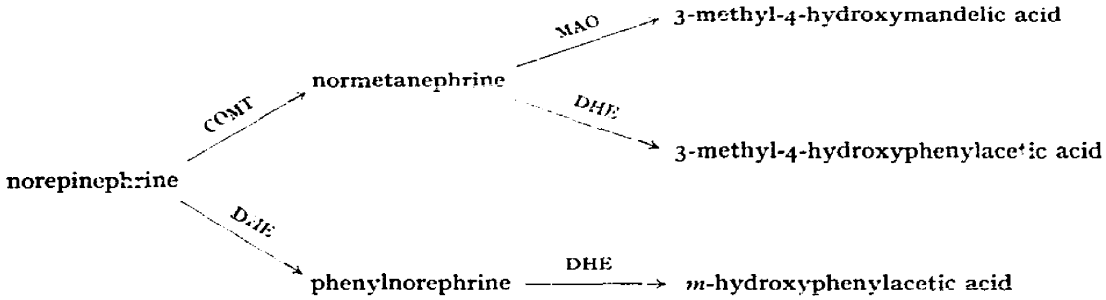
PN 1184

Dehydroxylation of norepinephrine

DL-[7-^{3}H]Norepinephrine has been found to be converted almost quantitatively to an unidentified phenolic acid in the guinea-pig treated with tolbutamide[1]. In order to obtain suitable quantities of this new catabolite for qualitative studies, a tracer dose of DL-[7-^{3}H]norepinephrine was mixed with 100 mg of *dl*-normetanephrine and the mixture injected intraperitoneally every 30 min in 10 divided doses. The guinea-pig was given 200 mg/kg tolbutamide (sodium salt) 1 h before injection of the amines and a similar dose 4 h later. A portion of the 24-h specimen of urine, made acid to pH 1, was extracted into ethyl acetate. Chromatograms developed in 2 dimensions were prepared from this extract and 4 compounds were revealed after spraying with diazo reagents. Three of these compounds were readily identified from their color reactions and by their R_F's as 3-methoxy-4-hydroxymandelic acid, unconjugated 3-methoxy-4-hydroxyphenylethyl glycol, and 3-methoxy-4-hydroxyphenylacetic acid. The latter is normally derived from dopamine and its appearance as a catabolite of norepinephrine and normetanephrine must have involved dehydroxylation of the aliphatic side chain. FELLMAN[2] has shown that the ethanolamine side chain of norepinephrine may be regarded as a glycol and subject to a pinacol–pinacolone rearrangement with loss of water. In support of this view he was able to obtain dihydroxyphenylacetaldehyde by heating either norepinephrine or epinephrine in strong acid. FELLMAN's view was extended to include the catechol group which conceivably might undergo dehydroxylation to yield *m*- or *p*-hydroxylphenylacetic acid. A sample of *m*-hydroxyphenylacetic acid was obtained through the courtesy of Dr. M. D. ARMSTRONG. Identity with *m*-hydroxyphenylacetic acid of the compound isolated from the urine was demonstrated by the finding of the same specific activity as a mixture of the isotope with carrier in eluates of paper chromatograms which had been developed in 3 solvent systems. Homogeneity was shown by the finding of similar specific activities in each eluate from 4 strips cut across the radioactive areas on each chromatogram. Reaction with diazotized *p*-nitroaniline to yield a reddish color was used to quantitate *m*-hydroxyphenylacetic acid in the eluates. The specific activities of *m*-hydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid and 3-methoxy-4-hydroxymandelic acid present in the urine were determined by counting eluates of each compound from 2-dimensional chromatograms, located by reaction with diazotized sulfanilate. The quantities of the compounds were determined by comparison with standards placed on the paper. 3-Methoxy-4-hydroxymandelic acid was found in a concentration of 225 μg/ml (specific activity 38 counts/sec/μg); 3-methoxy-4-hydroxyphenylacetic acid, 150 μg/ml (specific activity 5.3 counts per sec/μg); and *m*-hydroxyphenylacetic acid, 57 μg/ml (specific activity 88 counts per sec/μg).

The finding of low specific activity of 3-methoxy-4-hydroxyphenylacetic acid suggests that a greater proportion of inactive normetanephrine was converted to 3-methoxy-4-hydroxyphenylacetic acid than was norepinephrine. Removal of a phenolic hydroxyl may proceed more rapidly than the aliphatic hydroxyl since radioactive *m*-hydroxyphenylacetic acid and not 3-methoxy-4-hydroxyphenylacetic acid is the major new product of norepinephrine.

The results of these preliminary experiments can be summarized in a scheme proposed as an alternate pathway for norepinephrine inactivation in guinea-pigs.



(COMT, catechol:*O*-methyltransferase; MAO, monoamine oxidase (EC 1.4.3.4); DHE, dehydroxylating enzyme)

These reactions represent a novel mechanism for catecholamine inactivation. Deamination of norepinephrine may be either enzymic or non-enzymic as indicated. Conceivably, these reactions take place in man as suggested by the presence of both *m*- and *p*-hydroxyphenylacetic acids in urines of normal persons[3]. Traces of the latter have been tentatively identified in urines of guinea-pigs given large doses of normetanephrine and norepinephrine. Therefore, *m*- as well as *p*-dehydroxylation may take place.

Since tolbutamide somehow facilitates removal of aliphatic and aromatic hydroxyls it is tempting to consider that its effect on blood sugar may be related to dehydroxylation of carbohydrates. An enzyme system obtained from the livers of guinea-pigs is being prepared to test this interesting possibility.

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Note added in proof: Ring dehydroxylation of catechols appears to require a reactive side chain as suggested by the finding that dihydroxyphenylalanine, dihydroxycinnamic acid, but not dihydroxybenzoic acid are converted to *m*-hydroxy derivatives[4]. In view of this it seems likely that dehydroxylation of the norepinephrine side chain precedes loss of the phenolic hydroxyl. Phenylnorephrine therefore may not be the hypothetical intermediary between norepinephrine and *m*-hydroxyphenylacetic acid. (Received November 23rd, 1962)

Department of Psychiatry and Neurology, New York University Medical Center, New York, N.Y. (U.S.A.) ALFRED A. SMITH

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