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THE INFLUENCE OF DRUGS ON CILIARY ACTIVITY

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It is well known that the ciliary mechanism plays an important part in protecting the respiratory mucosa against invasion by bacteria (see, for example, Payling Wright, 1954), and a depression of its functional efficiency may have an important bearing on the occurrence of respiratory infections. Kordik, Bülbring & Burn (1952) have reported the effects of a number of drugs on ciliary activity. They made the interesting observation that tubocurarine had a marked inhibitory effect on ciliary activity *in vitro*. The concentration involved was of the same order (1–10 μg/ml.) as the plasma concentration that is used clinically to produce muscular paralysis (Marsh, 1952). In view of the importance of these findings to the anaesthetist it was felt that a fuller investigation of this effect was needed. However, the inhibitory effect described by Kordik *et al.* (1952) could not be demonstrated. The reason for this discrepancy is not certain; but a critical examination of the experimental technique has revealed that artifacts easily arise. Once these are eliminated the ciliated mucous membrane is seen to be remarkably unaffected by tubocurarine even in concentrations as great as 1 mg/ml.

METHODS

The 'activity' of a cilium depends both on its rate (i.e. beats/sec) and its 'force' (i.e. momentum per beat). In the transport of particles these two factors are inseparable, and the combined function is estimated here, by measuring the rate of movement of particles sprinkled on the surface of a ciliated mucous membrane.

The technique used in the preliminary experiments was almost identical with that described by Kordik *et al.* (1952). The preparation, either frog's oesophagus or rabbit's trachea, was dissected from a freshly killed animal. (No drug was used—frogs were pithed, rabbits killed by injecting air intravenously.) The tube was split down the mid dorsal line, and pinned out flat, ciliated surface upwards, on a small waxed cork board, many pins being used to obtain even tension. The preparation was kept moist by dropping physiological saline on to it at intervals so that a capillary layer of fluid covered the mucous membrane all the time. For the frog, Ringer's solution was used, having the following composition (mM): NaCl 115, KCl 2·0, $CaCl_2$ 1·8, buffered with Na phosphate 2 mM, to pH 7·0; for the rabbit, mammalian Ringer's solution (mM): NaCl 145, KCl 5·77, $CaCl_2$ 2·16, buffered with Na phosphate 3 mM to pH 7·2. Carborundum particles were

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sprinkled on to the surface of the mucous membrane, and observed through a binocular dissecting microscope; the time taken for individual particles to traverse the distance between two cross-wires in one eye-piece was taken with a stopwatch. Twelve particles were timed in each set of observations. Carborundum particles were chosen because they are of uniform size, inert, and very easily followed microscopically. The experiments with the frog's oesophagus were done at room temperature (20–24° C); in those with the rabbit's trachea the temperature was maintained at a higher level (35–37° C) by placing the dish containing the preparation on a thermostatically controlled warm stage.

RESULTS

Two typical results obtained with this technique are shown in Fig. 1 *A* and *B*. Each point on the graph represents the mean transit time of twelve particles and is expressed in sec/cm (i.e. reciprocal of velocity). The reason for using particle transit time instead of particle velocity will be discussed later. After each set of observations fresh Ringer's solution was dropped on to the preparation, except that, where indicated, the Ringer's solution contained pure crystalline tubocurarine (Burroughs Wellcome, freshly dissolved); the buffering power of the Ringer's solution was such that the pH of the solution was unaffected by the addition of tubocurarine.

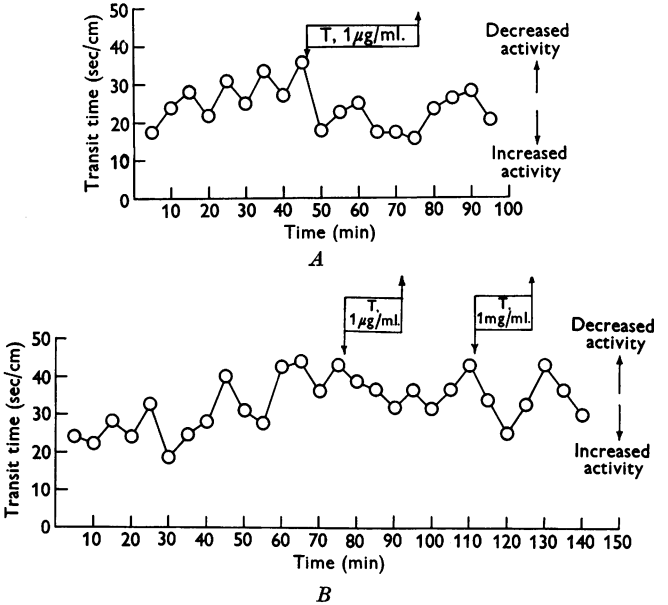

Fig. 1. Frog's oesophagus: *A* and *B* are different preparations. *T*, tubocurarine in concentration indicated, in Ringer's solution, applied during time shown. For further details see text.

Fig. 1 does not reveal any clear depressant effect of tubocurarine (contrast it with Fig. 5, p. 74, of Kordik *et al.* 1952); on the contrary, there seems to be a slight acceleration, though the fluctuations in the base line make it difficult to decide whether this effect is genuine or fortuitous. The fluctuations arise from several causes which will now be discussed; they were largely eliminated

in the later experiments by suitable modification of technique. A statistical treatment was then applied to the results to obtain an accurate estimate of experimental variation. It is clear that a number of seemingly minor details of technique have an extremely important effect on the result obtained.

Counter-currents. The capillary layer of fluid which covers the surface of the mucous membrane is raised above, and therefore isolated from, the capillary layer of fluid on the surface of the cork. Thus, if a current is set up by ciliary action in any particular region of this isolated volume, there must eventually be an equal and opposite current in some other region. This effect is clearly observed in practice, for streams of particles can be seen moving at very different speeds, and even in opposite directions, in different regions of the preparation. Even considering only a particle seen to be moving in the

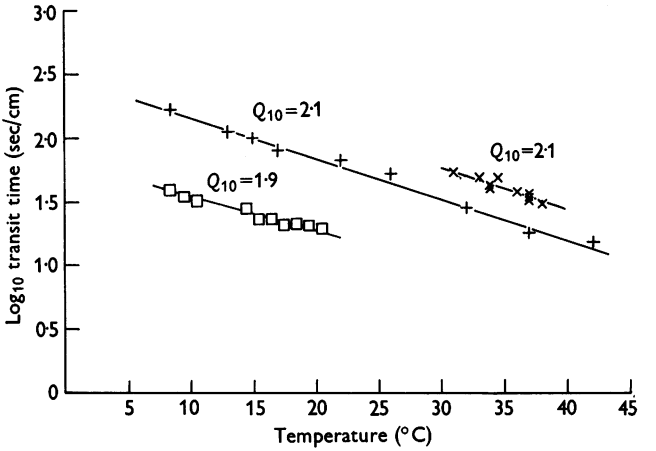

Fig. 2. Temperature coefficient of ciliary activity; semilog. scale. +, Plotted from data for horse's trachea, L. Hill (1928); ×, rabbit's trachea; □, frog's oesophagus.

known direction of the ciliary beat, its velocity will be the resultant of forward propulsion by ciliary activity and movement of fluid in the opposite direction produced by counter-currents near it. The truth of this explanation is confirmed by immersing the preparation 2 or 3 mm below the surface of a bath of Ringer's fluid so that the counter-currents can flow in the bulk of the solution away from the beating cilia and the indicating particles. All evidence of counter-currents then disappears: the forward velocity of particles is more or less uniform at all points on the surface. This modification of technique was adopted for all later experiments. L. Hill (1928) used a similar immersion technique, but he did not discuss his reasons for doing so.

Temperature control. Ciliary activity has a relatively large temperature coefficient. L. Hill (1928) gives data for the influence of temperature on ciliary activity in the horse's trachea which have been plotted in Fig. 2. From the

slope of the line, $Q_{10}=2{\cdot}1$ (i.e. activity increases 7·6%/° C temperature rise). My own experiments on the rabbit's trachea and the frog's oesophagus give similar values for the temperature coefficient (Fig. 2). In view of the high Q_{10} it is clear that special precautions must be taken to ensure that the temperature remains constant. This was accomplished by enclosing the preparation in a double-walled glass chamber with well-stirred water between the walls. This kept the temperature fluctuation to less than $\pm 0{\cdot}5°$ C throughout each experiment.

Mechanical stimulation. Cilia are sensitive to mechanical stimulation (Stewart, 1948; Lucas, 1933). Thus their activity increases if the ciliated surface is lightly brushed, if the solution is agitated or changed; even the addition of test particles may produce a disturbance. Since such disturbance cannot be avoided in these experiments, it was repeated regularly so as to form a stable background to the measurements (e.g. fresh particles were added before each measurement whether they were needed or not).

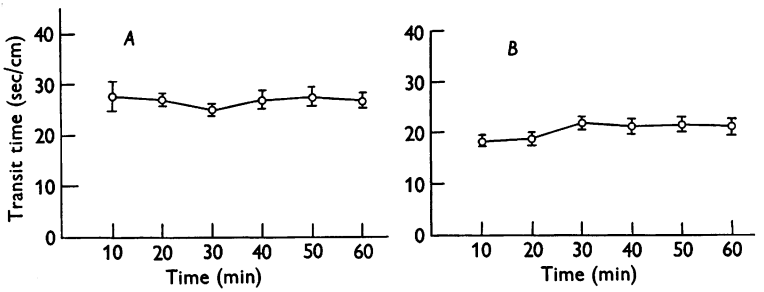

Fig. 3. *A*, rabbit's trachea; *B*, frog's oesophagus. Control series: 5% confidence limits drawn to either side of the means; σ calculated from individuals.

Statistical arrangement. In spite of these precautions there was still some variation between sets of observations in a control series (i.e. no drug applied) on any one preparation, though the variation was now small—compare Fig. 3 with Fig. 1. Part of the residual variation arises from slow changes within the $\pm 0{\cdot}5°$ C limit to which the temperature was controlled. This would be expected, from the Q_{10}, to give rise to a slow variation between means of about 8%. In addition, in some experiments of long duration (see, for example, Fig. 6*B*) there was a small decline in activity over a period of hours.

One must now decide whether this variation in the control series is large enough to obscure a genuine effect of the drug studied. In a number of experiments the standard deviation and standard error of the mean were calculated for each set of 12 observations, so that a 5% confidence limit could be drawn at $2{\cdot}201 \times$ S.E. to either side of the mean ($t=2{\cdot}201$ for 11 degrees of freedom). All the confidence limits in Figs. 3–6 were calculated in this way.

The confidence limit establishes the precision of each estimate of the mean. Consider Fig. 3: *A* and *B* are both controls; the means show genuine fluctuations, since the variation between one mean and the following mean is greater than that set by the confidence limit more often than 1 in 20 times (about 1 in 6 times in Fig. 3). This makes it impossible to lump together all the observations as though they came from the same population; thus in testing for the effect of a drug, it is impossible to apply a simple *t* test to assess whether

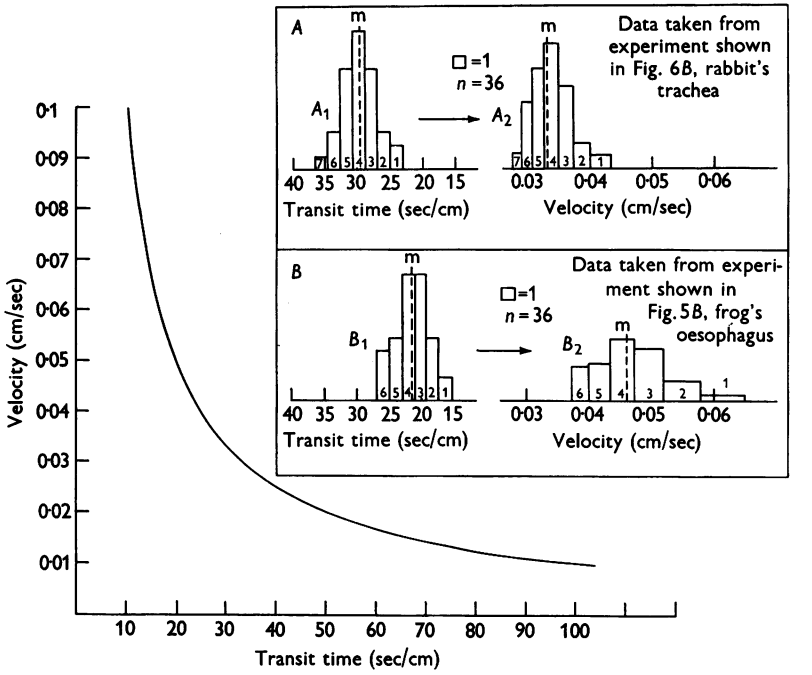

Fig. 4. Relation between velocity and transit time. Inset, frequency distribution diagrams. Area, frequency: abcissae: A_1, B_1, transit time, sec/cm; A_2 B_2, same data after transformation into velocity, cm/sec; data for *A* taken from experiment shown in Fig. 6*B*, rabbit's trachea; data for *B* taken from experiment shown in Fig. 5*B*, frog's oesophagus.

the drug has altered the 'population' mean. Nevertheless, the confidence limit gives a useful impression of the precision of each estimate. The slight variation from one moment to another may conceal a slight effect of the drug, but can hardly conceal an effect large enough to be of pharmacological interest. The biggest effect that tubocurarine 1 mg/ml. could possibly exert is certainly less than $\pm 5\%$ of the total activity.

The measurement obtained experimentally is one of particle transit time, which can, if desired, be converted to particle velocity by referring to Fig. 4. This conversion is only permissible after all statistical tests have been completed. The reason for this is shown clearly in Fig. 4 (inset): transit time is

distributed normally (A_1, B_1) but skewness is introduced by conversion to velocity (A_2, B_2).

Figs. 5 and 6 show typical experiments in which the statistical treatment outlined above was applied. It is obvious from these figures that tubocurarine did not have any marked effect on activity.

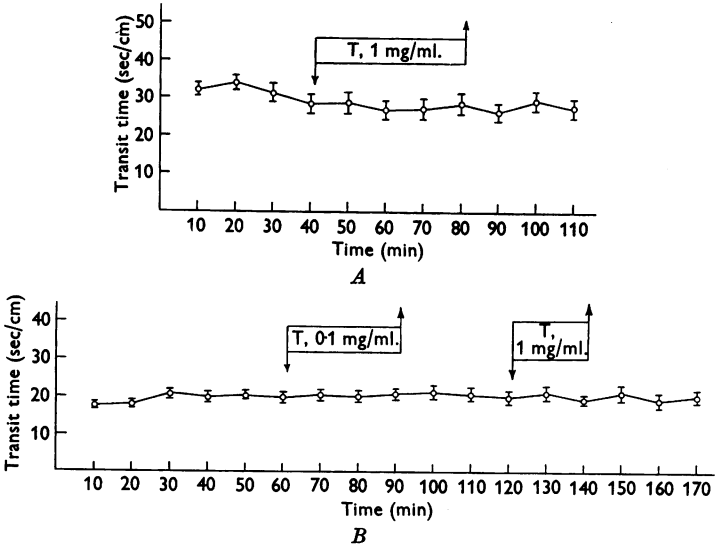

Fig. 5. Frog's oesophagus. T, tubocurarine in concentration indicated, in Ringer's solution, applied during time shown. 5% confidence limits drawn to either side of the means; σ calculated from individuals.

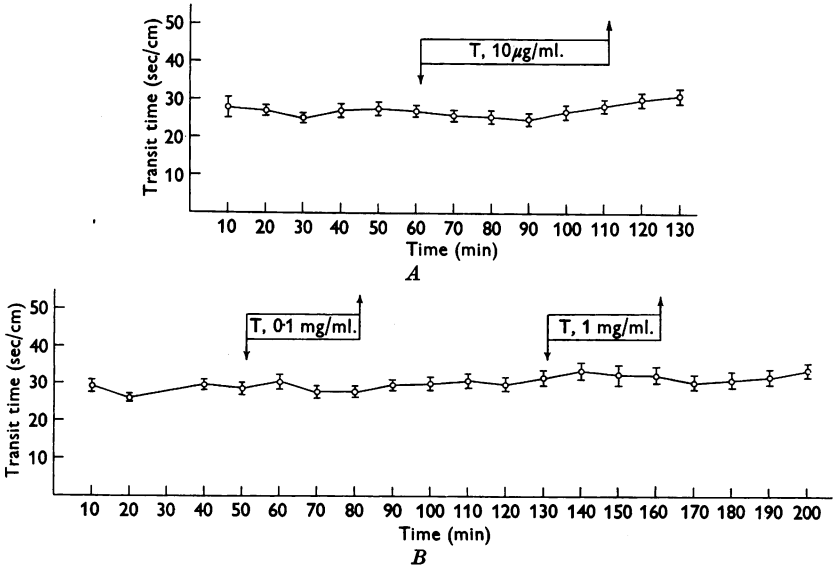

Fig. 6. Rabbit's trachea. Conventions as for Fig. 5.

Since the coefficient of variation is rather stable from time to time and from one preparation to another, with a mean value of about ±3%, the confidence limits for the means in later experiments were based on this figure. The mean was as before, based on twelve observations. This procedure has the advantage that one does not need to note individual times, but only the summed time (an adding stopwatch was used). Sets of observations could thus be made at much shorter intervals than before (every 3–5 min, instead of every 10 min) so that, if the drug had only a transient effect, this would not be missed.

Tubocurarine has been tested on both types of preparation in concentrations of 1, 10 μg/ml, 0·1, 1 and 2 mg/ml., but in no experiment did any concentration of tubocurarine produce significant ciliary inhibition.

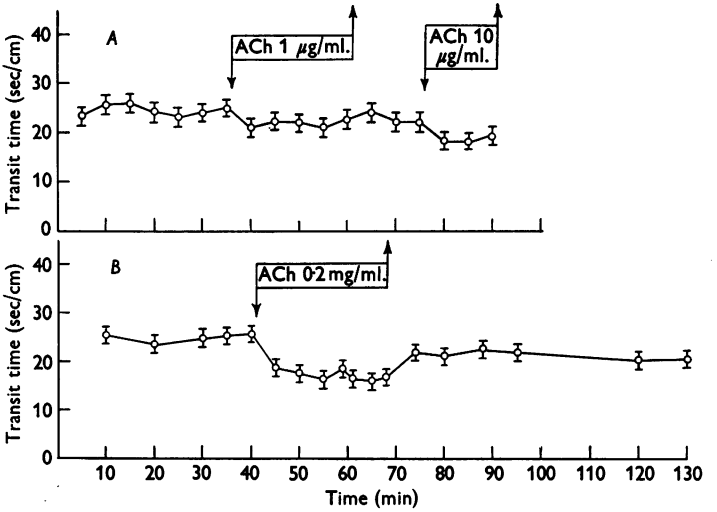

Fig. 7. Frog's oesophagus. Conventions as for Fig. 5. ACh in concentration indicated, in Ringer's solution, applied during interval shown. 5% confidence limits in this case based on mean coefficient of variation—see text.

On the frog's oesophagus further experiments showed that (1) there was no essential difference between the rates of transport of poppy seeds and carborundum particles, whether tubocurarine was present or not; (2) tubocurarine had no inhibitory effect when only a capillary layer of fluid covered the mucous membrane, though the results were more difficult to interpret because of counter currents; (3) tubocurarine was still without effect when either unbuffered Ringer's fluid or Ringer's fluid buffered with bicarbonate and CO_2 was used.

A few experiments were done in which the effect of acetylcholine on the activity of frog's oesophagus cilia was tested (see Fig. 7). (The salt was used, bromide and chloride both gave similar results.) No appreciable effect was obtained with concentrations of 1μg–0·1mg/ml.; at a concentration of

0·2 mg/ml. however this drug did have a significant accelerating effect (see Fig. 7). Removal of the drug solution was not followed by a transient reduction in ciliary activity to below its control level such as was reported by Kordik *et al.* (1952). The possibility that acetylcholine had been destroyed was excluded by testing the solutions on a frog's rectus at the end of the experiment.

DISCUSSION

A reliable method for estimating ciliary activity is of great practical importance to the study of the physiology of cilia. Many attempts have been made to use the rate of transport of particles over the surface of a ciliated mucous membrane as an index of its activity; see, for example, Lommel (1908), Henderson & Taylor (1910), Hach (1925), L. Hill (1928), Lucas & Douglas (1934), Barclay & Franklin (1937), Stewart (1948). However, the results obtained were generally unsatisfactory; only Hill and Stewart seem to have obtained fairly consistent quantitative results. The difficulties experienced have led to some confusion and a good deal of discussion about the best technique to use: for example Dalhamn (1956), in a recent extensive study, was led to abandon experiments with isolated membranes, apparently because of inconsistency in the results. However, once the factors that influence ciliary activity have been adequately controlled, consistent results can be obtained *in vitro*. Indeed the consistency of the measurements obtained is very striking. The variation between different preparations is not greater than the variation during a single experiment. Even more remarkable, the absolute velocity is about the same in the frog's oesophagus and the rabbit's trachea so long as both are at their normal working temperature (18–20° C and 35–37° C respectively). Perhaps at this velocity (0·03–0·05 cm/sec) the transfer of power from beating cilia to fluid is most effective. L. Hill (1928) gives similar figures for optimal velocity.

The isolated mucous membrane has the added advantages of convenience and ease of preparation; also when using it to test the effect of drugs on ciliary activity one is not bothered by any possible action of the drug on the local circulation.

The discrepancy between the results reported here and those of Kordik *et al.* (1952) seems to arise from a small difference in the experimental conditions. In their experiments the mucous membrane is kept in a moist atmosphere, and Ringer's fluid is applied to its surface from time to time; whereas in mine the mucous membrane is completely immersed in Ringer's fluid. In the latter case particle transport continues for at least three days; while in the former, activity declines fairly rapidly. In Dalhamn's (1956) experiments a rat's trachea was exposed to an atmosphere saturated with water vapour, and two independent estimates of ciliary activity were made almost simultaneously by observing both mucus flow rate and ciliary beating (by high

speed photomicrography). When all ciliary activity had apparently stopped as shown by cessation of mucus flow, ciliary beating was unchanged (see his tables, pp. 51 and 61). Cessation of mucus flow occurred generally within 14 min of extirpation in experiments *in vitro* and about 2 hr after opening the trachea in experiments *in vivo*.

It seems likely that the viscosity of the mucus increases in spite of the moist atmosphere until it becomes so tenacious that the cilia are unable to propel it along, though they continue to beat actively. The fact that Dalhamn (1956) did not moisten the surface of the mucous membrane might account for the rapidity with which the mucus flow stopped. Moistening the mucous membrane as in the experiments of Kordik *et al.* (1952) would certainly retard an increase in viscosity of mucus but might not prevent it altogether.

Since ciliary activity was unaffected by tubocurarine, it is highly improbable that this drug will produce ciliary depression when given clinically to produce muscular relaxation during anaesthesia. Ciliary activity appears to be relatively unaffected by many drugs that have powerful physiological effects. This resistance of ciliated mucous membranes to the action of drugs has been noted in the past. The pioneer investigations of Purkinje & Valentin (1835) more than a century ago showed that 'the ciliary motions are not affected by prussic acid, extract of aloes or belladonna, catechu, musk, acetate of morphia, opium, salicin, strychnine, nor by decoction of capsicum, even though most concentrated solutions of these substances be used'. L. Hill (1928) reported that adrenaline, acetylcholine and histamine had no effect on tracheal cilia. Cocaine solutions also have no effect on ciliary activity (Lierle & Moore, 1935; Kordik *et al.* 1952). Many of these drugs act predominantly at the cell surface, and the fact that they have no action on ciliary activity suggests that the ciliary contraction, unlike that of muscle, is not initiated at the cell surface.

SUMMARY

1. Ciliary activity in the frog's oesophagus and the rabbit's trachea was measured *in vitro* by a particle transport method.

2. When the factors which influence ciliary activity were properly controlled consistent measurements were obtained. The preparation must be fully immersed to prevent counter currents which cause serious inconsistencies.

3. Tubocurarine had no demonstrable effect on ciliary activity, even in a concentration as great as 2 mg/ml.

4. Acetylcholine had no clear action on ciliary activity in the frog's oesophagus in concentrations from 1 μg to 0·1 mg/ml., but at a concentration of 0·2 mg/ml. ciliary activity was slightly increased.

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