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SPONTANEOUS SQUIRTING OF AN ASCIDIAN, *PHALLUSIA MAMMILLATA* CUVIER

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(Text-figs. 1–16)

INTRODUCTION

The simple monascidian *Phallusia mammillata* Cuvier was found by the author to exhibit rhythmical spontaneous activity during long periods of observation in the laboratory (Hoyle, 1952) The movements consist principally of quick contractions of the whole body-wall together with water ejection from the branchial and atrial cavities. The water movements are of the nature of squirts similar to those which the animals give in response to a violent external stimulus, and are accompanied by siphon-rim closure as an inevitable consequence of the contraction of the body-wall musculature. The closures can be recorded without disturbing the animals unduly by a method which was described in the same paper. A slight inward movement of the test can be observed as the total volume of the animals diminishes during the squirts. The present work is a study of the frequency patterns of the spontaneous squirting of *Phallusia*, together with a brief study of the physiological mechanisms involved in its production and a consideration of the possible functional significance of the activity.

The phenomenon of spontaneous activity in ascidians has not attracted much attention. Polimanti (1911) observed spontaneous opening and closing of the siphons of *Ciona intestinalis* at high temperatures (30° C.) but does not describe spontaneous movements under more normal conditions. Several earlier workers on ascidians observed activity only in relation to the definite ejection of foreign particles, faeces or sexual products (Magnus, 1902; Fröhlich, 1903; Jordan, 1908). The phenomenon was first clearly described by Hecht (1918) working with *Ascidia atra* from the Bermuda coast. He found spontaneous contractions of the branchial siphon at intervals of about 5 min. at unspecified temperatures. He observed contractions visually in a colonial species *Ecteinascidia turbinata*. In considering the functions of the rhythm Hecht examined the possibilities that the movements may serve either to remove waste or to subserve respiration. He rejected both possibilities by argument based on his earlier observations on the ciliary current (Hecht, 1916). He finally suggested that the rhythm may be 'the degenerate remains of an activity homologous with the rhythmic pulsations of the salps', and that 'no function can be ascribed to this rhythmic occurrence'. This suggestion begs the question of phylogenetic relationships within the Tunicata and assumes the salp activity to be a primitive locomotory mechanism. The work of Hecht was followed by that of Day (1919), who observed spontaneous contractions in amputated branchial siphons of *Ciona*. This observation indicated that the nerve ganglion is not necessarily responsible for the rhythmicity, but Day did not observe spontaneous activity in the intact animal.

The problem of the nature of the activity and its relation to the ganglion was more completely investigated by Yamaguchi (1931), who showed that in *Styela clava* the rhythmicity affects both siphons synchronously. Marked contractions occur at intervals of about 8 min. in running sea water, together with smaller contractions at irregular intervals (published record). The rhythmicity became much more marked and also more frequent after a period in filtered sea water. The interval was then reduced to about 3 min. After ganglion extirpation the siphons still showed spontaneous activity, but the co-ordination was lost and the frequency was very variable. Moreover, the frequency was now markedly increased and the amplitude reduced. Yamaguchi also recorded spontaneous activity from both amputated oral and atrial siphons. According to Yamaguchi the normal spontaneous contractions of *Styela* are of two kinds. One concerns the circular muscles and results in water expulsion. The other consists of logitudinal muscle contractions and siphon closure which does not involve water expulsion. The latter is less frequent. Yamaguchi does not give measurements of the relative frequencies, but from his published records the ratio seems to be about 1 : 6. In his paper he makes no attempt to explain the functional significance of the rhythm.

Bacq (1935), in commenting on the spontaneous activity of *Ciona*, remarked that after ganglion extirpation he found the spontaneous activity more frequent and of reduced amplitude. Ganglion extirpation results in reduced muscle tone. Miss P. Kott has recorded spontaneous activity in *Ciona intestinalis*, *Ascidia aspersa* and *Molgula hattensis*, but unfortunately she has discontinued this unpublished work (see Report of the Council of the Marine Biological Association in *Journ. Mar. Biol. Assoc.*, Vol. 30, 1951). The most recent published comment on the activity was that of Young (1950), who suggested that the automaticity may be something in the nature of a hunger contraction.

PATTERNS OF SPONTANEOUS ACTIVITY

In Intact Animals

About sixty normal *Phallusia* have been observed altogether at either the spring or autumn of two seasons. All these animals showed spontaneous activity. With only two exceptions the contractions of both siphons were synchronous except for small movements caused by vibrational and other stimuli. Most of these animals were kept in tanks of the Plymouth aquarium under circulation for a few hours before observation. Some, however, were observed immediately after collection some 5 hr. previously. In the latter animals the siphons opened slowly, taking about 4 hr. to open completely. Spontaneous movement is apparent after the siphons begin to open in all instances and the contractions increase in amplitude as the siphons open more widely. The frequency of the contractions of these animals is high at first and gradually reduces in Plymouth tank sea water. Eventually a nearly constant rhythm of contraction is apparent, with spontaneous squirts at 6–9-min. intervals. All the observations were at temperatures from 11 to 14° C. Although this type of activity is most commonly recorded it changes at times to other equally regular patterns. These patterns include one in which large contractions alternate with a number of smaller ones (Fig. 10, lower record). Some of Yamaguchi's animals showed this pattern, and he described the large contractions as due to longitudinal muscles and the smaller ones to circular muscles. It is not possible to distinguish muscle types clearly in *Phallusia*, where the general muscle pattern appears to be very irregular. This type of activity is not common in *Phallusia*. Another type consists of bursts of squirts alternating with periods of rest. This type of variation recalls the familiar patterns of *Arenicola* preparations (e.g. Wells, 1949).

By far the commonest variations are simple increases or decreases of frequency. The changes to a different frequency occur fairly abruptly, sometimes very abruptly, and are often prefaced by a short burst of vigorous activity (Fig. 1 D). The new rate is almost exactly twice or one half the original one and is always maintained for a few hours. It is possible to distinguish slow, medium and fast rates of spontaneous squirting (Fig. 1, A–C). The animals were kept in 20–30 l. of sea water which was changed usually once a day. The value of intervals between squirts averaged approximately 16 : 8 : 4 min. The rate changes were not accompanied by any change in the amplitude of contraction. Sometimes, after a fresh supply of outside sea water, an animal will change to the slow rate after about 2–3 hr., maintain this for a further 4 hr., then transfer to an ultra-slow rate (intervals of about half an hour) for about 3 hr., during which time the amplitude gradually declines until the spontaneous contractions are only just perceptible. The first animal showing this behaviour was discarded in the belief that it was dying, but when another followed suit this was left overnight and was later found to have started again at the medium rate. Later, after a new supply of the same outside sea water, it repeated the performance. When the amplitude decreased, the branchial siphon declined first, the atrial later. When the amplitude increased again the branchial siphon increased before the atrial. The observations show that the decline in frequency is unlikely to be due to poor condition of the animals.

In Operated Animals

Since the squirting is rhythmical it is reasonable to suppose that there must be a pacemaker controlling the frequency. This may be situated in the

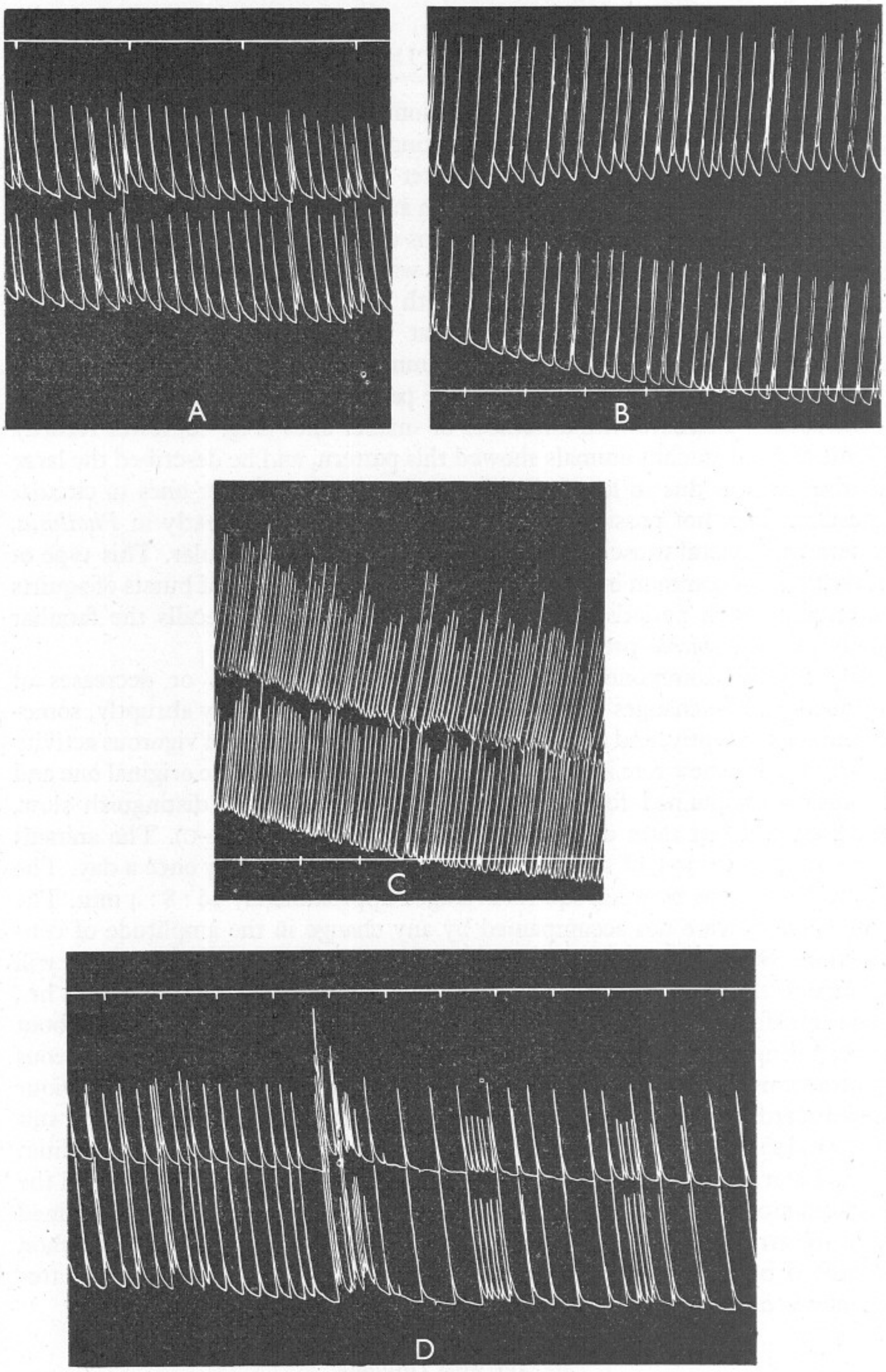

Fig. 1. A, the 'normal' 8 min. rhythm; B, the 'slow' 16 min. rhythm; C, the 'fast' 4 min. rhythm; D, spontaneous change-over from the 'medium' to the 'slow'. Time marks A–C, 1 hr.; D, 30 min. Upper record branchial siphon, lower record atrial siphon.

ganglion, in one or other of the siphons, or in some part of the body-wall. The purpose of the experiments described under this heading was to attempt to determine the site of the hypothetical pacemaker. The first experiments consisted of recording from the siphons of deganglionated animals. The method of operation to remove the ganglion has been described by Carlisle (1951). Animals in which the ganglion has been removed recover from the operation in a few hours and again show spontaneous activity. This is very variable from one animal to another. The amplitude of the contractions is invariably reduced. The atrial siphon tends to maintain the medium rate whilst the branchial siphon, although sometimes contracting at the same time as the atrial, also shows many other erratic contractions (Fig. 2). The activity is relatively constant over many hours regardless of the state of the bathing sea water and food supply, and the spontaneous changes of pattern seen in intact animals are not apparent. In other experiments the ganglion was left undamaged, but the nerves leaving it and passing to a siphon were cut close to their point of attachment to the ganglion. After this operation there is a general loss of tone as evidenced by the more sluggish responses of both the siphon-rims. There is a tendency at intervals for the denervated siphon to give a continuous series of closely spaced contractions. These summate to close that siphon and expel water from it almost completely.

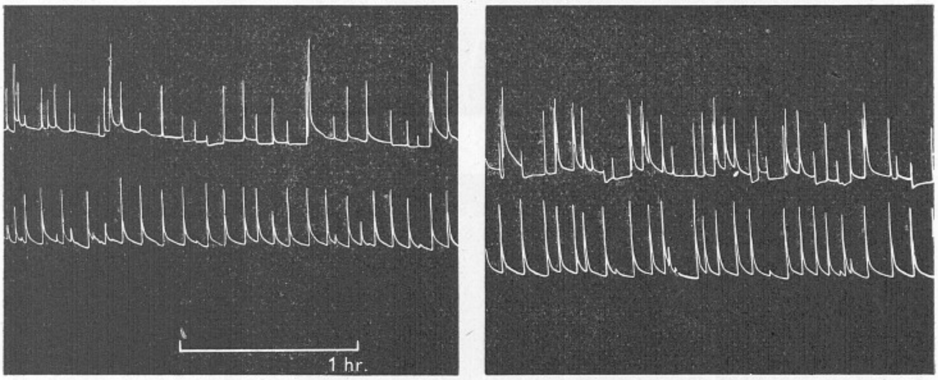

Fig. 2. Movements of the siphons of a deganglionated animal. Upper record branchial siphon, lower record atrial siphon. Filtered sea water. An interval of 10 hr. separated the two tracings. Note the occasional positive openings of the branchial siphon.

Whole siphons were amputated, and after about 6 hr. records were made of the activity of both the amputated siphons and the intact ones. No movements of amputated siphons occurred. The intact siphons, after remaining closed for a few hours following the operation gradually open. When fully open the intact siphons, both atrial and branchial, resume a fairly normal rhythmicity but with considerable amplitude variations (Fig. 3). The results are in general agreement with those of Yamaguchi (1931), but whilst both he and Day (1919) observed or recorded spontaneous movements in amputated pieces of siphon, no such movements are observed with *Phallusia* material. In this animal there is a strong body-wall attachment only at the siphon rims and contractions of the body-wall in the cut portion could not affect the siphon rim very much even if they were vigorous.

The general conclusion from all these results is that the pacemaker is not situated in either the ganglion or a particular siphon. So far only body-wall muscle from the intersiphonal region has been examined for the presence of purely local spontaneous activity. A strip of muscle 3 cm. long can easily be obtained from the intersiphonal region of the left side. Such strips have been dissected out and mounted vertically with the free end attached to an isotonic lever. These strips show spontaneous slow contractions on which quicker movements are superimposed. The movements of one of these strips were recorded at the same time as those of the siphons of a deganglionated animal and are illustrated in Fig. 7. The quick movements are irregular, but occur at similar intervals to the movements of the branchial siphon of the deganglionated animal. It is just possible that the isolated body-wall strip movements are myogenic: all parts of the body-wall may have a similar

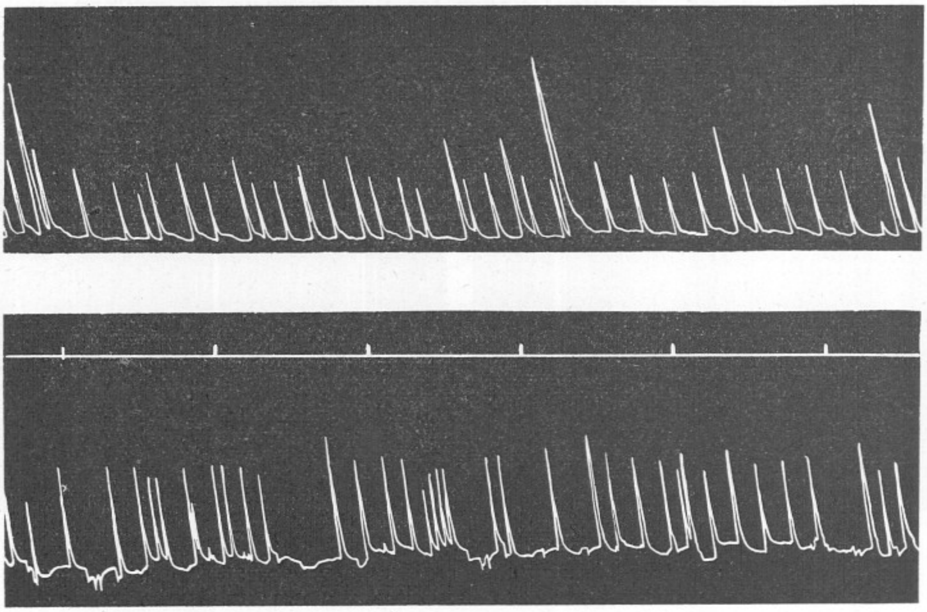

Fig. 3. Upper tracing from branchial siphon after amputation of the atrial. Lower tracing from an atrial siphon after amputation of the branchial siphon. Note the occasional positive openings of the atrial siphon. Time in hr.

activity, but this remains to be proved. Also, since Hunter (1898) has figured cell-bodies in nerve-muscle strips of an ascidian, and since the strips used undoubtedly included some fine nerve elements, the possibility must be included that diffuse nerve elements act as local pacemakers. However, the observations suggest that rhythmicity is a natural property of the body-wall. In the whole animal this is subject to considerable co-ordination and control by the ganglion, but there is still some co-operation even in the absence of the ganglion, especially within a siphon.

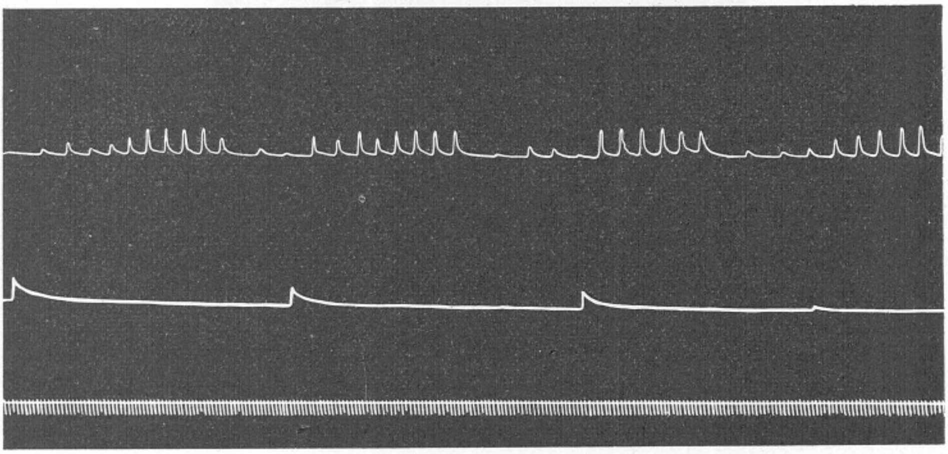

Fig. 4. Peculiar pattern of activity shown by one specimen in which the nerves to the atrial siphon had been cut. The amplitudes were quite small. Upper record branchial, lower record atrial. Time in min.

In fatigued and operated animals the spontaneity often presents peculiar patterns, but these always suggest that the basic phenomena are cyclical. One such pattern is shown in Fig. 4. The nerves to the atrial siphon had been cut, and the pattern illustrated soon developed and was maintained for several hours. The amplitude of the branchial siphon fluctuated in a periodic manner and the atrial siphon contracted at times corresponding only with the minima of the branchial siphon movements. Periodic fluctuation of the branchial siphon amplitudes is not uncommon in intact animals. The interval between the modulation peaks is then about 45 min. The mechanical response of a siphon to electrical stimulation is partially a function of the previous history of the animal, partly a function of the stage in the spontaneous activity cycle (Hoyle, 1952). A single excitation given just before a spontaneous squirt is due frequently elicits a large response. A single excitation given just after a spontaneous contraction tends to set up a series of closely spaced contractions. Both phenomena are present in operated animals. Thus, in Fig. 5, a single shock produced the latter effect although the nerves to the atrial siphon had been cut. After a short period of electrical stimulation the frequency of spontaneous squirting is reduced. The previous rate is regained fairly quickly (Fig. 6). All these phenomena must be due to some effects of external stimuli on the basic mechanisms which determine pacemaking in the body-wall.

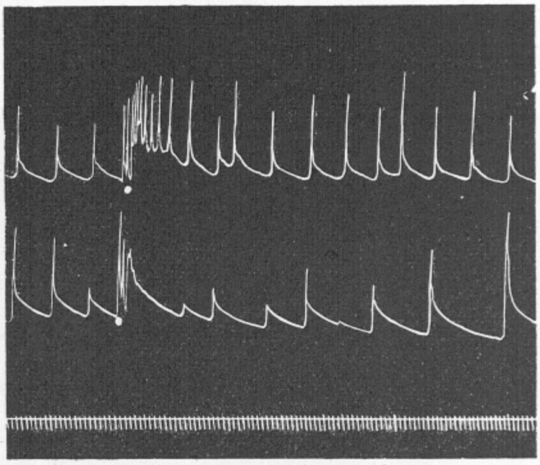

Fig. 5. The consecutive series of contractions in response to a single electrical stimulus given immediately after a spontaneous squirt evidenced in an animal with the nerves to the atrial siphon (lower trace) severed. Time in min.

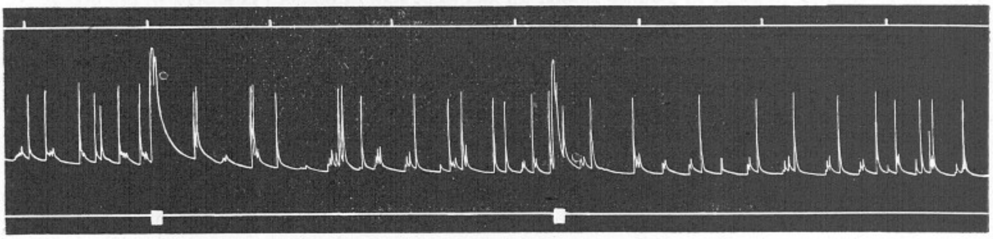

Fig. 6. The effect of a series of electrical impulses at 1 in 2 sec. intervals (indicated on the lower line) on the frequency of the spontaneous activity. Branchial siphon. Time in hr.

VOLUMES OF SEA WATER DISPLACED

The classical interpretation of feeding in sedentary monascidians is that they extract food particles from a continuous stream of water. This stream is maintained by the activity of cilia situated on the inside of ostia which perforate the branchial sac. The stream enters the branchial siphon, passes through the perforations and leaves via the atrial siphon (Fol, 1876; Roule, 1884; Orton, 1913). This current is supposed to be continuous, although no long-term observations have been made. Squirting, on the other hand, produces no flow through the pharynx; the water is expelled from the whole branchial and atrial sacs through the respective siphons at the same moment. It is of considerable value to know the actual volumes displaced by the two activities and to compare them. The only published observation on the rate of the through-current with which I am familiar, and one which is often quoted, is by Hecht (1916). Hecht fitted a glass tube into the atrial siphon of *Ascidia aspersa* and then fed the animal on carmine particles. When the particles appeared in the exhalent stream he measured the time taken for them to traverse the length of the tube. By a simple calculation (not specified), he deduced that a 100 g. animal passes 173 l. of sea water per day. This means an average output of 2 c.c./sec. Hecht's observations are open to some criticism. He appears to have multiplied the rate of movement of particles along the tube by its cross-sectional area in order to obtain rate of flow values. This assumes the rate to be uniform across the tube. In practice it is a function of the distance from the walls. There is no flow in the region in immediate contact with the walls. The rate increases towards the centre, where it is maximal, by a factor which is proportional to the square of the distance, i.e. the curve relating rate of flow to distance from the wall is parabolic. The Poiséille formula for the quantity of liquid, Q, flowing through a tube in unit time is

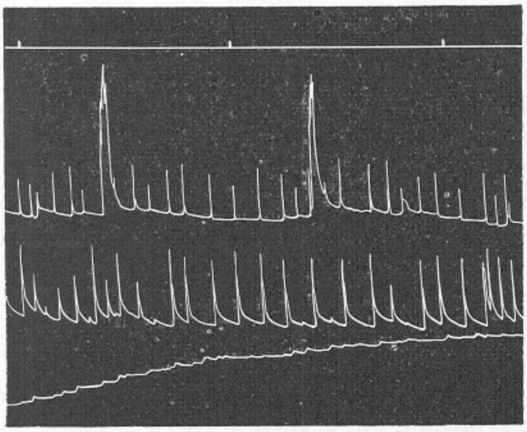

Fig. 7. Spontaneous movements of an isolated strip of body-wall from the intersiphonal region. The movements are the lowest tracing and are recorded at the same time as those of the siphons of a deganglionated animal. Upper record branchial, lower atrial. Time in hr.

$$Q=\frac{\pi}{8}\frac{pa^4}{\eta},$$

where p = pressure drop per unit length of tube, η = viscosity of liquid, and

a = diameter of tube. When the formula is derived assuming the velocity to be uniform it becomes

$$Q = \frac{\pi}{4} \frac{pa^4}{\eta}.$$

That is, Hecht's values are probably too large by a factor of ×2, since most of the particles will travel down the centre of the tube if the conditions for smooth flow are satisfied. In practice also, Hecht's higher rates are such that they give Reynold's numbers of the order of 2000. A Reynold's number of 1000 or greater is taken as an indication of the conditions for turbulent flow, which would make measurements by Hecht's method impossible. As a further check on the possible validity of these high figures, I have arranged a constant-flow device to deliver sea water containing carmine particles through a tube with the same dimensions as Hecht's and find difficulty in following the progress of any particles through the tube when the rate of flow is 2 c.c./sec.

The ciliary current of *Phallusia* has been measured in two different ways.

(1) The first method gave an intermittent record. A cannula with a wide tube and long, right-angled, narrow-bore side arm was inserted in the branchial siphon. The cannula and side arm were fixed in a horizontal position. At 2 min. following a spontaneous squirt, when the animal was quite undisturbed, the main arm was suddenly closed. The rate of water uptake was then measured by timing the movements of an aniline bubble along the side arm. With this method the greatest rate of flow observed was 4 c.c. per minute in Plymouth tank sea water at 14° C., during March 1952. The flow was not infrequently zero in the same individual. A rough average figure was 1 c.c./min.

(2) The second method was devised to give a continuous record of the rate of flow. It was similar to that used by Wells & Dales (1951) for recording the circulation of water in the tubes of Polychaete worms. A special cannula was gently inserted into an open atrial siphon. The bulb of the cannula was made just larger than the diameter of the siphon. The natural seal thus formed was capable of withstanding about 12 cm. pressure of sea water. The cannula was mounted vertically with its side arm horizontal. The wide vertical tube contained a float which was attached to a lever writing on a kymograph (Fig. 8). The side arm opened to the exterior through a short length of capillary tubing which could be by-passed for null-level determinations by a T-piece with pinchcock. The whole was covered with sea water (about 20 l.) to a level above the side arm. The device could be calibrated by calculation (see Wells & Dales), given the magnitude of the capillary, lever amplification, etc., or a constant-flow device could be inserted in place of the animal. The ciliary flow maintains a continuous depression below the null-level line, whilst the squirts are also recorded quantitatively as superimposed spikes. A typical atrial siphon record obtained by this method is shown in Fig. 10. The contractions appear slightly less regular in frequency than those recorded by the

siphon-rim closure method. Hence it is probable that the cannula constitutes a greater disturbance than the lever arms. Also, the method interferes to some extent with defaecation. However, one animal survived a week of this treatment without obvious deterioration. The method can also be used to determine branchial siphon activity by using a larger cannula and either removing or increasing the diameter of the capillary input resistance so that free access to the sea water is possible. The ciliary through-current is not then recorded.

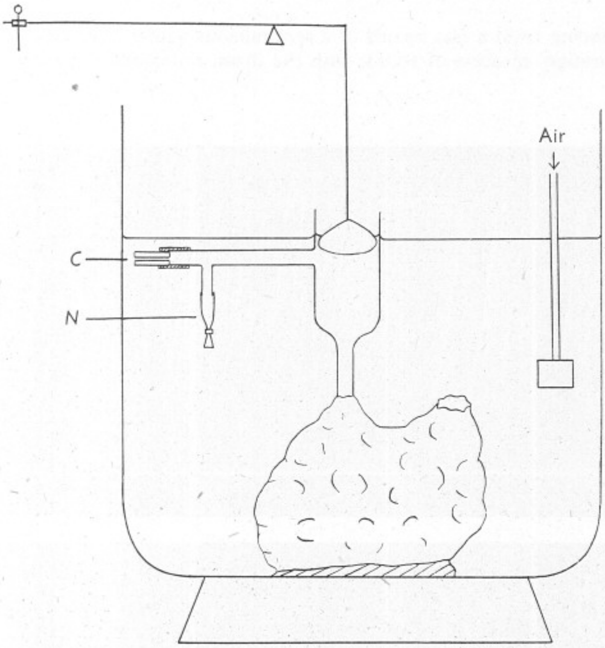

Fig. 8. Method of recording continuous flow and squirting simultaneously. *C*: capillary resistance; *N*: null-level indication pinchcock.

With the second method of recording it is seen that the branchial sac squirts about 12 c.c. and the atrial about 10 c.c. The average capacity of the branchial sac of a full-sized *Phallusia* is 17 c.c. The corresponding atrial sac holds about 14 c.c. These are necessarily rough values owing to the difficulties involved in making the determinations, but it is apparent that each sac expels some two-thirds of its contents on each squirt. A tracing from a fast record of a branchial siphon squirt is shown in Fig. 9. This was obtained by the float method. The water ejection is very rapid, occupying only $\frac{3}{5}$ sec. With the float method the continuous depression due to the ciliary current again shows it to have an average value of only 1 c.c./min. This represents a pressure in the side arm of 2 mm. water, which may be as large as the current is capable of producing.

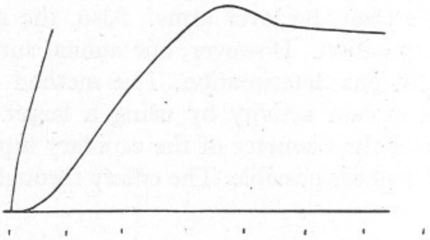

Fig. 9. Tracing from a fast record of a spontaneous squirt. The first stroke marks a calibration injection of 10 ml. with the drum stationary. Time in $\frac{1}{5}$th sec.

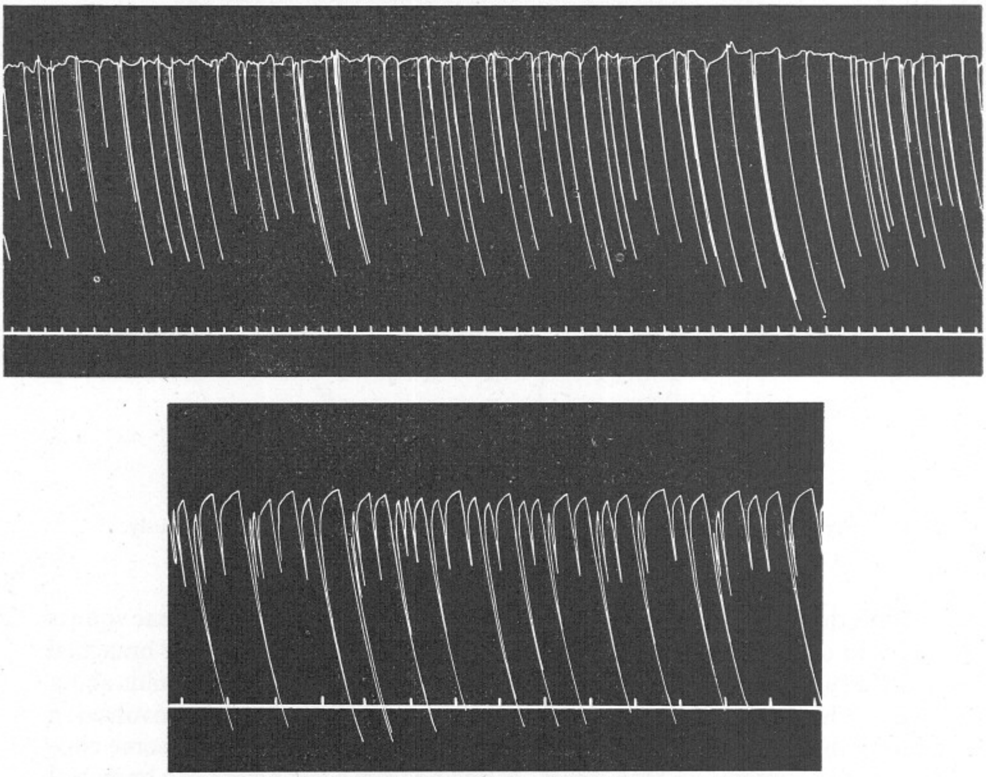

Fig. 10. Upper record: typical record of atrial siphon activity by the flow method. Time in 10 min. Lower record: variant of above showing alternate large and small contractions. Smaller diameter capillary than in upper record. Time intervals: 10 min.

Thus one might suspect that the real value might be larger than 1 c.c. However, the value was often less than 1 c.c./min., as recorded with the same method. We are therefore left with the problem of the still enormous difference between the *Phallusia* values for the through-current and the Hecht values which, even when allowance for possible errors in method are made, show the ciliary current to be some 100 times greater. Further, we must consider whether the observed *Phallusia* values would be adequate to subserve respiratory and nutritional requirements.

On the first of these points, an observation of considerable importance is that when carmine or graphite particles are fed to *Phallusia* they do not appear in the exhalant current. They are not again apparent until they are ejected as faeces. If very thick suspensions are added some of the particles are ejected from the branchial siphon at the next squirt. However, it is clear that the animals are very efficient at removing particles from the water going in between squirts. If Hecht's carmine particles were coming out of the atrial siphon almost immediately, how were they getting through the mucus film lining the branchial sac so quickly? Why were they not picked up by the feeding mechanism? Is this mechanism capable of selecting food particles and rejecting carmine? Some ascidians are known to have large slits connecting the anterior part of the pharyngeal sac directly with the atrial cavity. These slits were first described by von Kupffer (1875) and were called pharyngo-cloacal slits by Garstang (1891). Their function is uncertain, although Garstang offers the suggestion that they may serve to allow water from the contracting branchial siphon to flush through the atrial siphon and so help to remove accumulated faeces. I have observed carmine particles fed to *Ciona intestinalis* passing through such slits and passing directly out of the atrial siphon a few seconds after entering the branchial siphon. Clearly, the behaviour of these particles cannot indicate the fate of particles within the main body of the branchial sac which can presumably trap and remove them efficiently. Further, in view of the ease with which a contractile response can be elicited, it is just possible that Hecht's observations were unwittingly made on particles passing through these slits and subsequently being forcibly ejected by actual contraction of the body-wall.

The second point to be considered is the functional adequacy of the low values observed in the *Phallusia* experiments. Observations on the nutritional requirements of ascidians were made by Moore, Edie, Whitley & Dakin (1912). They found that a group of small unidentified ascidians of capacity 10 c.c. could be expected to obtain adequate oxygen and more than adequate food, on the assumption that metabolism is constant, from 150 c.c. Port Erin outside sea water per hour. If we assume a *Phallusia* to require 2–3 times this volume then the observed through-current is clearly inadequate. The water brought in by the current is not, however, the only source of food and respiratory gas. Respiration is also served by both the branchial and the atrial pumping. The former drives about 90 c.c./hr. and the latter about 76 c.c./hr. into contact with the internal body surfaces. Also, blood circulates through the test to the periphery and respiratory exchange may occur by diffusion across this surface. In all the experiments described, a large surface of sea water has been exposed to the air so that gaseous diffusion alone should serve to keep the water fully aerated, but vigorous air currents have also been added, to ensure both aeration and constant circulation. However, when the latter was deliberately omitted, the frequency of squirting was not affected. The total volume available for feeding is the sum of the through-current and the volume driven by the branchial siphon, i.e. about 150 c.c./hr. in normal animals. If we accept the data of Moore *et al.* this is only barely adequate for an animal living on outside sea water. The economy would be severely strained if for any reason the available food concentration became further reduced. However, since the volume driven by squirting is large compared with the through-current, it is possible that squirting constitutes an integral part of the feeding mechanism and the amount squirted can be varied by changing the frequency of spontaneous squirting, a phenomenon which has been observed in *Phallusia* as described above.

THE EFFECTS OF FEEDING AND STARVATION

This interesting possibility, that the squirting is part of a controlled feeding mechanism, has been tested in the following way using both siphon-closing and flow-recording methods. Animals were left to starve in filtered sea water and then fed with mixed flagellate cultures. Alternatively, they were kept in circulation in the Plymoth tank sea water with different rates of flow. The results of the experiments were consistent and precise. After a period of starvation lasting some 15 hr. the frequency changes from the normal to the fast (Fig. 11). The addition of food restores the normal frequency in about 2 hr. After continued starvation for 2 days the frequency is ultra-fast, of the order of one every 2 min. The amplitude is considerably reduced, possibly as a consequence of the increased frequency which may produce some fatigue. Another phenomenon now appears: the siphons slowly move about and it is difficult to keep the levers on the drums. On adding food the siphons become still, and both frequency and amplitude are restored to normal in about 3 hr. (Fig. 12). Control experiments with graphite particles (aquadag S) in place of food particles failed to produce an effect (Fig. 14). In one experiment (illustrated in Fig. 13) the food was added continually and in large measure following a short period of starvation. The animal quickly produced its normal and then its slow speeds. Later, the same phenomenon which had been observed in some freshly fed animals, namely the decrease in amplitude of the contractions with the branchial siphon leading, was again recorded. Some contractile activity was still present but the amplitude was almost completely suppressed. During this time the animal was relatively insensitive to external stimuli. After 2 hr. the food concentration was reduced by mixing with fresh sea water and the normal activity was quickly resumed.

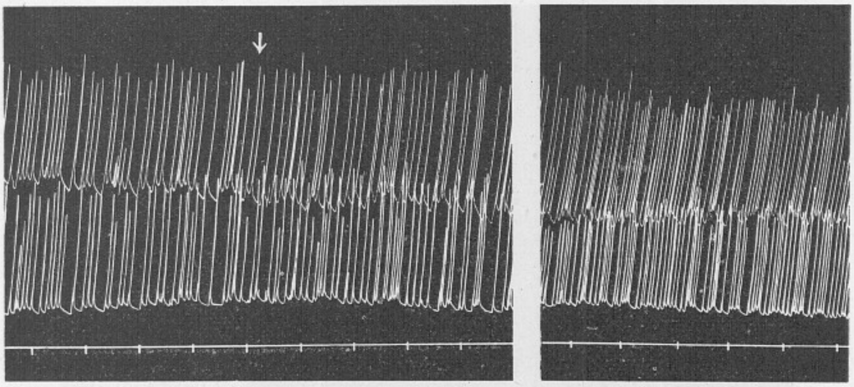

Fig. 11. Continuous records in the Plymouth tank circulation. The rate of flow was great at first and reduced at the point indicated by the arrow to a tiny trickle. An interval of 16 hr. separated the two parts of the record. Time in hr.

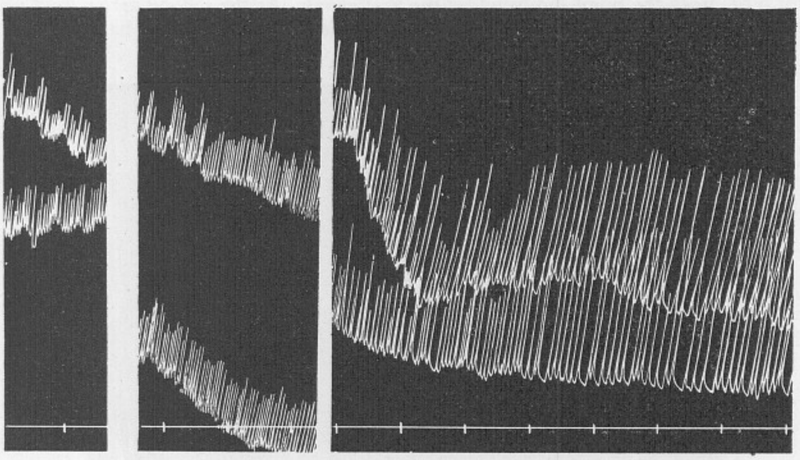

Fig. 12. Records of an animal after 48 hr. starvation in filtered sea water. Food was given to the animal towards the end of the second part of the record, and the third part is a direct continuation following lever adjustment. An interval of 10 hr. separated the first and second parts. Time in hr.

The records obtained by the flow method show similar results (Fig. 15). In addition, they show that the ciliary current is reduced (whether by aperture control in the branchial wall or change of ciliary beat cannot be decided) during starvation, and is restored remarkably quickly in the presence of food particles. If the branchial siphon is denied free access to fresh sea water and forced to resample the same volume continually the rate of squirting goes up rapidly (Fig. 16). The ciliary current was not restricted since a suitable capillary input was used and there was no interference with the atrial squirting, hence it is unlikely that the increased rate is due to interference with re-

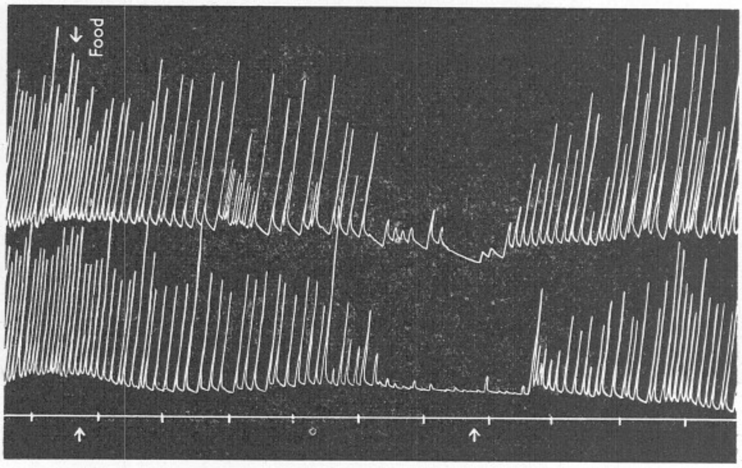

Fig. 13. Records of the activity of an animal starved for 14 hr. and given food continuously for 6 hr. from the point indicated by the first arrow. At the second arrow food was no longer given. A rich flagellate culture was used for feeding. Time in hr.

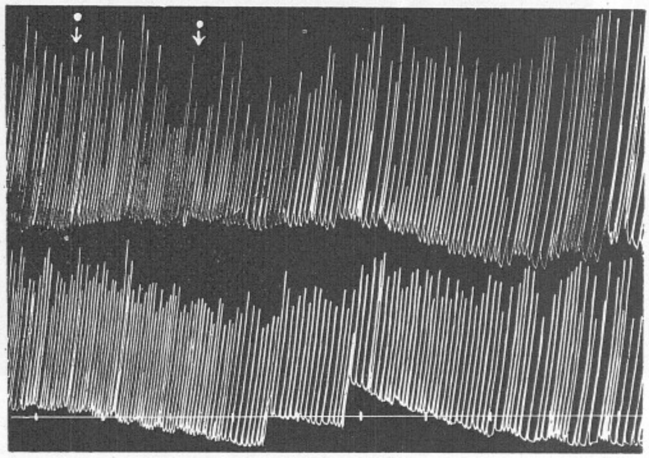

Fig. 14. Control experiments for the above (Fig. 13). The animal was fed only on graphite particles. It was given some food as well at the second arrow. Time in hr.

spiration, but is most probably due to the progressive depletion of food from the water. It is clear from all these experiments that the frequency of spontaneous squirting in *Phallusia* depends upon the concentration of food available in the branchial siphon. The frequency is low when there is a rich supply, high when the supply is impoverished. In this connexion the recent report of Collier, Ray & Magnitzky (1950) is interesting. They find that the volume of the effluent of oysters is proportional to the concentration of some unknown soluble organic material (measured as arabinose equivalents) in the sea water.

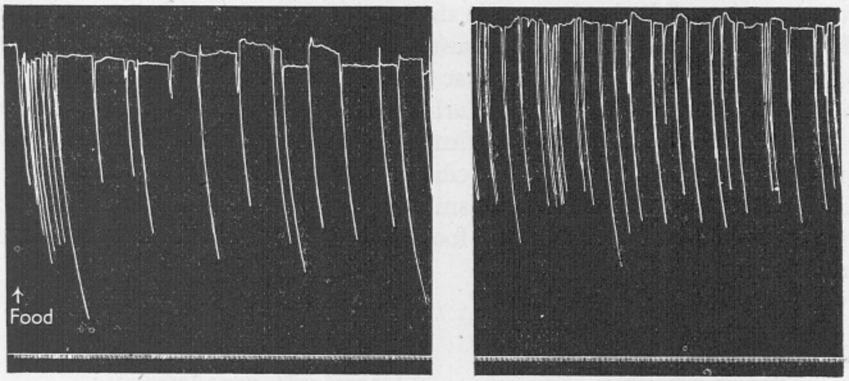

Fig. 15. Flow method recording of atrial siphon squirts immediately after feeding and 16 hr. later. The through-current is proportional to the depression below the upper edge of the record. In the left-hand figure the mean depression represents a continuous flow of 1 c.c./min.

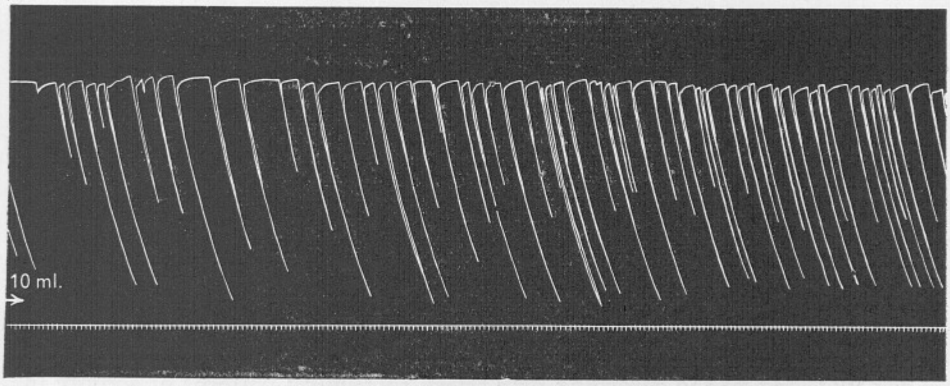

Fig. 16. Flow method recording of branchial siphon squirts showing the effect of restricting the sampling by introducing a capillary input. The arrow at the start of the record indicates a calibration depression of 10 ml. The frequency of the activity increases rapidly during the experiment. Time in min.

Data on the important question of relation between feeding habits and feeding frequency and the concentration of available food over long periods are almost non-existent.

The demonstration that the rate of squirting in an ascidian depends on the concentration of food substance in the branchial sac demands an inquiry into the nature of the receptor mechanism. It has been shown that some of the particles entering the branchial sac of an ascidian enter the mouth of the ciliated pit (van Weel, 1940). Carlisle (1951) has demonstrated the same phenomenon in a salp and confirmed the observation in a monascidian, finding the particles in both the ciliated pit and the neural gland. Carlisle found a gamete-releasing mechanism in *Phallusia* in response to the presence of eggs of the same species in the food. He suggests that the reflex pathways are as follows:

Stimulus	Chemical from ingested gametes
Receptor	Neural gland
Afferent pathway	Hormonal—gonadotrophin
C.N.S.	
Efferent pathway	Neural
Effector	Gonads
Response	Release of gametes

Nervous connexions between the ciliated pit and the ganglion have been demonstrated in two forms (Metcalf, 1895; Hunter, 1898) but have been denied in others (Julin, 1881). The nature of the afferent-efferent pathways needs investigation, but there is certainly a possible receptor mechanism for the food stimulus in the ciliated pit—neural gland complex.

Discussion

The work falls into two parts for the purposes of discussion: (1) the study of the physiological mechanisms of the spontaneous activity; (2) the consideration of its functional significance.

(1) The recent work of Batham & Pantin (1950) has drawn attention to the fact that long-period recording may reveal rhythmical patterns of activity in sedentary animals which on casual observation appear to be quite passive. The sea-anemone, *Metridium senile*, was shown to exhibit periodic contractions of the parietal musculature at intervals of about 10 min. The time sequence is, as Batham & Pantin say, quite low, and they compare it to the slow rhythm of the outbursts of activity of the *Arenicola* proboscis (Wells, 1949) and mammalian uterus. The normal 6–9 min. rhythm of the spontaneous squirting of *Phallusia* is also of this order and deserves equal comparison. The nature and site of the pacemaker presents a difficult problem. The nervous system is extremely simple in anemones and it is difficult to visualize how both pacing

and the co-ordination of muscular movements as complex as those described by Batham & Pantin can be effected by it. The *Arenicola* extrovert is paced by a region of the oesophageal wall (Wells, 1937). In *Metridium* a part of the body-wall appears to act as 'leader'. Other parts of the wall follow the 'leader's' contractions. Although a leading sector may continue to set the pace for a considerable time the site of the leadership may change from time to time. One important aspect of the activity is now clear for both the *Arenicola* and *Metridium* systems: the activity is inherent. It is modified by external stimulation but is not initiated from without. The chain-reflex theory of activity does not apply to either the gross activity cycles of the polychaetes or the slow movements of the anemone. The patterns of spontaneous activity of *Phallusia* clearly associate it with these animals in this respect. The activity is incessant under normal conditions, it is not determined by the periodic fluctuations of any environmental factor or by the gross products of its activity CO_2, faeces etc. It occurs in the absence of food over long periods.

In monascidians spontaneous activity can be recorded from isolated siphons (*Ciona* and *Styela*), and from isolated muscle strips (*Phallusia*). The inherent activity may, therefore, be myogenic with considerable neurogenic control. This situation is perhaps comparable with that found in *Metridium* where one part of the wall leads the rest. The nervous system need only be involved in so far as it effects co-ordination. The quick contractile activity in *Phallusia* involves the whole musculature synchronously, although each part probably has its own activity. The co-ordination is seriously impaired in ascidians in the absence of the ganglion, which also regulates muscle tone and some reflexes.

(2) Concerning the functions of the squirting rather more can be established. The spontaneous contractions of the body-wall serve regularly to clear the exhausted contents of the branchial sac. They must, therefore, assist in keeping the branchial mechanism clean and free from accumulated waste matter. They equally regularly clear the contents of the atrial siphon and this must assist in removing faeces and sexual products. The activity brings fresh supplies of sea water to the exposed internal body-wall and siphon surfaces and must assist in respiratory exchanges. The fresh sea water brought into the branchial siphon following squirts brings with it a supply of suspended food matter. In *Phallusia* the volume of water moved in this way is appreciably larger than that circulated by the ciliary through-current. The frequency of squirting varies inversely with the concentration of available food. It is therefore probable that the principal function of the spontaneous activity in *Phallusia* is to subserve feeding. The method works as follows. Following a spontaneous squirt a new sample of sea water, with various small organisms and food detritus in suspension is sucked into the branchial siphon. The particles are swept to the walls of the sac by the internal circulation of sea water produced by the activity of cilia lining the sac and by the current being drawn

through the apertures of the sac, also produced by ciliary activity. On the walls the particles are caught in the mucus stream proceeding upwards from the endostyle and driven towards the dorsal lamina. Here the twisted cords of mucus containing the particles are driven backwards into the oesophagus. Silt and mud which has not been picked up by the mucus during the few minutes elapsing between squirts is then ejected and the cycle is repeated.

The advantages of this method of feeding are fairly clear. *Phallusia* lives intertidally in sheltered places, or commonly offshore, where the food supply is at times very rich, but subject to considerable fluctuations and often liberally mixed with mud and silt. The branchial mechanisms are particularly liable to become clogged by this estuarine dirt. Squirting helps to remove the waste which would accumulate especially rapidly in an animal with a fast through-current. It also allows a degree of control over the rate of feeding. The continuous-current method does not allow careful control of the rate of feeding when food is plentiful and perhaps being taken in at a far greater rate than can be coped with adequately. The squirting method, since its frequency can be modified, permits a range of feeding volume from zero (when the activity is inhibited after heavy feeding) to about 300 c.c./hr. during starvation. The system is economical of energy. It is now known that the quick contractions of the salps are concerned principally with feeding rather than locomotion (Fedele, 1933; Carlisle, 1950). Some, at least, of the sedentary monascidians may be regarded as feeding by a somewhat similar method of gulping or rather squirting.

The existence of spontaneous activity in several invertebrate phyla has been clearly established, and this activity is apparently always inherent. It has been demonstrated by Wells (1950) that it serves a useful purpose in the lives of certain polychaetes. It is probably equally useful in actinians, although the evidence for this is only just being brought to light, and it is hoped that the present paper will establish its usefulness to certain ascidians. This type of rhythmic oscillation of the work output of certain muscles is widespread. In addition to the groups so far discussed it has also been described in the mantle of lamellibranch molluscs (Redfield, 1917), the *Aurelia* medusa (Widmark, 1913), the sabellid retractor muscles (Wells, 1951) and the retractor pharynx muscles of *Holothuria* (Pople, 1952). Wider investigation may be expected to reveal still more instances.

The work was carried out at the Plymouth laboratory during parts of the springs of 1951 and 1952 and the autumn of 1951, whilst occupying the London University table. I wish to thank the Director and staff of the laboratory for their kindness; Dr M. Parke for supplying the flagellate cultures; and Dr G. P. Wells and Prof. P. B. Medawar, F.R.S., for reading the draft.

SUMMARY

The previous work on spontaneous activity in ascidians is briefly reviewed: there is clear evidence for the existence of spontaneous contractions of the siphons of certain monascidians but no function has been ascribed to this activity. *Phallusia mammillata* exhibits spontaneous contractions of its siphons at intervals of 6–9 min. over long periods in Plymouth tank sea water. The siphon contractions are synchronous. The frequency of the contractions may shift fairly quickly under constant conditions to a higher or lower rate which is usually twice or one half the normal rate.

A method of recording the volume of water propelled by spontaneous activity is described. The method also records the ciliary through-current at the same time as the squirts.

The frequency of the squirting is increased during a period of partial or complete starvation. It is reduced again by adding food cultures to the water.

The physiological mechanisms involved in the spontaneous activity are investigated and comparisons are made with the spontaneous activity exhibited by *Metridium senile* and *Arenicola marina*. It is finally suggested that the spontaneous squirting of certain ascidians is an integral part of their feeding mechanism.

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