THE REACTIONS OF ISOLATED PARTS OF *SPIROSTOMUM*

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(With One Text-figure)

The determination of the reactions and behaviour of isolated fragments of a protozoan is of value to the study of three problems, namely, the relationship between the nucleus and behaviour, the detection of any area in the organism specialized for the reception of external stimuli, and in the case of a ciliate, the role played in normal swimming by the cilia of different regions of the body. Recently, an account has been given of the reactions of fragments of *Amoeba proteus* (Clark, 1944). The present paper is an extension of the work to include a ciliated form.

The behaviour of ciliate fragments was first studied by Balbiani (1889) and Verworn (1901). Neither author gives a detailed account of the work, but they agree that, in general, the fragments move and behave in much the same way as the entire organisms. Jennings & Jamieson (1902) examined the effects of chemical and mechanical stimuli on anterior and posterior fragments of *Stylonichia, Oxytricha, Stentor, Spirostomum* and *Paramecium*. They concluded that: 'As any portion of a crystal is organized like the entire crystal, so in the Ciliata any piece of the body, apparently, is organized so as to move and react to stimuli in the same manner as does the entire animal.'

Alverdes (1922, 1923) believes that in *Paramecium* the reception of thermal and chemical stimuli is confined to the extreme anterior end, and that *Spirostomum* and *Stentor* show sensitivity to thermal stimuli only at the anterior end but to chemical stimuli over the entire body surface. More recently, Horton (1935) determined the sensitivity of anterior and posterior halves of *Paramecium* to weak-acid stimulation but failed to confirm Alverdes's claim that only anterior fragments are responsive to chemical stimulation. He has also observed that, contrary to earlier accounts, posterior halves are more sensitive to acid than anterior halves, and attributes this to the greater permeability to acid of the newly formed pellicle at the anterior surface of the posterior fragment, as compared with the permeability of the undamaged pellicle at the front end of the anterior fragment. In connexion with the relative importance of regional cilia for swimming, he found that fragments lacking the oral cilia swim spirally like intact animals, indicating that the spiral track traced out by *Paramecium* '...is not dependent on the shape or on the action of the oral groove cilia,

but is produced by the co-ordinated activity of the body cilia'.

Two strains (S and SS) have been used in the present work, corresponding to the *major* and *minor* varieties of *Spirostomum ambiguum* (see Bishop, 1923). 'Wheat' cultures were used, to which two rat faecal pellets were added to every 30 c.c. of medium. Specht (1935) and Seyd (1937) have both reported favourably on the addition of mammalian faeces to cultures of *Spirostomum*.

To designate the levels at which cuts were made and localized stimuli applied, points were chosen at quarter-length intervals (see Fig. 1). Points 1 and 5 were the extreme anterior and posterior ends. Point 3 was the estimated middle of the body. The mouth generally occupied a position half way between points 3 and 4. Point 2 was estimated solely by comparison with points 1 and 3, there being at this level no morphological structure to which reference can be made. For further details on the morphology, reference should be made to the papers of Bishop (1923, 1928).

THE RESPONSES OF WHOLE ANIMALS TO GENERAL AND LOCALIZED MECHANICAL STIMULI

If an intact *Spirostomum* is subjected to a non-localized stimulus, e.g. by tapping gently against the edge of the container, the organism contracts strongly, darts back a little and then gradually relaxes, revolving on its long axis in a clockwise direction (facing the anterior end), and maintaining the reversal in the effective beat of the cilia for several seconds. When relaxation is complete, the anterior end swings out towards the aboral side, and forward swimming is resumed. If the stimulus is weak, temporary reversal may occur without any contraction of the myonemes.

Localized mechanical stimuli, applied by means of a glass needle, were separated by irregular time intervals of from one to five minutes and in different animals were applied according to different systems. In any one experiment, ten stimuli were localized at each of the points from 1 to 5, giving a total of 50. In some cases they were applied systematically, in a numerical sequence, i.e. ten at point 1, ten at point 2, ... and so to point 5. In others, they were

a applied systematically but in reversed order, commencing with ten at point 5, ten at point 4, until point 1 was reached. Finally, in a third series, the localization followed no regular system at all, each point receiving a total of about ten stimuli administered at random, according to which point was most conveniently situated with regard to the tip of the needle at the instant of stimulation.

A total of thirty individuals was examined in this manner and yielded quite consistent results. Generally, stimuli applied at points 1, 2 and 3 result in contraction and temporary ciliary reversal, while stimuli localized at points 4 and 5 produce contraction accompanied by swimming forwards. In a very small number of cases, as Jennings (1899a) has noted, a stimulus at the anterior end produces only contraction, the animal remaining stationary for a few moments while relaxation of the myonemes is taking place. The important point is that, in the

contraction and ciliary reversal both take place (e.g. as a result of a general stimulus, or one localized at points 1, 2 or 3) during the subsequent backward movement and relaxation, the rotation is clockwise. Precisely the same points hold for fragments. Rotation on the long axis is anticlockwise for all cases except that of contraction, followed by swimming backwards, when it is clockwise.

On contact with the glass thread, the organism contracts strongly. After the transection has been made, the anterior portion reverses and moves backwards in a wide spiral for a quarter of a minute or more. If the fragment is very small the path traced out is so compact a spiral as to be almost circular. On the other hand, larger fragments tend to move more in a straight line, revolving on the long axis the while. Owing to the slight curvature of the body, the posterior end of the fragment traces out a narrow spiral. Within a minute or so, the ciliary beat again
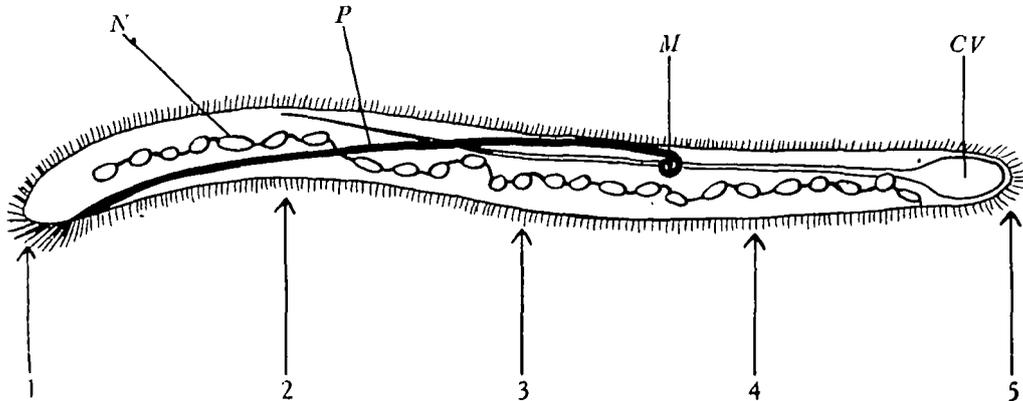

Fig. 1. Diagram of *S. ambiguum*, illustrating the essential morphological features and the points at which localized stimuli were applied and transections carried out. CV = contractile vacuole; M = mouth; N = macronucleus; P = peristome.

vast majority of cases, the organism definitely responds in relation to the localization of the stimulus. The response consists essentially of movement away from the source of the stimulus. Both Jennings (1899a) and Alverdes (1923) have noticed the greater sensitivity of the anterior end, which appears to have a lower threshold than the posterior end to a variety of stimuli. This feature, characteristic of many other ciliates, will be returned to when considering the reactions of anterior and posterior fragments.

THE RESPONSES OF FRAGMENTS TO MECHANICAL STIMULI

While swimming forward, the intact organism revolves slowly on its long axis in an anticlockwise direction, with the result that the anterior end traces out a narrow spiral. If a temporary ciliary reversal, or a contraction followed by forward movement takes place, rotation is still anticlockwise, but if

reverses and the fragment swims forward in a manner similar to that of an intact individual, differing only in that the tendency to follow a spiral course is more conspicuous.

The posterior fragment, immediately after transection, usually remains inactive for a few seconds, then suddenly shoots backwards for some distance, revolving only very slowly on the long axis. There is a slight tendency towards a spiral course, but this is certainly not so obvious as in the case of anterior fragments. Nor is the rate of movement so great in the posterior pieces and the reversal often lasts for a few seconds only. It is usually followed by a period of inactivity of about a minute's duration before forward swimming is resumed. In a small number of cases, the posterior fragment showed no ciliary reversal at all, simply moving forwards very slowly after transection. The chief difference, then, between anterior and posterior fragments immediately after cutting is the greater activity of the former and the tendency of the latter to remain inactive for a few

seconds before either continuing forwards, or reversing. Inactivity on the part of the anterior fragment is usually of only two or three seconds' duration.

Unfortunately, no earlier workers have indicated the age of the fragments used in their studies. Consequently, this point must be borne in mind when considering the discrepancies between their findings and my own, for, to anticipate the conclusion of this paper, fragments immediately after isolation from the whole do not show normal behaviour. On the other hand, regulation of form is accompanied by a return to normal in the behaviour and the responses to stimuli.

Jennings & Jamieson (1902) state. that both anterior and posterior halves move and react in the same way as the whole individual, revolving anti-clockwise when swimming and, when stimulated (general or localized?), backing and turning to the aboral side, the anterior fragment turning through a greater angle and swimming more rapidly. They also found that a posterior half or one-third, when subjected only to a mild stimulus, often oscillates back and forth, but reverses and turns to the aboral side if the stimulus is more severe. This is not in complete agreement with one of Jennings's earlier papers (1899a), in which he states that a posterior fragment reacts as the whole animal but fails to turn to the aboral side; from which he has concluded that 'the power of initiating a turn...is localized in some way in the anterior end'.

The behaviour of a fragment examined immediately after section has been found to be directly related to the position of the fragment in the animal as a whole. For example, a localized mechanical stimulus applied at points 1, 2 and 3 in an intact individual is followed by contraction and ciliary reversal. Therefore, a fragment taken from any region between points 1 and 3, or even the entire anterior half, responds to all stimuli by contracting and swimming backwards, regardless of whether the stimulus is localized at any point or is general. A stimulus localized at points 4 or 5 in the intact animal is followed by contraction with increased rate of swimming forwards. Precisely the same reaction is obtained from fragments taken from the posterior quarter of the whole organism, again regardless of the localization of the stimulus. A striking experi-ment is to bisect a *Spirostomum*, to allow the two halves to recover from the shock and to resume swimming forwards, and then to tap sharply against the edge of the watch glass. Provided the tap is severe enough, both fragments will contract, but while the anterior half darts backwards, the posterior swims forwards. They are responding in relation to their positions in the organism as a whole.

After a period of from 12 to 48 hr. has elapsed, the fragments show complete morphological regulation and, coinciding with this, commence to behave as whole animals, showing differential responses

towards localized stimuli and darting back●●ds when stimulated generally. Thus a statement of the normality or abnormality of the behaviour of a fragment is incomplete if there is no accompanying specification of the age of the fragment.

From the results of observations on responses to localized stimuli in more than thirty-five pairs of fragments, the general conclusion was drawn that the regulation of behaviour proceeds in both anterior and posterior fragments which are at least one-eighth the length of the original organism. In four experiments the fragments were less than a tenth of the original length, and all failed to show the reappearance of differential responses within 72 hr.

Fragments taken from the middle region of the body between points 2 and 4 are interesting in that they generally fail to show any locomotory response at all for several hours after section. Such fragments move very slowly and, when stimulated either generally or at some point on the body surface, merely contract and remain motionless until relaxation is complete, when they swim forwards again. Differential responses appear within 24 hr.

Jennings's contention (1899a) that a posterior fragment fails to turn to the aboral side after swimming backwards away from the source of a stimulus has been confirmed, and seems to be quite a constant feature of posterior fragments for several hours after they have been isolated from the whole organism. When differential responses appear, however, a turn towards the aboral side becomes just as much a feature of the avoiding reaction of a posterior fragment as of that of the normal animal. In the intact *Spirostomum* the power of initiating a turn to the aboral side seems to be localized near the anterior end, as Jennings has observed. The localization is not at the extreme anterior end, however, for cutting off the tip of the organism, at about a quarter of the distance between points 1 and 2, does not affect the ability of the hinder portion to turn to the aboral side. It is concluded that the localization is in the anterior quarter of the body but is not at the extreme tip.

The peculiar oscillation that Jennings observed when a posterior fragment is stimulated is also a common, but not constant, feature of recently isolated merozoa. It is most readily observed in posterior fragments resulting from section between points 2 and 3. When stimulated at its anterior end, such a fragment may contract and dart back a little, only to swim forwards a short distance and then swim backwards again. This may be repeated several times before normal swimming is resumed in the original direction.

Reference has already been made to the difference in the sensitivity of the anterior and posterior ends of certain ciliates, and which is described in some detail by Jennings (1906). In an attempt to obtain quantitative evidence for this difference the following experiments were carried out.

●gments were mounted in a moist chamber in su●● a way that, by means of an electromagnetic hammer striking against the chamber, they could be subjected to repeated general stimuli of constant intensity. The results of several experiments are grouped in Table 1, which gives the percentage of contractions produced in each individual by thirty constant intensity shocks spaced at 5 sec. intervals. The figures are given for the whole animal and for the corresponding anterior and posterior fragments immediately after section.

These figures illustrate a general feature, that the larger the fragment, the more sensitive it is, regardless of whether it is from the anterior or posterior regions of the whole animal. Hence, a large posterior fragment may appear to be more sensitive than a small anterior one (e.g. in Exps. SS 12 and 13). On the other hand, if the fragments are of nearly equal size, the anterior half definitely responds more

Table 1. *Responses to constant intensity shocks. Table of the percentage of contractions elicited from whole animals, and their resulting anterior and posterior fragments, by several mechanical stimuli of constant intensity. Each was stimulated thirty times, with a time interval of 5 sec. between successive stimuli*

No. of exp.	Level of section	Whole organism %	Anterior fragment %	Posterior fragment %
SS 13	1½	85	45	70
SS 12	2	95	40	85
SS 19	3	88	70	45
SS 11	3	85	60	10
SS 22	4	90	80	40
SS 16	4	70	75	25
SS 15	4½	85	95	0

readily than the posterior. Over a period of 36 to 48 hr., all the fragments show a gradual return to the sensitivity of the original organism.

In a second series of experiments, a determination was made of the minimal mechanical shock required to produce ten successive contractions, the time interval between the stimuli in each group being 30 sec. Typical results are presented in Table 2. Figures for the fragments were determined immediately after transection. Variation in the intensity of the mechanical shock was obtained by dropping small weights from a known height.

From these figures it may again be concluded that large fragments respond more readily than small, and that anterior fragments respond more readily than posterior ones. The threshold rises rapidly with decrease in the size of the fragment, but the rise is many times greater in posterior fragments, some of which often fail to respond regularly even towards extremely strong shocks (70,000–100,000 ergs). The threshold for all the fragments becomes smaller with

the lapse of time, tending towards about 15,000 ergs, which was found to be the minimal mechanical shock that would regularly produce contraction in a whole individual. This figure, of course, holds only for infrequent stimulation and presupposes no complications due to habituation.

It seems certain then, that there is a marked difference in the sensitivities of the anterior and posterior ends of *Spirostomum*. It will be remembered that Horton (1935) found that posterior halves of *Paramecium* are more sensitive to acid stimulation than anterior halves, and he explained this by assuming that the newly formed pellicle covering the cut surface is more permeable than that of the rest of the body. As the posterior fragment moves forwards, the first part of it to encounter a chemical stimulus is the newly formed pellicle, whereas, the

Table 2. *Table of the minimum, general mechanical shock found necessary to produce contraction of each individual on ten occasions, the stimuli being separated from each other by 30 sec. In the case of the posterior fragment of SS 35, a shock of 73,500 ergs produced a maximum of only 60 % of contractions. The minimum shock required to produce ten successive contractions (i.e. 100 %) must have been considerably greater than this and could not be determined without the risk of damaging the apparatus*

No. of exp.	Level of section	Intensity of shock. Producing 10 successive contractions		
		Whole organism ergs	Anterior fragment ergs	Posterior fragment ergs
SS 28	2	15,680	31,360	23,520
SS 2	3	14,700	17,600	29,400
SS 21	3	14,700	15,680	31,360
SS 17	3	14,700	14,700	58,800
SS 35	4	15,680	14,700	73,500 (60 %)

first part of an anterior fragment to encounter the chemical is the original, undamaged pellicle. Hence, any increase in the permeability of the pellicle would afford a ready explanation of apparently increased sensitivity on the part of the posterior fragment. Thus, Horton's observation does not necessarily conflict in any fundamental way with the current, widely held opinion that greater sensitivity is characteristic of the anterior end of ciliates. The following observations on the effects of cyanide solutions on fragments of *Spirostomum* are relevant.

It has been suggested by Pantin (1930), and demonstrated by Clark (1942), that the cytolytic action of a strong solution of potassium cyanide on amoebae is due to the action of the high concentration of potassium ions in a solution of high alkalinity. *Spirostomum* disintegrates readily in M/100 KCN, and whether the cytolysis is due to alkalinity alone

or to alkalinity plus potassium ions, the effect is essentially a surface one. If there is any difference between the permeabilities of the old and newly formed pellicles, immersion in cyanide should reveal it.

The individual to be examined was placed in a few drops of $M/100$ KCN contained on a depression slide, and covered quickly with a cover slip in order to prevent the rapid loss of HCN vapour from the solution. The time for cytolysis to occur was measured with a stop watch. The mean time for 50 intact spirostoma was found to be 270 sec., ranging from 46 to 367. With only one exception, the disintegration commenced at some point within the anterior third of the organism and proceeded caudally, illustrating a well-defined susceptibility gradient, which has already been described by Child (1924). Groups of anterior and posterior fragments were immersed in cyanide at varying intervals after transection, and in Table 4 the mean times for cytolysis are given. In all cases the organisms were cut at level 3, giving fragments of nearly equal size.

Table 3. *Table showing the relation between the mean time of cytolysis of fragments in $M/100$ KCN and the time elapsing since isolation*

Time after bisection hr.	Total number of fragments examined	Mean time for cytolysis sec.	
		Anterior	Posterior
0	20	18	16
1	20	150	35
12	10	277	257
24	10	294	305

The mean time for the cytolysis of a whole individual is 270 sec.

It will be seen that, following bisection, there is a very marked increase in the cyanide sensitivity, both anterior and posterior halves disintegrating rapidly. It is highly significant that, in every case, cytolysis commenced at the recently repaired surfaces, with the result that the anterior fragments disintegrated from their posterior ends. One hour after bisection there is a rise in the resistance of the fragments but cytolysis still commences at the newly formed surface, while 12 or 24 hr. after bisection, fragments resemble the whole animal both with regard to the order of magnitude of the time for cytolysis to occur, and to the appearance of an antero-posterior susceptibility gradient. These facts are most readily interpreted if we accept Horton's suggestion that the newly formed surface has a different permeability from the old.

THE RESPONSES TO A CHEMICAL STIMULUS

Using as a stimulus a dilute aqueous solution of sodium chloride, Alverdes (1923) found that both anterior and posterior fragments of *Spirostomum*

show an avoiding reaction, demonstrating th⬤e reception of a chemical stimulus is not confir⬤to the anterior portion of the body. Blaettner (1925) briefly states that he confirms this observation. To reinvestigate the matter, localized stimuli were applied by means of a micropipette, using a dilute solution of neutral red (0·05 %) in water as the stimulating agent. The use of a coloured solution is advantageous for it enables an estimation to be made of the degree of spreading of the stimulating agent from the point of application.

A second technique that has proved very valuable involves the use of a larger pipette with an orifice of about 0·1 mm. The ciliate is placed in a watch glass containing several drops of culture water and, when it is swimming in an approximately central position, the pipette is dipped beneath the surface of the water and a circle of dye solution is 'drawn' around the organism, which is thus enclosed within a small barrier. The diameter of the circle should vary from 2 to 10 mm., depending on the size of the organism. Soon the ciliate will reach the chemical barrier and, being stimulated at its anterior end, will move backwards. Provided the circle is of the correct diameter, this backward movement will bring the posterior end up to the dye of the opposite side of the circle and a response may again be observed. Thus the technique is a simple means of applying localized stimuli at the extreme ends of the animal. The experiment can be especially striking with organisms that move some distance backwards following a stimulus at the anterior end (e.g. *Spirostomum, Paramecium,* and *Frontonia*), but is naturally not suitable for organisms such as the hypotrichs, which move backwards only a short distance before turning to one side. Whole organisms and fragments were always examined individually, permitting a detailed observation of their reactions and movements. We will consider first the behaviour of whole animals, already described in some detail by Jennings (1899a, b) and Blaettner (1925).

On approaching the dye solution by the anterior end, *Spirostomum* hesitates, the tip may wave to and fro, as if testing the surrounding region, and then the ciliary beat is reversed. After moving back half a length, the animal swings out towards the aboral side through an angle of about 45°, and the direction of ciliary beat again changes and swimming forwards is resumed. In most cases the reaction is obtained before the anterior end actually reaches the coloured zone, indicating the detection of the chemical stimulus in a relatively high dilution. When enclosed within a circle of dye, *Spirostomum* may show this reaction as many as fifty times without contraction occurring. On the other hand, applying the dye locally at the anterior end by means of the fine pipette generally results in both ciliary reversal and contraction of the myonomes. Thus a weak chemical stimulus produces only ciliary reversal, while a strong stimulus elicits both reversal and contraction.

closed within a circle of dye, the anterior end of the animal is, repeatedly subjected to stimuli and, with each stimulus, there may be a gradual increase in the distance covered during the reversal of the ciliary beat. At the same time, the anterior end is turning through an appreciable angle after every reversal, and it is inevitable that sooner or later the posterior end of the animal will come in contact with the chemical barrier. No reaction is obtained unless the body definitely reaches the coloured zone, illustrating the relative insensitivity of the posterior end. When the posterior end does reach the dye, the intensity of the stimulus is relatively great and, as might be expected, contraction occurs and this is followed by a dart forwards.

As in the case of mechanical stimuli, the behaviour of fragments towards a chemical stimulus was found to be related to the degree of regulation that had occurred since transection was carried out. Fragments examined 24–48 hr. after isolation behave in exactly the same way as the whole animal. Consequently, the following account of aberrant behaviour concerns only fragments which had been isolated one or two hours before the experiments were made.

Anterior fragments less than three-quarters the length of the whole animal react to a chemical stimulus at either end by swimming backwards, contracting also if the stimulus is severe. If the stimulus is applied at the anterior surface, the reaction is fundamentally the same as that of the intact organism. A very small anterior fragment may show a slight difference in that, after reversing the direction of the ciliary beat, it may turn through two or three circles before swimming forwards again. Following a stimulus at the posterior surface, the reversal in the ciliary beat of course brings the fragment further into the dye. Contraction then occurs but the reversed swimming is maintained. Several more twitches may take place and if the dye is present in a very thick layer (as in the circle technique), streams of acid-reacting mucus are liberated from the body surface. Whether the fragment survives or not depends on the concentration of the dye and the thickness of the barrier that it constitutes.

A posterior fragment greater than about one-third the length of a whole animal' shows differential responses to localized stimuli, but after the reception of a stimulus at the anterior surface it fails to turn to the aboral side, although normal ciliary reversal takes place. After moving back a short distance the fragment swims forward along the original course. Again encountering the chemical, it reverses a short distance and then once more moves forwards. It may oscillate back and forth in this manner for several seconds (see also Jennings, 1899a).

A small posterior fragment behaves in an extremely interesting manner when stimulated at its anterior surface. If the stimulus is mild, reversal and oscillation are observed. On the other hand, if the stimulus is severe, contraction of the myonemes occurs and the fragment shoots forwards into the dye.

THE RESPONSES TO HEAT

Alverdes (1923) claims that the perception of thermal stimuli in *Spirostomum* is confined to the anterior end of the body. The technical difficulties involved when attempting to localize a thermal stimulus are so great as to be almost insuperable, for conduction and convection rapidly render the stimulus diffuse. Fairly satisfactory results can be obtained using the methods described by Jennings (1904, 1906). In an intact individual a stimulus applied at the anterior end elicits the usual avoiding reaction, while one localized at the posterior end produces an acceleration in the rate of swimming forwards. The latter response is probably a physical effect on the rate of ciliary movement, for a very strong stimulus applied at the same point (4 or 5) produces contraction accompanied by a dart towards the source of the stimulus. There is thus a failure to show a true differential response to heat.

Fragments that have undergone regulation show the same reactions as the whole animal, while recently isolated merozoa show variations similar to those already described in the previous two sections. More than thirty pairs of fragments have been examined and in no case did the posterior portion fail to respond to a thermal stimulus. This is not in agreement with the findings of Alverdes (1923), but I can offer no explanation for the discrepancy.

DISCUSSION

The main point demonstrated in this work is that while no area of the body surface in *Spirostomum* is differentiated to form a special sense organ, there is a definite antero-posterior gradient in the behaviour pattern. Mechanical, chemical or thermal stimuli, provided they are of sufficient intensity to equal or exceed the threshold, will elicit responses when applied to any point on the body surface. These responses are generally made with reference to the localization of the stimulus, and are differential in nature, although this has not been demonstrated for the case of a thermal stimulus. I am unable to offer an explanation of why Alverdes (1923) found that only the anterior end is sensitive to heat. Even extreme posterior fragments never failed to give an avoiding reaction towards a mild thermal stimulus encountered at the anterior surface. The 'mechanical shock' experiments have demonstrated in a quantitative way the greater sensitivity of the anterior end over the posterior, a difference described on several occasions by many writers, principally Jennings and Child. That the fragments also swim similarly to whole individuals supports the view that the spiral track taken by a swimming ciliate is due to the

co-ordinated activity of the body cilia, and not to the special action of the cilia of the mouth region. This is in general agreement with the views of Jennings & Jamieson (1902) and of Horton (1935), on the significance of the spiral track.

Jennings & Jamieson (1902) have expressed the opinion that a ciliate fragment behaves in the same way as the entire animal, and gave their 'crystal analogy' which was referred to at the beginning of this paper. They do not state what time elapsed between the isolation of the fragment and the application of the stimuli, and consequently criticism of their observations must be reserved, in so far as it will be valid only if they examined the fragments immediately after the animals had been bisected. If, however, several hours had elapsed, enabling the merozoa to regulate both morphological form and the nature of the responses, then the conclusion of these authors, that fragments respond like the whole organism, is correct. In the present paper, it is shown that, until regulation is complete, fragments behave with reference to the position they occupy as an integral part of the whole organism. In other words, Spirostomum, instead of being organized like a crystal, actually has a gradient pattern in its proto-plasm. After isolation from the whole, any one part of the organism at first behaves as though it were still part of the pattern, but eventually a process of reorganization takes place, similar to the morpho-logical reorganization that Dembowska (1925) and others have found during regeneration in the more highly organized hypotrichs. Regulation in the responses of a fragment to stimuli appears to be a further aspect of the tendency for an isolated portion of a morphogenetic field to reconstitute itself into a similar field of smaller size. Seyd (1937) studied the

process of morphological regulation·in fragmen of Spirostomum and has also expressed the opinion that a definite regulatory agency, in the nature of a field, functions in the isolated merozoa.

It would appear that Jennings's suggestion (1899a), that the power to initiate a turn in Spiro-stomum is localized in the anterior end, is correct, for it has been consistently observed that posterior halves, when swimming back from the source of any stimulus, fail to turn towards the aboral side unless they have already completed the regulatory process. Although Bishop (1928) failed to find in the neuro-motor system of Spirostomum any anatomical structure which could be called a 'motorium' or controlling centre, it would be of some interest to trace the reorganization and rearrangement of fibres occurring during regulation.

SUMMARY

1. Spirostomum shows differential responses to localized mechanical and chemical stimuli.

2. The entire body surface is sensitive to me-chanical, chemical and thermal·stimuli.

3. The spiral path followed during swimming is due to the co-ordinated activity of the body cilia.

4. Immediately after isolation, fragments do not behave like normal animals, but respond with reference to the position they occupied in the whole organism.

5. When morphological regulation is complete, the responses of the fragments are normal.

6. As revealed by the study of the responses of fragments, Spirostomum is not organized like a crystal, but seems to have a definite gradient pattern.

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