Brit. J. Pharmacol. (1959), **14**, 323.

THE ACTION OF TUBOCURARINE ON CILIARY MOVEMENT

BY

A. S. MILTON

From the Department of Pharmacology, University of Oxford

(RECEIVED JANUARY 21, 1959)

Further investigation of the effect of tubocurarine on the transport of particles by cilia of the frog oesophagus has confirmed that, when Ringer solution buffered with bicarbonate was used, tubocurarine inhibited the movement in a concentration of 10^{-6} g./ml. If, however, Ringer solution with a phosphate buffer was used tubocurarine was without effect. The difference in action is probably due to changes in calcium ion concentration caused by the phosphate in the Ringer solution.

Conflicting evidence has arisen from studies on the effect of tubocurarine on ciliary movement in the frog oesophagus. Kordik, Bülbring, and Burn (1952) found that tubocurarine in a concentration of 10^{-6} g./ml. inhibited ciliary movement. However, Hill (1957) described experiments in which tubocurarine had no effect on ciliary movement even in concentrations as great as 2×10^{-3} g./ml. Hill (1957) suggested that the results of Kordik *et al.* were artefacts arising from their experimental methods. As a result of the observations of Hill (1957), Burn and Day (1958) made a further study of the action of tubocurarine. They found that tubocurarine in a concentration of 10^{-4} g./ml. decreased the rate of ciliary movement in seven consecutive experiments, and in six of these the rate of ciliary movement returned to its initial value when the tubocurarine was removed. They also showed that it was possible to identify unknown solutions of tubocurarine and to distinguish them from Ringer solution by the action on ciliary movement.

Further experiments have now been made on the effect of tubocurarine in the concentration used by Kordik *et al.* (1952), namely 10^{-6} g./ml. Experiments have also been carried out to try to account for the conflicting results previously mentioned.

METHODS

The oesophagus of the frog was prepared in a way similar to that described by Burn and Day (1958), except for a few modifications in the dissection. The frog was decapitated, removing the upper jaw immediately behind the eye sockets. The lower jaw was left intact. The spinal cord was destroyed and the frog was placed prone on a small cork mat. The skin was slit along the mid-dorsal line and the vertebral column and dorsal body wall removed as far back as the sacral hump. The forelegs were removed with all but the central section of the pectoral girdle. The oesophagus was then cut down the mid-dorsal line and the cut continued a short distance into the stomach wall. The oesophagus was pinned out as a roughly rectangular sheet, being kept taut by pins. It was kept as level as possible by placing pieces of cotton wool soaked in Ringer solution beneath it. In female frogs the ovaries and oviducts were usually removed as these were liable to get in the way. In some experiments the whole of the hindquarters and the viscera were removed.

The rate of ciliary movement was measured by observing movement of particles. The preparation was placed inside a Perspex box similar to that previously described (Burn and Day, 1958). Particles were dropped on to the membrane through a slit in the lid and the time taken to travel 0.5 or 1.0 cm. was measured. This was facilitated by observing the particles as they travelled between three parallel lines on the lid, ruled 0.5 cm. apart. To avoid errors of parallax a sight was fitted above the lines. The particles were poppy seeds which could pass through a sieve of 40 mesh but were retained by one of 60 mesh and were therefore between 0.423 and 0.635 mm. in diameter. In the experiments with a totally immersed membrane, particles of garnet which could pass through a sieve of 60 mesh (0.423 mm.) were used as the poppy seeds often floated to the top. The Perspex box and all the solution were placed throughout the whole of each experiment in a water bath kept thermostatically at 20°.

To avoid errors, a strict sequence of events was adhered to. The membrane was rinsed with Ringer solution and, exactly 5 min. later, ten particles were individually timed over the measured distance. This took about 7 min. The membrane was then rinsed again with Ringer solution and exactly 5 min. later

ten more particles were timed. This sequence of events was continued until there was a steady rate of transport; the membrane was rinsed with Ringer solution containing the drug under test and exactly 5 min. later the particle transport time was measured. A further application of Ringer solution plus drug could then be made or the membrane rinsed with drug-free Ringer solution and particle transport time measured again. This sequence, alternating the administration of plain Ringer and Ringer solution containing the drug, could be continued for as long as was required. The average rate of particle transport for each set of 10 particles was expressed in cm./min.

The preparations usually remained active for about 24 hr., but measurements were made only on newly set up preparations. Measurements on particles falling near the cut edges or in the centre of the preparation near the glottis were not included in the results.

Experiments were also carried out using an isolated membrane immersed in Ringer solution and this preparation behaved in exactly the same way as the other.

The five solutions were as follows, the concentrations being mM.: (1) bicarbonate Ringer solution (Kordik *et al.*, 1952): NaCl 119, KCl 1.9, $CaCl_2$ 1.1, $NaHCO_3$ 2.4; (2) phosphate Ringer solution (Hill, 1957): NaCl 115, KCl 2.0, $CaCl_2$ 1.8, buffered with Na_2HPO_4 2.0 mM. to *p*H 7.0; (3) NaCl 115, KCl 2.0, $CaCl_2$ 1.8; (4) NaCl 115, KCl 2.0, $CaCl_2$ 1.8, $NaHCO_3$ 10.0; (5) NaCl 115, KCl 2.0, $CaCl_2$ 1.8, $NaHCO_3$ 10.0 buffered with 4.1% CO_2. Solutions (3) to (5) were also used by Hill (personal communication).

Results

The effect of tubocurarine (10^{-6} g./ml.) was observed in eight experiments using bicarbonate Ringer solution (Kordik *et al.*, 1952). In all, a decrease in particle movement was observed after the addition of tubocurarine. After the tubocurarine had been removed the rate returned to that observed initially (Fig. 1 and Table I).

TABLE I

RATE OF PARTICLE TRANSPORT BY CILIA OF FROG OESOPHAGUS IN BICARBONATE RINGER SOLUTION CONTAINING TUBOCURARINE (10^{-6} G./ML.)

The rates are given as cm./min.

Initial	In Presence of Drug	After Removal of Drug
1·07	0·62	0·91
1·16	0·92	1·17
1·17	1·05	1·34
1·34	1·14	—
1·39	1·03	1·78
1·58	1·27	1·53
1·98	1·46	1·85
2·29	1·86	2·29
Mean 1·50	1·17	1·55

If the initial rate in each experiment is taken as 100, the relative rates before, during the presence of tubocurarine and after its removal may be directly compared. The mean rate during the action of tubocurarine was 78% and after removing the tubocurarine it was 103%.

Hill (1957) was unable to demonstrate the stimulating action of low concentrations of acetylcholine and two experiments were therefore carried out using 10^{-6} g./ml. of acetylcholine. The stimulation was shown quite clearly (Table II).

TABLE II

EFFECT OF ACETYLCHOLINE 10^{-6} G./ML. ON RATE OF PARTICLE TRANSPORT

Rates are given as cm./min. in the two experiments.

Initial	Rate in Presence of Drug	After Removal of Drug	In Presence of Drug
0·24	0·45	0·27	0·40
1·24	1·52	1·01	

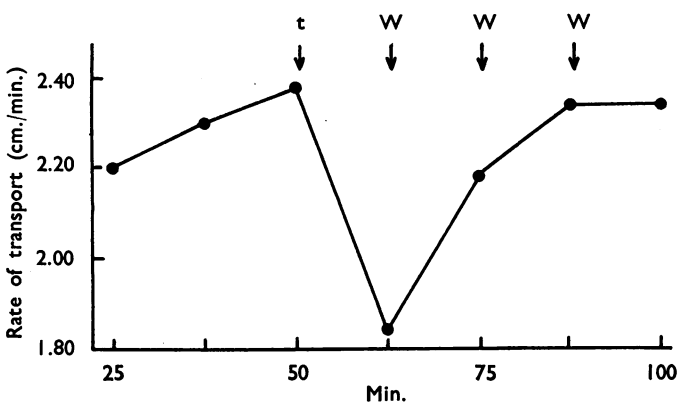

FIG. 1.—Action of tubocurarine (10^{-6} g./ml.) on ciliary movement in bicarbonate Ringer solution. Drug added at t. Washed with Ringer solution at W.

The results showed the inhibitory effect of tubocurarine and the stimulating effect of acetylcholine on ciliary movement. The failure of Hill (1957) to observe these effects was therefore puzzling and so an attempt was made to explain her results. She had suggested that artefacts had arisen in the experiments of Kordik *et al.* (1952) due to the presence of counter currents and the accumulation of mucus. In her experiments she used a membrane immersed in Ringer solution which, it was claimed, prevented artefacts. However, experiments with her method still showed the inhibitory action of tubocurarine.

A further study of the report of Hill (1957) showed one difference which had previously been overlooked. This was that, whereas Kordik *et al.* (1952) had used a solution containing bicarbonate, Hill (1957) usually used a solution containing phosphate. As this appeared to be the only real difference between the experimental procedures, observations were made using the solution described in her paper. In neither of the first two experiments had tubocurarine an appreciable effect (Table III).

TABLE III

RATE OF PARTICLE TRANSPORT SHOWING ABSENCE OF EFFECT OF TUBOCURARINE (10^{-6} G./ML.) IN PHOSPHATE RINGER SOLUTION

Rates are given in cm./min.

Initial Rate	In Presence of Drug	After Removal of Drug
1·59	1·57	1·34
2·35	2·39	2·24

Next, both the Ringer solutions were used on one preparation. In the first experiment, the bicarbonate Ringer solution was used initially and tubocurarine exerted an inhibitory effect; when the Ringer solution was changed for the one containing phosphate it was found after equilibration that tubocurarine was no longer inhibitory. In the second experiment, the phosphate Ringer solution was used initially and tubocurarine had no effect; on changing to bicarbonate Ringer solution the inhibitory effect of tubocurarine was readily observed (Fig. 2).

These results explained the inability of Hill (1957) to observe the effect of tubocurarine using the phosphate Ringer solution described in her paper. They did not, however, explain why this failure occurred. Further experiments were therefore carried out on the effect of *p*H.

The *p*H of the Ringer solution used by Hill (1957) was 7.0 whereas that of Kordik *et al.* (1952) varied between 7.6 and 8.3 owing to the very low buffering power of the bicarbonate. It should be remembered, however, that the *p*H of frog blood is about 8.0. Tubocurarine in concentrations of 10^{-4} g./ml. or less did not alter the *p*H of either solution.

In bicarbonate Ringer solution which had been brought to *p*H 7.0 with HCl, the cilia showed normal activity and were still active 20 hr. after being set up. Tubocurarine 10^{-6} g./ml. caused marked inhibition (33%). When the *p*H of the phosphate Ringer solution was raised to 8.0, visible precipitation of calcium phosphate gradually occurred. The cilia were so depressed and irregular in their rate of beat when using this solution that no measurements were possible.

These observations give no indication that differences of *p*H were responsible for the different results. However, the precipitation of calcium suggested that reduced calcium ion concentration due to binding with the phosphate might be responsible. In this connexion it was found that calcium-free bicarbonate Ringer solution caused an increase in rate at the start followed by slowing and irregularity in rate. Tubocurarine had no significant effect under these conditions.

The three other Ringer solutions used by Hill (1957) were also tested. With each, tubocurarine

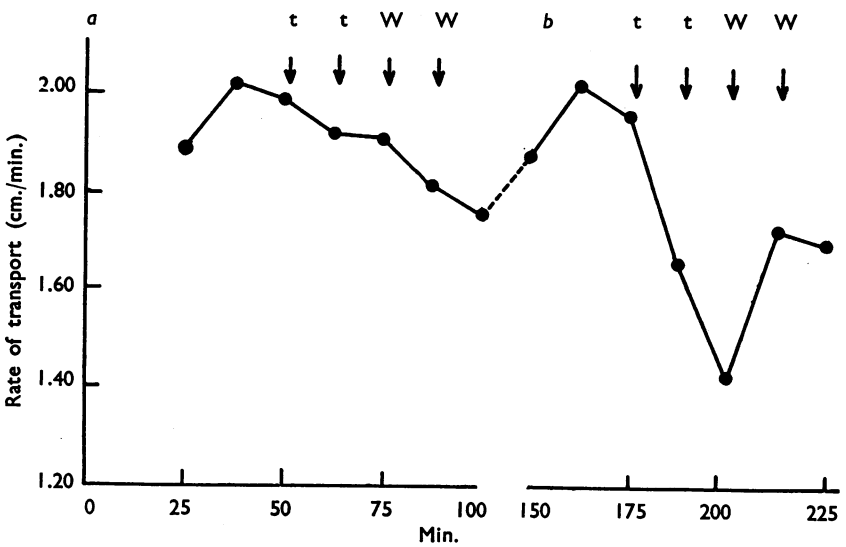

FIG. 2.—Action of tubocurarine (10^{-6} g./ml.) on ciliary movement on the same preparation, *a*, in phosphate and, *b*, bicarbonate Ringer solutions. Drug added at t. Washed with Ringer at W. Solution changed between 100 and 150 min.

caused a decrease in rate of particle transport (Table IV) and in two experiments the transport of particles was arrested altogether.

TABLE IV

RATE OF PARTICLE TRANSPORT IN PHOSPHATE-FREE RINGER SOLUTION (HILL, 1957)

Concentration of tubocurarine 10^{-3} g./ml. The rates are given in cm./min.

Sol. No.	Initial Rate	After Addition of Drug		Rate After
		3 min.	10 min.	
3	1·78	1·21	1·36	1·89
3	2·08	2·10	0	0
4	1·96	1·09	0	0
5	1·86	1·60	1·28	2·17

DISCUSSION

The results in this paper confirm the finding of Kordik *et al.* (1952) that tubocurarine (10^{-6} g./ml.) caused inhibition of the ciliary movement of the frog oesophagus. In the practical class of this Department, students observe the acceleration of ciliary movement by low concentrations of eserine and the inhibition by low concentrations of atropine. Frog Ringer solution containing bicarbonate as buffer is usually used, but on one occasion a solution containing phosphate as buffer, which had already been prepared for another purpose, was used instead. Eserine then showed no accelerating effect, though atropine was effective. This led to an investigation of the solution used by Hill (1957) which contained phosphate, and it was found that when phosphate was present the inhibitory action of tubocurarine was no longer seen; however, the action was restored in the same preparation when bicarbonate replaced the phosphate.

Dr. H. Blaschko pointed out that Alt (1930) had found that the phosphate interfered with tissue respiration. Thus even the inhibition of the respiration of kidney and liver slices by cyanide was reduced from 98% to 11% when bicarbonate buffer was replaced by a phosphate buffer. Alt (1930) suggested that this result might be due to the binding of calcium ions by the phosphate. This explanation might apply to the cilia, since, when calcium-free (bicarbonate) Ringer solution was used, a rapid depression followed an initial rise in rate and tubocurarine had no appreciable effect.

The use of Ringer solution containing phosphate in the majority of the experiments of Hill (1957) would therefore explain her failure to confirm the inhibitory action of tubocurarine. However, she also failed to observe the inhibitory action when using a bicarbonate Ringer solution. This failure together with the failure to observe the stimulant action of weak solutions of acetylcholine stands in contrast to the observations of Kordik *et al.* (1952), Bülbring, Burn, and Shelley (1953), Burn and Day (1958), and those reported here in which both these effects have been observed.

Attention has been drawn to the point that the rate of particle transport may be affected by mucus secretion. Hill (1957) stated that, during an experiment, the viscosity of mucus increases until it becomes so tenacious that the cilia can no longer propel particles. She suggested that the rapid cessation of flow observed by Dalhamn (1956) was because he did not moisten the surface. This could not account for the slowing with tubocurarine, for the irrigation of the mucous membrane whether with Ringer solution containing tubocurarine or with Ringer solution alone was performed at regular intervals of about 12 min., and moreover after removal of tubocurarine the original rate was restored.

Kordik *et al.* (1952) investigated the action of tubocurarine on salivary secretion in the cat and found that it had less than 1% of the paralysing action of atropine. Nevertheless tubocurarine was rather more potent than atropine in inhibiting transport by the cilia of the frog oesophagus. Bülbring *et al.* (1953) also reported that tubocurarine slowed the rate of the ciliary beat (observed by a stroboscope) on the edge of an isolated gill filament of *Mytilus edulis* washed by a flow of sea water. In these circumstances mucus secretion could not occur.

I wish to thank Professor J. H. Burn for his guidance throughout this work. I am indebted to Dr. H. Blaschko for his many helpful ideas.

REFERENCES

Alt, H. L. (1930). *Biochem. Z.*, **221**, 498.

Bülbring, E., Burn, J. H., and Shelley, H. (1953). *Proc. roy. Soc. B*, **141**, 445.

Burn, J. H., and Day, M. (1958). *J. Physiol. (Lond.)*, **141**, 520.

Dalhamn, T. (1956). *Acta physiol. scand.*, **36**, Suppl. 123.

Hill, J. R. (1957). *J. Physiol. (Lond.)*, **139**, 157.

Kordik, P., Bülbring, E., and Burn, J. H. (1952). *Brit. J. Pharmacol.*, **7**, 67.