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# LETTERS TO THE EDITOR

## Effect of sucrose on the spectrophotometric determination of cholinesterase activities

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In studies of the subcellular distribution of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) in sucrose fractions prepared from homogenates of retina we found (unpublished) that the apparent enzyme activities were significantly increased by the presence of sucrose when assayed by the spectrophotometric Ellman procedure (Ellman, Courtney & others, 1961). As we were unaware of any mention of this phenomenon in the literature it was examined in more detail.

Test cuvettes were prepared containing 0·1 ml 5,5-dithiobis-2-nitrobenzoic acid (DTNB—final concentration = $3 \times 10^{-4}$ M); 0·2–0·5 ml of sucrose samples; potassium phosphate buffer to give a final volume of 3·1 ml (final concentration = 0·1 M, pH 8·0). Specific inhibitors of either AChE or BuChE (1,5-bis(*p*-allyldimethylammonium-phenyl)-pentan-3-one dibromide (BW284C51), or ethopropazine hydrochloride (Parsidol) respectively) were included for the differential assay of the enzymes in tissue fractions. After a preincubation period of 10 min at 37° the reaction was initiated by the addition of substrate: either 0·1 ml acetylthiocholine iodide (final concentration = 2·5 mM) or 0·2 ml butyrylthiocholine iodide (final concentration = 10 mM). The reaction mixtures were then incubated for further periods of up to 10 min (37°) and the initial rate of change of absorbance, due to the production of the yellow anion of 5-thio-2-nitrobenzoic acid, measured at 412 nm on a spectrophotometer.

When test cuvettes containing substrate only were run against air as the blank, the rates of hydrolysis were 0·012 $\Delta A\ min^{-1}$ (2·73 nmol $min^{-1}$) and 0·029 $\Delta A\ min^{-1}$ (6·65 nmol $min^{-1}$) for acetylthiocholine and butyrylthiocholine respectively.

To examine the effects of sucrose on the assay, 0·2 ml (acetylthiocholine present) or 0·5 ml (butyrylthiocholine present) of sucrose solutions (BDH Analar grade sucrose) ranging from 0·4 to 1·8 M were included in the reaction mixtures containing the substrate indicated. These volumes were those normally assayed for the respective enzyme activity (unpublished). Inclusion of sucrose did not significantly alter the pH of the reaction mixtures. Sucrose was replaced with an equal volume of distilled water in the blank cuvettes. Thus the overall rate of reaction due to utilization of substrate in the test cuvettes was automatically corrected for buffer-mediated hydrolysis of substrate.

The results from such an experiment are depicted in Fig. 1. It can be seen that the presence of sucrose led to an increase in the rate of formation of product, 5-thio-2-nitrobenzoate ion. The rates of reaction increased with increasing sucrose concentration.

The omission of the specific inhibitors (employed in the assay of the enzymes in tissue fractions) had no effect on this phenomenon. In the absence of substrate no reaction was observed between sucrose and DTNB. Furthermore, these effects were consistently observed for several batches of sucrose. It was also found, using

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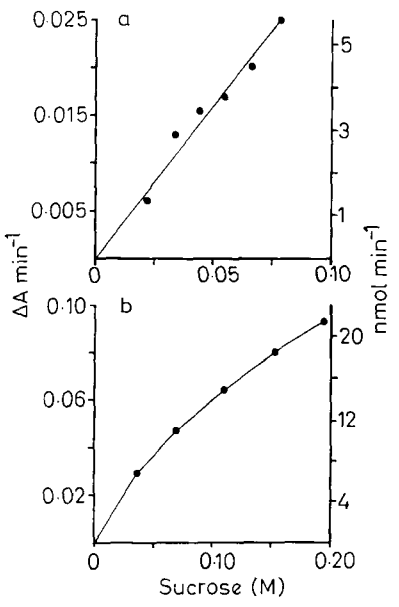


FIG. 1. The effect of sucrose concentration on the rate of non-enzymic breakdown of the substrates used to assay AChE and BuChE by the Ellman method. Incubation mixtures containing either (a) acetylthiocholine iodide (2·5 mM), or (b) butyrylthiocholine iodide (10 mM) were incubated (37°; pH 8·0) with different concentrations of sucrose, ranging from (a) 0–0·08 M, or (b) 0–0·2 M (final concentrations in cuvette). The initial rates of change of absorbance ($\Delta A\ min^{-1}$) at 412 nm were then determined spectrophotometrically. Values are corrected for buffer-mediated hydrolysis of the substrates, and each is the mean of duplicate determinations. Reaction rates are also given as nmol $min^{-1}$ substrate utilized.

pure AChE and BuChE, that high concentrations of sucrose had no effect on enzyme-catalysed hydrolysis of these thioesters after correcting for its effect on their non-enzymic breakdown.

These results show, therefore that the presence of sucrose in the cholinesterase reaction mixtures can give rise to high non-enzymic rates of reaction. As the sucrose concentration is increased the rate of substrate utilisation increases. This is presumably because the sucrose molecule itself is acylated by the substrates. Similar findings have recently been reported by Hebb, Mann & Mead (1975) in the radiometric assay of choline acetyltransferase (ChAc). In this instance sucrose appeared to be acetylated non-enzymically by one of the substrates, acetyl-CoA, giving rise to misleadingly high estimates of enzyme activity. Acetylation of sucrose is also a possibility in cholinesterase assays employing acetylcholine as a substrate (e.g. pH-stat, radiometric, Michel, Hestrin methods; see Holmstedt, 1971).

The present findings suggest, therefore that adequate controls should be carried out when determining the activity of cholinesterases in subcellular fractions, or other preparations containing sucrose, by the spectrophotometric Ellman procedure. This is especially important when measuring low levels of enzyme activity in the presence of high sucrose concentrations. Removal of the sucrose by dialysis or gel-filtration, or simply running parallel blanks containing concentrations of sucrose equivalent to those present in the enzyme reaction mixtures, are possible ways of overcoming this problem.

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# Bradykinin relaxes contracted airways through prostaglandin production

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Bradykinin is a potent constrictor of guinea-pig trachea and bronchus (Collier, Holgate & others, 1960). Paradoxically there have been a few reports where bradykinin has been shown to relax the trachea of guinea-pig (Ramos, Ramos & others, 1965; Iorio & Constantine, 1969), cat (Turker & Kiran, 1965; Turker & Ercan, 1976), dog (Turker & Khairallah, 1969) and rabbit (Fleisch & Calkins, 1976); and the bronchus of man (Mathé, Aström & Persson, 1971). Bradykinin has been reported to release prostaglandins (Damas & Deby, 1976; Erdos, 1976) and bradykinin-induced relaxation of cat trachea is blocked by aspirin (Turker & Ercan, 1976).

In a recent study in this laboratory, bradykinin ($10^{-7}$ to $10^{-5}$M) was found to contract trachea and bronchi of horse, ferret and guinea-pig. Bradykinin ($10^{-6}$ to $10^{-5}$M) relaxed the carbachol-contracted trachea and bronchi of dog, cat and rabbit, and the trachea, but not

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