NEUROID TRANSMISSION IN CILIATED EPITHELIUM.

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(Accepted for publication, January 17, 1925.)

I.

INTRODUCTION.

A study of cilia raises the question to what extent their activity is determined directly by the physicochemical conditions of the environment, and to what extent it is in physiological correlation with other activities of the organism. There is undoubted evidence that certain cilia act in relative independence of the rest of the animal to which they belong. Such cilia are indifferent to removal of the ciliated tissue, but show great susceptibility to changes of the environment.

Gray (1920a; 1920b) in two recent papers, has given an account of cilia on the gills of Mytilus. Cilia on excised gills continue undisturbed beating for several days when kept in sea water. Yet with change of the pH value of the sea water from 5.5 to 6.5 the cilia show an immediate suspension of beating. If this critical pH value is not overstepped, restoration of hydroxyl ions is followed by resumption of activity. If on the other hand the pH is carried above 9, the epithelium breaks up, though the cilia continue to beat. Furthermore, the presence of sodium, potassium, calcium, and magnesium in certain defined amounts is necessary for normal beating. Von Brücke (1916) demonstrated in a striking way the independence and autonomy of cilia of the epithelium of the frog's mouth. A piece of epithelium was removed, turned through ninety degrees, and replaced. The tissue so treated continued to live and its cilia to beat normally. They did not, however, orient themselves to their new position, but continued their old line of beat, at right angles to the surrounding cilia.

There is at the same time a coordination of all the cilia in the same row, whereby each cilium is slightly in advance in phase of the cilium next below it in the line of beat, regardless of the rate of the cilia. This condition has been studied by Kraft (1890) in the ciliated epithelium of the pharynx of the frog. In his observations he determined the rate of cilia by microscopic inspection, carrying out his experiments on the stage of the microscope. He confirmed Grützner's observation that
injury to Oberzellen (i.e., cells relatively high up in a row of beating cilia) caused inhibition of Unterzellen (cells below them in the line of beat), as well as the local inhibition. On the other hand he failed to find any inhibition of Oberzellen following injury to Unterzellen. He also substantiated König's work showing that facilitation of Oberzellen, produced by gentle stroking with a fine brush, was followed by increase in rate of beat of the Unterzellen, though similar stimulation of Unterzellen had a much more delayed and less marked effect on Oberzellen. Dropping a fragment of cork on the tissue likewise produced both local stimulation and stimulation of Unterzellen, though Oberzellen were scarcely affected.

In all these experiments cilia to one side of the row in question were uninfluenced. To answer the question whether this effect of Oberzellen on Unterzellen is merely mechanical or represents true physiological coordination, Kraft made use of a small box, 30 mm. by 10 mm. by 15 mm., with a glass bottom, brass sides, and a top of thin cover-glass. By two glass partitions at right angles to the longer side and to the bottom, the box was divided into three chambers, the middle one 5 mm. wide. The whole box could be placed on the stage of the microscope and water of desired temperatures kept circulating through the three chambers. A piece of epithelium could be then stretched over the cover-glass and the behavior of the cilia observed microscopically under different conditions. In one set of experiments the epithelium was arranged with its rows of cilia at right angles to the partitions, and water at 0°C. was passed through the middle chamber while water at 10-12°C. circulated through the end chambers. Under these conditions cilia over the end chambers maintained a slow beating; those over the middle chamber were quiescent. If now cilia over the upper end chamber (with reference to the line of beat) were stimulated by gentle stroking with a brush, cilia over the lower end chamber showed a corresponding increase of activity, though cilia over the middle chamber continued inactive as before. If a stimulating temperature of 15°C. in the upper chamber was substituted for the mechanical stimulus the results were the same except that in this case the cilia over the middle chamber also became active, though more slowly, and they themselves in turn produced further stimulation of the cilia over the lower chamber. In another set of experiments water of 0°C. was passed through both end chambers and water of 20°C. through the middle chamber. In this case the cilia over the lower chamber showed a marked increase of beat under the influence of the warm stimulus applied to the cilia above them, but cilia over the upper chamber showed only a very slight increase. As a result of these experiments Kraft concluded: (1) that cilia are not subject to the control of the nervous system; (2) that there is an inner, physiological conduction of the effects of a stimulus from Oberzellen to Unterzellen but not in the reverse direction nor laterally; and (3) that there is a slight mechanical effect on adjacent cilia produced by cilia under stimulation. He distinguishes "eine äussere, mechanische Leitung" and "eine innere, nervenartige Leitung der Eregung."
Engelmann (1898) in his article on cilia recognizes this physiological interconnection between ciliated cells, though he does not regard it as thus limited in direction. He says, "Il est évident que ce phénomène doit être rapproché de la conduction dans le cœur et dans les muscles lisses, où la conduction de l'excitant physiologique se fait par la propagation d'une action moléculaire entre cellules excitables, placées au contact les unes des autres."

Other evidence recently brought forward indicates that in some animals there is a control of cilia by the nervous system. Göthlin (1920), working on *Beroe* demonstrated that by use of mild electrical currents, 1 to 2 ma./ sq. cm., inhibition of the swimming plates could be produced without contraction of the meridional rows. The same effect was produced by gentle mechanical stimulation of the edges of the mouth and of the polar fields, and was observed even after removal of the sense body at the aboral end. Anesthesia with chloral hydrate, which did not interfere with the beating of the swimming plates, prevented this inhibition. Moreover swimming plates on fragments of epithelium separated from the underlying tissue showed no response to the inhibitory stimuli, though in cases where the underlying tissue was present inhibition was observed. From this Göthlin concluded that the swimming plates are under control of a nervous mechanism, and that the stimulus, acting directly on epithelial sense organs, affects the swimming plates only indirectly, by way of this nervous mechanism. Anesthesia or isolation, eliminating nervous processes, renders the swimming plates indifferent to the stimulus.

Copeland (1919, 1922) maintains that there is nervous control of cilia on the feet of certain mollusks. His observations are on *Alectrion trivittata, Alectrion obsoleta, Polinices heros*, and *Polinices duplicata*. He shows that the pedal cilia of these snails are associated with locomotion, and beat in coordination over the whole foot. In *Alectrion* stimulation of one of the pedal tentacles produces immediate activity of all the cilia on the foot. In *Polinices* the pedal cilia all become active simultaneously as if excited by an outside (nervous) impulse. In this animal cilia over an area isolated by cutting from adjacent cilia show no alteration in beat if their connections with the underlying tissue are not disturbed. On the other hand, isolation of such an area from the subjacent tissue, connection with the surrounding tissue being left intact, produces loss of normal activity. Copeland regards these facts as indicating nervous control of the pedal cilia of these snails.

Thus there are three groups of experiments which emphasize respectively (1) relative independence of ciliary activity of other organic processes, (2) interconnection of different parts of the same ciliated epithelium, and (3) relations of ciliary activity to the rest of the organism, in particular through the nervous system.
NEUROID TRANSMISSION

II.

Method and Apparatus.

In the following experiments a study was made of the correlation in rate of beat of the cilia on the gill of *Unio*, and of the supposed transmission by means of which such correlation is maintained, a transmission which Kraft described as "eine innere, nervenartige Leitung" and which together with some other types of primitive conduction has been more recently designated "neuroid transmission" (Parker, 1910). These cilia cover both surfaces of both inner and outer gills, serving to drive water through the gills and to convey the extracted food particles. Those on the outer gill beat upward, carrying the particles to the upper edge of that gill, where they are received by other, specialized cilia and swept forward in a groove-like depression to the mouth region, there to be received by the palps. Those on the inner gill beat downward, driving the food fragments to the lower margin of that gill, where they suffer a similar fate. In the following work only the inner gills were used since these were larger and since in the case of females, which predominated in the animals brought to the laboratory, the outer gills were filled with eggs. The gill to be examined was removed from the animal and allowed to remain half an hour or more in tap water until the excess mucus secreted in consequence of cutting was swept off and until the possible mechanical effects of handling might have subsided.

It was then tested for conduction of the stimulating effects of different temperatures with the apparatus and in the manner to be described. A rectangular depression, of length and width corresponding approximately with the dimensions of the larger gills, was hollowed out of a block of wood, lined with wax, and divided into two chambers by a slate partition at right angles to the longer sides (Fig. 1). Through the opposite walls of each chamber opened an inlet and an outlet formed of tight fitting glass tubing. The chambers were sealed on top either with a large cover-glass cemented down with a mixture of bees-wax and rosin or with a thin sheet of mica fastened down with shellac. This latter arrangement was found to be more satisfactory and was used in the later experiments. The chambers were thus rendered water-tight save for outlets and inlets. On the
outside around the edges of the cover-glass or sheet of mica a low bulwark of wax was erected so that the upper surface of the glass or mica might be flooded with a thin layer of water.

During an experiment, water of desired temperatures was syphoned through the chambers and any trapped air removed at the start by tipping the apparatus. The gill was laid in the desired position over the chambers and covered with a thin sheet of water to facilitate the

![Diagram of the apparatus](image)

**Fig. 1.** A wooden block partly hollowed out above and arranged to apply temperature changes to the gill of *Unio*. The two chambers, A and B, are separated by a narrow partition and sealed above by a thin sheet of mica or by a cover-glass. The piece of gill was laid on this mica or cover-glass and flooded with a small amount of water which was retained by the surrounding low barrier. The temperatures of the two chambers were maintained by currents of water led through them as indicated by the arrows. These temperatures affected the gill which was placed on the mica or cover-glass immediately above the two chambers and partly over each.

action of the cilia. A light fragment of a mm. rule was held over the surface of the gill, parallel to the rows of cilia, and grains of carmine were dropped near it on the gill. The time required for a particle to travel 1 cm. under the influence of the cilia was recorded with a stop-watch. An average of several such times was taken as representing the rate of the cilia under the given condition. Since the syphons could be changed without emptying the chambers the whole arrangement was left nearly undisturbed throughout the experiment.
III.

EXPERIMENTAL RESULTS.

In the first set of experiments an excised gill was laid on the cover-glass, overlapping both chambers and with its rows of cilia at right angles to the partition. The upper two-thirds of the gill, on which the rate of ciliary beat was observed, overlay the chamber kept at a constant low temperature. The lower third overlay the other chamber, whose temperature was alternated between warm and cold. It was found that the cilia under observation beat faster when the chamber under the lower third of the gill was warm than when it was cold. In another set of experiments the cilia on the lower two-thirds of the gill were placed over the constant chamber and their rate of beat was recorded during warm-cold fluctuations of the other chamber. The same changes in rate corresponding to changes in temperatures was observed as in the previous experiments. The changes in the observed rates and temperatures are shown in Figs. 2 and 3.

The gill was then placed, overlapping both chambers with its rows of cilia, parallel to the partition. Cilia overlaying the chamber that was kept at a constant low temperature were then observed while the temperature of the other chamber was varied between cold and warm. The distance of the rows of observed cilia from the partition was between 4 and 8 mm. The same fluctuations in rate corresponding to changes in temperature of the variable chamber were observed. The results are shown in Fig. 4. As in the two previous cases a study was made of the maximum distance from the partition at which this effect was observable. It was found that the fluctuations did not occur beyond 9 to 11 mm. from the partition. A typical set of three readings taken at points within 11 mm. of the partition is given in Fig. 5.

In order to check these results a gill was placed upon the cover-glass as before with its rows of cilia parallel to the partition. The rate of beat of cilia over the chamber kept at constant temperature was then observed in the usual manner during several alternations of the temperature of the other chamber. If the gill showed the usual changes in rate corresponding to the alternating changes in
FIG. 2. Changes in average rates of movement, cm. per minute, shown by particles of carmine driven by the cilia of the gill of *Unio* in which the region of the gill under observation was kept at 10°C. and an adjacent region lower in the line of ciliary beat was subjected to temperatures alternately low (about 10°C.) and high (about 30°C.). In no instance did the probable error of the observations upon which each average was based exceed 3.1 per cent of such average.

FIG. 3. Changes in average rates of movement, cm. per minute, shown by particles of carmine driven by the cilia of the gill of *Unio* in which the region of the gill under observation was kept at 7.5°C. and an adjacent region higher in the line of ciliary beat was subjected to temperatures alternately low (about 8°C.) and high (about 30°C.). In no instance did the probable error of the observations upon which each average was based exceed 2.7 per cent of such average.
FIG. 4. Changes in average rates of movement, cm. per minute, shown by particles of carmine driven by the cilia of the gill of Unio in which the region of the gill under observation was kept at 8°C. and an adjacent region to one side of the line of ciliary beat was subjected to temperatures alternately low (about 10°C.) and high (about 30°C.). The near edge of the observed region was from 5 to 8 mm. distant from that of the other region. In no instance did the probable error of the observations upon which each average was based exceed 4.6 per cent of such averages.

FIG. 5. Changes in average rates of movement, cm. per minute, shown by particles of carmine driven by the cilia of the gill of Unio in which the region of the gill under observation was kept at from 10-12°C. and an adjacent region to one side of the line of ciliary beat was subjected to temperatures alternately low (about 11°C.) and high (about 30°C.). The near edge of the observed region was distant from that of the other region in one set of observations (A) 20 to 22 mm., in a second (B) 14 to 16 mm., and in third (C) 9 to 11 mm. In no instance did the probable error of the observations upon which each average was based exceed 2 per cent of such averages.
temperature it was regarded as normal. Thereupon, a cut was made completely across the gill, parallel to the partition and a little to one side of it over the constant chamber so as to sever all organic connection between the region of alternating temperatures and the cilia under observation. The alternations of temperature on the variable side were continued, and the readings were made as usual on the same cilia as before. Under these conditions there was no longer any significant change in ciliary rate corresponding to changes in temperature of the variable chamber. Typical readings are given in Fig. 6.

![Graph showing changes in average rates of movement, cm. per minute, driven by the cilia of the gill of Unio in which the region of the gill under observation was kept at 9°C. and an adjacent region to one side of the line of ciliary beat was subjected to temperatures alternately low (about 10°C.) and high (about 28°C.). After six observations had been made the gill was cut in two between the region of observation and the other region and five more observations were made. The cut is indicated on the graph by the vertical double line. In no instance did the probable error of the observations upon which each average was based exceed 2.3 per cent of such average.

Throughout these experiments the temperature of the constant chamber had been low, equal to the low temperature alternating with the high, on the variable side. The question remained open, therefore, whether it was the effect of the cold, or the warmth, or both that was transmitted across the gill to produce the fluctuations observed. To test this point the temperature of the constant chamber was made the same as the warm alternating temperature. In this case it was assumed that cilia directly subjected to the warm stimulus would not be further excited by the same stimulus applied at a distance and acting only indirectly, so that any alternations of the rate must be due to the effects of the cold chamber, transmitted
FIG. 7. Changes in average rates of movement, cm. per minute, shown by particles of carmine driven by the cilia of the gill of *Unio* in which the region of the gill under observation was kept at the relatively high temperature of 29°C. and an adjacent region to one side of the line of ciliary beat was subjected to temperatures alternately low (about 8°C.) and high (about 28°C.). The near edge of the observed region was from 5 to 8 mm. distant from that of the other region. In no instance did the probable error of the observations upon which each average was based exceed 3.9 per cent of such average.

FIG. 8. Changes in average rates of movement, cm. per minute, shown by particles of carmine driven by the cilia of the gill of *Unio* in which the region of the gill under observation was kept at the medium temperature of 15°C. and an adjacent region to one side of the line of ciliary beat was subjected to temperatures alternately low (about 4.5°C.) and high (about 30°C.). The near edge of the observed region was from 5 to 8 mm. distant from that of the other region. In no instance did the probable error of the observations upon which each average was based exceed 3.6 per cent of such average.
through the gill. The results showed that under these conditions there was no significant fluctuation in ciliary rate corresponding with the temperature alternations of the variable side (Fig. 7). If, however, the constant temperature was midway between that of the cold and the warm the usual fluctuations were observed: Typical results are given in Fig. 8.

IV.

DISCUSSION.

It may be objected that these results are vitiated by the possibility of a spread of the warmth through the cover-glass, or gill, or both to the supposed constant area under observation. An answer to this objection was made possible by the kindness of Dr. F. G. Benedict, who offered the use of his thermopile for the measurement of the temperature gradient both on the bare cover-glass and on the gill over the region concerned in the experiment. These measurements showed a greater temperature uniformity on each side of the partition in the case of the bare cover-glass than in the case of the gill, but in either case uniformity sufficient to warrant confidence in the results. In the case of the bare cover-glass, if the temperature of the cold side at a point 8 mm. from the partition is represented by C, the gradient taken at 2 mm. intervals across the glass surface to a point 8 mm. from the partition on the warm side may be indicated as follows: cold side, 8 mm. C, 6 mm. C +0.18°, 4 mm. C +0.11°, 2 mm. C +0.5°; over the partition, C +3.5°; warm side, 2 mm. C +8.0°, 4 mm. C +9.0°, 6 mm. C +9.7°, 8 mm. C +10°. In the case of the gill, by using the same notation, the gradient may be expressed thus: cold side, 8 mm. C, 6 mm. C −0.21°, 4 mm. C +0.14°, 2 mm. C +2.21°; over the partition, C +4.76°; warm side, 2 mm.: C +13.6°, 4 mm. C +14.42°, 6 mm. C +15.19°, 8 mm. C +15.54°. It is evident that points more than 4 mm. from the partition on the cold side are not appreciably affected in temperature by the warm water circulating through the opposite chamber. It was at such points that the positive results were obtained; these results are not, therefore, invalidated by a spread of heat. The uniformity of the warm side is somewhat less pronounced, but as the results here were
negative *despite* any possible spread of heat they may be safely accepted.

These results reveal the fact that the temperature difference between the two sides of the cover-glass, as well as between the two sides of the gill on the cover-glass, is considerably less than the difference between the two streams of water circulating through the two chambers, so that the effective stimulus is less than what would be supposed from an inspection of the tables. This is due to the fact that the upper surface of the cover-glass or gill is in contact with the air, a condition which tends to equalize temperature differences on the two sides.

In judging these results it should be kept in mind that the rate of the cilia, as measured by the speed of the carmine particles, shows considerable variation, probably due to interference of mucus, even when the conditions of the environment are unchanged. The fluctuation of rate of beat accompanying the regular alternation of cold and warm is thus superposed on an irregular base line and itself appears irregular, though in reality it may not be so. In addition, variations in size and shape of the particles of carmine probably introduce further irregularities. Allowance must be made for these factors.

As to the nature of the conduction little may be said except that it is an example of the type of transmission termed "neuroid." A similar condition may be observed in a finger of the sponge *Stylostella*. If a cut be made on a finger of this sponge within 1.5 cm. of the osculum, the osculum is observed to close after a brief delay, indicating the reception of an impulse spreading out from the region of injury. The sponge is, however, without nervous tissue (Parker, 1910).

The conduction recorded in these experiments differs from that investigated by Kraft (1890) in the epithelium of the frog, for it is unlimited in direction, whereas that of the frog proceeds only from *Oberszellen* to *Unterszellen*. The two are alike, however, in their relation to temperature. By both the facilitating effects of warmth are transmitted but not the inhibitory effects of cold. Kraft does not make this statement explicitly in his paper, but it is evident from his results. For when the upper of his three chambers was kept
between 12° and 15°, the middle one between 0° and 2°, and the lower one between 12° and 15°, cilia over the upper and lower chambers showed a sluggish beating and those over the middle chamber remained nearly quiescent. But when the temperature of the upper chamber was raised to 20°, cilia over both upper and lower chambers showed rapid increase of beating. Here cilia over the lower chamber were influenced by two stimuli applied above them, one cold and one warm, each differing by the same number of degrees from the temperature of the lower chamber, the cold, however, being nearer than the warmth. Nevertheless the warmth is the effective one. Moreover, at the beginning of the experiment cilia over upper and lower chambers have the same rate of beat, though the cold of the central chamber can be transmitted only to the Unterzellen, if it is transmitted at all, according to Kraft's results. Hence Kraft's results also point to the transmission of the effects of warmth but not of cold.

In this connection it may be noted that in the case of the thermoreceptors in the skin of man there are two sets of end organs, one responsive to cold and the other to heat.

The results obtained on the gills of Unio may be explained after analogy with the vertebrate heart, where the fastest beating chamber, the venous sinus, controls directly the rate of beat of the other chambers, which are inherently slower. In the same way the fastest beating cilia seem partially to determine the rate of adjacent, naturally slower cilia. This may be attributed to the stimulation of adjacent cells by the action current of their more active neighbors. Such a view is supported by work of R. S. Lillie (1914) on the swimming plates of ctenophores. In these animals successive waves of coordinated beating arise from the aboral end of a row of plates and pass down over the entire row. If, now, such a row with underlying tissue, be excised and placed in sea water, it will curl up so that the opposite ends are juxtaposed though no organic connection exists between them. Under these conditions Lillie maintains that the wave of beating, arising from the aboral end and passing down the row, jumps across the gap, and continues round and round in this way indefinitely. He explains this jump across the interruption as due to the stimulating effect of the action current of the swimming.
plates of the oral end of the row on the adjacent plates of the aboral end. This explanation of conduction in ciliated epithelium would not, however, be in accord with the observation of Kraft that the excitatory effect of a stimulus may be transmitted through a region of quiescent cilia to excite other cilia beyond. This fact demands some other explanation of the mechanism of conduction, at least in the frog.

V.

CONCLUSIONS.

Evidence from several sources indicates that there is present in certain types of ciliated tissue a primitive form of conduction regulating the rate of beat of the cilia. (Kraft, 1890; Engelmann, 1898). A similar type of conduction has been observed by Parker (1910) in the sponge Stylotella. In the foregoing experiments a study was made of such conduction in the ciliated tissue of the gills of the clam Unio. Observations were made on the effects of temperature changes on the rate of beat of cilia adjacent to areas not themselves directly influenced. The following results were obtained.

1. The transmission through the gill of the effects of warmth applied locally is apparent through increased rate of ciliary beat on adjacent gill tissue in all directions from the region of application. Effects are not observed laterally at a distance greater than 9 to 11 mm. from the nearest edge of the stimulated area. The narrowness of the gill makes it impossible to determine the vertical limits of the transmission.

2. Effects of low temperatures are not observable beyond the limits of the region of direct application.

These results differ from those of Kraft in tissue from the frog's pharynx, where conduction was shown to take place only down a row of beating cilia. On the other hand they agree with his results in indicating that the effects of warmth only are transmitted.

The phenomenon might be explained by the stimulating effect of the action current of the directly excited cilia on the neighboring relatively quiet cilia. A similar explanation has been offered by Lillie for waves of coordinated beating in the rows of swimming plates of
ctenophores. Such an explanation, though in accord with the work on *Unio*, is inconsistent with certain of the observations of Kraft on the tissue from the frog's pharynx.

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