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THE SMOOTH MUSCLE CONTRACTING EFFECTS OF VARIOUS SUBSTANCES SUPPOSED TO ACT ON NERVOUS STRUCTURES IN THE INTESTINAL WALL

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Recently, Ambache (1946) has analysed the contractions of the isolated mammalian intestine produced by acetylcholine, histamine, potassium and barium ions. On the basis of his results, he concluded that the contractions produced by small doses of acetylcholine, by potassium ions and by barium ions were not due to a direct effect of these substances on the muscle fibres but to an action on the nervous elements present in the preparations. Small doses of acetylcholine were said to act like nicotine by stimulation of the nerve cells of the plexus of Auerbach, whereas barium and potassium ions were said to act by release of acetylcholine from the nerve fibres of Auerbach's plexus. Histamine was stated to have an action like potassium and barium in addition to a direct smooth muscle stimulating effect. The importance of such a new conception of the pharmacological effects of these substances on the isolated intestinal preparation made it desirable to repeat the experiments. When this was done no conclusive evidence was obtained in favour of the theory that these substances owe their muscle contracting property to an action on nervous structures in the intestinal wall.

The conclusion that small doses of acetylcholine have a nicotine-like action on the intestine was based by Ambache on the observation that the sensitivity of the gut to acetylcholine diminishes greatly after paralysing doses of nicotine. No controls, however, were made to ascertain if this change in sensitivity was specific for acetylcholine. The conclusion that the effects of histamine, potassium and barium ions was 'indirect' was based primarily on experiments with cooled preparations. According to Ambache, cooling the intestinal preparation reduces its sensitivity to histamine and abolishes the response to potassium and barium, whereas it does not affect the sensitivity to substances which stimulate smooth muscle directly. However, his experiments do not provide evidence for the second conclusion since it was not shown that the response to substances stimulating muscle directly remains unaffected by

cooling. The only substance used for this purpose was acetylcholine. A diminished response was obtained with this substance after cooling the intestine, but was attributed to a loss of the assumed nicotine action and not to impairment of the muscle fibre itself.

The hypothesis that acetylcholine is released by histamine, by potassium ions and by barium ions was supported by two additional observations: (1) histamine and barium ions were found to accelerate the synthesis of acetylcholine; and (2) histamine, potassium ions and barium ions were found to act more strongly when given with eserine. On repetition of these experiments, no evidence could be obtained that histamine and barium ions accelerate the synthesis of acetylcholine and the results obtained with eserine, when carried out with necessary controls, were found to supply no evidence in support of Ambache's hypothesis.

In the present experiments the hypothesis of a release of acetylcholine by histamine, potassium and barium was also tested by studying the response to these substances by the muscle when paralysed either by atropine or by benadryl (dimethylaminoethylbenzhydryl ether hydrochloride).

METHODS

The experiments were carried out on the isolated preparation of the guinea-pig's ileum and the rabbit's jejunum suspended in 10 c.c. Mg-free Tyrode's solution. The contractions of the fibres of the longitudinal muscle layer were recorded by a Lovatt Evans frontal writing lever. The bath was emptied by overflow and the substances were added in 0·2–0·4 c.c. volume with a syringe; air was bubbled continuously through the Tyrode solution, the temperature of which was between 30 and 35° C.

The cooled preparations were kept in Mg-free Tyrode's solution at 0–2° C. for varying times. Before use, any secretion accumulated in the lumen was pressed out gently.

Acetylcholine and choline were used as chlorides, histamine as dichloride and pilocarpine as nitrate. The values refer always to the salt. The values for barium chloride refer to $BaCl_2$, $2H_2O$. A sample of ethylal-propanediol-trimethyl ammonium iodide (2268 F) was kindly supplied to us by Sir Henry Dale. This substance acts like muscarine and has no nicotine-like effects on ganglion cells (Fourneau, Bovet, Bovet & Montezin, 1944).

Synthesis of acetylcholine. The method used was essentially the same as that described by Ambache. The small intestine of guinea-pigs was removed, washed with saline solution and slit open longitudinally. It was cut into pieces about 1 cm. long and these were distributed among all the samples. For instance, when six samples were set up a piece of intestine about 6 cm. long was cut into six strips and one placed in each flask; then another piece of the intestine was cut up and the strips distributed similarly amongst the flasks. In this way the whole small intestine was used up and the same procedure was adopted with the intestine of a second and third guinea-pig. The strips of each sample were dried between filter-paper and the total tissue of each sample weighed. The amounts of tissue used for each sample varied between 3 and 3·5 g. They were suspended in 5 or 10 c.c. of saline solution with the following composition: 9·2 g. NaCl, 0·42 g. KCl, 0·24 g. $CaCl_2$, 1 g. dextrose in 1 l. H_2O. Eserine sulphate was added to give a concentration of 1 in 200,000 in the samples. In one experiment (No. 5, Table 2) the solution was buffered with phosphate. The incubation was carried out at 37° C. for 40 min. in either air or oxygen. During this time, the samples were shaken continuously. For the extraction 4 c.c. N/3-HCl were added to each sample which was then ground with sand and boiled for 1 min. The samples were cooled and centrifuged;

the residue was washed twice, and the supernatant with the washings made up so that 10 c.c. corresponded to 1 g. of tissue. The samples could be kept in this condition overnight in the refrigerator. The acetylcholine content of the samples was assayed, after neutralization, on the eserinized frog's rectus muscle against solutions of acetylcholine to which had been added equivalent amounts of the same extract after it had been boiled for a moment in alkaline solution and neutralized. This procedure is necessary to obtain reliable quantitative results (Feldberg, 1945).

RESULTS

The response of the isolated intestine to drugs during nicotine paralysis

When a large dose of nicotine is given, a strong contraction ensues, followed after a few minutes by relaxation, despite the fact that the nicotine remains in the bath. When it is washed out and replaced at once by a fresh dose of

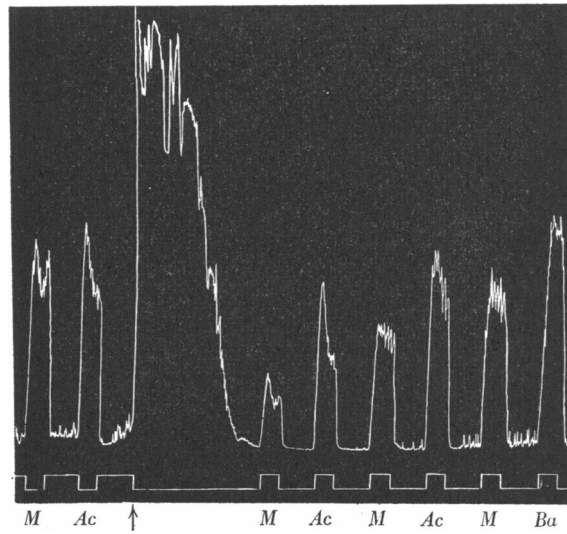


Fig. 1. Contractions of the isolated guinea-pig's intestine in 10 c.c. bath. At *M* 0·025 μg. 2268 F, at *Ac* 0·038 μg. acetylcholine, at *Ba* 1 mg. $BaCl_2$. All substances kept in the bath for 1 min. From the arrow (↑) till the end of experiment the bath fluid contained nicotine tartrate 1 in 50,000.

nicotine, no further contraction occurs, suggesting that the ganglion cells are now paralysed. During the initial phase of the paralysis the response to acetylcholine is sometimes, but by no means always, reduced; but, if so, the responses to other drugs like histamine, pilocarpine or 2268 F are reduced to the same extent. The period of reduced sensitivity is of short duration; within 10–20 min. the preparation gradually regains its original sensitivity to acetylcholine, histamine, pilocarpine and 2268 F, although the nicotine is kept in the bath and the ganglion cells remain paralysed. These results were obtained on the isolated intestine preparation of the rabbit and of the guinea-pig. Fig. 1 is from an experiment on the guinea-pig's intestine and illustrates (*a*) an initial phase of reduced sensitivity after paralysis with nicotine, but for 2268 F as well as for acetycholine; and (*b*) the gradual recovery of the

original sensitivity to both substances, although the ganglion cells remain paralysed. Hence there is no evidence that small doses of acetylcholine owe their stimulating effect on the intestine to an action on ganglion cells. The experiment shown in Fig. 1 also illustrates that nicotine paralysis does not abolish the stimulating action of $BaCl_2$ on the intestine.

Changes in the sensitivity to drugs after cooling the intestine

We have not been able to observe that cooling the rabbit's intestine affects the response to various drugs differently but have found that differences may be detected, although by no means regularly, on the cooled guinea-pig's intestine.

Rabbit's intestine

The cooled preparation, when suspended in the bath, started to contract gradually after an initial period of relaxation. During the initial relaxation, which lasted between 30 and 60 min., the muscle was often insensitive to any drug examined, at least when the cooling had been carried out for several days.

When the contraction started to develop, the muscle became sensitive to the various substances, but there was no clear or regular difference in the degree of the response of the muscle to the various substances. Sometimes it appeared as if the sensitivity of the muscle had been reduced to a greater extent for one substance, sometimes for another. A typical experiment showing such irregularities is given in Fig. 2. Tracing *a* shows the contractions produced on the fresh preparation by 0·1μg. of acetylcholine, 1 mg. of $BaCl_2$, 300μg. of histamine, 2·5μg. of pilocarpine and 10 mg. of KCl. The tracing *b* is taken from an adjacent piece of intestine which had been cooled for 4 hr. From the responses obtained during the initial period of relaxation it would appear that 4 hr. of cooling had affected the sensitivity to acetylcholine and to pilocarpine more than that to $BaCl_2$. When KCl was tested, the muscle had started with its gradual contraction; KCl had practically no effect. It might, therefore, appear as if there were a fundamental difference between the actions of $BaCl_2$ and of KCl, but a different impression was gained when these drugs were retested a little later. Between the two parts of *b* there was an interval of 15 min. during which the muscle contracted to a new level. Now all five substances produced small contractions. Tracing *c* is taken from another piece of the same intestine after 5 days of cooling and after 2½ hr. suspension in the bath. During the initial period of relaxation none of the five substances had caused contraction. Later, when the muscle had partly contracted, it responded again to the different substances but the effect was small as compared with the original effect of these substances. There was practically no difference in the response to 0·1μg. of acetylcholine, 1 mg. $BaCl_2$, 10 mg. KCl and 400μg. of histamine; the sensitivity of the muscle to these substances had been reduced to practically the same extent. Pilocarpine alone was wholly inactive in the original dose (2·5μg.); it caused a small contraction only when four times the

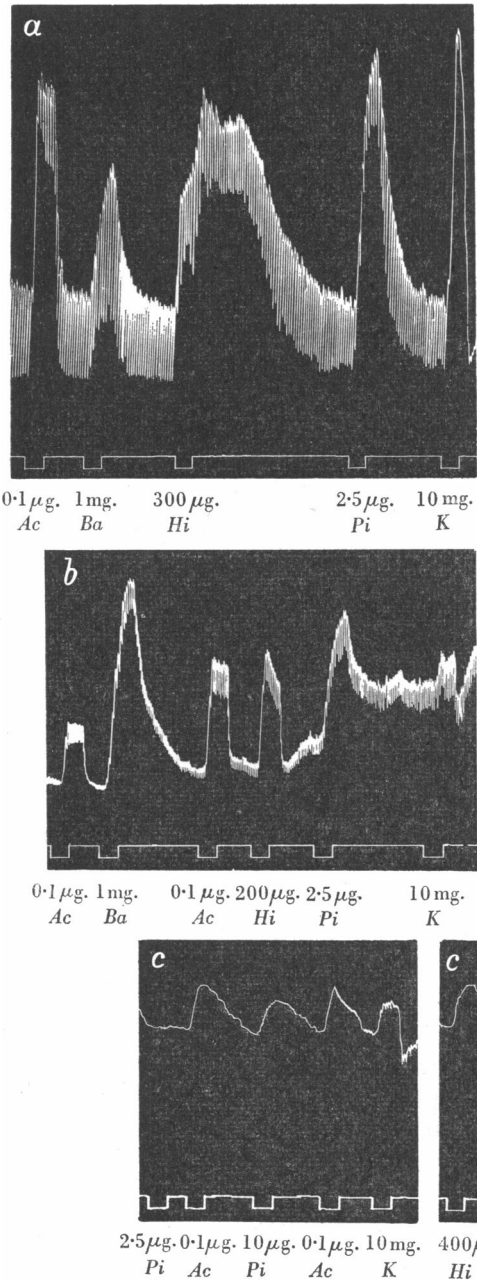


Fig. 2. Effect of cooling rabbit's intestine, suspended in 10 c.c. bath, on sensitivity to acetylcholine (*Ac*), $BaCl_2$ (*Ba*), histamine (*Hi*), pilocarpine (*Pi*) and KCl (*K*). (*a*) fresh preparation; (*b*) after 4 hr. cooling; (*c*) after 5 days' cooling. The distance between the base line and the record from the intestine gives the degree of contraction of the muscle. (For details see text.)

amount was given. This result was not observed in all experiments. After 7 days of cooling the intestine had lost its ability to contract to any of the five substances even when they were given in larger doses and after a long period of suspension in the warm bath.

Guinea-pig's intestine

Unlike the rabbit's intestine preparation, that of the guinea-pig only rarely showed a spontaneous gradual increase in tone when examined after cooling. There was, however, an initial phase, lasting 20–60 min., in which the suspended preparation was more or less insensitive to drugs. Therefore, tests had to be carried out after this initial phase had passed. A quantitative comparison of the effect of cooling on the sensitivity of the muscle to various drugs was found to be extremely difficult and sometimes even impossible on account of the following facts:

(*a*) The sensitivity to a drug like acetylcholine varies even on the same fresh preparation; first it increases and later it decreases, but these changes are by no means regularly obtained and vary in degree and time sequence from one experiment to another.

(*b*) The sensitivity of the fresh muscle varies differently in the course of the experiment with different drugs so that a certain dose of one drug may have at one time a stronger, at another a weaker action than a specified dose of another drug.

(*c*) When the amount of a drug tested is doubled, the increase in the response varies with different drugs. This is shown for KCl and acetylcholine at the beginning of the tracing in Fig. 9*a*.

(*d*) Although it is possible to obtain comparable contractions with repeated administration of the same dose of acetylcholine and histamine, apart from the gradual changes in sensitivity of the muscle, other substances like barium and potassium may give greatly different responses when given repeatedly. A particularly striking instance is shown in the case of barium in Fig. 7*a*. $BaCl_2$ was given fourteen times in 2 mg. doses, only every third response being reproduced in the figure. In Fig. 6 are shown several different responses to 10 mg. of KCl, and in the experiment of Fig. 9, 5 mg. of KCl had no effect at the beginning of the experiment, but produced strong contractions of the muscle at the end. Such great differences in the response to KCl may occur even when it is given in succession at 4–5 min. interval.

Despite these difficulties, the response to barium and potassium was found in some experiments to be affected to a greater extent by cooling than that to acetylcholine, histamine and pilocarpine. The experiment of Fig. 3 is given as an illustration, but it must be emphasized that the responses of the cooled muscle varied greatly from experiment to experiment. In detail the results for the different substances were as follows.

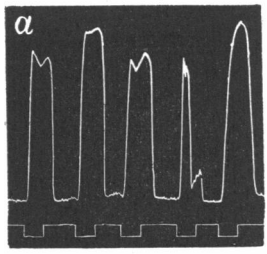
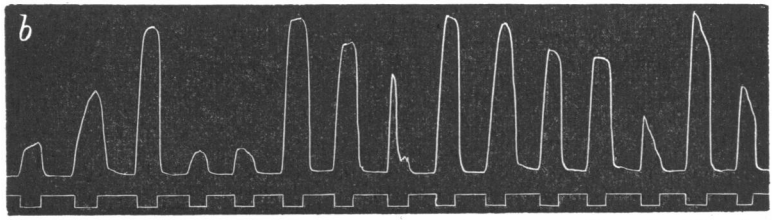
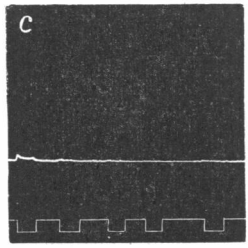
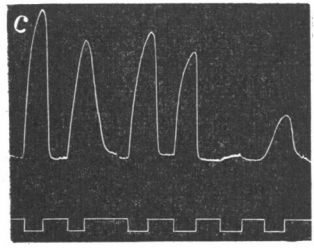
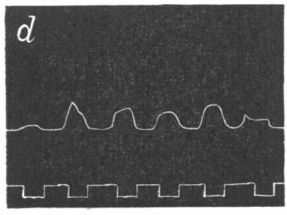


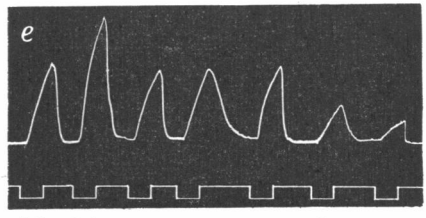
Fig. 3. Effect of cooling the guinea-pig's intestine, in 10 c.c. bath, on sensitivity to histamine (*Hi*), acetylcholine (*Ac*), $BaCl_2$ (*Ba*), KCl (*K*) and pilocarpine (*Pi*). (*a*) fresh preparation; (*b*) adjacent piece after 4 hr. cooling and suspension for 35 min.; (*c*) same piece as in (*b*) after 3 days' cooling and suspension for 20 min.; between the first and second part of (*c*) an interval of 70 min.; (*d*) third piece of same intestine after 5 days' cooling and suspension for 100 min.; (*e*) same piece as in (*a*) after 6 days' cooling and suspension for 60 min. (For details see text.)











Acetylcholine. Table 1 illustrates the fact that cooling for 24–48 hr. may depress the sensitivity of the muscle to acetylcholine. It gives the results from two experiments. A number of contractions were obtained with 0·1 μg. of acetylcholine, first on the fresh, and later on the cooled preparation. The

TABLE 1. Effect of cooling the guinea-pig's intestine on the sensitivity of the muscle to acetylcholine and KCl

Days of cooling	Percentage of original response to 0·1 μg. acetylcholine (*Ac*) and to 10 mg. KCl			
	Exp. 1		Exp. 2	
	(*Ac*)	(KCl)	(*Ac*)	(KCl)
0	100	100	100	100
1	68	34	—	—
2	8	12	70	24
4	—	—	4	4

average height of the recorded responses was measured and expressed as the percentage of the response obtained on the fresh preparation. The contractions on the cooled preparation were obtained after it had been suspended in the warm bath for at least 30 min. It will be seen that even 24 hr. of cooling may depress the sensitivity of the muscle to acetylcholine. When the dose of acetylcholine was increased it was possible to obtain strong contractions even from the depressed cooled preparation. These results agree with those reported by Ambache, since he used much greater doses of acetylcholine (increases up to 100 times) on cooled than on the fresh preparations. In the experiment of Fig. 3, this depression of the acetylcholine response was only slight even after 3 days of cooling; it became pronounced after 5–6 days' cooling, and after 7 days' cooling the muscle had become insensitive to acetylcholine.

Histamine. As long as the muscle was sensitive to acetylcholine it also responded to small doses of histamine. Sometimes the cooled preparation responded less readily to histamine, sometimes to acetylcholine, and on the same preparation, first the response to one, and later that to the other, might appear to be the more depressed. In general, however, the muscle, on prolonged cooling, became simultaneously less sensitive and finally insensitive to both drugs. This is seen in Fig. 3. The responses to both drugs are reduced only slightly after 3, but greatly after 5–6 days' cooling. After 4 hr. cooling, the response to the first dose of histamine (Fig. 3*b*) is much more reduced than that to acetylcholine given a few minutes later. This result could easily be taken as evidence for a more pronounced effect of cooling on the response to histamine than to acetylcholine. It is explained, however, by the fact that the muscle was still recovering from its initial phase of insensitivity to drugs, when the record was started. The difference, in fact, disappeared when the two drugs were retested later on in the experiment.

Pilocarpine usually resembled histamine and acetylcholine in the way its action was affected by cooling the muscle. In some experiments, however, the response to pilocarpine was reduced to a much greater extent, and this result was not dependent on the time for which the muscle had been cooled. For instance, in the experiment of Fig. 3 the response to 0·2μg. of acetylcholine and to 10μg. of pilocarpine were comparable on the fresh preparation and after 4 hr. cooling; after 3 days' cooling the effect of 10μg. of pilocarpine was slightly less than that of 0·2μg. of acetylcholine. After 5 days' cooling, 100μg., and after 6 days' cooling only 30μg., were needed to produce the same small effect as 0·2μg. of acetylcholine.

Potassium. The results were not uniform. In some preparations potassium behaved like acetylcholine. The response of the muscle to both substances became gradually smaller on prolonged cooling. This is illustrated in Table 1. It is true that the sensitivity to KCl decreased to a greater extent than that to acetylcholine. Since the concentration gradient of the two substances when given in increasing doses on the fresh preparation is different, such a result is not surprising. The experiment of Fig. 3, on the other hand, illustrates a result in some ways resembling those obtained by Ambache. Cooling for 4 hr. seemed to have depressed the response to 5 mg. of potassium to a much greater extent than that to 0·2μg. of acetylcholine. When the dose of potassium was increased to 7·5 mg. a greater response was obtained than with 0·2μg. acetylcholine. In addition, it was found, in this as well as in many other experiments, that the sensitivity to potassium could be partly restored when it was given in successive doses (see end of tracing *b*). After 3 days' cooling 15 mg., and after 5–6 days' cooling 20 mg. KCl were required to match approximately the response to 0·2μg. of acetylcholine. The response to acetylcholine, however, is also greatly reduced and it must be emphasized that the reduction in the response to KCl was always associated with a general reduction in the sensitivity of the muscle to all drugs examined.

Barium. There is no doubt that cooling affects the response of the muscle to $BaCl_2$ more than that to any other substance so far examined. This is evident from the experiment of Fig. 3. On the cooled preparation a ten-fold increase or more in the dose of $BaCl_2$ only produced a small contraction, but again it will be seen that the effect of cooling on the response to $BaCl_2$ proceeds gradually. In addition it must be stated that, even on the fresh preparation, the effects of very small doses of barium cannot be obtained with regularity when it is given in successive doses.

Eserine

According to Ambache, eserine potentiates the effect, on the isolated fresh intestine, of histamine, KCl and $BaCl_2$. These results were taken as further proof for the view that the three substances release acetylcholine. His experimental procedure, which was imitated closely in the present experiments, was

as follows. Eserine, in a concentration of about 1 in 3·5 millions, was added to the bath and followed usually, after 10–15 sec., by either histamine, $BaCl_2$ or KCl; eserine and the substance to be tested were then kept in the bath for as long as 2 min.

We can confirm Ambache's observation that under these conditions the substances he has examined act more powerfully, but this 'potentiation' by eserine occurred with all muscle-stimulating substances and is no evidence for the theory that they cause a release of acetylcholine.

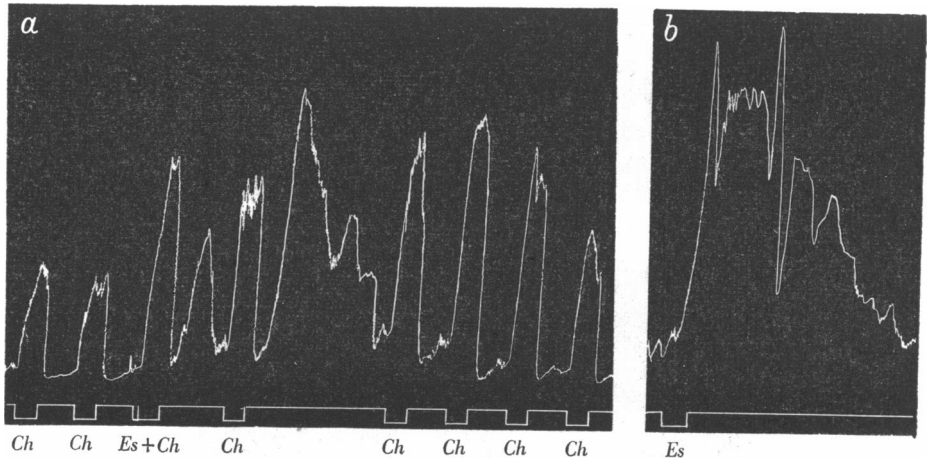


Fig. 4. Contractions of the isolated guinea-pig's intestine in 10 c.c. bath. At (*Ch*) 0·3 mg. choline kept in bath for 1 min.; 15 sec. before the third choline dose 2·5μg. eserine sulphate (*Es*) added to bath and washed out after 75 sec. with the choline. Record (*b*) effect of 2·5μg. eserine added alone and kept in bath for 75 sec. (For details see text.)

When eserine 1 in 4–5 millions, which is even weaker than the concentration used by Ambache, is added to the bath and kept there for 75 sec. there ensues, usually after a latency of 20–40 sec., a gradually developing contraction of the guinea-pig's intestine which may last for several minutes (see Fig. 4*b* and Fig. 5*c*). When a muscle-stimulating substance is added to the bath 15 sec. after the eserine, its effect therefore appears to be much greater. This is illustrated for choline in Fig. 4*a*. If the tracing had been reproduced in such a way as to give the immediate effect of choline only, the tracing would resemble those obtained by Ambache for histamine, barium and potassium. In some experiments the motor activity of the eserine is so pronounced within the first 75 sec., that there is no immediate relaxation when the eserine and the respective muscle-stimulating substance are washed out. This is illustrated for the muscarine-like substance 2268 F in Fig. 5*a*, *b*. The so-called 'sensitization' by eserine thus results from the fact that the eserine causes accumulation of acetylcholine, the effect of which is added to that of the respective muscle-

stimulating substance. In Ambache's experiments themselves, there is evidence that the accumulation of acetylcholine by eserine was so great as to produce, by itself, contraction of the intestine. He reproduces three figures (Figs. 8, 11, 16) in which the muscle did not relax completely after the eserine and the respective substance had been washed out.

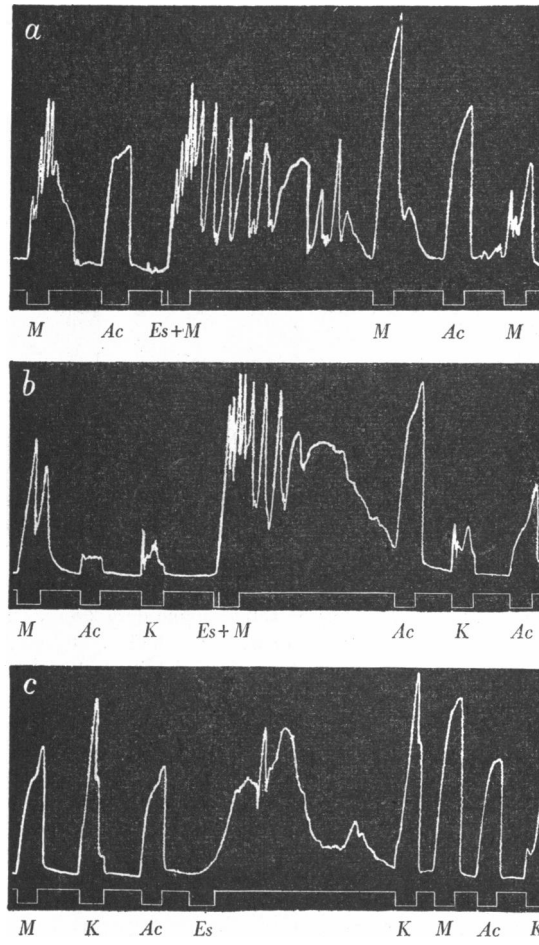


Fig. 5. Contractions of the isolated guinea-pig's intestine in 10 c.c. bath. At *Ac* 0·1 μg. acetylcholine, at *M* 0·1 μg. 2268 F, at *K* 5 mg. KCl kept in bath for 1 min. At *Es* 2 μg. eserine sulphate kept in the bath for 75 sec. but followed, in tracing (*a*) and (*b*), after the first 15 sec. by 0·1 μg. 2268 F. (For details see text.)

The effects of various substances have been examined also after the eserine contraction had more or less subsided, and in this condition it was again found that all the muscle-stimulating substances tested produce an increased response which was followed by a period of depression. This is shown in Fig. 5

for 2268 F, acetylcholine and potassium. The 'after sensitization' is almost equally strong with all substances so far tested. Such sensitizations can be explained on lines similar to those suggested for the initial sensitization. The action of eserine has not completely worn off; some accumulation of acetylcholine is still going on, at a rate insufficient to stimulate the muscle. When a muscle-stimulating substance is given during this period, the subthreshold amounts of acetylcholine are sufficient to augment its stimulating effect.

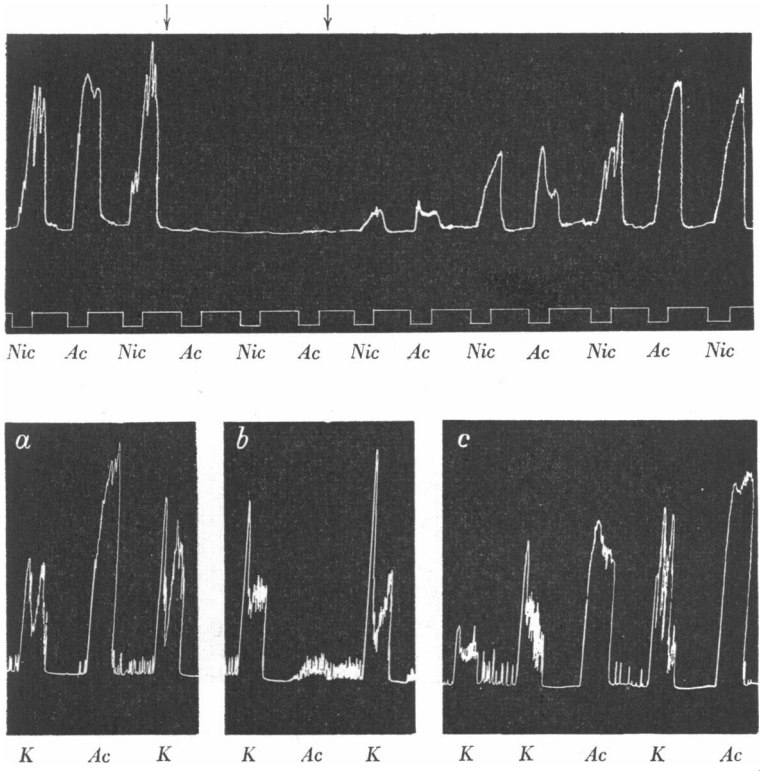


Fig. 6. Contractions of the isolated guinea-pig's intestine in 10 c.c. bath; upper and lower tracing from different intestines. Effect of atropine, 1 in 200 millions on nicotine and potassium contractions. At *Ac* acetylcholine (0·1 μg. in upper and 0·2 μg. in lower tracing); at *Nic* 200 μg. nicotine, at *K* 10 mg. KCl. In upper record the atropine was kept in the bath between the two arrows and in the lower record during (*b*). (For details see text.)

Atropine

Atropine was tested in concentrations just low enough to abolish completely the stimulating effects of small doses of acetylcholine on the intestine. The use of greater concentrations of atropine has been avoided because they render the muscle insensitive to nearly all drugs examined. For instance, Feldberg (1931), found that atropine in concentrations sufficient to abolish an acetylcholine contraction of the guinea-pig's intestine depressed slightly an equally strong

histamine contraction; with higher concentrations of atropine the histamine effect too was abolished (see also Bernheim, 1931).

It might be argued that low concentrations of atropine can abolish the effect of the acetylcholine applied to the outside of the muscle, but not that of the acetylcholine released by the action of drugs from the nervous structures

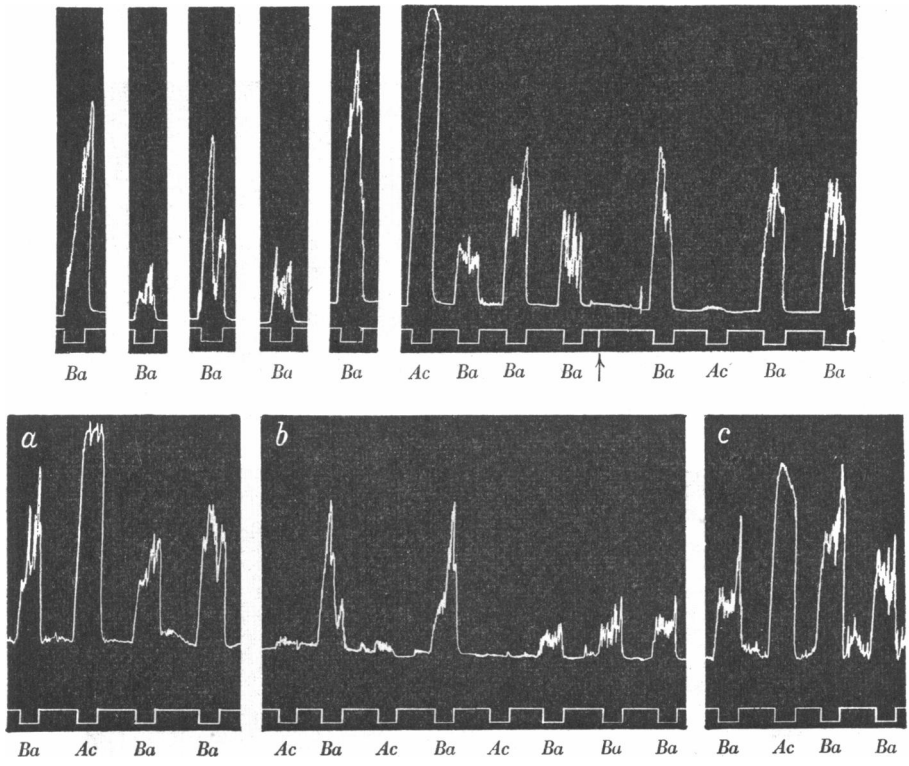


Fig. 7. Contractions of the isolated guinea-pig's intestine in 10 c.c. bath; upper and lower tracing from different intestines. Effect of atropine, 1 in 200 millions, on $BaCl_2$ contractions. At *Ba*, $BaCl_2$ (2 mg. upper and 0·8 mg. lower tracing). At *Ac*, 0·2μg. acetylcholine. Atropine given in upper tracing at the arrow (↑) and kept in the bath till end of record and in lower tracing during (*b*). (For details see text.)

present in the intestinal wall. This argument does not hold good. For instance, in the experiment of Fig. 6*a*, atropine, 1 in 200 millions, abolished the equally strong contractions produced by 0·1μg. of acetylcholine and by 20μg. of nicotine; when the atropine was washed out the sensitivity of the muscle to both drugs returned simultaneously. Thus atropine has an identical quantitative effect on the response to acetylcholine, released by the stimulating action of nicotine on the nerve cells in the intestinal wall, and on the response to acetylcholine added to the bath and acting from the outside of the muscle.

Unlike the effect of nicotine, that of potassium is usually resistant to small doses of atropine. In the experiments of Fig. 6*b* atropine, 1 in 200 millions, had no action on the potassium contraction. In fact, the smallest effect with potassium was obtained several minutes after the atropine had been washed out and the muscle had nearly recovered its original sensitivity to acetylcholine. In some experiments the effect of potassium was not maintained and was reduced after atropine, but, since these changes were not obtained regularly, were not always reversible after washing out the atropine and, in addition, occurred sometimes spontaneously, it is difficult to relate them to the atropine. These results are in agreement with earlier statements on the atropine-resistent action of potassium. They do not support the view that potassium owes its stimulating action on the intestine to the release of acetylcholine.

The great variations in the response of the intestine to repeated administration of barium made it difficult to obtain reliable and regular results. In some experiments, such as the one illustrated in Fig. 7*a*, the barium contractions were not affected by the atropinization; in others, such as the one illustrated in Fig. 7*b*, the barium contractions were definitely reduced by the atropine, and, when it was washed out, the muscle regained its sensitivity to barium and to acetylcholine simultaneously. In other experiments, the barium contractions were reduced during atropinization, but not restored when the atropine had been washed out and the muscle had again become sensitive to acetylcholine. There was no parallelism between the effect of atropine on the response to acetylcholine or nicotine, on the one hand, and to barium, on the other. It is not even certain that the irregular depression of the barium response during atropinization must be regarded as an effect of atropine.

Benadryl

Benadryl in low concentrations abolishes the effect of histamine. It is known, and could be confirmed, that such concentrations do not affect the response to acetylcholine. For instance, in the experiment of Fig. 8, 0·15μg. of histamine gave a stronger contraction of the guinea-pig's ileum than 0·1μg. of acetylcholine. Benadryl, 1 in 125 millions, practically abolished the response to histamine, but had no effect on the response to acetylcholine. If histamine were to act partly by the release of acetylcholine this result would be difficult to understand. It would then be necessary to assume that the mechanism of the release of acetylcholine by histamine were affected by benadryl. There is no justification for such an assumption, for the benadryl had no effect on the response to 20μg. of nicotine, the muscle-contracting effect of which equalled that of 0·15μg. of histamine. Since nicotine acts by stimulating the ganglion cells, and thereby releasing acetylcholine, this result shows that the mechanism of the release of acetylcholine is not affected by benadryl, at least not in the concentration used in this experiment.

According to Loew, McMillan & Kaiser (1946), benadryl also inhibits the action of acetylcholine and of barium on the guinea-pig's intestine, but the concentration required for acetylcholine was 1 in 2·5–4 millions, that for barium about 1 in 100,000. In our own experience a concentration of benadryl which inhibited the response to a small dose of acetylcholine also depressed that to a small dose of barium. For instance, in the experiment of Fig. 9, benadryl, 1 in 1 million, abolished the contraction produced by 0·15μg. of acetylcholine and nearly abolished the equally strong one produced by 1 mg. of barium chloride; 2 mg. of barium chloride, however, produced a much stronger contraction than even 0·6μg. of acetylcholine. It is possible that when large doses of either barium or acetylcholine are used, a much stronger concentration of benadryl is required for antagonizing the effect of barium than that of acetylcholine, but it appears that there is no great difference in the antagonizing action which benadryl exerts on the responses to small doses of acetylcholine and barium respectively on the guinea-pig's intestine.

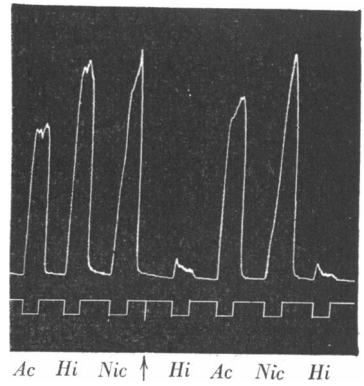


Fig. 8. Contractions of isolated guinea-pig's intestine in 10 c.c. bath. Effect of benadryl, 1 in 125 millions (from arrow till end of tracing), on the contractions produced by 0·1μg. acetylcholine (at *Ac*), 0·15μg. of histamine (at *Hi*) and of 20μg. of nicotine (at *Nic*).

Fig. 9 also illustrates the fact that benadryl, 1 in 1 million, antagonizes the action of potassium. Instead of the strong contraction produced by 10 mg. of KCl at the beginning of the experiment there was only a relatively small effect, and the contraction was not maintained. Doubling the dose of potassium did not increase the response, but it was interesting that 5 mg. of KCl, which had had only a slight effect when given before the benadryl, was not wholly ineffective when added to the bath containing the benadryl. Such results illustrate the difficulty of an exact quantitative comparison of the action of benadryl on the responses to different substances. It seems certain, however, that benadryl is a more powerful antagonist of acetylcholine than of potassium. This difference becomes particularly clear after the benadryl had been washed out. During the ensuing period of gradual recovery (Fig. 9*c*) the sensitivity of the muscle to potassium returned earlier than that to acetylcholine. At a time when the response to acetylcholine was still reduced, the muscle was found to be more sensitive to potassium than it had originally been, and to contract strongly to as little as 5 mg. of KCl. Fig. 9 illustrates further that nicotine, unlike potassium and barium, was affected by benadryl to approximately the same extent as was acetylcholine. The experiments with benadryl, therefore, do not support the view that the contractions produced by potassium and barium result from a release of acetylcholine.

Synthesis of acetylcholine in strips of the guinea-pig's intestine

According to Ambache, histamine and $BaCl_2$ accelerate the synthesis of acetylcholine which occurs in strips of the guinea-pig's small intestine incubated

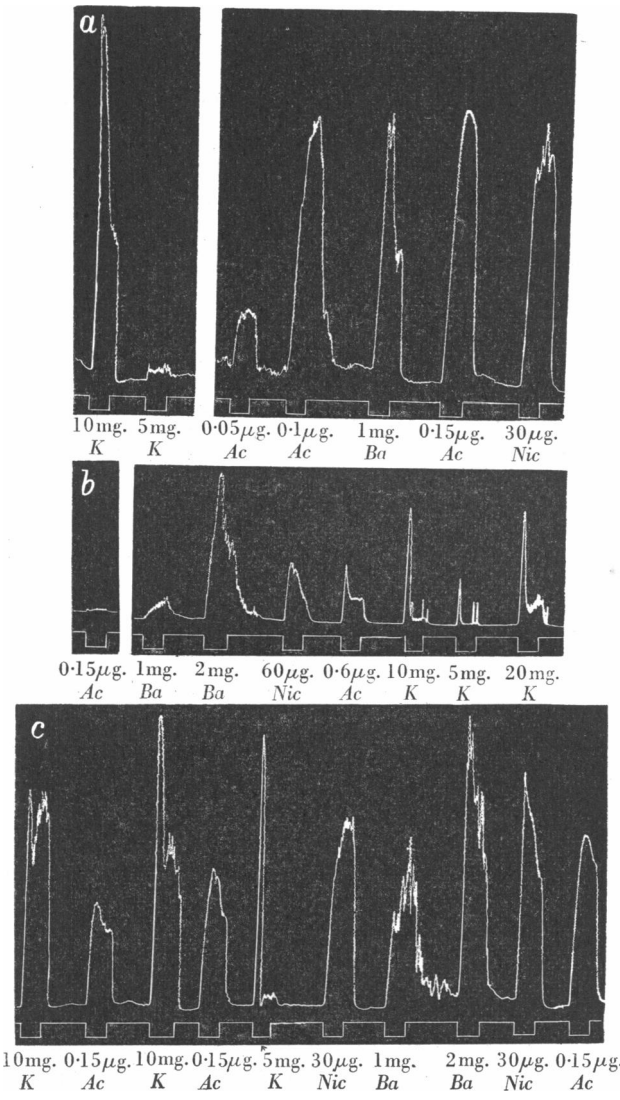


Fig. 9. Contractions of isolated guinea-pig's intestine in 10 c.c. bath. Contractions produced by acetylcholine (at *Ac*), KCl (at *K*), $BaCl_2$ (at *Ba*) and nicotine (at *Nic*) before (*a*), during (*b*) and after (*c*) benadryl, 1 in 1 million. (For details see text.)

in bicarbonate-free Tyrode's solution. Using similar conditions we have been unable to confirm his results (Table 2). In each experiment three control

samples were set up. One was extracted without incubation to give the acetylcholine content of the intestinal strips before incubation, and two were incubated without either the addition of histamine or barium chloride. From the acetylcholine content of these samples it can be seen (*a*) that synthesis of acetylcholine occurs in the intestinal strips during incubation, and (*b*) that the amounts synthesized vary in individual samples. For instance in Exp. 4, synthesis of acetylcholine during the 40 min. of incubation, amounted in the one sample to 1·4, but in the other to 5·2 μg./g. On the other hand, the addition of either histamine or barium chloride did not increase the yield of acetylcholine on incubation. The $BaCl_2$ was added either every 10 min. in amounts of 1 mg./c.c. (col. 6) or once at the beginning of incubation in amounts of 4 mg./c.c. (Exp. 3, col. 7) or of 8 mg./c.c. (Exp. 5, col. 7). Histamine was added every 5 min. in amounts of 0·2 or 1 μg./c.c. Similar amounts were used by Ambache in his experiments.

TABLE 2. Effect of histamine, potassium and barium ions on synthesis of acetylcholine in intestinal strips

	μg. acetylcholine per g. tissue								
				Incubated with					
								Histamine	
	Not incubated	Incubated controls		3 mg./c.c. KCl	5 mg./c.c. KCl	$BaCl_2$		0·2 μg./c.c.	1 μg./c.c.
Exp.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1	6·0	8·5	9·0	10·3	—	8·6	—	8·0	—
2	6·0	8·1	8·7	8·8	—	8·7	—	8·3	—
3	7·0	8·8	8·7	12·0	—	5·0	12·4	12·4	7·4
4	7·4	8·8	12·6	9·9	11·0	—	—	—	—
5	9·0	9·9	11·1	12·5	12·0	13·0	12·9	—	—

The effect of potassium ions on the synthesis of acetylcholine was also examined, since they are known to have an accelerating effect in slices or brei of brain tissue. It was found, however, that potassium was ineffective or accelerated the synthesis in one out of five experiments only. We must therefore conclude that the spontaneous synthesis of acetylcholine which occurs in incubated intestinal strips cannot be accelerated by changes in the incubation medium which might have an effect on tissue slices or brei. The difference between our results and those of Ambache, may be explained by the fact that he used only one control sample in each of the few experiments carried out at all, and that the effects of histamine and barium which he observed are accounted for by individual variations in the spontaneous synthesis.

DISCUSSION

The results of the present experiments do not support the conception that small doses of acetylcholine owe their stimulating effect on the small intestine to a nicotine-like action on the nerve cells in the intestinal wall, as stated by

Ambache. We could not confirm his results on which this statement is based, that the sensitivity of the intestine to acetylcholine is reduced during nicotine paralysis. It is true that the strong contractions produced by large doses of nicotine are sometimes followed by a period of reduced sensitivity of the muscle to acetylcholine, but this period is of short duration and the muscle kept paralysed by nicotine regains its original sensitivity to acetylcholine. In addition, during the period of reduced sensitivity to acetylcholine, the muscle is also less sensitive to other muscle-stimulating substances. The temporary depression is not a specific after-effect of a nicotine contraction but, according to Cantoni & Eastman (1946) follows any maximal contraction, except that produced by KCl. Ambache came to his conclusion because he failed to carry out two necessary controls: (*a*) testing drugs other than acetylcholine during nicotine paralysis, and (*b*) keeping the muscle paralysed long enough for the immediate after-effect of the strong nicotine stimulation to wear off.

Our results again do not support the view that the stimulating action on the intestine of barium and of potassium is due to a release of acetylcholine from nerve fibres originating from the plexus of Auerbach, and that this mechanism accounts for part of the stimulating effect of histamine. Ambache based his conclusions mainly on the assumption that cooling the intestine preparation for a few days inactivates the nervous structures in the intestinal wall, without impairing the sensitivity of the muscle fibre itself. Cooling the intestine for such periods, however, was found to reduce the sensitivity to direct muscle-stimulating drugs, and there is no significant difference between the reduction in sensitivity to either acetylcholine, pilocarpine or histamine. On the guinea-pig's intestine, but not on that of the rabbit, some evidence was found that cooling had a stronger effect on the response to potassium, and particularly to barium, than to the other drugs examined. It is difficult at present to explain this difference satisfactorily. The following facts, however, have to be taken into account. Since the dose-response curve varies with different substances it is difficult to compare quantitatively the effect of cooling on the response to various drugs. In addition, as far as KCl and particularly $BaCl_2$ are concerned, comparable responses are difficult to obtain even on the fresh preparation with repeated administration of the same dose of either. Nevertheless, we could confirm Ambache's observation that a condition of the cooled muscle can be obtained in which it is practically insensitive to large doses of $BaCl_2$, but responds to acetylcholine, at least when added in large doses. In this way $BaCl_2$ resembled nicotine to some extent, but no evidence could be obtained that $BaCl_2$ acted on the nerve cells in the intestinal wall. During nicotine paralysis, the preparation responded well to $BaCl_2$. This result in itself does not exclude a ganglionic action of $BaCl_2$ since it is known that nerve cells may be paralysed specifically to one but not to another drug. For instance, after curare the sympathetic ganglion cells become insensitive to acetylcholine, but

not to potassium (Brown & Feldberg, 1936*a*). If $BaCl_2$ were to act, however, like nicotine by stimulating the nerve cells in the intestinal wall, the effect, like that of nicotine, should be abolished by atropine. Atropine had no regular action of this kind.

The conclusion that $BaCl_2$—and the same applies to KCl and histamine—acts on nervous structures in the intestinal wall was based in addition on results obtained with eserine and on experiments concerning the synthesis of acetylcholine. Ambache had observed that eserine sensitizes the response to histamine, KCl and $BaCl_2$. It could be shown, however, that in the conditions of his experiments eserine had a similar action on the response to other drugs such as choline, 2268 F or acetylcholine. Ambache had omitted to show that the sensitization was specific. The effect of eserine can easily be explained as follows. Eserine, by its cholinesterase-inhibiting effect, leads to accumulation of acetylcholine, the effect of which sums with that of the substance to be tested on the intestine. In fact, under the conditions of Ambache's experiments, the accumulation of acetylcholine was so great as to produce contraction of the muscle by itself, so that the increased responses which he obtained with histamine, $BaCl_2$ and KCl was a combined effect of these substances with the accumulated acetylcholine. Even when the accumulation of acetylcholine is proceeding at a rate insufficient to stimulate the muscle by itself, it can still augment the response to any other stimulating drug. This condition was obtained in the present experiments when the effect of eserine was wearing off. The so-called eserine sensitization obtained by Ambache does not, therefore, supply evidence for an indirect mechanism of drug action. Nor could any evidence be obtained in favour of his theory by examining the responses to the various substances on the intestine paralysed by small doses of atropine or benadryl. The use of benadryl, in fact, enabled us to exclude conclusively the theory that a release of acetylcholine participates in the muscle-contracting effect of histamine, because with benadryl it is possible to abolish the response of the intestine to histamine without impairing that to acetylcholine, when it is either added to the bath or released from nervous structures in the intestine.

No evidence of an increased yield of acetylcholine on incubation of intestinal strips with either $BaCl_2$ or histamine could be found in our experiments. These results are contrary to those of Ambache who, however, carried out only a few experiments and did not make allowances for the individual variations which may occur in different samples of intestinal strips. His method of incubating relatively large pieces of intact intestinal tissue seems in fact unsuitable for studying the effect of substances, added to the incubation medium, on the synthesis of acetylcholine which occurs within the tissue. Even potassium, which is known to have an accelerating effect on the synthesis of acetylcholine, only occasionally affected the synthesis under these circumstances. It is possible that the substances added to the incubation medium are unable to

penetrate to the site of synthesis, when it occurs in pieces of intact intestinal tissue.

Therefore, the evidence given by Ambache for an indirect mechanism of action on the intestine of histamine, $BaCl_2$ and KCl cannot be accepted. This theory can certainly no longer be accepted for histamine. In the case of KCl, it is known that it can release and accelerate the synthesis of acetylcholine, but as far as its effects on structures other than the intestine are concerned, they are independent of the release of acetylcholine. It has been shown (Brown & Feldberg, 1936*b*), for instance, that the stimulating effect of KCl on sympathetic ganglia is independent of the acetylcholine metabolism, because it occurs also on the denervated ganglion which contains no acetylcholine. There is at present no evidence that a release of acetylcholine by KCl contributes to its muscle-stimulating effect on the intestine; if such a mechanism were involved in the muscular contraction observed after KCl its contribution could be small only, and it would be difficult to detect it without being able to abolish the direct stimulating action of KCl.

The response to $BaCl_2$ was certainly affected by cooling the guinea-pig's intestine to a greater extent than that to other drugs. This discrepancy between $BaCl_2$ and other drugs, however, was not observed on the cooled rabbit's intestine. It is unlikely that the mode of action of $BaCl_2$ is fundamentally different in the two preparations. Before the theory that $BaCl_2$ owes its stimulating action on the gut to the release of acetylcholine can be accepted it is necessary to show that it can in fact release acetylcholine from perfused tissues containing cholinergic nerve fibres. At the present stage of our knowledge we are not justified in attributing such an effect to $BaCl_2$, and it seems rather dangerous to interpret the changes which occur in the sensitivity of the intestine to drugs after cooling solely to inactivation of nervous elements, since cooling impairs the muscular tissue as well. Similarly, in our opinion, it is not possible to postulate a nervous origin for the rhythmic contractions of the longitudinal muscle of the intestine simply because they disappear in the cooled preparation.

SUMMARY

1. No evidence could be obtained in support of the theory that small doses of acetylcholine owe their muscle-contracting property on the isolated intestinal preparation to a nicotine-like effect on ganglion cells. Paralysing doses of nicotine were not found to affect the sensitivity of the preparation to small doses of acetylcholine, as was stated by Ambache. It is true that in some preparations the strong contraction, which is produced by a large dose of nicotine and precedes the stage of paralysis, renders the preparation less sensitive to acetylcholine. But in this condition the preparation is also less sensitive to other muscle-stimulating drugs; this condition lasts for a short period and is independent of the nicotine paralysis.

2. No evidence could be obtained in support of the theory that the muscle-contracting effects of $BaCl_2$ and KCl on the isolated intestine result from a release of acetylcholine from the nervous structures in the intestinal wall and that such a mechanism accounts also for part of the contraction produced by histamine. This theory of Ambache was based on the assumption that cooling an intestinal preparation inactivated only the nervous structures of the preparation. We have found, however, that the treatment also impaired the muscle fibres.

3. The theory of Ambache was supported by experiments which were meant to show that $BaCl_2$ and histamine increase the yield of acetylcholine in incubated strips of intestinal tissue, and that eserine sensitized the preparation to these substances. No evidence could be found for an accelerating effect of $BaCl_2$ or histamine on the synthesis of acetylcholine under the conditions used by Ambache, and his results can be explained by the fact that no allowance was made for the great individual variations which occur in different samples of incubated intestinal strips. The sensitizing effect of eserine observed under the conditions of Ambache's experiments was found to occur with all muscle-stimulating substances and is explained as follows. Eserine by its cholinesterase-inhibiting action leads to the accumulation in the intestinal wall of acetylcholine, the effect of which sums with that of any muscle-stimulating substance added in this condition.

4. When histamine, $BaCl_2$ and KCl were examined on the intestinal preparation paralysed by small doses of atropine or by benadryl no evidence could be obtained in favour of Ambache's theory.

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