bzw. Pyroninophilie, welche stärker ist als in den übrigen Abschnitten des Syncytiotrophoblasten, besonders auch stärker als in den den Epithelplatten benachbarten Syncytiumknoten, zeigt ebenfalls, dass die Syncytiumsprossen in bezug auf ihren Stoffwechsel etwas Besonderes darstellen. Aus dem höheren Gehalt an Lipiden sowie der stark positiven Reaktion auf 3-β-ol-Steroid-Dehydrogenase, einem Enzym, welches bei der Synthese der Östrogene eine Schlüsselstellung einnimmt[5], muss der Schluss gezogen werden, dass die Syncytiumsprossen denjenigen Ort im Syncytiotrophoblasten darstellen, welcher für die Östrogenproduktion spezialisiert ist. Weitere histochemische, besonders aber auch elektronenmikroskopische Untersuchungen zur Stützung dieser Auffassung sind im Gange.

*Summary.* The syncytial sprouts of the human placenta were found to give a distinctly stronger reaction for glucose-6-phosphat-dehydrogenase, DPNH-diaphorase and steroid-3-β-ol-dehydrogenase than other regions of the trophoblastic syncytium. Besides they show a more intensive basophilic reaction and higher contents of fat than other parts of the syncytium. Therefore the syncytial sprouts can be regarded as the areas of the trophoblastic syncytium that are specialized for estrogen production.

K. S. Ludwig

*Anatomisches Institut der Technischen Hochschule Aachen (Deutschland), c/o Anatomisches Institut der Universität Basel (Schweiz),*
*2. Februar 1968.*

[5] H.-J. Staemmler, *Grundriss der gynäkologischen Endokrinologie* (Thieme, Stuttgart 1965).

## Morphological and Functional Changes in the Distal Hypothalamo-Neurohypophysial System of the Grass Frog (*Rana pipiens*) after Transection of the Proximal Neurohypophysis

An increased amount of material[1] with the same staining affinity as neurosecretory substance has been reported to occur proximal and distal to the site of damage after electrocoagulation[2,3] or transection of the proximal neurohypophysis[4,5]. The present study was undertaken to investigate this phenomenon by light and electron-microscopy and to correlate the morphological findings with the pressor activity of neurohypophysial extracts. A total of 506 grass frogs kept at either 18 °C or 8–12 °C environmental temperatures was used. In 291 animals the proximal neurohypophysis was transected; 215 animals served as controls.

As early as 15 min after transection in the higher temperature group, a slight increase in PAF + substance is observed in the distal stump. With increased time after the operation the augmentation becomes more apparent (Figure 1). Herring-bodies occur distal to and in the vicinity of the lesion (Figure 1). Around the sixth postoperative day, the nerve fibers and Herring-bodies gradually lose their staining affinity and become depleted of PAF + substance.

In the lower temperature group, the first increase in PAF + substance occurs about 20–22 h after the transection. During the first 2 days morphological changes are the same as those observed in the higher temperature group. Later, however, a mushroom-like appearance of protruding nerve fibers is characteristic for the distal stump (Figure 2). Even farther distal, the nerve fibers contain large masses of PAF + substance. The Herring-bodies tend to be associated with the ependymal cells

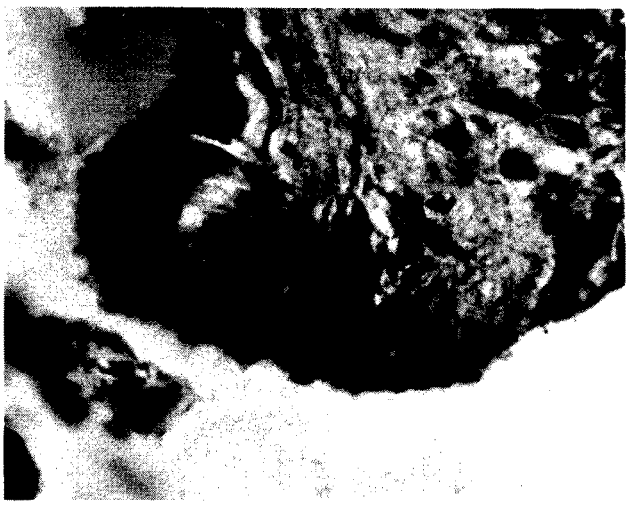

Fig. 1. Distal stump of the transected proximal neurohypophysis 2.5 days after the transection in the higher temperature group, Gomori's method, × 550. Notice the enlarged nerve fibers at the transection site and their beaded aspect distal to it.

Fig. 2. Distal stump of the transected proximal neurohypophysis 10.5 days after the transection in the lower temperature group, Gomori's method, × 550. Protrusion of neurosecretory nerve fibers and pituicytes proximal to the transection site.

sometimes appearing to be directly protruding into the third ventricle. No changes in the staining affinity could be observed during our present observation period of 14 days.

At the fine structural level the majority of the axons at the transection site are nearly or entirely filled with normal (600–4000 Å) or modified large granular vesicles (up to 6000 Å) or heterogeneous material (Figure 3), 12 h after the transection in animals kept at 18 °C. The enlargement of these vesicles was most prominent during the first 48 h, their granular content becomes less electron dense, and light and dark granules can be easily differentiated (Figures 3 and 4). Often the axon contains small dark and light granules which appear identical to the vesicular granules (Figure 4). This observation, together with vesicles incompletely surrounded by a membrane or membrane remnants, suggests the possibility that the vesicles rupture and empty their contents into the axon. The fusion of 2 or more large vesicles, surrounded by a membrane (Figure 3), is considered a preliminary step toward the aforementioned condition.

In the axons of the control animals the micro tubules parallel each other and are longitudinally directed. 12 h and 24 h after transection the Herring-bodies (Figure 5) and many parts of the axon contain an increased number of these tubules with no definite direction or orientation. The tubules are increased in size, sometimes forming vesicular distensions which are occasionally filled with granular material. Dense lamellar bodies and degenerative bodies appear. Some axons are either empty or contain only a few microtubules, small dense lamellar bodies, and some mitochondria, whereas others are filled with many large and small empty vesicles. Intraaxonal lipid and glycogen inclusions, small and large extremely electron-dense granules and irregularly shaped dense bodies, dense lamellar bodies and autolytic bodies are frequently found. With increased time interval between transection and observation, more and more large granular vesicles fuse and become emptied of their contents. The membranes of these vesicles seem to participate in the formation of multilamellate bodies. The mitochondria increase considerably in number and size. Around 84 h after the transection some of the mitochondria start to disintegrate, the cristae mitochondriales form tubules and

[1] Subsequently referred to as PAF + substance (paraldehyde fuchsin positive).

[2] J. F. CHRIST, *Neurosecretion* (Eds. H. HELLER and R. B. CLARK; Academic Press, New York 1962), p. 127.

[3] J. F. CHRIST and H. NEMETSCHEK-GANSLER, Z. Naturf. [B] *20*, 278 (1966).

[4] H.-D. DELLMANN and H. E. DALE, Anat. Rec. *154*, 336 (1966).

[5] H.-D. DELLMANN, H. E. DALE, L. F. ELDRIDGE and P. A. OWSLEY, Proc. XVIII Int. Veterinary Congress, 1967, p. 724.

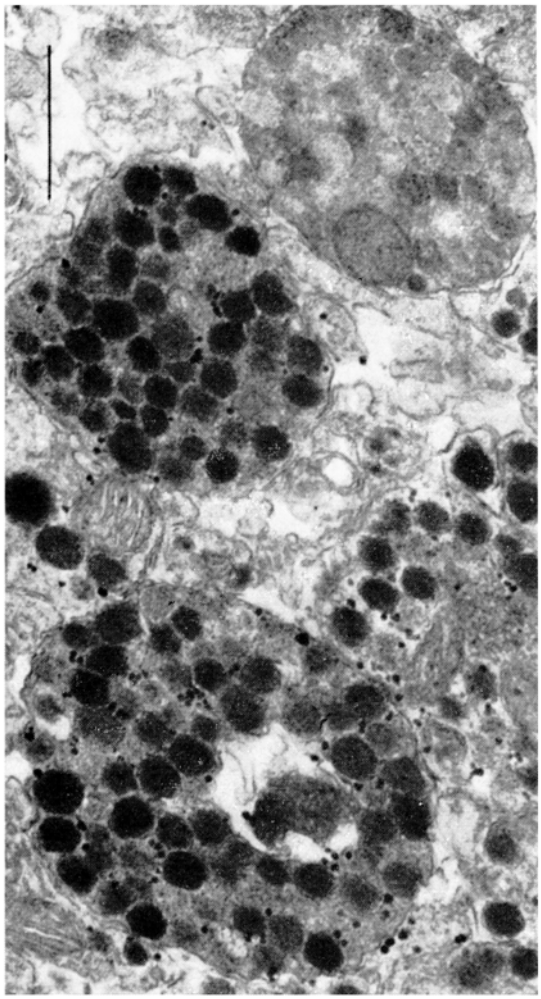

Fig. 3. Electron micrograph of neurosecretory nerve fibers in the distal stump of the transected proximal neurohypophysis in the vicinity of the lesion 1 day after the transection. For explanations see text. × 41,200, bar represents 0.5 $\mu$.

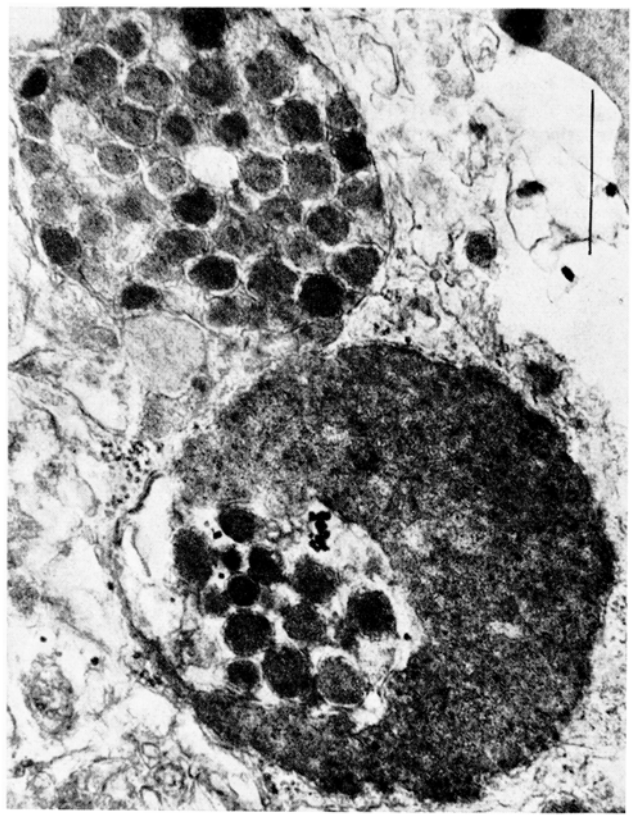

Fig. 4. Electron micrograph of neurosecretory nerve fibers in the distal stump of the transected proximal neurohypophysis 1 day after the transection. × 56,500, bar represents 0.5 $\mu$.

vesicles which closely resemble the microtubules and vesicles described above.

The bioassay of the vasopressor activity[6] of the neurohypophysis distal to the transection revealed a significant increase ($p < 0.05$) during the first 36 h in the higher temperature group. The values at 48, 60 and 72 h after the transection were also higher than the control values (Figure 6); after 72 h a constant decrease of the hormone activity was observed. In the lower temperature group the vasopressor activity decreased on days 1 and 3 after hypophysotomy, but increased at days 6 and 8 (Figure 7); the high values at days 6 and 8 are significantly higher ($p < 0.05$) than the low ones at days 1 and 3.

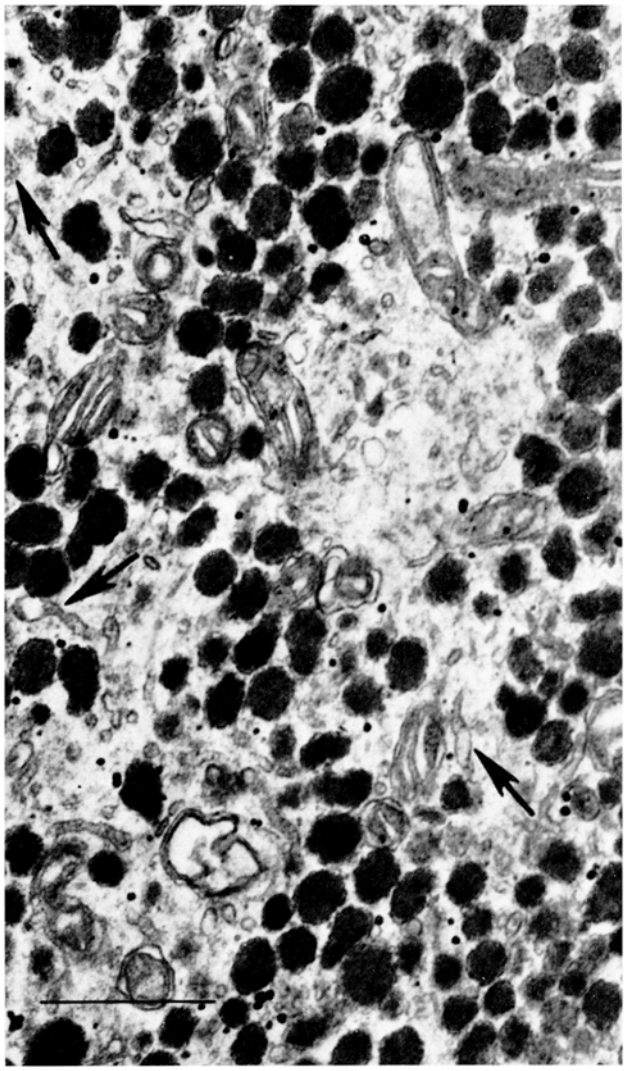

Fig. 5. Electron micrograph of part of a Herring body in a non-transected proximal neurohypophysis. Note the vesicular distensions (arrows) and irregular arrangement of the microtubules. × 46,800, bar represents 0.5 $\mu$.

It has been repeatedly contested whether or not the PAF + substance found in the distal stump of the transected neurohypophysis actually represents neurosecretory material. In the present investigation an increased amount of normal or modified granular vesicles (elementary neurosecretory granules) of variable density were observed, however, the origin of these vesicles remains to be determined.

The classical concept of protein synthesis involving the endoplasmic reticulum and the Golgi apparatus of the perikaryon has been adopted widely for the formation of the neurosecretory substance, although local axonal synthesis has also been suggested more than once[2,3,7–11]. Our morphological data in the normal as well as in the transected axons support the hypothesis that the process of polypeptide synthesis which is initiated in the perikaryon continues within intraaxonal tubular and vesicular structures, which are considered a continuation of the Golgi apparatus[11,12]. The axon reacts to the transection by an increase in these membranous systems and in the number of mitochondria, whereby the process of local packing and pulling together of polypeptides (precursor substances) into active hormones (vasotocin, oxytocin) may be increased proportionally. The presence of an augmented vasopressor activity occurring simultaneously with an increased number of dense vesicles would support this hypothesis. The fact that the increase in activity occurred later after the transection in the animals kept at lower temperatures is readily explained by slower reactions due to the cold exposure.

In the experimental animals, some pituicytes contained vesicles with the same morphology as the large granular vesicles found in the neurosecretory nerve fibers, which

[6] J. Dekanski, Br. J. Pharmac. *7*, 567 (1952).
[7] H. M. Gerschenfeld, J. H. Tramezzani and E. de Robertis, Endocrinology *66*, 741 (1960).
[8] J. D. Green and D. S. Maxwell, *Comparative Endocrinology* (Ed. A. Gorbman; John Wiley and Sons, New York 1962), p. 125.
[9] R. Diepen, *Neurosecretion* (Eds H. Heller and R. B. Clark; Academic Press, New York 1962), p. 111.
[10] H.-D. Dellmann, J. Hirnforsch. *5*, 249 (1962).
[11] F. G. W. Knowles, Proc. R. Soc. [B] *160*, 360 (1964).
[12] H. A. Bern and F. G. W. Knowles, *Neuroendocrinology* (Eds L. Martini and W. F. Ganong; Academic Press, New York 1966), p. 139.

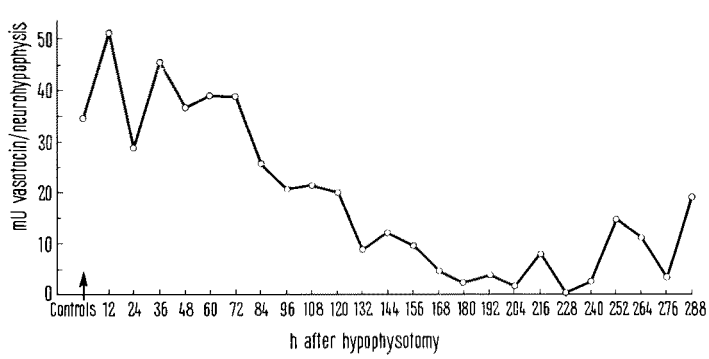


Fig. 6. Vasopressor activity of the distal part of the transected neurohypophysis in animals kept at 18 °C expressed in mU vasotocin/neurohypophysis plotted against hours after hypophysotomy.

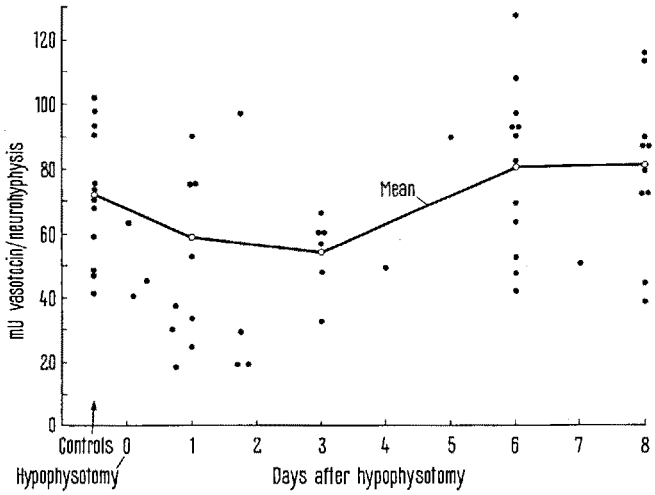


Fig. 7. Vasopressor activity of the distal part of the transected neurohypophysis in animals kept between 8° and 12 °C expressed in mU vasotocin/neurohypophysis plotted against days after hyposotomy.

confirms observations by FRIDBERG et al.[13], and others[12]. The exact nature of these vesicles remains to be determined. Even if it is assumed that the primitive neurohypophysial ependymal and neuroglial cells can elaborate granules with hormone activity, it seems very unlikely, due to their small number, that the increase in vasopressor activity could be attributed to the activity of these glial cells.

On the basis of our light and electron microscopic findings, we conclude that the increased amount of PAF plus substance in the distal stump of the transected neurohypophysis is neurosecretory material. A local synthesis of this material is suggested by the increased vasopressor activity found in our bioassays.

*Zusammenfassung.* Nach Durchtrennung der proximalen Neurohypophyse wird im distalen Stumpf eine gegenüber Kontrolltieren erhöhte Menge paraldehyd-fuchsinpositiver Substanz gefunden, die im Elektronenmikroskop einwandfrei als Neurosekret identifiziert werden konnte. Die im Tierversuch ermittelte erhöhte, blutdrucksteigernde Aktivität des distalen Teils der Neurohypophyse spricht für eine lokale Synthese des Neurosekrets.

H.-D. DELLMANN[14], H. E. DALE,
P. A. OWSLEY and L. F. ELDRIDGE

*Department of Veterinary Anatomy and Department of Veterinary Physiology and Pharmacology, University of Missouri, Columbia (Missouri 65201, USA), 28 September 1967.*

[13] G. FRIDBERG, R. S. NISHIOKA, H. A. BERN and W. R. FLEMING, J. exp. Zool. *162* (1966).

[14] Supported by NIH Grant No. NB 06641 and the Space Sciences Research Center of the University of Missouri.

## DNA Synthesis in the Follicular Cells of *Carausius morosus* Br. (Phasmidae)

A study was carried out to determine the synthesis modality of desoxiribonucleic acid in the nuclei of the follicular cells of *Carausius morosus* using tritiated thymidine. The follicular cells of this insect vary considerably in shape and size during ovocyte growth[1]. At first, when the ovocytes are still very small, the follicular cells are flat and multiply actively by mitotic processes. During the later stages, which can be observed in the larger ovocytes, the nuclei of follicular cells increase in size considerably and arrange themselves perpendicularly on the surface of the ovocyte. The growth of the nuclei in follicular cells is the consequence of repeated endomitotic processes which take place at the same time as the ovocytic growth. Adult individuals of *C. morosus* were injected in the abdomen with tritiated thymidine (Amersham: specific activity 3000 mC/m*M*). Each subject received a dose of 5 $\mu$C. The ovaries were removed at intervals varying from 1 h to 4 days after injection and fixed in Carnoy. The slices, 5 $\mu$ thick, were coloured with Feulgen and then subjected to autoradiographic processing using liquid emulsion (Kodak NTB2).

*Results.* The study of the incorporation was carried out on ovocytes 800–1200 $\mu$ long. The sections used were mainly those cut perpendicularly to the length of the nuclei. Cylindrical nuclei look round in these sections and their diameters, in one and the same ovocyte, differ visibly. The follicular cells of the operculum, which are of a different shape and size from the other follicular cells, were not taken into consideration here.

The ovocytes examined were divided into 3 classes according to the different length and nuclear volumes of the follicular cells calculated for each class. These volumes are shown in the form of histograms (Figures 1–3). The dotted lines refer to the nuclei which are labelled. The arrows in the histograms indicate the nuclear volume corresponding to certain classes of ploidy. These volumes were calculated by assuming a linear relationship between volume and the level of ploidy and taking the calculated

[1] L. P. PIJNACKER, Experientia *22*, 158 (1966).