

EXPERIMENT NO.: 8

DATE:

AIM: TO STUDY THE EFFECT OF DRUGS ON CILIARY MOTILITY OF FROG OESOPHAGUS

INTRODUCTION:

- Cholinergic drugs causes contraction of cilia leading to increased movements.
- Anticholinergics drugs causes paralysis of cilia leading to decreased movements.

OBJECTIVE:

- To find out the action of certain drugs on the ciliary motility of frog oesophagus.

MATERIALS:

- Frog.
- Poppy seeds.
- Frog wooden board.
- Stop watch
- Drugs & Solution:
 - A) Acetylcholine 10%: Cholinergic agonist.
 - B) Physostigmine 10%: Anti-choline esterase inhibitor.
 - C) Atropine 0.1%: Non-selective muscarinic agonist, or Cholinergic antagonist.
 - D) Frog Ringer: Nutrient solution to keep the tissue viable.
 - E) d-Tubocurarine: Muscle relaxant



Frog wooden board



Poppy seeds

METHOD:

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/wooden board, 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.

RESULT:

Eserine sulphate:

An experiment illustrating the effect of eserine sulphate in concentration of 10^{-4} (Figure 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 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.

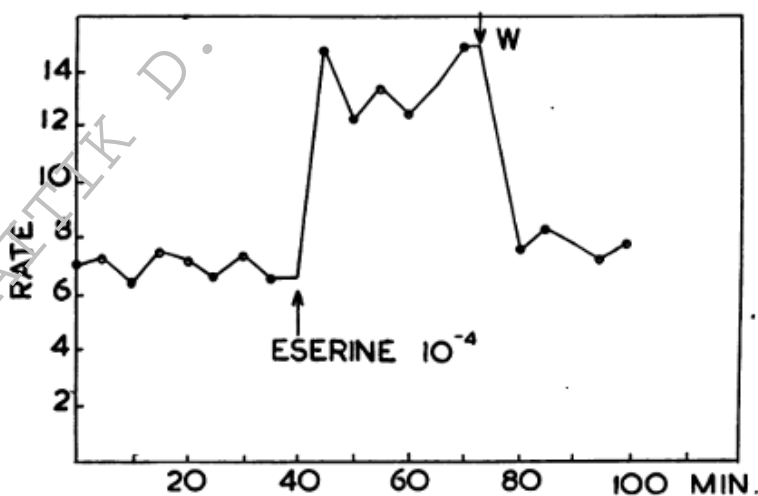


FIG. 1.—Action of 10^{-4} eserine on ciliary movement in the oesophagus of the pithed frog. Ordinate is rate in mm./30 sec. Each point is the mean of 10 observations. At W membrane washed with diluted Locke solution.

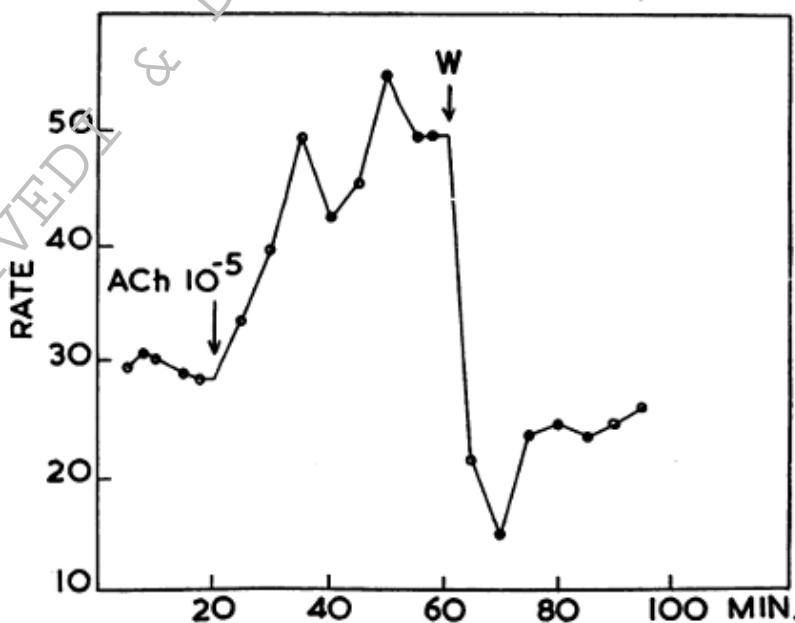
EFFECT OF ESERINE ON CILIARY MOVEMENT IN THE FROG OESOPHAGUS
(Each figure is taken from a separate experiment)

		Percentage change in rate at concentrations indicated			
		10^{-5}	10^{-4}	2×10^{-4}	4×10^{-4} eserine
Mean ..		+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
		+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 sometime 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.

SOURCE: CILIARY MOVEMENT AND ACETYLCHOLINE BY PAMELA KORDIK, E. BULBRING, AND J. H. BURN, from the Department of Pharmacology, Oxford University (Received October 2, 1951), Brit. J. Pharmacol. (1952), 7, 67.