LETTER

UPTAKE OF ^3H -DL-LEUCINE AND ^3H -URIDINE BY THE CAUDAL NEUROSECRETORY SYSTEM OF THE LOACH (*MISGURNUS ANGUILLICAUDATUS*) MAINTAINED *IN VITRO*

To the Editors:

The caudal neurosecretory system of fish has been investigated extensively since the structure was elucidated as a neurosecretory organ (Enami, 1955). The distinguished and large perikarya and the aggregation of their bulbous endings to form a storage depot (depot) at the caudal aspect of the spinal cord facilitates the radioautographic study of protein and RNA synthesis in the neurosecretory cells. By this technique, transportation of the material which is synthesized at the perikarya can be demonstrated *in vivo* as previously reported on the loach, *Misgurnus anguillicaudatus* (Inoue and Sato, 1966). In this experiment we demonstrated that newly synthesized proteinous substance, labelled with H^3 leucine in the perikaryon, migrated caudal to the terminal storage depot through the axon. This is the same line of the observation demonstrating accumulation of neurosecretion at the proximal to the transection when surgical transection of the spinal cord of the loach was performed just anterior to the depot (Enami, 1956; Inoue, 1959). No such migration of the labelled substance was seen when H^3 uridine was administered.

In an attempt to elucidate the factor(s) controlling synthetic activity in the neurosecretory cells and migration of the proteinous substance from the perikaryon to the depot, the caudal neurosecretory system of the loach was cultivated *in vitro*. The following observations are based on 68 explants, histologically stained with either hematoxylin and phloxine or Mal-

lory's trichrome after fixation in Bouin's fluid and sectioning at 5μ . For radioautography, the strip film method was utilized after staining the preparations with hematoxylin and phloxine. In the first experiment, the caudal neurosecretory system was excised and cultured on a stainless steel mesh in a specially designed organ culture dish (Falcon Plastics) containing chemically defined culture medium (medium 199, Difco Labs). The atmospheres used were air, 5% CO_2 (Dessicator with candle; Merchant *et al.*, 1964) or mixture of 95% O_2 and 5% CO_2 , respectively. Culture media were changed every third day. Although these conditions was not favorable for maintaining the cultures, quite a few cells were observed to be healthy 3 days after incubation when the cultures were kept in 5% CO_2 . The favorable condition for maintaining the explant in subsequent experiments involved the utilization of equal volumes of medium 199 and horse serum, containing 500 mg% of glucose, in 5% CO_2 and 26°C . The explants maintained in air also showed viable morphology; whereas in mixture of 95% O_2 and 5% CO_2 the explants showed earlier degeneration. These results indicated that higher O_2 tension is not favorable for maintaining the caudal neurosecretory system *in vitro*, as the case of some mammalian and chick embryos (New, 1966). After 19 hrs. of incubation in 5% CO_2 , the neurosecretory cells in the explants retained their characteristic morphology in a healthy condition (Fig. 1), although the neurosecretory cells tended to decrease in number. Most of the ependymal cells and glia cells in the ex-

Fig. 1. The perikarya of the caudal neurosecretory system after incubation for 19 hrs. A small number of medium neurosecretory cells (NC) and ependymal cells (EC) appear healthy. $\times 430$. Fig. 2. The perikarya after incubation for 4 days. A number of small neurosecretory cells (SNC) and ependymal cells (EC) are seen in a healthy state. $\times 430$. Fig. 3. The perikaryon after incubation for 3 days in the medium containing $30 \mu\text{Ci}$ H^3DL -leucine per 1 ml. Note the strong uptake by the ependymal cell layer (EC) and the slight uptake by the axon near the cell body (arrow). $\times 870$. Fig. 4. The terminal storage organ (depot) after incubation for 3 days, showing uptake of H^3DL -leucine. Note the strong uptake by the ependymal cell layer (EC) and the slight uptake by the depot (DEP). $\times 430$.

plant were similarly viable. Reissner's fiber observed in the central canal of the explant appeared normal during this period. Although the larger neurosecretory cells showed degeneration after 3 or 4 days of incubation, a number of small and medium-sized neurosecretory cells retained their morphological characteristics and appeared healthy (Fig. 2). Similar to the shorter incubation period, the other cellular components seemed to be biologically sound. By the 6th or 8th day of incubation, a considerable number of viable neurosecretory cells were seen to remain.

The uptake of H^3DL -leucine and H^3 uridine in the cultured neurosecretory system was observed radioautographically at 2 hrs., 3, 4 and 8 days after incubation. The explants were incubated in a medium (equal portions of medium 199 and horse serum with 500 mg% glucose) containing H^3DL -leucine ($30 \mu\text{Ci}/\text{ml}$) and H^3 uridine ($30 \mu\text{Ci}/\text{ml}$) respectively. Two hrs. after incubation, the perikarya of the neurosecretory cells took up H^3DL -leucine to a greater extent than the ependymal and glial components similar to the earlier *in vivo* experiment. However, 3 days after incubation,

uptake of the isotope by the neurosecretory cells was less than that of the ependymal cells which differed from the previous *in vivo* experiment (Fig. 3). *In vivo*, the neurosecretory cells exhibited a higher level of uptake than the ependymal cells. The decrease of isotope uptake *in vitro* indicates that protein synthetic activity of the neurosecretory cells diminishes more rapidly than the other cellular components. A moderate uptake of isotope was exhibited along the axon near the cell body as is demonstrated (arrow) in figure 3. In contrast to the results of the *in vivo* experiment, the uptake of H^3 DL-leucine by the depot was always much weaker than that of the cell body throughout the present experiment (Fig. 4). This fact suggests that the substance being labelled with H^3 DL-leucine in the perikaryon might migrate caudal to the storage depot only in small amounts. This is strikingly different from the *in vivo* results in which a considerable amount of labelled substance moved caudal to the storage depot by 24 hrs., 2 and 4 days. The reason for this difference could possibly be due to the inadequate cytoplasmic activity of the neurosecretory cells which convey the synthesized material from the perikarya to the storage depot due to (1) sub-optimal culture conditions or (2) to a lack of appropriate stimulation from the other parts of the body (e. g. endocrine organs and hypothalamus) or (3) both.

At the end of 2 hrs. after the incubation with H^3 uridine, silver grains over the perikarya were mainly restricted to the cell nucleus with strong accumulation in the nucleolus and sparse distribution in the cytoplasm. This was similar to the findings *in vivo*. Furthermore, pretreatment of the explants with actinomycin D ($10\mu g/ml$ medium) for 30 mins. inhibited H^3 uridine concentration over the cell nucleus after one hr. of incubation. Three days after incubation, concentration of H^3 uridine into the perikarya varied, being heavily concentrated

in some while sparsely concentrated in others. However, the isotope tended to concentrate in the cell nucleus which was different *in vivo* observations. *In vivo* silver grains were distributed randomly through the perikarya 2 or 4 days after administration of isotope. This may reflect the maintenance of RNA synthetic activity by the neurosecretory cells *in vitro* and/or continuous supply of H^3 uridine from the medium at any given time during cultivation.

Using the organ culture technique, in summary, the caudal neurosecretory cells of the loach can be maintained *in vitro* for several days. Moreover, the perikaryon is able to take up H^3 DL-leucine and H^3 uridine. However, the transportation of H^3 DL-leucine labelled material in large amount, from the perikaryon to the terminal storage organ, previously demonstrated *in vivo*, could not be confirmed in this study.

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