On the use of ethanol as a scavenger in storing labelled dopa and 5-hydroxytryptamine in aqueous solutions

Radiation decomposition is a serious problem when using radioactively labelled compounds. In this laboratory, decomposition of tritium-labelled L-dopa has presented a special problem (Persson & Waldeck, 1970). Self-irradiation decomposition of tritium-labelled compounds in aqueous solutions can be reduced by the addition of ethanol which serves as a scavenger or radical trap (Evans, 1966; Bayly & Evans, 1968). Among labelled compounds supplied in aqueous solutions containing ethanol are L-dopa and 5-hydroxytryptamine (5-HT) (see current catalogues of manufacturers of labelled compounds).

Exposure of ethanol to ionizing radiation in the presence of water results in the formation of *inter alia* acetaldehyde (Swallow, 1953; 1960). Since dopa appears to condense easily with acetaldehyde, forming tetrahydroisoquinoline derivatives (Cohen & Collins, 1970), the choice of ethanol as a scavenger in storing aqueous solutions of labelled dopa may be questionable. This may be true also of 5-HT which condenses with aldehydes forming tetrahydro-β-carbolines (Corrodi & Jonsson, 1965). Both reactions are special cases of the general Pictet-Spengler reaction (see review by Whaley & Govindachari, 1951). The following experiment illustrates the hazard.

Radiochemically pure [^{3}]dopa (L-3,4-dihydroxyphenylalanine-*ring*-2,5,6-^{3}H, specific activity 1 Ci mmol^{-1}, The Radiochemical Centre, Amersham), 1 μCi, and 40 μg unlabelled L-dopa were mixed in 0·05M phosphate buffer, pH 6·5 or in 0·01N hydrochloric acid, pH 2, the total volume being 50 μl. Finally, 10 μl acetaldehyde was added and the mixture allowed to stand for 15 min at room temperature (25°). After that time an aliquot was put on a Munktell S 302 chromatographic paper and developed in isopropanol–2N hydrochloric acid (65 : 35) by descending chromatography for about 20 h. The procedure was repeated using 0·1 μCi of radiochemically pure [^{14}C]-5-HT (5-hydroxytryptamine-2-^{14}C, specific activity 17·2 mCi mmol^{-1}, NEN Chemicals,

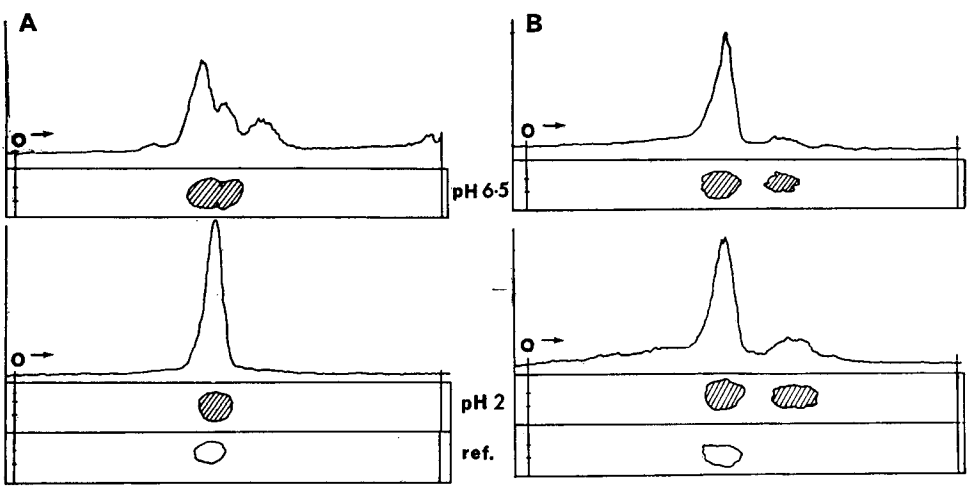

FIG. 1. Radiopaper chromatography of reaction products formed from [^{3}H]dopa (A) or [^{14}C]-5-HT (B) in the presence of acetaldehyde. [^{3}H]Dopa or [^{14}C]-5-HT were mixed with unlabelled dopa and 5-HT respectively, in 0·05M phosphate buffer, pH 6·5, or in 0·01N hydrochloric acid, pH 2. Acetaldehyde was added and 15 min later aliquots of the reaction mixtures were put on chromatographic papers and developed in isopropanol–2N hydrochloric acid, 65:35. The papers were stained with diazotized *p*-nitroaniline. The ordinate represents the amount of radioactivity; O = origin. In A, ref. = dopa; in B ref. = 5-HT.

Dreieichenhein bei Frankfurt/Main) and 40 μg unlabelled 5-HT. Authentic L-dopa and 5-HT, respectively, were run in parallel with the samples on the same papers.

After the development, the papers were stained with diazotized *p*-nitroaniline and scanned for radioactivity in a Frieseke & Hoepfner, FH 452 strip-scanner with a windowless gas-flow detector.

At pH 6·5 three distinct peaks of radioactivity, with a slight indication of a fourth, appeared (Fig. 1). [^{3}H]Dopa upon storage in an ethanolic, neutral aqueous solution shows a similar pattern of radioactive contaminants (unpublished). Two of the peaks were accompanied by spots with the same reddish-violet colour as L-dopa, visible after staining. These two spots, although close to the position of authentic L-dopa, could not be fully characterized. However, it does seem unlikely that the two spots are artifacts due to double-spot formation (Beckett, Beaven & Robinson, 1960) since this phenomenon is unlikely to occur (and has never been observed by us) under the strongly acidic conditions used.

When the reaction with acetaldehyde was performed at pH 2, one single peak of radioactivity and one spot appeared, both with the same position as authentic L-dopa. This is analogous with the previous finding (Cohen & Collins, 1970) that acidification stops condensation reactions between catecholamines and aldehydes. Thus, a slight acidification should improve the stability of labelled dopa in ethanolic aqueous solutions. However, acidification may increase the rate of tritium loss by exchange (cf. Waterfield, Spanner & Stanford, 1968), but from a biochemical point of view THO is a less serious contaminant than are derivatives of [^{3}H]dopa which are difficult to detect and separate analytically and which may interfere biochemically.

Also 5-HT appeared to react with acetaldehyde, forming a new compound, as indicated by the second peak of radioactivity, and the appended spot which had the same reddish-violet colour as authentic 5-HT but a higher R_F. In this case the reaction occurred also at pH 2. The radioactive peak formed was recognized from radio-paper-chromatograms of [^{3}H]-5-HT subject to radiodecomposition upon storage in an ethanolic aqueous solution (unpublished).

In view of these results care should be taken in the selection of ethanol as a radical scavenger for storing aqueous solutions of tritiated compounds which are likely to react with acetaldehyde.

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Department of Pharmacology,
University of Göteborg,
Fack,
S-400 33 Göteborg 33, Sweden.

BERTIL WALDECK

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REFERENCES

BAYLY, R. J. & EVANS, E. A. (1968). *Storage and stability of compounds labelled with radio-isotopes.* RCC Review 7. The Radiochemical Centre, Amersham, Bucks, England.

BECKETT, A. H., BEAVEN, M. A. & ROBINSON, A. E. (1960). *J. Pharm. Pharmac.*, **12**, *Suppl.*, 203T–216 T.

COHEN, G. & COLLINS, M. (1970). *Science*, **167**, 1749–1751.

CORRODI, H. & JONSSON, G. (1965). *Acta histochem.*, **22**, 247–258.

EVANS, E. A. (1966). *Nature*, **209**, 169–171.

PERSSON, T. & WALDECK, B. (1970). *Acta pharmac. tox.*, **28**, 466–476.

SWALLOW, A. J. (1953). *Biochem. J.*, **54**, 253–257.

SWALLOW, A. J. (1960). *Radiation Chemistry of Organic Compounds.* Oxford: Pergamon Press.

WATERFIELD, W. R., SPANNER, J. A. & STANFORD, F. G. (1968). *Nature*, **218**, 472–473.

WHALEY, W. M. & GOVINDACHARI, T. R. (1951). In: *Organic reactions.* Coll. Vol. 6, pp. 151–206. Editor: Adams, R. New York, N.Y.: John Wiley and Sons, Inc.