dependent animals by parenteral injection of morphine, 3–4 times daily, for a period of several weeks (Seevers & Deneau, 1963; Halbach & Eddy, 1963), assessing the dependence liability of a new drug by the extent to which it prevents symptoms after the withdrawal of morphine. A more convenient means of inducing dependence was described by Maggiolo & Huidobro (1961) and Way, Loh & Shen (1969), who used pellets of morphine base, implanted subcutaneously in mice, to provide a continuous dosage. However, this technique has the drawback that to produce withdrawal symptoms it is necessary to excise the pellet or to administer an opiate antagonist.

The method demonstrated enables a rat to be exposed continuously to morphine by the single daily administration of morphine solution into a subcutaneously implanted reservoir. Each reservoir consists of a 3 cm length of silicone rubber tubing (I.D. 4·8 mm, O.D. 7·9 mm) at one end of which is fixed a cellophane membrane, which allows slow outwards diffusion of drug. The reservoir is filled by means of an inlet and an outlet tube again made of silicone rubber (I.D. 1·0 mm, O.D. 2·2 mm) which are sealed onto the opposite end of the reservoir. An advantage of the method is that with minimal interference with the rat, morphine can be administered and withdrawn, or a novel analgesic compound substituted simply by washing out the reservoir with an appropriate solution. After dosing with morphine hydrochloride (30 mg/ml) in this way for only 9 days, characteristic withdrawal symptoms occur on removal of the drug from the reservoir. These are prevented by replacing morphine with solutions of known addictive analgesics such as codeine, methadone and pethidine.

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Tolerance to neostigmine

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Experiments in our laboratories demonstrate the development of tolerance to neostigmine in rats.

The experiments were designed to identify any adaptative changes which occur during chronic treatment with neostigmine bromide, and ultimately to observe any differences in the activities of enzymes associated with the cholinergic system.

The effects of neostigmine were observed in anaesthetized rats (pentobarbitone sodium 60 mg/kg intraperitoneally) after injection of the neostigmine methyl sulphate into the foot pad. When a dose of neostigmine (0·4 μmol/kg) was injected into control rats the typical effects observed were muscle fasiculations and salivation, commencing within 6 min of injection and lasting for 35 min. Carbachol (0·25 μmol/kg) similarly injected produced salivation; the mean weight of this secretion from a group of six rats being (0·45 g/rat)/8 min.

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