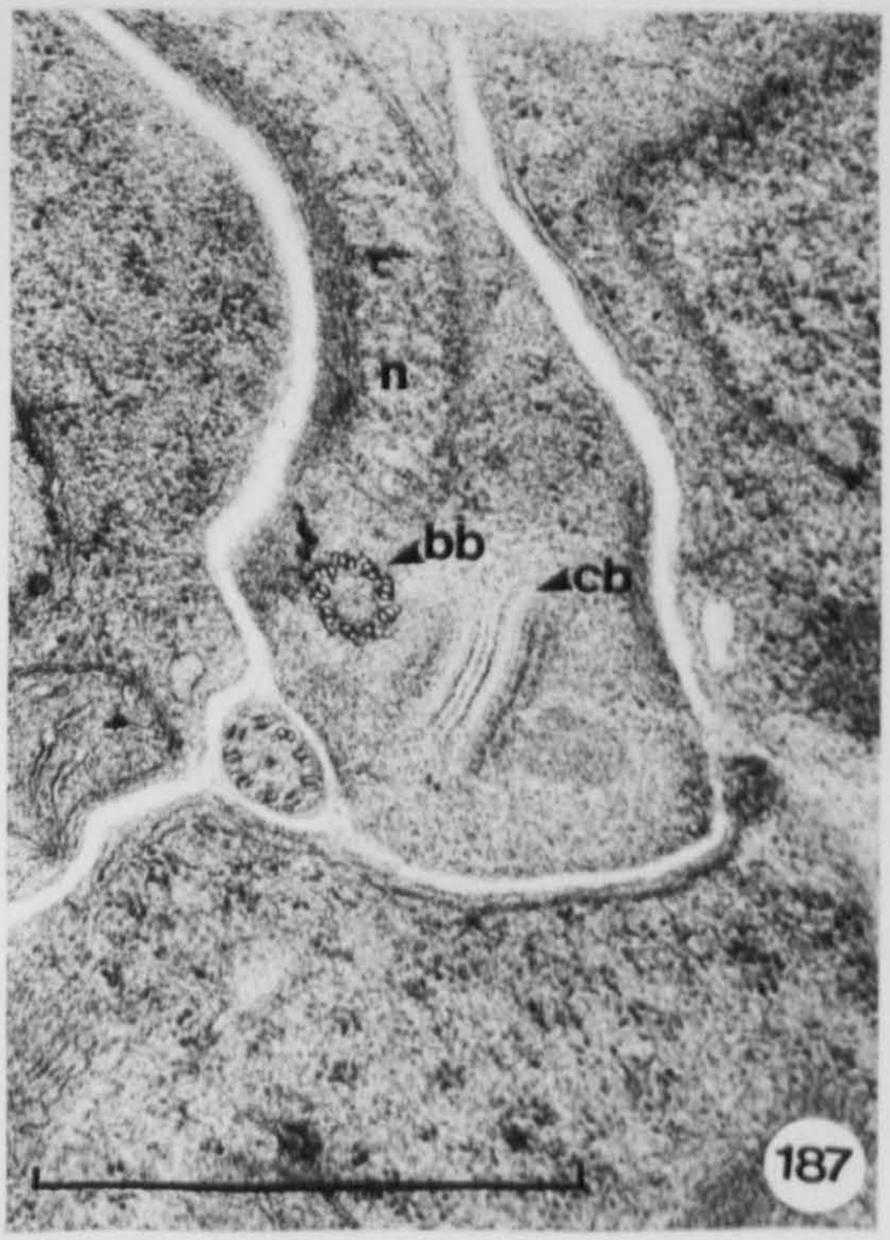

n
bb
cb
187

Plate 188 48 hours in vitro culture.

The elongated nucleus of the spermatid contains strands of condensed chromatin (cn) and is cut many times in cross section.

Spermatids in various stages of development are present.

Plate 189 4 days in vitro culture.

This section through the periphery of the testes shows mature sperm (s). Four small dense areas can be seen on either side of the two groups of peripheral microtubules (arrowed).

Inset to Plate 189

An axial filament in cross section, showing the nine pairs of doublet tubules (pd) connected to the central core (co) by radial "spokes" (rs) to give the typical "9+1" configuration.



188



189

Plate 190 4 days in vitro culture.

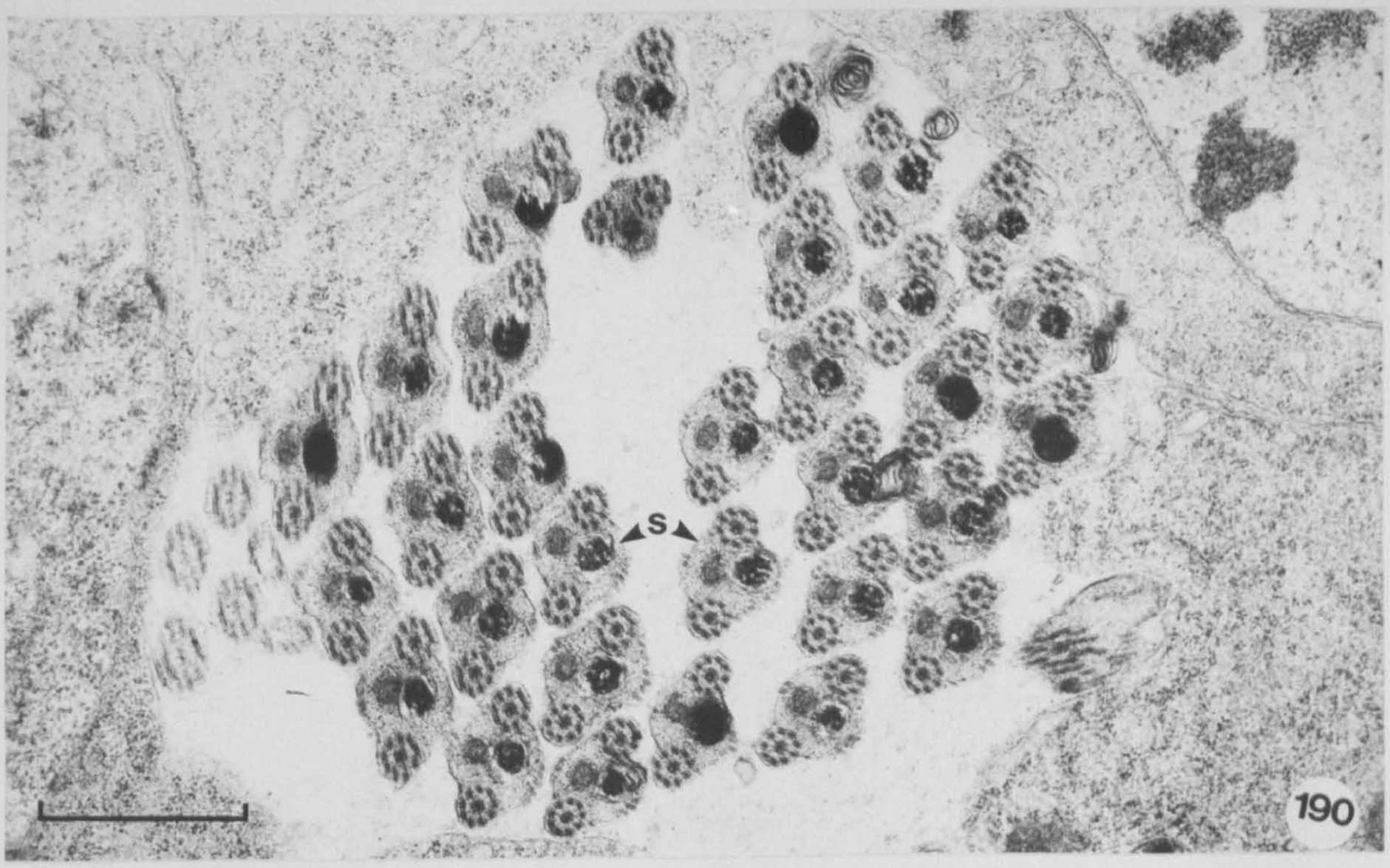
Cross sections of mature sperm (s) showing the "typical" Digenean pattern of two "9+1" axial filaments, a nucleus with condensed chromatin, a mitochondrion and two sets of peripheral microtubules.

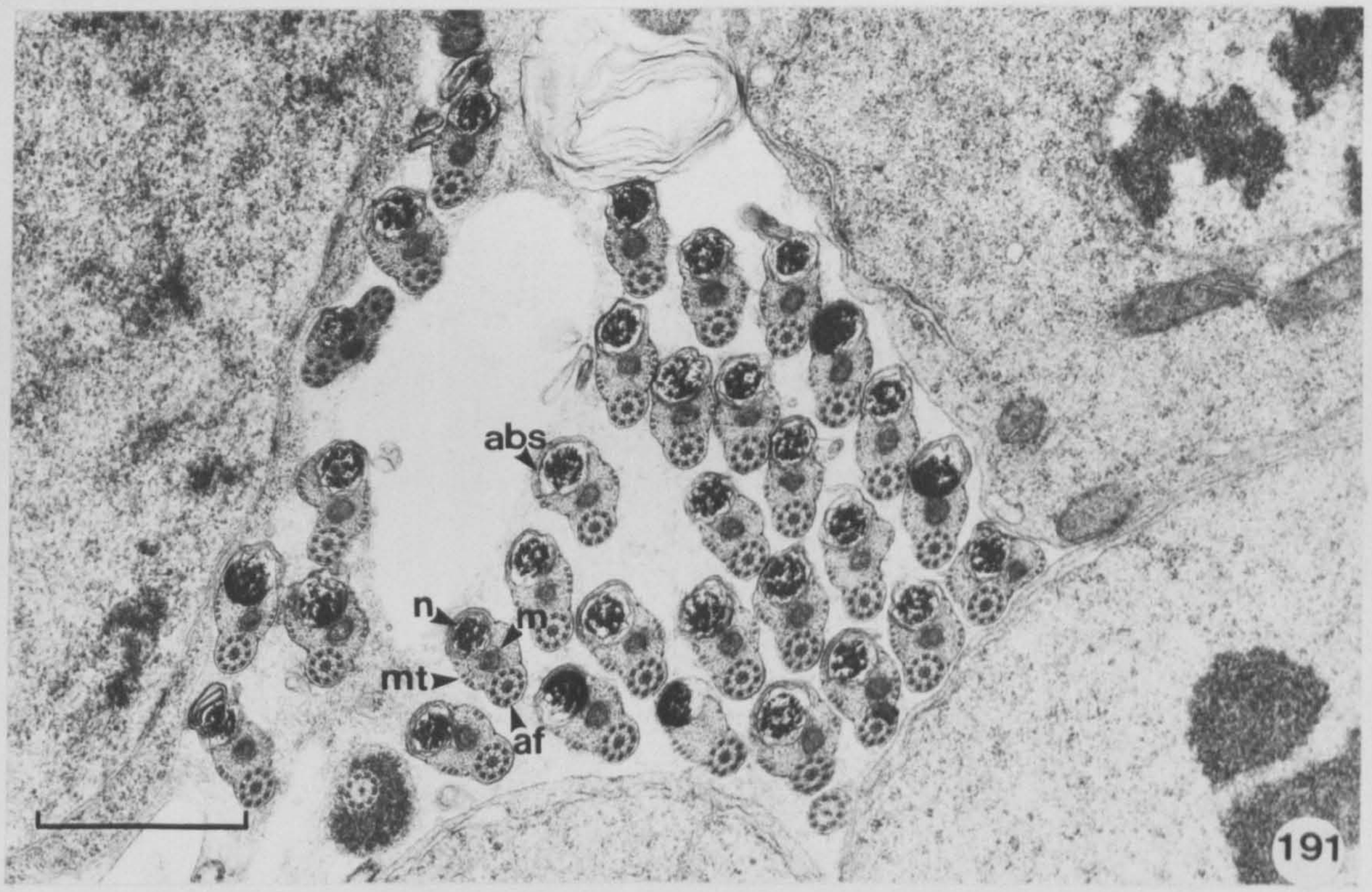
Plate 191 4 days in vitro culture.

Cross sections of "abnormal" or atypical sperm (abs) where there is only one axial filament and the nucleus occupies the position where the second filament is in the"typical" sperm.

Plate 192 4 days in vitro culture.

This section shows some sperm in the vesicula seminalis. One of the sperm (1) shows the "typical" Digenean structure, another (3) is similar to those seen in Plate 191 whilst another (2) contains four axial filaments instead of the "typical" two.


S
190


abs
n
m
mt
af
191

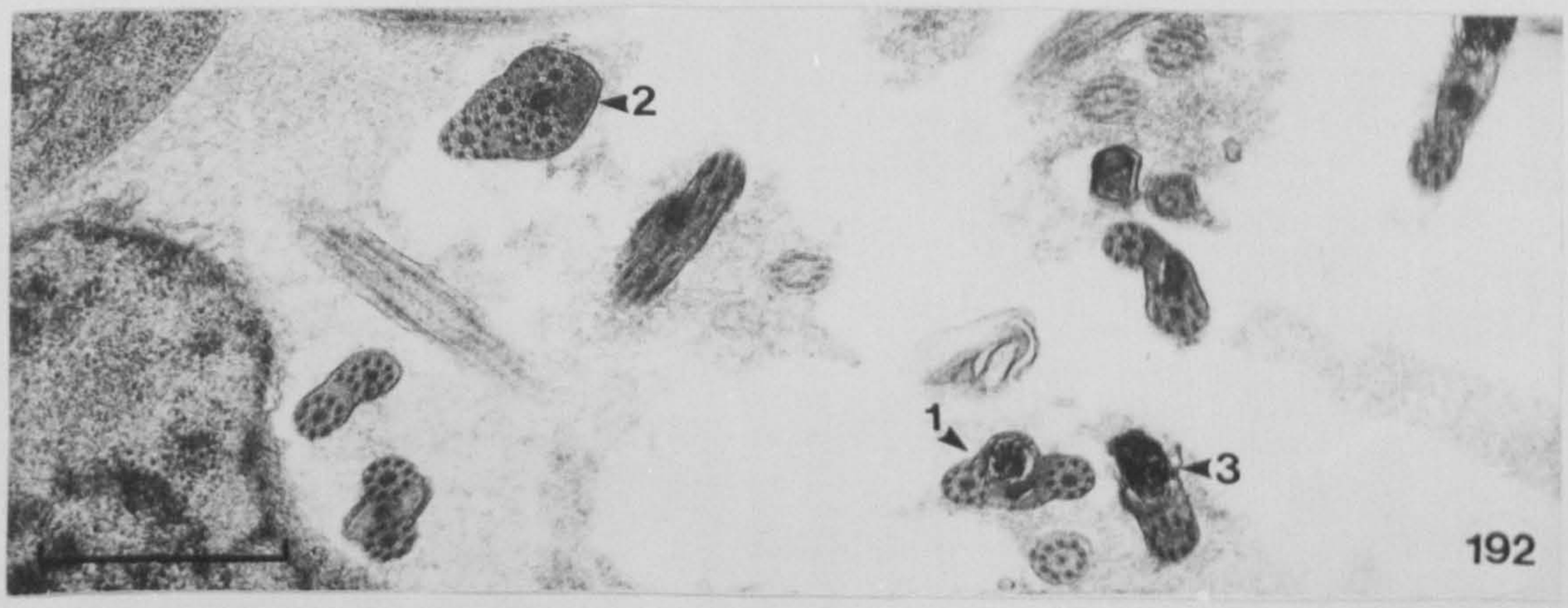

2
1
3
192

Plate 193 48 hours in vitro culture.

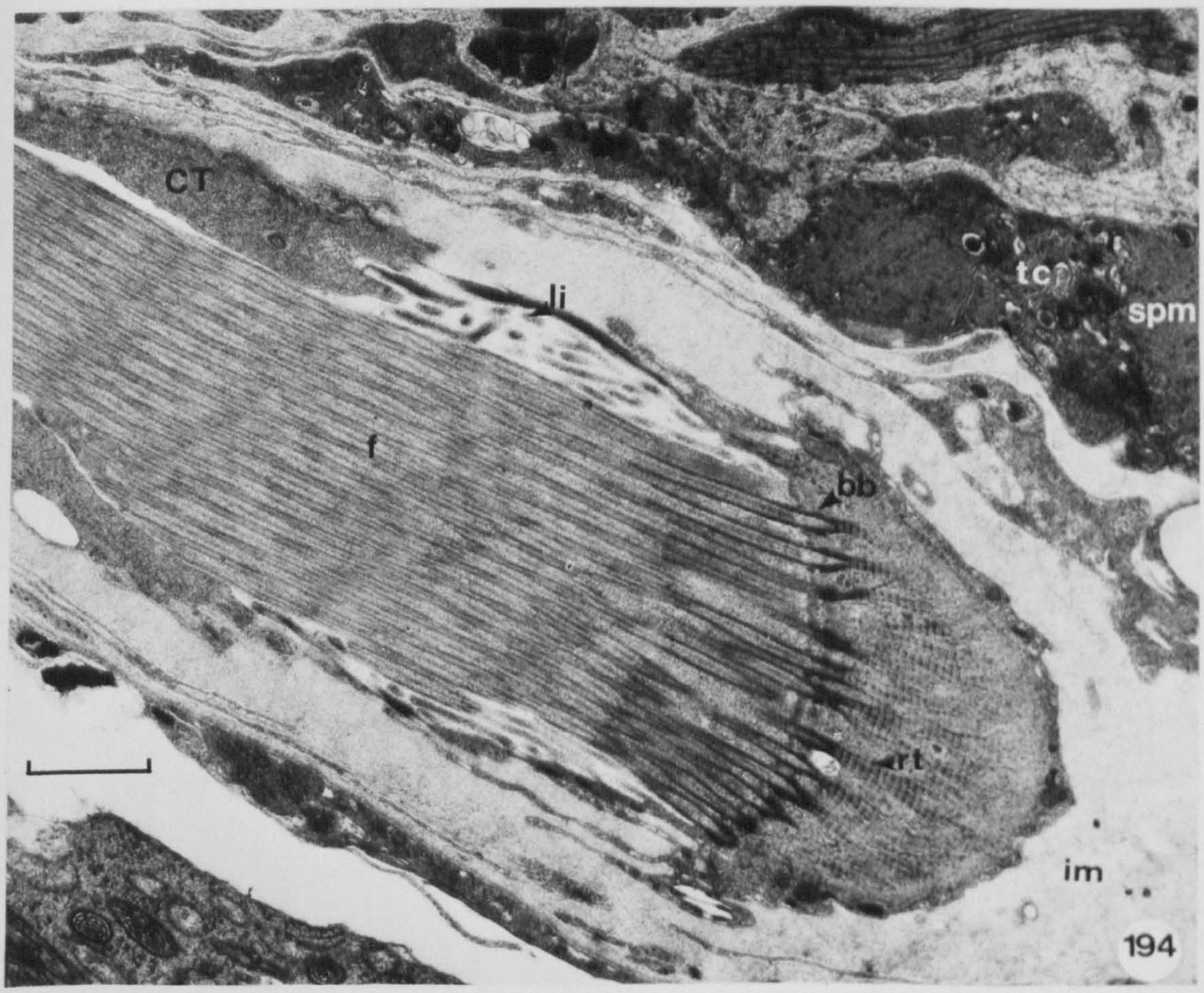
The ductus ejaculatoris (de), which passes through the musculature of the male papilla (mp), is lined by a syncytium (syn). In the lumen of the duct are several groups of dense granules (pgs) which may be secretions from the prostate gland.

Plate 194 48 hours in vitro culture.

This section shows a longitudinal section through a flame cell. The cup-shaped portion of the flame cell body containing the ciliary basal bodies (bb) and branched striated rootlets (rt),and the cell of the conjoined tubule (CT) can be seen. In the lumen of the flame cell are the cilia of the "flame" (f) and some internal leptotriches (li). The flame cell is embedded in a thick layer of interstitial material (im).



193



194

Plate 195 4 days in vitro culture.

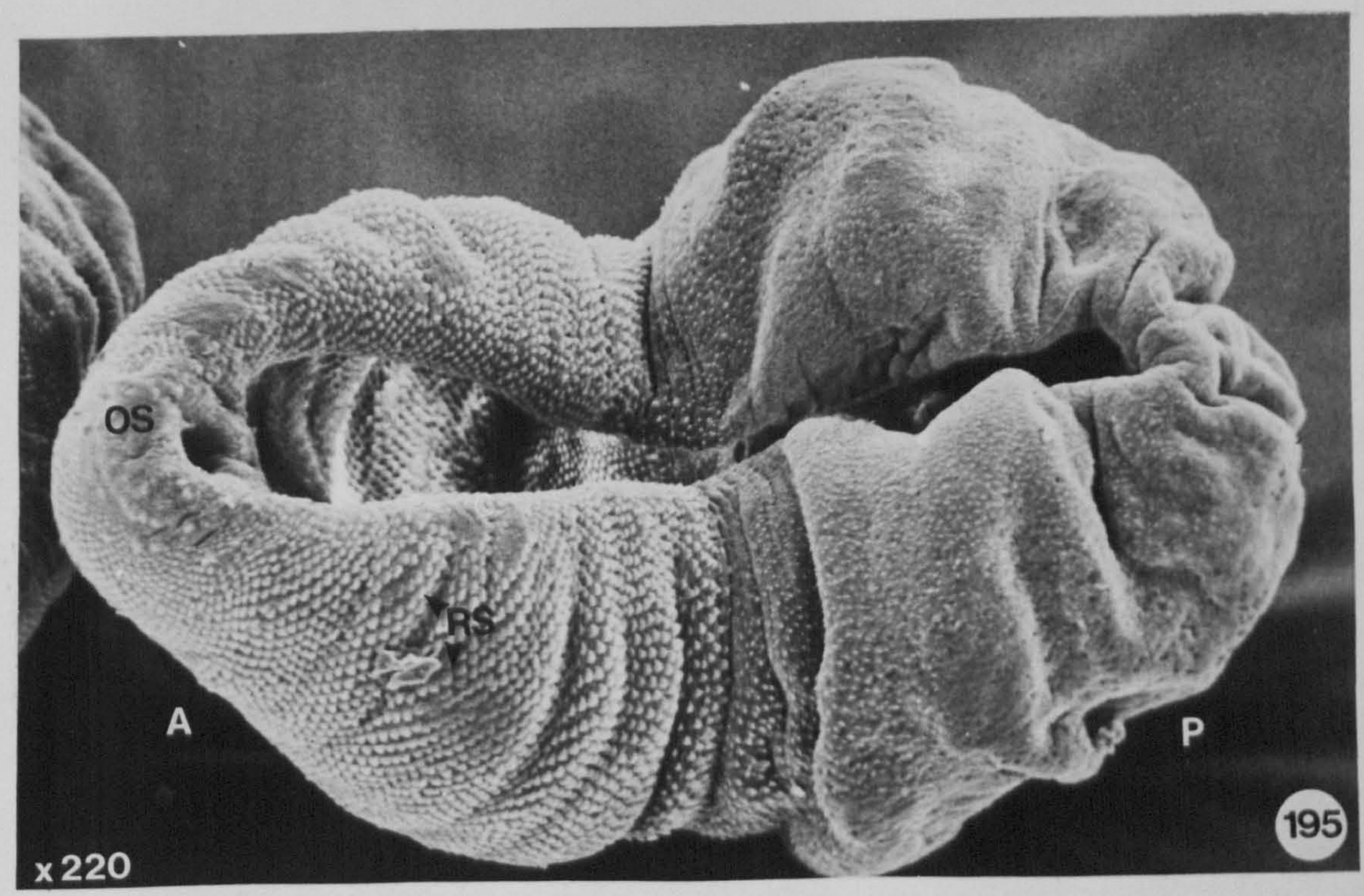
This view shows the typical shape of cultured flukes with the lateral surfaces curled ventrally. The different types of surface on the anterior (A) and posterior (P) portions of the body can be clearly seen.

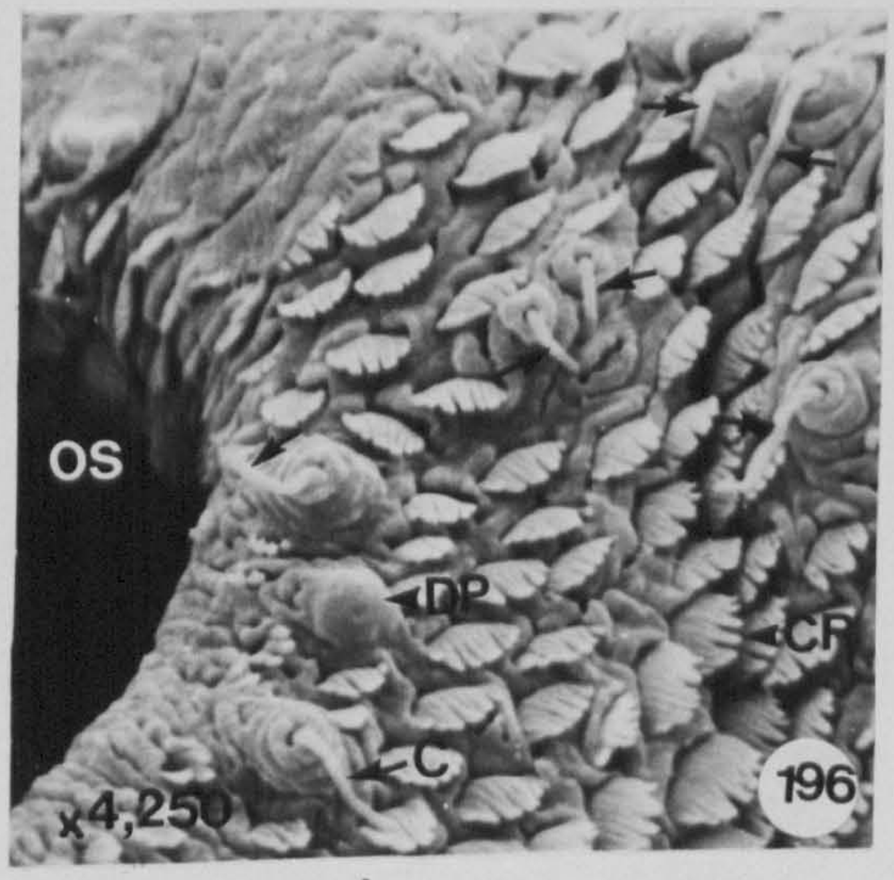
Plate 196 4 days in vitro culture.

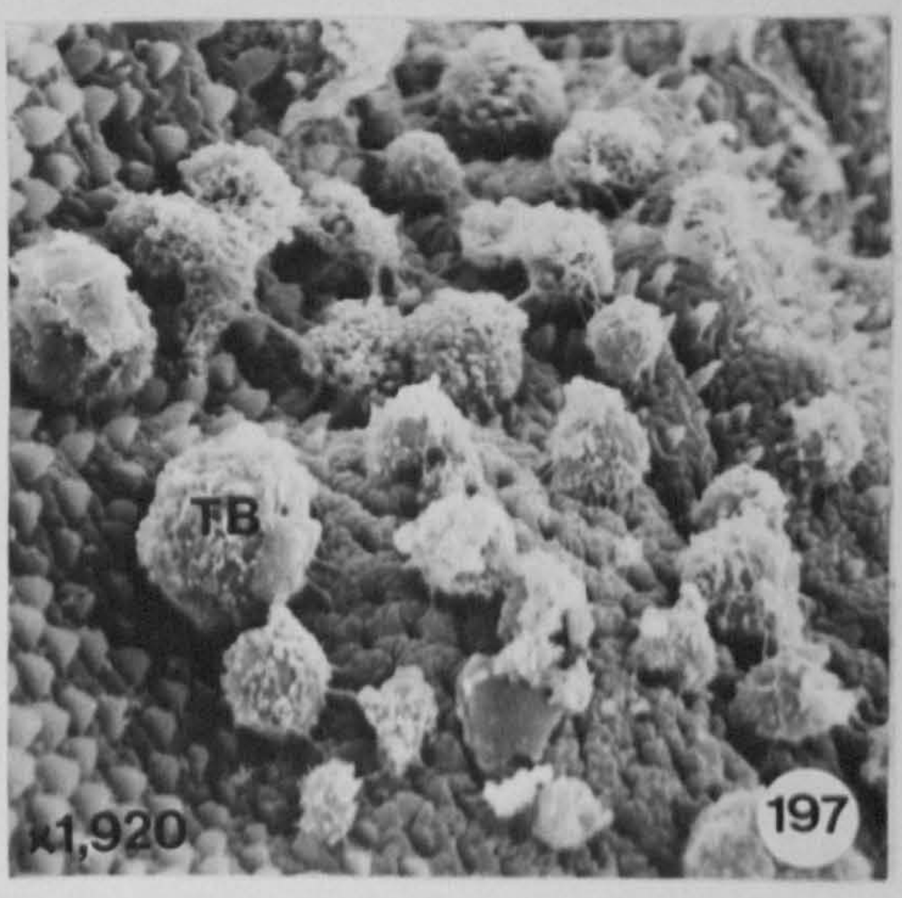
In the region of the oral sucker (OS) two types of sensory papilla can be seen. The most frequent type is the ciliated papilla and the cilia (C) often protrude from the bulb of the papilla for a considerable distance (arrowed). The other type of papilla is the "domed" type (DP) which bears no visible cilium.

Plate 197 4 days in vitro culture.

"Tegumental bubbles" (TB) are frequently seen on the surface of cultured flukes.


OS
RS
A
P
x 220
195


OS
DP
CP
C
x4,250
196


TB
x1,920
197