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).

cerebrospinal fluid) were carried through the entire procedure in every experiment. This is important, not only for the calculation of the recovery, but also for the qualitative determination, because the presence of organic material usually causes small changes in the Rf-values. By this method spots have been observed corresponding in position and colour to HVA and VMA added to cerebrospinal fluid. The amounts of the acids were estimated semiquantitatively on the chromatograms. The recoveries of HVA and VMA were about 40%. After correction for this, the normal concentration of HVA in cerebrospinal fluid was estimated to about 75 ng/ml and of VMA to about 25 ng/ml. DOPAC and DOMA were not found on the chromatograms. Nor was it possible to detect them spectrophotofluorimetrically after condensation with ethylenediamine[3]. The recoveries of added DOPAC and DOMA varied between 50 and 60%.

The catecholamines in the central nervous system have two inactivating pathways. Dopamine and noradrenaline are transformed by monoamine oxidase (MAO) to dihydroxylated phenolic acids, DOPAC and DOMA, respectively. This reaction seems to take place mainly in the synthesizing cell[4], but the enzyme can probably degrade also extracellular amines. The catecholamines can also be inactivated by catechol-O-methyl transferase (COMT), which seems to be localized far away from the synthesizing sites[4]. At their passage to the ventricles and subarachnoidal space, the 3-O-methylated catecholamines and dihydroxylated phenolic acids are converted to the corresponding 3-O-methylated acids by MAO and COMT, respectively. The catecholamines and their metabolites are normally so slightly lipid soluble that they can only with difficulty diffuse through the lipid-like blood-brain barrier. This may be the reason why HVA and VMA occur in the cerebrospinal fluid in such high concentrations. They probably leave the central nervous system by filtration through the arachnoidal villi[5]. An active transport from cerebrospinal fluid to blood in the choroid plexus may also be possible[6,7].

Zusammenfassung. Es wird papierchromatographisch gezeigt, dass Cerebrospinalflüssigkeit gesunder Menschen sowohl Homovanillinsäure als auch 3-Methoxy-4-hydroxymandelsäure enthält. Die Konzentration der ersteren ist etwa 75 ng/ml und die der letzteren etwa 25 ng/ml.

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[6] J. R. PAPPENHEIMER, S. R. HEISEY, and E. F. JORDAN, Amer. J. Physiol. *200*, 1 (1961).
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[8] *Acknowledgment.* This work has been supported by grants from Leo Ltd., Hälsingborg, and from the Medical Faculty, University of Göteborg (Sweden). For technical assistance we are indebted to Mrs. I. OLOFSON.

The 'brown spots' (bsp) Character of *Drosophila melanogaster* and its Relation to Copulation

The *'brown spots'* (*bsp*) character arose spontaneously in the wild stock *Aspra 52* of *Drosophila melanogaster* in 1959.

The phenotypical manifestation is strictly limited to the female sex; it consists in the formation of brown-coloured areas, variable in shape and size, localized in the pleurae; the pigmentation (probably due to melanin) (DI PASQUALE[1]) affects the hypodermic cells only (DI PASQUALE[2]).

The character is transmitted by the male as well as by the female; it is due to the presence of one or more recessive factors localized in the 2nd chromosome, and segregates normally in F_2 (DI PASQUALE[3]). The penetrance varies in time from 60% to 90%.

The phenotypical manifestation shows a clear relation to mating. Females kept apart from males, and females isolated from them when all the phases of courtship are complete but copulation has not yet taken place, never show spots.

A high incidence of manifestation (63.3% sp. and 21.6% d.)[4], on the other hand, is found when females are allowed to pair with males and are removed from them soon after the first copulation. Moreover, repeated copulations barely increase the degree of spotting.

bsp females mated with males of nine different stocks (*cn*, Chieti-*v*, *Cyl/Pm*, Varese, *tu* B3, *tu* A2, Oregon, Urbana, *y w*) show in all cases typical spots generally with a high frequency; significant differences are, however, noticeable to a greater or lesser extent in the incidence in the *bsp* stock; the mating with *y w* males, in which the incidence of the spots is particularly low (9.5% sp. and 2.2% d.), is a peculiar case.

Males of two different stocks, X/Y^{Lc} and X/O, sterile because of the non-motility of spermatozoa, the factors contained in the short arm of the Y chromosome being absent, also determine the appearance of spotting in *bsp* females. The same result is also obtained by pairing *bsp* females with X/X; *tra/tra* individuals. These, genotypically females, are transformed into males owing to the presence of *'transformer'*, a recessive gene localized in the 3rd chromosome (STURTEVANT[5]); they possess external genitalia and secondary sexual characters of male type, copulate regularly with females, have normal paragonia and rudimentary testes, and therefore do not produce spermatozoa.

Interspecific mating between *bsp* females and *Drosophila simulans* males also produces spotting, but with a low incidence (10.9% sp. and 1.1% d.).

These investigations, therefore, prove that the observed response to mating is a general phenomenon; it is also independent of the sterility of the male, whether due to the non-motility of the spermatozoa or to their absence.

Recent experiments using paper chromatography (CHEN and DIEM[6]) emphasize the presence of a ninhydrin-

[1] A. DI PASQUALE, Atti A.G.I. *7*, 138 (1962).
[2] A. DI PASQUALE, Atti A.G.I. *5*, 117 (1960).
[3] A. DI PASQUALE, Atti A.G.I. *6*, 233 (1961).
[4] In these experiments it was preferred to classify separately the females with spots (sp.) and with very small pigmented areas (dottings, d.).
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