

DATA TO THE KNOWLEDGE OF SUBMUCOUS GLAND CELLS OF THE AVIAN PROVENTRICULUS

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Among the anatomical peculiarities of the avian digestive system the structure of the stomach is the most conspicuous one, having two well separable parts i. e. the glandular stomach (proventriculus) and the muscular stomach (ventriculus). The main task of proventriculus is to produce gastric juice while that of ventriculus is to grind the food. Consequently, their histological structure is particular.

In this paper we wish to deal with the light and electron microscopic structure of secreting cells of the compound gland in the tunica submucosa of the proventriculus.

Materials and Methods

Our observations were performed on the ventriculus partly on granivorous species (Balkan laughing dove: *Streptopelia decaocto* FRIV.; domestic pigeon: *Columba domestica* L.) partly on raptorial species (black-headed gull: *Larus ridibundus* L.; peewit: *Vanellus vanellus* L.).

For the histological examination the freshly dissected material was elaborated in two ways. Partly it was fixed in Bouin's and Carnoy's fixing mixture, then after having been dehydrated and embedded in paraffin, the section series were stained with haematein-eosin, methylgreen-pyronin and toluidineblue-floxine.

Partly, for investigating it with electron microscope, we embedded the dehydrated material — fixed in 1 p. c. OsO₄ buffered according to MILLONIG (1962) — into araldit. Contrasting it in the block, we used 3—4 p. c. uranyl-acetate, at sections according to REYNOLDS's (1963) procedure. The sections were prepared with an ultramicrotome Tesla BS 478, the photographs with a table electron microscope Tesla BS 242 D.

Apart from studying paraffin-embedded and ultrathin sections, we made semi-thin preparations, as well, staining them according to RÜDEBERG (1967) with metileneblue-tionine.

Results

The most characteristic feature of the histological structure of fowl proventriculus is the compound tubular gland, filling in the tunica submucosa nearly in its full thickness, whose structural composition corresponds to that of glands of similar type. The cells performing secretion are cubic while those forming the wall of the efferent canals are cubic initially but in the main efferent canal they turn into pyramidal epithelia (Table I, Fig. 1).

In the histologically stained paraffin sections, the secretory cells do not separate (Table I, Fig. 2). In the ultra-thin sections and in the semi-thin ones, investigated complementarily, two cell types can be distinguished („A” and „B”) that may be separated clearly from each other and are showing some differences not only in their structures and staining but also in their position. The majority of cells forming the wall of the exocrine gland tubules are type „A”. Wedged in them, near outer wall of the tubule we find type „B” cells (Table II).

The characteristic of „A” cells is a strong interdigitation (Table III, Fig. 1, 3) that can be observed only scarcely when coming into contact with the cells of type „B” (Table V). At the base of cell, the membrane protrusions create a complicated system whose average thickness is 2600 Å (Table III, Fig. 3). The part of membrane near the lumen is smooth in a state of rest; in active cells, however, it is rich in microvilli.

From the cytoplasmatic organella of „A” cells, the number and size of mitochondria, the orientation of mitochondria and of the granulated endoplasmatic reticulum deserve attention. The average size of mitochondria found in the cells in large numbers is 1–1.5 μ . They can be observed particularly densely in the basal part of the cell and the very same area is covered with the densest network of the granulated endoplasmatic reticulum, as well (Table III, Figs. 1, 3). Often, even the nucleus of cell is enclosed by a mitochondrial ring. The endoplasmatic reticulum surrounds in a characteristic way almost every mitochondrium with a densely granulated canal (Table III, Fig. 3). The apical part of cell is poorer in granulated endoplasmatic reticulum, beside it we can observed the enrichment in vacuola with an average diameter of 0,16 μ (Table III, Fig. 2).

Cells „B” fall very short both in number and in size of those of type „A” (Tables II, IV, V, VI). These cells are situated in the basal membranes of the gland tubule and their apical part never reaches the lumen of tubule. It shows differences from the previous type that are important in ultrastructural respect,

ABBREVIATIONS

A	— cell type „A”	L	— lipid granule
B	— cell type „B”	Lu	— lumen of the secreting canal
BM	— basement membrane	M	— mitochondrium
C	— capillary	N	— nucleus
CT	— connective tissue	NL	— nucleolus
End	— endothelial cell	R	— ribosoma
GER	— granulated endoplasmatic reticulum	RBC	— red blood cell
Go	— Golgi apparate	SER	— smooth endoplasmatic reticulum

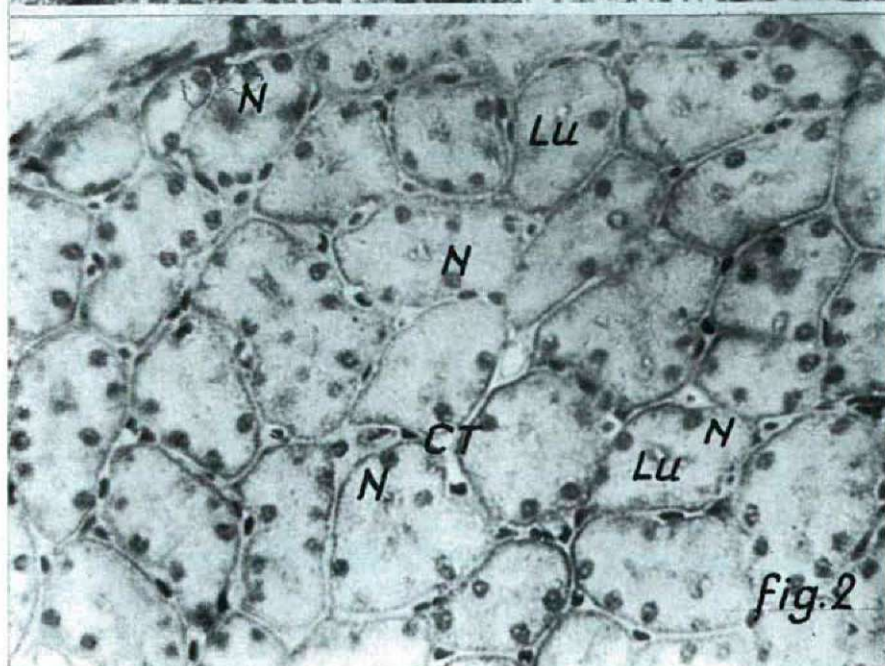
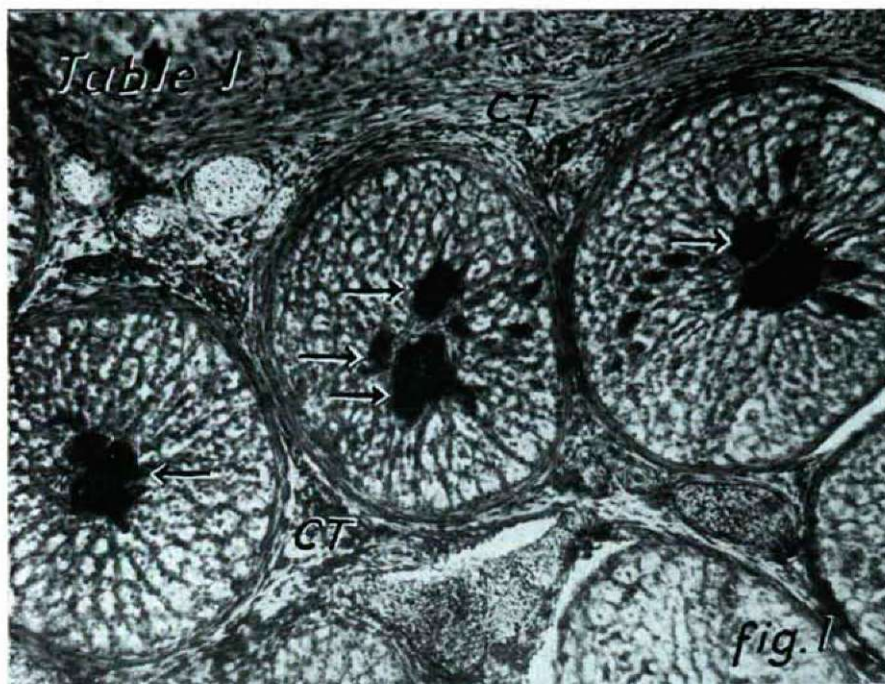
Table I

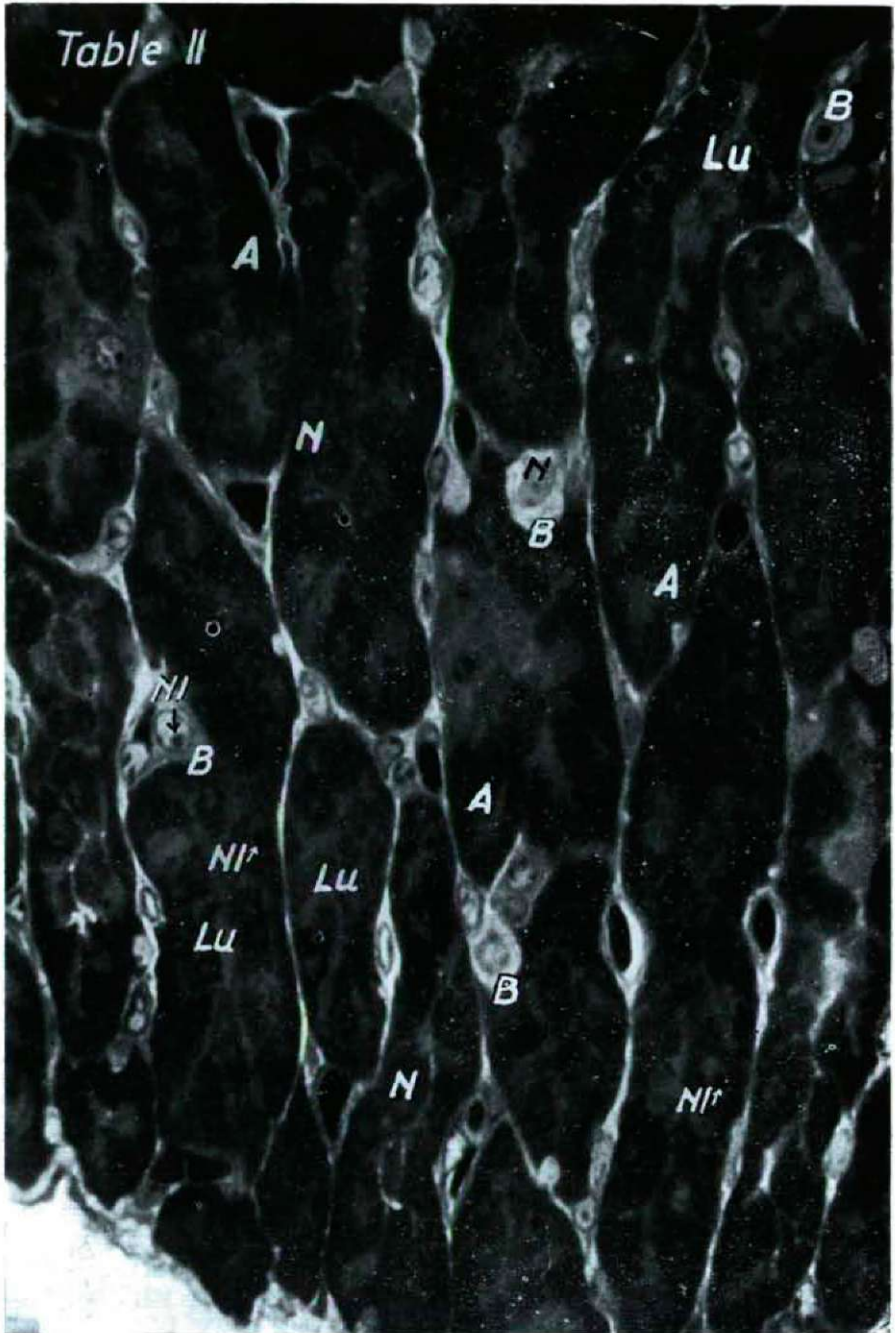
Fig. 1. *Larus ridibundus*: proventriculus. Sectional drawing of submucosa glands. The cells of outlet and the secretion (arrows) are strongly stained. Toluidineblue-floxine staining. x 315.

Fig. 2. *Larus ridibundus*: proventriculus. The cells forming the wall of secretory tubules are equally stained. Toluidineblue-floxine staining. x 1200.

Table II

Columba domestica: A detail of the submucosa gland of proventriculus. Semi-thin section, methyleneblue-thionine staining, light microscope photograph. The wall of gland tubules inconstucted mainly of cells of type „A” wedged between them some cells of type „B”. Among the gland tubules several blood vessels can be observed. x 1700.





too. A characteristic of their membranes is the absence or deficient development of interdigitation. The tiny cell is filled almost fully by the centrally situated nucleus giving — contrary to the previous cells — a considerable part of the mass of cell (Tables II, IV, VI). In the narrow cytoplasm border surrounding the nucleus of the cell there are but few mitochondria and little granulated endoplasmatic reticulum — contrary to cells of type „A”. At the same time, Golgi apparatus is more developed and there can frequently be observed granula of various density and size, varying between 700—2400 Å (Tables V, VI).

Discussion of results

Literary date concerning the gland cells of the submucosa of proventriculus are comprising the discussion of cell structure and function. The researchers have established that these cells have in their structure the attributes both of the parietal and of the main cells of the mammalian stomach (HALLY, 1959; VASSALLO, SOLCIA and CAPELLA, 1969). Similar conclusions were obtained also by others during studying the gland cells of frog stomach (VIAL and ORREGO, 1960; SEDAR, 1961a; 1961b), inferences were drawn that these cells were equally capable of producing acid and zymogen-like matter (BATT, 1924; AITKEN, 1958; TONER, 1963a; 1963b).

A part of the literary data attribute a double function to these cells although the degree of importance is as yet unsettled. Accordingly, BATT (1924) and AITKEN (1958) emphasise the production of acid, while VONK, BRINK and POSTINA (1949) give account of their observations about acid production taking place not so much in the proventriculus but rather in the ventriculus.

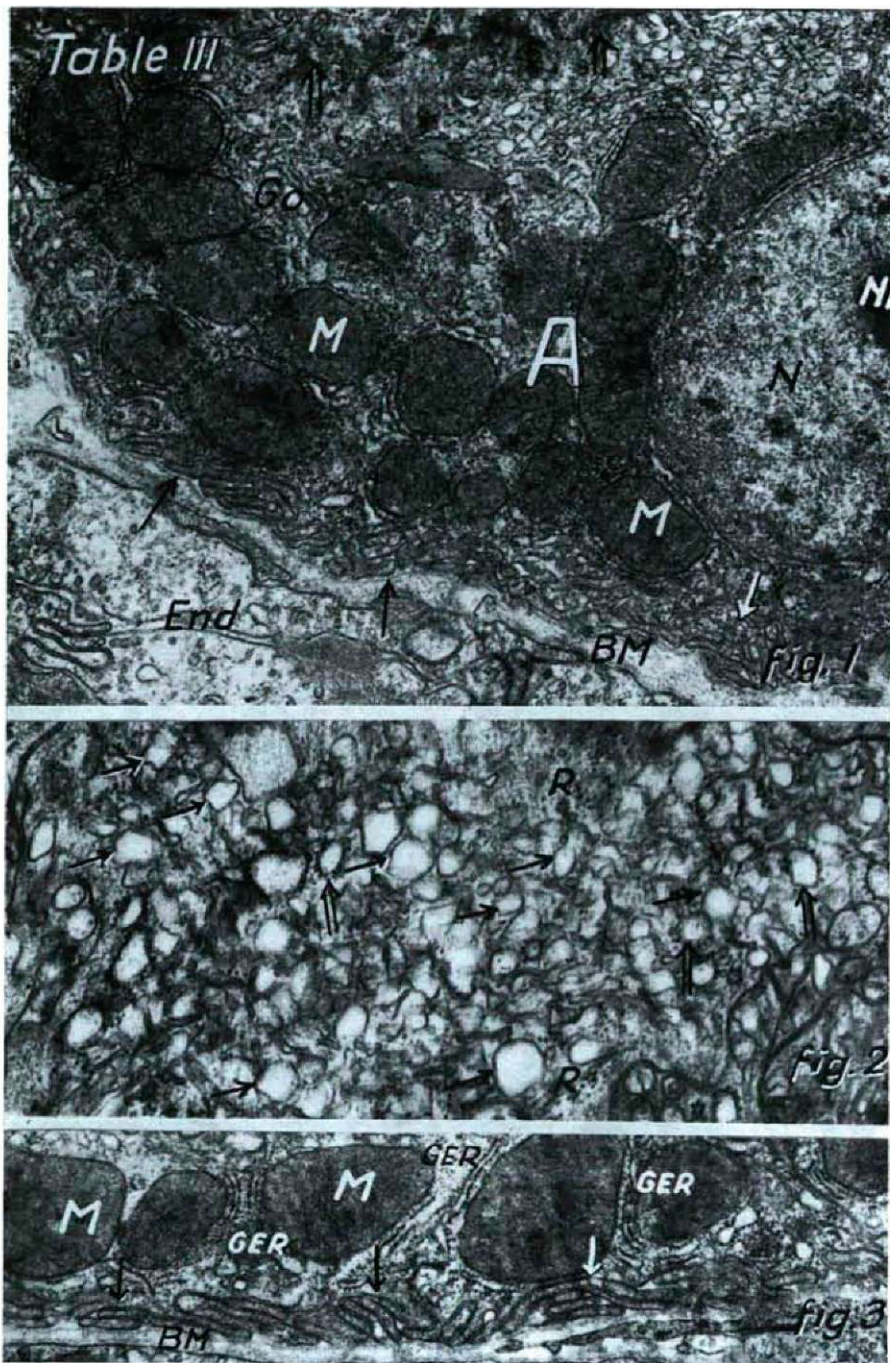
On the basis of our investigations we can establish that the submucous glands of the proventriculus are built up by two cell types. These cells have

Table III

- Fig. 1. Electron microscopic picture of a cell of type „A”. The cell is lying on a basal membrane, surrounded wholly by a complicated system of protrusions (arrows) forming an interdigitative connection with the adjacent cells of type „A” (double arrows). The nucleus is situated basally. Mitochondria appear in high numbers primarily close to the basal membrane and round the nucleus. The endoplasmatic reticulum is conspicuously rich; there are several free ribosoma and those attached to membranes. x 11 700
- Fig. 2. Part of a cell of type „A” from the lumen. The cytoplasm is filled mainly with smooth endoplasmatic vesicles (arrows) and, in addition, with those of granulated surface (double arrows). Free ribosoma can be observed sporadically. x 32 800
- Fig. 3. Basal part of a cell of type „A”. A rich network of cytoplasm protrusions rests on the basal membrane (arrow). Mitochondria are characteristically surrounded by the canals of the granulated endoplasmatic reticulum, and the bulk of ribosoma is connected with it. x 16 800

Table IV

Picture of a cell of type „B”. The cell is resting on the basal membrane; a further area of its surface is adjacent to a cell of type „A”. Its cytomembrane is smooth, its nucleus is comparatively large. The cell is poorer in mitochondria, and these are smaller, too, than those in type „A”. x 51 000





a structure that is similar to that of the secretory cells of tunic mucosa in the mammalian stomach as stated by other authors, as well (BATT, 1924; AITKEN, 1958; TONER, 1963a; 1963b).

Summary

The proventriculus of birds of various nutrition has been investigated with the morphological methods of light and electron microscope. It was established that the gland cells of submucosa can be divided structurally into two groups:

one marked with „A”, which are similar to the parietal cells of the mammalian stomach, the other with „B” corresponding to the main cells of the mammalian stomach.

References

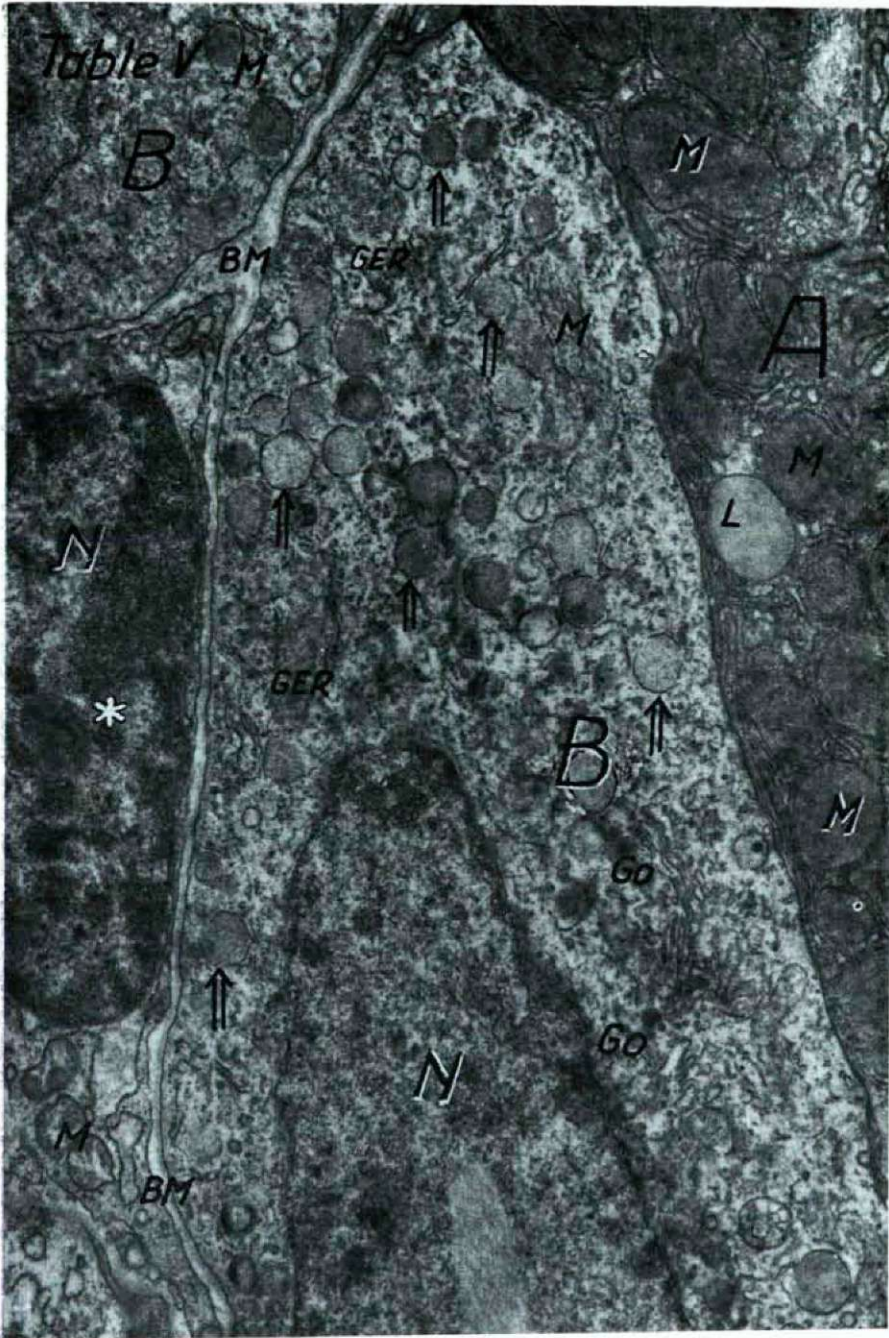
- AITKEN, R. N. C. (1958): A histochemical study of the stomach and intestine of the chicken. — *J. anat.* **92**, 453—466.
- BATT, H. E. (1924): A study of the normal histology of the fowl. — Report of the Ontario Veterinary College, 21—31.
- HALLY, A. D. (1959): The fine structure of the gastric parietal cell in the mouse. — *J. anat.* **93**, 217—225.
- MILLONIG, G. (1962): Further observation on a phosphate buffer for osmium solutions in fixation. Fifth Internat. — Congress for Electron Microscopy, Philadelphia.
- REYNOLDS, E. S. (1963): The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. — *J. Cell Biol.* **17**, 208—212.
- RÜDEBERG, C. (1967): A rapid method for staining thin sections of vestopal w-embedded tissue for light microscopy. — *Experientia*, **23**, 792—794.
- SEDAR, A. W. (1961a): Electron microscopy of the oxyntic cells in the gastric glands of the bullfrog (*Rana catesbiana*). I. The non-acid secreting gastric mucosa. — *J. biophys. biochem. Cytol.* **9**, 1—18.
- SEDAR, A. W. (1961b): Electron microscopy of the oxyntic cells in the gastric glands of the bullfrog (*Rana catesbiana*). II. The acid-secreting gastric mucosa. — *J. biophys. biochem. Cytol.* **10**, 47—57.
- TONER, C. P. (1963a): The fine structure of resting and active cells in the submucosal glands of the fowl proventriculus. — *J. anat.* **97**, 575—583.
- TONER, C. P. (1963b): Postosmication of submucosal gland cells in the fowl proventriculus. — *Z. Zellforsch.* **60**, 204—212.

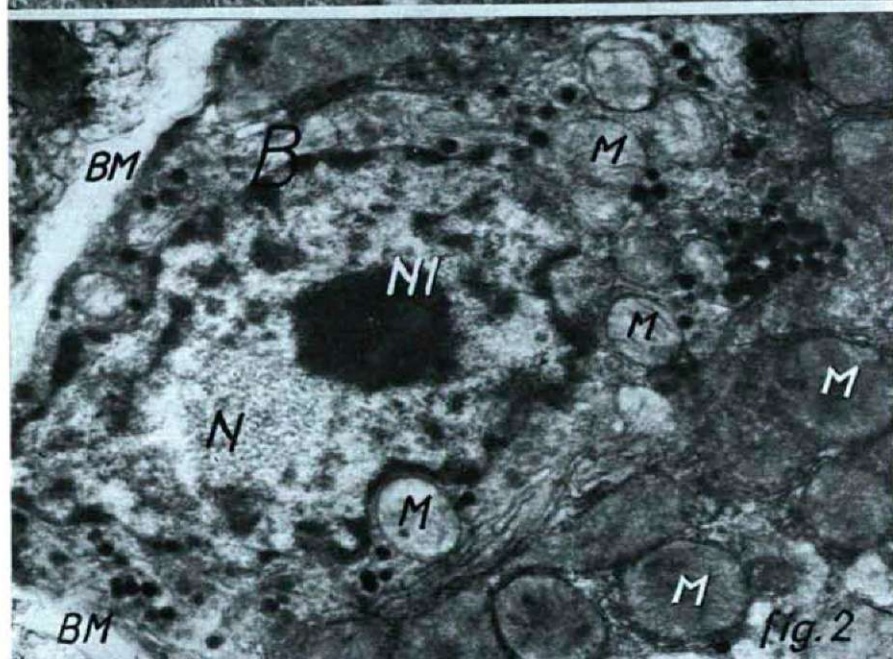
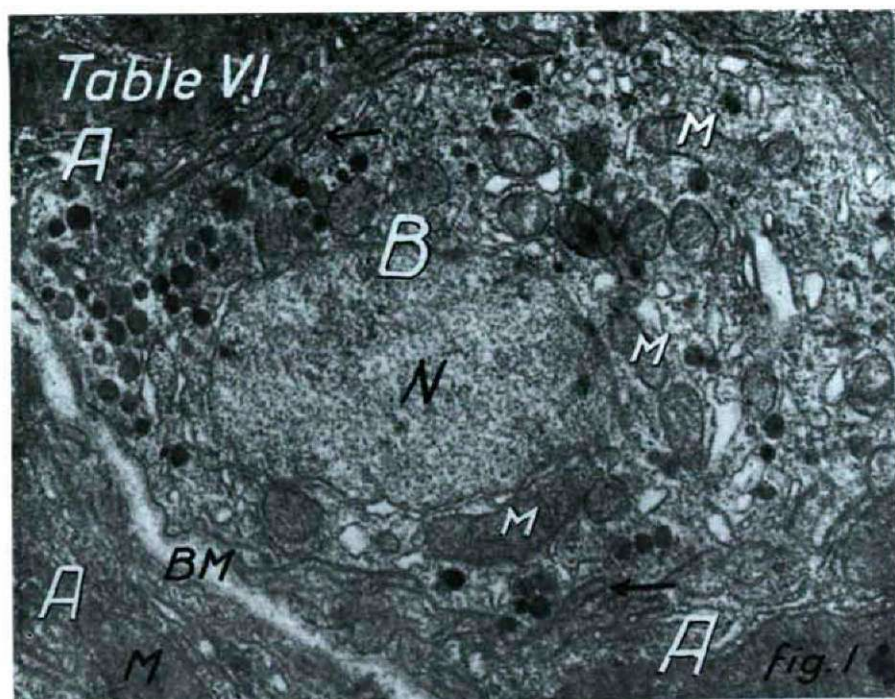
Table V

Picture of a secreting cell of type „B”. Apart from the Golgi-apparatus and a few canals of the granulated endoplasmatic reticulum, secretion drops of various density of a size similar to, or somewhat smaller than, that of mitochondria are seen (double arrow). Cell „B” touches an „A” cell on the right, while on the left, it is separated by the narrow strip of the basal membrane from a cell having a very thin cytoplasm (asterisk) supposedly a fibrocyte. x20 000

Table VI

Figs. 1, 2. Secreting cells of type „B”. In some places, the cytoplasm of „B” cells performs interdigitation with a cell of type „A” (VI/1, arrows). The density of secretion is inversely proportional to their size (double arrows), i. e., inspissate. x 28 000





- VASSALLO, G., SOLCIA, E. and CAPELLA, C. (1969): Light and electron microscopic identification of several types of endocrine cells in the gastrointestinal mucosa of the cat. — *Z. Zellforsch.* 98, 333—356.
- VIAL, J. D. and Orrego, H. (1960): Electron microscope observations on the fine structure of parietal cells. — *J. biophys, biochem. Cytol.* 7, 367—372.
- VONK, H. J., BRINK, G. and POSTINA, W. M. (1949): Digestion in the stomach of birds. I. The acidity in the stomach of young chickens. — *Proc. K. Akad. Wet. Amst.* 49, 972—982.

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