

# A NOTE ON THE RELATION BETWEEN THE RESTING RELEASE OF ACETYLCHOLINE AND INCREASE IN TONE OF THE ISOLATED GUINEA-PIG ILEUM

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When the Magnus preparation of the guinea-pig ileum treated with diisopropylphosphorodiamidic fluoride (Mipafox) was suspended in Krebs solution, the increase in tone with time was found to be proportional to the acetylcholine output. Use may be made of this phenomenon to monitor the state of the preparation in experiments involving the resting release of acetylcholine.

THE isolated ileum of the guinea-pig has little inherent tone compared with that of the rabbit. The "tone" of an ileal segment suspended for periods of time in Krebs solution increases and the magnitude of this increase depends on the duration of incubation.

The experiments reported in this paper were made to discover a possible connection between this increase in tone and the resting release of acetylcholine.

## METHODS

Adult guinea-pigs were killed and the ileum was excised. The terminal ileum was cut into 3 cm. segments which, along with a 10 cm. length from the middle region, were incubated for 75 min. in 200 ml. aerated Krebs solution containing the organophosphorus anticholinesterase diisopropylphosphorodiamidic fluoride (Mipafox) in a concentration of  $1 \times 10^{-5}$ .

A 3 cm. segment from the middle region so treated was suspended in a 3 ml. organ bath of Krebs solution aerated with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide and arranged to record longitudinal contractions isotonicly. The lever had a magnification of ten times with a load of about 250 mg.

The bathing fluid was changed by means of a 3-way tap and samples for assay were obtained by removing the entire bath volume in a 5 ml. syringe. Samples were removed after incubating for 5, 10, 20 and 40 min.

The resting release of acetylcholine was assayed by the method described by Birmingham (1961) on the terminal ileum.

## IDENTIFICATION

The spasmogenic substance released was inhibited by hyoscine, destroyed by boiling with normal sodium hydroxide but was not affected by boiling with normal hydrochloric acid. These tests eliminated histamine, 5-hydroxytryptamine and adrenaline as possible agents and it was concluded that the substance was an ester of choline with an order of potency identical to acetylcholine.

The Krebs solution used throughout the experiments contained choline in a concentration of  $1 \times 10^{-6}$  (Bligh, 1952).

## RESULTS

*Increase in Tone*

Throughout the periods of incubation in Krebs solution there was a progressive increase in tone of the ileum which was recorded as a slow contraction. The magnitude of the response depended on the duration of incubation (Fig. 1). The responses were tabulated as per cent of the 40 min. response (Table I). Cooling to 25° and reduction of the calcium

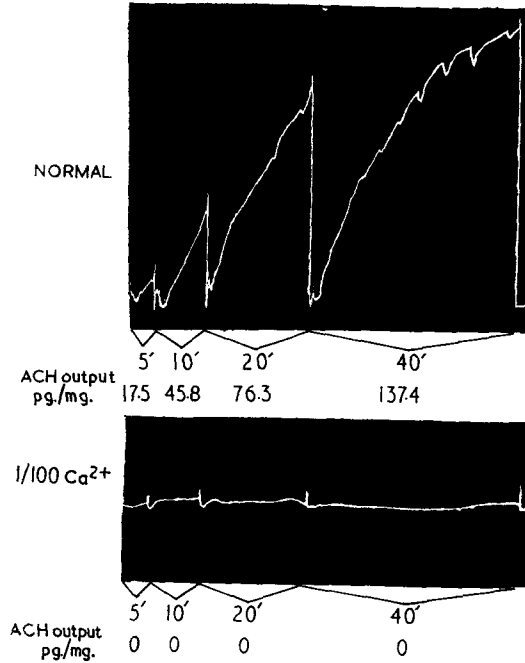


FIG. 1. Upper trace. The relation between increase in tone and acetylcholine output in pg./mg. with time, for the resting ileum in Krebs solution at 37°. Lower trace. The effect of a hundred-fold reduction in the calcium content of the Krebs solution. The reduction in calcium ions inhibits the resting release of acetylcholine, and also prevents the increase in tone.

TABLE I

THE INCREASE IN TONE OF THE GUINEA-PIG ILEUM RECORDED DURING PERIODS OF 5, 10, 20 AND 40 MIN. AS PER CENT OF THE 40 MIN. PERIOD. THE AMOUNT OF ACETYLCHOLINE FOUND BY ASSAY FOR THESE FOUR PERIODS IS GIVEN IN PG./MG. OF WEIGHED WET ILEUM AND IS ALSO CALCULATED AS PER CENT OF THE 40 MIN. PERIOD. EACH RESULT IS A MEAN OF 10 EXPERIMENTS AND ITS STANDARD ERROR

Incubation period, min.	Increase in tone mean $\pm$ S.E. as per cent 40 min. period	Acetylcholine release, pg./mg. $\pm$ S.E.	Acetylcholine release mean $\pm$ S.E. as per cent 40 min. period
5	19.3 $\pm$ 3.7	34.3 $\pm$ 3.3	29.4 $\pm$ 2.8
10	40.1 $\pm$ 3.9	50.6 $\pm$ 4.7	43.4 $\pm$ 4.0
20	70.8 $\pm$ 4.4	70.6 $\pm$ 12.3	60.6 $\pm$ 10.6
40	100	116.5 $\pm$ 23.1	100 $\pm$ 19.8

## RESTING RELEASE OF ACETYLCHOLINE

content of Krebs solution to one hundredth of its normal value abolished the increase in tone.

### *Resting Release of Acetylcholine*

The resting release of acetylcholine per unit weight of intestine was found to be directly proportional to the time of incubation. The results of 10 experiments are given in Table I. Fig. 2 demonstrates the correlation of the resting release of acetylcholine and the increase in tone of the preparation. The graphs are not statistically significantly different. If the experiment is made at 25° or with the calcium content of the Krebs solution reduced to one hundredth of its normal value, the resting release of acetylcholine is reduced to a level below the limit of sensitivity of the assay.

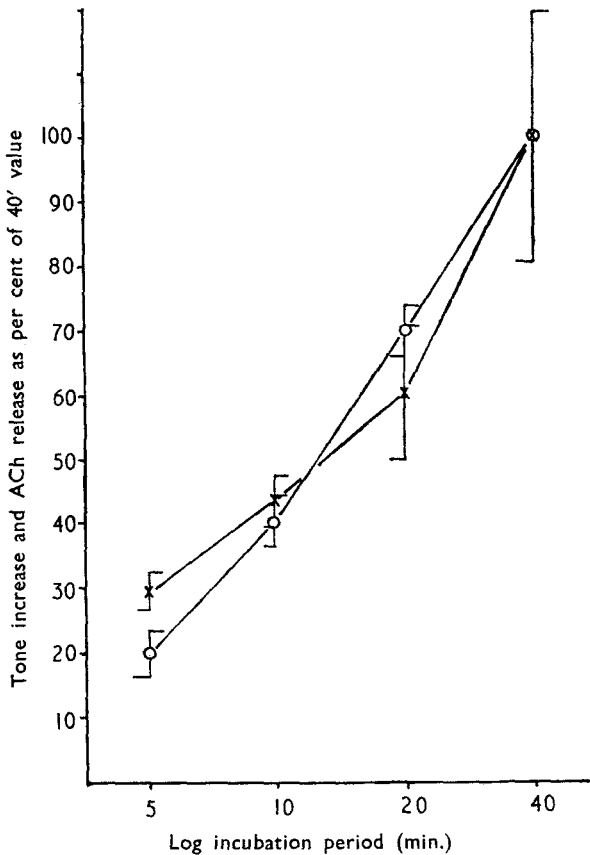


FIG. 2. The relation between the resting release of acetylcholine (x) and the increase in tone (O) for four periods of incubation. Each result is a mean of ten experiments together with the standard error. There appears to be a simple relationship between the acetylcholine release and the increase in tone; both are related to the length of the incubation period.

## DISCUSSION

The property of tone in smooth muscle is ill understood and has been attributed to many factors. The rhythmic activity and tone of the longitudinal muscle of the intestine have been regarded as inherent properties of the muscle fibre on the one hand, and as dependent on the intrinsic innervation on the other hand. It is well known that acetylcholine and choline are liberated continuously from the guinea-pig ileum at rest, but no one has yet successfully related this release with tone.

The origin of the resting release of acetylcholine has been the subject of much discussion; Feldberg and Lin (1949, 1950) favoured the release from non-nervous structures and Schaumann (1957) claimed that it was released mostly from nerve endings.

My results demonstrate that the increase in tone of the resting ileum is directly proportional to the release of acetylcholine. When the intestine is cooled to 25° or its Krebs solution replaced by that containing a reduced calcium content (Fig. 1), there is no increase in tone at rest, and no measurable release of acetylcholine. This means that in experiments made to investigate the resting release of acetylcholine the tone of the ileum can be used to indicate the state of the preparation, an increase in tone signifying an increase in the output of acetylcholine.

Feldberg and Lin (1949) accepted the muscular contractions produced by eserine on the intestine as evidence for the spontaneous release of acetylcholine. Harry (1962) showed that eserine induced contractions of both circular and longitudinal muscle of the guinea-pig ileum, and considered this to be a muscarinic effect. This means that the effects of eserine do not result only from cholinesterase inhibition. The responses in Fig. 1 are slow contractions (unlike those of Feldberg and Lin (1949) which developed after a few seconds) and represent the effects of a gradual increase in the resting output of acetylcholine.

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