the hypothalamus during sexual activity are related to a direct effect of gonadotrophins on this nervous structure.

Since the oxidative metabolism, in the central nervous system, is one of the principal sources of the high energy compounds which are needed for protein synthesis, and considering the probable peptide nature of the hypothalamic releasing substances[9], it has been proposed[5] that the metabolic changes of hypothalamus are directly related to the formation of these peptides. If such an hypothesis is consistent, it is probable that the increased oxygen uptake of hypothalamus of hypophysectomized rats is related to the increase of gonadotrophin-releasing factors described in such animals[10]. Nevertheless, further evidence is needed before a conclusion can be reached on this point.

Resumen. En el presente trabajo se ha estudiado el consumo de oxígeno y la producción de ácido láctico en hipotálamo anterior, medio y posterior en animales hipofisectomizados. Los resultados obtenidos indicaron que la hipofisectomía produce un significativo incremento en la actividad oxidativa del hipotálamo anterior y posterior sin modificar el hipotálamo medio.

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Autoradiographic Studies on the Neurosecretory Hypothalamo-Hypophysial System of the Grass Frog (*Rana pipiens*) After Disconnection of the Distal Hypophysis

In 1949, with the discovery of the applicability of Gomori's chromium hematoxylin phloxin method for the study of neurosecretory phenomena[1], research in neurosecretion entered a new phase. Since then 2 concepts have prevailed as to the possible origin of the neurosecretory substance: the classical concept considers the neurosecretory material to be produced in the perikaryon of the neurons of the supraoptic and paraventricular nuclei and conveyed by a proximo-distal axoplasmic flow in the neurohypophysis; on the other hand the concept of a synthesis of neurosecretory granules within the axon was put forward[2–4]. Recently, however, in the light of new electron microscopic findings, the latter hypothesis was modified to conceive a packaging and pulling together of amino acids and/or polypeptides and proteins within the neurotubules, considered to be continuous with the Golgi apparatus, to take place in the distal parts of the neurons[5–7]. SACHS and collaborators[8,9] suggest that a precursor molecule which contains vasopressin in a biologically inactive form precedes the activation of the hormone in the more distal part of the neuron. Our current experiments were designed to yield additional information on the possible existence of a distal hormone synthesis, a packaging and/or activation of a precursor molecule; they were carried out on 23 grass frogs which received 10 or 20 μc of S-35 L-cysteine hydrochloride via i.p. injection immediately after transection of the proximal hypophysis. They were sacrificed at various time intervals between 1 h and 4 days after transection; at the same time 11 control animals were sacrificed. In addition 350 experimental and 167 control animals were available for comparison studies using standard methods for the demonstration of neurosecretory material and electron microscopy.

One hour after the injection of the labeled amino acid into the control animals a slight darkening can be observed in the distal lobe of the neurohypophysis; a higher uptake is noticed in the distal and intermediate lobes of the adenohypophysis. 12 h after the injection both the distal and the proximal parts of the neurohypophysis are characterized by an increased uptake of labeled substance; as expected, due to the few neurosecretory granules which are found in neurosecretory nerve fibers of the proximal neurohypophysis and the ventral hypothalamus, there are only a few black granules observed here making it impossible to demonstrate the neurosecretory pathway by this method. At the same time a much higher uptake is observed in the distal and intermediate lobes of the adenohypophysis. A slight intensification of the phenomena observed at 12 h occurs at 24 h; conditions remain essentially the same after this period (Figure 1).

Six hours after transection of the proximal hypophysis the first appearance of radioactive material can be observed in the proximal as well as in the distal parts of the neurohypophysis, but there is a definite concentration at the distal end of the proximal stump. The distal lobe and especially the intermediate lobe of the adenohypophysis have a higher uptake than the distal neurohypophysis. 12 h after the surgical intervention a higher uptake, as compared to the control animals and

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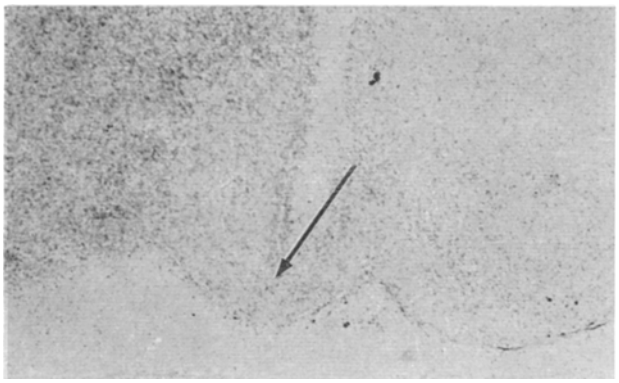

Fig. 1. The ventral hypothalamic region, the proximal neurohypophysis (arrow at the site of transection), the median eminence and the distal lobe of the adenohypophysis in a control animal 42 h after injection of 10 µc of S-35 L-cysteine hydrochloride. Note the relatively poor uptake in the neurohypophysis as compared to the adenohypophysis. × 130.

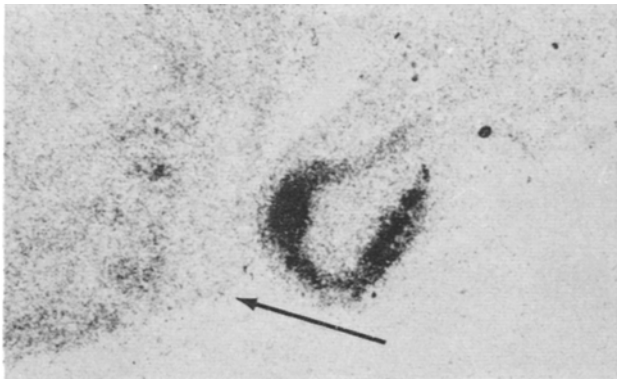

Fig. 2. The same regions as in Figure 1 30 h after transection of the proximal hypophysis and injection of 10 µc of S-35 L-cysteine hydrochloride. In the proximal stump which was bent forward accidentally (compare with Figure 3) the neurosecretory axons are filled with labeled neurosecretory material whereas the proximal end of the distal stump (arrow) is devoid of it. × 145.

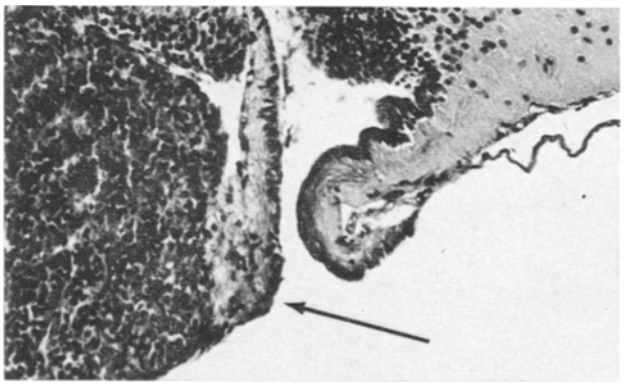

Fig. 3. Section taken from the same animal as in Figure 2, haematoxylin-eosin staining, × 130. Note the presence of neurosecretory material in the proximal and distal stumps (arrow) and compare with Figure 2.

the adjacent distal lobe of the adenohypophysis and the neurohypophysis, is observed in the intermediate lobe as well as an increased concentration of granules in the distal portion of the proximal stump. Subsequently conditions in the disconnected hypophysis remain essentially the same in the autoradiographic picture in spite of the observation of an increased amount of neurosecretory material at the proximal end of the distal stump by classical histological methods (Figures 2, 3). Proximally, however, the distal ends of the interrupted axons become blackened over a distance which increases with increasing time interval between transection/injection and sacrifice of the animal (Figure 2).

Based upon the observation in the light microscope[3,6,7,10–12] of an increased amount of neurosecretory material in the distal stump of the transected or coagulated proximal neurohypophysis as well as on investigations of the ontogenetic appearance of the neurosecretory material it was postulated[13] that a local synthesis takes place in the distal part of the neurosecretory neurons. The fact that previous autoradiographic investigations[14,15] have failed to demonstrate a simultaneous blackening in the neurosecretory nuclei and the neurohypophysis and the absence of ribosomes at the electron microscopic level proved that this hypothesis could no longer be maintained; the current results conclusively demonstrate that even under experimental conditions a local de novo synthesis does not occur. Obviously even a higher incorporation rate in the highly active neuroglial cells at the site of transection does not take place. The blackening which occurs in the distal neurohypophysis as early as 1 h after the injection of the labeled substance is probably due to an uptake by the pituicytes which have been seen to have a high activity under normal and experimental conditions[16].

The fact that labeled neurosecretory substance can be observed at the site of transection about 6 h earlier in the experimental than in the control animals indicates that there is obviously an acceleration of the axoplasmic flow after interruption of the axon.

What explanation can be given for the accumulation of neurosecretory granules in the proximal end of the distal stump and the temporary increase of vasopressor activity of the disconnected distal neurohypophysis[6]? Recently we have shown[16] that an increase in size of the neurosecretory granules occurs as well as a coalescence of several granules in the distal part of the disconnected axon which can account for the increased amount of neurosecretory material observed in the light microscope; furthermore a backflow of granules[11] toward the site of least resistance is supposed to further contribute to the accumulation of neurosecretory material. Even though the neurotubular system has been shown to be the possible site of a local packaging[5,6] recent observations[17] make it rather improbable that it contributes

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extensively to the phenomenon of increased neurosecretory substance. The question remains to be answered in how far subelectronmicroscopic particles are packaged within the distended neurotubules.

The current concept of the mechanism of protein synthesis[18] undoubtedly favors the concept of the existence of a biologically inactive precursor molecule of vasopressin[8,9] which is likely to be activated in the disconnected neurohypophysis by local enzymes which have been shown to increase considerably in the distal stump of transected axons[19].

Incidentally, the high uptake of labeled substance in the intermediate lobe beautifully demonstrates the increased activity of this lobe following withdrawal of the inhibitory hypothalamic influence[20].

Zusammenfassung. Nach Durchschneidung der proximalen Hypophyse wird im proximalen und distalen Stumpf der Neurohypophyse ein erhöhter Gehalt an neurosekretorischen Granula festgestellt. Die Tatsache, dass nur proximal S^{35}-L-Cystein-Hydrochlorid nachgewiesen werden kann, schliesst die immer wieder vorgebrachte Hypothese einer distalen Neurosekretsynthese endgültig aus.

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Effect of Mitomycin-C on the Development of the Eye-Disks in *Drosophila melanogaster*

It is well known that mitomycin-C has a intensive effect, as well as a specific inhibition, on DNA formation in the bacteria, *Escherichia coli*[1–4]. Previously it has been found that mitomycin-C also inhibits the development of *Drosophila* larvae. Above all, the facet-formation of the compound eyes was markedly inhibited by the treatment of this chemical during their larval stage[5].

Nevertheless, in our previous studies of the Bar eye in *Drosophila*, it was pointed out that acid amides have a strong action in accelerating the facet-formation of the mutant Bar eye. When these chemicals were administered, the Bar eye became larger than that of the wild type in extreme cases[6–8].

In the present work, the autoradiographic analysis of the mechanism of the development in the Bar eye was carried out in respect to the nucleic acid metabolism.

The 60-h larvae were treated after hatching with the tracer (^{3}H-acetamide 5.64 μC/ml or ^{3}H-thymidine 100 μC/ml) for 6–8 h with or without mitomycin-C (60 μg/g) and then transferred to normal medium until the end of larval stage. The larvae were fixed in Carnoy, embedded in paraffin, sectioned at 3 μ, and then finally dipped in nuclear emulsion. Autoradiographic exposure for ^{3}H-acetamide was 7 days and that for ^{3}H-thymidine, 3 days. Number of grains were the counts/25 μ^2, taken as a unit area of the tissue.

The results of ^{3}H-acetamide incorporation treated with or without mitomycin-C are summarized in Table I.

^{3}H-acetamide incorporation was marked in those of the eye disks, of the fat bodies and of the salivary glands.

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Table I. Results of autoradiographic grain counts for ^{3}H-acetamide incorporation treated with or without mitomycin-C

Treatment	Average grain No.[a] per 25 μ^2		
	Eye disk	Fat body	Salivary gland
^{3}H-acetamide (8 h)	30.3	27.3	24.0
^{3}H-acetamide + mitomycin-C (8 h)	18.8	23.4	19.5
^{3}H-acetamide (6 h) → mitomycin-C (6 h)	17.1	18.9	17.1
Mitomycin-C (6 h) → ^{3}H-acetamide (6 h)	15.4	16.3	15.3

[a] Mean of 15 samples.

Table II. Results of autoradiographic grain counts for ^{3}H-thymidine incorporation treated with or without mitomycin-C

Treatment	Average grain No.[a] per 25 μ^2		
	Eye disk	Fat body	Salivary gland
^{3}H-thymidine (8 h)	29.5	29.1	25.7
^{3}H-thymidine + mitomycin-C (8 h)	18.7	16.8	15.4

[a] Mean of 15 samples.