LETTERS TO THE EDITOR

The Extraction of Acetylcholine in Small Samples of Cerebral Tissue

SIR,—The extraction of acetylcholine (Ach) and acetylcholine-like substances (Florey, 1961) can be made by one of several methods (Abdon, 1944; Bentley and Shaw, 1952; Crossland, 1961; Lewis and Smallman, 1958; Smallman and Fisher, 1958; Birks and MacIntosh, 1961.

The substance is assayed usually either on the frog rectus abdominis or on the cat blood pressure preparation with the precautions indicated by Felberg (1945). Also, the guinea-pig ileum may be used (Blaber and Cuthbert, 1961; Birmingham, 1961).

With some of these procedures it is not always possible to assay extracts with low concentrations of Ach, since they are prepared from small samples of tissue, such as certain parts of the central nervous system of, for example, rats or guinea-pigs. These animals, on the other hand, are useful for the analysis of the zonal distribution of Ach and the modification brought about in this by drugs or experimental procedures.

We now describe our method of overcoming the problem of estimation.

Guinea-pigs of either sex, weighing 270–330 g., are decapitated and the skull is opened with scissors. 50–100 mg. of cerebral tissue (even 20–30 mg. if necessary) is rapidly excised, weighed and immersed for 2–3 min. in 3 ml. of McIlvaine citric acid-disodium phosphate buffer, ph 4; 0·014 *M*, at 98°–99° contained in glass homogeniser tubes in boiling water. The standards are prepared by adding known amounts of Ach (30–100-300 ng.) to 50–100 mg. (or 20–30 mg.) of nervous tissue, previously boiled for 10 min. at pH 9·5–10. After cooling, the tissue is carefully homogenised under ice and kept at 18° for 15 min. Then the pestles and the side of tubes are washed with 3 ml. of Tyrode solution without glucose and bicarbonate, but with a double concentration of salts, to obtain an isotonic medium. The supernatant is diluted 2:5 or 1:5 with the normal Tyrode solution and assayed (1–2 ml.) on the guinea-pig ileum against either the standards or freshly prepared solutions of Ach.

The terminal ileum is set up in 3 ml. of the normal Tyrode, oxygenated at 30°, containing diphenhydramine 2×10^{-8} to improve the selectivity, and morphine 5×10^{-8} to reduce the motility and increase the discrimination within smaller responses. The lower sensitivity limit is 1×10^{-10}, as Ach final concentration. Our experimental conditions differ slightly from those of Blaber and Cuthbert (1961) and Birmingham (1961); they used the guinea-pig ileum sensitised with organophosphorus anticholinesterases, to assay amounts of Ach smaller than those present in our extracts.

The assay is done at intervals of 90–120 sec. The contractions are recorded with an isotonic lever, 1:20 magnification, loaded at 0·40–0·50 g., writing on a smoked drum. At the end of the assay the usual controls are made: alkaline hydrolysis of the sample and treatment of the terminal ileum with atropine 2×10^{-8}. The amount of Ach in the extract is obtained by bracketing its response between that of 2 known doses of Ach and of 2 standards.

The average recovery of the standards, against known solutions of Ach, was 101·3 $\pm$ 17 per cent (s.d.): so it is clear that our extraction method releases only small amounts of interfering substances (Feldberg, 1945) and does not cause loss.

In the Table, the Ach content detected in the olfactory bulbs, in the posterior part of the parietal area and in the anterior part of verme cerebellaris is compared using the above method and also that of Lewis and Smallman (1956).

Also the extracts prepared according to the second method were assayed on the terminal ileum. The average recovery of the standard is 90 ± 22 per cent (s.d.).

TABLE I

ACETYLCHOLINE CONTENT OF GUINEA-PIG BRAIN
(ACH CHLORIDE μG./G. FRESH TISSUE ± s.d.)

Method	Animals No.	Olfactory bulbs	Cerebral cortex	Cerebellum
Lewis and Smallman (1956) ..	16	2·017 ± 0·447*	2·207 ± 0·425*	0·498 ± 0·172
Citric acid disodium-phosphate buffer	16	2·053 ± 0·419†	2·454 ± 0·490†	0·485 ± 0·132

* = The difference between these two values is not statistically significant.
† = The difference btween these two values is statistically significant. (P = 0·01.)

Although the results do not differ significantly, only with our extraction procedure is the Ach content of the cerebral cortex significantly higher than that of olfactory bulbs. (P = 0·01.)

It is probable that the immediate treatment with heat at pH optimum for the stability of Ach and the use of a strongly hypotonic medium, favour both the inactivation of the esterases and the diffusion of the neural hormone from the tissue.

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