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EPENDYMAL NEUROSECRETION. II.

GOMORI-POSITIVE SECRETION IN THE PARAVENTRICULAR ORGAN AND THE VENTRICULAR EPENDYMA OF DIFFERENT VERTEBRATES

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The subcommissural organ of vertebrates is situated in the third cerebral ventricle below the posterior commissure; it consists of modified ependymal cells and of the hypendyma which abounds in glial cells, fibres and capillaries.

Stutinsky, demonstrating in 1950 a Gomori-positive substance, brought the organ within the scope of neurosecretory investigation. The subsequent studies of Bargmann, Schiebler [3], Wislocki, Leduc [20], Ollson [14] and others suggest, in agreement with our own findings [19], that in the subcommissural organ there is a Gomori-positive secretion which resembles hypothalamic neurosecretion.

In previous studies we have compared the secretory function of the organ in different vertebrates and established a certain interrelationship between the intensity of secretion and the animals' oecological conditions. We found that the organ's ependymal cells are in different stages of secretion.

The subcommissural organ is one of the ependymal organs of the third ventricle. It is supposed that not only one of these organs is able to produce Gomori-positive secrete. Therefore, in the present investigation, the paraventricular organ and the ependyma of the ventricle wall were studied.

The paraventricular organ is situated symmetrically at the side wall of the third cerebral ventricle. It has first been described by A. Kappers in 1921 and its name, "paraventricular organ", has been given by Roussy and Mosinger [16]. Later it was studied in more detail by Charlton [5], Legait [11, 12], Papez [15] and Fleischhauer [8]. Like the subcommissural organ, it consists of an ependymal and a hypendymal portion. The former is composed of multiple rows of long ependymal cells, the latter is made up of the processes of these cells, glial cells and a remarkable quantity of capillaries (Fig. 1).

Materials and methods

The following species were studied:

Fish	Lebistes reticulatus
	Brachydanio rerio

Urodela	Triturus cristatus
	Triturus vulgaris
	Pleurodeles waltlii
	Amblystoma mexicanum
Anura	Rana esculenta
	Bombina bombina
Reptilia	Lacerta viridis
	Lacerta agilis
	Natrix tesselatus
	Natrix natrix
Birds	Passer domesticus
	Columba domestica
Mammals	Felis domesticus
	Myotis myotis
	Rhynolophus hypposideros
	Epimys norvegicus

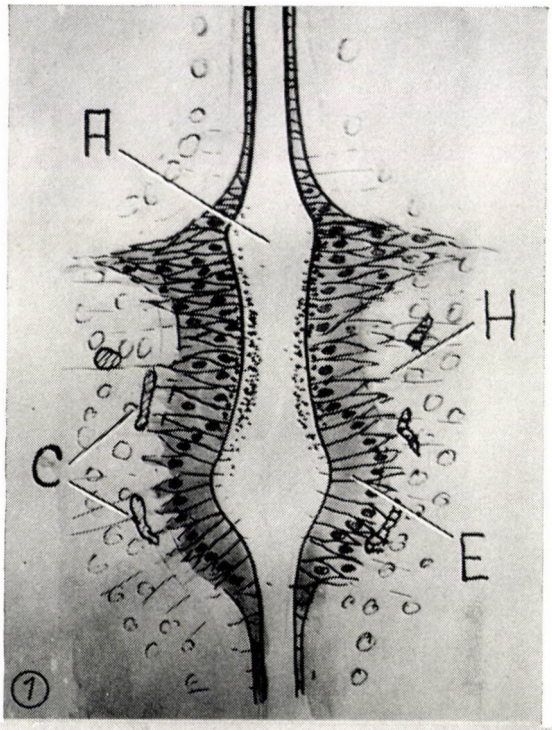
The animals were decapitatad and the organs were fixed in Bouin's fluid, embedded in paraffin, and serial sections, 6 microns in thickness, were stained with Gomori's chromalaunhaematoxylin-phloxin modified according to BARGMANN, and with Gabe's paraldehyde-fuchsin. A total of 200 animals was used. All examinations were carried out in the autumn.

Results

In *Lebistes reticulatus* the paraventricular organ was situated at the side wall of the cerebral ventricle (Fig. 2). His ependymal cells were characteristically elongated. The nuclei of the cells appeared in the conically tapering basal portion of the plasma. The ventricle's surface of the cells is ciliated. The ependymal cells were arranged in one or two layers and showed a gradual transition to those of the subjacent cerebral substance. The staining with chromalaunhaematoxylin-phloxin and paraldehyde-fuchsin showed the presence of Gomori-positive granules in the cells. In the third cerebral ventricle beside the paraventricular organ there were albuminous coagulates which frequently appeared as elongations of the superficial cell processes. The ependyma constituting the wall of the third cerebral ventricle resembled in some areas the paraventricular organ which itself did not contrast as sharply with the surrounding ventricular ependyma as in more differentiated species. The organ was traversed by many capillaries.

In the ependyma cells of the ventricle wall Gomori-positive granules could also be found. They often occurred more abundantly than in the cells of the paraventricular organ. There is a dense colloidal substance on the surface of the ependyma cells of the ventricle which stained electively with both, chromalaunhaematoxylin-phloxin and paraldehyde-fuchsin (Fig. 3).

The paraventricular organ of *Brachydanio rerio* resembled the one just described, implying the appearance of Gomori-positive secretion in the ependyma of the ventricle wall. There was more secrete within the cells and on their surface than in any of the more differentiated species. The dense Gomori-positive substance often filled the whole of the ventricle lumen (Fig. 4).



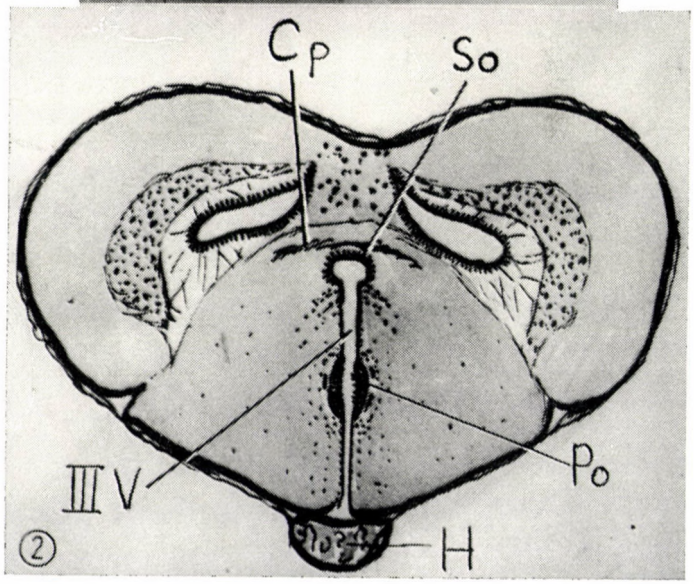


Fig. 1. Diagram of the paraventricular organ. A = ventricle of the brain; H = hypendyma; E = ependymal cell; C = capillaries

Fig. 2. Brain of guppy *(Lebistes reticulatus)* in frontal section (diagram). Cp = posterior commissure; Po = paraventricular organ; III V = third ventricle; So 2 = subcommissural organ

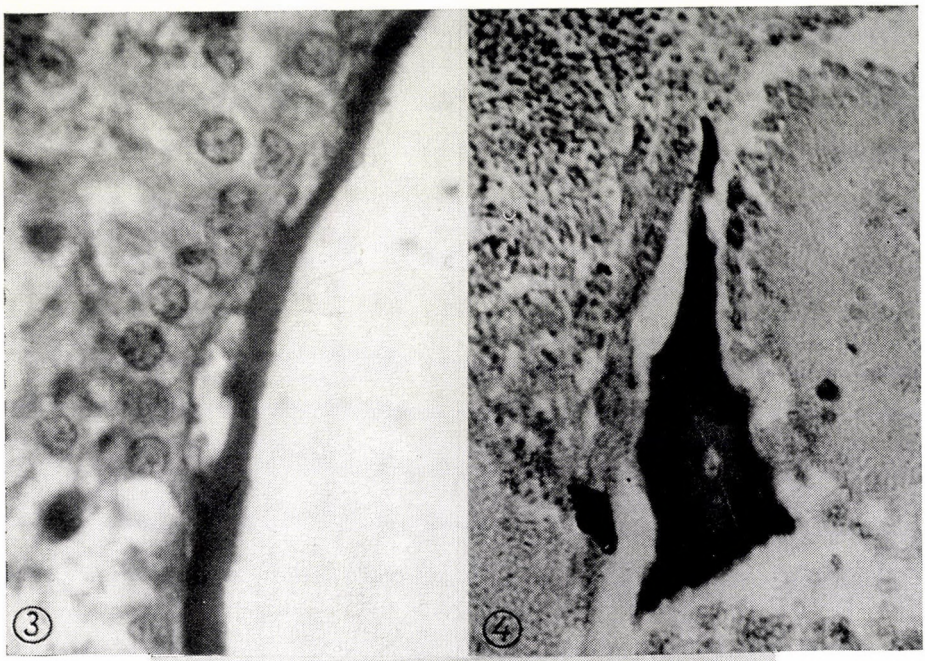


Fig. 3. Dense Gomori-positive colloid substance on the surface of the ependymal cells in guppy *(Lebistes reticulatus)*. Chromhaematoxylin-phloxin stain. 800×

Fig. 4. Gomori-positive colloid substance filling the lumen of the ventricle in zebra-danio *(Brachydanio rerio)*. Paraldehyde-fuchsin stain. 120×

Fig. 5. Brain of crested newt *(Triturus cristatus)* in frontal section (diagram). III V = third ventricle; Po = paraventricular organ; So = subcommissural organ

In *Triturus cristatus* the cells of the paraventricular organ were arranged in two or three rows, showing a gradual transition into the surrounding ependyma (Fig. 5). The basal location of the cell nuclei and the abundance of elongated protoplasmic processes could be regarded, like in the studied fish, as distinctive features of the organ. His cells contained Gomori-positive granules. A further analogy with fish consisted in a protein-like coagulate presenting itself in the ventricle at the same level as the organ. The hypendyma abounded in capillaries.

There were numerous Gomori-positive granules in the ependyma cells of the ventricle wall. The granules were not confined to the third cerebral ventricle but extended as far as the aquaeduct of the fourth ventricle and the ependymal epithelium of the lateral ventricles. The ependymal cells often presented vacuoles which did not contain Gomori-positive material (Fig. 6).

The paraventricular organ and ventricular ependyma of *Triturus vulgaris*, *Pleurodeles waltlii*, and *Amblystoma mexicanum* showed much resemblance to the one just described.

In *Rana esculenta* the paraventricular organ was weakly developed and most of it located infundibularly (Fig. 7). Characteristically, the protoplasmic processes on the cell surfaces were very numerous and considerable in length like in fish and caudate amphibia. A conspicuous feature though common with some other species was the presence of protein coagulate in the ventricle above the organ. The Gomori-positive granules in the cells appeared as a fine powder. A great number of capillaries presented themselves at the basal sides of the cells.

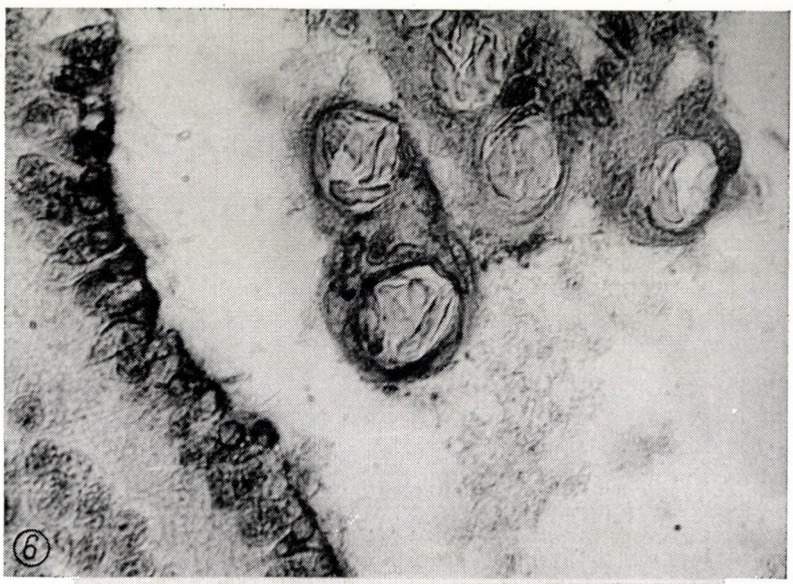
Far greater in number and different in size were the Gomori-positive granules in the ependymal cells which made up the ventricle walls (Fig. 8). The granules were located apically between the ventricular surface and the cell nuclei. On this side of the nucleus where the secrete was situated the nucleus was polymorphous (Fig. 9). Secretion occurred not only in the ependyma of the third and fourth ventricle but also in that of the lateral ventricles and the aquaeduct.

The basal portions of the cells, too, were frequently containing secrete; moreover the ependymal processes, mainly those of the fourth ventricle, extended as chains of fine Gomori-positive droplets, a long way into the cerebral substance.

The secretion was not confined to the ependyma. Large, swollen cells, oval or spherical in shape, were scattered at the basal portion of the ependyma, their cytoplasm overreplete with Gomori-positive substance.

Much the same was the picture in *Bombina bombina.*

In *Lacerta viridis* the paraventricular organ was highly developed, more than in the less differentiated vertebrates so far discussed, and extended under the subcommissural organ in mid-height of the side of the third ventricle



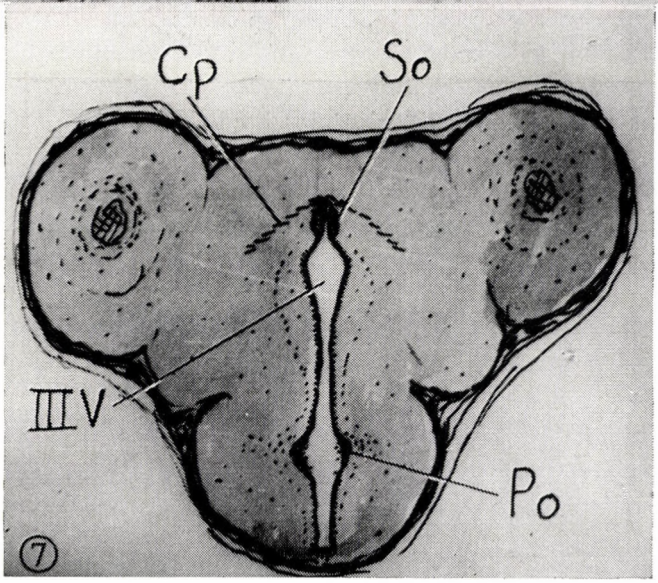
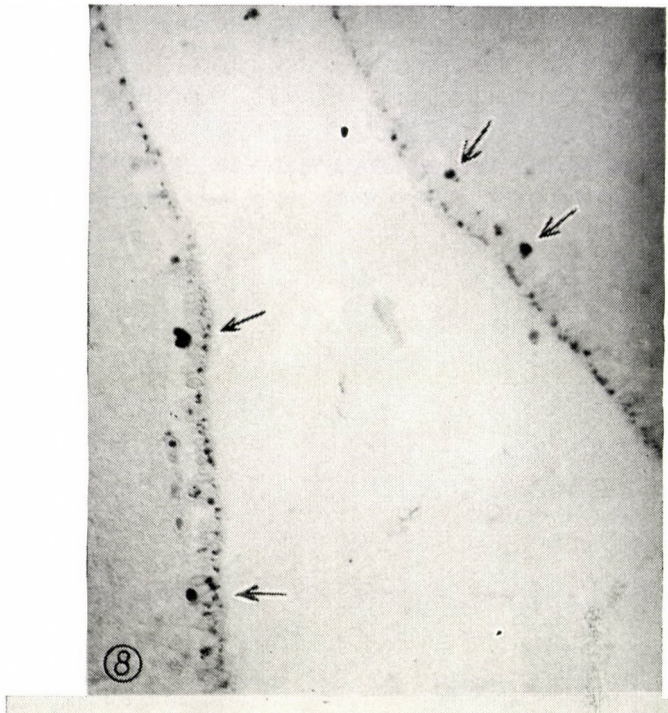


Fig. 6. Ependymal cells in the third ventricle of the crested newt *(Triturus cristatus)*. Gomori-positive granules in the apical part of the cells. Arrows point to vacuoles. 600×

Fig. 7. Brain of bull frog *(Rana esculenta)* in frontal section. III V = third ventricle; Po = paraventricular organ; Cp = posterior commissure; So = subcommissural organ



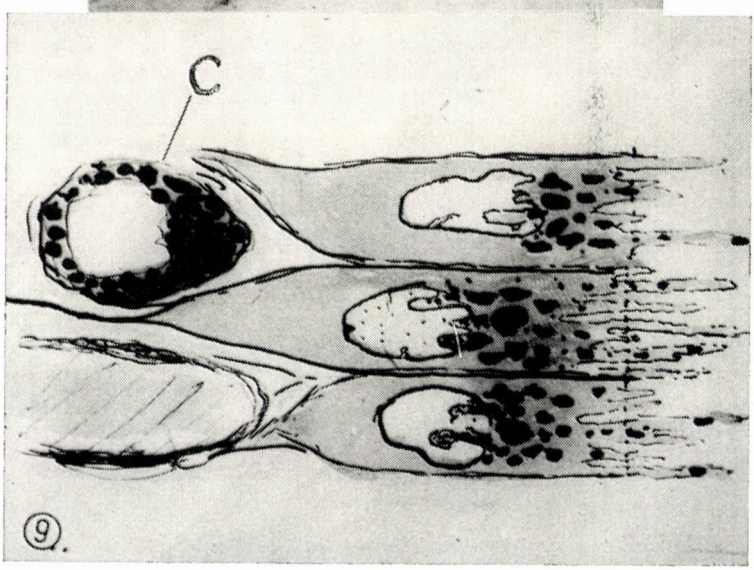


Fig. 8. Ependyma in the third ventricle of bull frog *(Rana esculenta)*. Gomori-positive granules in the apical part of the ependymal cells. Arrows point to round cells at the base of the ependymal cells, filled with secretion. Paraldehyde-fuchsin stain. 200×

Fig. 9. Outlay of ependyma in the ventricle of bull frog *(Rana esculenta)*. Gomori-positive granules in the apical, polymorphous part of the cell nuclei and in the protoplasmic processes. C = basal cell stuffed with Gomori-positive granules

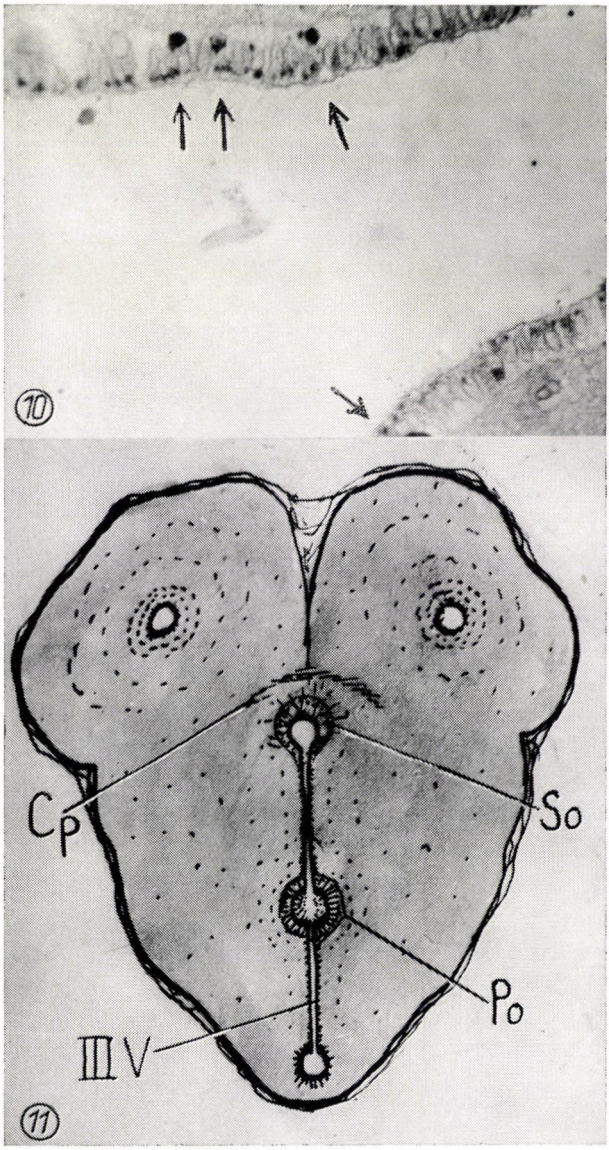


Fig. 10. Ependyma in the ventricle of bull frog *(Rana esculenta)*. Each of the cells contains Gomori-positive granules. Arrows point to round basal cells filled with Gomori-positive granules. 320×

Fig. 11. Brain of European green lizard *(Lacerta viridis)* in frontal section. III V = third ventricle; Cp = posterior commissure; Po = paraventricular organ; So = subcommissural organ

(Fig. 11); it presented a sharp contrast to the circumjacent lower ependymal epithelium. On frontal sections it appeared, just like the subcommissural organ, as a circular structure built up of two regular semicircles set against each other. The cerebral ventricle itself was observed to reach into the organ, forming a pair of small semi-circular bulges. Conspicuously, the protoplasmic processes were nearly equal in length with the more superficially located ependymal cells they emerged from, and seemed almost to fill up the small ventricular sinus. Studying with immersion enlargement it seemed as if the spaces between the plasm processes would deeply draw into the internal of the cell as invagination (Fig. 12).

The ependyma covering the ventricle wall consisted of cells flatter than those in the less differentiated species, with a moderate amount of granular secrete in it.

Very similar were our findings concerning the paraventricular organ and the ventricular ependyma in *Lacerta agilis.*

In *Natrix tesselatus* and *Natrix natrix* the ventricular ependyma showed a close resemblance in structure to the corresponding organ of the lizards. So did the paraventricular organ except that the radial arrangement of the cells was less regular.

Even more developed than in reptiles was the paraventricular organ in *Passer domesticus* (Fig. 13). It consisted of cuboid cells arranged in 5 to 10 instead of 2 to 3 rows. The superficial cell row was conspicuous, like in the less differentiated species, with nuclei situated basally and the numerous cytoplasmic processes emerging from the surface. Cells containing Gomori-positive substance were more frequent than in the corresponding organs of fish, amphibia and reptiles. Most of the secrete appeared in elongated cells tapering to a point at either end (Fig. 14). These cells, unlike the more cuboidal ones of the ependyma, showed strong Gomori-positivity and were found chiefly in the median and hypendymal portions of the organ. An abundant network of capillaries was observed.

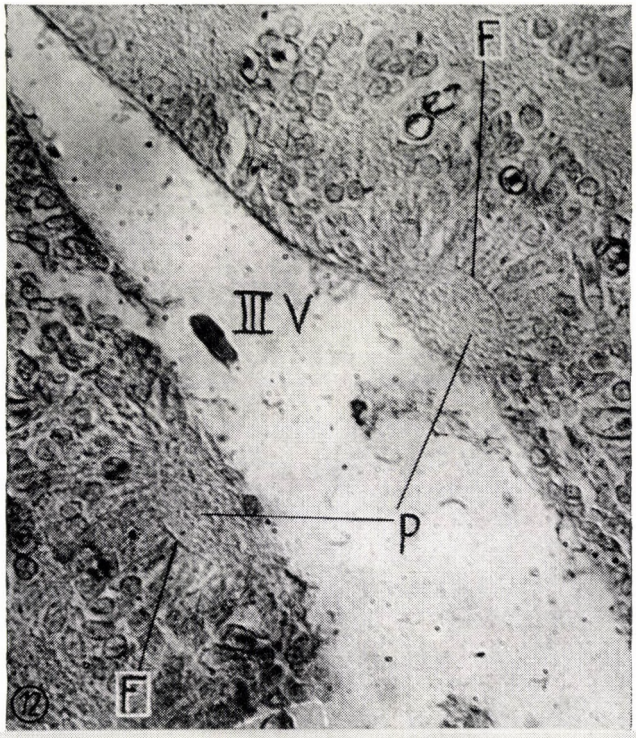
Most of the ependyma lining the ventricle consisted of flattened or cuboid cells with a smaller amount of Gomori-positive granules than in the lower species.

Much the same was the picture in *Columba domestica.*

The paraventricular organ in the brain of *Felis domesticus* showed a lacunar structure, a characteristic of mammals (Fig. 15). His epithelium was enlarged, and formed protuberances and recesses. The hypendyma was highly developed. The ependyma cells contained Gomori-positive granules (Fig. 16).

The ventricular ependyma, however, was for the most part flattened and of regressive character, showing rare Gomori-positivity.

In *Epimys norvegicus* the organ was undulated on the surface and pseudoglandular in structure. The hypendyma was well-developed. Neither this nor



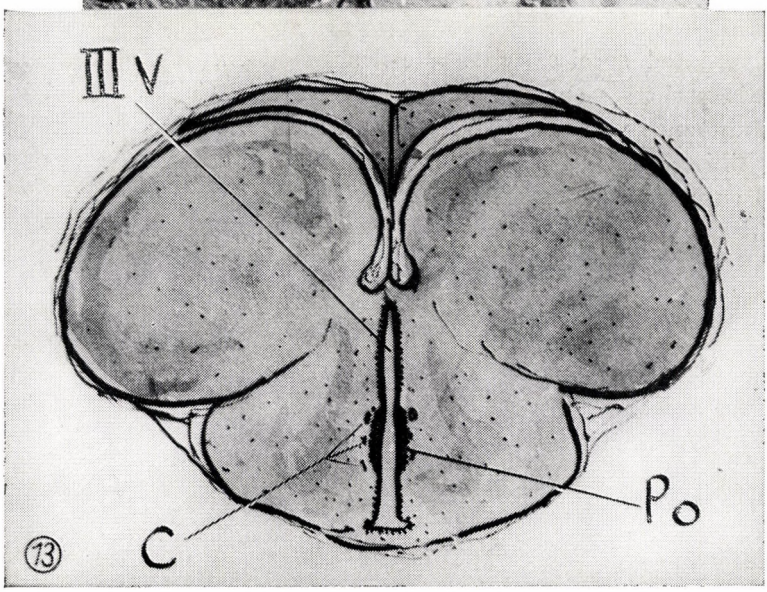


Fig. 12. Paraventricular organ of European green lizard *(Lacerta viridis)*. III V = third ventricle; F = ventricular surface of paraventricular cells; P = mass of protoplasmic processes. 700×

Fig. 13. Brain of house sparrow *(Passer domesticus)* in frontal section (diagram). III V = third ventricle; Po = paraventricular organ; C = capillaries

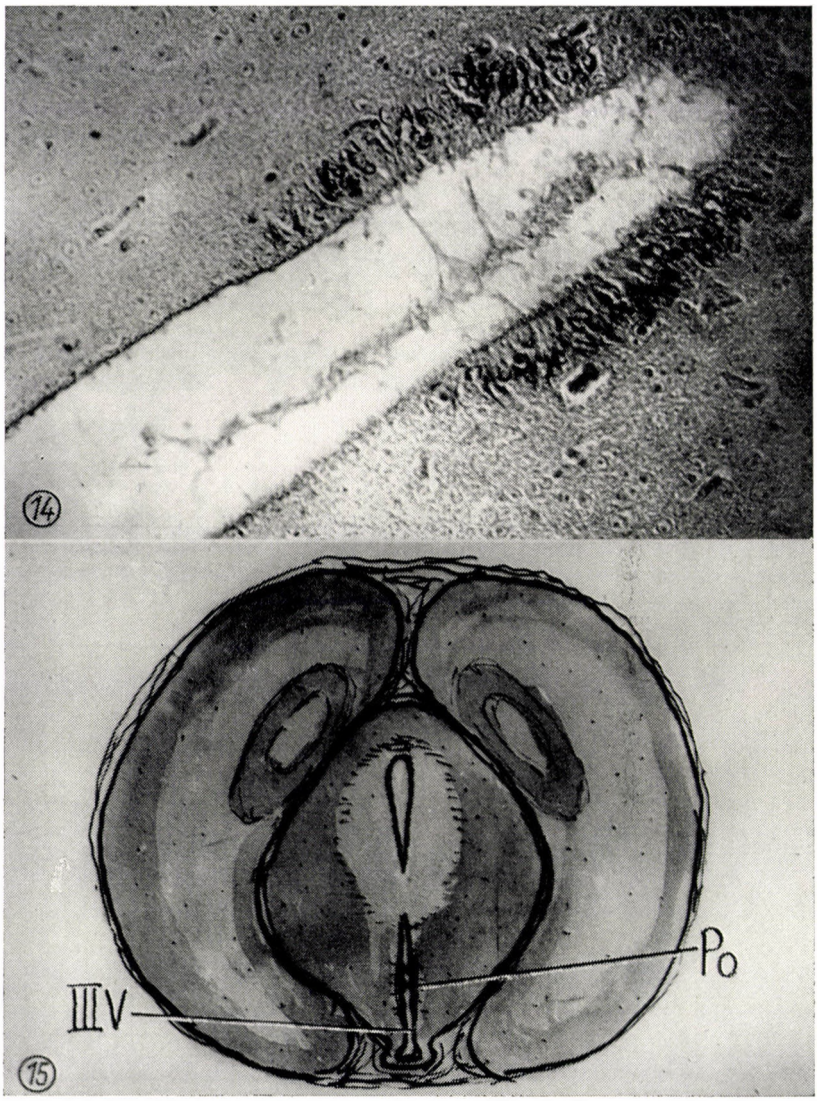


Fig. 14. Paraventricular organ of house sparrow *(Passer domesticus)*. Note cell engorged with Gomori-positive matter. Paraldehyde-fuchsin stain. 320×

Fig. 15. Brain of domestic cat *(Felis domesticus)* in frontal section (diagram). III V = third ventricle; Po = paraventricular organ

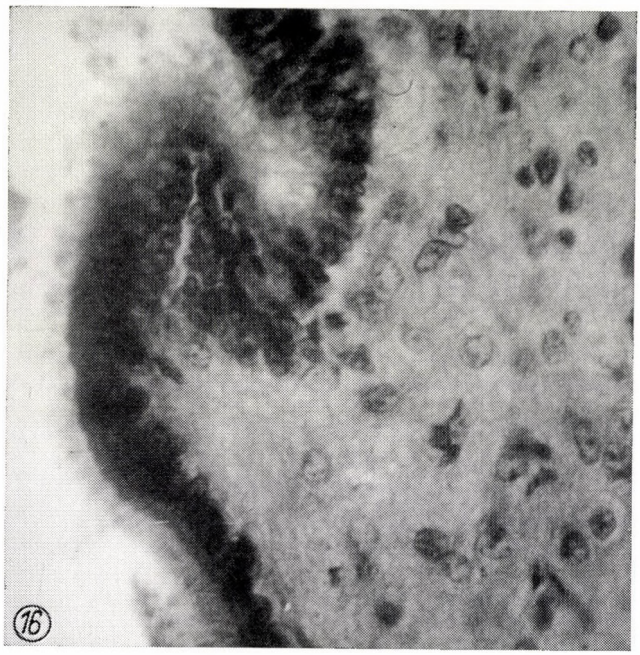


Fig. 16. Detail from the paraventricular organ of a domestic cat *(Felis domesticus)*. Note mass of Gomori-positive granules in the ependymal cells. Gomori's chromhaematoxylin-phloxin stain. 120×

the ventricular ependyma presented any features essentially different from those described in *Felis domesticus*.

The structures observed in *Myotis myotis* and *Rhynolophus hypposideros* were very similar to that of the rat.

Discussion

A comparison of the paraventricular organs in different vertebrates revealed that the number of cell rows increases as we proceed from lower to higher species. In fish and amphibia the organ consists of one or two layers; in birds there are 5 to 10 of them. In mammals the organ presents recesses on protuberances probably in consequence of an enlargement of the surface. These findings suggest that the cell rows multiply according to the rise in degree of differentiation. Analogous to this interrelationship were our earlier observations regarding the subcommissural organ in different vertebrates [19].

In higher species the paraventricular organ shows a remarkable contrast to the flat and reduced ependyma but in less developed species it contains one or two layers only, and is sometimes hardly distinguishable from the

largely undifferentiated environment. There are, however, always two factors present also at higher species. One is that the nuclei of the superficial ependymal cells are always situated basally far from the ventricular surface, leaving room for a cytoplasmic zone of some width; the other is the appearance of numerous cytoplasmic processes emitted from the cell surface.

Gradually as the paraventricular organ is reaching higher stages of development, the picture of the ventricular ependyma shows a retrogressive tendency. An analogous interrelationship seems to exist between the two regions in the amount of Gomori-positive secretion, which abounds in the ventricular ependyma of fish and amphibia, in contrast to that of birds and mammals. Relative to the paraventricular organ the situation is contrary.

In agreement with LEGAIT's findings in several species [17, 18, 19] we could establish the presence of an albuminous Gomori-negative coagulate in the ventricle above the paraventricular organ in each species.

LEGAIT is of the opinion that the albuminous secretion released by the organ is a constituent of the cerebrospinal fluid. The presence of Gomori-positive material however, allows to infer that the cells are producing two types of secrete, one Gomori-positive, the other Gomori-negative; accordingly the organ's function must be a complex one.

LEGAIT furthermore reports to have found chains of Herring bodies in the height of the paraventricular organ which he believes to have derived from the hypothalamic neurosecretory nuclei. However, Gomori-positive substance may be formed in the ependyma and the secrete may spread in the way of basal ependyma cell processes similar to the chains of Herring bodies [19]. We ask the question whether the Herring bodies may not be a result of the ependyma secretion. The extensive surface formed by the cytoplasmic processes of the paraventricular organ and the multitude of capillaries are indicative, at any rate, of a high metabolic activity which calls attention to the importance attaching to the organ and recommends a further inquiry into its function.

The most remarkable features revealed by our comparative studies were the abundance of secrete in the ependyma of the ventricle wall, respectively the general formation of Gomori-positive material in the ependymal cells.

Experimental results achieved in the last few years have somewhat changed our former notion of the neurosecretory function [1, 2, 4, 10, 18, 21]. It has made it evident that the ability to secrete is not limited to the hypothalamic nuclei but is shared by the majority of nerve cells. What is secreted may be either lipoids or different mediating substances or Gomori-positive material. It also appears that hypothalamic neurosecretion is just a specialized form of the general function. Furthermore our experiments have revealed a resemblance between the cells of the subcommissural organ and the ependyma in the general capacity to produce a secrete similar to the one discharged by

the hypothalamus. Thus the studies that the ependymal cells produce Gomori-positive substance — which we would term "ependymal neurosecretion" or "ependymosecretion" — have furnished some new data to the modern perception of neurosecretion.

The ependymal cells are the least differentiated elements in the nervous system. So the secretory function is not only a general feature of the nervous system but an ancient one.

The morphological tests, however, could only go so far as to prove the ability of the ependyma to produce a Gomori-positive secrete. The further question whether or not the ependymal secretion has any hormonal effect similar to that of the hypothalamo-hypophyseal colloid, has remained undecided and it must be left for the physiological examinations now in progress to give the answer.

The next question to appear in a new light was that of hydroencephalocrinia, first described by COLLIN [6] as the transfer of neurosecrete to the cerebral ventricles. So far, this phenomenon was mostly so explained that the Gomori-positive material of the cerebrospinal fluid takes its origin from the hypothalamic secretion [7, 13]. Now that the general ability of the ependymal cells to produce Gomori-positive substance has become manifest, the explanation suggests itself that these cells discharge their secrete into the ventricles.

On the basis of our experimental results, greater importance has to be attached to the ependymal cells than has been done so far. The ependyma appears both genetically and histologically as the most central part of the nervous system, comprising the least differentiated cells of the embryonal neural tube. The fact that it shows frequent mitoses even in adult age is a clear sign of its ability to regenerate. Its cell processes which enmesh the whole of the nervous system establish interconnections between the capillaries, the nerve cells and the liquor. Recent electronmicroscopic findings [8, 9] do not favour the view to regard the ependymal processes merely as supporting elements but prove that the fibres are always enwrapped in plasm sheaths. As pointed out in one of our previous studies [19], it is hardly possible that any change in the metabolism of the nerve cells should go without corresponding changes in the blood, the liquor space and the closely related ependymal elements. LEGAIT's observation (13, page 81) of a resemblance in the morphological changes of the nerve cells of the hypothalamus and those of the cerebral ventricle and the ependyma seems now completed with our findings that it is a general ability of the ependyma to produce a Gomori-positive substance which occurred, though with different intensity, in the ventricle of each brain region. We suppose that there is a certain relation between the ependymosecretion in the various cerebral regions such as the telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon, and their metabolic states and functions.

But the role played by the "ependymal neurosecretion" on the nervous system and on the function of the whole organism, is still unknown and await elucidation.

Summary

The paraventricular organ and the ventricular ependyma in fish, amphibia, reptiles, birds and mammals were subjected to comparative studies.

A Gomori-positive granulo-colloidal substance occurred in the ependyma of each examined species.

The process of differentiation of the paraventricular organ and the ependyma of the ventricle and the presumable importance of the Gomori-positive secretion have been discussed.

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ЭПЕНДИМАЛЬНАЯ НЕЙРОСЕКРЕЦИЯ II.
ГЁМЁРИ-ПОЛОЖИТЕЛЬНАЯ СЕКРЕЦИЯ ПАРАВЕНТРИКУЛЯРНОГО ОРГАНА И ЖЕЛУДОЧКОВОЙ ЭПЕНДИМЫ У РАЗЛИЧНЫХ ВИДОВ ПОЗВОНОЧНЫХ

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Авторы исследовали и сравнивали паравентрикулярные органы и желудочковую эпендиму рыб, земноводных, пресмыкающихся, птиц и млекопитающих.

В эпендиме каждого исследованного вида позвоночных можно выявить Гёмёри-положительное, гранулоколлоидальное вещество.

Трактуется процесс дифференциации паравентрикулярного органа и желудочковой эпендимы, и дается предположение о значении паравентрикулярной и эпендимальной Гёмёри-положительной секреции.

EPENDYMALE NEUROSEKRETION. II.

Die Gomori-positive Sekretion des Paraventrikularorgans und des Kammerependyms bei verschiedenen Wirbeltieren

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Das Paraventrikularorgan sowie Kammerependym von Fischen, Amphibien, Reptilien, Vögeln und Säugern wurde untersucht und verglichen.

Bei sämtlichen untersuchten Arten war Gomori-positive granulokolloidale Substanz im Ependym anzutreffen.

Der Differenzierungsprozeß des Paraventrikularorgans und Kammerependyms sowie die wahrscheinliche Bedeutung der paraventrikulären und ependymalen Gomori-positiven Sekretion werden besprochen.

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