Rats receiving neostigmine bromide [(10–40 mg/rat)/day] in the drinking water over a period of 4 weeks did not salivate or show fasciculations after injection of neostigmine (0·4 μmol/kg) into the footpad. The salivation produced by these chronically treated rats after carbachol (0·25 μmol/kg) was (0·19 g/rat)/8 min; this value is significantly less than for the controls ($P<0·05$).

After neostigmine was withdrawn, it was found that the tolerance to carbachol and neostigmine injections was maintained for up to 3 weeks while plasma cholinesterase levels returned to normal within 1 week. These results suggest that although inhibition of cholinesterase may be involved in the development of tolerance to neostigmine, there is no direct relationship between the effects.

Tolerance to neostigmine has not previously been studied in any detail, but much work has been reported on the tolerance to organophosphate anticholinesterases. Some of these reports (Brodeur & Dubois, 1964; McPhillips & Dar, 1967; McPhillips, 1969) suggest that tolerance to organophosphates could be due to decreased sensitivity of cholinergic receptors or other postsynaptic systems. These conclusions might also be applicable to our results with neostigmine.

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Simultaneous determination of choline acetylase and cholinesterase activity

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The study of cholinergic neurones by the quantitative measurement of cholinesterase alone is open to error since levels of this enzyme do not always run parallel to levels of acetylcholine. It is desirable, therefore, to study cholinacetylase in addition to cholinesterase. In many instances the relative levels of the two enzymes are not parallel in different sites (Heading, 1969).

We have modified existing radiometric methods so that choline acetylase and cholinesterase activity can be measured in the same sample of tissue. The advantages of the method are (i) precise comparison of the two enzymes; and (ii) economy of material, time and expense.

In the first stage of the procedure, in which ChE activity is determined, the tissue sample is incubated with [^{3}H]-acetylcholine in 0·2 M phosphate buffer (pH 7·4) usually for 30 min. The reaction is then stopped by the addition of 10^{-4}M neostigmine bromide, which is part of the buffer/substrate for the subsequent determination of choline acetylase. The other components of the final incubation solution are those described by Buckley, Consolo, Giacobini & McCaman (1967) with the omission of eserine. After a suitable incubation period, usually 30 min, the reaction is stopped

by the addition of 5 volumes of ice-cold 0·2 M NaH_2PO_4 solution and the reaction tubes transferred to ice.

The [^{14}C]-acetylcholine is extracted by adding 10 volumes of 3-heptanone containing 2·5 mg/ml of Kalignost (Fonnum, 1969). All of the organic layer is removed for liquid scintillation counting. The [^{3}H]-acetate is extracted into toluene/iso-amyl alcohol after the incubation solution had been acidified with 0·2 N HCl (Potter, 1967). The scintillation fluid used was that described by Buckley & Heaton (1968). The 2-channel liquid scintillation spectrometer (Packard Tri-carb 3002) was set so that one channel counted [^{14}C] with an efficiency of 20% and [^{3}H] with an efficiency of 0·1%. The other channel counted [^{3}H] with a 12% efficiency and [^{14}C] with a 60% efficiency. [^{14}C] or [^{3}H] n-hexadecane was used as an internal standard.

Results will be presented to illustrate the use of the method for cholinesterase and choline acetylase determination in freeze-dried sections of rat nervous tissue.

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Determination of the iontophoretic release of adenosine diphosphate from micropipettes

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The application of ionized substances to blood vessels in the microcirculation by micro-iontophoresis was first described by Duling, Berne & Born (1968). This method permits accurate control of the site so that reactions of different types of small vessel can be determined. We describe here a technique for quantitating the dose by measuring the iontophoretic release from micropipettes of substances available at high specific radioactivities.

The adhesion of circulating platelets in blood vessels may be initiated by adenosine diphosphate (ADP) released from damaged cells (Born, 1962). We have, therefore, been investigating the mechanism of this adhesion by applying ADP iontophoretically to small vessels in the hamster cheek pouch (Begent & Born, 1970a, b). As a step towards determining the lowest effective concentration of ADP, the rate has been measured at which radioactive ADP is released from micropipettes by iontophoretic currents. Micropipettes (tip 1–3 μM) were filled with 10^{-2}M [G-^{3}H]-ADP (specific activity, 44·3 Ci/mol, purity >95%) and connected by an external circuit to a silver silver-chloride reference electrode.