Pharmacological Inhibition of Acetylcholine Synthesis

SCHUELER[1] has designated as the "hemicholiniums" a series of quaternary bases[2] which exhibit an unusual kind of toxicity. Chemically they are characterized by the incorporation of a choline (or choline-like) moiety into a six-membered ring through hemiacetal formation. Their most striking pharmacological action is respiratory paralysis: this is central in origin and late in onset, and when the dose is not too large can be prevented by the administration of either eserine or choline. Schueler noted that hemicholinium intoxication presents several features reminiscent of poisoning by botulinus or tetanus toxin, and suggested that it might be due to interference with some cholinergic mechanism. We thought that a substance having such effects might be a specific poison of acetylcholine synthesis, and through Dr. Schueler's kindness we have been able to verify this notion with compound No. 3 of his series, here called *HC*3.

*HC*3 (2×10^{-5} *M*), added to the eserinized plasma perfusing a cat's superior cervical ganglion, did not affect the rate at which acetylcholine was released into the perfusion fluid during repetitive stimulation of the preganglionic nerve trunk for 1–2 min. With more prolonged stimulation, however, the rate of acetylcholine release rapidly declined, and coincidentally the ganglion lost its ability to transmit impulses and also most of its store of preformed acetylcholine. The acetylcholine synthesized by a ganglion during an hour's stimulation at 20/sec. was reckoned by comparing its residual acetylcholine content with that of its unperfused fellow and correcting for the acetylcholine discharged into the perfusion fluid. In five experiments with *HC*3 the amount synthesized was $0{\cdot}10 \pm 0{\cdot}09$ μgm. (mean ± S.D.), and in five experiments without the drug it was $1{\cdot}21 \pm 0{\cdot}17$ μgm. The effects of *HC*3 could be antagonized by raising the choline content of the perfused plasma. When the ganglion was stimulated during perfusion with eserinized Locke's solution instead of with plasma, it synthesized acetylcholine much more slowly, and synthesis was still further reduced if *HC*3 was present, or alternatively, if the concentration of eserine was increased. Evidence that eserine may interfere with acetylcholine synthesis has already been presented by Perry[3] and by Shelley[4].

Analogous results were obtained with *HC*3 in experiments on mouse brain, minced and incubated in eserinized bicarbonate–Locke as described by Mann *et al.*[5]. Under our experimental conditions this preparation synthesized acetylcholine at the rate of about 11 μgm./gm. (wet weight) in 4 hr.; *HC*3 (10^{-4} *M*) diminished the synthesis by at least 75 per cent; choline increased the synthesis and partly (2×10^{-5} *M*) or wholly (10^{-3} *M*) reversed the inhibitory effect of *HC*3. The release of acetylcholine from tissue to medium, which proceeded slowly throughout the period of incubation, appeared to be unaffected by *HC*3 or by choline, except in so far as the level of bound acetylcholine was altered.

*HC*3 was tested for its ability to inhibit the synthesis of acetylcholine by an extract of acetone-dried rat brain powder in a medium containing choline (10^{-2}–10^{-5} *M*), acetyl–coenzyme *A* (2×10^{-4} *M*) and cysteine (2×10^{-4} *M*). In this system it also acted as an inhibitor and its effect was again antagonized by choline; but its action was here so weak (for example, about 50 per cent inhibition by 10^{-2} *M* *HC*3 in the presence of 10^{-4} or 10^{-5} *M* choline) as to seem unrelated to its pharmacological activity. It is unlikely, furthermore, that *HC*3 acts by preventing the acetylation of coenzyme *A*: for (*a*) such an action might be expected to interfere with many essential metabolic processes, whereas the toxic effect of *HC*3 appears to be highly specific; and (*b*) we have found that the ability of homogenized pigeon liver to acetylate sulphanilamide, which depends on coenzyme *A*[6], is well maintained in the presence of 10^{-2} *M* *HC*3. It is conceivable that *HC*3 acts on intact nervous tissue by poisoning choline acetylase (that is, the enzyme that transfers acetyl groups from acetyl-coenzyme *A* to choline), but that the enzyme on being extracted from tissue becomes less susceptible to poisoning by *HC*3. An alternative explanation would be that *HC*3, and other hemicholiniums, may compete with choline for transport by a specific carrier system to intraneuronal sites of acetylation.

This work was supported by grants from the National Research Council of Canada and the Defence Research Board of Canada. We wish to thank our colleague Prof. A. S. V. Burgen for his advice.

F. C. MACINTOSH
R. I. BIRKS
P. B. SASTRY

Department of Physiology,
McGill University,
Montreal.
Aug. 27.

[1] Schueler, F. W., *J. Pharmacol.*, **115**, 127 (1955).
[2] Long, J. P., and Schueler, F. W., *J. Amer. Pharm. Assoc.* (Sc. Ed.), **43**, 79 (1954).
[3] Perry, W. L. M., *J. Physiol.*, **119**, 439 (1953).
[4] Shelley, H., *J. Physiol.*, **131**, 329 (1956).
[5] Mann, P. J. G., Tennenbaum, M., and Quastel, J. H., *Biochem. J.*, **33**, 822 (1939).
[6] Lipmann, F., *J. Biol. Chem.*, **160**, 173 (1945).

Chromatography and Spectrophotometry of Fused Salts

WE have recently observed chromatographic adsorption on alumina (Merck's reagent grade aluminium oxide) of transition metal cations dissolved in fused salts.

Solutions of Fe^{+++}, Co^{++}, Ni^{++}, Cu^{++}, UO_2^{++} in lithium-potassium nitrate eutectic mixture (lithium-potassium nitrate, 43 mol. per cent lithium nitrate, m.p. 132° C.) were placed in a jacketed 'Pyrex' tube (10 mm. internal diameter, 20 cm. long) filled to a height of 10 cm. with alumina. The temperature of the tube was maintained at 150° C. by circulating silicone oil ('Dow-Corning 710') through the jacket. The solutions were all approximately $0{\cdot}01$ *M* with respect to the transition metals and had been prepared by dissolving appropriate amounts of the anhydrous chlorides in lithium-potassium nitrate. All the transition metal cations listed are adsorbed on the column. They may be eluted with lithium-potassium nitrate containing the complex-forming anions Cl^-, $SO_2^=$, or CN^-. We are investigating the elution properties as a function of the particular complexing anion, its concentration and the temperature of operation of the column.

Essentially similar observations have been made using anhydrous ammonium formate (m.p. 118° C.) as a solvent.