RESULTS

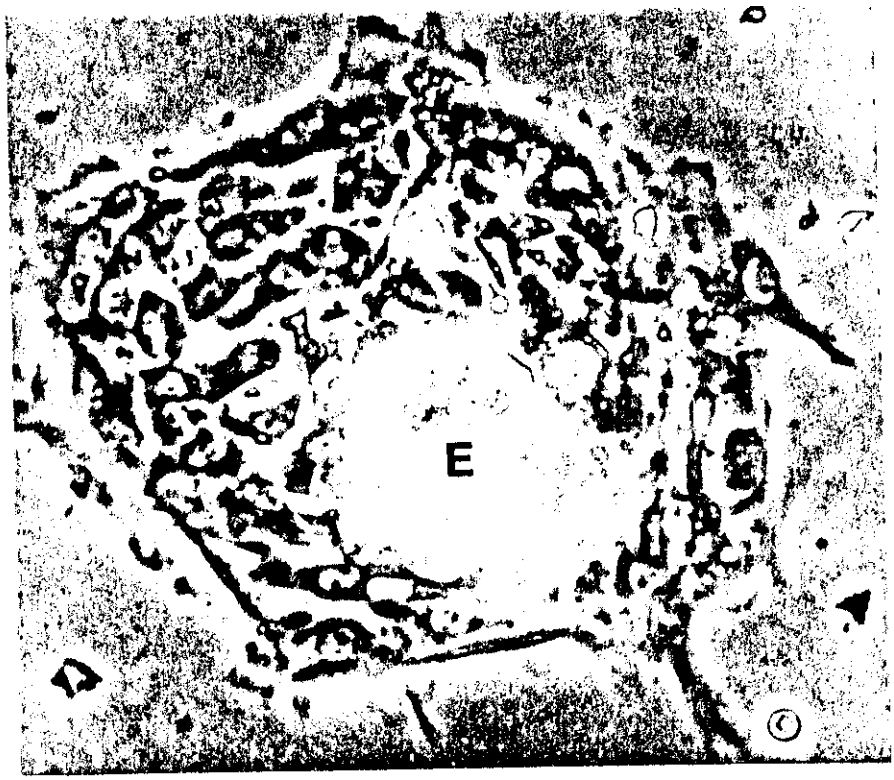
RESULTS

1. Effect of different culture media

a) Light microscopy and histochemistry

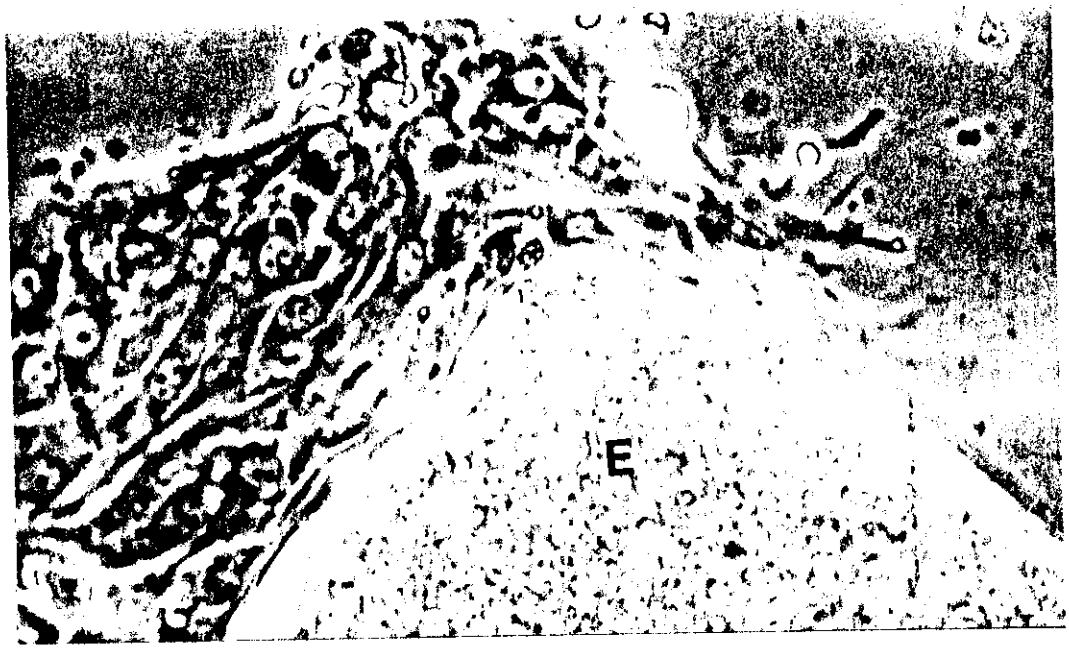
Comparative experiments were performed to study gallbladder growth in eight different culture media. Guinea pig gallbladders were cultured in these media for one week before termination for study by light microscopy. In all media, there was morphological evidence of satisfactory survival of gallbladder epithelial cells for seven days and there was no marked morphological difference between the growth in these different media (Fig. 1 - 6, 12 - 27).

Gallbladder explants attached readily to either the coverslips or the bottom of petri dishes and cellular outgrowth was observed after 1-2 days (Fig. 1). This cellular outgrowth appeared either surrounding the whole explant or coming from one or more sides (Fig. 2). The outgrowth consisted primarily of epithelial-like cells growing as tight colonies of polygonal, closely apposed cells (Fig. 3,4). At that early stage of culture, small isolated epithelial cell colonies were also seen in some petri dishes (Fig. 3). Mitoses were observed (Fig. 5, 6). Fibroblast-like cells, identifiable by their spindle shape and their disorganised growth pattern, were occasionally seen in small numbers.



Figures 1 - 6 are phase contrast photographs of explants of guinea pig gallbladder maintained in tissue culture for varying times in media I or V.

Fig. 1 (above): Outgrowth surrounding the explant (E) after one day of culture in medium I. X 320.



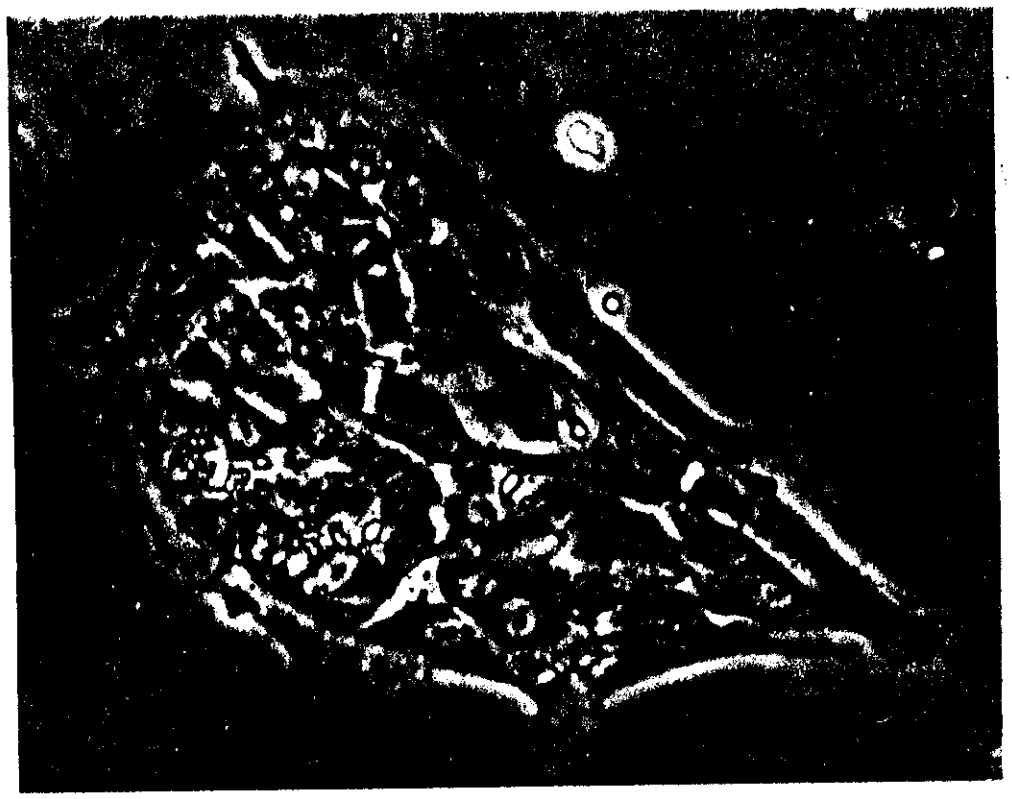


Fig. 2 (above): Epithelial cell outgrowth migrating from one side of the explant (E) after one day of culture in medium I. X320.

Fig. 3 (below): An epithelial cell island after one day of culture in medium I. X320.

Fig. 4 : Epithelial cell colony after two days of culture in medium V. X600.

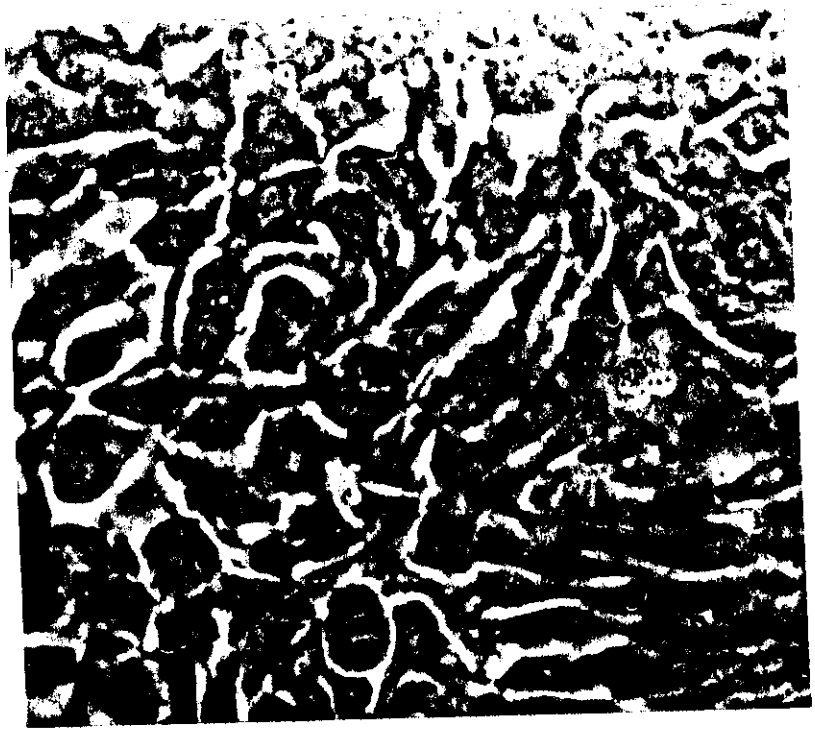
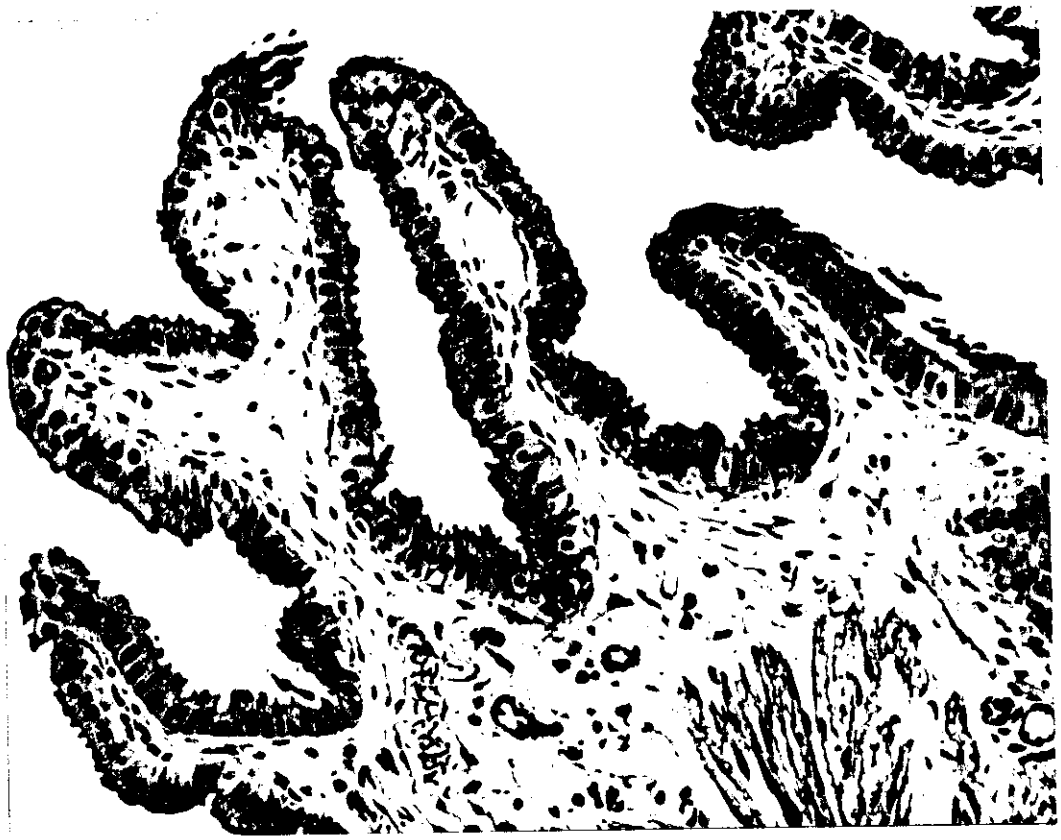


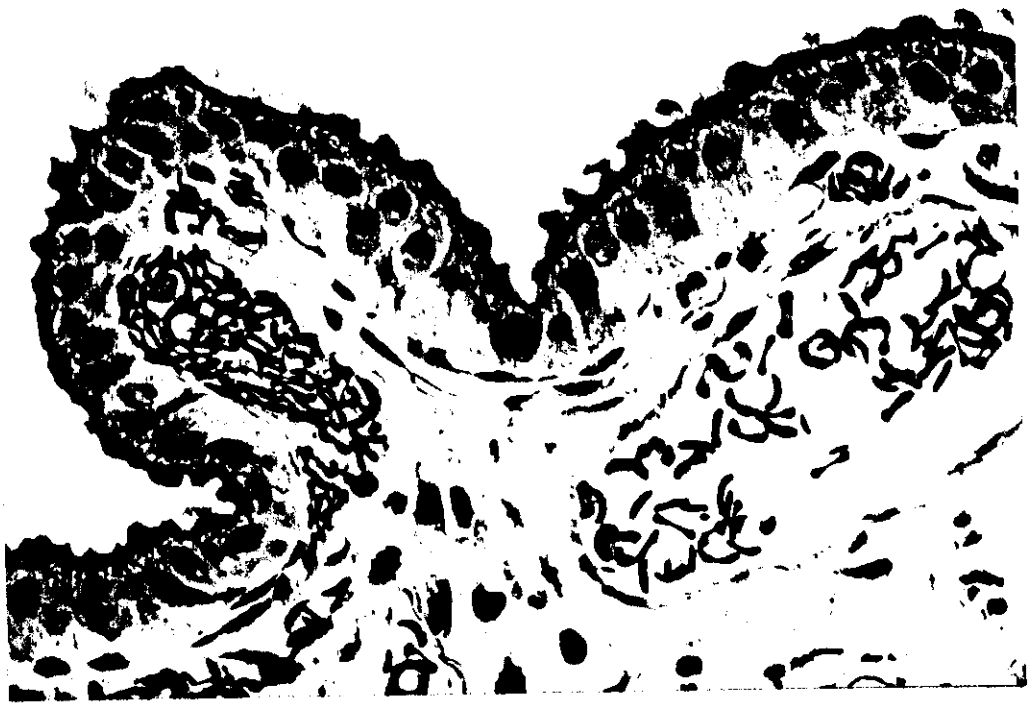
Fig. 5 (above): Epithelial cell colony after two days of culture in medium I showing mitosis (arrowhead). X600.

Fig. 6 (below): Epithelial cell outgrowth showing mitosis (arrowhead) after 7 days of culture in medium I. X320.

The mucosa of uncultured gallbladder fragments (control) consists of a single layer of columnar epithelial cells resting on a loose connective tissue base forming folds (Fig. 7, 8). Only the surface epithelium survived as tall columnar cells and cells at the bottom of some infoldings of the mucosa died (Fig. 21). Sections of explants examined after one day of culture showed some mechanically damaged cells and cell necrosis near the edge of the explant. Even at this early phase of culture some thinner epithelium was seen apparently migrating over the surface of the underlying connective tissue. The cells had a clear cytoplasm and in some cells there were perinuclear eosinophilic granules. After two days of culture, Karyorrhectic bodies were seen in the underlying connective tissue.

Diastase sensitive PAS positive glycogen was limited to the apical cytoplasm of the uncultured control cells (Fig. 9). Also in control cells, granules of acid mucosubstances were limited mainly to the apical cell membranes and to a lesser extent to the apical cytoplasm (Fig. 10, 11). After one day of culture, diastase sensitive PAS positive glycogen was seen throughout the cytoplasm and this remained until the end of the culture period (Fig. 21 - 27). At this early phase of culture, acid mucosubstances were limited only to the apical cell membrane.

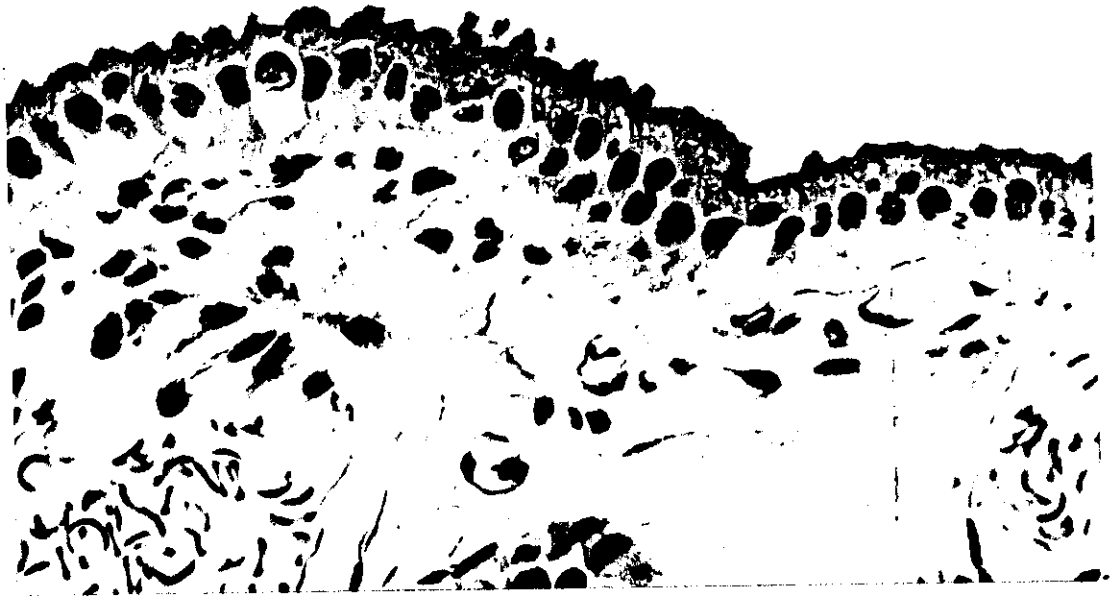




Figures 7 - 11 are plastic sections of guinea pig gallbladder explants before culture (control) stained by the following techniques : haematoxlin and eosin, PAS, Alcian blue (pH 2.5) and high iron diamine.

Fig. 7 (above) and Fig. 8 (below) showing the single layer of columnar epithelium resting on a connective tissue base. Haematoxlin and eosin. X245 (above) X600 (below).

Fig. 9 : Diastase sensitive PAS positive glycogen is limited to the apical cytoplasm (arrowheads). PAS. X600.



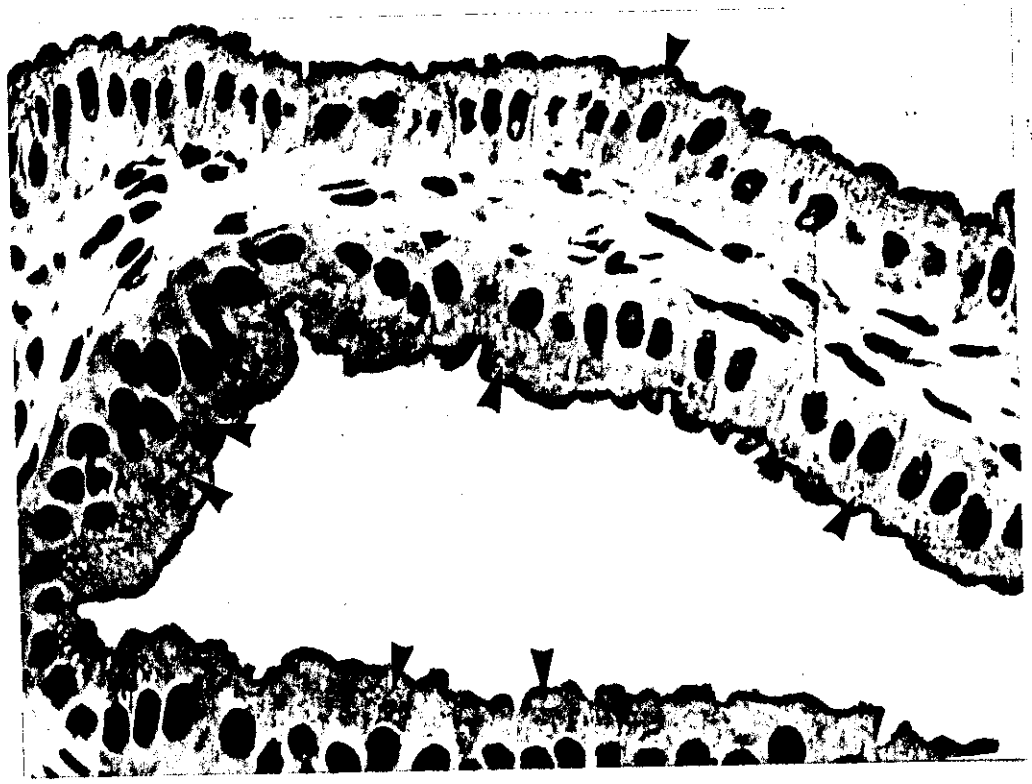
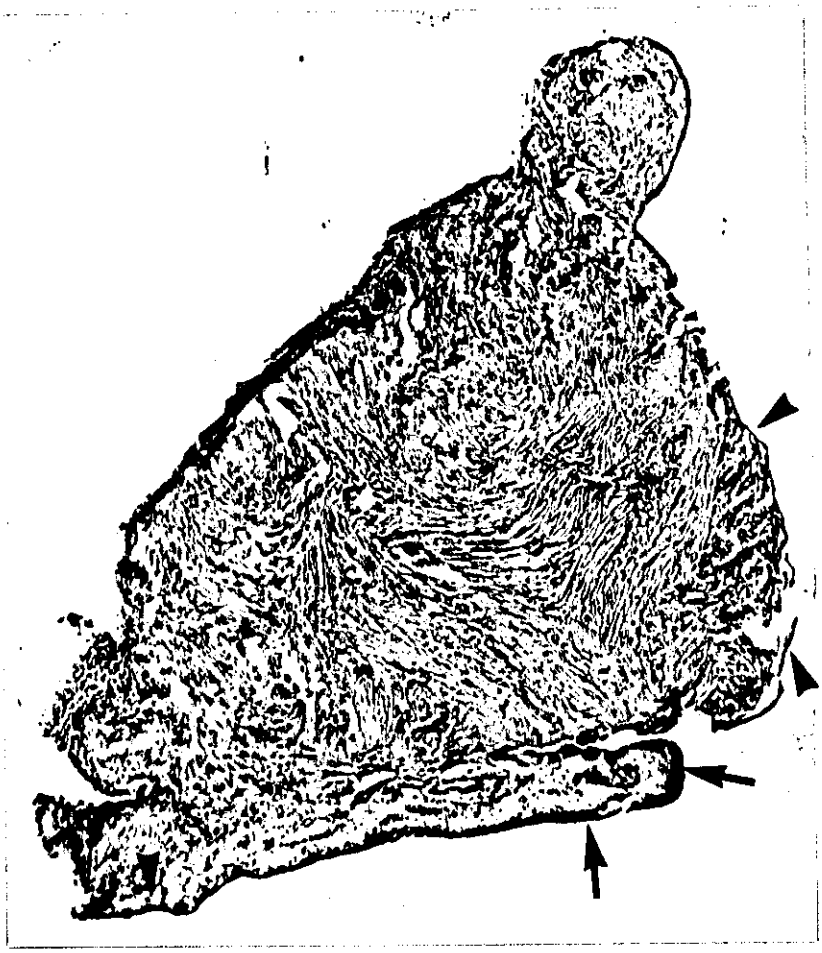


Fig. 10 (above) and Fig. 11 (below): Acid mucosubstances are limited to the apical membrane and to a smaller extent in the apical cytoplasm (arrowheads). High iron diamine (above) and Alcian blue (below). X600.



Figures 12 - 20 are sections of explants of guinea pig gallbladder grown for varying periods in different culture media stained by haematoxlin and eosin.

Fig. 12 (above): Thin cells have migrated over the explant (arrowheads). Note the tall epithelial cells (arrows). Six days of culture in medium VI. X150.

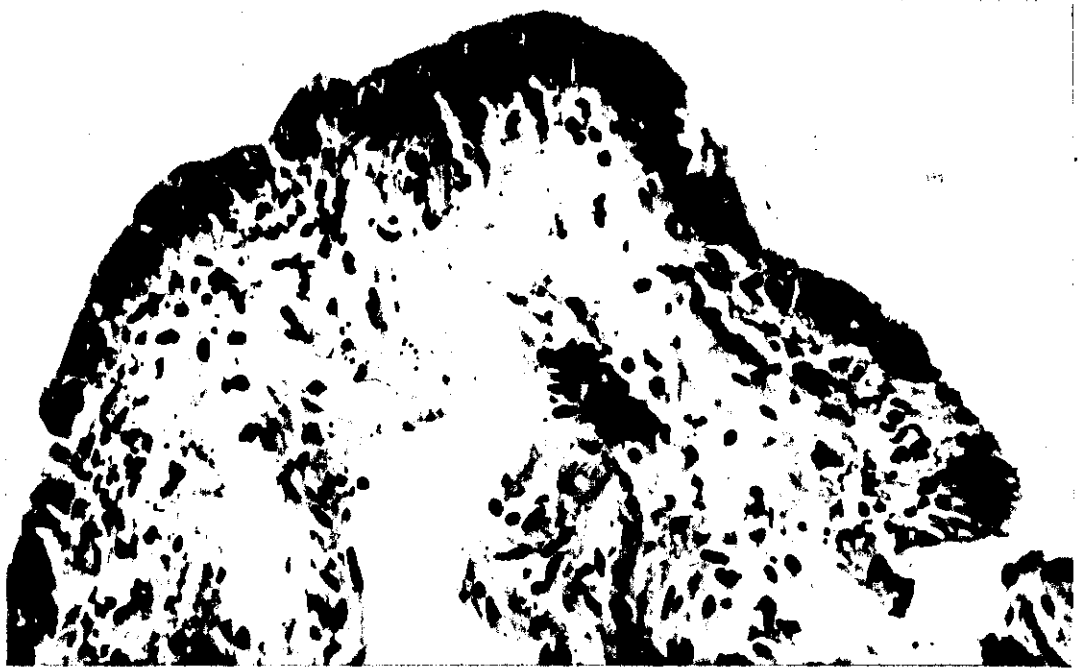
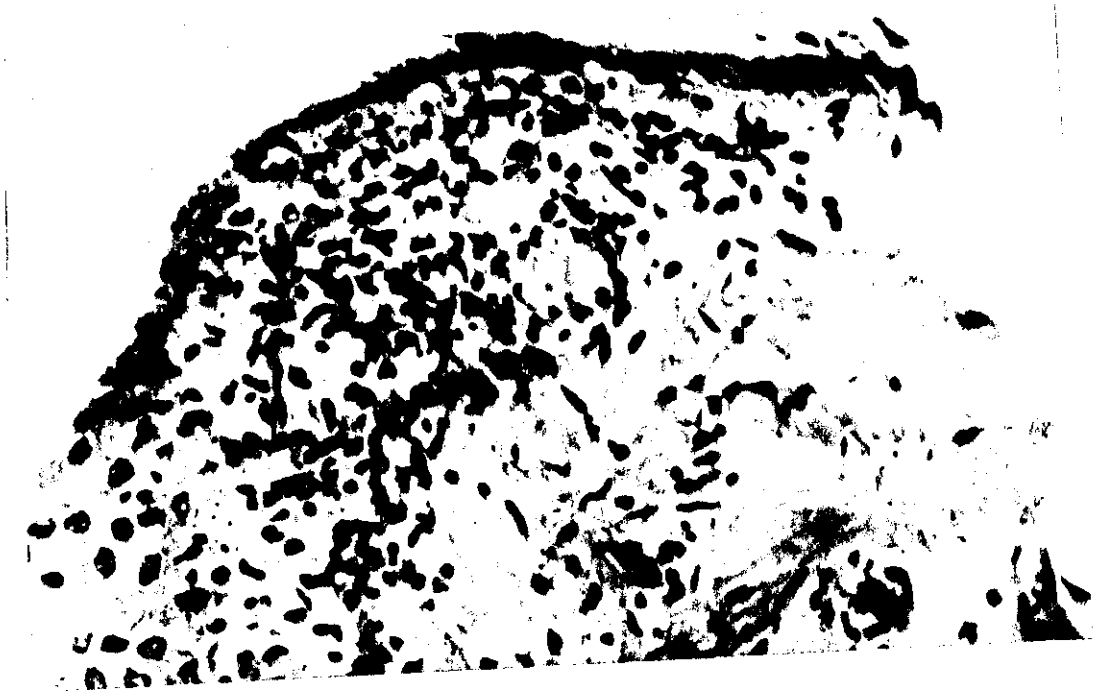


Fig. 13 (above): Epithelial cells growing onto overlying coverslip. Two days of culture in medium IV. X600.

Fig. 14 (below): Three days of culture in medium II. X375.



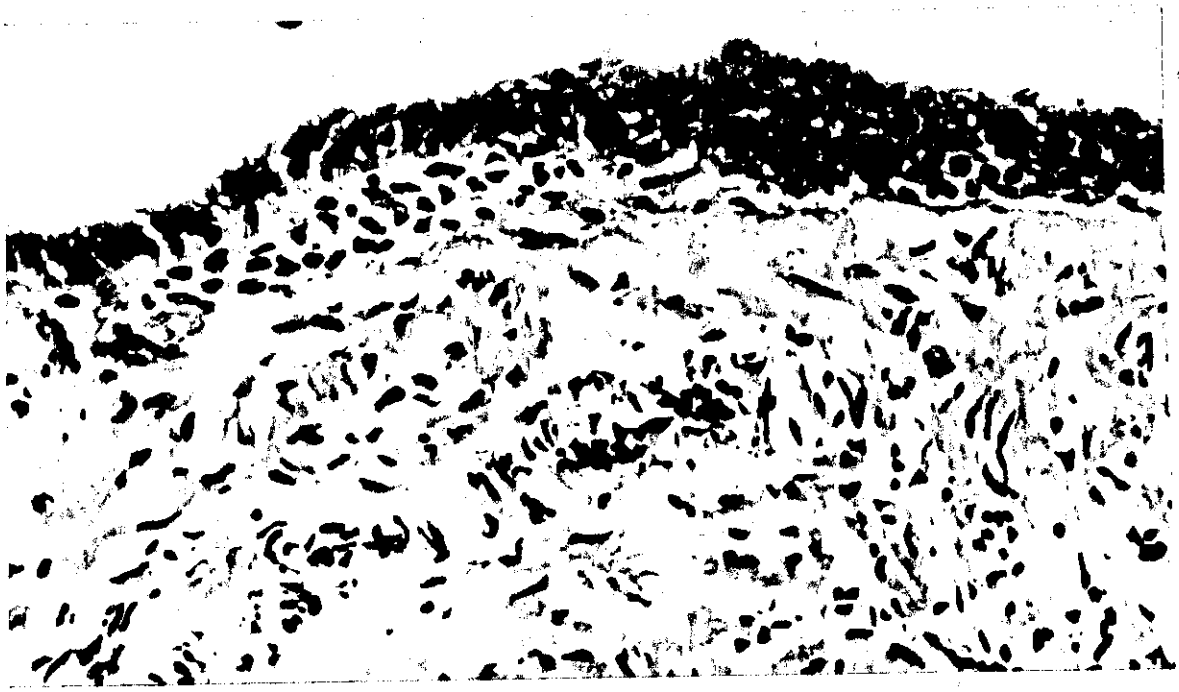


Fig. 15 (above): Four days of culture in medium III. X375.

Fig. 16 (below): Seven days of culture in medium III. X375.

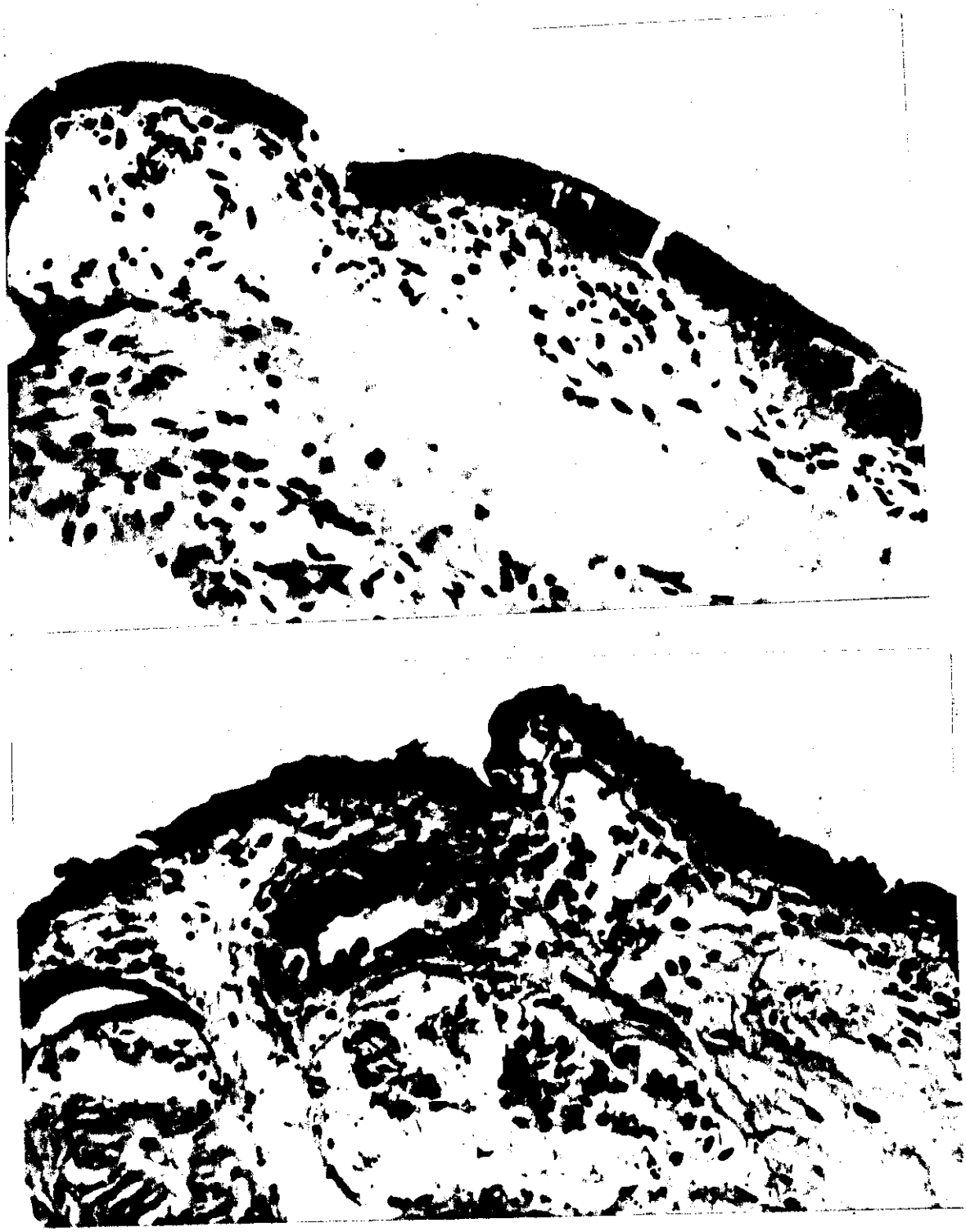


Fig. 17 (above): Two days of culture in medium VII. X375.

Fig. 18 (below): Four days of culture in medium III. X375.

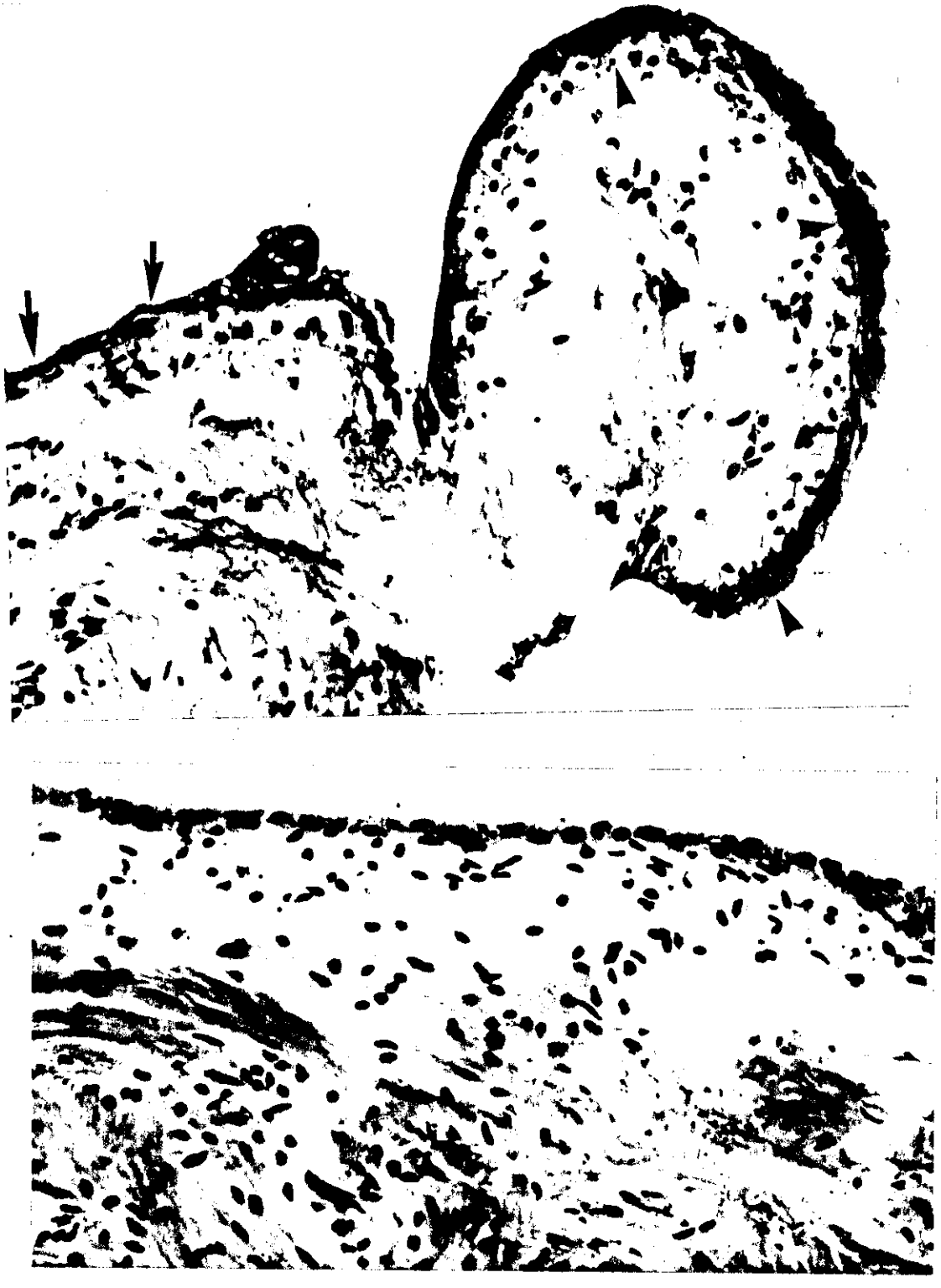
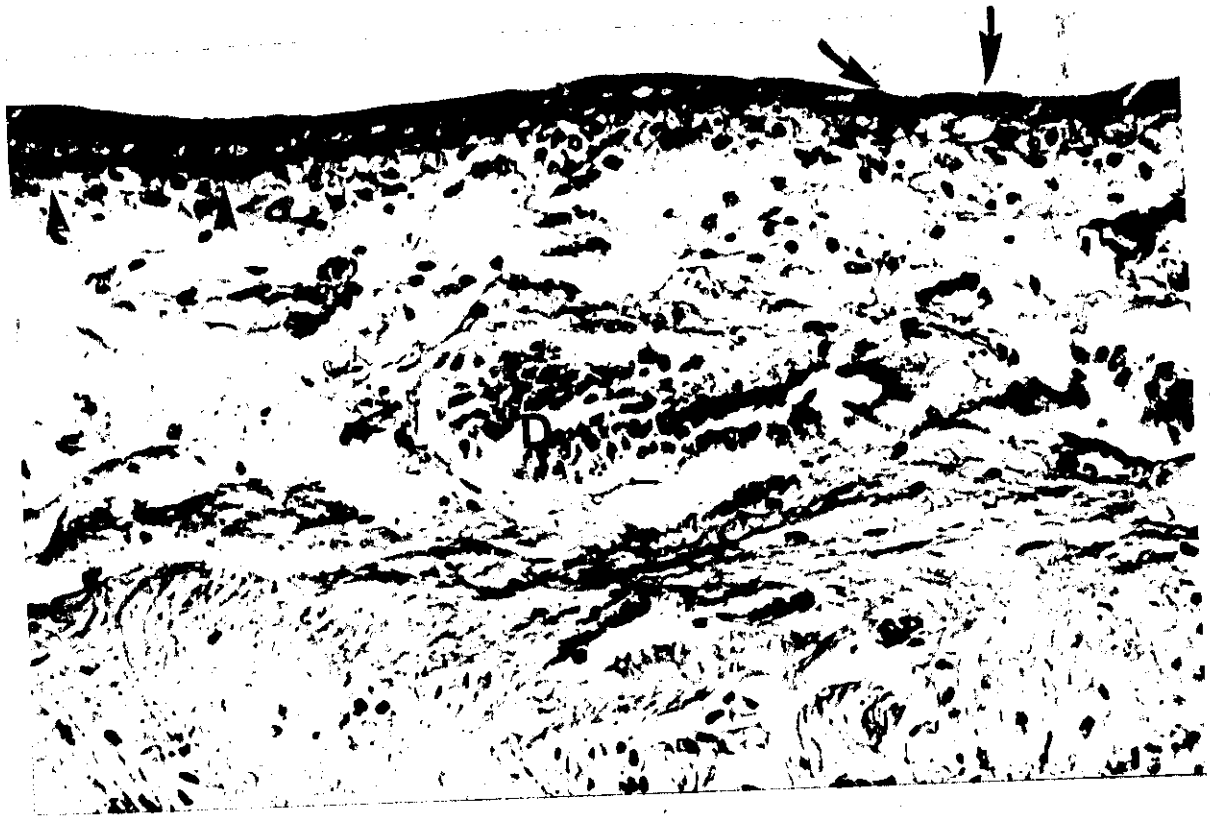


Fig. 19 (above): Showing tall epithelial cells (arrowheads) and flattened cells (arrows). Four days of culture in medium II. X375.

Fig. 20 (below): Note the cuboidal epithelial cells. Seven days of culture in medium VIII. X375.



Figures 21 - 27 are light microphotographs of explants of guinea pig gallbladder grown for different periods in different media stained by PAS. The PAS + ve diastase sensitive glycogen fills the cytoplasm.

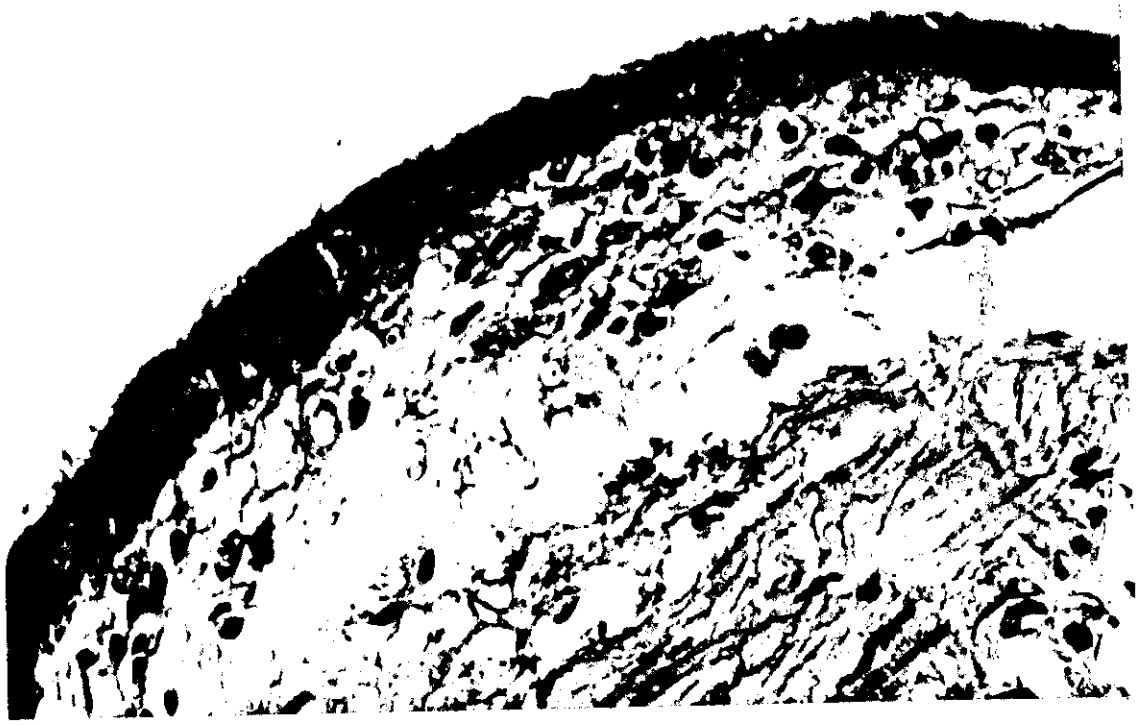
Fig. 21 (above): Seven days of culture in medium II. Note the tall epithelial cells (arrowheads) and cuboidal epithelium (arrows) Debris (D) from degenerated epithelium folded deeper into the gallbladder. X375.

Fig. 22 (above): Four days of culture in medium I. X375.

Fig. 23 (below): Four days of culture in medium III. Note the tall epithelial cells. X600.

Fig. 24 (above): Six days of culture in medium IV. X375.

Fig. 25 (below): Seven days of culture in medium V. X375



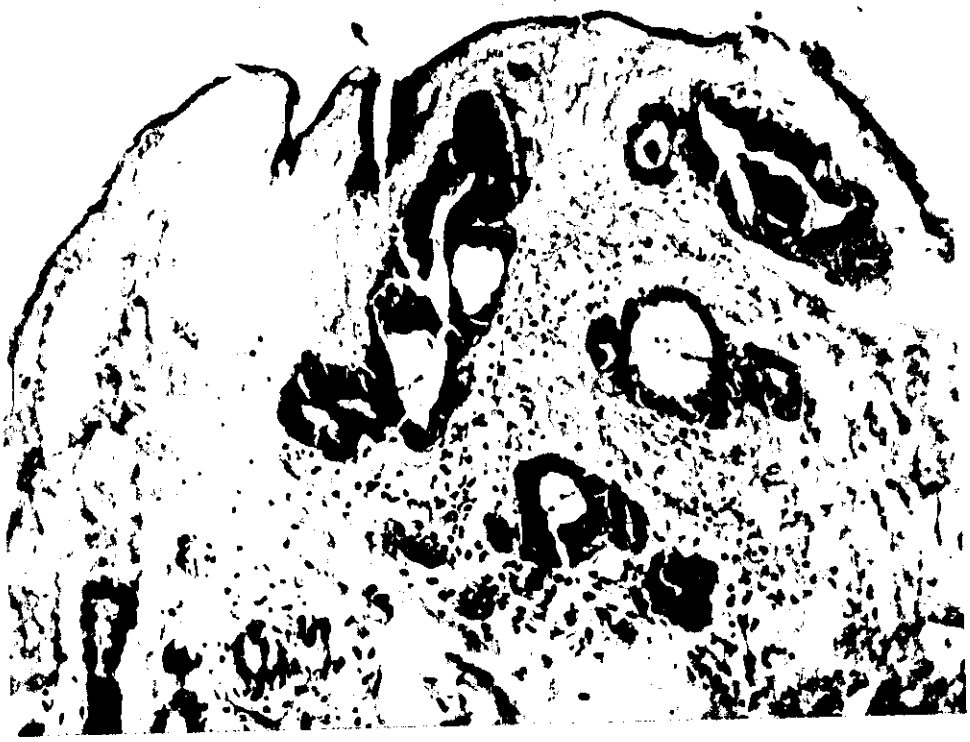


Fig. 26 (above): Seven days of culture in medium III. X600.

Fig. 27 (below): Two-days of culture in medium VI. Abnormal glandular acini were occasionally developed with this medium. X375.

After seven days of culture, tall columnar cells were still seen although cuboidal and flatter cells were also in evidence, often apparently colonising much of the explant surface (Fig. 16, 20, 21, 25, 26).

Abnormal glandular acini were occasionally seen with medium VI (Fig. 27) but otherwise there was no morphological difference between the growth in different media.

b) Transmission electron microscopy

Ultrastructural preservation of epithelial cell morphology including organelles and cell junctions was maintained up to 14 days (the tried culture period at this stage) in the two media used. The surviving cells fell broadly into two groups: tall epithelial cells resembling the original cells and flatter migratory cells.

Explants examined after one day showed mechanical damage of epithelial cells at the edge. After this time a sheet of thin cells started to migrate over the surface of the connective tissue of the explant. Cells in culture were held firmly together by tight apical junctions (Fig. 31, 44) while elsewhere the apposed intercellular surfaces displayed electron-dense cytoplasmic condensation resembling desmosomal adhesion points (Fig. 31, 36, 39). The lateral cell walls displayed numerous folds which interdigitate

with folds of the adjacent membranes (Fig. 32, 44, 45). Well preserved mitochondria (Fig. 30, 35, 36), and Golgi apparatus (Fig. 59) were in evidence.

During the culture period, various changes occurred at different times. In controls the apical cytoplasm contains secretory droplets (Fig. 28, 29). Over the first 24 hours, a most striking change was loss of secretory vesicles from the cell apices (Fig. 30, 32, 43). The thin migrating cells had also no secretory droplets. Only very occasional vesicles were seen at subsequent times. This loss of secretory vesicles coincided with an increase in glycogen rosettes, sometimes forming glycogen fields (Fig. 32) and, at other times, widely dispersed in the cytoplasm itself (Fig. 33). This increase continued throughout the culture period corresponding to the increase in diastase sensitive PAS positive material seen by light microscopy. After three days of culture autophagic vacuoles containing glycogen were also seen (Fig. 60).

Over the same period lipid droplets appeared in the cultured epithelium (Fig. 39, 41). These are not normally present in the control material (Fig. 28, 29). At the same time, lipid droplets were found in various cells in the underlying lamina propria (Fig. 32, 40).

Microvilli with well developed glycocalyx were maintained (Fig. 31, 32), although some variations in the length and number of microvilli were noted. Some microvilli were longer than normally observed

(Fig. 41). Some surviving cells had bald areas and those microvilli that remained were sparsely distributed (Fig. 40, 46). These changes are more obvious in the scanning electron micrographs (Fig. 53-55), and after 14 days of culture (Fig. 46).

By four days, many of the thinner cells contained microfilaments (Fig. 36, 37) and tubules (Fig. 33, 35) which became gathered together compared with the control cells (Fig. 28, 29). These elements were mostly arranged around the cell periphery although sometimes gathered in bundles in the cytoplasm. Coated pits were also more obvious in cultured cells (Fig. 33, 34, 35) than in the control cells (Fig. 28, 29). Coated pits were often at the basolateral cell aspects (Fig. 33, 34). Near the cell surface numerous pinocytotic vesicles were also observed (Fig. 38, 41).

The basal lamina of some cultured cells was apparently thrown into a series of folds with accumulation of debris in the pars rara interna of the basal lamina (Fig. 39, 47).

Many of the tall cells retained their normal morphology up to seven days (Fig. 42). Elsewhere sheets of thin epithelial cells could be found. By 14 days of culture, most cells were in sheets with preservation of epithelial cell morphology (Fig. 44, 45) although some evidence of dedifferentiation was also noted as some cells had become flattened

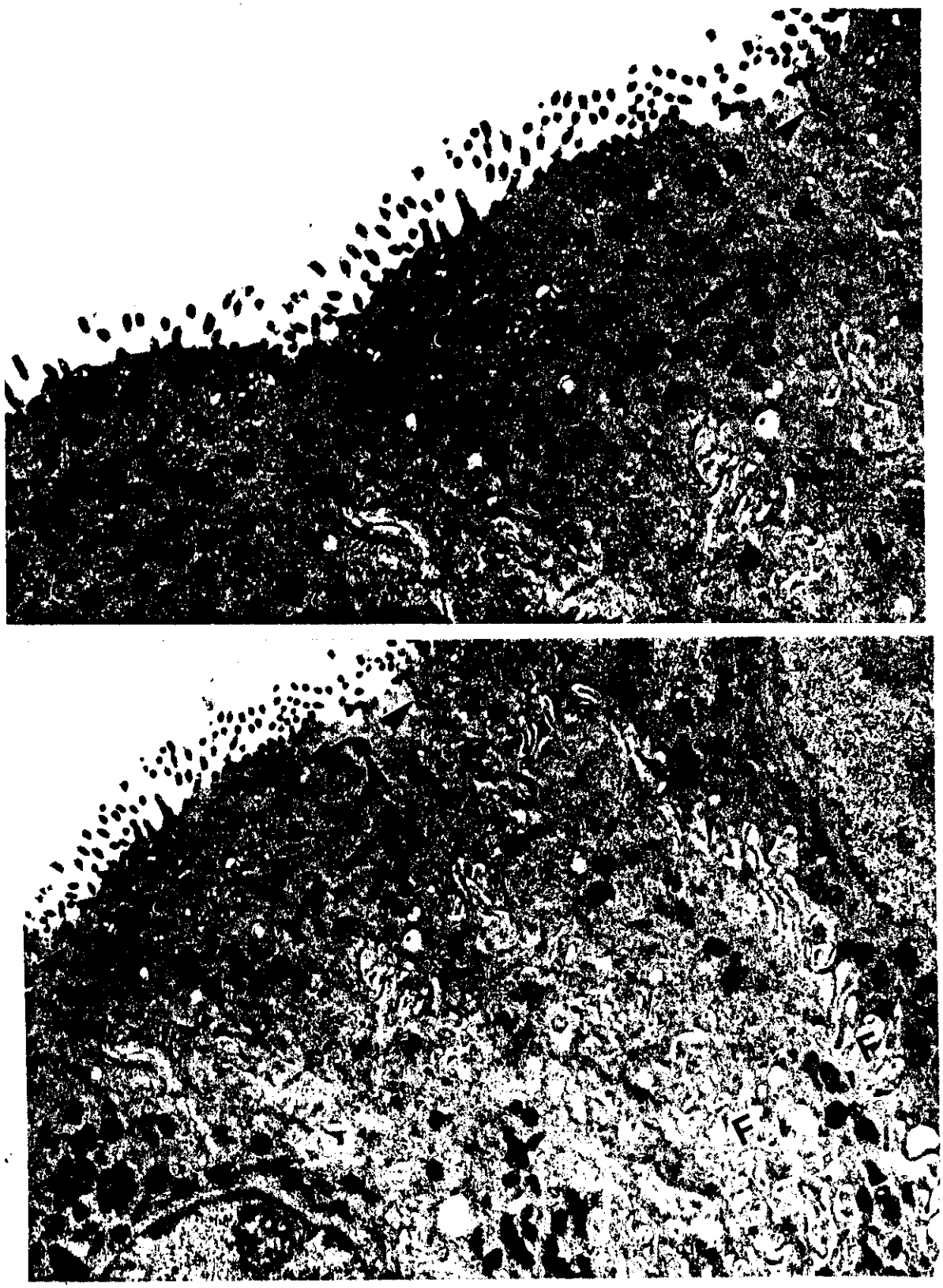
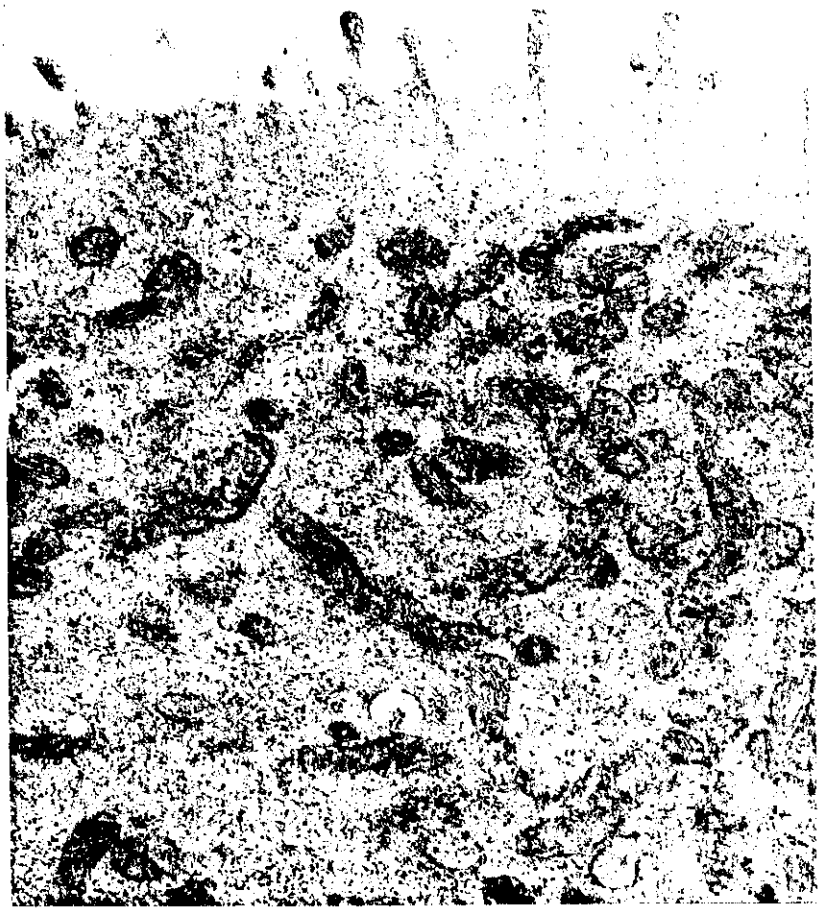


Fig. 28 (above) and Fig. 29 (below) are transmission electron micrographs of guinea pig gallbladder cells before culture (control). Note cell junctions (arrowheads), secretory vesicles (arrows) and the lateral interdigitating folds (F). X9,650 (above) X7,000 (below).



Figures 30-49 are transmission electron micrographs of guinea pig gallbladder cells cultured for varying periods up to 14 days in media I or V.

Fig. 30 (above): Apex of an epithelial cell showing good preservation of mitochoncria and microvilli. Note loss of secretory droplets. One day of culture in medium I. X25,000.

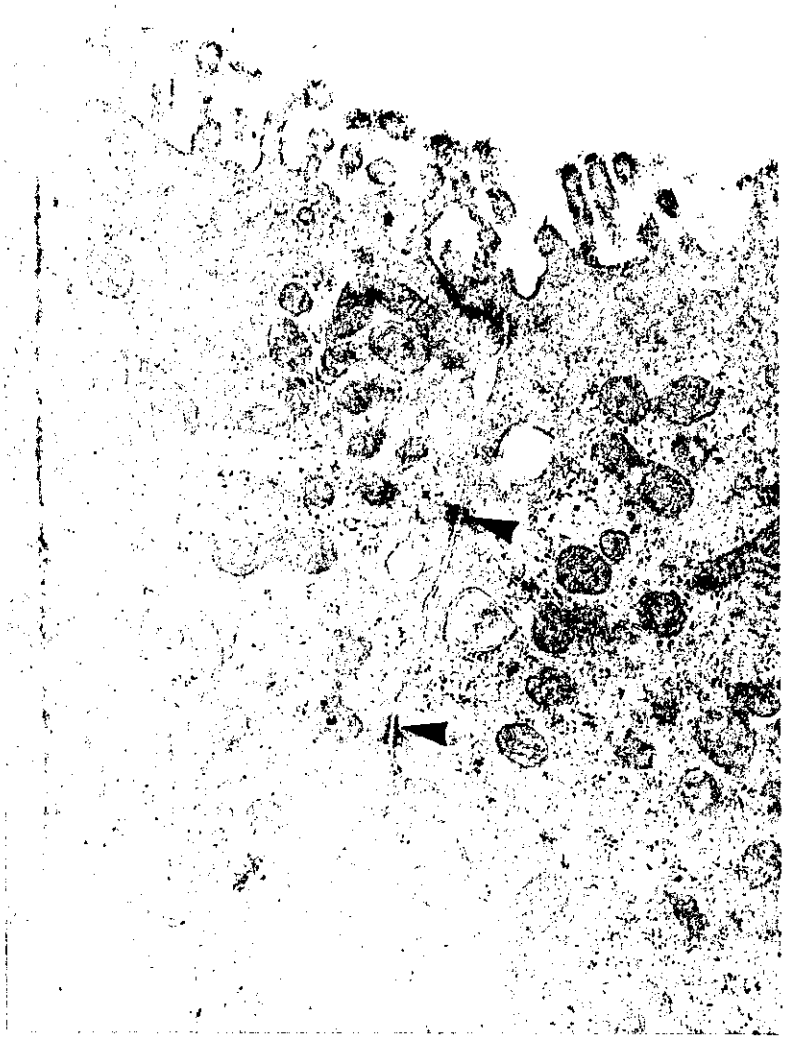


Fig. 31: Apical part of two epithelial cells showing good preservation of organelles. The cell apices are held together by well formed tight junctions while elsewhere desmosomal junctions (arrowheads) are seen interlocking the adjacent cells. Three days of culture in medium I. X28,000.

Fig. 32: Portion of two epithelial cells showing glycogen fields (G). Microvilli covered with well developed glycocalyx. No secretory droplets are seen. Note lipid (L) in connective tissue cells and the elaborate interwoven folds of lateral cell membrane forming a labyrinth of interconnected intercellular spaces. Three days of culture in medium V. X12,000.

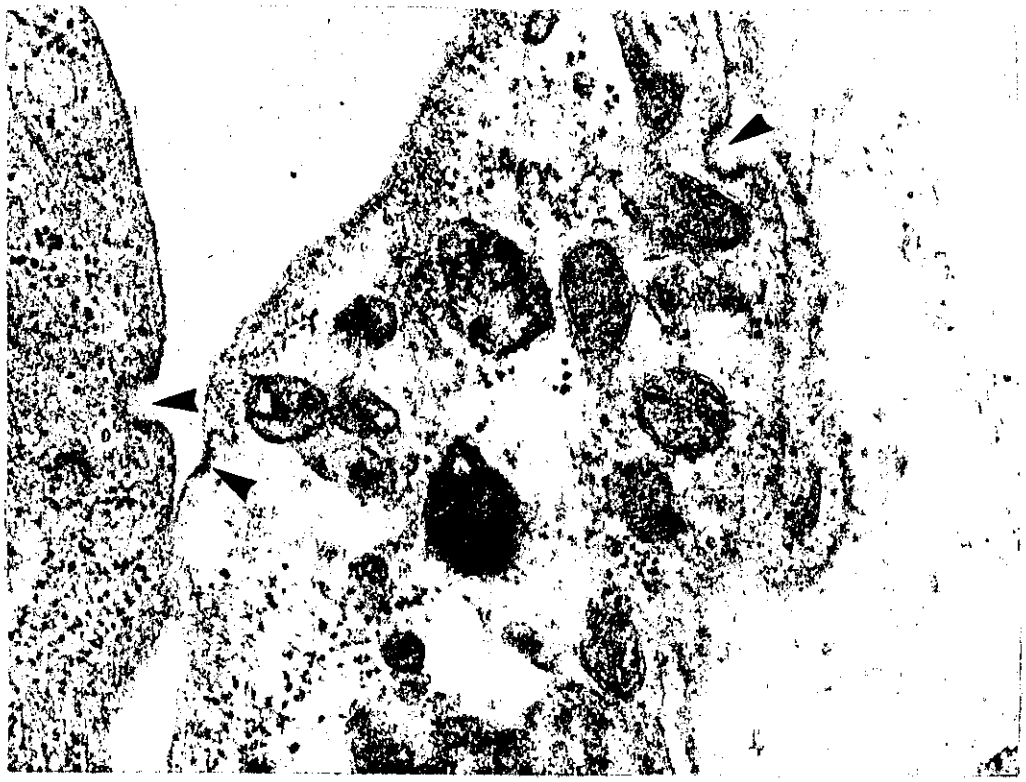


Fig. 33 (above): Basal aspect of an epithelial cell showing coated pits (arrowheads) and microtubules (arrows). Note the glycogen (black dots). Seven days of culture in medium I. X35,000.

Fig. 34 (below): Basolateral aspect of two epithelial cells showing coated pits (arrowheads). Six days of culture in medium I. X35,000.

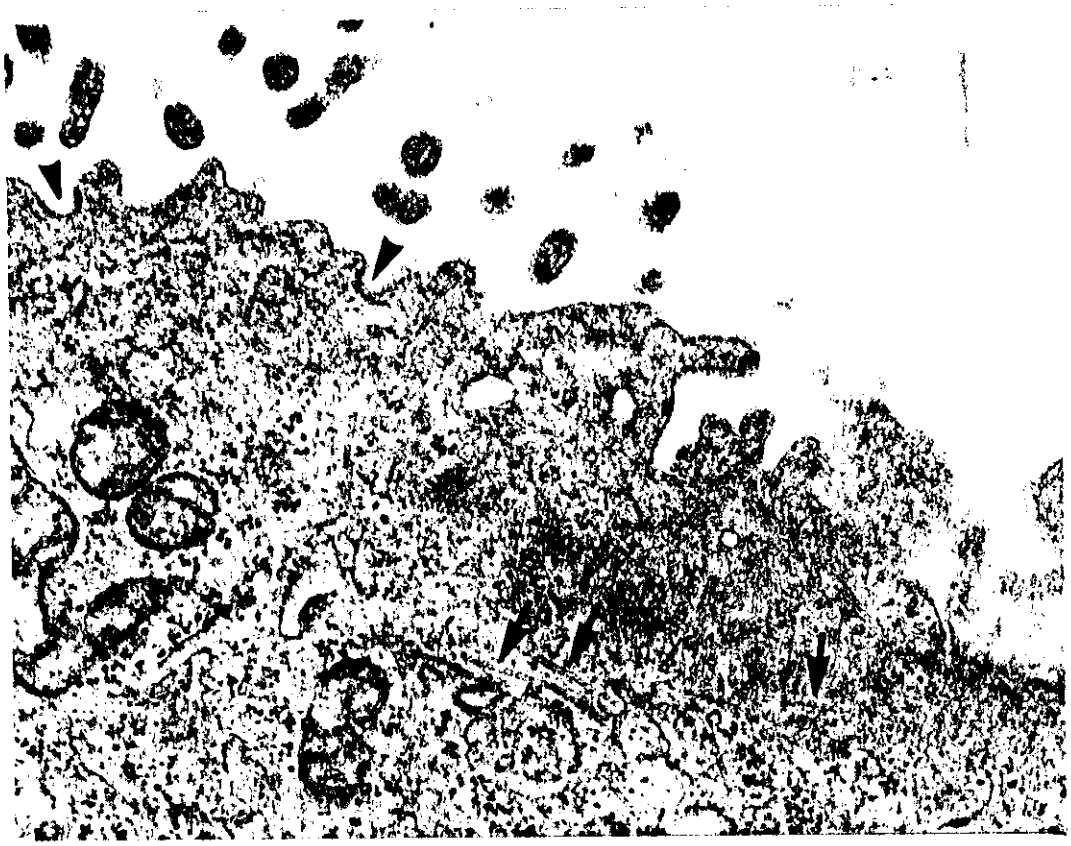


Fig. 35: Coated pits (arrowheads), microtubules (arrows) and well preserved mitochondria after six days of culture in medium V. X30,000.

Fig. 36: Bundles of microfilaments (arrows) are present throughout the epithelial cells, but especially near the cell surface and at sites of cell-cell contact. Desmosomal junctions (arrowheads) are also seen. Seven days of culture in medium I. X25,000.

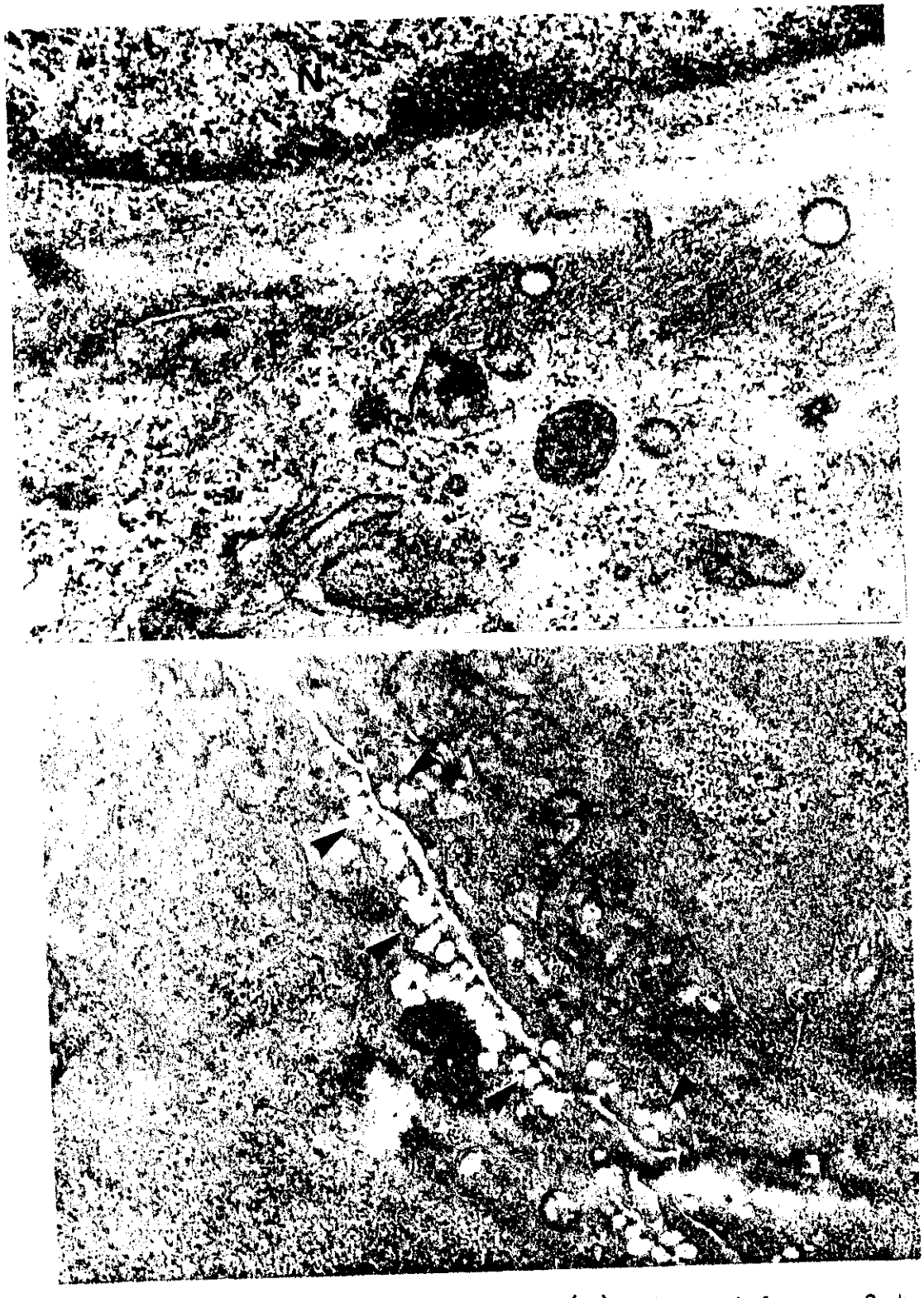


Fig. 37 (above): Microfilaments (F) at periphery of two epithelial cells. The edge of nucleus (N) is also seen. Seven days of culture in medium V. X80,000.

Fig. 38 (below): Portion of epithelial cells showing pinocytotic vesicles (arrowheads) near the cell surface. Six days of culture in medium V. X15,000.

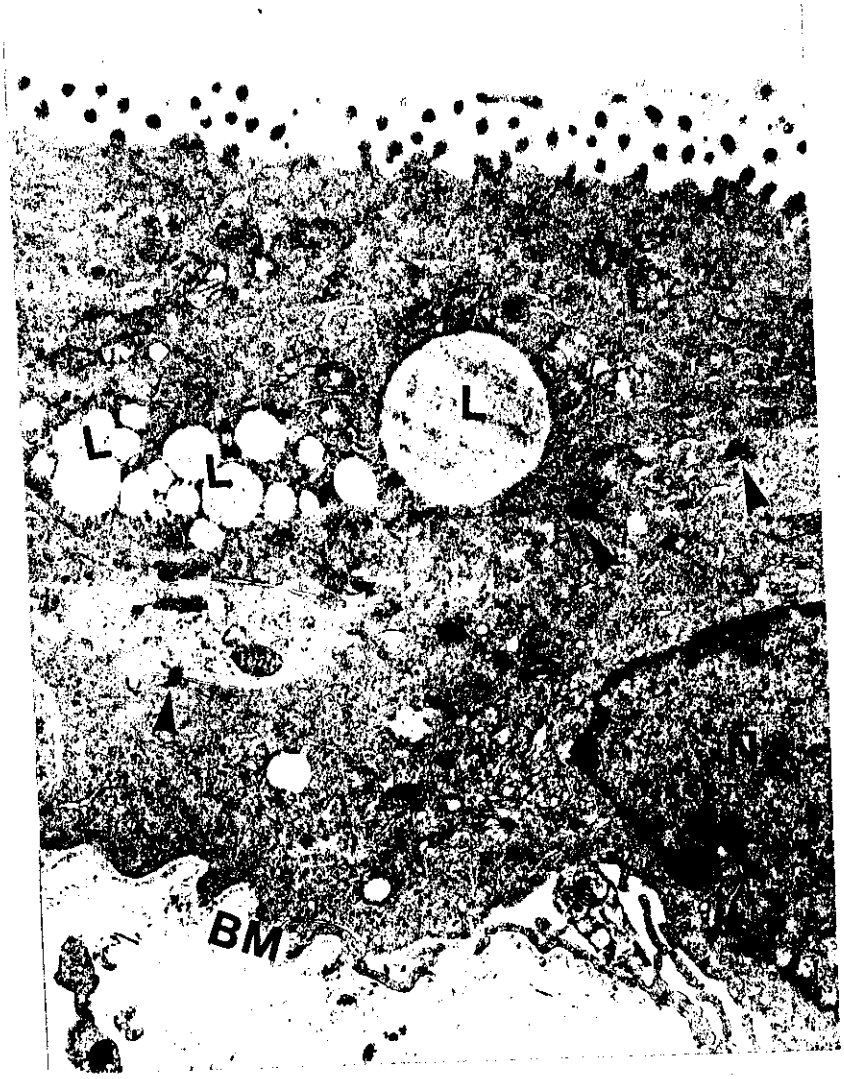


Fig. 39: Epithelial cells showing numerous lipid droplets (L) in the cytoplasm, desmosomal junctions (arrowheads) and folded basement membrane (BM). A portion of cell nucleus (N) is seen. Three days of culture in medium V. X12,000.

Fig. 40: Thin epithelial cells largely devoid of microvilli. One connective tissue cell, probably macrophage, contains lipid droplets (L). The fibroblast (F) contains no lipid. Seven days of culture in medium I. X7,750.

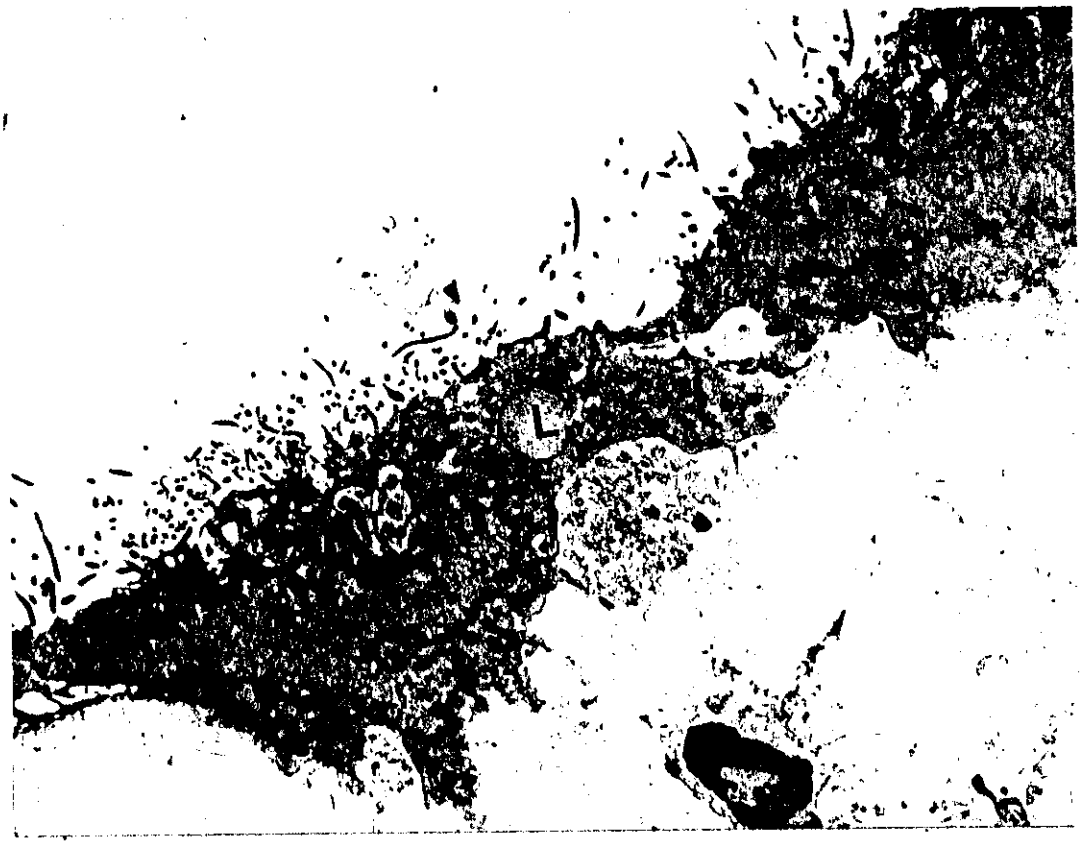


Fig. 41: Thin epithelial cells showing numerous microvilli of varying lengths, lipid droplet (L) and pinocytotic vesicles (the small white dots near the cell surface). Three days of culture in medium V. X8,000.

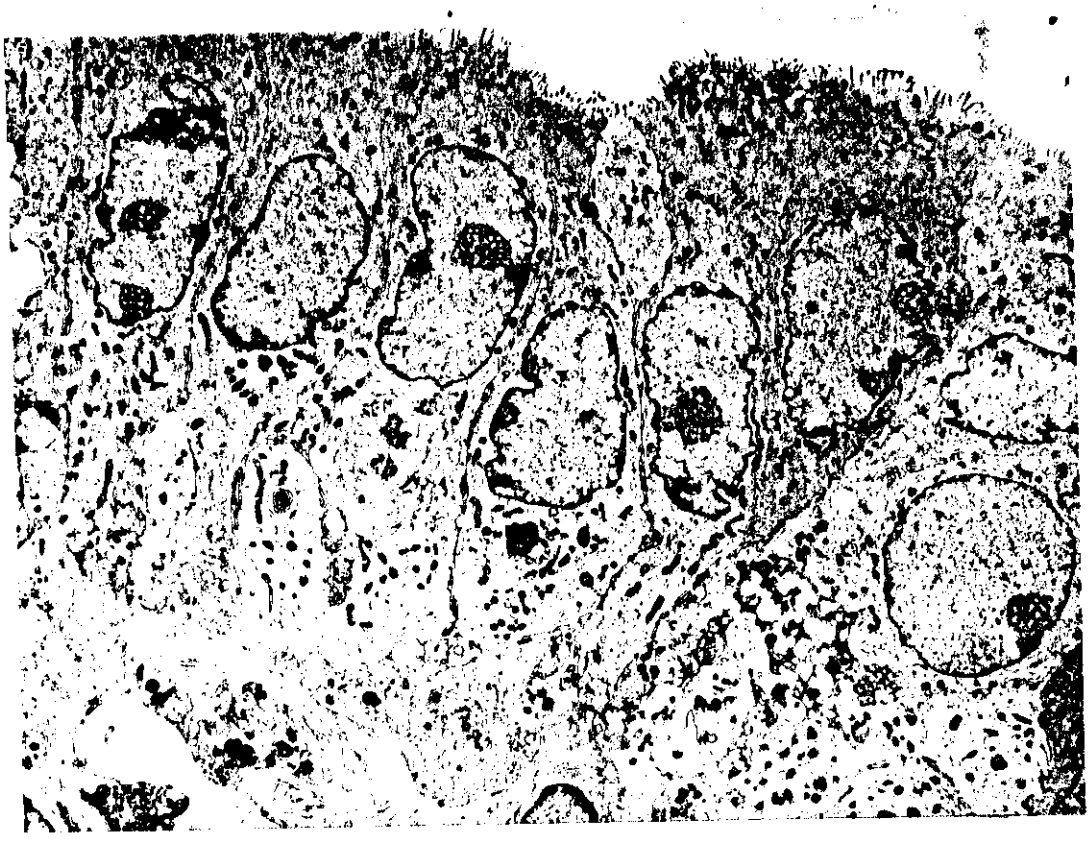


Fig. 42: Tall epithelial cells in a good condition after seven days of culture in medium I. Note the regular microvilli. X1,500.

Fig. 43: Portion of an epithelial cell showing well marked glycocalyx on stunted microvilli. Note dilated Golgi apparatus (arrowhead), absence of secretory droplets and the well preserved mitochondria. Ten days of culture in medium I. X20,000.

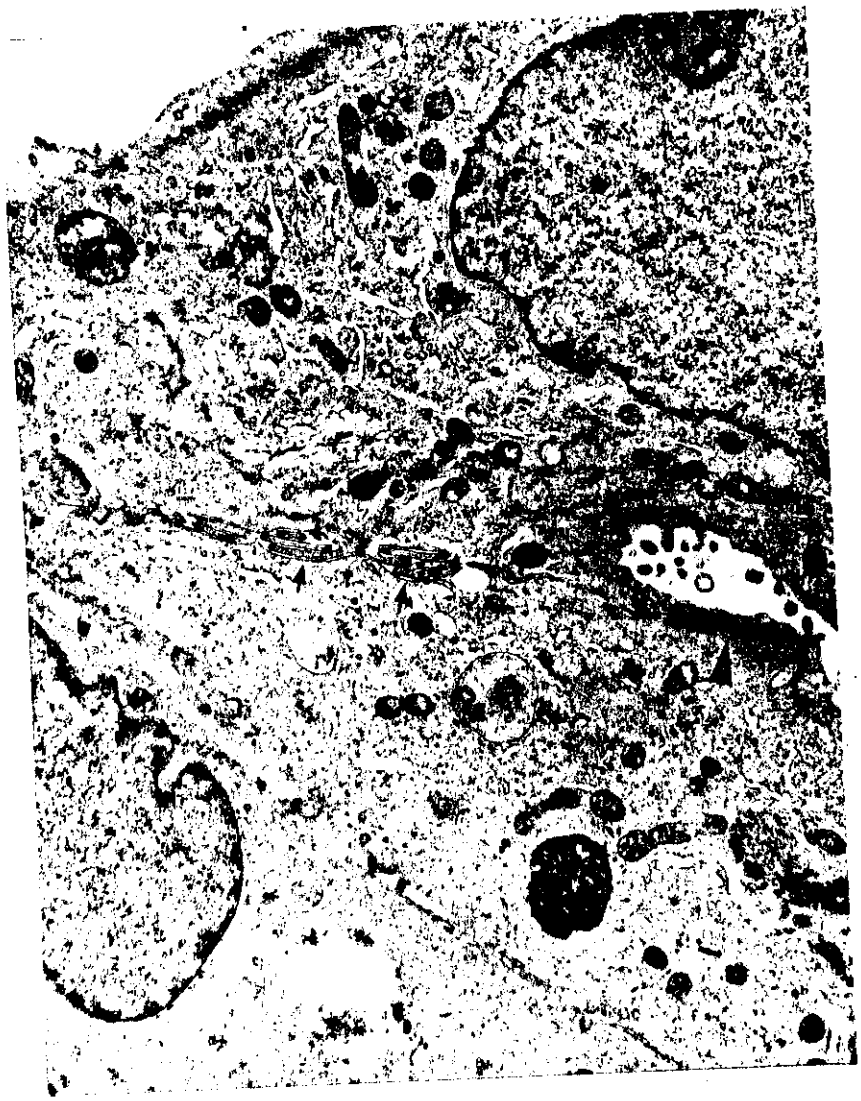


Fig. 44: Epithelial cells showing tight apical junction (arrowhead) and lateral interdigitating folds (arrows). Fourteen days of culture in medium V. X12,000.

Fig. 45: Epithelial cells showing shortened microvilli covered with glycocalyx, well preserved mitochondria, intercellular junctions (arrowheads) and lateral interdigitating folds (arrows). Fourteen days of culture in medium I. X26,000.

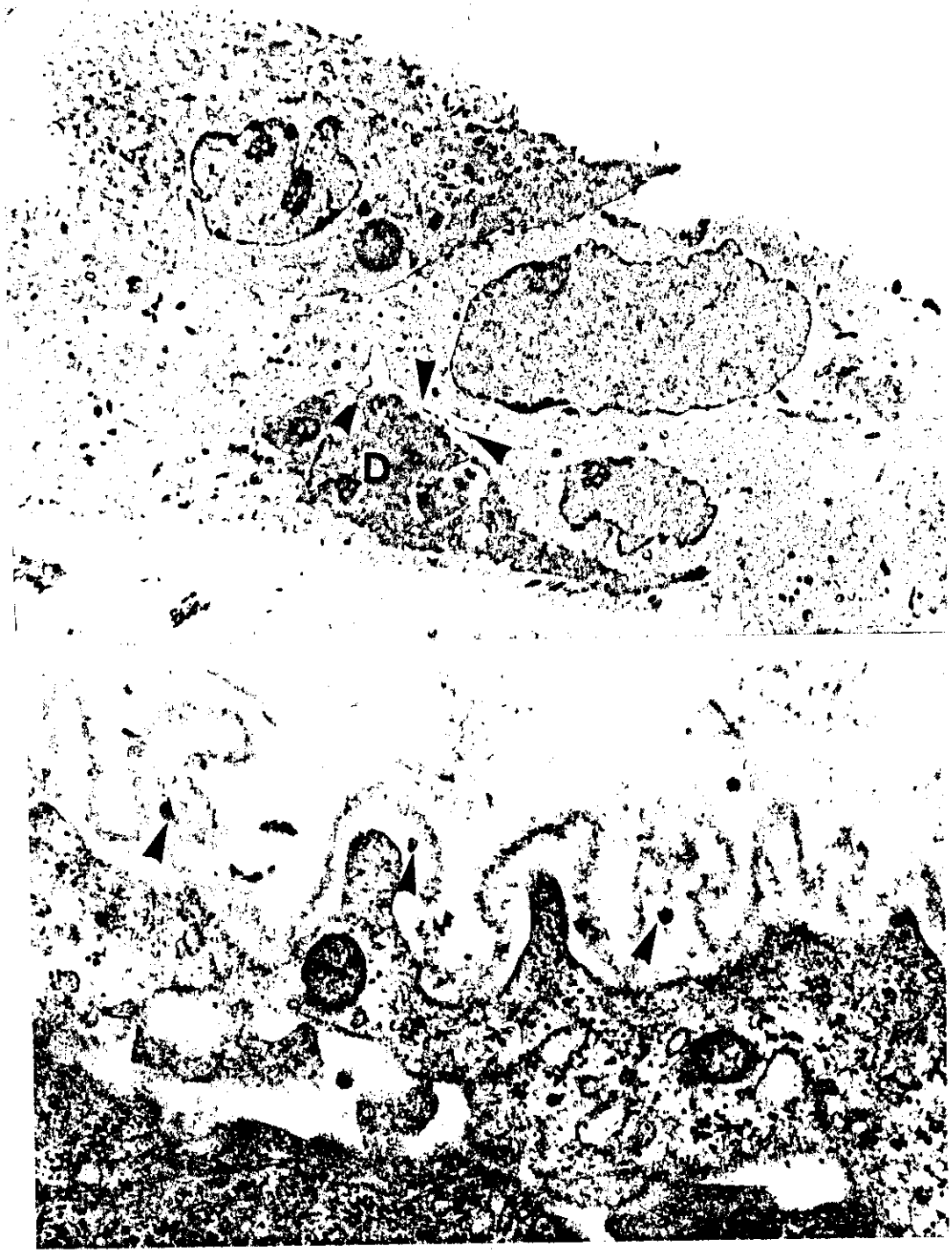


Fig. 46 (above): Epithelial cells after 14 days of culture in medium I showing some dedifferentiation. Microvilli are preserved in some areas while others are bald. Generally microvilli are less in number although elongated microvilli (arrowheads) can be seen. The deeper cell looks damaged (D). Note folding of the basement membrane. X3,500.

Fig. 47 (below): The basement membrane thrown into a series of apparently redundant folds. Note the particulate debris in the pars rara interna (arrowheads). Fourteen days of culture in medium V. X22,800.

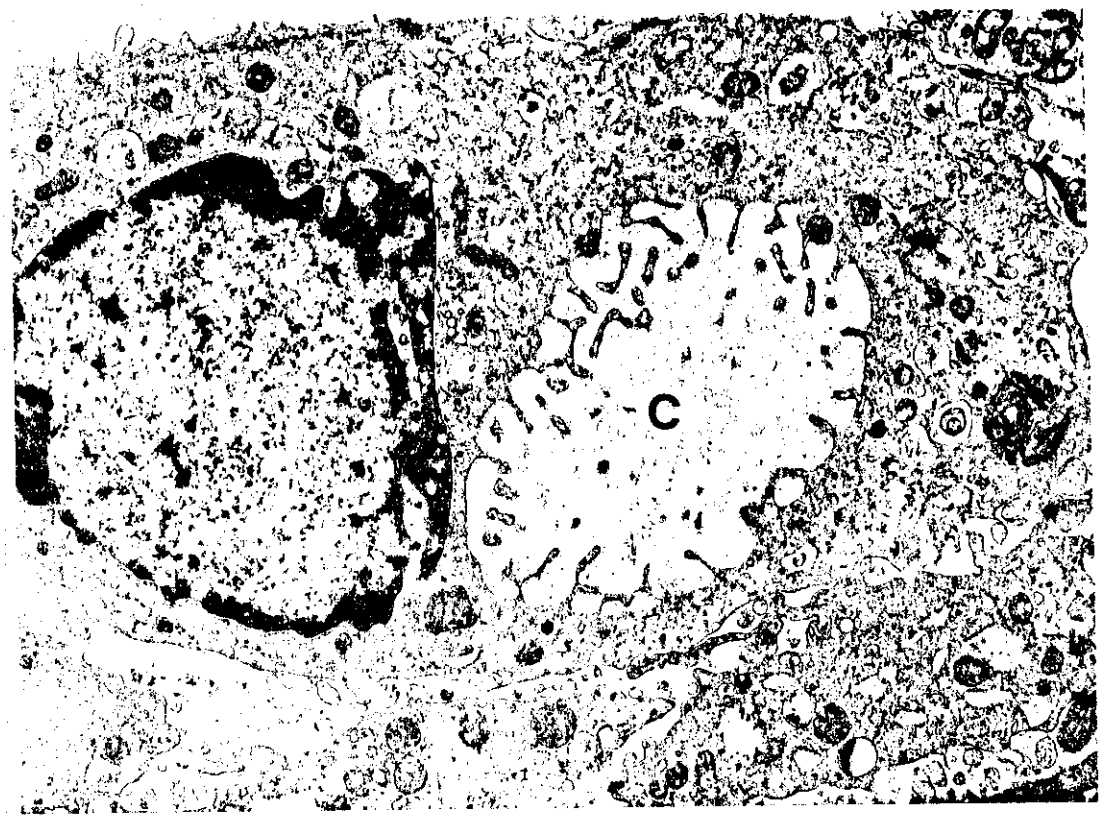


Fig. 48 (above): Intracellular canaliculus (C) with long microvilli along its inner surface. Six days of culture in medium V. X13,000.

Fig. 49 (below): Debris from damaged cells (probably traumatic). Note the numerous damaged mitochondria with electron dense inclusions. Secretory droplets are also present (arrowhead). Nuclear remnant (N) is seen. Two days of culture in medium I. X15,000.

with loss of polarisation and some loss of microvilli (Fig. 46).

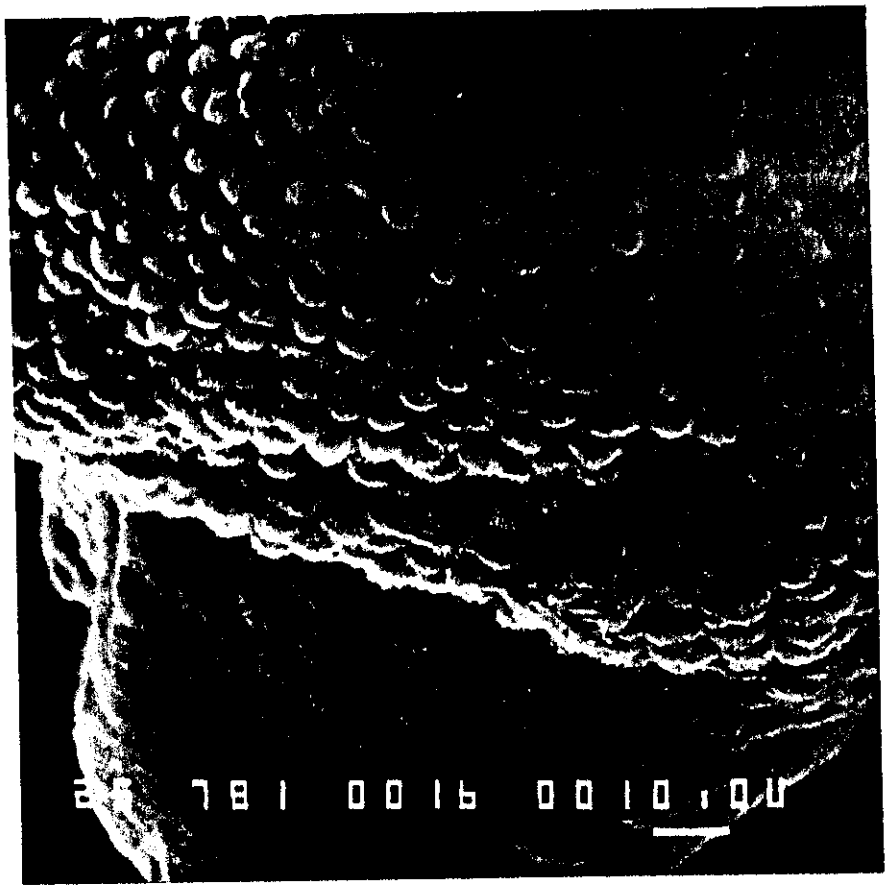
On three occasions after six days of culture in the two media used (medium I and V) a peculiar cell type was seen which showed the development of an intracellular canaliculus or lumen. In each case there was a better developed glycocalyx over the canalicular microvilli than over the cell surface (Fig. 48).

c) Scanning electron microscopy

The scanning electron microscopy findings amplified and extended the observations made on sectioned material by light and electron microscopy. After several days of culture the migrating cells formed a sheet sweeping over the underlying connective tissue (Fig. 52). The microvilli were preserved in some cells while in others there were bald areas and sparse microvilli (Fig. 54, 55) following seven days of culture. Another feature which developed during culture was very long microvilli (Fig. 53).

2. Effect of foetal calf serum

Medium I was tested with a random sample of F.C.S. and found to support gallbladder growth. Experiments were then performed to compare three different batches of sera with medium I. The



Figures 50-55 are scanning electron micrographs of guinea pig gallbladder cells before culture (control) and after culture in medium I.

Fig. 50 (above): Epithelial cells (control) showing the polygonal outlines of the cells and the surface microvilli which can just be distinguished at this magnification. the bar is 10 μM long.

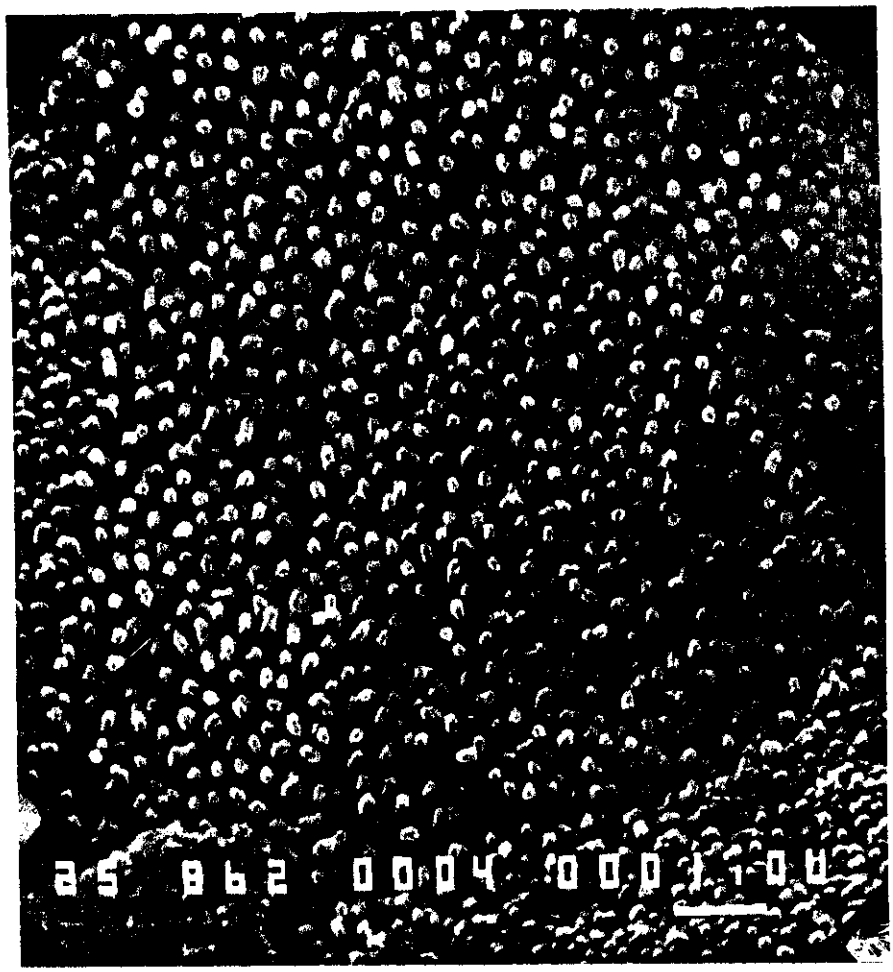


Fig. 51: Epithelial cells (control) showing dense and regular microvilli. The bar is 1 μM long.

Fig. 52: A sheet of migrating cells is seen sweeping over the serosal surface. Note folding of the sheet. Seven days of culture. The bar is 100 μM long.

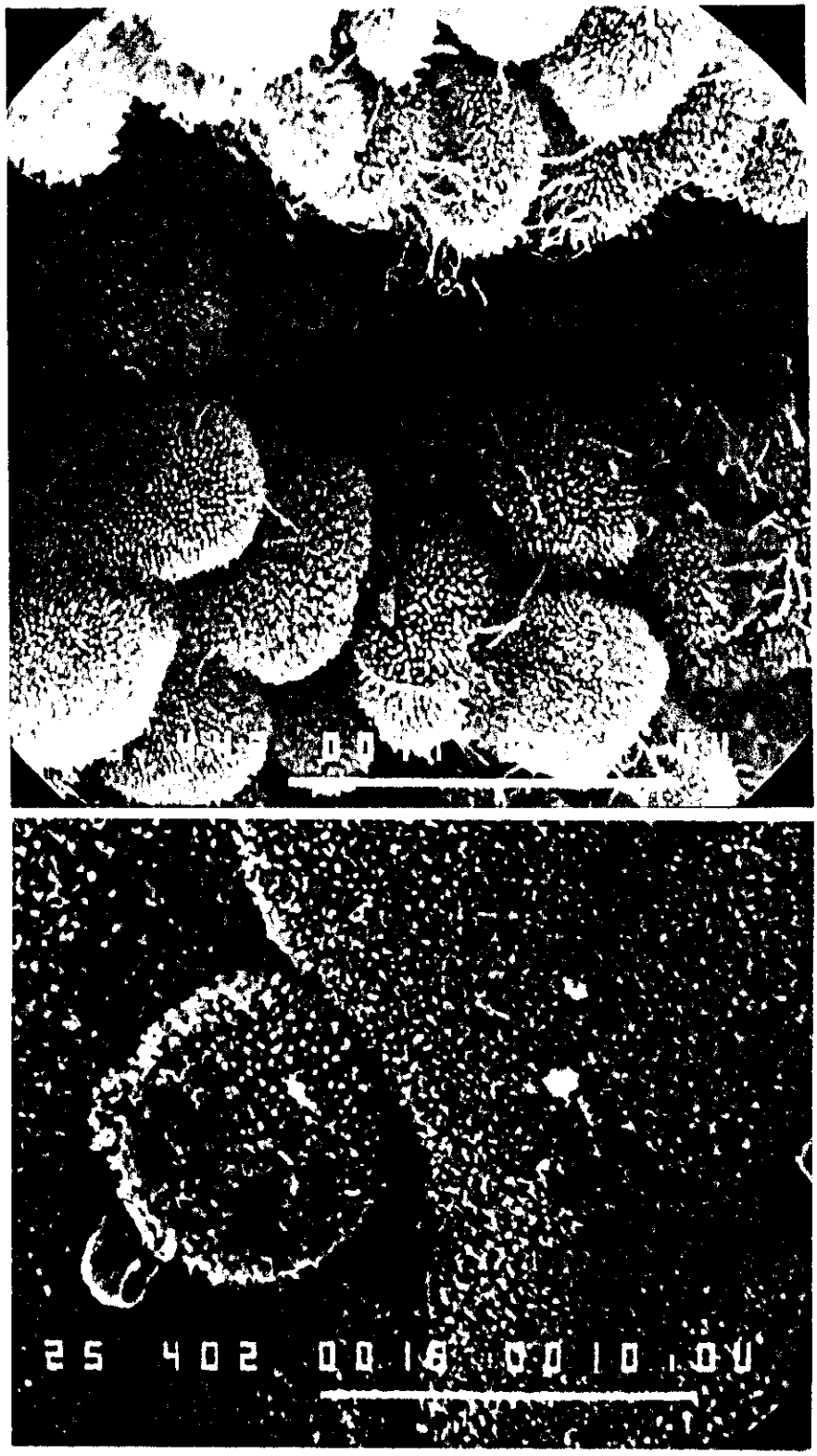


Fig. 53 (above): Some elongated microvilli have developed on the otherwise normal epithelial cells. Three days of culture. The bar is 10 µM long.

Fig. 54 (below): Note the bald areas (arrowheads). Three days of culture. The bar is 10 µM long.

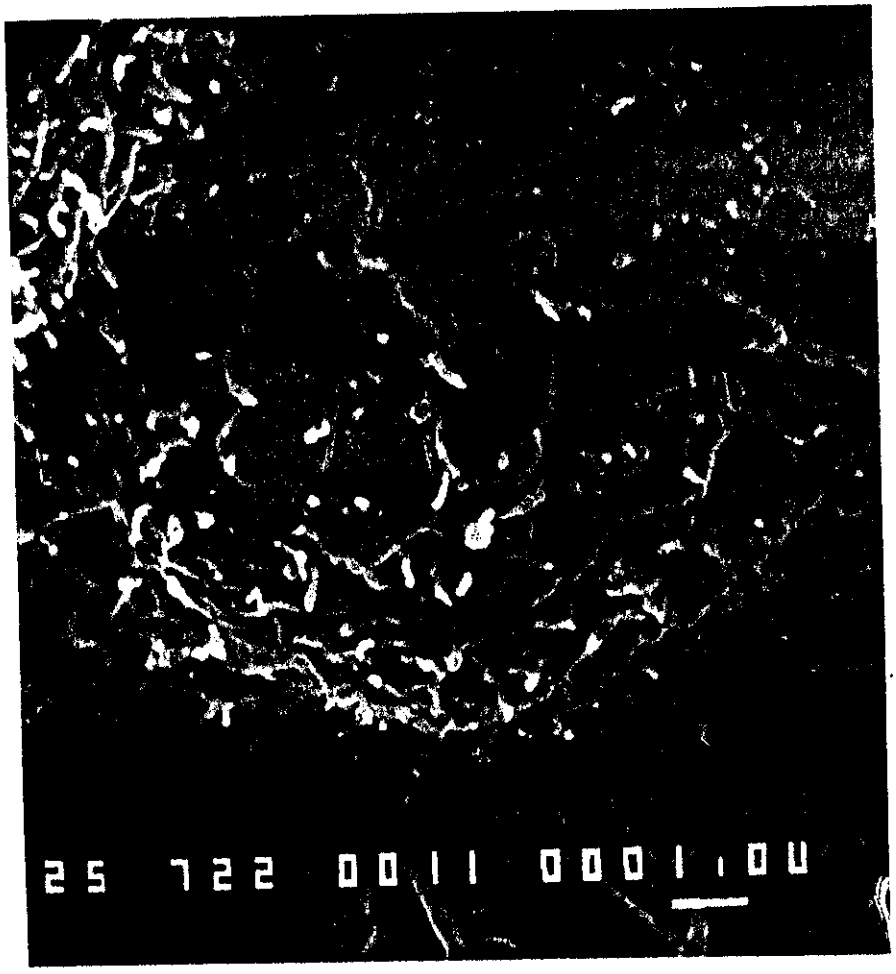


Fig. 55: Epithelial cells showing microvilli of varying length, some short and some long. Note the decrease in microvilli number. Seven days of culture. The bar is 1 μM long.

batch which promoted the outgrowth of epithelial ce from guinea pig gallbladder explants was selected.

3. Effect of medium exchange

Medium renewal on alternate days during the first week of culture made no improvement on the ultrastructural characteristics.

4. Effect of insulin, hydrocortisone, collagenase and trypsin

Adding insulin, hydrocortisone or collagenase to the culture media or treatment of tissue fragments with trypsin before culture in the presence or absence of collagenase failed to show any improvement in either growth characteristics or the ultrastructural features.

5. Absorption of cationized ferritin

Gallbladders cultured for between two and ten days responded in the same way to Cat. F. which was bound to the apical cell membrane and microvilli. This Cat. F. showed clumping (Fig. 56) while prolonged exposure produced mats of the marker on the cell surface (Fig. 57). Vesicles were seen in the apical cytoplasm which were either small (< 100 nm) or larger (100-400 nm). In tissue which had been

cultured in the presence of Cat. F. both sizes were labelled (Fig. 56-58) although the larger vesicles tended to carry proportionately more particles of Cat. F. These large vesicles were observed close to the apical surface apparently recently loaded (Fig. 56). Labelled vesicles were seen close to the lateral intercellular space and later, the marker was found in the space.

In specimens receiving a one hour pulse of Cat. F. vesicles were seen near the Golgi apparatus, although this later organelle did not become labelled with Cat. F. apart from an occasional particle (Fig. 59).

Prolonged cultures, following one hour pulse, led to deposition of Cat. F. in lysosomes (Fig. 59) and autophagic vaccules (Fig. 60). Further extension of the culture period showed labelling of residual bodies (Fig. 61, 62).

At the same time, Cat. F. appeared in the lateral intercellular space, mostly as a series of clumps (Fig. 61) but sometimes to form a linear pattern with varicositis (Fig. 62).

Within one hour of exposure to Cat. F. the marker was found initially in irregular clumps between the base of the epithelial cells and the underlying basement membrane (Fig. 63). After eight hours, this formed a regular array (Fig. 66). By this

Figures 56-69 are transmission electron micrographs of guinea pig gallbladder cells cultured for 2, 4, 6 or 10 days in medium I then exposed to Cat. F. either as a pulse (one hour) or continuously up to 24 hours. All electron micrographs are uncounterstained.

Fig. 56 (above): Apex of an epithelial cell (pulsed with Cat. F.) showing labelling of the apical cell membrane and microvilli with clumping of Cat. F. (arrow) in some places. Some apical vesicles (v) contain the label while one (arrowhead) appears to be separating from a pit. Two days of culture. X82,000.

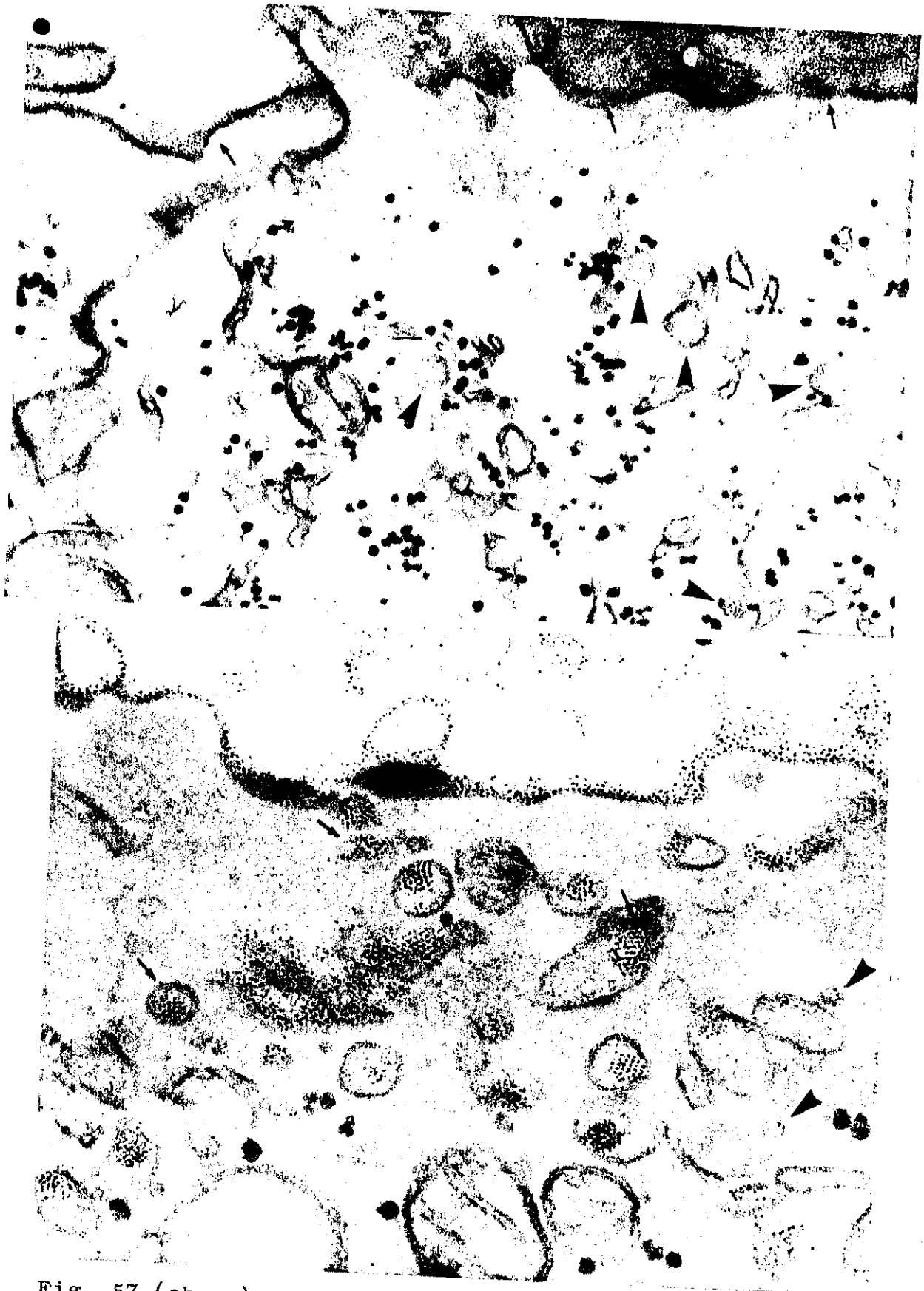


Fig. 57 (above): Variation in Cat. F. binding between cells. The marker produced a mat on cell surface (arrows). Several labelled vesicles are seer (arrowheads). Six days of culture, pulsed with Cat. F. X63,000.

Fig. 58 (below): An epithelial cell which has been exposed to Cat. F. for two hours. Numerous vesicles ar labelled, heavier in the case of the large vesicles (arrows) than smaller vesicles (arrowheads). Six days of culture. X104,000.

Fig. 59: Portion of epithelial cell pulsed with Cat. F. showing labelled vesicles (arrowheads). One is seen near the Golgi apparatus and another one is attached to a lysosome (L). No marker is seen inside the Golgi apparatus. Six days of culture. X63,000.

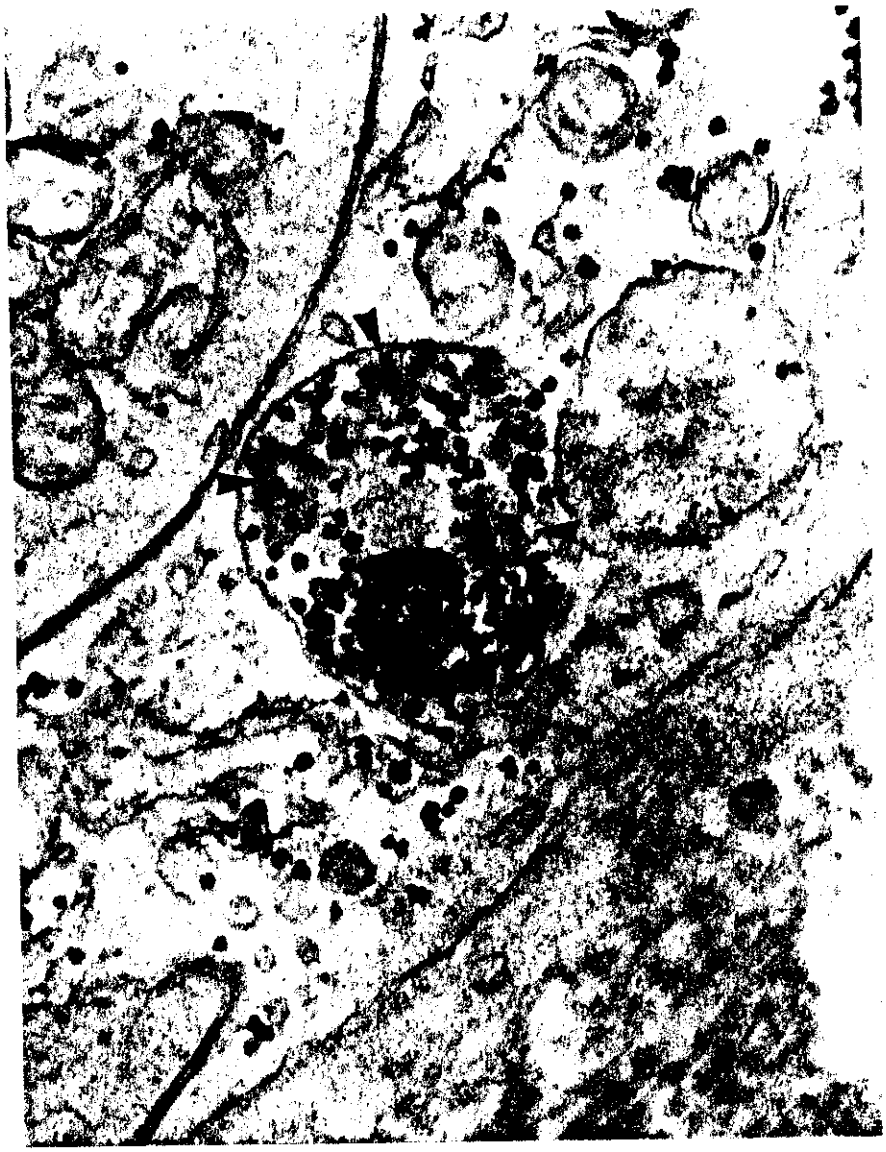


Fig. 60: Labelling of an autophagic vaccule with Cat. F. (arrowheads). Glycogen (large black dots) is seen in the cytoplasm and in autophagic vaccule. Six days of culture. Pulsed with Cat. F. X63,000.

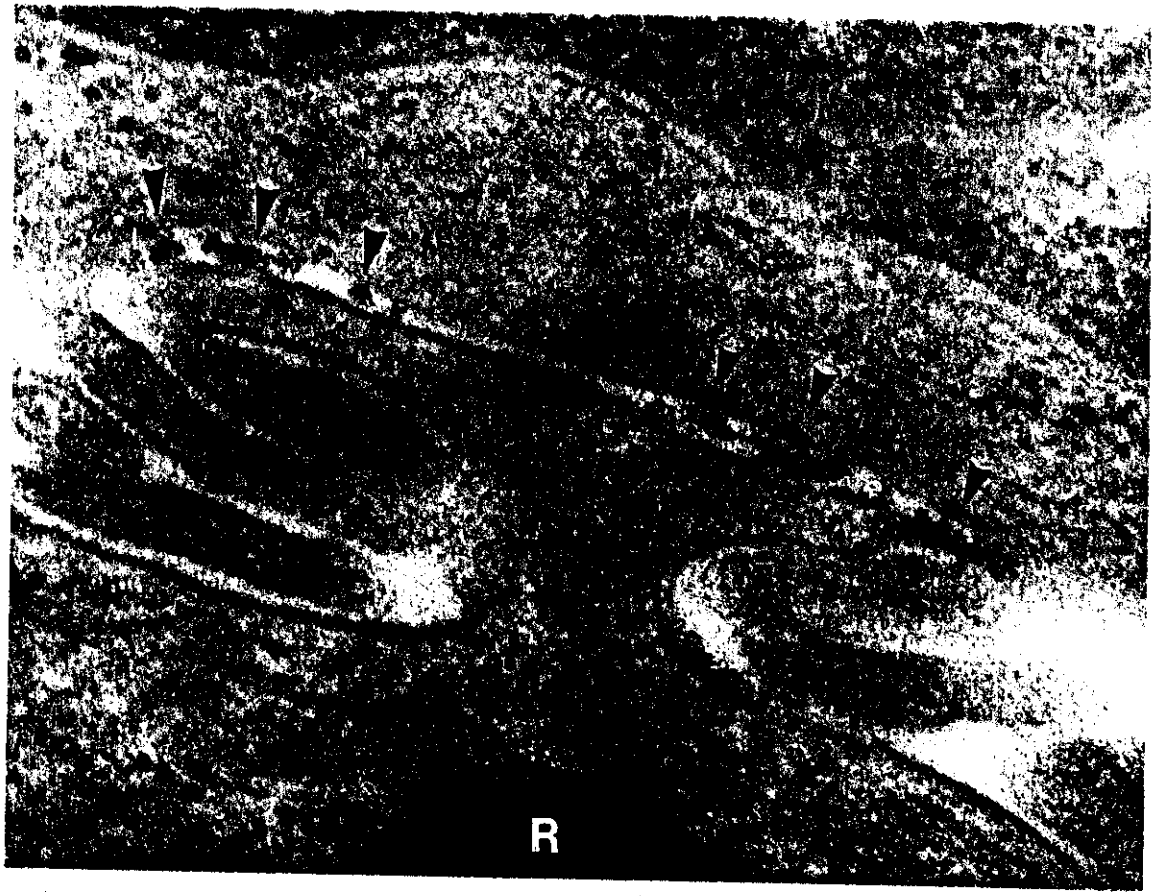


Fig. 61: Cat. F. appears in the intercellular space as a series of clumps (arrowheads). Residual body (R) is labelled. Note the cell junction (J). Six days of culture exposed to Cat. F. for eight hours. X104,000.

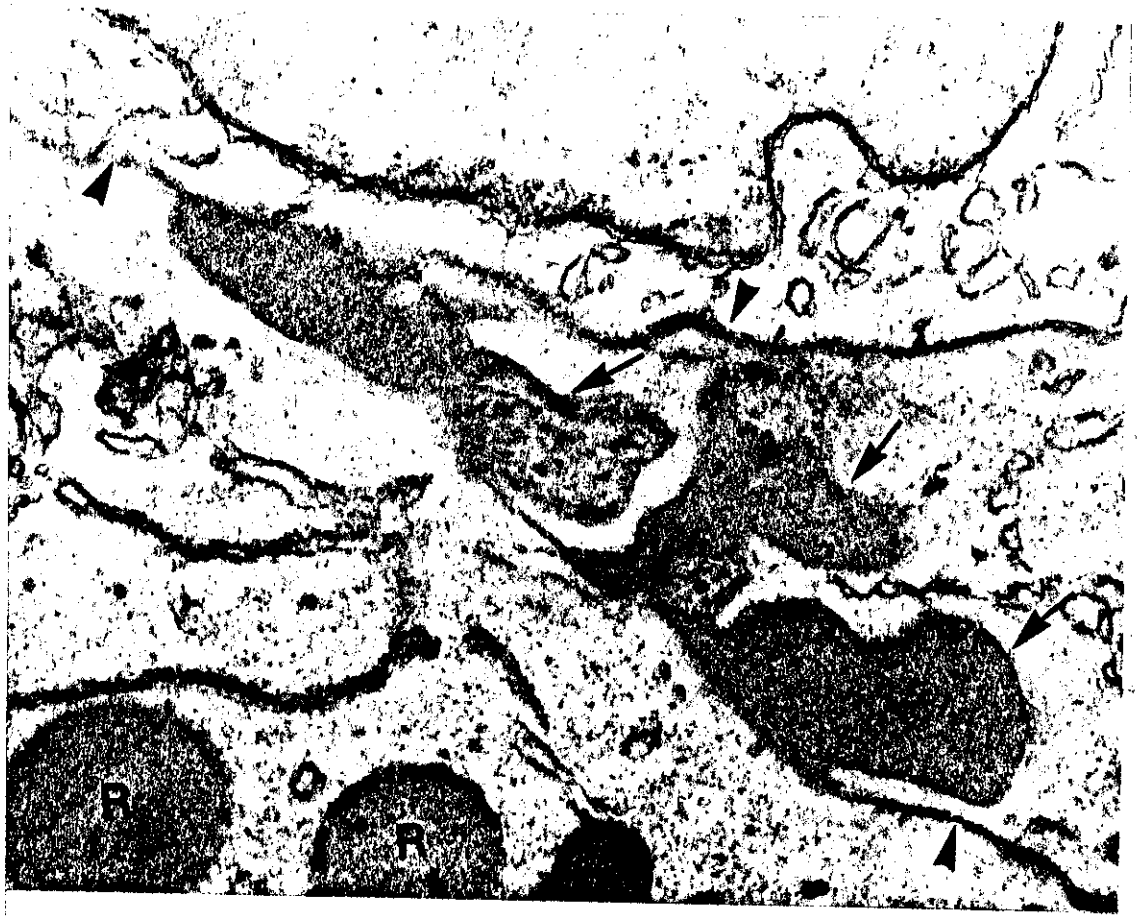
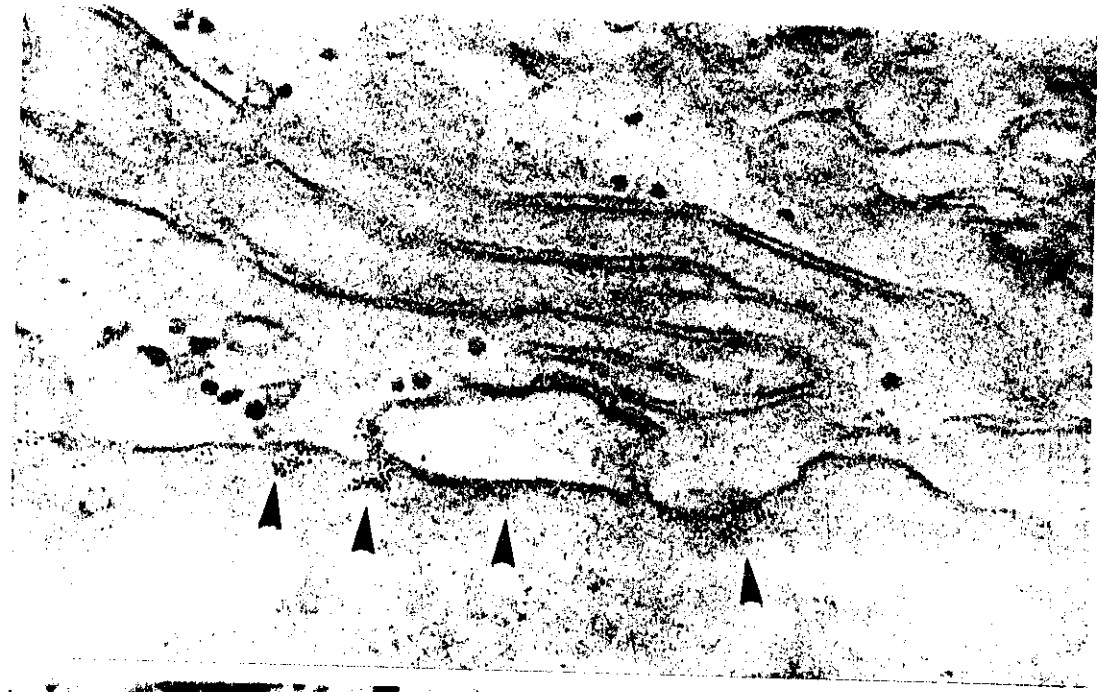


Fig. 62: Epithelial cells exposed to Cat. F. for 16 hours showing accumulation of the marker in large amounts in some dilatations between lateral cell membranes (arrows). Elsewhere, the cell membranes are closely apposed with small numbers of marker particles between them (arrowheads). Portions of residual bodies (R) contain Cat. F. Six days of culture. X33,000.



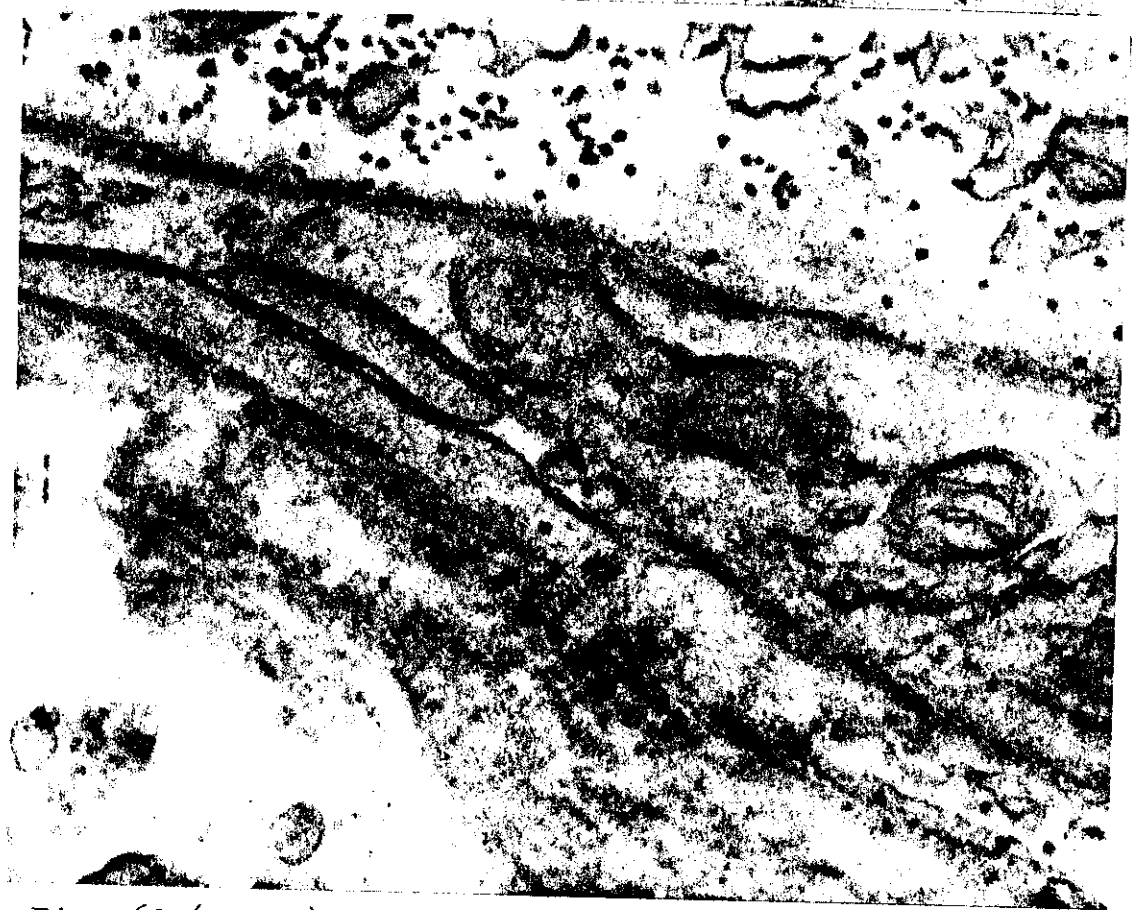


Fig. 63 (above): Basal aspect of epithelial cells pulsed with Cat. F. showing the marker (arrowheads) in the basement membrane after one hour. Ten days of culture. X82,000.

Fig. 64 (below): Basal aspect of epithelial cells exposed to Cat. F. for two hours showing collection of marker in the basement membrane. Two days of culture. X63,000.

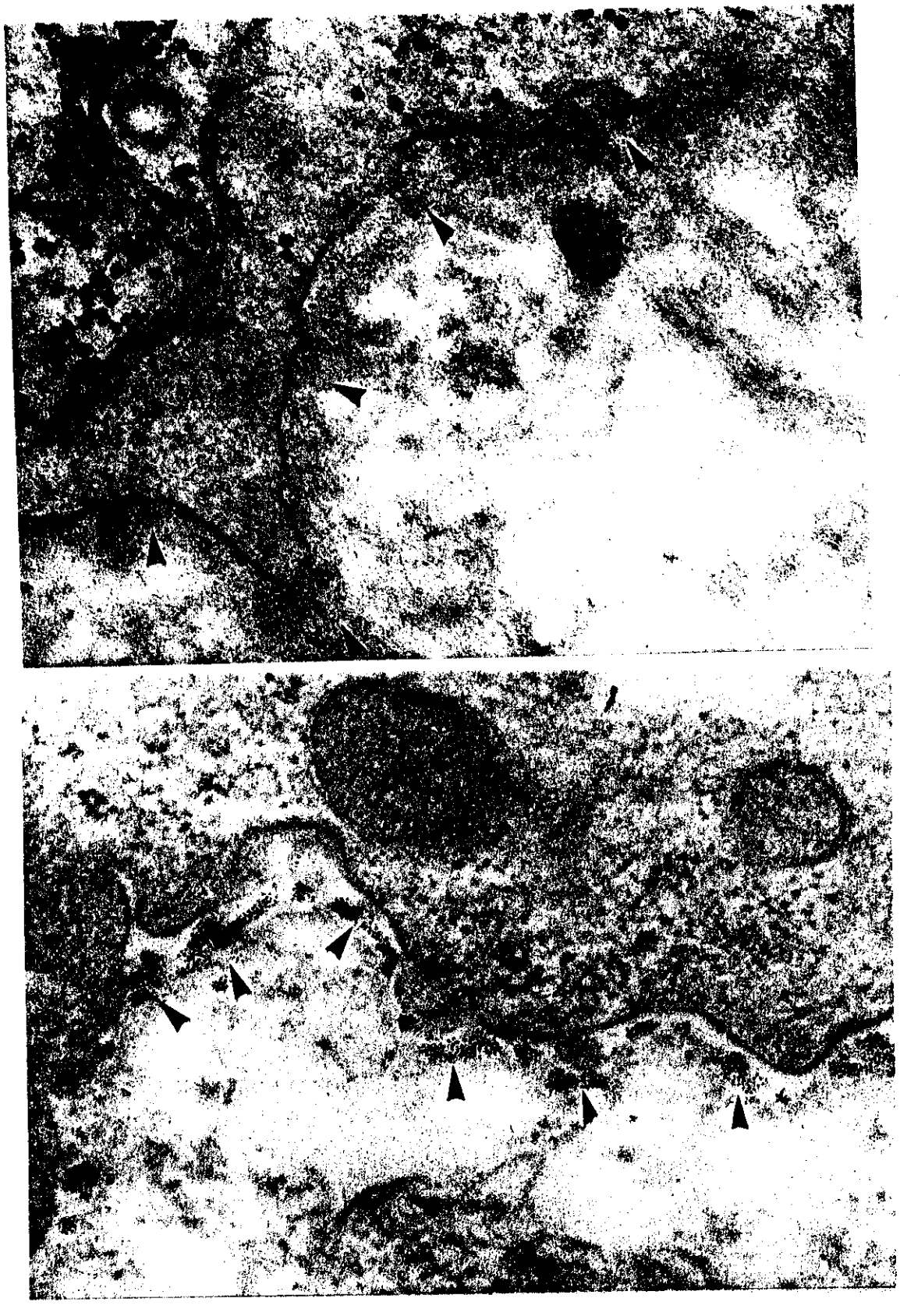


Fig. 65 (above): Base of an epithelial cell exposed to Cat. F. for four hours. More clumps of the marker (arrowheads) are seen in the basement membrane and take on a quasi-regular array. Ten days of culture. X82,000.

Fig. 66 (below): Epithelial cells exposed to Cat. F. for eight hours showing a quasi-regular and extensive accumulation of Cat. F. particles (arrowheads) in the basement membrane. Six days of culture. X82,000.

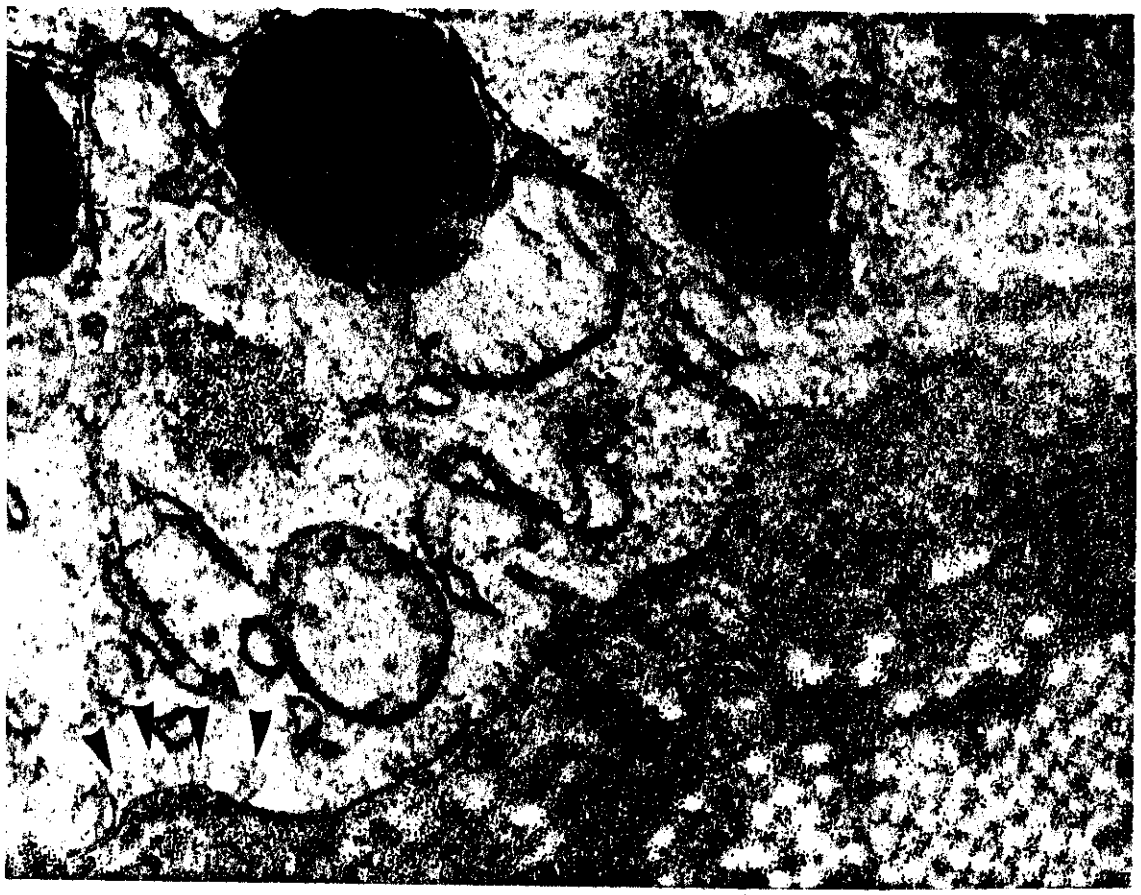


Fig. 67: Epithelial cells exposed to Cat. F. for 16 hours. The marker is seen in pits at basal aspect of cells (arrowheads). Extensive amount of Cat. F. is seen in the connective tissue (CT) which may be the origin of Cat. F. seen in basal pits. Two days of culture. X80,000.

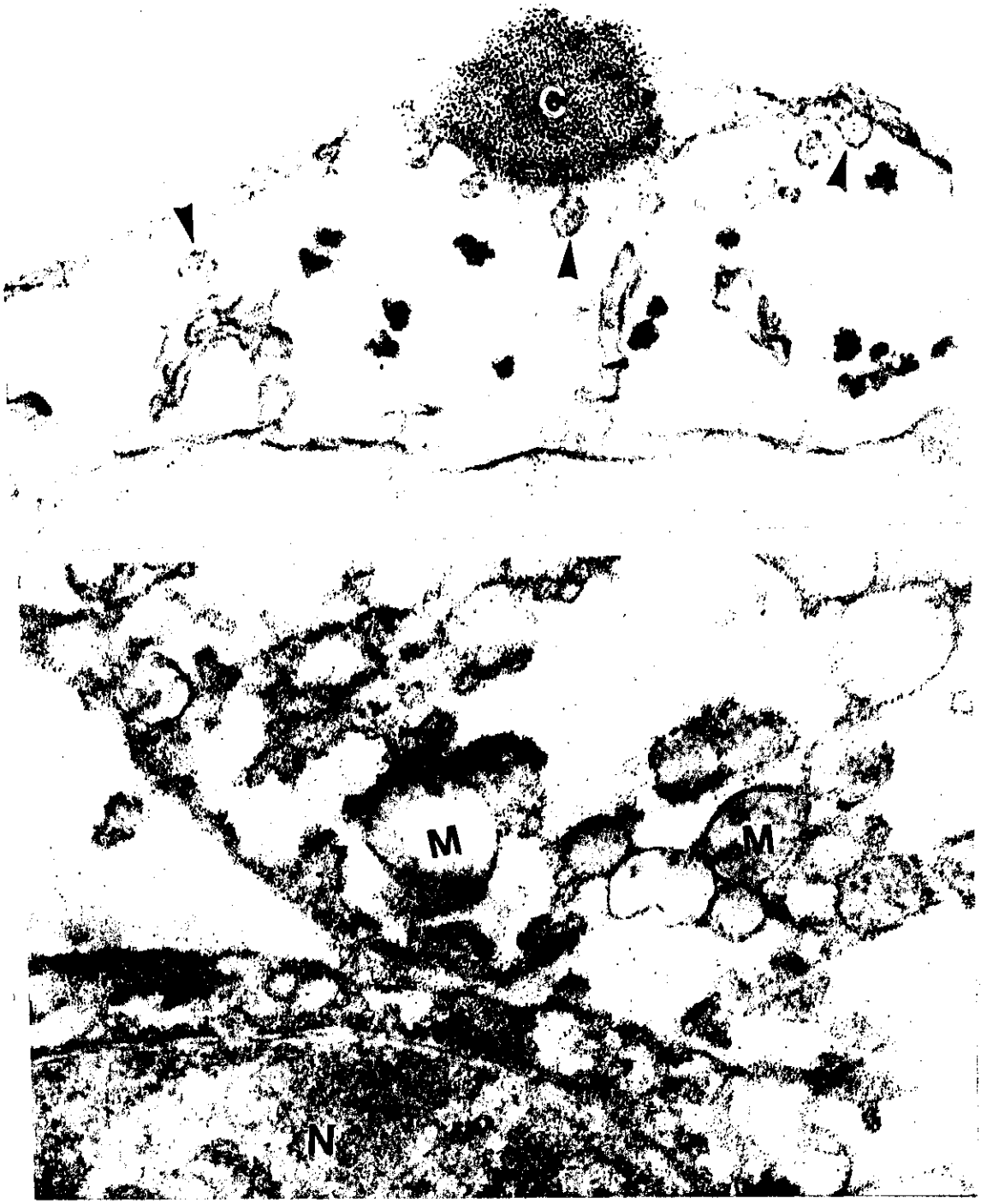


Fig. 68 (above): An endothelial cell showing Cat. F. in pinocytotic vesicles (arrowheads) and small collection of marker (C) on the abluminal surface. Six days of culture, pulsed with Cat. F. X82,000.

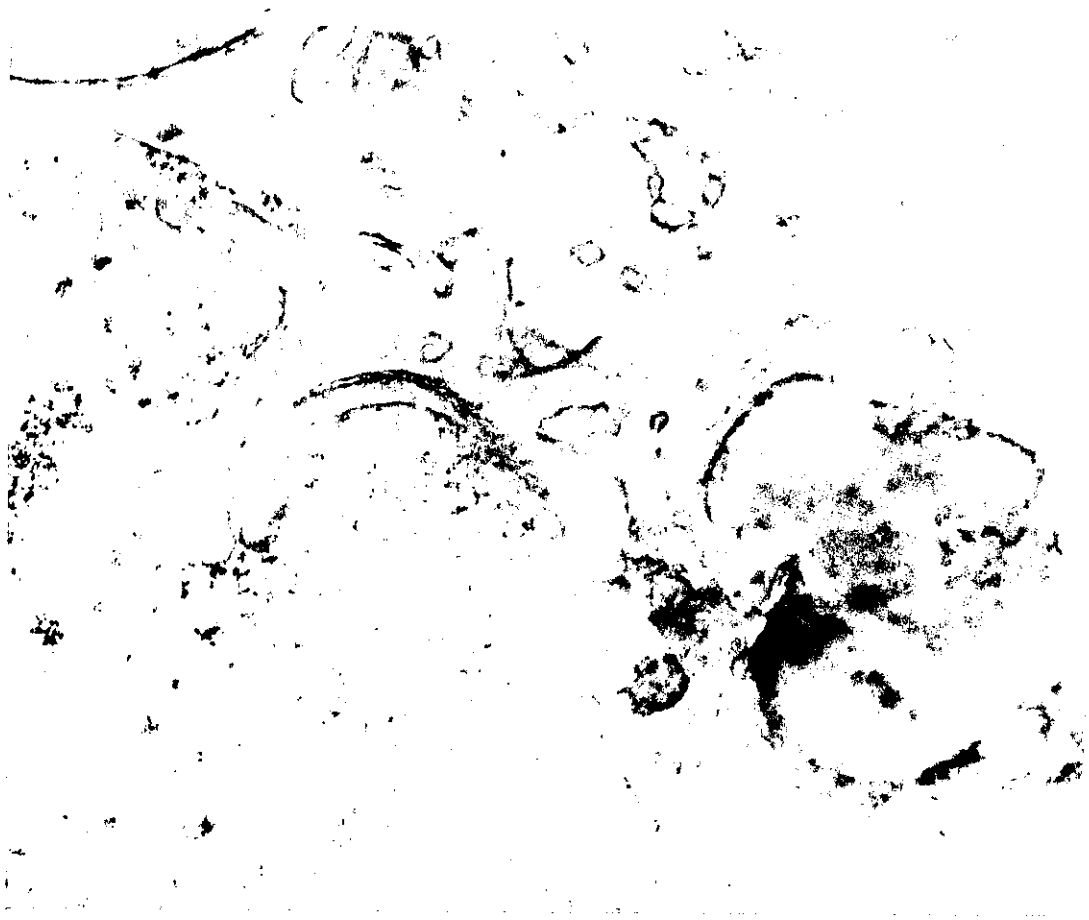
Fig. 69 (below): Cell debris decorated with Cat. F. The various mitochondria (M) exclude the label from the interior as does the nucleus (N). X50,000.

time, Cat. F. was also seen in the underlying connective tissue although in some instances this had diffused directly into the lamina propria. Small pits, some labelled, were seen on the basal membrane of cells which had been incubated in Cat. F. for four hours or longer (Fig. 67). In one or two instances, the endothelium of capillaries showed uptake of Cat. F. with labelling of the pinocytotic vesicles (Fig. 68) and small collections of marker on the abluminal surface.

Native ferritin was not bound or internalised by the gallbladder epithelium.

6. Effect of tripotassium dicitrato-bismuthate (TDB)

TDB was visualised when it was added to the culture medium at 10% (V:V) while at 2.5% or 5% was not detected. Cultured guinea pig gallbladder cells grown in the presence of TDB showed no signs of damage. TDB permeated through the lamina propria in a similar manner to other uncharged markers such as dextran and glycogen and there was no particular alignment relative to the collagen fibres nor did TDB become attached to the basement membrane in any regular array (Fig. 70-74). Although there was no evidence of binding to the apical cell membrane nor was it found in apical vesicles, TDB crystals were seen at the periphery of lipid droplets (Fig. 73-75) and no where else in the cytoplasm. TDB was seen also in the intercellular space (Fig. 72, 73).



Figures 70-75 are transmission electron micrographs of guinea pig gallbladder cells cultured for two days in medium I then exposed to TDB continuously up to 24 hours.

Fig. 70 (above): Small crystals of TDB (black dots) are seen throughout the lamina propria. Four hours exposure to TDB. X50,000.

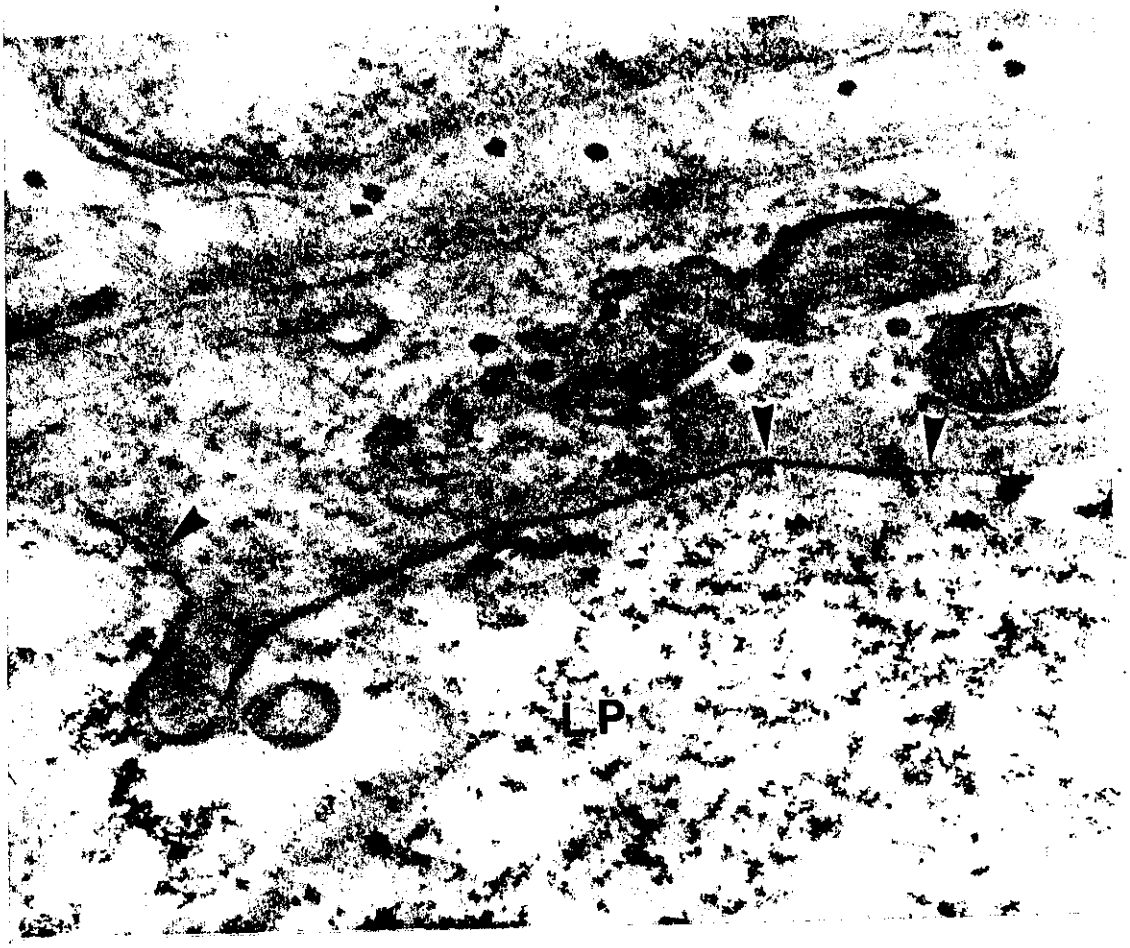


Fig. 71: Basal aspect of epithelial cell exposed to TDB for four hours. TDB crystals can be seen dispersed throughout the lamina propria (LP). Some have apparently crossed the basement membrane (arrowhead). X66,000.

Fig. 72: TDB crystals can be seen in the lamina propria (LP) and in the lateral intercellular space (arrowheads). Four hours exposure to TDB. X20,000.

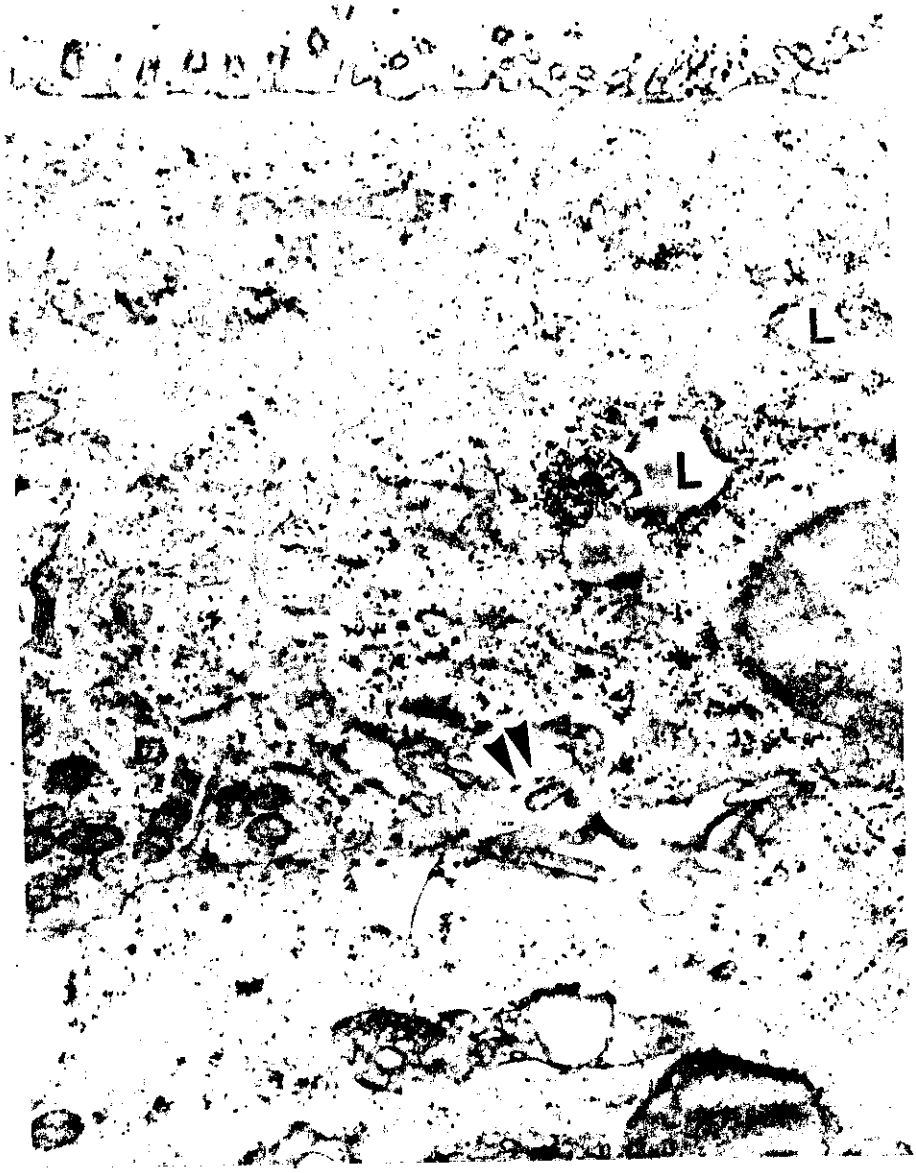


Fig. 73: Epithelial cells exposed to TDB for four hours. TDB crystals can be seen at the periphery of lipid droplets (L) and in the intercellular space (arrowheads). Some crystals are present at the apical cell membrane but not in any apical vesicles. X 27,000.

Fig. 74 (above): Epithelial cell showing TDB crystals throughout the lamina propria (LP) and at the periphery of a lipid droplet (L) after four hours of exposure to TDB. X60,000.

Fig. 75 (below): Lipid droplets with TDB at the periphery. Four hours exposure to TDB. X60,000.

7. Enzyme studies

a) Enzyme activities

Under the conditions of assay alkaline phosphatase, acid phosphatase and β-glucuronidase activities, shown b the release of 4-methylumbelliferone, were linear for at least 90 minutes of incubation at a protein concentratio in the range of 0.05 - 2 mg/ml homogenate. Enzyme activities were related to the homogenate protein and expressed as mu/mg protein where one unit is defined as the amount of enzyme liberating 1 μmol of 4-methylumbell ferone in 15 minutes (for phosphatases) or 30 minutes (fo β-glucuronidase), the respective incubation times.

M.A.O. activity was linear for at least 20 minutes of incubation at a protein concentration in the range of 0.25 - 0.75 mg/ml homogenate. Enzyme activity, again related to the homogenate protein, was expressed as mu/m protein where one unit is the amount of enzyme liberatin 1 μmol of hydrogen peroxide in 15 minutes.

These four enzymes were estimated in three culture sets but no uniform trends were apparent.

i) Alkaline phosphatase

Alkaline phosphatase activity remained comparatively constant in two sets while in the third set there was a transient rise at two days followed by a fall and constant activity between day 3 and day 6 (Fig. 76).

ii) Acid phosphatase

Acid phosphatase showed higher activity than alkaline phosphatase activity and, in two sets, an initial rise was followed by a fall and relatively constant activity after day 4. In the third set the rise occurred at day 4, followed by a decrease to starting value by day 7. This is shown in Fig. 77.

iii) β-glucuronidase

β-glucuronidase was constant in one set but in another set after constant activity for four days there was a rise between day 5 and day 6 followed by a decline towards the initial value. In the third set there was a general decline in activity for three days and then it remained more or less constant up to six days (Fig. 78).

iv) Monoamine oxidase

M.A.O. was relatively constant in two sets, although in the third set M.A.O. activity showed a general decline until day 4 after which it remained constant up to day 7 (Fig. 79).

b) Precision

Coefficient of variation of each enzyme studied was found to be less than 6%.

Fig. 76: Alkaline phosphatase activity shown in three different guinea pig gallbladder culture sets. Cells harvested from 25 petri dishes, each containing 6-8 tissue fragments, were used for each assay.

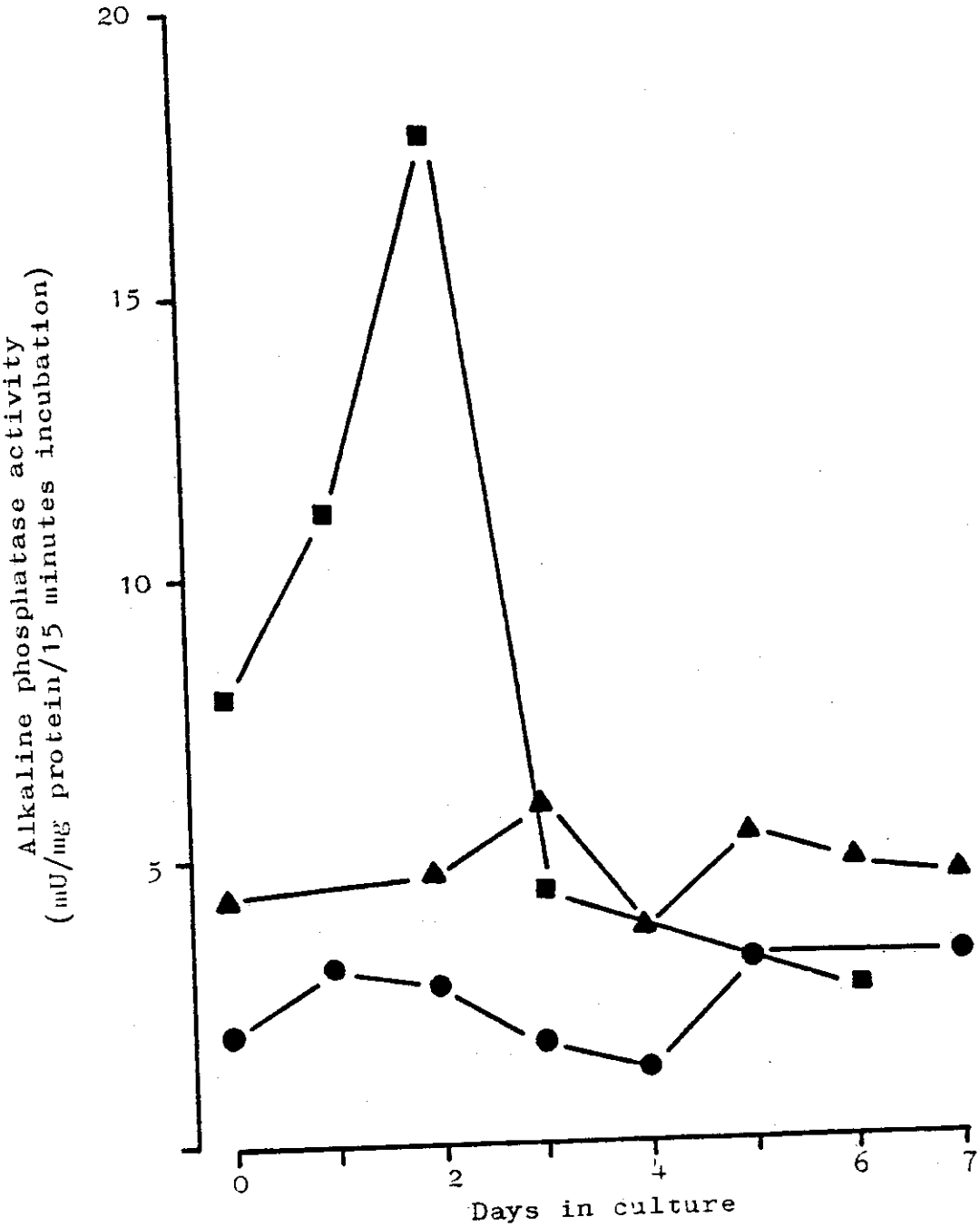


Fig. 77: Acid phosphatase activity shown in three different guinea pig gallbladder culture sets. Cells harvested from 25 petri dishes, each containing 6-8 tissue fragments, were used for each assay.

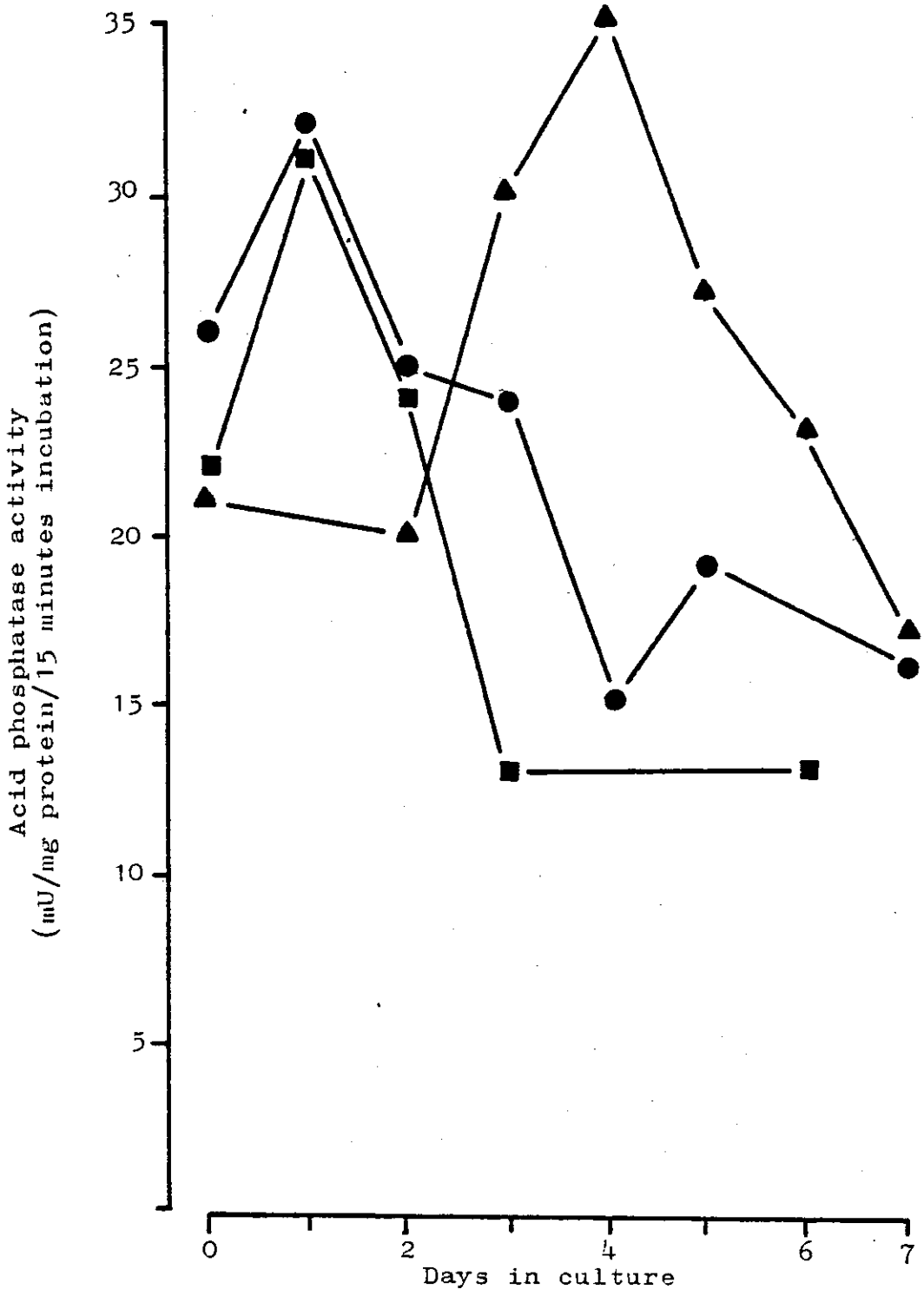


Fig. 78: β-glucuronidase activity shown in three different guinea pig gallbladder culture sets. Cells harvested from 25 petri dishes, each containing 6-8 tissue fragments, were used for each assay.

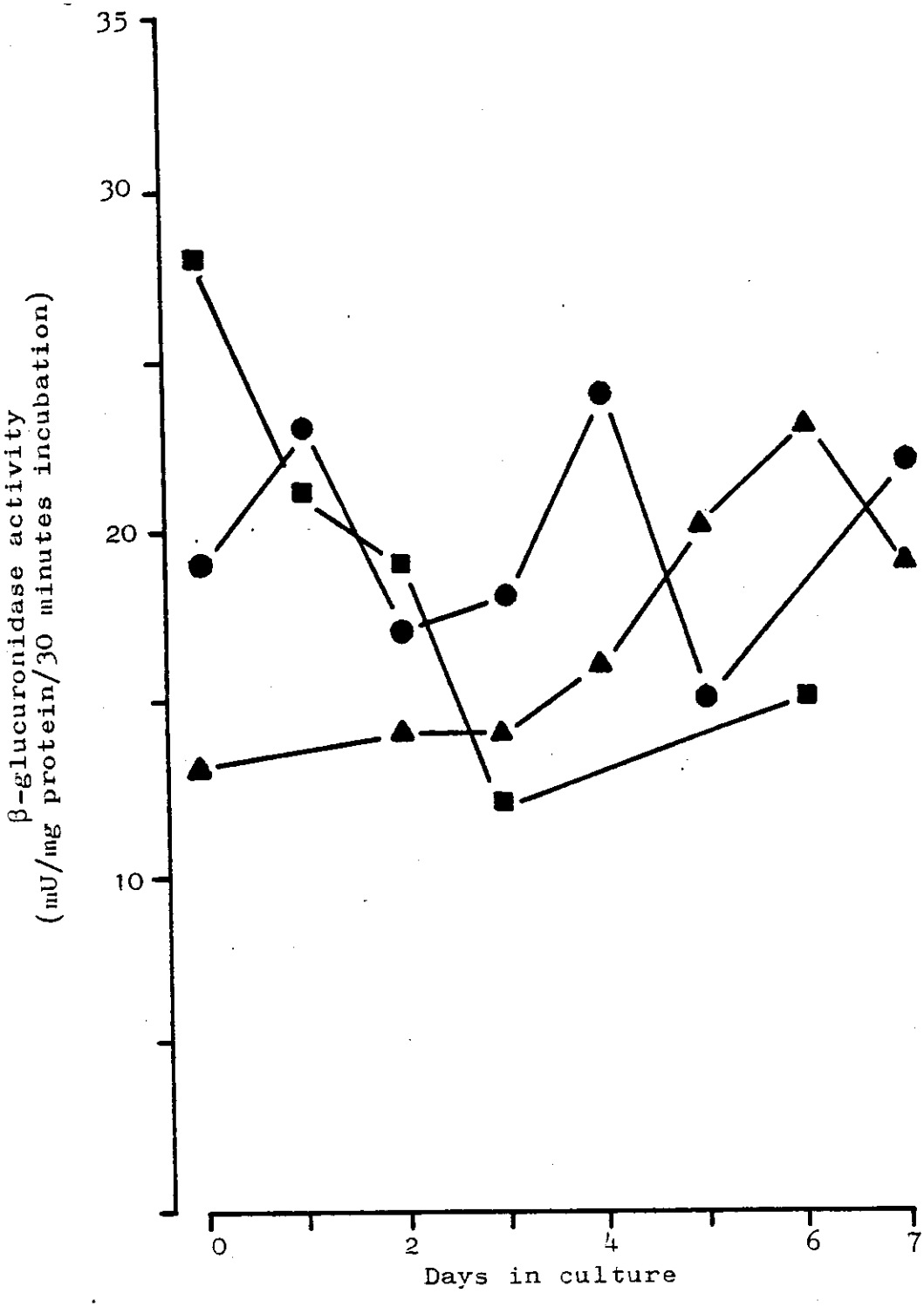
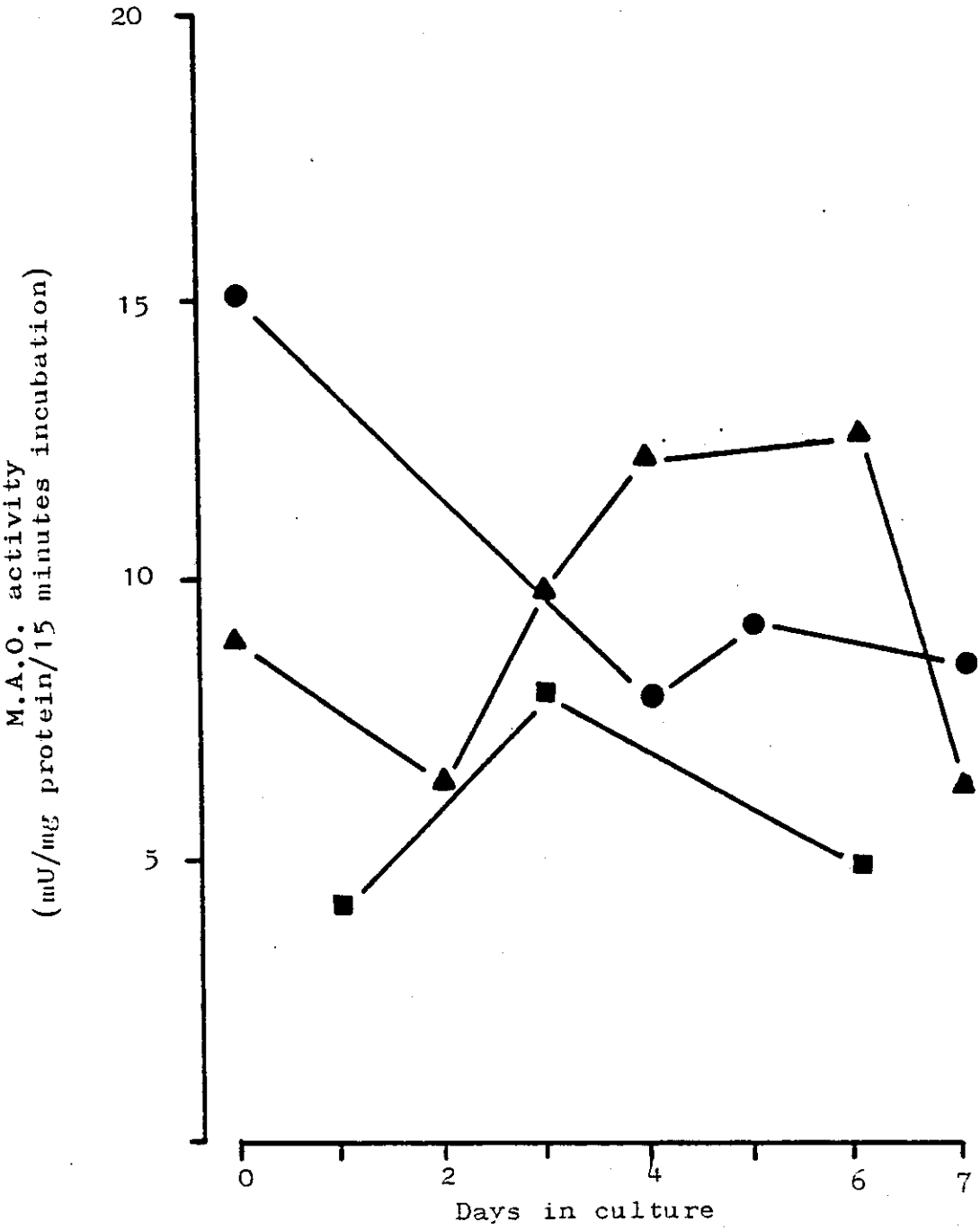


Fig. 79: M.A.O. activity shown in three different guinea pig gallbladder culture sets. Cells harvested from 25 petri dishes, each containing 6-8 tissue fragments, were used for each assay.



8. DNA assay

Under the conditions of assay, DNA concentration gave a linear response at a DNA concentration in the range of 10-100 ng. DNA content was estimated from a calibration graph included with each assay.

9. DNA synthesis

At varying periods of culture, [^{3}H] thymidine was added as a pulse for one hour in the presence of deoxycytidine. Radioactivity incorporated into DNA was expressed as dpm/μg DNA. In the two cultures studied [^{3}H] thymidine incorporation showed a gradual increase until it reached a maximum between 2.5 - 3 days of culture and then it remained more or less constant up to seven days (Fig. 80).

10. Protein synthesis

At varying culture periods [^{3}H] leucine was given as a pulse for one hour. Radioactivity incorporated into protein was expressed as dpm/μg protein. Both culture sets showed the same pattern of radioactivity incorporation as [^{3}H] leucine increased to a maximum after one day and remained more or less constant up to seven days of culture (Fig. 81).

11. Effect of bile

Guinea pig gallbladder cultured in the presence

Fig. 80: DNA synthesis by cultured guinea pig gallbladder cells following a one hour pulse of [^{3}H] thymidine. Cells harvested from 15 petri dishes, each containing 6-8 tissue fragments, were used for each assay. Each line represents a different culture set.

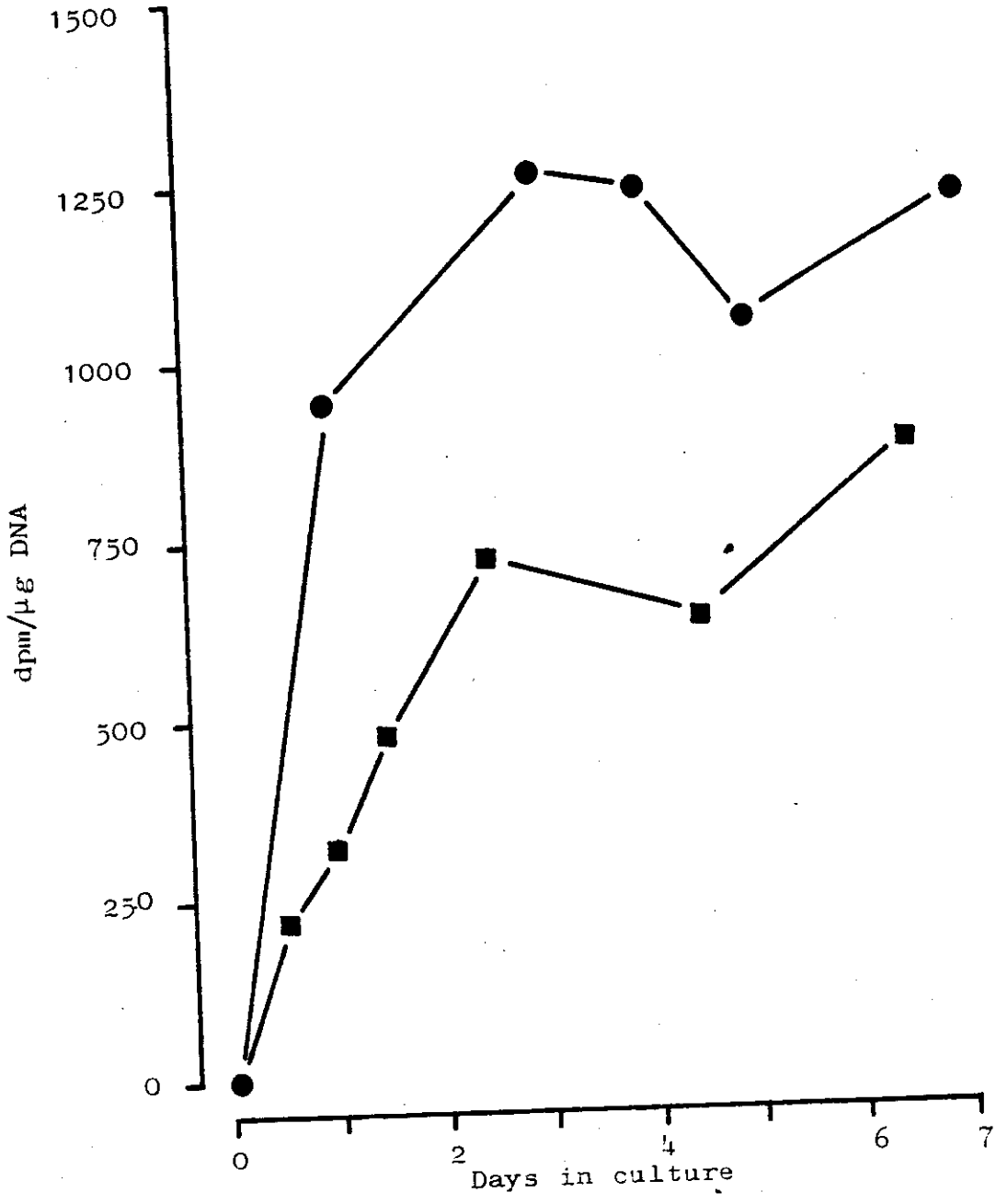
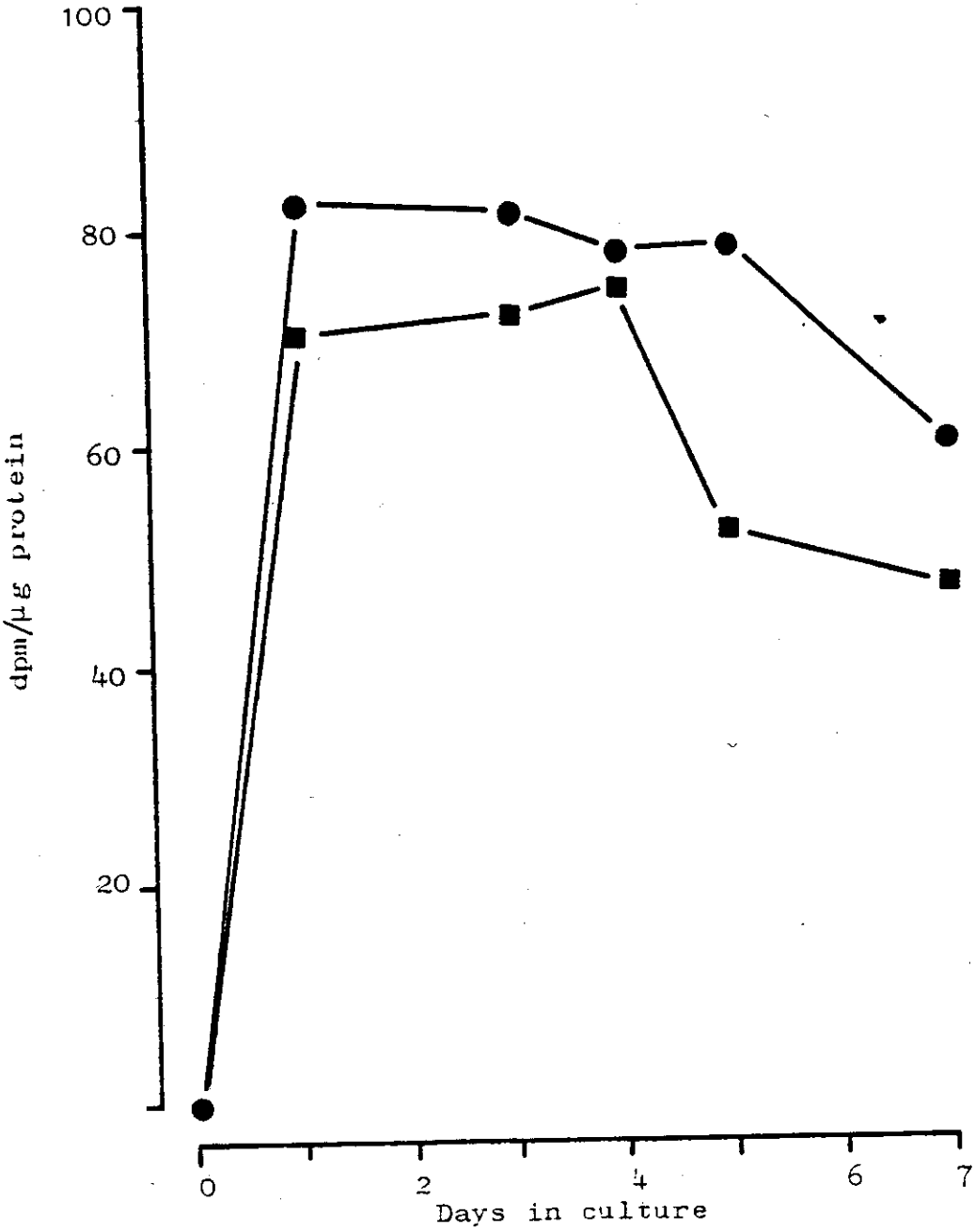


Fig. 81: Protein synthesis by cultured guinea pig gallbladder cells following a one hour pulse of [^{3}H] Leucine. Cells harvested from 10 petri dishes, each containing 6-8 tissue fragments, were used for each assay. Each line represents a different culture set.



of bile showed oedema in many epithelial cells and damage to the organelles (Fig. 82).

12. Human gallbladder

a) Culture

As in guinea pig gallbladder culture, epithelial cell outgrowth was observed within 1-2 days of culture. Good ultrastructural preservation of epithelial cell morphology including organelles, cell junctions and microvilli with a well developed glycocalyx (Fig. 86-93) was maintained up to seven days, the culture period tried. Lateral interdigitating folds (Fig. 89), microfilaments (Fig. 90) and folded basement membrane (Fig. 88) were also observed. Glycogen in fields (Fig. 91) and in dispersed form in the cytoplasm (Fig. 93) were noted after one day of culture. At this time, lipid droplets were also observed (Fig. 87). In contrast to guinea pig, mucous droplets were maintained in cultured human gallbladder cells for up to seven days (Fig. 91).

b) Absorption of cationized ferritin

One human gallbladder cultured for two days was exposed to Cat. F. for up to 24 hours. The marker was bound to the apical cell membrane and microvilli which showed clumping (Fig. 94, 95). Mats of the marker

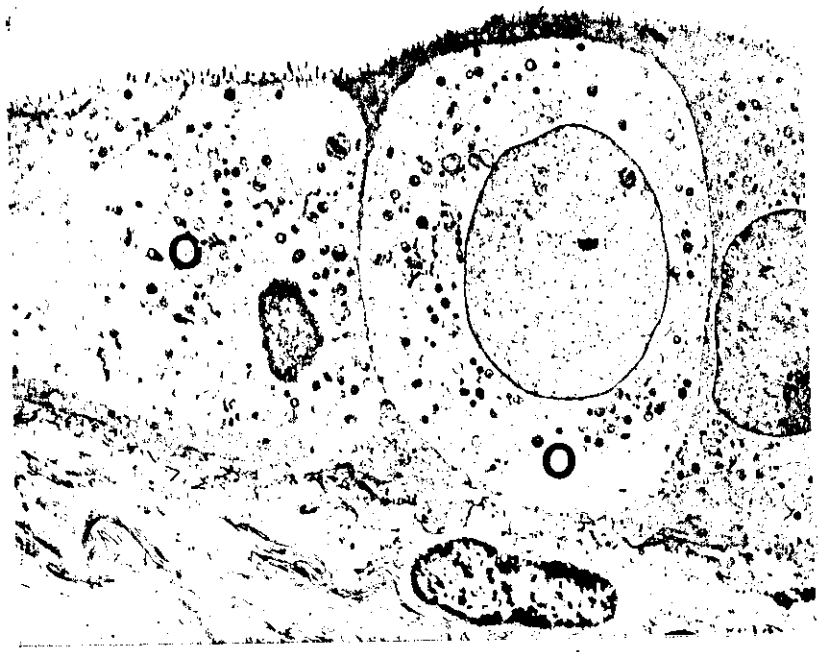
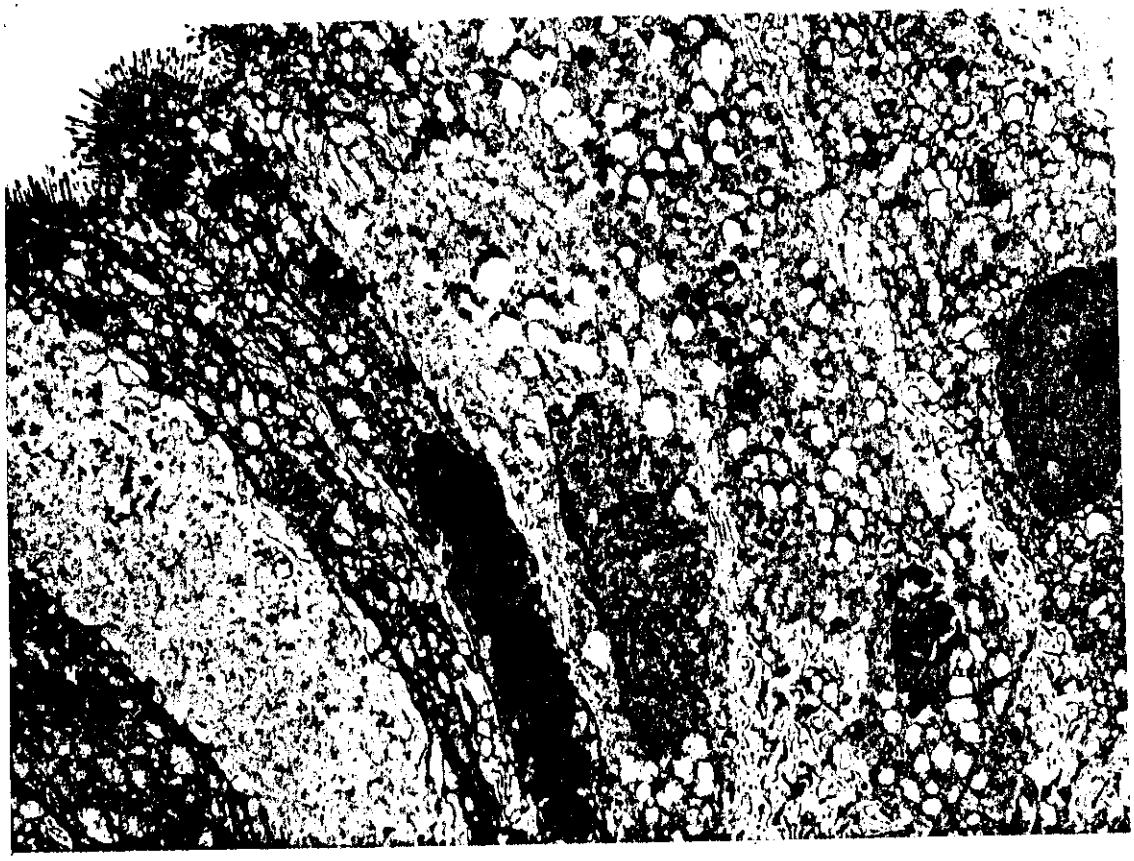


Fig. 82: Gall bladder epithelium grown in the presence of bile for one day. Several epithelial cells are oedematous (O) and the organelles are damaged. X2,500.



Figures 83-93 are transmission electron micrographs of human gallbladder cells before culture (control) and after culture for various periods up to seven days in medium I.

Fig. 83 (above): Epithelial cells (control) showing general damage which may be due to anoxia at surgery. X1,600.

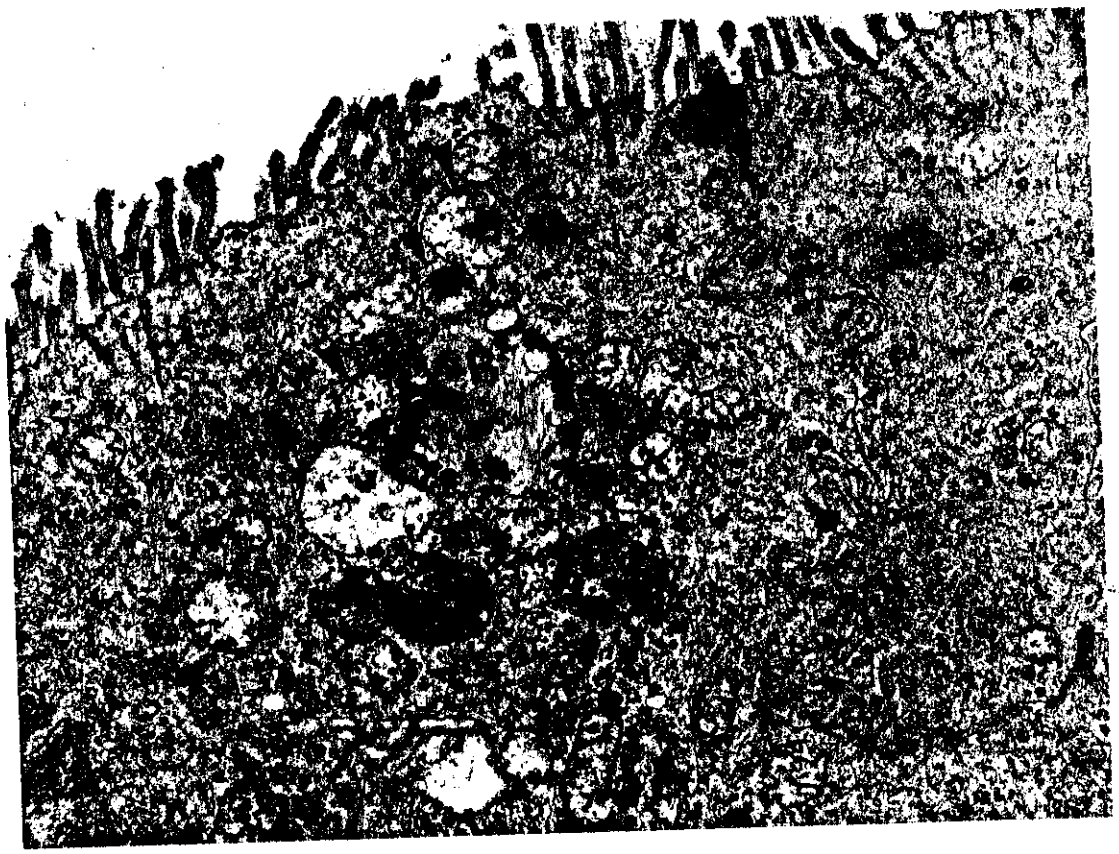


Fig. 84: Epithelial cells (control) showing severe damage to mitochondria (arrowheads). Residual body (R) with microfilaments is seen. X30,600.

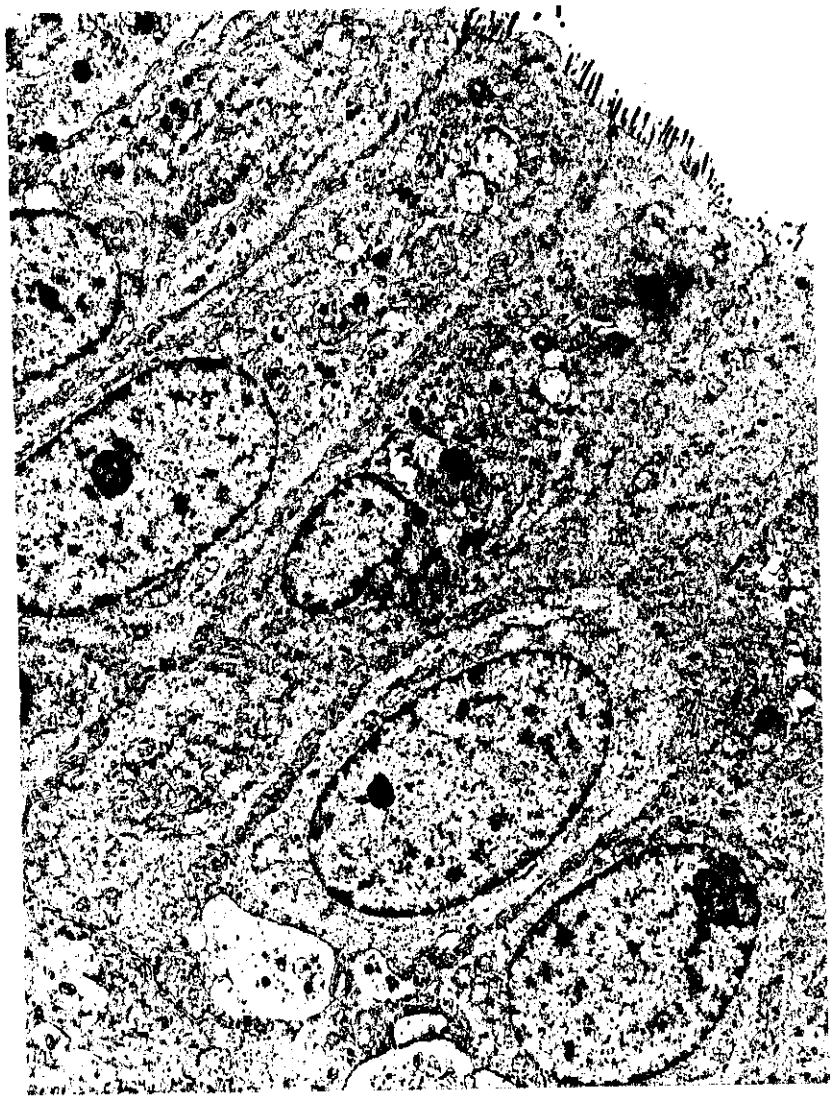


Fig. 85: Epithelial cells (control) with little mitochondrial damage. X24,000.

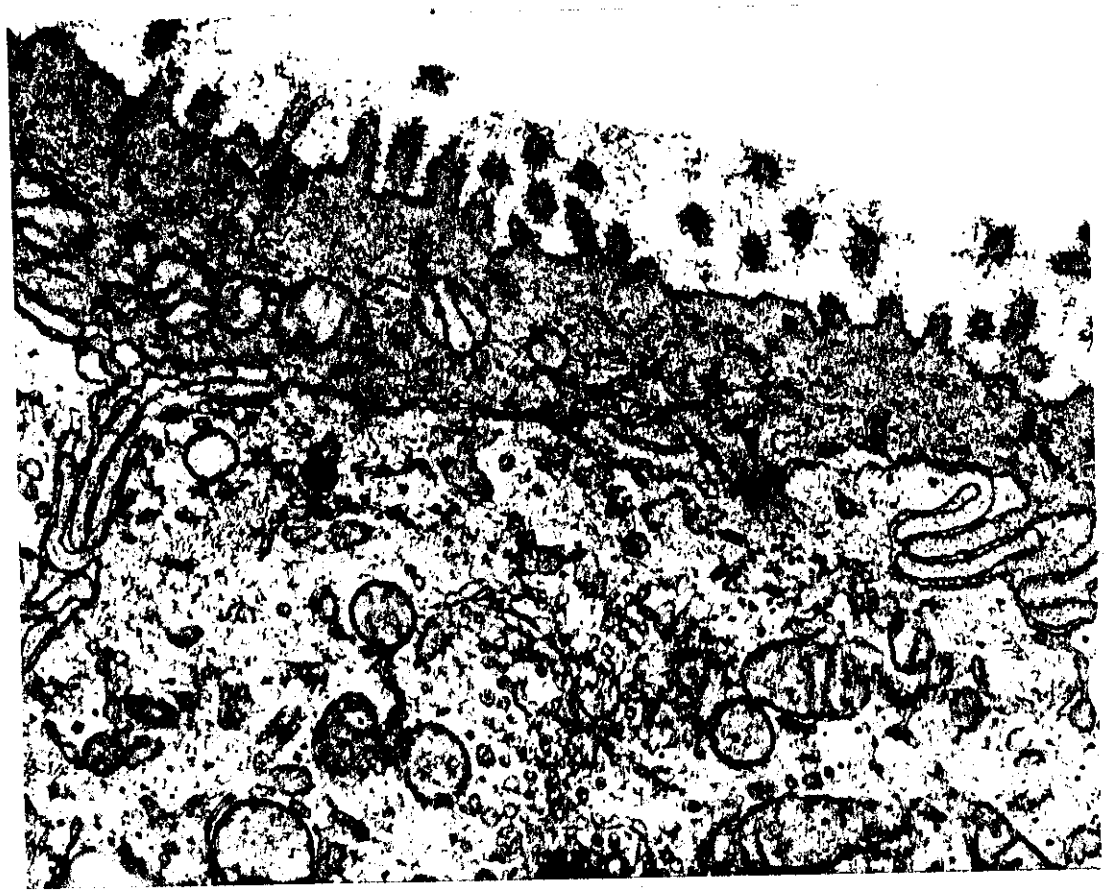


Fig. 86: Oblique section of epithelial cells after one day of culture showing microvilli with well marked glycocalyx, well preserved mitochondria and cell junctions (arrowheads). X17,000.

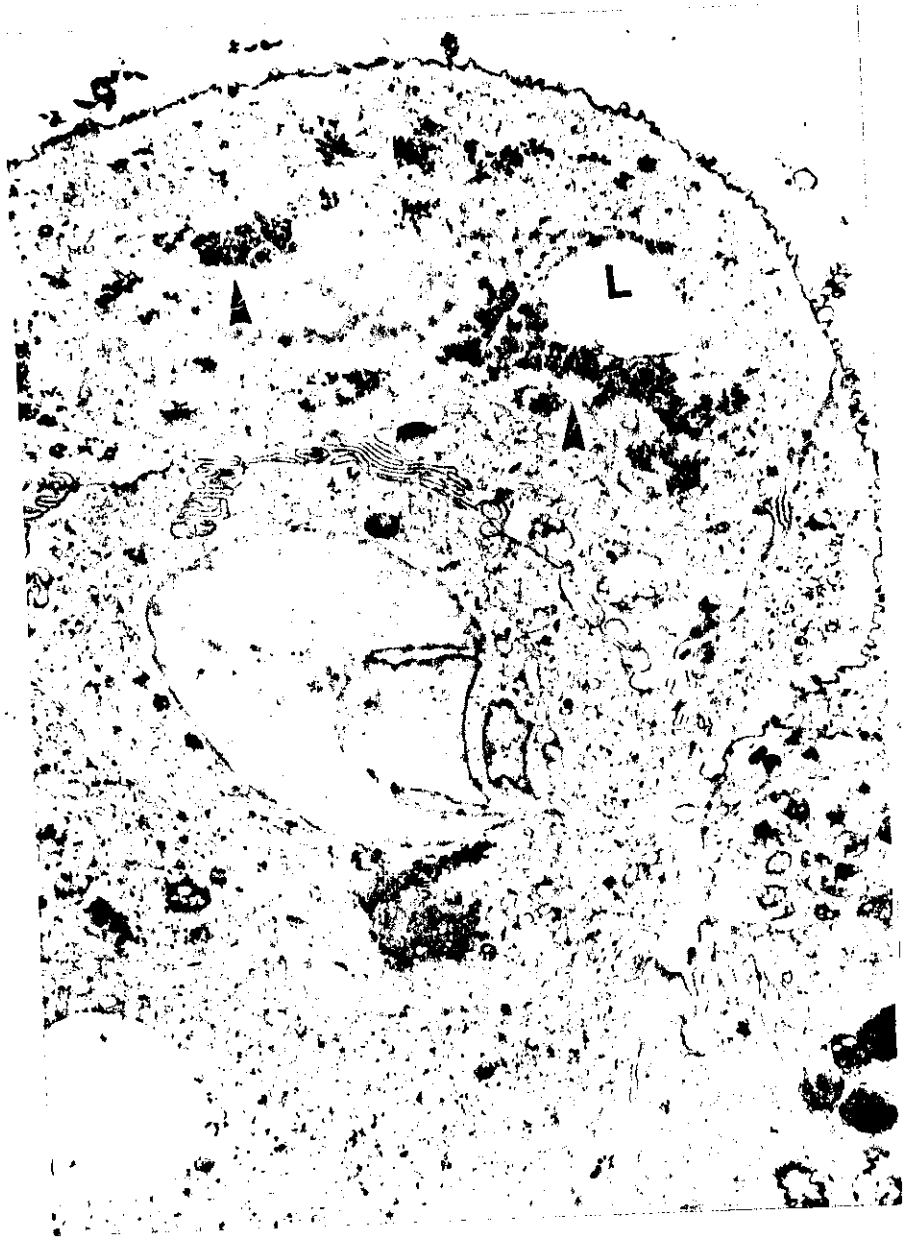


Fig. 87: Epithelial cells cultured for two days showing glycogen aggregates (arrowheads) and lipid droplet (L) in the cytoplasm. X6,000.

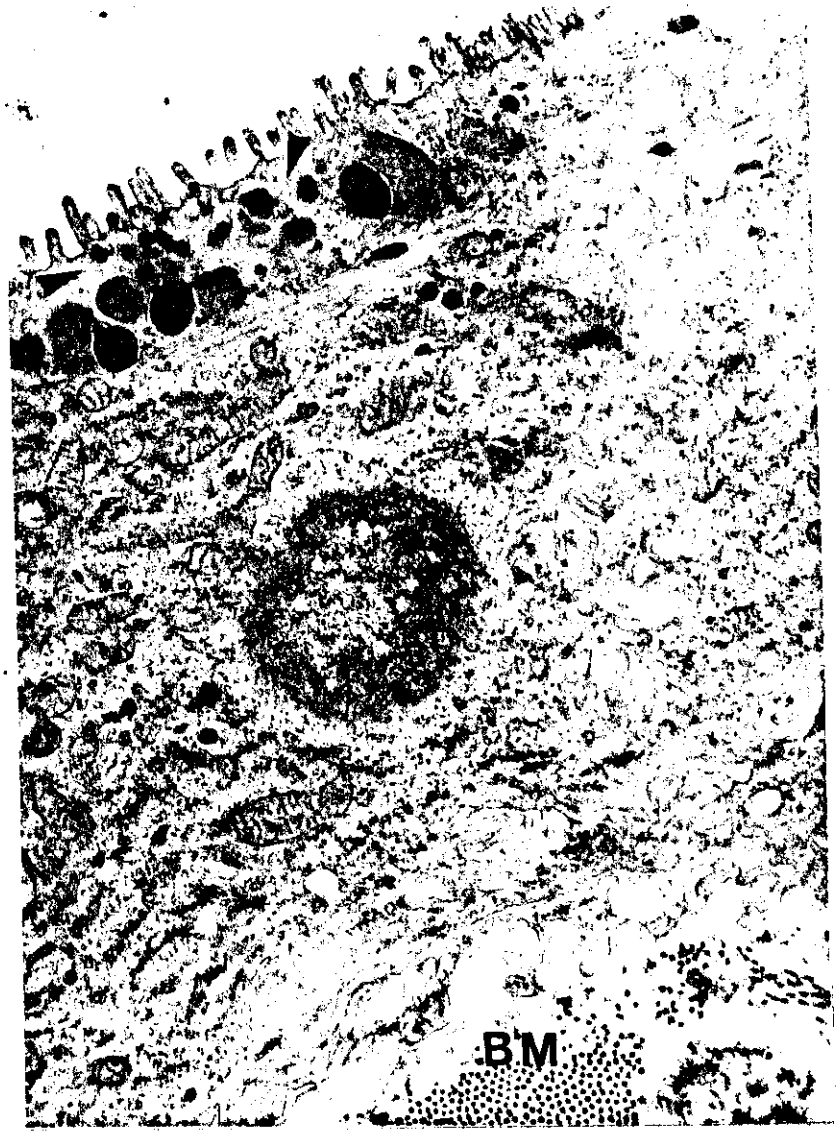


Fig. 88: Epithelial cells cultured for two days showing well preserved microvilli and mitochondria. Note the apical secretory droplets (arrowheads) and folded basement membrane (BM). X75,000.

Fig. 89: Epithelial cells showing well preserved microvilli, mitochondria, tight apical cell junction (J), Golgi apparatus (G) and the lateral interdigitating folds (F). Membranous whorls in residual body (arrowhead) which may represent cellular recovery from damage. A portion of worled body (W) is also seen. Two days of culture. X11,000.

Fig. 90: Epithelial cells showing well preserved microvilli with fuzzy coat and a desmosomal junction (arrowhead). Three days of culture. X19,000.

Fig. 91: Glycogen is seen in fields (G) and in dispersed form (black dots) in the cytoplasm. Apical secretory vesicles (arrowheads) are also seen. Note the well preserved mitochondria and microvilli. Seven days of culture. X16,000.

Fig. 92 (above): Thin migrating epithelial cells showing good preservation. Note the apical cell junction (arrowhead), secretory droplets (arrows), glycogen (small black dots) and the lateral interdigitating folds (F). Seven days of culture. X6,800.

Fig. 93 (below): Thin migrating epithelial cells showing well preserved organelles, cell junctions (arrowheads). Glycogen (black dots) is also seen. Seven days of culture. X17,200.

on the cell surface (Fig. 95) together with labelled vesicles (Fig. 96) and residual bodies (Fig. 97) were also observed. After one hour exposure, the marker appeared in the intercellular space (Fig. 98) and within four to eight hours was seen in the basement membrane (Fig. 99) indicating a transport of this marker across the human epithelial cells in a similar way as in guinea pig gallbladder cells.

Figures 94-99 are transmission electron micrographs of human gallbladder cells maintained in tissue culture for two days in medium I then exposed to Cat. F. continuously for up to 24 hours.

Fig. 94 (above): Apex of an epithelial cell showing labelling of the plasma membrane and vesicles (arrowheads) after one hour exposure to Cat. F. X15,000.

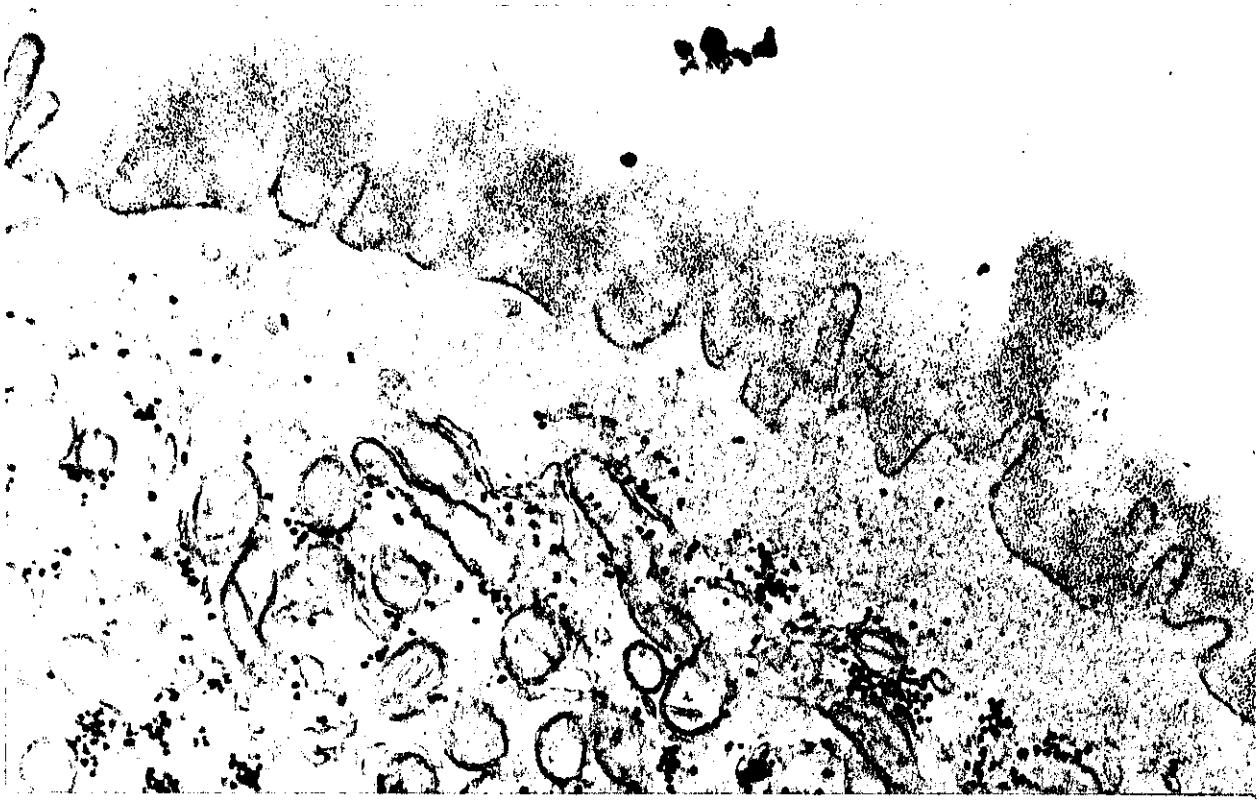
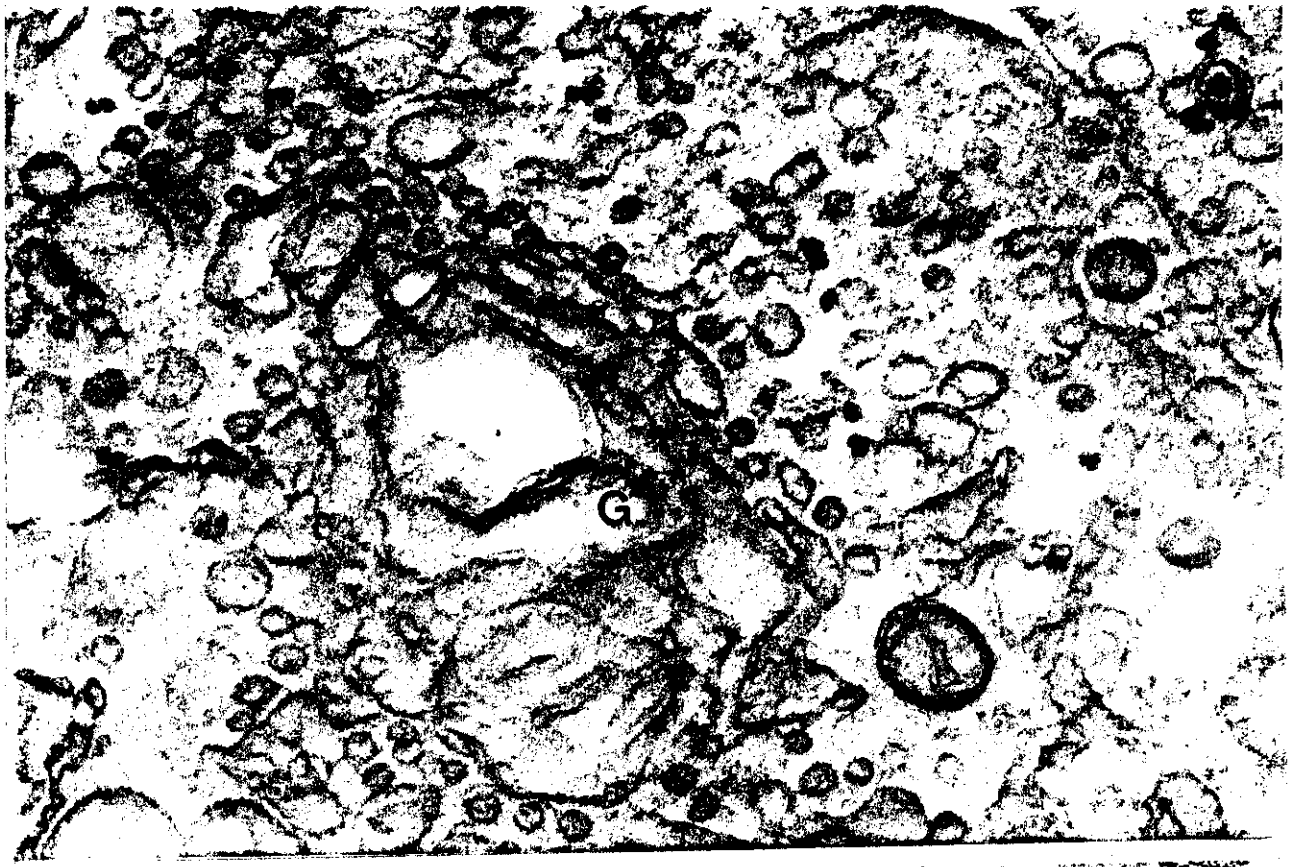


Fig. 95: Cat. F. produced a mat on the plasma membrane. Clumping of the marker is also seen. Numerous vesicles are labelled (arrowheads). One hour exposure to Cat. F. X13,000.



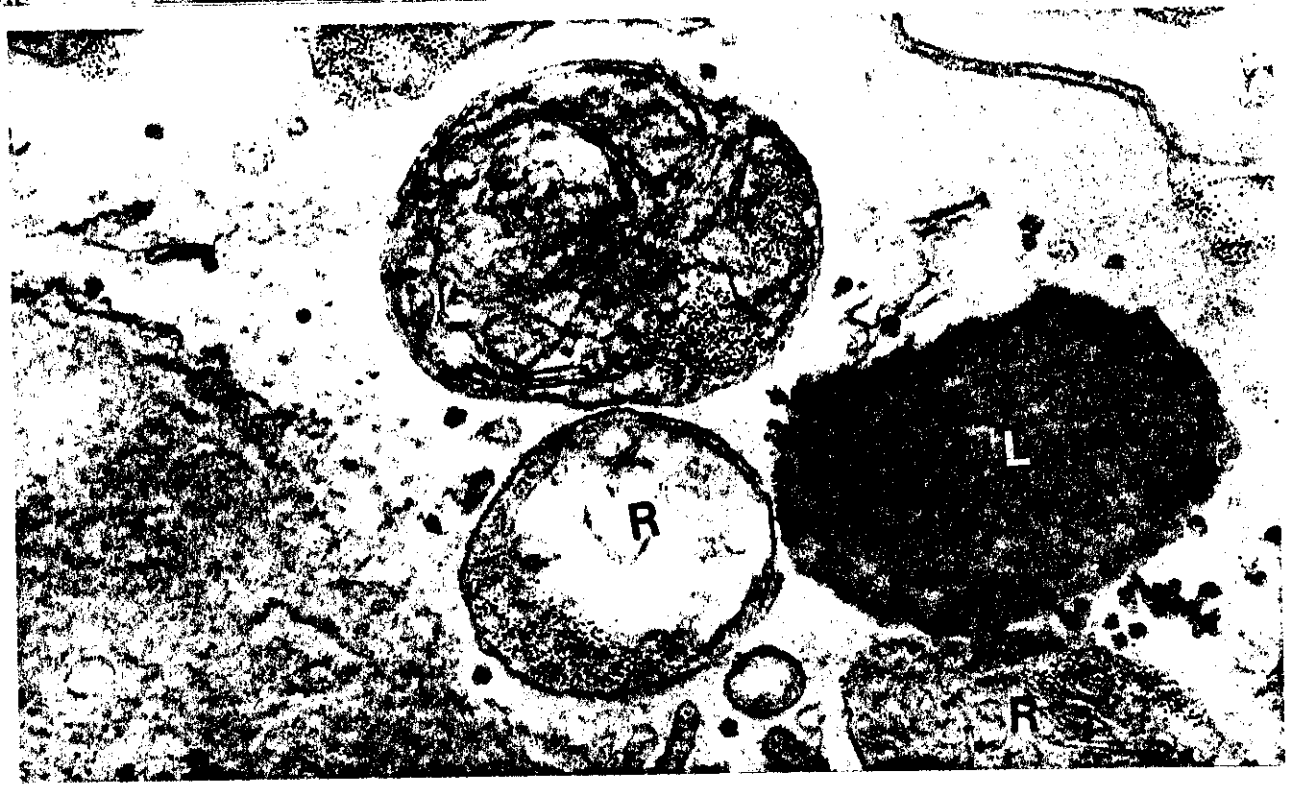


Fig. 96 (above): Portion of an epithelial cell exposed to Cat F. for two hours showing labelled vesicles (arrows). Two labelled vesicles fused with each other (arrowhead). Golgi apparatus (G) is not labelled. X72,000.

Fig. 97 (below): Two hours exposure to Cat. F. showing the marker in residual bodies (R) and between cells (arrowheads). Large lipid droplet (L) containing no marker is also seen. X72,000.

Fig. 98: Clumps of Cat. F. (arrowheads) are present in the intercellular space after one hour exposure to Cat. F. Basement membrane (BM) and portion of cell nucleus (N) are seen. X72,000.

Fig. 99: Basal aspect of epithelial cell exposed to Cat. F. for eight hours showing clumps of the marker (arrowheads) in the basement membrane (BM). X72,000.