An Electron Microscopic Study on the Toad Subcommissural Organ.*

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In spite of numerous investigations, the nature and function of the subcommis-
sural organ (SCO) located beneath the posterior commissure was not clear owing to poor
staining methods, and for a long time this organ was regarded as one of the most mys-
terious organs. However, since recently a variety of special staining methods includ-
ning GOMORI's chrome alum hematoxylin and other histochemical techniques was
introduced in studies of this organ, remarkable advances have been made in this field
by many investigators, and the fact that the SCO throughout the series vertebrates
displays either rudimentary or intensive secretory activity and that the stainable sec-
retory substances which are presumed to contain mucopolysaccharide-protein com-
plex are discharged from the apical portion of the cell into the ventricle lumen has been
established. On the other hand, it has been also confirmed that in most of the lower
vertebrates, occasionally even in a few mammals, besides the apical secretion the other
mode of transport of secretory substances occur by which they are released via cell
processes into capillaries or the subarachnoidal lumen (OKSCHE 1956, OKADA 1956,

Though a large amount of light microscopical studies about it were performed,
investigations of the SCO using electron microscope have been hitherto published only
by a few authors (AFZELIUS and OLSSON 1957, MURAKAMI 1959 and MURA-
KAMI et al. 1962).

MURAKAMI and his collaborators (1962) studied the SCO of histamin treated
Gecko with the electron microscope and stated that the secretory substances are pro-
duced in the rough surfaced endoplasmic reticulum located in the supranuclear region.
But the problem to what extent the GOLGI complex is involved in the formation of
secretory substances remained unsolved.

In the present paper the fine structure of the toad subcommissural cell as well as
of the hypendymal cell was observed and at the same time the origin of secretory sub-
stances was discussed.

I. Material and methods.

All the experiments were carried out for a period of 2 months from August to

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September. Tissues were obtained from mature toads, *Bufo vulgaris*, of both sexes. The toads were killed by decapitation. The brain was exposed and small blocks containing the subcommissural organ were removed. The blocks were cut under the binocular microscope into several smaller ones with razor blade. The smaller blocks obtained were fixed in a 2 per cent osmium tetroxide solution adjusted at pH 7.4 with CAULFIELD’s veronal acetate buffer containing sucrose (1957), and then were washed briefly with distilled water, dehydrated in a graded series of acetone and embedded in Epon according to LUFT (1961).

Sections were cut on a PORTER-BLUM microtome equipped with self-made glass knife, mounted on copper girds covered with a formval and doublestained with uranyl acetate and lead hydroxide. Electronmicrographs were taken in a JEM-4 and HS-6 at initial magnification of 3,000—9,000 times and enlarged photographically as desired.

For light microscopy the tissues were fixed either in ROSSMAN’s or BOUIN’s solution, embedded in paraffin, cut into 6 μ thick serial sections and stained with paraldehyde-fuchsine-phloxin or with periodic acid-SCHIFF staining.

II. Observation and discussion.

*Subcommissural cells (SCO cells).*

There are considerable differences in the extent of differences of the SCO cells between the central and the peripheral portion of the organ: in the former the SCO cells are well differentiated and provided with characteristic features described below, in the latter most of them are not well differentiated and are similar in many structural respects to the ependymal cells covering the rest of the ventricles.

Therefore the observation here shall be confined to the typical SCO cells located in the central portion of the organ.

The SCO cells bordering the ventricle consist of 1—2 rows of the columnar cell with a long peripheral process, as described already by many lightmicroscopists. They are similar in form to the ependymal tanycytes existing commonly in the other regions of brain, but somewhat larger in size than the latter. The nucleus of the SCO cell is rounded or elongated oval in shape and located frequently in the basal portion of the cell. The intercellular space formed between adjacent SCO cells is generally narrow near the ventricle, measuring about 200 Å in diameter, and runs parallel to the lateral cell membrane, but with approaching the base of the cell it becomes wider and wider to be finally continuous with the vast extracellular space surrounding the neuropils (Text-fig. 1, Fig. 1).

The terminal bars characterized by increased thickness and density of the cell membrane are present just below the apex between adjacent cells. On the opposing cell membranes of two adjacent cells are also found desmosomes which can be seen frequently to be arranged in row as illustrated in Fig. 2. The desmosomes are built up of dense amorphous material, through which bundles of fine fibrils suggestive of keratin run parallel to the lateral cell membrane. Some of these keratin-like fibrils

Fig. 1. Survey picture of the supranuclear region of the Subcommissural cell. At lower magnification. In this region well-developed GOLGI complexes and abundant paired membranes of the rough surfaced endoplasmic reticulum can be seen. Intercellular space reveals occasional dilatation, within which microvilli-like small processes from the subcomissural cell are found. Beneath the free surface membrane of the cell a few sectory granules are accumulated. At the upper left corner of this picture ventricle lumen. $\times 12,600$
extend into the cytoplasm, where they are randomly oriented.

Since the SCO cell, as generally known, occupies the relatively wide brain territory extending from the border of the ventricle across the posterior commissure to the outer brain surface, it seems to be convenient for description to divide the cytoplasm of the cell into the following four parts characteristic in structure respectively: the supranuclear, nuclear, basal regions and a process.

Supranuclear region.

The apical cell membrane of the SCO cell is generally even and tends to be slightly convexed towards the ventricle lumen. Microvilli small in number and size are poorly developed in this cell. Cilia are only occasionally found in it and composed of 9 peripheral filaments and 2 central ones. Rootlet fibers with cross-banded structure are seen to radiate away from the basal part (basal corpuscle) of a cilium (Fig. 3). Though cilia of this cell show the typical internal structure of normal kinocilia, they can scarcely be seen. Therefore the cilia may not be thought to have any function in the sense of promoting the circulation of cerebrospinal fluids, unlike those of the usual ependymal cells. They seem to be mere embryological remnants indicative of differentiation from the ependymal glia.

According to cell, as shown in Fig. 4, occasional cytoplasmic projections from the free surface of the cell into the ventricle lumen, which contain not cellorganelles, such as the mitochondria and the GOLGI complex, but a large number of vesicles and dense granules, are encountered. They resemble the protrusions which are characteristic of apocrine secretion as described by several investigators in various glands (KITAMURA 1958, KUROSUMI et al. 1959 and 1961, SCOTT and PEASE 1959, ITO 1961 and PARKS 1962). They are, however, so rare in this cell that it is doubtful whether they represent true apocrine secretion or not, and even if that is the case, such mode of secretion as this is unlikely to be representative of the SCO cell.

In the portion just below the apical cell membrane are present a large amount of dense granules about 1,500—3,000 Å in diameter (Fig. 5). They appear to be homogeneous or granulated and are surrounded by the smooth membrane, from which some of the granules are separated by a narrow space. Many of the granules are round or oval in shape, but sometimes rod-like or irregularly shaped granules are observed among them. Though in a few cases, some of the granules are to be in close apposition or in contact with the surface cell membrane. Fig. 6 may suggest that one of the granules is open to the ventricle lumen at the cell surface, where the limiting membrane of the granulum facing the ventricle lumen disappears presumably by fusion with the cell membrane. These granules also can be seen dispersed randomly throughout the supranuclear region, though abundant in the GOLGI area, but few of them are found in the other cytoplasmic regions.

The appearance as well as the distribution of these granules entirely correspond to the finding obtained with light microscopy that the paraldehyde-fuchsin-phloxin stainable secretory substances are accumulated most numerously in the supranuclear region, above all, near the free surface of the SCO cell. Therefore, it might be reasonable to conclude that the granules represent one of the secretory substances characteristic of the SCO cell and that they are discharged from the cell surface into the ventricle lumen to form possibly the REISSNER' s fibers in the same way as described.
above, although no structures suggestive of REISSNER's fibers were observed in our study.

The GOLGI complex is considerably well developed in this cell and mostly located in the supranuclear region (Fig. 1). It is composed of the following three components, as generally accepted, GOLGI-lamellae, GOLGI-vacuoles and GOLGI-vesicles. They are embedded in the GOLGI area showing somewhat higher density than the rest of the cytoplasm. In the GOLGI area, especially in its periphery dense granules surrounded by the limiting membrane are abundantly encountered. Most of them are rounded or oval-shaped, but some appear rod-like or irregular in shape. Their size ranges from 1,000 Å to 1,500 Å in diameter (Fig. 7). From their close spatial relationship with the GOLGI complex, these granules are assumed to have been produced in the GOLGI complex and their envelopes to have been originated from the wall of the GOLGI components, possibly of the GOLGI vesicles. These granules resemble in structure and shape closely the above-mentioned secretory granules accumulated beneath the apical cell surface, though somewhat small in size as compared with the secretory granules.

From this, it can be concluded that the granules found in the GOLGI area may be nothing but the immature form of the secretory granules. Thus the secretory granules may be revealed at first within the GOLGI components, especially in the GOLGI vesicles, transported from the GOLGI field through the cytoplasm into the apical portion of the cell, becoming larger in size and accumulated finally in the portion beneath the free cytoplasmic membrane as mature granules.

In the wall lizard such osmiophilic granules (GOLGI granules) are also observed near the GOLGI area of the SCO cell, as previously reported (MURAKAMI et al. 1962). In the lizard, however, the granules are very small in number and size in comparison with those of the toad and no clusters of the granules are observable in the portion below the free cell surface.

AFZELIUS and OLSSON (1957) investigated the SCO cell of the hagfish with the electron microscope and found in the apical region of the cell some rows of osmiophilic particles, about 0.7 μ in diameter, which lack the distinct limiting membrane. The authors presumed that particles might be identical with the chrome alum hematoxylinophilic granules which are characteristic of the SCO cell. On the contrary, in the toad we could not recognize such particles described by them.

The paired membranes of the rough surfaced endoplasmic reticulum are abundant in the supranuclear region as well as in the nuclear and basal region. They are arranged in closely parallel lamellae in the supranuclear portion near the nucleus. However, in the apical portion below the free cytoplasmic membrane their arrangement becomes

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Fig. 2. Paranuclear region of two adjacent subcommissural cells. At the junction of the two cells a row of dense desmosomes can be seen, through which bundles of fine filaments suggestive of keratins run longitudinally. ×13,500

Fig. 3. Apical portion of the subcommissural cell showing rootlets of cilia. A cross-banded structure of the rootlets is obviously seen surrounded by mitochondria. ×19,200

Fig. 4. Apical portion of the subcommissural cell. The apical cytoplasm is considerably swollen and protrudes into the ventricle lumen. Within this cytoplasmic projection a number of vesicles and dense granules are observed, but no cellorganelles such as mitochondria. In the ventricle lumen a few cross-sections of cilia are also visible. ×11,500
irregular and vacuole- or tubule-like profiles of the endoplasmic reticulum increase in number. They are provided with only a few ribosomes, i.e. most of them are partly granular, but some entirely smooth (Figs. 4 and 6).

The contents of the cavity of the endoplasmic reticulum are of either uniformly low density or empty.

Mitochondria are scattered randomly in the supranuclear region of the cell and more abundant than in the rest of the cytoplasm. They exhibit oval or rod shaped profiles, and bifurcated ones are also occasionally observable. In the apical portion the matrix of the mitochondria sometimes reveals high density as compared with the other portion of the same cytoplasmic region. Therefore, in this case, it may be difficult to distinguish the mitochondria from the secretory granules mentioned above (Fig. 5). AFZELIUS and OLSSON (1957) suggested that the secretory droplets in the SCO cell of the hagfish are transformed from the mitochondria. But in the toad the direct involvement of this cellorganelle in the production of secretory granules was not confirmed, though the necessity of mitochondria as an energy source in producing the granules is undeniable.

Free ribosomes are found distributed abundantly through the supranuclear region of the cell as individual granules or small clusters of granules.

Nuclear region.

The nucleus is filled with diffusely distributed fine granules containing desoxyribonucleic acid. The nucleoli are only occasionally found within the nucleus eccentrically. The nuclear envelope consists of the inner and outer nuclear membranes enclosing a narrow space called perinuclear space. The outer nuclear membrane is studded with fine granules presumed to be ribosomes.

In some places, the perinuclear space appears to be continuous with the cavities of endoplasmic reticulum situated near the nucleus, as reported in the same organ of the wall lizard (MURAKAMI et al. 1962). Nuclear pores formed by fusion of the two nuclear membranes are not clearly demonstrable in this cell. The perinuclear portion sandwiched between the lateral cell membrane and the outer nuclear membrane is very narrow and shows a slight convexity towards the outside in accordance with the expansion of the nucleus. The greater part of this portion is occupied exclusively by the paired membranes of endoplasmic reticulum closely arranged in parallel lamellae, which are associated with those in the supranuclear region of the cell. Very few mitochondria are present in this region.

Fig. 5. Apical portion of the subcommissural cell. Beneath the surface cell membrane a large amount of the dense secretory granules with the limiting membrane are accumulated. Many of them are oval or round in shape, but some of them are rod-like. ×31,500

Fig. 6. Apical portion of the subcommissural cell. Below the surface cell membrane the secretory granules and vacuoles provided with a few ribosomes are scattered. Arrow shows one of the secretory granules, which is about to open into the ventricle lumen. ×28,000

Fig. 7. Supranuclear region of the subcommissural cell showing the GOLGI complex. In the GOLGI area a large number of GOLGI vesicles filled with moderate dense material occur. In the periphery of the GOLGI area a few dense granules of somewhat larger size than the GOLGI vesicles are observed. They are enveloped by the limiting membrane and similar in structure and form to the secretory granules located in the apical portion of the cell. Near the GOLGI area a bifurcated mitochondrion is seen. ×27,000
**Basal region.**

The characteristic of this region, as illustrated in Figs. 8–11, is the appearance of a concentrically arranged lamellar body of the paired membrane bearing fine granules. The body contains occasionally a central core of dense material. This whorl-like structure of the body is round or elongated oval in shape and its size is so large, 6 μ, that the basal region of the cell appears to be commonly occupied by only one whorl. Most of the paired membranes comprising the whorl are large in number and closely arranged each other. In some cases, however, they are in small number and coarsely arranged and presumably represent the initial stage of formation of this structure. The cavities of the paired membrane are generally of constant width, measuring about 200 to 300 Å. But sometimes they exhibit the cisternal or bead-like shaped dilatation of various sizes. Each of the paired membranes does not always form a closing ring, but shows an occasional anastomosis and turn-up with adjacent pairs. The cavities of the paired membrane are usually low dense, while the dilated ones optically empty.

Many of this whorl-like structure occur solitarily in the basal region. However, in sections, their edge is found to be continuous with the membranes of the rough surfaced endoplasmic reticulum extending from the nuclear region. This rises the possibility that such whorl-like structures are nothing but a modification of the rough surfaced endoplasmic reticulum, so that fine granules attached to the outer surface of the paired membranes comprising the structure can be said to represent granules of ribosomes. Similar concentric or whorl-like lamellae of the paired membrane studded with fine granules have been found hitherto either in the pathological or in the normal cell of various kinds (HAGUENAU 1958, HYMER et al. 1961, SHIMAZAKI 1962, HERMAN and FIZGERALD 1962 and KUROSUMI et al. 1962), though their functional significance is not yet clear.

Concerning the functional significance of these concentric lamellae of the rough surfaced endoplasmic reticulum, we speculate that these structures do not suggest any degenerative process of this organelle or artefacts caused by fixation, but may participate positively in synthesis of certain, possibly secretory substances, including formation of the endoplasmic reticulum itself. The finding obtained by light microscopy that a large amount of paraldehyde-fuchsin or PAS positive granules are accumulated in the basal region as well as in the supranuclear region of the cell, might support the rightness of our speculation.

This structures are not only limited to the basal region, but are observed also frequently in the proximal portion of a cell process.

In relatively rare cases, the irregularly shaped sacs of large size occur in the basal region (Fig. 18). On the inner side of the limiting membrane bordering the sac con-
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Densation of dense material can be encountered and its cavity is diffusely filled with fine granules of less density. At some places, the sac appears to be open to the extracellular space surrounding the cell (not figured). Therefore, it is presumed to contain certain substances which differ in property from those originated in the Golgi complex or in the endoplasmic reticulum. Very few mitochondria are observed in this region.

**Cell process.**

The basal region of the cell continues peripherally without any abrupt decrease in diameter into the cell process, whose matrix appears to be somewhat electron lighter than that of the other cytoplasmic regions. Long wavy filamentous structures in parallel array run longitudinally through the process (Figs. 10 and 12). They are divided into two kinds: one of these is a tubular filament about 100 Å in diameter, the other is finer one of compact structure, about 30 Å wide, suggesting the keratin. These two kinds of filaments are also present in the supranuclear region of the cell, but their appearance is most conspicuous in the process where a number of vacuoles of variable sizes and form are encountered. Many of the vacuoles are tubular or oval in shape and distributed randomly throughout the process. Most of the wall of the vacuoles are provided with only a few granules presumed to be ribosomes (Figs. 12 and 13). Accordingly, such vacuoles are thought to have been formed by segregation of the endoplasmic reticulum in the same way as those of the apical portion beneath the free cell surface.

On the other hand, it is well-known that the process of the SCO cell of the amphibian contains abundant granules stained with paraldehyde-fuchsin or PAS through its entire length.

From these findings it can easily be postulated that such vacuoles correspond to the carrier of paraldehyde-fuchsin positive granules, which are on the way of transport to capillaries or to the outer brain surface, though the cavities of vacuoles are of homogeneously low density or appear electron transparent, possibly due to dissolution of contents.

Mitochondria in the process are generally small in size and number. Sometimes, in the periphery of the process the swelling of mitochondria accompanied with the disappearance of cristae occurs, whose significance and cause are not clear. Between the cell processes as well as between the neuropils the wide extracellular spaces continuous with each other are found (Text-fig. 1). These spaces extend from the intercellular space between adjacent SCO cells just below the terminal bar to the perivascular space enclosing the capillary. They are interrupted finally by bundles of nerve fibres constituting the posterior commissure.

Since the extracellular space is recognizable in all the sections examined including sections in which fine structures of the tissue are well preserved, it might not be artefacts caused by unsuitable fixation or by other procedures.

One of the striking features of the process of the SCO cell is the appearance of

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Fig. 10. Proximal portion of the process of the subcommissural cell. The cavities of the paired membrane comprising the lamellar body reveal cisternal or bead-like dilatation. ×12,000

Fig. 11. Proximal portion of the subcommissural cell. The figure shows a concentric lamellar body presumably at the developing stage. The paired membranes comprising this body are coarsely arranged and small in number. Note the turn-up of the paired membranes (arrow). ×25,000
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microvilli-like small processes about several $\mu$ in length and 0.2 $\mu$ in width. These small processes project from the lateral surface of the cell process perpendicular to its long axis into the extracellular space mentioned above. They differ from usual microvilli in the following respect. Each process contains a chain of small vesicles and long tubules probably formed by fusion of the small vesicles and both of them run through the process longitudinally. Most of the cavities of such vesicles and tubules are optically empty, but some of them are of uniformly low or high density. The above-described extracellular space surrounding the process of the SCO cell are filled almost with a large number of such small processes running in random direction (Figs. 12 and 13). Small processes provided with the same structure were reported recently by EAKIN and WESTFALL (1962) in the ependymal cell of the amphioxus infundibular organ and by DOWLING and GIBBONS (1962) in the pigment epithelium of the albino rat.

As to the function of these small vesicles, the latter authors stated that the vesicles might be involved in migration of certain substances, for instance, vitamin A. From our finding obtained, we also postulate that these vesicular systems occurring in the small processes may indicate micro-pinocytosis. If that is so, the extracellular space surrounding the process may be thought to have an important physiological significance for the metabolism of the SCO cell as a passway or a lake of metabolic substances similar to the ventricle lumen.

A few profiles of unmyelinated nerve fiber can also be seen in the extracellular space. Between the nerve fiber and the process of the SCO cell synapses are occasionally found, which are characterized by the accumulation of small vesicles, possibly presynaptic vesicles, in the nerve ending and by thickening of opposing two cell membranes (Fig. 12).

According to OKSCHE (1962) the SCO organ of the frog is innervated by the N. pinealis from the pineal organ and both organs form functionally a close connection with each other. However, it cannot be decided here whether his opinion is right or not.

**Connection between the SCO cell and the blood capillary.**

The process ending of the SCO cell resting on the capillary is somewhat enlarged in volume and contains a number of vacuoles derived from the endoplasmic reticulum, where the wall of most vacuoles is no longer provided with ribosome granules.
of these vacuoles show dilatation of variable degrees, occasionally such dilated vacuoles are seen to come in contact with the plasma membrane of the process ending (Fig. 14). This finding may suggest the release of the contents of the vacuole from the process ending into the capillary.

Between the process ending of the SCO cell and the capillary intervenes the perivascular space of highly variable width, which appears in general optically empty, but contains bundles of collagen fibrils randomly oriented, pericytes and small portions of the process of the SCO cell, whereas in the same tissual territory of the wall lizard no perivascular space was encountered (MURAKAMI 1959).

Both sides of the perivascular space are limited by a layer of the inner and outer basement membrane respectively, which is of homogeneously moderate density and rather thin, measuring about 150 Å. The inner basement membrane is not directly subjacent to the plasma membrane of the SCO cell process, but between them is found a narrow empty spacing of about 200 Å in diameter, the inner cement layer (PEASE 1955), which is continuous with the extracellular space described above.

The 'outer cement layer' similar in structure and width to the inner one is also formed between the outer basement membrane and the capillary endothelium.

The capillary lumen is bordered continuously by a single layer of the endothelium about 0.4 µ in thickness. The cytoplasm of the endothelium appears somewhat dense and is filled with a large amount of vesicles, tubules and vacuoles. In sections, some of the vacuoles can be seen to push up the apical plasma membrane to the capillary lumen (Fig. 15). Thus, the abundant occurrence of vesicular system indicative of pinocytosis may suggest that in the endothelium some sorts of the lively activity of metabolism including possible discharge of the secretory substances of the SCO cell occur.

**Hypendymal cell.**

Subjacent to the base of the SCO cell cells of glial nature, different from the SCO cell in many respects, are observed. Since they are round or oval in shape and possess a remarkably large nucleus in proportion to their cytoplasm, they are easily distinguished from the overlying SCO cell even at optically lower magnification (Fig. 16). In view of its situation, such cells are considered to correspond to the so called 'hypendymal cell' reported by several authors (s. OKSCHE 1961).

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**Fig. 14.** Capillary nourishing the subcommissural organ. The endothelium of the capillary contains small vesicles and long tubules lying parallel to the surface membrane of the endothelium. Endfoot of the subcommissural cell resting on the capillary shows slender dilation, in which vacuoles of varying size and shape are found scattered. Some of these vacuoles dilate cisternal and occasionally are in contact with the cytoplasmic membrane of the endfoot (arrow). The capillary endothelium and the endfoot of the subcommissural cell are separated by a broad perivascular space, where pericytes, portions of the process of the subcommissural cell and dotlike cross-sections of the collagen fibrils are recognizable. ×20,700

**Fig. 15.** Portion of the capillary endothelium of the subcommissural cell. The cytoplasm is filled with a large number of vesicles and vacuoles of variable sizes and appearances. Cell-junction between adjacent two endothelia (arrow). ×14,000

**Fig. 16.** Survey picture of the hypendymal cell. At lower magnification. Note the large nucleus of the cell for its cytoplasm. The greater part of the cytoplasm of the hypendymal cell is directly surrounded by an extracellular space. At the lower left corner transverse section of an myelinated nerve fiber possibly branched from the posterior commissure. ×14,000
The nuclei of the hypendymal cells are rounded or transversally elongated oval-shaped in accordance with its cytoplasm and occupy the major portion of the cell. They are filled with fine granular chromatin substances distributed diffusely within. The nucleoli are rather often encountered as compared with those of the SCO cell. However, their localization in the nucleus varies from cell to cell. The nuclear membranes enclosing the nucleus are distinct and the outer nuclear membrane is provided with fine ribosome granules like the membrane of the rough surfaced endoplasmic reticulum. The cytoplasm of the hypendymal cell appears rather optically light. A few paired membranes of the rough surfaced endolasmic reticulum are also encountered. Most of them are tubular or cisternal in shape and no endoplasmic reticulum forming lamellae is found. At some places, the cavity of the endoplasmic reticulum is continuous with the perinuclear space formed between the two layers of the nuclear membrane. The GOLGI complex is poorly developed and present near the nucleus. This organelle consists only of GOLGI membranes and GOLGI vesicles of small number and the formation of GOLGI vacuoles is obscure in this cell (Fig. 17).

Mitochondria are found scattered sparsely in the cytoplasm. Some of them reveal swelling as seen in those of the process of the SCO cell (Fig. 19). The free ribosome granules are distributed through the cytoplasm. Dense granules suggestive of secretory phenomena, such as found in the SCO cell, are observed neither in the GOLGI field nor in the rest of the cytoplasm.

Although not in all the sections examined here, but in the hypendymal cell such irregularly shaped sac as found in the SCO cell (Fig. 18) and a few uniformly dense bodies presumed to be lipid are encountered.

The surface membrane of the hypendymal cell is partly applied closely to the adjacent one of the SCO cell, but its major part is exposed directly to the extracellular space.

OKSCHE (1961) reported recently that in the frog both the SCO cell and the underlying hypendymal cell contain abundant secretory granules stained with chrome alum hematoxylin, and are connected closely with each other by their processes. On the basis of this light microscopic finding he stated that both cells are not only spatially, but also functionally in close relationship. Any evidence, however, which may support enough his opinion, has not been obtained by us.

III. Conclusion.

The subcommissural organ of the toad was studied with the electron microscope and the following results were obtained.

1. The GOMORI-positive secretory granules, characteristic of the subcommissural

Fig. 17. Portion of the cytoplasm of the hypendymal cell. The cytoplasm of this cell appears rather light and its cellorganelles are poorly developed as compared with those of the subcommissural cell. In this cell any dense granules suggestive of secretory phenomena are not recognized. Close to the nucleus of the hypendymal cell cross-sections of two centrioles facing each other are present. G Golgi complex. \( \times 13,000 \)

Fig. 18. Cytoplasm of the hypendymal cell showing an irregularly shaped sac, which is characterized by homogeneous central contents and a dense periphery. \( \times 24,400 \)

Fig. 19. Cytoplasm of the hypendymal cell showing swelling of mitochondria. \( \times 16,000 \)
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cell, are made up of two kinds of the substance different in appearance and site of their origin: One of these consists of dense granules measuring about 1,500—3,000 Å in diameter, which are produced in the GOLGI complex and located in the supranuclear region of the cell. The other is small vacuoles with low dense or empty contents, which are derived from the rough surfaced endoplasmic reticulum found in the perinuclear and basal region of the cell.

The former dense granules are thought to pass the apical cytoplasm and be released from the free surface of the cell into the ventricle lumen. On the other hand, the latter vacuoles may be discharged from the process ending of the SCO cell into the capillary lumen.

2. In the basal region of the subcommissural cell, a concentric lamellar body of the rough surfaced endoplasmic reticulum occurs. Its functional significance was discussed.

3. The process of the subcommissural cell is provided with a large amount of microvilli-like small processes containing rows of vesicles and tubules suggestive of micropinocytosis.

4. Between the processes of the subcommissural organ and the capillary nourishing this organ intervenes a wide perivascular space. The endothelium of the capillary has no pores.

5. The hypendymal cell is rounded or transversally elongated oval-shaped and the cellorganelles of this cell are generally poorly developed. This cell does not show any features suggestive of secretory phenomena.

6. In the subependymal layer the extracellular space continuous with each other are formed. They are presumed to display an important role in the metabolism of the subcommissural organ.

内容抄

脳の交通下器官が電顕的に検索された。

1. 交通下上衣細胞内に充満しているGomori陽性分泌物は電顕的には生産と放出の場と性状により異なった2種類のものに区別される。1つはGolgi装置内に出現する限界膜で包れた好オスミウム性の粒子である。この粒子は根上部にあるGolgi野から細胞の胞体内を上行して細胞遊離表面の直下に集積し、最後にそこから第三脳室内に放出される。脳室内に経路におけるReissnerの線維は恐らくこれ等粒子の凝固によって生じたものであろう。他の一つの分泌物は細胞内の特に核の周囲を占める粗面小胞体の分離によって生じた電子密度の低い液腔であり、この液腔は主として細胞突起中を下行して附近の毛細血管に放出される。本細胞の核下部には粗面小胞体の同心円状に密に配列したものが認められる。このものは上記2種の分泌物並びに粗面小胞体自身の生産に与えるものと考えられる。交通下上衣細胞及び周閉の神経毛群の相互間には互いに連絡した細胞外腔が存在しておりこの内に交通下上衣細胞の突起からの微細毛様の突起が多数突出している。これ等的小突起はいずれも胞飲（バイノサイトーシス）を意味すると思
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References.