

Incorporation of ^{35}S -Labelled L-Cysteine and L-Methionine in the Ependyma of the Subcommissural Organ in the Thyroidectomized Rat

SLOPER et al.¹ have reported conspicuous incorporation of ^{35}S -labelled cysteine in the ependyma of the subcommissural organ (SCO). Previous results² also suggest that a change of thyroid function in rats treated with thiouracil or excess thyroxine is accompanied by a change in the incorporation of ^{35}S -labelled cysteine in the SCO. No studies on the incorporation of ^{35}S -labelled methionine in the SCO have been made so far.

The function of the SCO is still obscure. The physiological significance of its potential secretory activity is not clarified. Numerous experimental studies³ exist on the morphology and metabolism of the SCO. Histochemically, the presence of protein-bound SH and SS groups in the ependyma of the SCO has been noted⁴. This sulphur-rich protein is claimed to be part of the 'secretory material' of the organ. This material is selectively stainable by the histological methods which also stain the 'neurosecretory material' in the hypothalamic-hypophyseal system.

The hypothesis that cysteine is incorporated in the 'secretory material' has resulted in attempts to use the intensity of incorporation of labelled cysteine as an indicator of the secretory or metabolic activity of the SCO. It is likely that a high proportion of the labelled cysteine is incorporated in protein. Methionine, too, is probably incorporated in protein. GAITONDE and RICHTER⁵ showed that, after injection of labelled methionine, some 80% of the label found in the brain of the rat are present in protein. The protein fraction labelled with ^{35}S after injection of labelled methionine has not been studied. It can be assumed, however, that like cysteine, labelled methionine is also incorporated in the 'secretory material'.

The principal aim of the present study was to investigate the effect of thyroidectomy on the incorporation of ^{35}S -labelled cysteine and methionine in the SCO. The difference in the effect of thyroidectomy on the incorporation, if any, was expected to throw some light on the question whether labelled cysteine and methionine are incorporated in the same or in different proteins.

Material and methods. A total of 72 male adult albino rats were used. All rats were kept in the same room during the test period. They received standard pellet diet and tap water ad libitum. 36 of the animals were

thyroidectomized under anaesthesia, while the other 36 served as controls. 8 weeks later, 18 thyroidectomized animals and 18 animals from the control group received an average dose of 150 μCi of ^{35}S -labelled cysteine (L-Cysteine-S 35 hydrochloride, The Radiochemical Centre, Amersham, Bucks., England) by i.p. injection. 18 thyroidectomized and 18 control rats received a dose of 150 μCi of ^{35}S -labelled methionine (L-Methionine-S 35 hydrochloride, The Radiochemical Centre, Amersham, Bucks., England). 6 animals from each group were sacrificed by rapid decapitation 30 min, 6 h and 24 h, respectively, after injection.

The paraffin-embedded brains were cut sagittally in serial sections at 7 μm . Autoradiograms were made by the emulsion method, using Kodak NTB 2 emulsion. The slides were stored in light-tight boxes for 32 days at 4°C and developed in Kodak D 11 developer, and fixed with Kodak rapid fixer. The sections were stained with haematoxylin-eosin.

Grain counts were made using an eye-piece micrometer with $\times 10$ eye-piece and $\times 100$ objective. The area counted measured $150 \times 75 \mu\text{m}$. 10 areas of the subcommissural ependyma of each rat were counted.

From the series of counts thus obtained, the mean values and their standard deviations were calculated. Student's *t*-test was applied in comparing the means, by pairs⁶. The mathematical calculations were made by the Computer Centre of the Helsinki University.

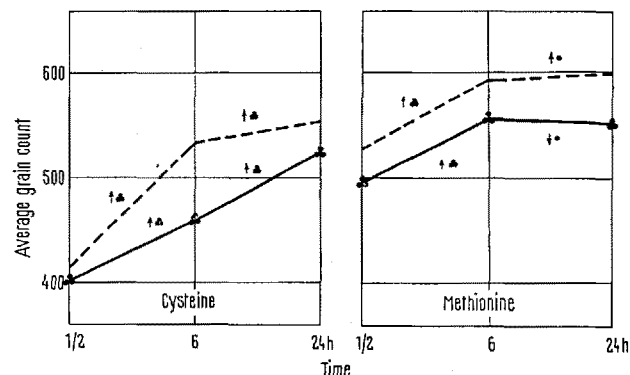
Results and discussion. The results are presented in the Figure. Intense incorporation of both cysteine and methionine in the ependyma of the SCO was seen. However, the incorporation of cysteine seems to differ from that of methionine. On the whole, the counts were markedly lower in the thyroidectomized rats, compared with the controls.

The metabolism of cysteine in the ependyma of the SCO is obviously different from that of methionine. This is suggested by the differences in incorporation and by the differences in the effect exerted by thyroidectomy. Nothing can be said concerning the character of the proteins labelled by the ^{35}S of the amino acids examined. Kinetic analysis of the results might constitute an approach to this problem. Such studies are in progress⁷.

Zusammenfassung. Nach Thyreoidektomie wurde die Inkorporation von i.p. verabfolgtem und mit ^{35}S markiertem Cystein und Methionin im Ependym des Subcommissuralorgans der Ratte untersucht. Die Cystein-Aufnahme war stärker reduziert als diejenige von Methionin.

S. TALANTI

Department of Anatomy and Embryology,
College of Veterinary Medicine, Helsinki 55 (Finland),
28 December 1970.



Diagrams showing the grain counts (mean of 6 animals) found at different times after injection of the isotope in the ependyma of the subcommissural organ. ---, control group; —, thyroidectomized. Triple, single dots: $P < 0.001$, 0.05 (difference of mean from corresponding mean of controls; change of mean between 2 times of observation; with arrow indicating direction of change).

¹ J. C. SLOPER, D. J. ARNOTT and B. C. KING, *J. Endocrin.* 20, 9 (1960).

² S. TALANTI, *Life Sci.* 7, 1245 (1968).

³ J. PALKOVITS, *Morphology and Function of the Subcommissural Organ* (Stud. biol. Hung. Akademiai Kiadó 1965), vol. 4.

⁴ S. TALANTI, *Annls. Med. exp. Biol. Fenn.* 36 suppl. 9, 1 (1958).

⁵ M. K. GAITONDE and D. RICHTER, *Proc. R. Soc. B* 145, 83 (1956).

⁶ R. A. FISHER, *Statistical Methods for Research Workers* (Oliver and Boyd, Edinburgh 1950).

⁷ This study was financially supported by the National Research Council for Medical Sciences, Finland and the Sigrid Jusélius Foundation, Helsinki (Finland).