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CILIARY MOVEMENT AND ACETYLCHOLINE

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Acetylcholine is established as a substance which is released at nerve terminations to initiate the contractions of skeletal muscle and to augment the contractions of some forms of smooth muscle. Evidence has also been obtained that the capacity of the isolated auricles of the rabbit heart for maintaining rhythmic activity is closely related to the rate of synthesis of acetylcholine within the auricles (Bülbring and Burn, 1949), and thus the suggestion has arisen that acetylcholine may act as a local hormone to maintain rhythmic movement. The work of Feldberg and Lin (1950) indicates that the formation of acetylcholine in the intestine maintains pendular movement and mucous secretion. If acetylcholine, in addition to being the humoral transmitter of nervous impulses, is also a substance which controls rhythmic movement in various types of muscle, then it might be possible to demonstrate that the activity of the ciliary epithelium is related to acetylcholine production. To test this suggestion we have carried out experiments on the ciliary movement in the oesophagus of the pithed frog and also in the excised trachea of the rabbit.

Early observations on the ciliary movement in the dog's trachea *in situ* were made by Lommel (1908). He determined the rate of transport of lycopodium seeds, which he found to be about 0.33–0.5 mm./sec. He observed that cocaine in 10 per cent solution did not affect the rate until sufficient was absorbed to produce anaemia, and then the rate was slowed to less than 0.1 mm./sec. Lommel recorded that the section of both vagi had no effect on ciliary movement, which continued unmodified after denervation. Rozansky (1926), however, stated that although ciliary movement is autonomous, it can be increased by vagal stimulation. Most of the observations on the effect of stimulation of autonomic nerves or on the application of autonomic drugs on ciliary movement have been made on frogs. McDonald, Leisure, and Lenneman (1928) stated that stimulation of sympathetic fibres accelerated and that stimulation of parasympathetic fibres inhibited the movement of the cilia in the mouth of the frog. They found in accordance with this that adrenaline and ephedrine accelerated ciliary movement, and that pilocarpine inhibited it ; the effect of atropine was sometimes in one and sometimes in the other direction. Pohle (1931) obtained results with adrenaline, which varied according to the temperature ; thus at 16° C. it depressed ciliary movement, but at 29° C. it quickened it. Choline, on the other hand, quickened ciliary movement at 16° C., but depressed it at 29° C. Pohle also observed that atropine inhibited ciliary movement. Plattner and Hou (1931) found that 10^{-4} or 10^{-5} acetylcholine quickened ciliary movement and 10^{-2} choline also had a slight accelerator action. Eserine (10^{-4}) had no effect or caused some inhibition, but when combined with 10^{-5} acetylcholine it caused an

acceleration of ciliary movement greater than that due to 10^{-5} acetylcholine alone. Plattner and Hou also found atropine to be without effect by itself, but in a concentration of 10^{-5} it prevented the effect of acetylcholine and of pilocarpine ; they found that 10^{-5} adrenaline caused acceleration, and this was also prevented by atropine.

In 1934 Lierle and Moore studied ciliary movement in the intact turbinate of the guinea-pig, in mucosa from the dog's nasopharynx, and in pieces of mucosa from the human frontal sinus. When such pieces were placed at once in Locke's solution, and irrigated with it, ciliary movement was vigorously maintained for 77 hr., and there was still some activity at 121 hr. The prolonged washing with Locke's solution resulted in the removal of the protective mucous sheet, but they state that it did not impair the activity of the cilia. The application of cocaine in 5 per cent solution slowed ciliary movement in only 4 out of 13 trials. Adrenaline (10^{-3}) caused arrest or slowing of the movement.

Recently Blaich and Klar (1950) have studied ciliary movement in the frog's oesophagus in order to determine the action of penicillin. They examined eserine in a concentration of 10^{-4}, and found it to have no effect or to be inhibitory, but together with penicillin there was a 35 per cent increase in rate. Penicillin alone had a slight augmenting action, and this was cut short by giving atropine, together with the penicillin. Penicillin, together with adrenaline, as well as with acetylcholine, likewise increased the rate, and this effect was also cut short by atropine. Both adrenaline and acetylcholine were found to increase the rate. Finally Seaman and Houlihan (1951) have studied the effect of eserine and of DFP on the mobility of *Tetrahymena*, and also on the rate of movement of carborundum particles in the frog oesophagus. These anticholinesterases in a concentration of 10^{-3} M immobilized an increasing percentage of *Tetrahymena* and slowed the rate of ciliary movement in the frog oesophagus. The authors failed to observe any accelerating action of eserine.

Observations on Ciliary Movement

Methods

Frog's oesophagus.—The preparation was made by passing one blade of a pair of scissors into the mouth of the frog so as to cut off the head, leaving the floor of the mouth and lower jaw intact. The spinal cord was destroyed and the frog pinned on a cork mat, dorsal surface uppermost. The skin of the back was divided down the midline, and the posterior body wall was removed by cuts parallel to the midline for 3 cm. The oesophagus was thus exposed and was opened dorsally from the buccal cavity to the stomach. It was laid out so that its inner surface was nearly horizontal, using one or two pins. The cork mat with the frog was then placed in a perspex chamber and covered with a perspex lid. A piece of cotton-wool on which hot water was poured to keep the air moist was placed on each side of the frog. There was a small slot in the lid at right angles to the midline of the oesophagus, and small particles, the movement of which was to be observed, were dropped on to the oesophagus through the slot. We used poppy seeds, selected by passing them through a sieve of mesh 40, and then retaining those which would not pass through a mesh 60. A line marked on the perspex lid indicated the distance to be travelled by the particles. We used a distance of 8 mm.

Recording ciliary movement.—The preparation was irrigated at intervals of 5–20 min. with diluted Locke's solution (10 vol. solution to 14 vol. distilled water). Readings consisted in taking the time for 10 seeds to travel the 8 mm. distance, from which the mean

time was calculated, and from this the distance travelled in 100 sec. was further calculated. When determinations of the rate of transport had thus been made for a period of 30–40 min., the oesophagus was irrigated with a solution of the drug in the diluted Locke's solution. The irrigation was repeated at intervals between readings as before. The effect of the drug having been observed, irrigation was then resumed with diluted Locke's solution only, and a return of the rate of transport to about the initial value occurred. In some experiments the oesophageal mucous membrane was removed from the frog, and observations were made when no possibility remained of a circulation through the membrane.

Rabbit trachea.—The trachea was removed from a freshly killed rabbit by cutting it out from below the larynx to the bifurcation. It was slit open along the mid-dorsal line and stretched on a cork with pins. The cork was placed in the perspex chamber used for the frog. Boiling water was poured over cotton-wool at each end of the chamber, and the temperature was maintained at 35°–37° C. by a lamp above it which also served for illumination. The temperature remained fairly constant indefinitely.

Recording ciliary movement.—The surface of the mucous membrane was washed with Locke's solution taken from a bath at 38° C. by applying about 5 ml. with a capillary pipette at intervals of 20 min. or less. Particles of charcoal were scattered over the mucous membrane by a fine camel-hair brush. Their movement was observed through a binocular microscope, magnifying 10 times, which had a scale in one eyepiece ; on the scale 7.7 divisions were 1 mm. The distance travelled (in scale divisions) during 30 sec. was then recorded for each of 10 particles, and the mean distance was calculated. Only particles of charcoal which moved were considered, and variations in the proportion of moving particles to particles which remained still were ignored. All solutions of drugs were prepared in Locke's solution, and were kept at 38° C. About 5 ml. of a solution was applied by a capillary pipette, and the application was repeated at intervals of about 5 min. Fresh charcoal was scattered after every application whether of Locke's solution or of a solution of a drug.

Ciliary movement was well maintained in these preparations for periods up to 9 hr. In the frog's oesophagus *in situ* there appeared to be some secretion of mucus from time to time which might have been responsible for some of the results obtained. Since, however, the same results (for example, with eserine and with *d*-tubocurarine) were also obtained in both the isolated frog's oesophagus and the rabbit's trachea, and since in these isolated preparations there was no secretion of mucus, we felt satisfied that the effects on ciliary movement we observed were not secondary to effects on mucous secretion.

RESULTS

Frog oesophagus

Eserine sulphate.—An experiment illustrating the effect of eserine sulphate in concentration of 10^{-4} is shown in Fig. 1, in which the transport of poppy seeds is shown as ordinate. By observations during 35 min. it was established that the rate was about 7 mm. per 100 sec. During this period the mucous membrane was washed with diluted Locke's solution every 5 min. Eserine was then applied, and 5 min. later the rate had risen to about 14 mm. per 100 sec., where it remained for the 30 min. during which the membrane was regularly washed with eserine solution. At the end of the 30 min. period the membrane was once more washed with diluted Locke solution. The rate of transport soon returned to about the initial value.

A series of experiments was performed with different eserine concentrations, and the changes in the rate of ciliary movement were expressed as a percentage of the

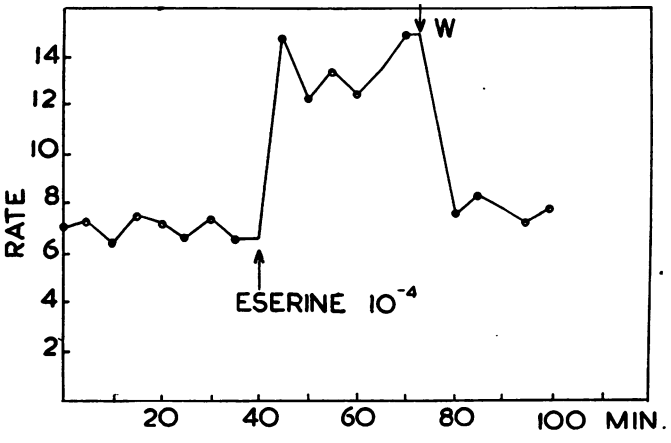

FIG. 1.—Action of 10^{-4} eserine on ciliary movement in the oesophagus of the pithed frog. Ordinate is rate in mm./30 sec. Each point is the mean of 10 observations. At W membrane washed with diluted Locke solution.

initial rate. In each experiment the initial rate was taken as the mean of all rates observed before the application of eserine, and the rate after applying eserine was taken as the mean rate during the whole period of application. In all experiments the removal of eserine resulted in a return to the initial rate. The results are given in Table I, in which it will be seen that, as the concentration of eserine rose, the mean effect rose up to a maximum at 10^{-4}, beyond which it declined ; 4×10^{-4} eserine did not increase the rate of ciliary movement but depressed it.

TABLE I

EFFECT OF ESERINE ON CILIARY MOVEMENT IN THE FROG OESOPHAGUS

(Each figure is taken from a separate experiment)

	Percentage change in rate at concentrations indicated			
	10^{-5}	10^{-4}	2×10^{-4}	4×10^{-4} eserine
	+55	+145	+78.5	−20.5
	+40	+79.5	+14.5	+38
	+24	+83	+27.5	−43
	+35.5	+81.8	+53	−16.8
	+52	+87	+19.6	−13.2
Mean ..	+41.3	+95.2	+38.6	−11.1

Acetylcholine.—The application of acetylcholine, like that of the lower concentrations of eserine, caused an increase in the rate of ciliary movement. The only concentration used was 10^{-5}, and that produced increases in five experiments of 79, 25, 44, 54, and 56 per cent respectively, the mean increase being about 50 per cent. In three of the experiments it was observed that the removal of the acetylcholine was followed by a transient slowing of the rate to much less than the initial rate. The effect is illustrated in Fig. 2 ; in view of the earlier work on the auricles it suggests that in the presence of applied acetylcholine the natural production of acetylcholine was inhibited, and was only resumed at the initial rate some time after the applied acetylcholine had been removed.

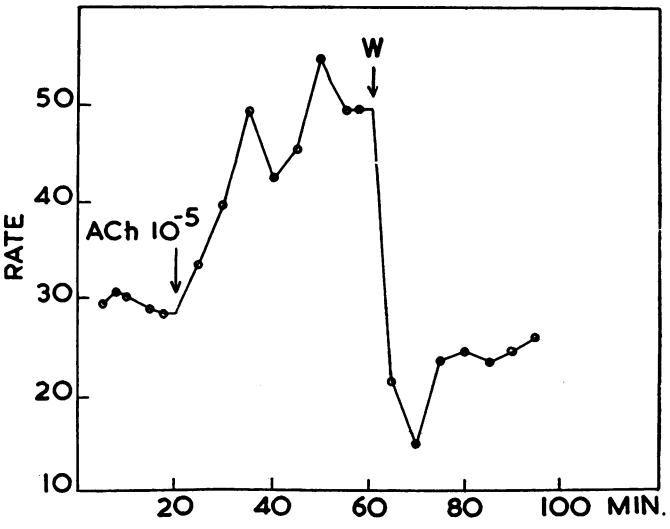

FIG. 2.—Action of 10^{-5} acetylcholine on ciliary movement in the oesophagus of the pithed frog. Ordinate is rate in mm./100 sec. Note the drop in rate after the acetylcholine had been removed by washing at W; the rate fell to less than the initial value and then slowly recovered.

Atropine sulphate.—Atropine in a concentration of 10^{-6} was found to inhibit ciliary movement. In three experiments the rate was diminished to 18, 64, and 40 per cent of its original value, and when the atropine was removed the rate was rapidly restored.

d-*Tubocurarine.*—*d*-Tubocurarine in a concentration of 10^{-6} was also found to inhibit ciliary movement to the same extent as atropine. The effect was seen not only in the oesophagus *in situ*, but also in the isolated preparation.

Rabbit trachea

Eserine sulphate.—Nine experiments were carried out on the mucous membrane of the rabbit's trachea with eserine sulphate, and the results are given in Table II.

TABLE II

EFFECT OF ESERINE ON CILIARY MOVEMENT IN RABBIT TRACHEA

	Percentage change in rate at concentrations indicated		
	10^{-5}	10^{-4}	2×10^{-4} eserine
	+85	+63	movement stopped
	+54	+114	,, ,,
	+56	+116	,, ,,
Mean ..	+65	+98	,, ,,

The effect of the concentrations 10^{-5} and 10^{-4} was similar to the effect on the frog's oesophagus, for they quickened the rate to a similar extent. The concentration of 2×10^{-4}, however, abolished ciliary movement, though in two of the experiments there was an initial quickening, as shown in Fig. 3. Fig 3 demonstrates very clearly the two phases of eserine action when it is present in sufficient concentration. The ciliary movement was resumed when the eserine was washed away.

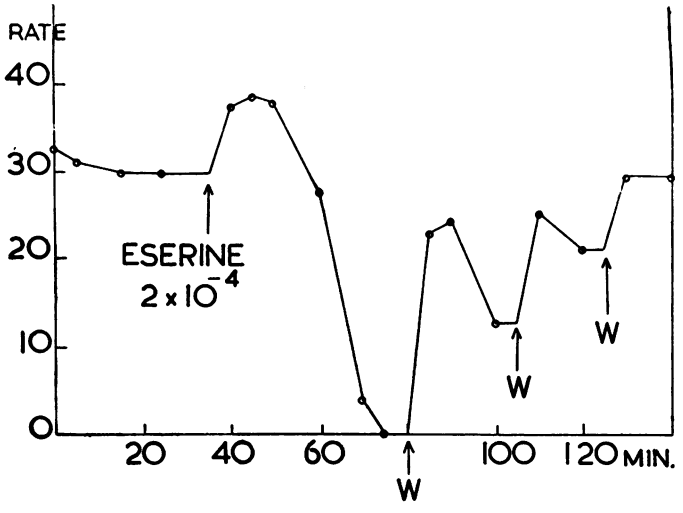

FIG. 3.—Action of 2×10^{-4} eserine on the ciliary movement in the isolated rabbit trachea. Lower concentrations had the effect seen in Fig. 1. Ordinate is rate in scale units/30 sec. Note the initial stimulation followed by depression to standstill.

Acetylcholine.—Sixteen experiments were made with acetylcholine, the results of fourteen of which are given in Table III. The lower concentrations quickened

TABLE III

EFFECT OF ACETYLCHOLINE ON CILIARY MOVEMENT IN RABBIT TRACHEA

	Percentage change in rate at concentrations indicated			
	10^{-5}	2×10^{-5}	5×10^{-5}	10^{-4} acetylcholine
	+32	+36	−15	−35
	+27	+26	−5	−32
	+32	+30		−34
	+17	+40		−29
Mean ..	+27	+33	−10	−33

the ciliary movement, while the higher ones slowed it, as shown in Fig. 4. The effects of acetylcholine were, however, less than those of eserine. Thus the application of 10^{-4} eserine doubled the rate, while the maximum mean quickening after acetylcholine was only 33 per cent above the initial rate. Similarly eserine in concentration of 2×10^{-4} depressed the rate to zero, while acetylcholine in concentration of 10^{-4} depressed the rate to 67 per cent. The effects of acetylcholine were not so well maintained throughout the period of application as those of eserine. Increase of rate became less, and when concentrations of acetylcholine were used which depressed the rate, the depression also became less. In the two experiments, indeed, which are not included in Table III, there was an initial depression with the concentration 5×10^{-5}, and then later the depression gave way to increase of rate. We are inclined to think that, when acetylcholine is applied to the mucous membrane, it causes a diminution of the amount of acetylcholine which is being formed within the membrane, and thereby reduces its direct effect on the ciliary movement.

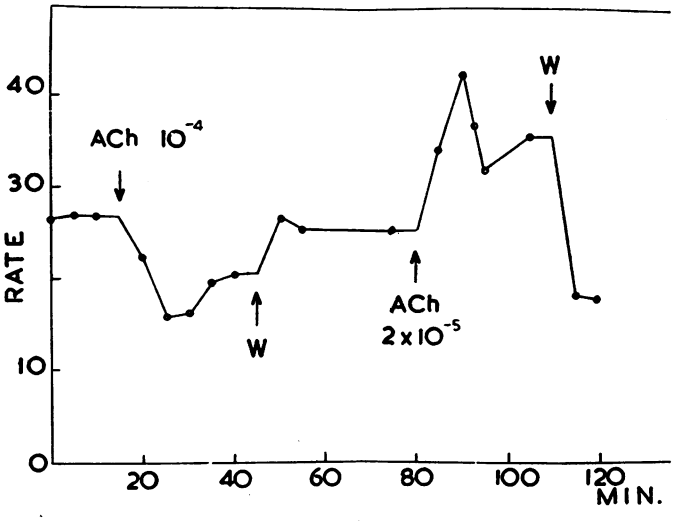

FIG. 4.—Ciliary movement in the isolated rabbit trachea, showing the depressant action of 10^{-4} acetylcholine and the stimulant action of 2×10^{-5} acetylcholine. Ordinate is scale units/30 sec.

Atropine sulphate.—Atropine was applied in a concentration of 10^{-6} in five experiments. In each of these the rate of ciliary movement was decreased respectively to 55, 45, 44, 74, and 67 per cent of the initial value during the period of application.

d-*Tubocurarine.*—*d*-Tubocurarine acted like atropine, and diminished the rate of ciliary movement. In a concentration of 10^{-6} the rate was decreased to 43 per cent of the initial; in concentrations of 10^{-5} and 10^{-4} the ciliary movement was arrested altogether, but was restored when the tubocurarine was washed away (see Fig. 5). These results with tubocurarine made it clear that the effects on ciliary movement which we observed were not secondary to effects on mucous secretion, since tubocurarine has no appreciable effect on mucous secretion. In a cat under chloralose in which the salivary secretion was recorded, atropine in a dose of 12 μg. blocked the effect of chorda stimulation and of 40 μg. acetylcholine. Tubocurarine chloride in a dose of 0.8 mg. diminished the effect of chorda stimulation, but did not block it, and failed to modify the effect of 40 μg. acetylcholine; in a dose of 1.6 mg. it diminished the effect of 40 μg. acetylcholine but did not block it. Thus tubocurarine has less than 1 per cent of the paralysing action of atropine on salivary secretion. Yet when compared directly with atropine on the cilia of the frog's oesophagus, tubocurarine was found to be rather more potent than atropine.

Cocaine hydrochloride.—We were anxious to determine whether ciliary movement was modified by cocaine, because of the possibility that the production of acetylcholine in the mucous membrane depended on nervous elements present in it. We applied cocaine in concentrations of 10^{-4}, 10^{-3}, and 10^{-2} in different experiments without observing any change. We then applied a concentration of 2×10^{-2} in three experiments and, having again observed no change, tested the effect of eserine in the first two and of atropine in the third experiment. We observed that these substances produced their ordinary effect, though that of eserine was less than usual. Finally, we tested cocaine in another experiment in 10 per cent solution; this appli-

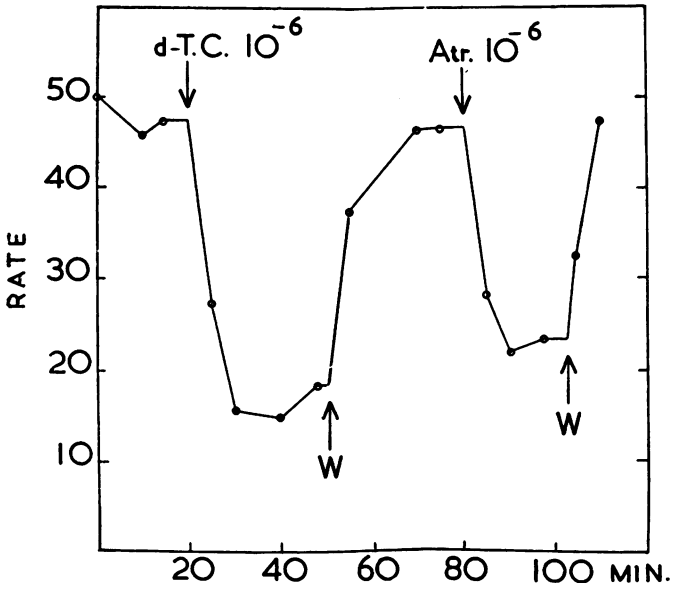

FIG. 5.—Ciliary movement in isolated rabbit trachea as in Figs. 3 and 4. The depressant action of 10^{-6} *d*-tubocurarine and of 10^{-6} atropine is shown.

cation did perhaps reduce the rate of ciliary movement slightly, but the effect was uncertain. We conclude that cocaine has no significant effect on the ciliary movement of the tracheal mucous membrane.

Adrenaline and noradrenaline.—Adrenaline was found to have a slight effect in stimulating ciliary movement, but noradrenaline was without effect. Six experiments were made in which adrenaline was tested ; in concentrations of 10^{-5} and 2×10^{-5} it had no action, but 5×10^{-5} increased the rate of ciliary movement by 23 per cent, and 10^{-4} increased it by 33, 32, and 24 per cent. In four experiments 10^{-4} noradrenaline was without effect, and in one it was without effect in twice this concentration ; in this experiment adrenaline 10^{-4} then caused an increase in rate.

Histamine.—Histamine was ineffective in concentrations of 10^{-5}, 10^{-4}, and 10^{-3}. Through the kindness of Dr. H. Schild we were able to test the antihistaminase Grewe diamine, which we used in concentrations 10^{-5} and 10^{-4}. This also had no effect.

Nicotine.—Nicotine in a concentration of 10^{-5} had no influence on ciliary movement.

ACETYLCHOLINE IN EXTRACTS OF TRACHEAL MUCOUS MEMBRANE

The acceleration of ciliary movement produced by eserine indicated that the mucous membrane probably contained acetylcholine. We therefore made extracts and tested them for the presence of acetylcholine by various methods ; we also tested them for histamine. The number of experiments carried out was limited by the fact that the mucous membrane from 6–10 rabbits was required for each experiment.

The rabbits were injected with 1 mg. eserine sulphate 5 min. before they were killed. The mucous membrane was scraped free from cartilaginous tissue ; the collected material was weighed and transferred as soon as possible to a cooled mortar containing 0.5 ml.

0.1 N–HCl for the mucous membrane of each trachea. The tissue was cut up with scissors, and ground with a small quantity of silica ; 0.2 ml. was added for the mucous membrane of each trachea, and the extract was boiled and then rapidly cooled. Before use the extract was centrifuged and the volume adjusted so that it contained the activity of 100 mg. tissue/ml.

Extracts were also made using 10 per cent (w/v) trichloroacetic acid, of which 1 ml. was taken per 100 mg. tissue. The extract prepared by grinding in a mortar was centrifuged, and the supernatant was poured off into a stoppered measuring cylinder. Ether was added and pipetted off after shaking thoroughly ; this was repeated 4 times. The last of the ether was blown off with nitrogen. 0.1 ml. 0.33 N–HCl was added for each 1 ml. extract, and the extract was stored at –15° C. Before the extract was tested, it was nearly neutralized in the presence of universal indicator, and the volume adjusted to contain the activity of 100 mg. tissue per ml.

TABLE IV

ACETYLCHOLINE AND HISTAMINE IN THE TRACHEAL MUCOUS MEMBRANE OF THE RABBIT

Ach = acetylcholine; H = histamine

Exp.	Extracting agent	Test preparation					
		Frog heart	Frog rectus	Guinea-pig ileum		Cat blood pressure	
		μg. Ach/g.	μg. Ach/g.	μg. Ach/g.	μg. H/g.	μg. Ach/g.	μg. H/g.
1	Acid saline	1.06	2.8	1.0	—	1.4	—
2	,, ,,	1.9	2.5	—	—	—	—
3	,, ,,	2.04	—	1.9	—	1.7	4.15
4	Trichloroacetic acid	0.23	0.4	—	4.94	—	4.85

Estimations of acetylcholine were made on the frog rectus muscle, the isolated perfused frog heart, the guinea-pig ileum, and on the blood pressure of the cat under chloralose. Estimations of histamine were made on the guinea-pig ileum and on the blood pressure of the cat. Results for three extracts made with acid saline and for one extract made with trichloroacetic acid are given in Table IV. It will be observed that the amounts of acetylcholine found in the trichloroacetic extract were much less than those found in the acid saline extracts. We think that acetylcholine was lost in the ether extraction of the trichloroacetic acid extracts. No such discrepancy was observed in the amounts of histamine extracted by the two methods.

The tests on the frog heart and the cat blood pressure for the acetylcholine in one of the acid saline extracts are illustrated in Fig. 6. The upper record on the frog heart shows that 0.03 ml. of the extract S produced inhibition greater than that of 0.003 μg. acetylcholine and less than that of 0.004 μg. acetylcholine. Hence 1 ml. S was equivalent of 0.12 μg. acetylcholine. Both the inhibitory effect of S and of acetylcholine was abolished by atropine. The lower record on the cat blood pressure shows that the depressor action of 0.05 ml. S was similar to that of 0.03 μg. acetylcholine, and rather less than that of 1.0 μg. histamine. An antihistamine substance, Lergigan, was then injected in the dose of 0.25 mg., and then, when the effect of 1.0 μg. histamine was abolished, 0.25 ml. S was equivalent to 0.04 μg. acetylcholine ; that

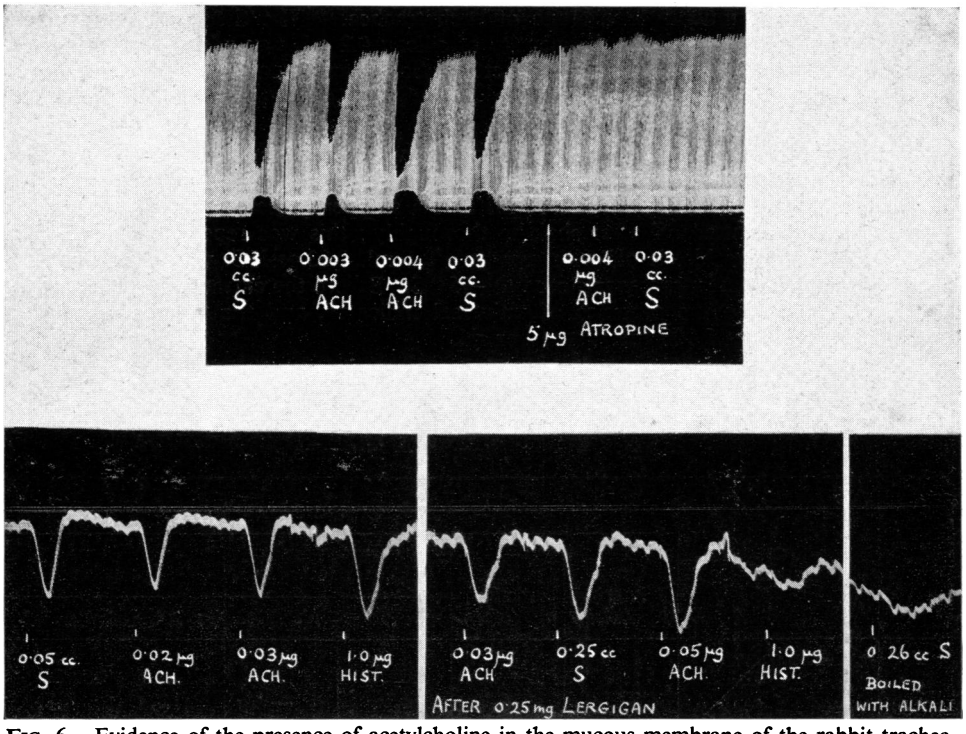

FIG. 6.—Evidence of the presence of acetylcholine in the mucous membrane of the rabbit trachea. The upper record compares the effect of an extract (S) of the mucous membrane with that of acetylcholine on the isolated frog heart. The effects of both were abolished by atropine. The lower record compares the effect of the same extract on the blood pressure of the cat before and after giving an antihistamine compound. In the presence of the antihistamine the relation of the extract to acetylcholine is similar to that on the frog heart (see text).

is to say, 1 ml. S was equivalent to 0.16 µg. acetylcholine, which is a result agreeing reasonably well with that on the frog heart. Finally, it was seen that when the extract was boiled with alkali for a few minutes, the depressor action of 0.26 ml. was absent. These results, together with those on the frog rectus and guinea-pig ileum, leave no doubt of the presence of acetylcholine in the extract.

SYNTHESIS OF ACETYLCHOLINE BY TRACHEAL MUCOUS MEMBRANE

The presence of acetylcholine in the tracheal mucous membrane made it probable that the membrane would be found to contain choline acetylase. A series of experiments, in each of which the mucous membrane from 5 or 6 rabbits was taken, were therefore carried out to determine the presence of this enzyme. The method was that described by Feldberg and Mann (1946) for brain tissue, and previously used by us for auricles (Bülbring and Burn, 1949). An acetone-dried powder was prepared, and observations were made on 50 mg. portions of the powder. In each experiment we also prepared an acetone-dried powder of rabbit brain since the enzyme is present in brain, and the value obtained for brain gave us an estimate of

the efficiency of our procedure. In making estimations of acetylcholine on the frog rectus the incubated solutions were compared, not with solutions of acetylcholine, but with control solutions containing all the ingredients used in the incubation, except the tracheal powder. These solutions were placed in the thermostat alongside the test solutions, and finally known amounts of acetylcholine were added to these control solutions for the comparison. The results obtained are given in Table V,

TABLE V

ACETYLCHOLINE SYNTHESIS BY CHOLINE ACETYLASE

Exp.	μg. acetylcholine/g. powder/75 min.	
	Tracheal mucous membrane	Brain
1	30	400
2	22.5	330
3	20	275
4	25	600
5	38	600
6	31	470
7	22.5	550
8	50	850
Mean	30	510

which shows that acetylcholine was synthesized by the tracheal mucous membrane in each of the eight experiments. The results obtained for brain were low compared with those of Feldberg and Mann, and compared with our earlier observations ; as a rule the synthesis under the conditions we then used varied from 800 to 1,000 μg./g./hr. Such a result was obtained only in the last experiment in Table V, and in this experiment the highest figure for the tracheal mucous membrane was obtained. Hence we consider it probable that our mean figure 30 μg./g./75 min. errs by being too low.

Cholinesterase in Tracheal Mucous Membrane

We examined the tracheal mucous membrane for cholinesterase in four experiments. The mucous membrane was put into a previously cooled mortar, cut up with scissors, and quickly frozen to −15° C. It was then ground up until it thawed again. A small amount of silica was added to complete the grinding, and 10 ml. of Krebs's bicarbonate Ringer was added to each gramme of tissue. The extract was stored at −15° C. The activity was measured by the Warburg technique, and was expressed as microlitres CO_2 per hr. per g. wet tissue. The substrates used were acetylcholine bromide, benzoylcholine chloride, and acetyl-β-methylcholine chloride. Both substrates and extracts were made up in Krebs's bicarbonate Ringer, and the gas mixture used was 95 per cent nitrogen and 5 per cent CO_2. Acetylcholine and benzoylcholine were used in a final concentration of 0.015 M, and acetyl-β-methylcholine was used in a concentration of 0.03 M. The results are given in Table VI. They show that the so-called " true " or " specific " form of cholinesterase was present.

TABLE VI

CHOLINESTERASE IN RABBIT TRACHEAL MUCOUS MEMBRANE

Substrate	μl. CO_2/g. fresh tissue/hr.			
	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Acetylcholine	2,730	—	1,935	2,500
Benzoylcholine	—	156	—	320
Acetyl-β-methylcholine	—	1,350	1,860	1,780

DISCUSSION

The results which have been obtained in the course of the work which has been described are all consistent with the view that ciliary movement is controlled by the production of acetylcholine, which when produced keeps in being the rhythmic activity. The accelerating action of eserine has only one likely explanation, namely, that it is due to the inhibition of cholinesterase, and as a result acetylcholine accumulates in greater concentration than before. Thus the action of eserine is by itself evidence that the ciliary movement is controlled by the production of acetylcholine.

This evidence has been supported by three further findings: the presence of acetylcholine in the tracheal mucous membrane, the presence of choline acetylase in the mucous membrane, and, third, the presence of "true" or "specific" cholinesterase in the mucous membrane. Consistent with the view that ciliary movement is controlled by the production of acetylcholine is the further evidence that ciliary movement is abolished by atropine and also by *d*-tubocurarine; these substances share the property of antagonizing the action of acetylcholine, but only atropine depresses the secretion of mucus.

Both effects of acetylcholine are to be seen when it is itself applied to the mucous membrane of the rabbit trachea. The lower concentrations accelerate the movement of the cilia, while the higher ones depress the movement. It has been questioned whether acetylcholine ever produces inhibition in peripheral tissues. So far as ciliary movement in the mucous membrane of the rabbit trachea is concerned, there is no doubt of this. The same twofold effect is also to be observed with eserine, from which it is to be concluded that acetylcholine synthesis in the membrane can proceed so far that, if the cholinesterase is completely inhibited, the accumulation of synthesized acetylcholine can block the receptors on which it acts.

The question next arises in what structures is acetylcholine synthesized. Is it produced in nerves, or is it produced in non-nervous tissue? Ciliary movement is generally believed to be autonomous in vertebrates. Moreover, in the preparation of the frog, the central nervous system was destroyed, and the ciliary movement was observed to be vigorous for many hours. There was no difference in ciliary movement, or in the action of drugs on it, whether the oesophagus was left *in situ* or was excised. In the preparation of the rabbit's trachea, the tracheal mucous membrane was isolated from the body and there was no circulation in it. We are greatly indebted to Dr. P. Glees, of the Department of Physiology, for examining the preparation histologically. He found that ganglion cells were entirely absent, and that

there were a few nerve fibres in the submucosa which appeared to be sensory fibres. These were, of course, severed from the centre during the observations. It appears to us most improbable that these divided nerve fibres could be the source of the acetylcholine which maintained the ciliary movement, especially in view of the fact that cocaine hydrochloride in a concentration as high as 1 in 50 had no effect whatever on ciliary movement, and even in a concentration of 1 in 10 produced only the slightest depression. We are thus led to the conclusion that acetylcholine is probably synthesized in tissue which is not nervous, and consider that the action of acetylcholine in promoting ciliary movement in the tracheal mucous membrane is an example of a property distinct from that of a humoral transmitter, namely, the property of a local hormone.

Summary

1. Ciliary movement has been studied in the oesophageal mucous membrane of the pithed frog both *in situ* and when isolated. It has also been studied in the isolated mucous membrane of the rabbit trachea.

2. In both preparations low concentrations of eserine increase the rate of movement, while higher concentrations depress it.

3. In both preparations low concentrations of acetylcholine increase the rate of movement and high concentrations depress it.

4. Both atropine and *d*-tubocurarine depress or arrest ciliary movement.

5. Adrenaline increases the rate of ciliary movement in the rabbit trachea, though noradrenaline is without effect.

6. Cocaine in 2 per cent concentration has no effect on ciliary movement.

7. Acetylcholine and histamine are both present in the mucous membrane of the rabbit trachea.

8. The rabbit mucous membrane contains both choline acetylase and "true" cholinesterase.

9. As there are no ganglion cells in the tracheal mucous membrane, acetylcholine is considered to be produced by non-nervous tissue and to control ciliary movement as a local hormone.

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