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MICROSCOPICAL STUDY ON THE PURPLE GLAND OF *TETHYS PUNCTATA* CUV.

BY

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(With 4 Text-figures and 1 Plate)

It is a well known fact that *Tethys punctata* CUV. upon irritation discharges a purple secretion in remarkable amount from its purple gland. Many papers have been published in which the secretion is discussed from the chemical viewpoint, but there are only a few dealing with the matter from the histological point of view. As a matter of fact, the manner of the formation of the purple secretion in the gland cells is still quite unknown. The reasons why the histological studies on the gland have been thus delayed may probably be due first to the superficial simplicity of its structure and secondly to the difficulty of obtaining the younger animals in which the gland is developing.

The present study was undertaken by the author with the purpose of making clear the process of the formation of the secretory granules in this gland by both the histological method and by experiments.

The investigation has been carried on at the Mitsui Institute of Marine Biology under the guidance of Prof. Kan OGUMA to whom the writer wishes to express his sincere gratitude for many kind suggestions and helpful criticisms.

Material and Methods

Tethys punctata CUV. is abundantly found in littoral waters along the coasts of the south-western parts of Japan from February

till June. They migrate from the deeper sea to shallow waters up to tide marks in order to find places on which their cordons of eggs are to be attached. The matured animal is brown to black in colour with many white spots, having a length of about 10–20 cm. when fully extended.

The rich purple secretion characteristic to this animal is produced from unicellular glands situated on the under side of the free edge of the mantle (BABA '28, '29), as a response to mechanical irritation on the mantle.

By rubbing the surface of the under side of the mantle-skirt with the fingers one can easily obtain an empty condition of the gland by discharge of secretion to an extreme degree. Then the colour of the surface changes into yellow. The animals, thus operated, were put into a tide-pool marked with short threads attached at the end of their soles to distinguish them from other un-operated ones. Every one hour after operation they were fetched into the laboratory and their mantles were cut off into pieces with a pair of scissors, then the pieces were thrown into the fixatives. The dissection had to be done as rapidly as possible, otherwise the secretion accumulated by that time might be discharged by operative irritation. The secretion is produced very slowly, yet the author ascertained that the under side of the mantle becomes black again about six hours after the extreme discharge of the secretion.

Before determining the fixatives to employ in the present study, the reaction of the secretion to various kinds of reagents was tested. As indicated in every paper dealing chemically with the secretion, it dissolves itself out of the tissue in water, alcohol, and acetic acid. In accordance with McMUNN (see SAMUELY'S chapter in ABDERHALDEN '22), the author could precipitate the secretion with water saturated with ammonium sulphate.

Among fixatives employed in this study such as "Susa", ZENKER'S, BOUIN'S and BOUIN-ALLEN'S mixture minus urea, the last one evidently proved to be most suitable for the study of the general

appearance of the tissue. The same mixture containing urea brings about a strong shrinkage of connective fibres. Although the purple secretion dissolves into the fixatives to some extent, some constituents of the secretion still remain in the sections. As the control the author adopted the freezing method in parallel to the ordinary methods. For staining, MANN's methyl blue-eosin method was chiefly applied either for the topographical or for the cytological observation. (In the present paper 'M-E' indicates MANN's method). Sections were made 7 micra in thickness. The freezing sections were made after fixing the material with water saturated with ammonium sulphate. For those sections, staining with methyl green (0.1% aqueous solution) gave better results, as compared with neutral red or fuchsin. Most of other dyes, however, seemed unusable as they were precipitated by ammonium sulphate.

For the purpose of demonstration of mitochondria the author used CHAMPY-KULL's fixative followed by chromification in the ordinary way. The sections were stained by ALTMANN's acid fuchsin-picric acid method and counterstained with light green, the sections being 3 micra in thickness. Very interesting to say, chromic acid bleaches the purple colour of the secretion.

In order to detect the crystals of urea xanthidrol reaction¹⁾ was tried, but no fragments of crystals were found as explained later on. No reaction could be noted when 9 c.c. of 0.1% aqueous solution of pilocarpin were injected into the hemocoel beneath the neck.

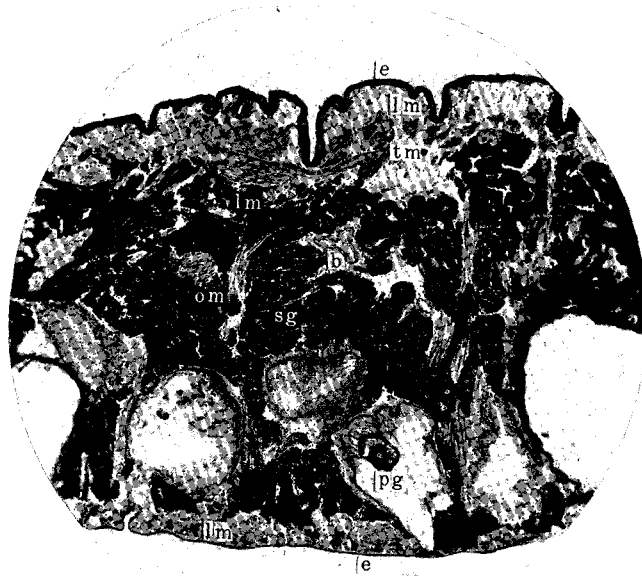
General Structure of the Gland

As already stated this gland is situated on the under side of the mantle-skirt, forming a black lunar shaped organ (BABA '28, '29). Histological studies of this gland have been published by only a few authors such as GOODSIR ('42), BLOCHMANN ('83) MAZZARELLI ('89), among whom BLOCHMANN ('83) shows the most precise results on

1) After ROMEIS: "Taschenbuch der mikroskopischen Technik." 13. Auflage, 1932.

the histological anatomy not only for the present species but also for the other closely allied ones. The remaining two authors inform merely some fragmental observations on the gland.

The material was always cut transversally. The upper side of the mantle-shelf is covered by one layer of tall pigmented epithelium cells (Text-fig. 1, e and Text-fig. 2, e). Large mucous cells

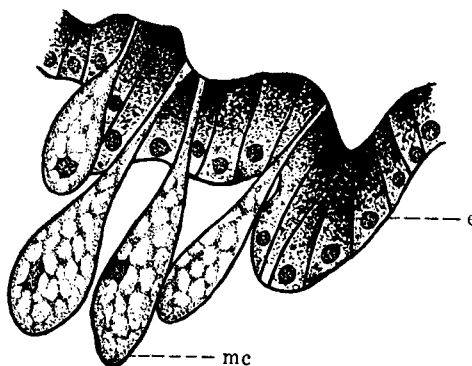


Text-fig. 1. Cross section through the mantle-shelf. Photomicrograph. $\times 50$; e epithelium, lm longitudinal muscle, tm transverse muscle, om oblique muscle, pg purple gland, sg small glandular cells, b blood-vessel.

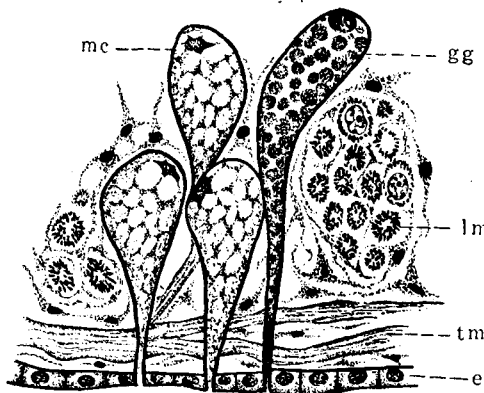
grow into the subepithelial tissue from the epithelium (Text-fig. 2, mc). Beneath the epithelium muscular bundles are arranged into strata forming a thick muscular coat (Text-fig. 1, tm and lm). The bundles are to be grouped into two kinds, transverse and longitudinal. The latter is again separated into two, lying on the upper and under sides of the former (Text-fig. 1, tm and lm).

The under surface of the mantle-skirt is covered with epithelium consisting of flat cells without pigment (Text-fig. 1, e and Text-fig. 3,

e). Beneath the epithelium there are also found two muscular coats, the outer being transverse and the inner longitudinal (Text-fig. 3, tm and lm). Mucous cells and albuminoid glandular cells with eosinophilic granules have their openings among the epithelial cells (Text-fig. 3, mc and gg). It is in this region where the large unicellular glands with voluminous nuclei, the purple glands, are to be found abundantly, and also they have their mouths on this surface of the shelf (Text-fig. 1, pg). The purple gland is coated with muscular fibres, which come into such close contact with the cell as one can hardly distinguish the cell-boundary from the fibre. This muscular coat is very thick while the cell is free from purple secretion and is very thin during full activity of secretion. From what is known about changes in thickness it is plainly to be concluded that the muscles are provided for discharging the secretion by their contraction.

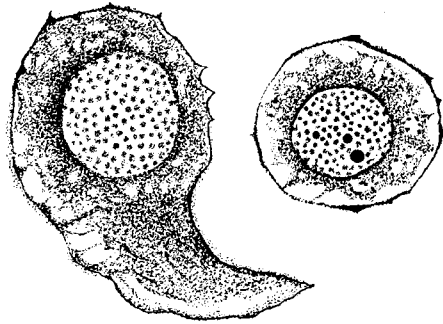


Text-fig. 2. Transverse section of pigmentated epithelium covering upper surface of mantle-shelf. $\times 680$; mc mucous cell, e epithelium cell.



Text-fig. 3. Transverse section of under side of mantle-shelf, where purple gland is not indicated. $\times 680$; mc mucous cell, gg gland with granulations, tm transverse muscle, lm longitudinal muscle, e epithelium cell.

The whole inner part of the mantle is occupied by small glandular cells (Text-fig. 1, sg)²⁾ and connective tissue (Fig. 9, c). Those small glandular cells correspond with those previously considered as the connective tissue cells by BLOCHMANN (bdg. in BLOCHMANN '83). But those cells are clearly different from the true connective tissue cells, because the former have somewhat larger nuclei stained blue by M-E and very often contain granules of the purple secretion in their cytoplasm as explained in the next paragraph, while the latter have smaller nuclei stained red with M-E. Across the area where the small glandular cells appear the muscular fibre bundles run obliquely



Text-fig. 4. Two nerve-cells, one of which has its axon. $\times 680$.

in connection with both upper and under surface of the mantle-shelf (Text-fig. 1, om).

Among the aggregation of those small glandular cells are found the nerve cells of characteristic large size (Text-fig. 4), in addition to the blood capillaries (Text-fig. 1, b). The nuclei of the

nerve cells are very large, and they have a considerable amount of chromatin and contain 2-3 nucleoli in each nucleus.

The Secretions after Experiments

The author has discovered that the small glandular cells produced the purple secretion after his experiment, as stated in detail later, but the speed of this production was very slow. The great amount of the purple secretion contained in the purple gland may have already been made during their infancy. As the purple glands

2) In the present paper the name 'small glandular cells' always denotes these cells, while the name 'purple gland' means the large unicellular gland as above described.

developed from the epithelium cells (BLOCHMANN '83), the small glandular cells around them might collapse into the purple glands.

Prior to the commencement of the cytological observation of the gland, the animals had previously been so operated as almost completely to free the glands from the secretion accumulated up to that time. This condition (Fig. 5) is easily obtained, as mentioned already, by rubbing the under surface of the mantle-shelf with the fingers. In the course of time after such experiment the new secretion gradually appears in the gland and accumulates very slowly. The secretion can be differentiated into three kinds, which will be described as follows.

1) *Purple secretion.*—This secretion is characterized by its purple colour in living cells as well as in sea water, but it is changed to brown by almost all sorts of ordinary fixatives except those containing chromic acid, which bleach the colour completely. Sections of the material set free for six hours after their discharge, revealed the course of formation of the secretion (Fig. 6). Upon examining the sections carefully by microscope under high magnification yellow granules can be found in some small glandular cells around the purple gland. Those granules have strong resistance against every reagent.³⁾ The cells containing those granules migrate into the cavity of the purple gland through the interspaces of muscle fibres (Fig. 6), then the colour of the granules changes gradually into brown. In the course of the migration the cells seem to die and disintegrate, and only mere secretory granules are seen falling into the cavity, where they fuse with one another to form larger purple globules. The formation of the secretory granules will be traced more in detail in the smaller glandular cells (Figs. 9–12).

The first step of secretion is indicated by the appearance of granules staining blue (Fig. 10). At the next step of development the granules grow larger and become liable to stain red with eosin (Fig. 11). Then they lose all the staining affinity to M-E, and they

3) The microscopic detection of urea was tried in connection with the production of those granules, but the result was negative.

continue their growth further, thus altering finally into the yellow granules with high refracting index to light (Fig. 12). In parallel to the changes in the secreting granules, the nuclei of the cells also show changes in their staining capacity, from blue to red, and they gradually increase in volume. In other words, the nucleus becomes oxyphilic in contrast to its original basophilic nature. Throughout all the stages a large round nucleolus stained red is found in every nucleus. While the changes are taking place in the small glandular cells as mentioned above, the cytoplasm of the purple glands seems to produce the same kind of secretion, as the brown granules are not only scattered in the cytoplasm but also aggregated on the surface of the nucleus (Fig. 8). But the relationship between the purple secretion, or brown globulous particles in the preparations, and those nucleoli is still obscure.

2) *Thin albuminoid secretion.*—This kind of secretion is sharply distinguished from the purple matter in respect to its staining light blue by M-E and seems to be more dilute than the latter (Fig. 2). This secretion very often occupies the whole area of the glandular lumen instead of the characteristic purple secretion. The formation of this thin albuminoid secretion is closely similar to that of the preceding one. Some small glandular cells first migrate into the cavity where they collapse (Fig. 7). In the cavity, their nuclei become picnotic and the cytoplasm becomes gradually translucent and dissolves finally into the characteristic fluid, which often slowly changes its chemical nature—blue stain to red stain by M-E (Fig. 2).

3) *Thick albuminoid secretion.*—The name 'thick albuminoid secretion' is given to the secretion of more viscous nature than that described above. The fluid is so viscous that artefact slips are always produced around the coagulated masses (Fig. 3). The formation of this secretion cannot be observed. As there exist, however, intermediate kinds of secretion between the thin and the thick ones, it may be concluded that the latter is in all probability derived from the former. The most striking character of this kind of secretion is its strong staining capacity for eosin.

The glands containing thin and thick albuminoid secretions are also found in the sections from unoperated material, but they are found more frequently in the animals after operation.

4) *Watery secretion*.—This secretion contains a small amount of coagulable material; yet one sometimes finds some clouds of fleecy coagulation stained blue by methyl blue (Fig. 4). This coagulation reminds one of the albuminoid secretion, and the formation of this secretion must be closely related to that of the albuminoid ones. In many cases the cavities of the purple glands are filled up with this watery content, especially in the sections of the animals kept for a long time in the aquarium. In those sections the decrease of the small glandular cells may be noticed; most of them have probably been consumed for the formation of the watery secretion. Sometimes the purple gland expands so greatly by the enormous accumulation of fluid that the nuclei of the gland become compressed against the gland wall.

It can be proved that the latter three kinds of secretion include no trace of purple secretion when the material fixed with ammonium sulphate are cut by means of freezing method and the sections are stained with methyl green.

Mitochondria in Relation to the Secretion

With a purpose to know the relation, if any, between the secretory granules and mitochondria contained in the cytoplasm of the small glandular cells, the writer tried first to demonstrate the mitochondrial elements by employing ALTMANN's method in combination with light green. As stated already, the secretory granules, which show beautiful purple in the natural state are entirely bleached by chromic acid and absorb picric acid taking on a yellow colour (Fig. 16; Fig. 17, *g*). In strong contrast to this kind of granules, the mitochondria are to be distinguished by staining with acid fuchsin as well as by having remarkably small size (Fig. 13). If mitochondria of such appearance are compared with the secretory granules of

yellow colour as above mentioned, there is noticed scarcely any intimate relationship between these two formative elements in cytoplasm. But in reality, it is the author's belief that the latter is directly derived from the former by transformation of shape and changes in chemical nature as described in detail as follows.

In the cell, in which the secretion has not yet taken place, the mitochondria present the ordinary shape as granules or short filaments which may curve in any way uniformly distributed in the cytoplasm (Fig. 13; 17, *a* and *b*). The secretion initiates in mitochondria of such shape. First of all, vacuolization occurs, thereby they grow larger and become converted into small spherules, of which the peripheral part still takes acid fuchsin intensely enclosing the paler interior part (Fig. 14; 17, *c*). This latter pale part, then acquires the same intensity of colour as the periphery, thus presenting a more compact appearance, and it grows (Fig. 17, *d*). The growth continues still further and the staining reaction gradually changes from red to green (Fig. 15; Fig. 17, *e* and *f*), then pale green to yellow at last (Fig. 17, *g*). While such a transformation of mitochondria is taking place, the cell body itself grows too and considerably increases its volume (Fig. 16).

From what could be directly observed, as stated above, in the cytoplasm of the small glandular cell, the author tends strongly to the view that the purple matter secreted by the purple gland is the direct descendant of the mitochondria. In regard to the secretion of various kinds of glands, the mitochondria are believed in general to play an important rôle either directly or indirectly. Especially in the case of zymogen granules their direct changes have been confirmed by many authors (see DUESBERG '12 and HEIDENHAIN, M. '07). The formation of the secretory granule of purple matter seems consequently to belong in the same category with the case of zymogen granules, at least in mode of transformation which is possible to observe under microscope.

Summary

1. The secretions in the purple glands of *Tethys punctata* CUV. can be classified into four categories: the purple, the thin albuminoid, the thick albuminoid and the watery secretion; the latter three kinds of secretion are very often met with after the discharge of the dye.

2. By experimental study the cells which have been regarded as the connective tissue cells are proved to be glandular in nature; they migrate into the cavity of the purple gland and turn into the secretion.

3. The secretory granules of purple colour are produced directly from the mitochondria.

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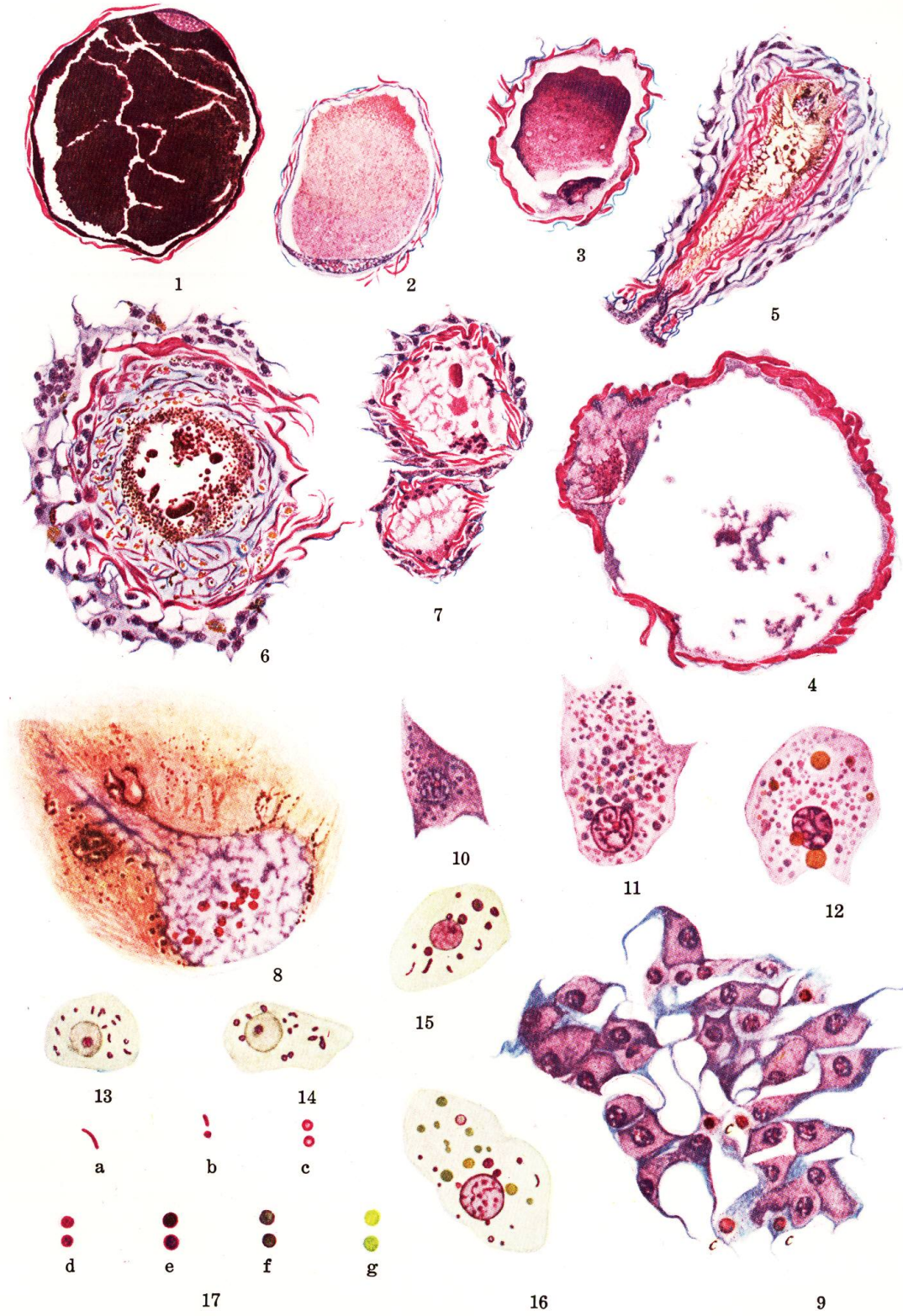
Plate XIV

Explanation of Plate XIV

All the figures of the plate were drawn at the level of the stage of the microscope, with the aid of ABBE's drawing apparatus. Figs. 1 to 7 were drawn with LEITZ objective 3 and ocular 10, magnification being 85 times. Fig. 8 was drawn with LEITZ objective 6 and ocular 10, the magnification being 600 times. Figs. 9 to 17 were drawn with LEITZ aplanatic objective apart. 1.30 and ocular 6, t. 1. 200 m.m. The magnification is about 1750 times. Fig. 17, *a-g* are schematic figures.

Figs. 1 to 12 are drawings from the sections of the material fixed with ALLEN-BOUIN's fixative minus urea and stained with methyl blue and eosin. Figs. 13 to 17 are drawings from sections of the material fixed with CHAMPY's mixture followed by chromification in the ordinary way and stained with ALTMANN's acid fuchsin-picric acid method and light green.

- Fig. 1. Gland full of purple secretion.
- Fig. 2. Gland full of thin albuminoid secretion.
- Fig. 3. Gland full of thick albuminoid secretion.
- Fig. 4. Gland full of watery secretion.
- Fig. 5. Empty gland immediately after the discharge.
- Fig. 6. Gland into which the small glandular cells collapse with their purple secretions.
- Fig. 7. Gland producing the thin albuminoid secretion.
- Fig. 8. Nucleus of the purple gland surrounded by the deep purple granules.
- Fig. 9. Small glandular cells in resting stage of the secretion. *c*, connective tissue cells.
- Fig. 10. Small glandular cells at beginning of secretion.
- Fig. 11. Small glandular cells, advanced in secretion.
- Fig. 12. Small glandular cells, with some yellow secretory granules, which are in the stage just before the production of the purple secretion.
- Figs 13-16. Successive mitochondrial changes in the small glandular cells at the formation of the purple secretion, where mitochondria turn into secretory granules.
- Fig. 17, *a-g*. Successive stages in the change of the mitochondria into the yellow granules in Figs. 13-16.



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