MORPHOLOGY AND PATHOMORPHOLOGY

A HISTOCHEMICAL STUDY OF THE DEVELOPMENT OF THE NEURONES OF THE SUPRA-OPTIC NUCLEUS OF THE HYPOTHALAMUS AND POSTERIOR HYPOPHYSEAL LOBE IN RATS

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The developement of the neurosecretory hypothalamo-hypophyseal system has been studied histologically in various animals [3, 6, 8-10]. However, nothing more has been done than to demonstrate the neuro-secretion without any attention being paid to possible changes in the chemical processes within the neurones themselves during the period in which the secretion is being elaborated, and during the process of secretion; neither has any account been taken of changes in the pituicytes of the posterior hypophyseal lobe. We have carried out work of this kind on rats [1].

We decided to continue the investigation in order to establish what kind of chemical changes take place in the fundamental structures of the supraoptico-hypophyseal system during development. For this purpose, we made a simultaneous study of the neurosecretion and of the amount of nucleic acids, proteins, carbohydrates, and fats present.

EXPERIMENTAL METHOD

The material was fixed in Susa, stained with aldehyde-fuchsin and chrome hematoxylin to identify the neurosecretion; it was treated by Danielli's method for protein for demonstration of the tyrosine, tryptophane, and histidine (TTH) which it contained, by Barnett and Zeligman's method for carboxyl protein groups, as well as by the PAS-reaction. After fixation in Carnoy the material was stained with gallocyanin or by methyl green-pyronine for nucleic acids (RNA and DNA). Fats were revealed by Maximow's osmium method, or secretions were stained with Sudan, and fixed by Chiachchio's method. The experiments were performed on rats.

EXPERIMENTAL RESULTS

During the course of development there is a marked change in the reactions and cytoplasmic structure of the cells of the hypothalamo-hypophyseal system. We observed no essential changes in the nuclei or nucleoli. For this reason the results below apply only to the cytoplasm.

The change from intra-to extra-uterine life represents an important stage in the development of the supra-optico-hypophyseal system. It is at the moment of birth that it first becomes possible by staining with aldehyde-fuchsin, to demonstrate neuro-secretory granules standing out against a pale colorless cytoplasm in the nerve cells of the supra-optic nucleus. Even before that, during the last days of fetal development, some indistinct granules masked by the background could be distinguished. The diffuse tint of the cytoplasm cannot be taken as specific, especially because in the neurones of the supra-optic nucleus 1-2 days before the appearance of the neurosecretory granules it becomes so intense that even at low magnification the supra-optic nucleus stands out among the other regions of the hypothalamus. The same state of affairs was found at the 21-22nd day of embryonic development in the demonstra-

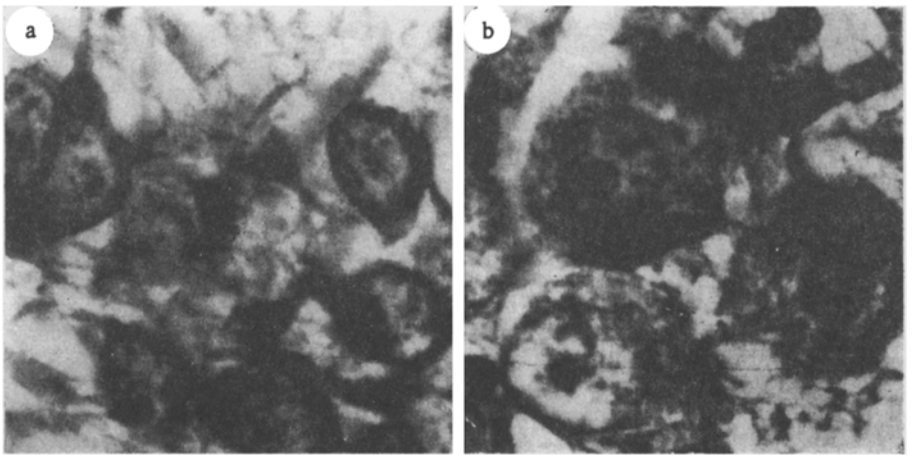

Fig. 1. Neurone of supra-optico nucleus of embryo (a) and adult stage (b). Reagent is TTH. Objective 90×, ocular 10×.

tion of TTH and carboxyl groups. This marked increase in the reaction occurs immediately before the appearance of the neurosecretory granules.

Whichever of the various staining methods is used the cytoplasm and outgrowths stain almost homogeneously at the early stages, and the same is true in all neurones (Fig. 1a). Subsequently the color becomes increasingly differentiated: the various neurones within a single nucleus begin to react differently, and within the cytoplasm of a single cell areas stand out as more intensely or less intensely stained, and the cytoplasmic processes cease to be stained. Finally at the age of approximately 2 weeks the cytoplasm assumes the appearance of a reticulum whose strands are stained at moderate intensity, and do not differ in their reaction from the other hypothalamic neurones. Such an appearance is also characteristic of the sexually mature animal (Fig. 1b). The intensity of the reaction is reduced, although the neurones continue to elaborate the neurosecretion. It appears as though the intensification of the reaction for TTH and carboxyl groups is not associated with the elaboration of the neurosecretion but rather with the early stages of a mechanism which makes possible the formation of this secretion.

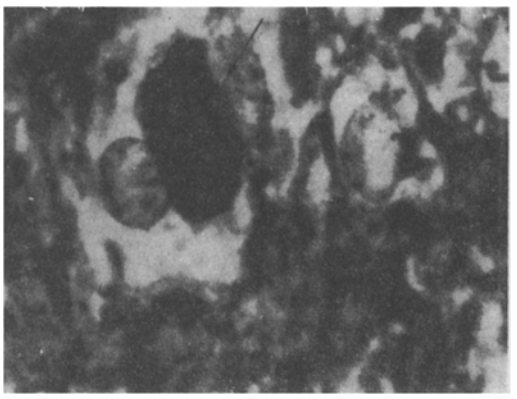

Fig. 2. Reverse side of hypophysis, adult stage. Reagent is the carboxyl group. Objective 90×, ocular 10×.

The neurones of the supra-optic nucleus of the embryo are rich in RNA granules, which are evenly distributed throughout the cytoplasm. The appearance of the neuro-secretory granules is not associated with any noticeable change in the RNA; all that happens is that the clumps of RNA disappear from the perinuclear zone (the zone which contains the neuro-secretory granules). Possibly the clumps are simply pushed out towards the periphery by the granules.

A weakly positive reaction for carbohydrates is found in occasional neurones at various times during the whole of the embryonic period. We consider this to be a chance occurrence. After birth the positive PAS-reaction in the cell is always closely associated with the presence of the neurosecretion. At first this diffusely pink region of the cytoplasm is in the zone of the neurosecretory granules; later, in sexually mature rats, the positive PAS-reaction is given by the granules themselves. No traces of fat were found in the neurones.

The development of the pituicytes proceeds in a very different way. The first change is an alteration in the amount of cytoplasmic RNA. In the embryonic period the cytoplasm is filled with clearly seen pyroninophil clumps. After birth when the neurosecretion first appears in the posterior lobe, pituicytes appear containing no RNA clumps, and pituicytes with very small vacuoles are found in the periphery of the cells. During the first days after birth the granules of RNA disappear almost completely, and the vacuoles increase in size. Subsequently, ribonucleoproteins appear only in the

nucleoli of the pituicytes. The nature and intensity of the reaction of the pituicytes for TTH and carboxyl groups changes but little with age. The reaction for TTH is most intense in the nuclei and in the intra-nuclear structures, and is weakly shown in the neuro-secretory fibers. In the reaction for carboxyl groups all the structures are stained approximately equally, except for the cytoplasm of the pituicytes, which may be unstained, and which is optically clear: quite often it has a reticular structure, and is difficult to distinguish from the surrounding neuro-secretory fibers; finally it may be quite homogeneously stained. The reaction of the neuro-secretory fibers increases with age. In the adult rat, some large Hering bodies give a very intense reaction for carboxyl groups (Fig. 2), and for TTH.

At this stage we are confronted with the nonhomogeneous chemical constitution of the neurosecretion. Previously [1] we have been able to show that the neurosecretion of the cellular bodies, unlike that of the neuronal processes contains a carbohydrate component and has only a weak affinity for Gomori's chrome hematoxylin. Consequently the neurosecretion may be distinguished both by both its TTH and carboxyl groups.

During the transition from the pre- to the postnatal stage, vacuolization of the cytoplasm of the pituicytes is not directly related to the accumulation of osmiophil granules [2, 7]. These granules appear earlier than the vacuoles, their number increases less rapidly, and they are not confined to the periphery of the cell but extend throughout the whole cytoplasm. Finally in certain pituicytes, both vacuoles and osmiophil granules can be made out.

In attempts to find a method which would enable a thin section to be obtained, which would retain fatty substances in the tissue, and which would not require the use of expensive and scarce osmic acid, we have used Chiachchio's fixative, applying it as a means of demonstration of total fat. In preparations stained with Sudan Black after chroming, in the cytoplasm of the pituicytes, besides fatty droplets, striking reticular structures, quite often of remarkable size could be seen in the dorsal part of the posterior lobe where there were very few fatty granules in the bodies of the pituicytes. The ventral portion of the posterior hypophyseal lobe was much richer both in neurosecretion and in fatty droplets than was the dorsal part. Here, the pituicytes were packed with granules. The accumulations of fat in the ventral part of the posterior lobe and in the infundibulum were so great that quite frequently one could not be certain that they were intra-cellular structures. The connection between these masses of fat and the neurosecretion is not clear. But the relationship between the pituicytes and the neurosecretion stands out extremely clearly, and we can see extensive visual evidence leaving not the slightest doubt that whether or not they contain fat droplets, pituicytes, and the neurosecretion represent a single system.

The results we have described show that the development of the supra-optico-hypophyseal system is associated with a change in the structure and constitution of its elements. The chemical properties of the neuro secretion alter during development, and with the movement of the neurosecretion from the supra-optic nuclei towards the posterior hypophyseal lobe.

SUMMARY

In the development of the supra-optico-hypophyseal system of rats, changes in the chemical composition of the neurosecretion, the cellular elements, and the cytoplasm occur. On the 20-22nd day of embryonic development the cytoplasmic reaction of protein containing tyrosine, tryptophane, and histidine, and of the carboxyl group proteins is intensified, as is also the chromatophilia.

These changes seem to be associated with the development of the neurosecretory mechanism. The chemical properties of the neurosecretion continue to change with age. While the pituicytes are developing, at the same time that the neurosecretion appears, the cytoplasmic ribonucleoproteins in the posterior hypophyseal lobe disappear, and vacuoles develop in the peripheral parts. Fat droplets accumulate in the cytoplasm during pituicyte development. There is more fat and neurosecretion in the pituicytes in the ventral part of the posterior lobe than in the dorsal part.

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