Absence of Spasm in a Sensitive Assay for Acetylcholine

SIR,—One of the difficulties encountered by Blaber and Cuthbert (1961) in the use of di-isopropylphosphorofluoridate (dyflos, DFP) for increasing the sensitivity of the isolated guinea-pig ileum to acetylcholine is the tendency of the preparation to go into spasm at 20–30 min. intervals. Neostigmine and ethyl pyrophosphate (TEPP) have similarly been found to produce spasm.

Experience with the organophosphorus anticholinesterase mipafox (*NN'*di-isopropylphosphorodiamidic fluoride) in this department (Harry, 1961) has shown it to be almost devoid of this property and suggested its suitability for increasing the sensitivity of the guinea-pig ileum to small concentrations of

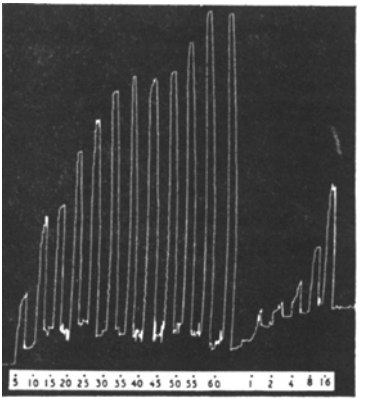

FIG. 1. Isolated guinea-pig ileum; mipafox 1×10^{-5}; morphine sulphate 5×10^{-6}; Krebs's solution at 37°; a dose-response curve to acetylcholine 5 to 60 pg. added to a bath of 10 ml.; followed by a dose-response curve of 1 to 16 pg.; 2 min. cycle, 15 sec. contact; total duration about 50 min. with absence of spasm.

acetylcholine. Our experimental conditions differed sufficiently from those of Blaber and Cuthbert to emphasise the general usefulness of their application of the use of morphine.

The ileum was suspended in 10 ml. of Krebs's solution (Gaddum, 1959) at 37° containing 5×10^{-6} morphine sulphate. The tissue was incubated with 1×10^{-5} mipafox for 1 hr., then excess mipafox was washed out. Using a 2 min. cycle and 15 sec. contact time the preparation regularly gave a good response to 100 pg./ml. of acetylcholine and occasionally responded to 1 pg./ml.

Slight fluctuations in baseline which occur are not troublesome, and spontaneous spasms are not seen (Fig. 1).

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