Succinyl adenosine, a new substance in the human cerebrospinal fluid

In the course of an examination of the cerebrospinal fluid for free nucleotides, nucleosides, and similar substances, the paper-chromatographic technique revealed the presence of a substance (occasionally two) not identical with those previously reported.

Hydrolysis in 1 *N* HCl at 100° for 1 h transformed the constantly occurring substance (X) into the compound which was occasionally found (Y). The reaction of X with orcinol is positive, but it does not contain phosphate. It is retarded in paper chromatography with 95% ethanol–1 *M* ammonium acetate, pH 7.5 (7.5:3, *v/v*) as solvent[1] when the latter is saturated with borate[2].

Hydrolysis of X in 3 *N* HCl at 100° for 5 h will form substances identified chromatographically as well as spectrophotometrically as adenine, hypoxanthine and aspartic acid.

Y contains no phosphate, it is not retarded by saturation of the solvent of PALADINI AND LELOIR[1] with borate, and it does not give a positive orcinol reaction. The HUNTER[3] reaction which is positive with succinyl adenine[4] gave a positive reaction with Y. Chromatography and spectrophotometry showed that Y was identical with succinyl adenine synthesized by the method of CARTER[5]. It is concluded that X is succinyl adenosine and Y the corresponding aglycone.

So far as we are aware, succinyl adenosine has not been found before in animals and the two substances have not been demonstrated previously in the cerebrospinal fluid.

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Mammalian hydroxylation in the 6-position of the indole ring

Many pathological human urines contain the sulphate ester of a hydroxyskatole[1,2]. Enzymic hydrolysis of this substance gives the unconjugated hydroxyskatole, which is an unstable substance. The persulphate oxidation of skatole gives a product identical with that excreted by man, together with 5- and 7-sulphatoxyskatoles, and other products including 3-methyloxindole[1,3]. The oral administration of skatole to the rat results in the excretion of a mixture of sulphate esters of hydroxyskatoles similar to that resulting from the persulphate oxidation reaction. Enzymic sulphation of the hydroxyskatoles resulting from the iron–ascorbic acid hydroxylation of skatole gives a similar mixture, containing the human sulphate ester, along with 5- and 7-sulphatoxyskatoles[1].

The products of the iron-ascorbic acid oxidation[4,5] of skatole have now been separated and identified. The oxidation was carried out in open flasks at room

temperature in aqueous acetone. After 12 h the greater part of the unreacted skatole was removed by extraction with cyclohexane, and the oxidation products were then removed with ether. The products were separated by chromatography on silica (60–80 mesh) with 6 % benzene–chloroform (v/v) as the eluting solvent. Six products were found; these included N-formyl-*o*-aminoacetophenone, 3-methyloxindole, and four hydroxyskatoles which were readily differentiated by their characteristic ultraviolet spectra in neutral and alkaline solution, and by their colour reactions with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde in HCl) and Van den Bergh's reagent (diazotized sulphanilic acid). The latter reagent gave strikingly different and distinctive colours with each of the position isomers.

One of these isomers was chromatographically identical with the enzymic hydrolysis product from the sulphate (isolated by an ion-exchange method) from human urine, and this identity was confirmed by comparison of the ultraviolet spectra (in neutral and in alkaline solution) for the human hydroxyskatole and the synthetic oxidation product. The distinctive absorption spectrum in alcohol was practically identical with that reported for 6-hydroxyindole[6], and this suggested that the human excretion product was 6-sulphatoxyskatole. Authentic 6-hydroxyskatole was prepared by the sequence *m*-nitrophenol → *m*-benzyloxynitrobenzene → *m*-benzyloxyaniline → *m*-benzyloxyphenylhydrazine → propionaldehyde *m*-benzyloxyphenylhydrazone → 6-benzyloxyskatole → 6-hydroxyskatole (m.p. 149–151°. Found: C, 73.65; H, 6.22; N, 9.61. Calc. for C_9HO_3N: C, 73.45; H, 6.16; N, 9.52). The ultraviolet and infrared spectra, colour tests, and chromatographic behaviour observed for this substance were identical with those found for the product obtained by direct (iron-ascorbic acid) hydroxylation. Enzymic sulphation of the synthetic material gave 6-sulphatoxyskatole (chromatographically identical with the human product) and this was also excreted by the rat after administration (stomach tube) of 6-hydroxyskatole.

It is noteworthy that the major route of hydroxylation of skatole in man is through the 6-position, a pathway for indole metabolism in humans that has not hitherto been found.

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