Investigations into the Structure and Function of the Exocrine Pancreas of Lampreys, by Hans Luppa and Armin Ermisch. XXXXXXXXXX. (1967)

1. Introduction and Setting Out of the Problem.

As representatives of the most primitive of recent vertebrate groups, lampreys show fundamental differences in different features of organisation to the species of the remaining classes of vertebrates.

The topical distinction between exocrine and endocrine pancreas is also considered among the morphological peculiarities of Petromyzontes (BARGMANN 1939, ERMISCH 1966 et al.). Certain cells in the anterior epithelium of the middle intestine of lampreys are comparable to the secretory pancreatic cells of higher vertebrates, as was observed by BRECHET (1897) and whose histological description was completed in particular by MASKELL (1930 "glandular cells"), BARRINGTON (1936, 1942), WASILEWA (1961), SCHIRNER (1963) and ADAM (1963), with Myxine) as well as YAMAMOTO (1965). LUPPA (1964) was able to confirm, through spesial histochemical evidence, that the "glandular cells" of the epithelium of the middle intestine of lamprey cross-section correspond to the exocrine pancreas cells of other vertebrates with respect to their function as manufacturers of special digestive enzymes. From the results of this investigation, the secretory production is not however the exclusive function of the exocrine pancreas cells, but a simultaneous process of reabsorption is probable.

The following work will contribute to a further explanation of this problem with the help of electro-optical and histochemical methods of research.
electron and optical microscopy
It is also of interest with regard to the systematic organisation of lampreys, to improve further the cytological comparison between the pancreas cell, which must be construed as primitive, of this animal and the secretory pancreatic cells of higher vertebrates.

2. Material and Methods.

Animals: the larvae of 50 brook lampreys of 45-172 mm. long out of the Plane at Niemegk. In order to exclude the possibility of alterations taking place through handling conditions, the animals were killed by decap-

itation either on the day of capture or on one of the following days, while
the intestines were removed at once and placed in the appropriate fixing
liquid (information on the individual fixing medium in connection with the
histochemical process of preparation). Part of the material was freeze-dried
or dissected in frozen sections of 10 to 15μm thickness after fixing in
formalin.

Histochemical evidence.

Inorganic substances: iron; Prussian blue reaction (modified after
FENTON et al. 1964); Turnbull-blue method on paraffin sections after formalin
and formalin-alcohol fixing, or on freeze-dried material. Micro-ash
(HINTZSCHE 1956); and optical evaluation of and the following subsequent Prussian blue reaction
(FENTON et al. 1964). Calcium: gypsum crystal method (PEARSE 1960), phthalo-
cyanin method on paraffin sections of material fixed in formalin-alcohol
(KISZELY-POSALAKY 1964). Zinc: dithizon reaction after YOSHINAGA (1963)
on paraffin sections and on spodograms (YOSHINAGA et al. 1965). Proteins
and amino acids: ninhydrin-SCHIFF reaction after YASUMA and ICHIKAWA (1953);
histidin: procedure according to BACHMANN and SEITZ (1961).

Enzyme: unspecific acid phosphatase: Azokupplungs (= azo-dye coupling)
method (Naphthol-AS-BI-phosphate, Sigma Chemical Company St. Louis; Echtrot-
salz ITR, Fast Garnett GBC) after BURSTONE (1961) on frozen sections fixed in
formalin-calcium. Unspecific esterase: azodye coupling methods after SHNITKA
and SELIGMAN (1961, naphthol-AS-acetate, Echtblausalz BB). Cytochrome
oxydase oxydase: after BURSTONE (1959, 1960) with naphthol-AS-L3G (Pfister Chemical
Works, Ridgefield). Dehydrogenases: succinate-dehydrogenase, TPN-dependent
glucose-6-phosphate-dehydrogenase, DPN-dependent glutamic acid-dehydrogenase,
DPN-dependent glycerophosphate-dehydrogenase, DPN-dependent lactate-dehydrog-
enase (all dehydrogenase evidence with Nitro-BT; methodical data with BARKA
and ANDERSON (1963). Monoamino-oxydase: procedure after GLENNER, BURTNER
and BROWN (1957) with Nitro-BT. The collected dehydrogenase evidence was
accomplished on natural frozen sections 10 to 15μm thick. Before slicing
the material was frozen partly with dry ice, partly with liquid nitrogen
(10 to 20 s). Controls: incubation without substrate. The section was
boiled for 10 min. before the incubation enzyme inhibitor: 0.02 sodium

fluoride for unspecific acid phosphatase, 10^{-5} m E 600 (diethyl-p-nitrophenyl-phosphate; paraoxon, VEB Philopharm Quedlinburg) for unspecific esterase, . 0.01 m Marsilid-phosphate La Roche in 0.1 m phosphate bumper (?) pH = 7.2 for mono-amino-oxydase (pre-incubation of the section at 37°C).

In order to investigate the reabsorptive activity of the epithelial cells of the middle intestine we injected 0.2 ml "In-fonutrol" (Cottonseed Oil B.P. 15% w/v, Anhydrous Dextrose B.P. 5% w/v, lecithin 1.2% w/v, poly-oxy-ethylene-oxypropylene 0.3% w/v^2) into the cavity of the bowel. Control animals received the corresponding amounts of distilled water. The animals were killed 30, 45 or 60 minutes after the application and the middle intestine was fixed with formalin-calcium after BAKER. The dye Sudan-black-B was applied to the 15μm -thick frozen sections for evidence of lipids.

Electromicroscopic technique: fixing and further handling of the pieces of tissue followed after the method of WOHLFAHRT-BOTTERMANN (1957), the embedding in Vestopal W. Some objects had been fixed previously with 5% glutaraldehyde in TYRODE-solution for 10 minutes. Thin sections with the ultramicrotomes after SCHWARTZE, Jena, and v. ARDENNE, Dresden. The electromicroscopic investigations were carried out at 60 kV with the SEM 3 of the VEB Fernsehelektronik, Berlin-Oberschöneweide.

Next to the preparations of river lamprey larvae there were available already existing series of sections through intestinal sections of the metamorphic stages and imagines of the brook lamprey (Bachneunauge) as well as of the adult river lampreys (Flussneunauge) (LUPPA 1964, ERMISCH 1966). Moreover we prepared a series of sections through the liver and the adjacent intestinal region of Myxine glutinosa (formalin fixing, haemalum-eosin dye, PAS-reaction, evidence of tryptophane after GLENNER 1957). Through this a comparative hisological treatment was made possible.

3. Results.

Histological summary: the single-layer epithelium of the anterior middle intestine section of lamprey larvae consists essentially of two cell groups (Figs. 1,2,3); the apically pronounced granulated "glandular cells" (MASKELL 1930) and ungranulated "columnar cells". In the middle intestine there also

appeared isolated cell groups with distinct ciliation   In the neighbourhood
of the basal membrane further cell types are found in the epithelial region.

The glandular cells are most numerous in the front section of the anterior
intestine, and are particularly concentrated in the Fornices, as shown by
sections across this region.

In the epithelial section lying on the spiral fold fewer glandular cells
appear. Where the spiral fold at its widest projects against the intestinal
opening (Ridge of the spiral fold, BIZZOZERO 1893) the glandular cells are
almost completely missing on the adjacent epithelium.

Longitudinal sections through the middle intestine of the larval lamprey
show that the number of glandular cells diminishes towards the caudal. Finally
they disappear completely, and with this begins by definition the "posterior
intestine" (BRACHET 1897, PIQUET 1913, MASKELL 1930, BARRINGTON 1936).

In adult river lamprey the "glandular cells" are often found in a group
at the base of folds of the epithelium of the middle intestine, as they
develop during the metamorphosis with the formation of a villi relief. (Fig. 4).

In the intestinal epithelium of animals which had been caught during
the anadromous spawning phase, the columnar cells outstripped the glandular
cells numerically many times over.

There are signs that the number of the cells active in secretion is
already reduced through the process of decay, as they terminate in the intest-
inal epithelium in connection with the assimilation of food employed.

"Glandular cells" are also found in the epithelium of the middle intestine
of Myxine glutinosa. According to the results of staining with haemalum-Eosin
and the PAS-reaction the glandular cells of Myxine correspond to the zymo-
genic cells of lampreys in all cytological particulars. This is also shown
very clearly by the evidence of tryptophan in a series of sections through
the intestine of Myxine. The granules in the glandular cells are especially
rich in tryptophan.

In the liver or in the Ligamentum hepatogastricum (cf. SCHIRNER 1963)
no cells could be identified which were comparable with the "glandular cells"
in the intestinal epithelium or which on account of their special features
had had to be identified as exocrine pancreas cells.

Cytology of the excrine pancreas cells and columnar cells.

Exocrine pancreas cells: the exocrine pancreas cells are slender and club-shaped. Their nuclei lie largely in the lower third of the cell. The apical cell section is filled with secreted granules, with the exception of the area directly bordering on the nucleus. In the *electron* microscope the granules appear to be filled up with electron-thick material of varying concentration. On the outside they are covered in a membrane (Figs. 5 and 9). The basal section of the cell contains thick ergastoplasm, in the neighbourhood of the basal membrane against it a narrow layer of finely granulated substance (v. figs. 2 and 6). The ergastoplasmic lamellae lie thickly packed in the basal part as well as near the nucleus and are packed with ribosomes (Fig. 7). Mitochondria are lacking in this ergastoplasm structure. In the direction of the apex of the cell the number of ergastoplasmic tubes decreases, while at the same time their lumina become increasingly dilated. In the upper third of the cell are noticeable only the rest of the cellular lamella system between the zymogen granules which are dominant in the picture.

The spherical or longitudinally oval nuclei of the glandular cells ("glandular cells", MASKELL 1930; "BARRINGTON Cells") possess one or two large, powerfully tingierbar (?) nucleoli which vary openly according to the secretion phase of the cell.   *probably refers to staining reaction .*

The apical cell region requires particular attention (Figs. 5,6,10,11), as their structural arrangement is of essential significance for the phylogenetic and functional assessment of the Barrington cells. The rod column ascertained by ~~light~~ *optical* microscope (LUPPA 1964)consists electro-optically of finger-shaped microvilli, completely covered by a plasmalemma, whose number and form can vary considerably under functional conditions which have yet to be determined. Lamellar material inside the microvilli extends in rootlets into the superficial cytoplasm. Nearby fine granules are to be recorded within the microvilli (Figs. 5 and 10).

The "granular zone" at the base of the microvilli, recognisable by optical microscope, is seen in the electro-optical picture as a fine-grained demarcation. Beneath this follows a thick, mitochondria-free zone with the finest filaments, occurring overwhelmingly as transverse (so-called "terminal

tissue"). Under certain functional conditions vacuolar formations could be observed here, although in no case sections of the endoplasmic reticulum. The region adjacent to the base contains - as already remarked - varying numbers of zymogen granules, the channels of the endoplasmic reticulum occupied by ribosomes, and mitochondria of the crista type with mostly granular inclusions. The abundant incidence of mitochondria in the region of the secretory concentration is a remarkable fact in comparison with the circumstances in the exocrine pancreas cell of higher vertebrates. A second concentration of mitochondria is found in the neighbourhood of the basal membrane. Hence for this cell a polar arrangement of the mitochondria is significant. It appears also that the constituent amount of mitochondria and ergastoplasm is proportional in inverse ratio.

Surprisingly the lysosomes in the supranuclear cell framework known as bearers of hydrolytic enzymes could not be proved with certainty. The well-developed Golgi apparatus lies in the supranuclear cell region (Fig. 8). Its structure corresponds with that which has already been described in the exocrine pancreas cells of higher vertebrates (SJÖSTRAND and HANZON 1954, HIRSCH 1960, 1961, PALADE 1959, PALADE, SIEKEWITZ and CARO 1962, SJÖSTRAND 1962).

The plasmalemma forming the border of the cell body shows clearly visible desmosomes) in the optical microscope, in the apical region of the lateral cell wall.

The parts of the supposed secretion cycle of the Barrington cells which can be reconstructed from the electronmicroscope pictures are diagrammatically reproduced in figures 11a to f. Fig. 11a shows a cell filled with secretion, which possesses clearly developed microvilli at the rejuvenating cell apex. In fig. 11b (Fig. 5a) a hood-shaped prominence with a granular content at the cell apex is recogniseable. Here there comes a loss of microvilli and zymogen granules. Fig. 11c demonstrates a condition where the cell apex is again completely occupied by microvilli and the zymogen granules are concentrated on the upper cell section. Mitochondria are localised in relatively greater amounts in the supranuclear cell region. Fig. 11d (Fig. 10) illustrates the phase where with complete loss of the zymogen granules there is formed a cup-shaped extension at the cell apex. The mitochondria spread out in the zone between the cell nucleus and the terminal tissue. The cell

illustrated in Fig. 11e resembles a columnar cell completely in the supra-
nuclear cell region. Fig. 11f shows the start of the fresh formation of
secretion. From Figs. 11c to 11e a continuous expansion of the cell apex
is to be observed. With an increasing concentration of secretion a rejuv-
enation at the cell apex is registered against it. Here the supranuclear
cells receive a more barrel-shaped form. It can be taken as almost certain
that in the diagram of fig. 11e the illustrated cell stage exercises a
reabsorptive function. Reabsorption experiments with infomutrol lead to an
increase of sudanophile material in the apical region of the epithelium,
against control animals. It could not however be made out with certainty
whether the increase in lipids also followed in the glandular cells.

Columnar cells: as can be seen from Fig. 6, the columnar cells do not
essentially deviate from the neighbouring glandular cells in relation to
structure. The mitochondria-free terminal tissue follows on the finger-
shaped microvilli, which continue in "rootlets". This is joined at the base
by a region reaching to the top of the nucleus, in which mitochondria of the
crista type, free ribosomes, channels of the endoplasmic reticulum and of
the supranuclear Golgi apparatus thickly occupied by ribosomes, occur in a
relatively stronger concentration. The basal section of the cells extensive ly
resembles the basal part of the glandular cells.

Histochemistry of the exocrine pancreas and the columnar cells of the exocrine pancreas cell.

Inorganic substances: after reduction of the section to ashes and a
subsequent evaluation against a dark background with the phase contrast pro-
cedure, inorganic substances can be shown in the "glandular cells" of the
epithelium of the anterior middle intestine. These substances are not evenly
distributed over the cell, but are particularly concentrated at the cell apex
in the zone of the secretory granules and localised beneath the nucleus in
the mitochondria region (Fig. 12). The area directly above the nucleus con-
tains few minerals.

Zinc could be shown on paraffin sections and on spodograms in the gland-
ular cells with the help of the dithizon reaction (YOSHINAGA 1963, 1965).

There the zinc is concentrated principally in the secretory granules.

The exocrine pancreas cells contain ionised or "occult" iron only in traces, since a clear reaction was obtained neither with the Prussian blue reaction in the modification after FENTON (1964) nor with the Turnbull blue method on fixed and freeze-dried paraffin sections nor in correspondingly handled spodograms (FENTON 1964).

Clear evidence of calcium could be shown neither with the gypsum crystal method nor with the phthalocyanin method.

Proteins and amino-acids: According to the investigations of LUPPA (1964) the following amino-acids could be detected in the granules of the "Barrington" cells: tyrosin, tryptophan, arginin, cystein, cystin, α-amino groups, terminal carboxyl groups. The reaction result of the evidence for tryptophan is there at its strongest and roughly elective (=elektiv?). A distinct tryptophan reaction is also given - as already mentioned - by the granules in the "glandular cells" of the epithelium of the middle intestine of Myxine glutinosa.

In continuation of the histochemical analysis of the structure a histidine reaction was attained in the granules of the glandular cells with the method of BACHMANN and SEITZ (1961), corresponding in reaction strength and electivity (?) with the tryptophan reaction (Figs. 13, 14). Specificity ?

The intergranular cytoplasm yields a relatively weak, diffuse reaction with the evidence of the amino-acids mentioned. The infranuclear cytoplasm structures by themselves gave a stronger reaction outcome.

Enzymes

Hydrolases: unspecific alkaline phosphatase is localised in the rod column of the glandular cells, whereas unspecific acid phosphatase appearing in corpuscular form is distributed irregularly in the cytoplasm (Fig. 15). The detection procedure for unspecific esterase resulted in a positive reaction in the secretory granules (Fig. 16). With the optical microscope it can be determined with a fair degree of certainty that unspecific acid phosphatase and unspecific esterase do not correspond in their localisation.

Oxireductases

Succinate-dehydrogenase and cytochrome oxidase show a relatively strong reaction in the apical and basal cell sections. The evidence for isocitric acid-dehydrogenase gave a strong reaction at the cell base of most animals, but a diffuse reaction in the other cells. Likewise the evidence for β-hydroxy-butyric acid dehydrogenase and glycerophosphate-hydrxaga dehydrogenase gave positive results, where the β-hydroxy-butyric acid dehydrogenase reaction does not precipitate strongly at the basé of the cell, but weakly at the apex of the cells. The lactate-dehydrogenase belonging to the EMBDEN-MEYERHOF cycle resulted after 20 min. incubation in a reaction product of granular appearance.

With the evidence of the mono-amino-oxydase a different reaction result was observed: at the apex and base of the cells the reaction product is granular and strongly concentrated. In the region of the zymogen granules, however, there came about a diffuse violet colouring.

Columnar cells.

Inorganic substances: in contrast to the pancreas cells, inorganic substances in the columnar cells occur in very poor amounts. The substances are mainly located in the region of the cell membrane. Also evidence of zinc in the columnar cells comes out practically zero. Ferruginous material which the exocrine pancreas cells – as mentioned previously – contain only in traces, is detectable in the columnar cells of the antibasal epithelial ? region in the form of coarse pellets (Fig. 17).

According to the material of our investigation, the reaction only takes place if ferruginous food particles are contained in the intestinal lumen of the lampreys. (cf.L-Grana von HEBIG 1961).

Proteins and amino-acids: from the amino-acid reactions obtained from the representation of the zymogen granules, the procedures on the representation of tryptophan and histidine in the columnar cells became almost negative. The evidence for tyrosin, amino groups, sulph-hydryl and disulphide groups as well as for arginine led to diffuse colour reactions in the supranuclear cell sections. In the infranuclear cell region, distinguished by a strong RNS

concentration the reaction outcome was regularly stronger than in the supra-nuclear zone.

Enzymes.

Unspecific esterase could not be shown in the columnar cells of the "anterior intestine" of lamprey larvae. Unspecific and alkaline phosphatases showed the same behaviour in strength of reaction and localisation as in the exocrine pancreas cells. No differences recognisable under the microscope were recorded for the oxydo-reductase evidence as a whole in reactivity and topic (?) between glandular cells and columnar cells.

localisation ?

Discussion.

On comparing the exocrine pancreas cells of the larvae of river lampreys with neighbouring columnar cells, a striking feature is the concentration of the mitochondria in the apical and basal cell region of both cell types, next to the structural correspondences in the framework of the cell apex. Such a polar arrangement of the mitochondria is characteristic of reabsoptive epithelium cells (LUDWIG and RICHTERICH 1953, SCHMIDT 1965). In this respect an essential significance is attached to the size of the chondriom for a consideration of the functional morphology of the pancreas cells, as the synthesis of energy-rich phosphates follows on the supply of energy content of a cell to an overwhelming part on the reppiratory chain phosphorylation. The glycolytic method of obtaining energy, which is shown through the occurrence of the lactate-dehydrogenase, should however play a subordinate roll. The correspondence in reaction strength of the intra- and intermitochondrial oxydo-reductases in pancreas cells and neighbouring columnar cells can thus show that the metabolical activity is/similar in both cell types.

From the detected hydrolases the lack of unspecific esterases in the columnar cells bordering the exocrine pancreas is of itself noteworthy, since this enzyme in reabsorptive thin intestinal cells belongs to the permanent enzyme supply (LUDIWG and RICHTERICH 1954, ARVY 1962, BARGMANN 1962, SHNITKA and SELIGMAN 1961). The location of the enzyme in the secretory granules accords with results already obtained on the exocrine pancreas cells of

mammals (SCHULTZE 1962).

In contrast to the unspecific esterase, the unspecific acid phosphatases in columnar cells and glandular cells could be detected in equal distribution. It is noteworthy, however, that in our electro-optical pictures the lysosomes generally taken as structures responsible for the acid phosphatases could not be made out. According to our present knowledge, the acid phosphatase does however count just as the criterion for the identification of granules as lysosomes (KOMNICK and WOHLFAHRT-BOTTERMANN 1964, SCHMIDT 1965).

Among the structuro-histochemical researches there stands out the strongly positive outcome of the tryptophan and histidin-evidence, through which the secretory granules are recorded. Both amino-acids were represented in equal electivity (?) also in the zymogen granules of the exocrine pancreas of higher vertebrates. The strong colouring of the zymogen granules with paraldehydefuchsin after is according to GABE (1953) definitely attributable to the presence of sulphydryl and disulphide groups.

As can be seen from the electro-optical pictures, morphologically the extrusion of secretions by the pancreas cells of lamprey larvae is very probably accomplished in the form of a hood-shaped prominence. In this context the microvilli are de reformed reads. In contrast to this the extrusion of the secretions in the pancreas cells of higher vertebrates follows through discharge of the granular content in the glandular process after fusion of the plasmalemma (?) and vesicle membrane (EKHOLM, ZELANDER and EDLUND 1962, SIEVERS 1965). The process of secretory formation, however, proceeds in the manner known from mammals (HIRSCH 1939, 1961, 1964, EKHOLM, ZELANDER and EDLUND 1962). The glandular cellxx again completely packed with microvilli after the extrusion of secretion at the cell apex, in this phase resembles a columnar cell completely morphologically. Although the experiments in reabsorption with infonutrol did not lead to an unequivocal confirmation of a reabsorptive function of the glandular cells with secretory granules, we consider that, after the results of the electro-optical and histochemical experiments, a function of this kind is very probable. Various signs also indicate that defined columnar cells can pass over to production of secretions. Further experiments, as well as a comparison with Myxine glutinosa, are necessary for a definite clarification of the questions; the pancreas cells of

the former correspond structurally with those of the lamprey larvae according to the electro-optical pictures of ADAM (1963).

YAMAMOTO (1965) distinguishes "striated border cells", "secretory cells", and "ciliated cells" on the intestinal epithalium of the lamprey (Lamptera japonica) according to electro-optical experiments. The secretory cells show a definite similarity with the pancreas cells of the river lamprey larvae, although a polar arrangement of the mitochondria of Lampetra japonica does not appear to exist. Also the form of the secretory extrusion varies from that of the pancreas cells of the river lamprey larvae under investigation. The striated columnar cells should exercise a reabsorptive and also a secretory function (after the occurrence of PAS-positive material in the vacuoles) on the base of the microvilli and vacuolar structures in the apical cell section. YAMAMOTO accepts that the striated columnar cells represent a step forward of the goblet cells of the fish intestine. A comparison between the results of experiments on Lampetra planeri and Lampetra japonica is complicated by the fact that data on the ecophase of the intestines investigated on Lampetra japonica are lacking.

Next to the comparative consideration of cytological detail and its functional connections, there has still to be investigated the question of whether the method of construction of the intestinal canal of lamprey larvae should be considered as phylogenetically primitive, or whether it should be regarded as the specialisation of a phylogenetic collateral line.

Indications that we are finding supposedly actual primitive circumstances in the lamprey can be deduced from functional and anatomical considerations as well as from ecophysiological points of view: the secretory cells in the anterior middle intestine epithelium of lampreys correspond functionally, according to existing data, to the exocrine pancreas cells of the higher vertebrates. Such cells are found in related animal groups, eg. in Myxinidae (SCHREINER 1957, SCHIRNER 1963, ADAM 1963). But zymogen cells can also be located occasionally or continuously in the epithelium of the middle intestine of animals which possess a pancreas formed as an appendant gland to the intestine. AL HUSSAINI (1949) and BARRINGTON (1957) describe zymogen cells in the intestinal epithelium of teleostei, strikingly resembling the pancreas

cells of Lampetra morphologically, and whidh possess a supposedly tryptic activity. There have been many references to accessory pancreatic glands in the human intestinal epithelium, (cf. PATZELT 1936). Histochemical methods could also be used to investigate whether specified morphological correspondences between the exocrine pancreas cells of lampreys and the acid-loving [acidophilic] glandular cells in the intestinal epithelium of Torpedo (HELLY 1905), of Acipenser and Teleostei (ROGOSINA 1928, 1930, BAECKER 1934) are traceable to phylogenetic relationships.

If the nutritional method of Ammocoetes (inner mucous filters) is still "primitive in the phylogenetic sense", this likewise indicates that the construction of the intestine of lampreys still depicts a simple stage of organisation, since a similar method of digestion has been positively shown for the Ostracodermi of the Ordovician and the Gottlandian (= Silurian?) (STERBA 1961). [probably correct.]

From the distribution of zymogen cells in the intestine of the lamprey, general conclusions can also be deduced on that point in our opinion, as the pancreas has arisen out of the glandular cell appendages in the intestinal epithelium as an anatomical unit, ie. as an appendant gland to the intestine, in the phylogenesis of vertebrates. The exocrine pancreas cells are especially frequent in the fornices [in the larvae] of river lampreys. The zymogen cells are still found only in the fornices of the Ammocoetes of the New Zealand lamprey genus Geotria (MASKELL 1930). The fornices of this species - especially the left one - are moreover transformed into long, tubular diverticula, a direction of development which is also visible in the Australian genus Mordicia (MASKELL 1932), where likewise an outward curvature of the intestine is found at the place mentioned. In such phenomena one can also see the rudiments of the concentration of pancreas cells and the morphological demarcation of the exocrine pancreas from [of] the intestinal canal.

The communication of SCHIRNER (1963) on three pancreas parts of Myxine must be discussed with the phylogenetic considerations on the pancreas of lampreys. SCHIRNER finds next to the zymogen cells in the intestinal epithelium a "pancreas intrahepaticum" and a "pancreas disseminatum". Information on cytological details was [were] not given. According to special observations of

lampreys and exploratory experiments on Myxine, however, the putative pan-
creas parts in the liver represent parts of the gall drainage system. This
view arises not only from the general histological picture - the zymogen
cells are quite unlike the cells in the liver under question -, but also
is supported by the results of the tryptophan reaction. In contrast to the
exocrine pancreas cells in the intestinal epithelium, the channels of the
liver contain no tryptophan. We could also determine no glandular cells in
the ligamentum hepatogastricum which might be comparable with the exocrine
pancreas.

Summary.

    The exocrine pancreas of the larvae of river lampreys was investigated
histologically, electro-optically as well as histochemically and compared with
the columnar cells of the ~~entariar~~ a section of the anterior middle intestine.
The reabsorptive function of the epithelium of the middle intestine was
examined through the application of infonutrol. Pancreas cells and columnar
cells are recognised through a polar arrangement of the mitochondria charac-
teristic of reabsorbent epithelial cells. The intra- and intermitochondrial
oxydo-reductases detected correspond in locality and reaction strength in
both cell types. The unspecific esterase located in the granules of the
pancreas cells, however, is not met in the columnar cells. The secretion
cycle of the pancreas cells which can be built up out of the electro-optical
pictures indicates a double function, ie. a secretory and reabsorptive per-
formance of this cell type. The phylogenetic aspects arising in connection
with the method of construction of the intestinal canal of lamprey larvae
are being discussed.

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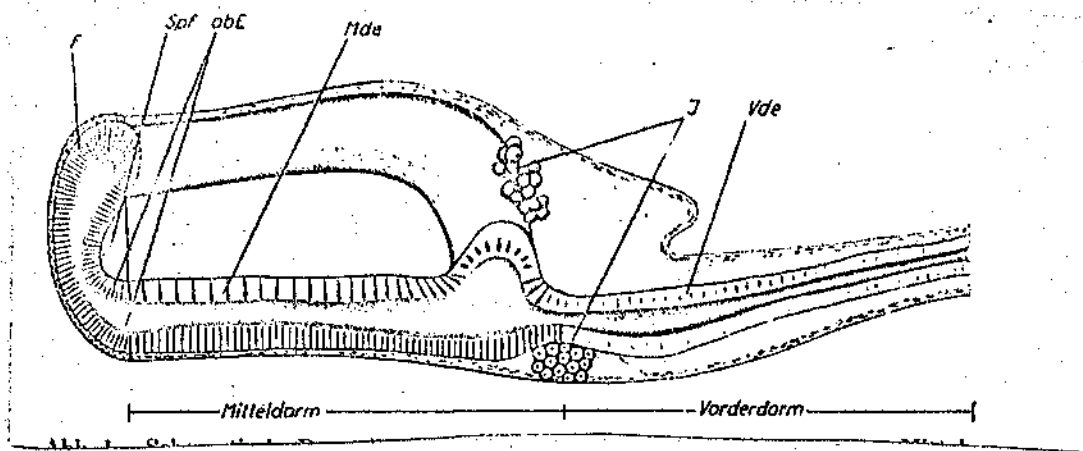701 Leipzig, Talstraße 33

Fig. 1 Schematic representation of the transitional region of the anterior
to middle intestine of the brook lamprey larva. Vde = epithelium of the
anterior intestine, I = islands, Mde = epithelium of the middle intestine,
abE = antibasal epithelium, F = fornix, Spf = spiral fold.

Fig. 2 Section through the epithelium out of the anterior middle intestine
section of a brook lamprey larva. DZ = glandular cells with strong formation
of ergastoplasm in the infranuclear cell section. Sz = columnar cells.
Fixing after BOUIN. Ferrohaematoxylin after HEIDENHAIN. 800:1.

Fig. 3 Section through the upper epithelial region from the anterior middle
intestine section. DZ = glandular cells with paraldehyde-fuchsin positive
granules. Sz = columnar cells show no reaction. Fixing after BOUIN. Paral-
dehyde-fuchsin after GABE (1953). 1200:1.
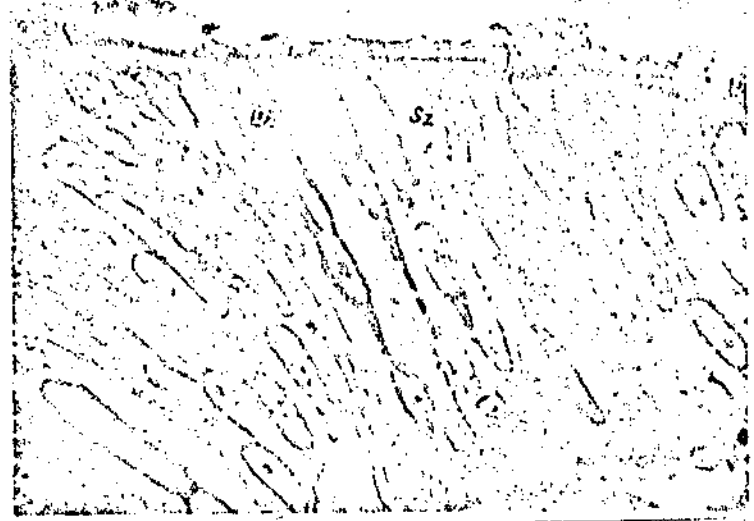
Fig. 4 Section through the middle intestine of an adult river lamprey. (correct)
Dz = glandular cells with paraldehyde-fuchsin reactive granules. Sz = columnar
cells. Fz = glitter cells. Fixing after BOUIN. Paraldehyde-fuchsin after
GABE (1953). 900:1.

Fig. 5a Longitudinal section through the supranuclear section of glandular
cells and columnar cells of a brook lamprey larva. Mv = microvilli, Tg =
terminal tissue, Zg = zymogen granules, Mi = mitochondria, Ds = ddsmose,
V = vacuoles, Sx = secretory extrusion. Electro-optical magnification 10,000:1.
Final magnification 22,000:1.
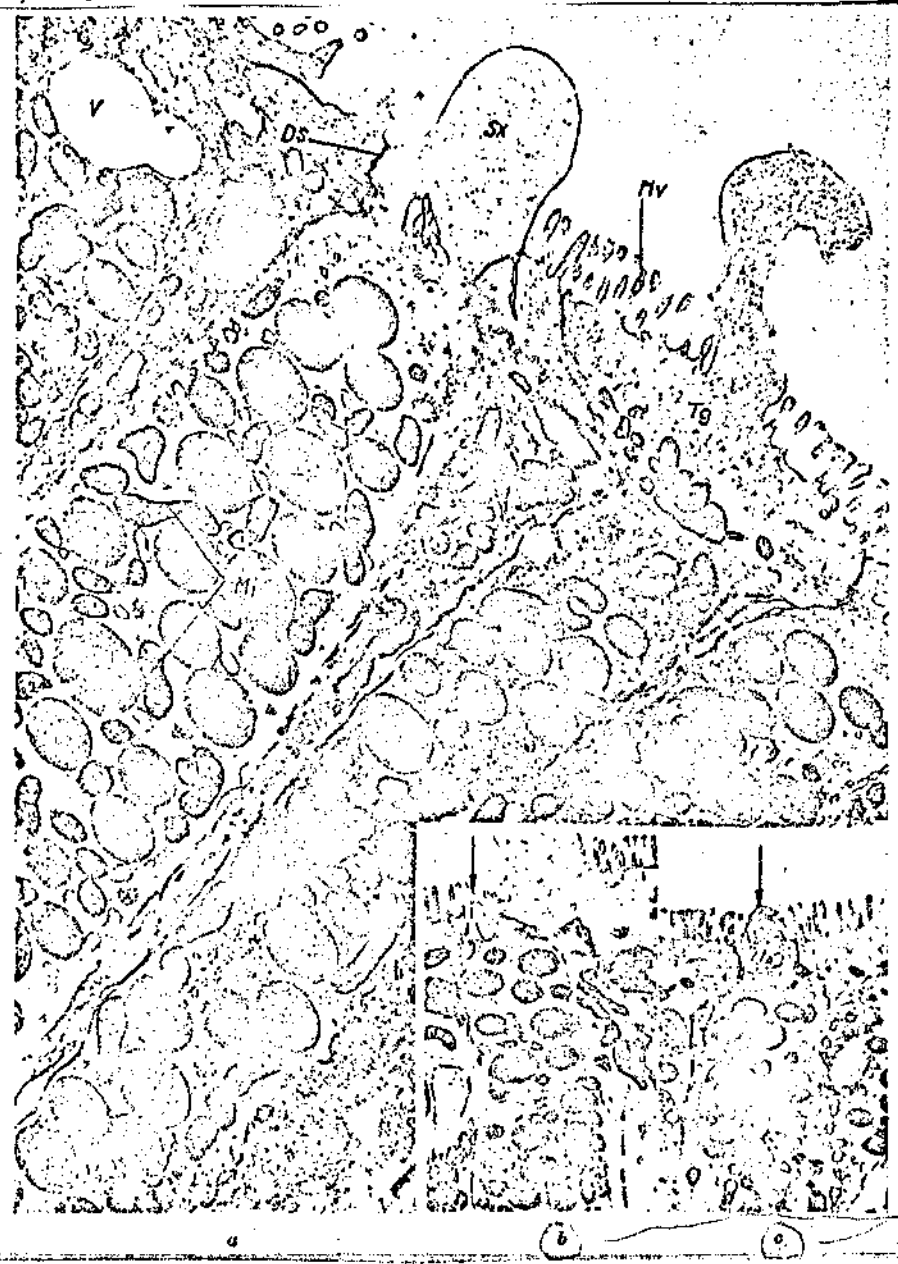
Figs. 5b and 5c Section as with 5a. Note the development of the secretory
extrusion (arrow!). Electro-optical magnification 6000:1. Final magnific-
ation 8000:1.
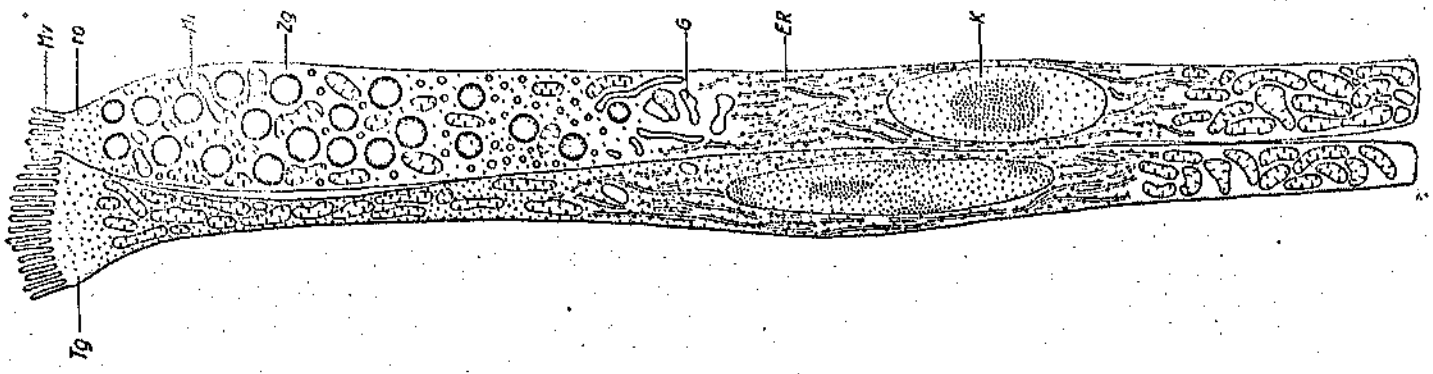
Fig. 6 Schematic representation of a pancreas cell and a columnar cell from the middle intestine epithelium of a brook lamprey larva. Mv = microvilli, ro = rootlets, Mi = mitochondria, Zg = zymogen granules, G = Golgi apparatus, ER = endoplasmic reticulum with ribosomes, K = nucleus, Tg = terminal tissue.
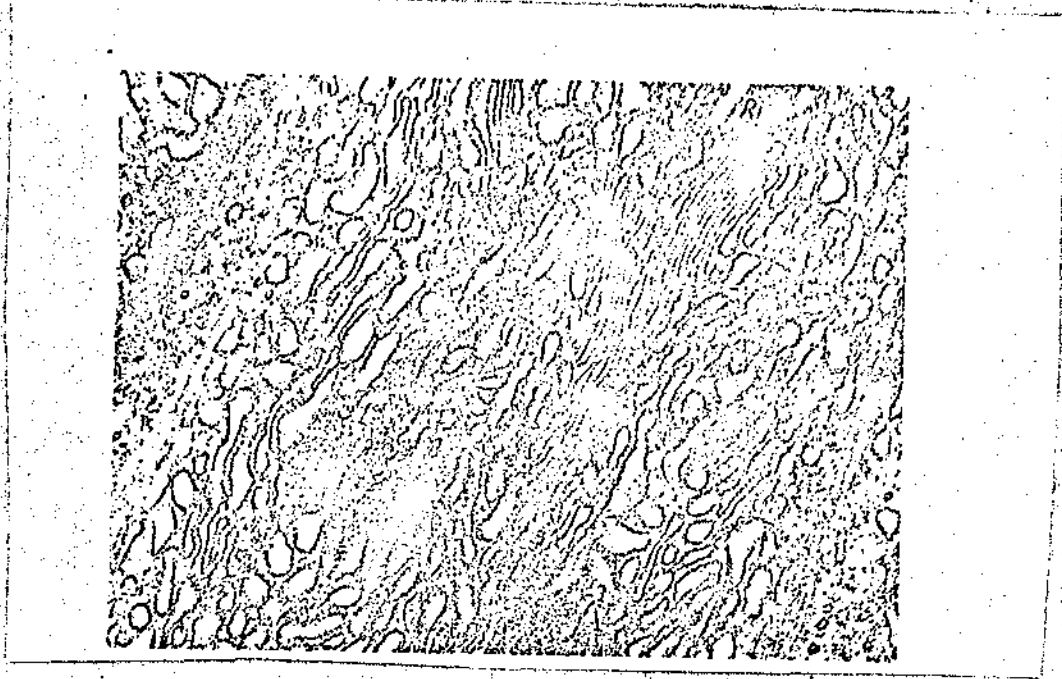
Fig. 7 Segment from the infranuclear section of a glandular cell with densely packed ergastoplasmic canals and free ribosomes Ri. Electro-optical magnification 10,000:1. Final magnification 30,000:1.
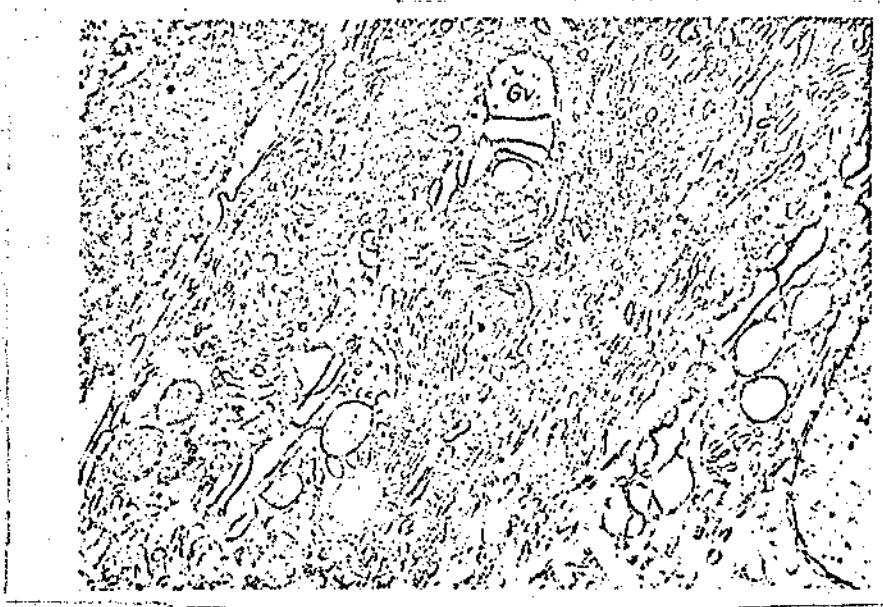
Fig. 8   Section from the Golgi region of a glandular cell. Developamant
of the pro-secretions in the Golgi vesicles, Gv. Electro-optical magnification
6000:1. Final magnification 17,000:1.

Fig. 9   Section from the supranuclear cell region with the formation (Se) of
secretions and their development (Pse). El.-opt. magnification 10,000:1.
Final magnification 22,000:1.

Abb. 10. Schnitt durch die apicale Zellregion des Mitteldarmepithels einer Bachneun-augenlarve. *Mv* Mikrovilli, *ro* rootlets, *Ds* Desmose, *Mi* Mitochondrien. *Sx* Sekret-extrusion. Beachte: Drüsenzelle ohne Sekretgranula und mit starker Mitochondrien-konzentration. Vergrößerung el. opt. 6000:1. Endvergrößerung 22000:1

Fig. 10 Section through the apical cell region of the middle intestine epi-thelium of a brook lamprey larva. Mv = microvilli, ro = rootlets, Ds = Desmose, Mi = mitochondria, Sx = Secretory extrusion. Note the glandular cells without secretory granules and with strong concentration of mitochondria. El.-opt. magnification 6000:1. Final magnification 22,000:1.
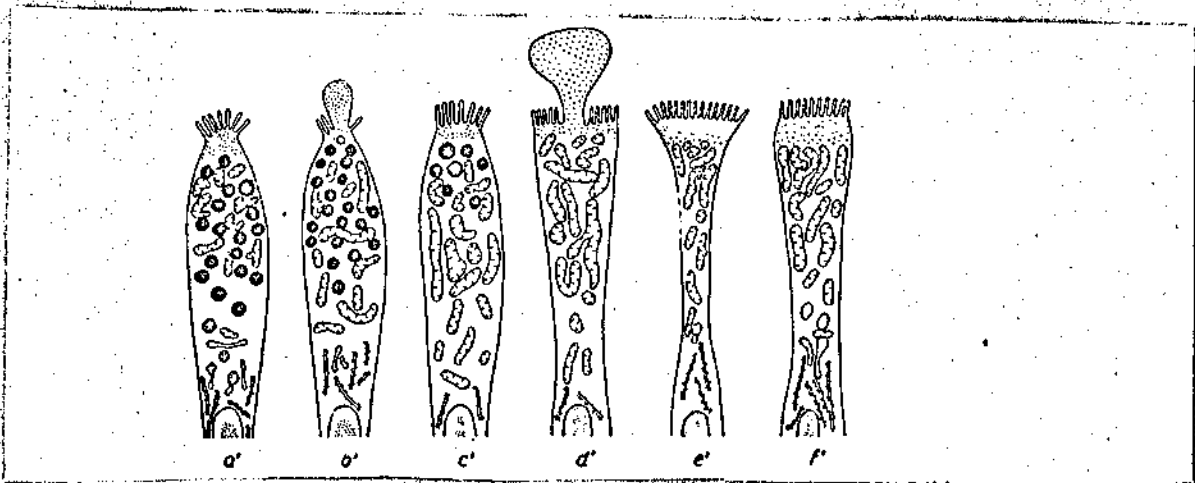
Fig. 11 Schematic representation of the reconstructed supposed secretory cycle of the glandular cells from the electromicroscopic pictures (explanation in the text).

Fig. 12 Spodogram of a section through the anterior middle intestine section of a brook lamprey larva. Concentration of the inorganic substances in the supranuclear region of the pancreas cells. Fix. formalin 10% neutral. Reduction of the section to ashes. 645:1.
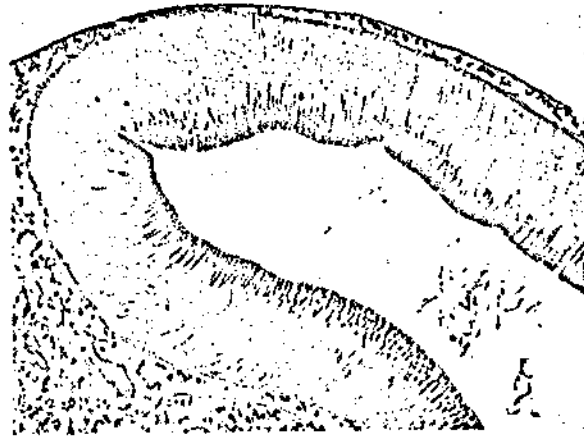

Fig. 13 Cross-sectional view from the anterior middle intestine section of a brook lamprey larva. Zymogen granules with strong histidin reaction. Freeze-drying. Evidence for histidin after BACHMANN and SEITZ (1961). 200:1.
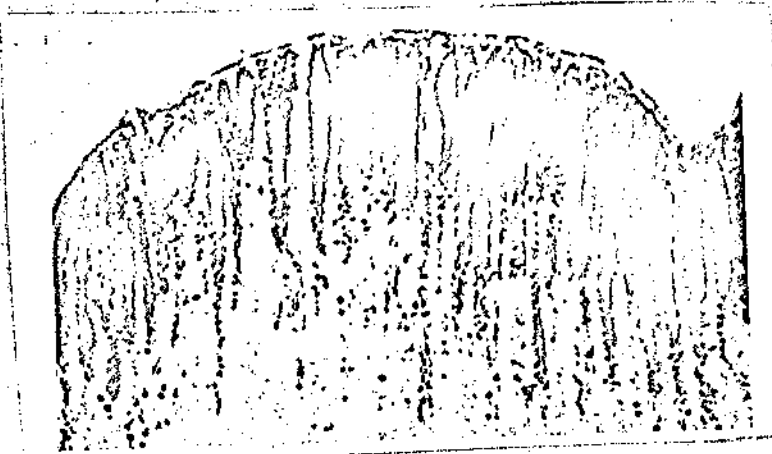

Fig. 14 Enlarged section of Fig. 13. Strong histidin reaction of the zymogen granules. Columnar cells unstained. Freeze-drying. Evidence for histidin after BACHMANN and SEITZ (1961). 600:1.
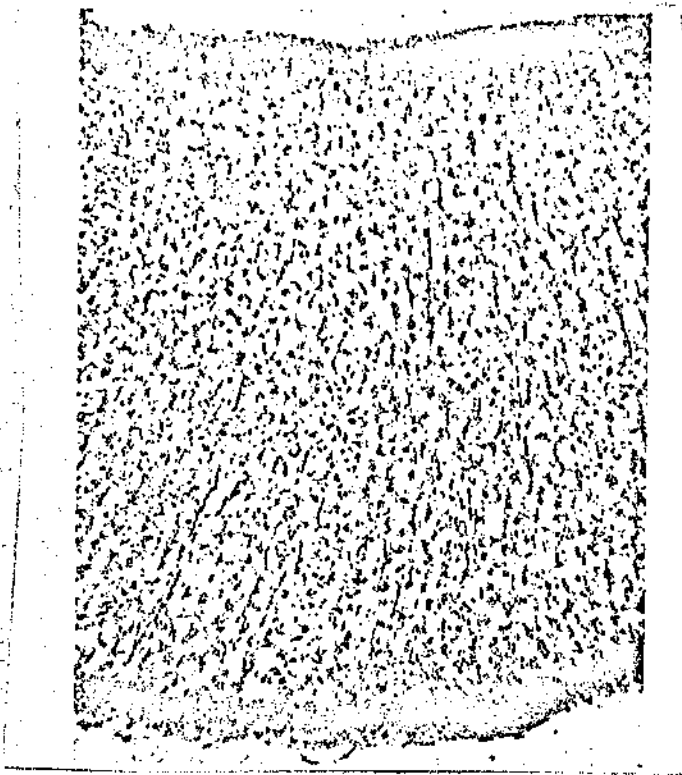
Fig. 15 Cross section out of the epithelium of the fornix of the anterior
middle intestine section of a brook lamprey larva. Acid phosphatase reactivity
in the form of granular particles diffusely scattered in the epithelium.
Fix. cold formalin-calcium after BAKER. Nitrogen-coupling (= Azokupplungs?)
method after BURSTONE (1961), nmaphthol-AS-BI-phosphate, Echtrotsalz ITR).
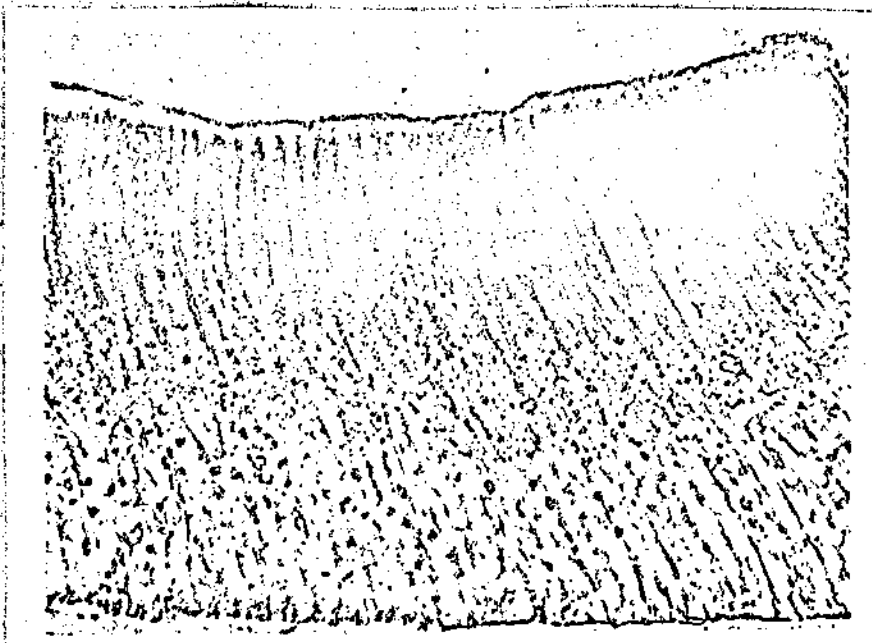1200:1.

Fig. 16 Cross section of the epithelium of the fornix of the anterior
middle intestine section of a brook lamprey larva. Activity of unspecified
esterase in the zymogen granules. Fix. cold formalin-calcium after BAKER
with sucrose after-treatment. Evidence for unspecific esterase after SHNITKA
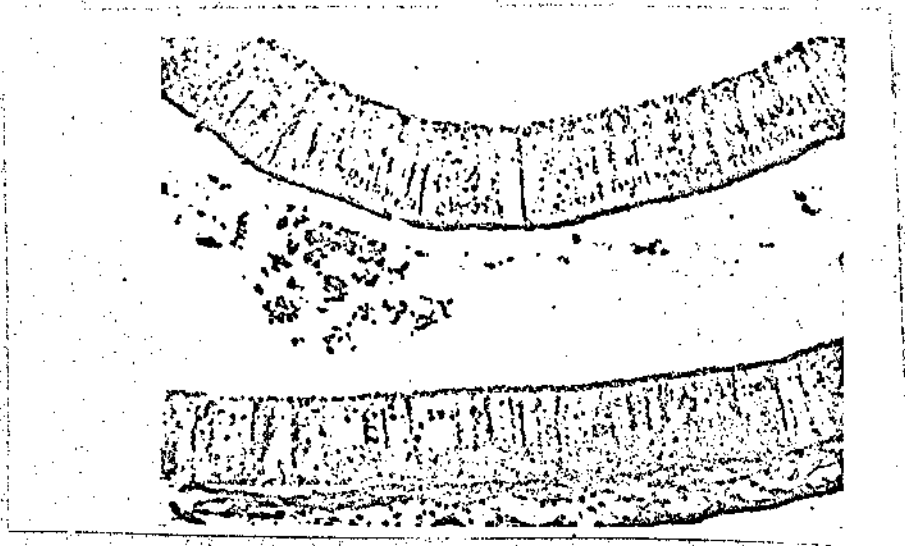and SELIGMAN (1961, naphthol-AS-D-acetate, Echtblausalz BB). 900:1.

Fig. 17   Section through the epithelium of the antibasal epithelial region
of a brook lamprey larva. Ferruginous material in the form of coarse globules
in the columnar cells. Fix. formalin 10% neutral. Turnbull-blue method
(PEABSE 1960). 150:1.

Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.