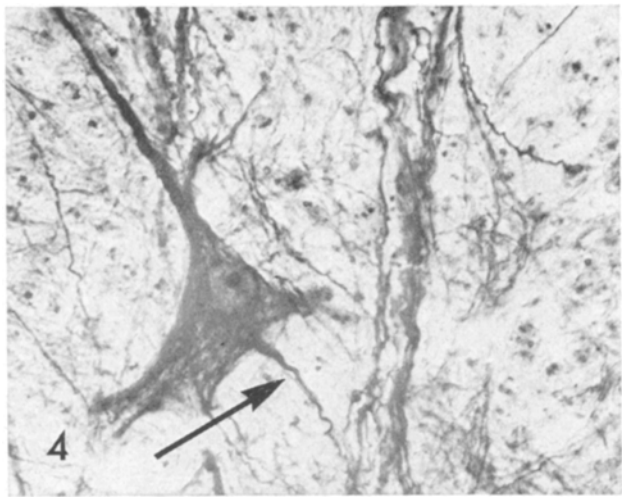


Fig. 1–4. Silver stained transverse sections from the spinal cords of chick embryos incubated for 18 days. The arrows in Figure 1 point to marginal cells while the arrow in Figure 4 indicates an axon extending towards the dorsal white matter.

type found in the substantia gelatinosa of the embryonic and adult avian spinal cord.

*Materials and methods.* Chick embryos incubated for 12–20 days and post hatched chicks up to 5 weeks of age were sacrificed and the spinal cords fixed in Carnoys B fluid for Nissl staining or in De Castros fixative for block Cajal staining. The cords were embedded in paraplast, sectioned serially at 15 μ and mounted on glass slides.

*Results.* In Nissl and silver stained sections of embryonic spinal cord the substantia gelatinosa forms a well defined band extending from the medial side of the dorsal cell column, around the apex and down the lateral side, ending near the base of the dorsal horn. It is composed primarily of small Nissl free neurons, however, an occasional large multipolar neuron, average perikaryal diameter 35–45 μ is also present (Figure 1). These large neurons have spherical nuclei with 1 or 2 darkly stained nucleoli and their cytoplasm contains large floccular Nissl granules. The large multipolar neurons, usually 1 per section occasionally 2 as shown in Figure 2, are located in fairly fixed positions. When only one is present, the cell body is located near the apex of the dorsal cell column (Figures 1–3). If a second cell is present it is usually found close to the lateral boundary of the substantia (Figure 2). The processes of these large multipolar neurons can be followed in various directions with the dendrites oriented towards the dorso-lateral aspect of the cord and the axons (arrow in Figure 4), extending into the ventro-medial area of the dorsal white matter.

*Discussion.* Although this report has dealt primarily with the embryonic spinal cord it must be stressed that the large multipolar neurons were also found in the substantia of 5-week-old post hatched chicks. The cells in question however attain their maximum size and are largest, relative to their surroundings, during the last 5 days of incubation. The maturation of these large neurons in the substantia, coincides with the onset of repetitive somatic movements of the chick prior to hatching and it is interesting to speculate that these cells may be part of a transient system concerned with the hatching behavior.

*Résumé.* On a trouvé chez le poulet, dans la substance gélatineuse de la moëlle épinière en voie de maturation ou adulte, de gros neurones multipolaires d'un diamètre moyen de 35–45 μ. Ces neurones ont des noyaux sphériques contenant un ou deux nucléoles fortement contrastés. Dans leur cytoplasme se trouvent des granules de Nissl, gros et à contour floconneux.

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## Incorporation of $^{35}$S-Labelled L-Cysteine in the Ependyma of the Rat's Subcommissural Organ and Choroid Plexus

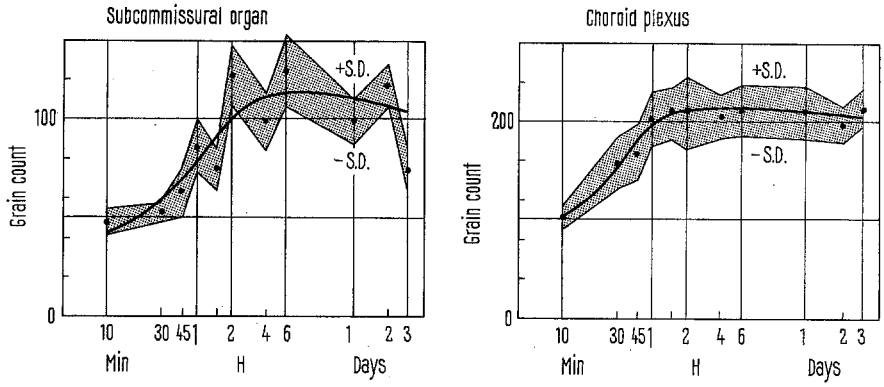
Conspicuous incorporation of $^{35}$S-labelled dl-cysteine in the ependyma of the subcommissural recess has previously been elicited by autoradiographs[1]. Observations have been made to the effect that changes in the function of the thyroid gland have an influence on this incorporation[2].

The problematic function of the subcommissural organ (SCO) has been a constant object of investigation. Endeavours have been made to find new methods of research for clarification of its physiological significance. Particular efforts have been exerted on the search for a morphological method to measure the activity of the SCO, in addition to the previously predominantly employed quantification of the selectively stainable 'secretion' and to karyometric measurement. The incorporation of cysteine has been used as indicator for the activity of the hypothalamic-hypophyseal neurosecretory system. Similarly, this incorporation may be thought to reflect also the secretory activity of the SCO. Closer analysis of the phenomenon was therefore considered appropriate. In order to obtain a suitable reference basis, the incorporation in the ependyma of the choroid plexus of the third ventricle was also studied in addition to that taking place in the ependyma of the SCO.

*Material and methods.* Altogether 31 adult male albino rats of 225 g average body weight were used. The animals received standard pellet diet and tap water ad libitum. All the rats were kept under identical conditions. Each animal was given, by i.p. injection, $^{35}$S-labelled L-cysteine ('L-Cysteine-S 35 hydrochloride', The Radiochemical Centre, Amersham, Bucks., England) at an average dose of 150 μCi. The injections were all administered on one day, about 09.00 h. The animals were divided into 11 groups, which were sacrificed at the following times after injection: 10, 30 and 45 min, 1, $1^1/_2$, 2, 4 and 6 h, 1, 2 and 3 days. There were 3 rats in every group but those sacrificed after 2 and 3 days, which had 2 members each. The rats were killed by rapid decapitation without anaesthesia.

The brains were embedded in paraffin after fixing in Bouin's fluid. Serial sections at 7 μ were made sagittally;

[1] J. C. Sloper, D. J. Arnott and B. C. King, J. Endocrin. *20*, 9 (1960).
[2] S. Talanti and V. Pasanen, Life Sci. *7*, 1245 (1968).




Means of the grain counts from autoradiographs of specimens from the subcommissural organ and choroid plexus of the third ventricle of the rat, plotted over logarithmic time after injection of $^{35}$S-labelled L-cysteine (dots), and ± S.D. range (shade area). The hand-drawn curves approximate the plots and remain almost entirely within the said range.

they were mounted on glass slides and coated with Kodak NTB 2 emulsion. After storage in light-tight boxes for 25 days at 4 °C, the slides were developed in Kodak D 11 developer, fixed with Kodak Rapid Fixer and stained with haematoxylin-eosin.

Grain counts were made with a × 10 eye-piece and × 100 objective, using the ocular micrometer. The area counted was 75 µ by 75 µ. The objects thus studied were the SCO and the choroid plexus of the third ventricle, ten areas of the ependyma of both objects being subjected to counting in each rat, but only one area of both objects in each section.

The mean values and standard deviations of the counts were calculated for each time after injection. Student's *t*-test was applied in comparing the means, two and two. These calculation were carried out by Computer Centre of the University of Helsinki.

*Results and discussion.* The results are shown in the Table and in the Figure, in which the means referring to various times after injection have been plotted together with the respective ± S.D. limits. The abscissae represent the logarithm of the time after injection. In the figure a curve has been drawn by hand for the SCO and one for the choroid plexus observations, which runs within the ± S.D. range and fits the plots fairly well in the former and quite closely in the latter instance. The results of the *t*-tests can be summarized to the effect that in both sets the means of the observation times under 1 h differ at a statistically significant or highly significant level from all later means and from each other, with few exceptions, while no significant differences exist upwards of 1 h in the observations of the choroid plexus, and in those made of the SCO they also appear entirely spurious.

The general trend is evident from the results that incorporation of the isotope in the ependyma of the SCO is rapid and reaches its maximum within 2–4 h. The maximal level seems to persist up to and perhaps even beyond 24 h. 3 days after the injection a considerable amount of activity is still noted.

Incorporation in the choroid plexus is even more rapid; its maximal level was reached within 1–2 h and persisted at virtually constant magnitude up to at least 2 days.

As could be expected on the basis of the earlier results of the investigation cited above, quite distinct incorporation of the isotope in the ependyma of the SCO was thus elicited. The absolute grain counts are different for the SCO and the choroid plexus (approximately double for the latter), and the rates of incorporation were different. This is thought to indicate qualitatively different function of the 2 ependymas.

A necessary prerequisite for cysteine incorporation to be usable as an indicator of the specific secretory activity of the SCO is that this amino acid is indeed metabolized to become part of the secretion of the SCO. Only indirect evidence of this exists. A fact in its favour is the histochemical observation that protein-bound SH and SS groups are localized in the area of the selectively stainable 'secretion' of ependymal cells[3]. If cysteine is adjoined to be part of the secretion, its incorporation reflects the synthesis and release of the secretion in the same way as it is considered to reflect the dynamics of neurosecretion when it is included as a component in the neurosecretory material[4].

Uptake of radioisotope by the ependyma of the subcommissural organ and of the choroid plexus of the third ventricle of the rat after i.p. injection of $^{35}$S-labelled L-cysteine, as indicated by grain counts made of autoradiographs

| Group | Time after injection | Grain counts Subcommissural Organ Mean | S.D. | Choroid Plexus Mean | S.D. |
|---|---|---|---|---|---|
| 1 | 10 min | 46.9 | 6.3 | 101.3 | 12.4 |
| 2 | 30 min | 52.8 | 5.0 | 157.1 | 25.8 |
| 3 | 45 min | 63.1 | 12.1 | 167.2 | 29.0 |
| 4 | 1 h | 87.7 | 14.1 | 201.9 | 23.7 |
| 5 | 1.5 h | 74.0 | 9.9 | 208.3 | 26.0 |
| 6 | 2 h | 123.1 | 15.7 | 208.5 | 37.9 |
| 7 | 4 h | 99.4 | 13.9 | 204.5 | 22.5 |
| 8 | 6 h | 125.7 | 18.4 | 211.1 | 24.6 |
| 9 | 1 day | 98.9 | 11.1 | 209.1 | 27.1 |
| 10 | 2 days | 117.9 | 11.4 | 196.2 | 17.2 |
| 11 | 3 days | 73.3 | 11.4 | 213.1 | 18.5 |

*Zusammenfassung.* Die Inkorporation von i.p. verabfolgtem, mit $^{35}$S gezeichnetem Cystein im Ependym des Subkommissuralorgans und des Plexus choroideus wurde bei normalen Ratten untersucht. Rasche und intensive Inkorporation fand statt, und das Isotop konnte in den Zellen noch nach 3 Tagen wahrgenommen werden.

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*Department of Anatomy and Embryology, College of Veterinary Medicine, Helsinki (Finland), 2 May 1969.*

[3] S. TALANTI, Ann. med. exp. biol. Fenn. *36* suppl. 9, 1 (1958).

[4] This investigation was supported by a grant from the National Research Council for Medical Sciences, Finland.