5-Hydroxytryptamine of the Spinal Cord Normally and after Transection

It has recently been observed, in the rabbit, that noradrenaline almost completely disappears in the thoracic, lumbar and sacral portions of the spinal cord after transection at the second thoracic segment[1]. This seemed to us to indicate that most of the noradrenaline in the cord was present in descending nerve fibres. This observation has prompted us to investigate if 5-hydroxytryptamine in the cord behaves in a similar manner as noradrenaline after transection.

The experiments were performed on adult rabbits, the spinal cords of which were cut at the second thoracic segment under ether anaesthesia. As large amounts of 5-hydroxytryptamine are held by the blood plateletes in this species, the animals were bled under nembutal anaesthesia from the carotid artery at the same time as a Ringer's solution of 37°C was infused into a jugular vein. The 5-hydroxytryptamine of the cord was determined as earlier described[2] with the modification that the tissue was re-extracted with the original volume of 0.4 *N* perchloric acid. The results are found in the Table. In some specimens of the cord, the cholinesterase activities were also determined[3].

As will appear from the Table, 5-hydroxytryptamine is present in the spinal cord of the rabbit at a concentration of 0.26 μg per g, the concentrations being about the same in cervical and the lower parts. A week after the transection the content in the portion below the section is only about 15% of that found in the cervical portion. This fact may be interpreted to mean that the 5-hydroxytryptamine in the cord, like noradrenaline, is mostly localized in nerve fibres descending from more centrally sited cell bodies. The section did not have any significant effect on the cholinesterase activity of the cord.

In a few experiments it was found that administration of L-dihydroxyphenylalanine or 5-hydroxytryptophan to rabbits 2 h after the transection caused a facilitation of the spinal reflexes of the hind legs. The flexor reflex and mass reflexes could be much more easily evoked after administration of either of the two drugs. As these are the precursors of the catecholamines and 5-hydroxytryptamine, it is probable that the substances of these groups present in the central nervous system may have a modifying effect on the reflex activity therein[4].

5-Hydroxytryptamine content of the spinal cord of the normal rabbit and after transection at the 2nd thoracic segment. The figures indicate μg/g

	Controls		Operated animals	
	above Th 2	below Th 2	above Th 2	below Th 2
	0.28	0.27	0.30	0.04
	0.27	0.30	0.38	0.05
	0.27	0.23	0.28	0.01
	0.22	0.22	0.31	0.09
Mean	0.26	0.25	0.32	0.04
± S.E.M.	± 0.013	± 0.020	± 0.021	± 0.015

Zusammenfassung. Der 5-Hydroxytryptamingehalt im Rückenmark des Kaninchens ist 0,26 μg/g. Querschnittsläsion durchs Rückenmark führt zu einer 85prozentigen Abnahme des 5-Hydroxytryptamingehaltes. Es wird darum angenommen, dass 5-Hydroxytryptamin in Nervenzellen des Zentralnervensystems lokalisiert ist.

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1 T. Magnusson and E. Rosengren, Exper. *19*, 229 (1963).
2 Å. Bertler and E. Rosengren, Exper. *15*, 382 (1959).
3 For this purpose about 100 mg tissue was homogenized in 1.5 ml 0.9% sodium chloride. To the homogenate 10 mg acetyl choline was added and the mixture made up to 10 ml with 0.9% sodium chloride. The sample was then put into the reaction beaker of an automatic titrator (Radiometer Titrigraph). The pH of the mixture was kept constant at 6.8 by addition of 0.025 *N* NaOH, the supply of the correcting base being continuously recorded on a chart.
4 We are indebted to Miss A.-Ch. Lilljeqvist for skilful technical assistance. The work has been supported by grants from the Office of Aerospace Research, United States Air Force, and the Swedish Medical Research Council.

On the Occurrence of Homovanillic Acid and 3-Methoxy-4-Hydroxymandelic Acid in Human Cerebrospinal Fluid

Judging by our investigations, as well as literature reports, the normal cerebrospinal fluid does not seem to contain 5-hydroxytryptamine, noradrenaline or dopamine in free forms. The turnover rates of these amines in the central nervous system may possibly be studied by determination of their degradation products in the cerebrospinal fluid. In a previous paper, the occurrence of 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid was reported[1]. As a consequence of this finding, we have now extended our investigations to acid catecholamine metabolites in this fluid.

The phenolic acids of 30–40 ml fresh, lumbar cerebrospinal fluid were extracted and purified according to the method for determination of 5-HIAA[1]. After transferring the acids from an ether phase to a phosphate buffer, pH 7, the latter was adjusted to pH 1 with metaphosphoric acid and saturated with sodium chloride. The apparent acids were extracted with ethyl acetate. This was evaporated to a small volume, which was chromatographed on Munktell S 302 filter paper (washed with ethanol), in an ascending *n*-butanol-pyridine-water (14:4:5) system. After about 18 h, the paper was dried and sprayed with diazotized *p*-nitroaniline[2]. The Rf-values for water solutions of homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-4-hydroxymandelic acid (vanillomandelic acid, VMA) and 3,4-dihydroxymandelic acid (DOMA) were: 0.60, 0.52, 0.43, 0.34, respectively. Internal standards (5 μg of each substance to 30–40 ml

1 B.-E. Roos, Life Sciences *1*, 25 (1962).
2 W. v. Studnitz and A. Hansson, Scand. J. clin. lab. Invest. *11*, 101 (1959).