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THE REACTIONS OF THE LIMPET, *PATELLA VULGATA* L., TO CERTAIN OF THE IONIC CONSTITUENTS OF SEA WATER

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(Text-figs. 1–8)

It has been shown (Arnold, 1957) that when exposed to air the common British limpet, *Patella vulgata* L., reacts to splashing with normal or moderately diluted sea water by raising the front edge of the shell, and that the amount of this movement can be correlated with the degree of dilution and with the tide level from which the animals are obtained. A basically similar result was obtained by the use of solutions of sodium chloride. The precise factors influencing the limpets were not identified. Additional data can now be presented on these and other reactions of *P. vulgata* as a contribution towards further definition of these factors.

MATERIALS AND METHODS

The limpets were obtained from a rocky ledge lying just below H.W.O.N.T. at the Pier Rocks, St Andrews, and were collected by chipping away fragments of the rock to which they were attached. In the laboratory they were placed in running sea water until they moved from the shattered rock. Each was then placed in a small polythene cup. Though they could easily climb out, the majority adhered firmly and remained within the cups for several days. Difficulties due to horizontal movement of the animals, encountered in the previous experiments, were thus largely avoided. Specimens which at any stage were found to be damaged were discarded. The shell length of those used ranged from 36 to 47 mm, mean length 41 mm.

As in the earlier work, vertical movements were normally recorded by means of a heart lever attached to a hook inserted beneath the front edge of the shell. An upward movement of the shell was thus recorded as an upward movement on the trace. The presence of a hook caused a local retraction of the mantle fringe, but seemed not to affect qualitatively the behaviour of the animal. A slower drum speed (0·048 mm/sec) and longer lever (magnification 35:1) were used than in the preceding experiments. Stimulation was provided by dropping about 2 ml. of fluid onto the apex of the shell, a small amount

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passing beneath its edge and coming into contact with the mantle fringe. The amount of liquid used in this manner was found not to be critical; presumably once the space beneath the shell had been filled any excess drained off without effect. Stimuli were customarily given at 5 min intervals, though in a few experiments 10 min intervals were used. After each stimulus the limpets were washed with about 5 ml. distilled water in order to remove any of the stimulating liquid still adhering to the shell. Holes drilled in the bases of the polythene cups allowed these to drain between each test.

The solutions used (sodium chloride, calcium chloride, magnesium chloride, potassium chloride, sodium sulphate, sodium bicarbonate and an artificial sea water composed of these salts) were made up from Analar reagents to the concentrations given by Pantin (1946) as approximately isotonic with sea water of salinity 34·6‰.

All experiments were performed in daylight during the first three days after collection of the limpets. Further details of the methods employed are given in the accounts of the experiments concerned.

PATTERN OF MOVEMENT OF UNSTIMULATED ANIMALS

Despite all care it was impossible fully to prevent vibrations from reaching the experimental animals. A number of records of otherwise unstimulated limpets were therefore made in order to assess how far they might be affected by these unavoidable mechanical stimuli. It was found that under the conditions generally prevailing in the laboratory the limpets, as upon the shore, slowly raised their shells so as to form a slight gap between the edge and the substratum, then remained quiescent except for a series of, usually small, downward movements. The periodicity and amplitude of these contractions varied between different animals (Fig. 1). Occasionally, those animals which had not been long out of water showed considerable activity, and for this reason a period of 8–10 h dryness prior to each experiment was adopted as standard procedure throughout the rest of the investigation. The occurrence of the movements made by the unstimulated limpets could not be related to the general vibrations of the laboratory. Indeed, almost identical traces were given by two animals, one recorded on a day when the laboratory was entirely empty, the other during a visit by a large party of school-children. Only jarring of the apparatus or of the bench upon which it stood produced an unequivocal response, invariably a sharp downward movement. However, care was needed to avoid sudden decreases in illumination, to which the limpets responded in a similar manner. The presence or absence of this shadow reaction formed a useful test for their general well-being during experiments.

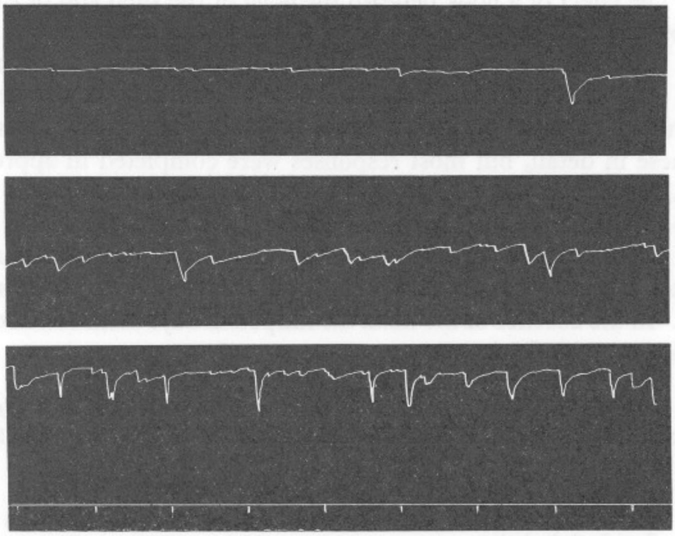

Fig. 1. Part of kymograph records of vertical movements of unstimulated limpets. Time marks at 15 min intervals. Shell lengths 43 mm (upper), 36 mm (middle) and 37 mm (lower).

MODIFICATION OF THE PATTERN THROUGH STIMULATION

Since the limpet is not fully contracted against the substratum when at rest, it may respond to splashing either by an upward movement of the front edge of the shell or by a downward movement. An upward movement was given to sea water, to isotonic solutions of sodium chloride, calcium chloride and magnesium chloride, and to artificial sea water; a downward movement was given to distilled and tap water and to solutions of sodium sulphate, potassium chloride and sodium bicarbonate isotonic with sea water.

Effect of experimental conditions

Since the conditions under which the experiments were performed might be expected to affect the behaviour of the limpets, a series of comparisons were made between animals left undisturbed within the collecting area and those brought into the laboratory, using the responses to sea and fresh water as a guide. It was found that animals upon the shore reacted in a manner qualitatively similar to those studied in the laboratory. Accurate measurements of their movements could not be made, however, and for this purpose a number of specimens attached to small boulders were brought into the laboratory and studied under a binocular dissecting microscope fitted with a micrometer eyepiece. It was thus possible, though tedious, to measure fairly accurately the heights to which the limpets rose upon stimulation, without removing them from their scars or disturbing them in any further manner. The effect of a hook beneath the edge of the shell and of the tension exerted by the heart lever could also be studied in this manner.

It was found that the magnitude of the responses to sea water, measured from the position of maximal contraction of the animal, showed great regularity, while their time relations were also regular. No attempt was made to record these in detail, but most responses were completed in approximately 2 min. Limpets left on their scars gave rather smaller responses than did those re-settled in polythene cups (Table 1). The presence of the hook naturally prevented complete contraction of the animal upon stimulation with fresh water and in these cases the downward movements might be somewhat irregular. In the absence of the hook the limpets always contracted completely when irrigated with fresh water.

TABLE 1. THE EFFECT OF EXPERIMENTAL CONDITIONS UPON THE RESPONSES OF *PATELLA* TO STIMULATION BY SEA WATER

	A	B	C	D
Number of stimuli	20	20	19	10
Mean height attained	1·0	1·5	2·6	1·8 mm
Standard deviation	0·24	0·27	0·22	0·11 mm

A. 38 mm limpet undisturbed on small boulder.
B. Same specimen attached to lever, but still on scar.
C. 40 mm limpet attached to lever and resettled in polythene cup.
D. 36 mm limpet, conditions as in C.
Measurements of A and B made under binocular microscope, those of C and D calculated from kymograph traces.

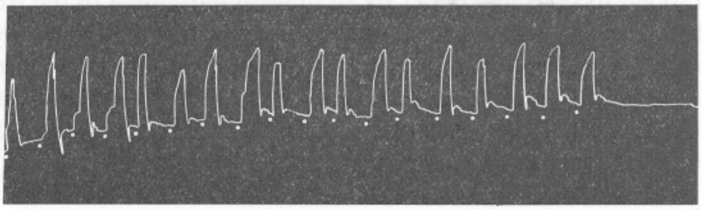

Fig. 2. Kymograph record of response to sea water. Stimuli every 5 min. Shell length 40 mm.

Response to sea water

The response to sea water had certain well-defined characteristics, the majority of which are shown in Fig. 2. The movement commenced abruptly and soon after stimulation and consisted of a smooth, rapid rise to a clear maximum, followed by an equally rapid fall. Occasionally this 'peak' response was preceded by a very small initial contraction of the order of 0·1 mm actual movement. A noticeable feature of the response was that the movements, except for the first one or two in a series, were extremely regular and showed but little variation in their form, general time relations or height attained (by no means the maximum height to which the animals were capable of moving) and the amount of the subsequent downward movement. Under continued stimulation, about half the limpets studied showed a tendency to slight progressive increase in the height of the responses, accompanied by a gradual diminution in the amount of the subsequent downward movements, while the others gave responses of even size. None showed decreased height of movement on repeated stimulation.

Effect of osmotic pressure

As described in the earlier account, the height of response given to sea water diminished with dilution. In order to determine whether this was due to alteration of the ionic concentration or whether it could be ascribed simply to alteration of the osmotic pressure, limpets were tested with normal sea

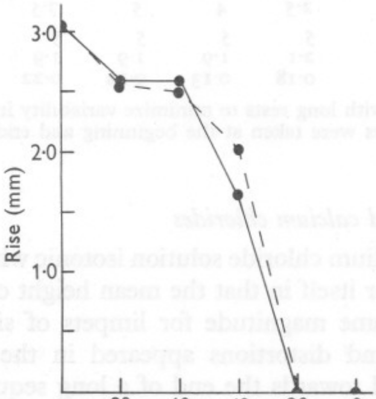

Fig. 3. Response to sea water diluted with distilled water (continuous line) or 0·9 M dextrose solution (broken line). Both distilled water and 0·9 M dextrose solution caused contraction when applied to the extended limpet. Shell length 39 mm.

water and with sea water the relative salinity of which had been reduced to 80, 60, 40 and 20% of normal by dilution with either distilled water or 0·9 M dextrose solution. Both series of dilutions, though of different osmotic pressures, elicited responses which showed considerable similarity in size and form at each level, while differences in height between the two series (Fig. 3) were not consistent.

Influence of pH

Since the reaction of sea water alters slightly upon dilution, while the other solutions used varied considerably in their pH, the influence of this factor was determined by comparison of the reactions to sea water and to isotonic sodium chloride solutions, the pH of which was varied by addition of small amounts of hydrochloric acid or sodium hydroxide. The results of a typical experiment are given in Table 2. In experiments where more alkaline media were used the responses remained regular up to pH 9·5. At pH 10 upward movements were given to the first 2–3 stimuli only, later stimuli eliciting downward movements. Above pH 10·5 the limpets always contracted strongly upon stimulation. If the pH was lowered by use of sulphuric in place of hydrochloric acid the limpets normally moved downward when stimulated and any upward movements given were always smaller than were the responses to sea water.

TABLE 2. EFFECT OF DIFFERENT pH VALUES UPON RESPONSES OF A SINGLE *PATELLA*

	Sodium chloride solutions. Approximate pH					Sea water	
	2·5	4	5	7·5	8		
No. of stimuli	5	5	5	5	5	5	5
Mean height attained	2·1	1·9	1·9	1·9	2·2	2·2	2·1
Standard deviation	0·18	0·13	0·22	0·22	0·22	0·18	0·13

Short series were used with long rests to minimize variability inherent in stimulation with NaCl. The sea-water series were taken at the beginning and end of the experiment. Shell length 40 mm.

Response to sodium and calcium chlorides

The response to sodium chloride solution isotonic with sea water resembled that given to sea water itself in that the mean height of a series of responses was usually of the same magnitude for limpets of similar size. However, many irregularities and distortions appeared in the records and became especially pronounced towards the end of a long sequence of tests (Fig. 4). Most of the upward movements obtained with this form of stimulation were peaks, but both they and the subsequent downward movements showed much greater variation in height than did those given to sea water, while after a time the rapidity of the response was also affected and the movements began to lose their well-defined form, thus giving a transition to a form of response more aptly to be described as a 'hump'. Sodium chloride alone was not an entirely favourable stimulus, since when the isotonic solution was placed upon a portion of the mantle fringe there was a sharp local contraction. After about the tenth stimulation by sodium chloride the shadow reaction was lost, while after about the twentieth stimulation the limpet ceased to react further to either sodium chloride or fresh water and reacted in a quite irregular and unpredictable manner when stimulated with sea water.

The use of isotonic calcium chloride solution as a stimulus resulted in an upward movement, but one of very different form to that elicited by sodium chloride or by sea water. The first few responses were all of the peak type, but thereafter the movements slowed and the maxima became less well-defined, while the subsequent downward movements became slower and smaller until after about the tenth stimulation the limpets failed to react further to either calcium chloride or fresh water (Fig. 5), remaining in an expanded condition with a wide gap between shell and substratum. Responses to calcium chloride were very nearly as regular in the height attained, measured from the level of maximum contraction, as were those given to sea water. The mantle fringe gave no local reaction to isotonic calcium chloride solution.

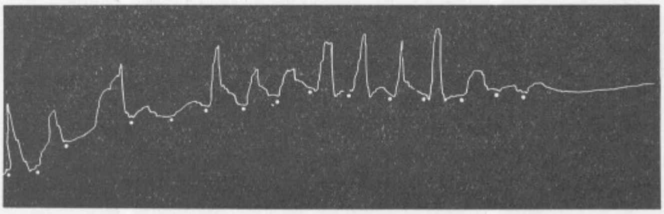

Fig. 4. Kymograph record of response to isotonic NaCl solution. Stimuli every 5 min. Shell length 40 mm.

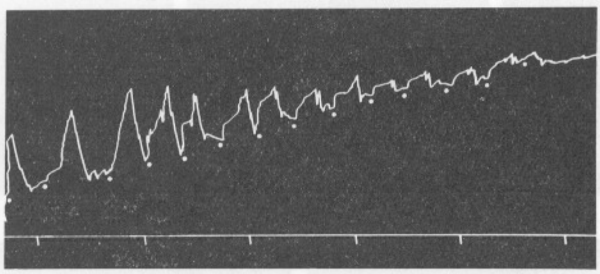

Fig. 5. Kymograph record of response to isotonic $CaCl_2$ solution. Stimuli at 5–10 min intervals. Time mark every 15 min. In this and following records the level of the time mark has been adjusted to the position of maximum contraction of the limpet. Shell length 42 mm.

Since the response to sodium chloride solution resembled that to sea water in its general form, while that to calcium chloride solution did so in its regularity of height attained, it was of interest to examine the effect of mixtures of these two solutions. This proved a matter of some difficulty. The continued use of either one alone was already known to result in characteristic anomalies of behaviour and ultimate cessation of response, while during a long series of tests upon the same animal fatigue and the possible residual influence at each stage of the experiment of the mixture used in the preceding stage had to be considered. The method finally adopted was to group the stimuli in sets of 6 for each test and to alternate each set with a similar group of sea-water stimuli as control. Thus each result could be immediately compared with the behaviour to natural sea water and the stability of this behaviour during the course of the experiment could be assessed. Since such an experiment would involve some scores of stimuli, the limpet was left untouched for 30 min between each set of responses. The combinations of chlorides used were made up from the original solutions isotonic with sea water and contained 1·5, 2·5, 3, 5 and 20% calcium chloride.

An example of a series of traces obtained from a single limpet is given in Fig. 6. The response to sea water showed the same pattern throughout the experiment. The height above base, indicated by the time-trace, remained

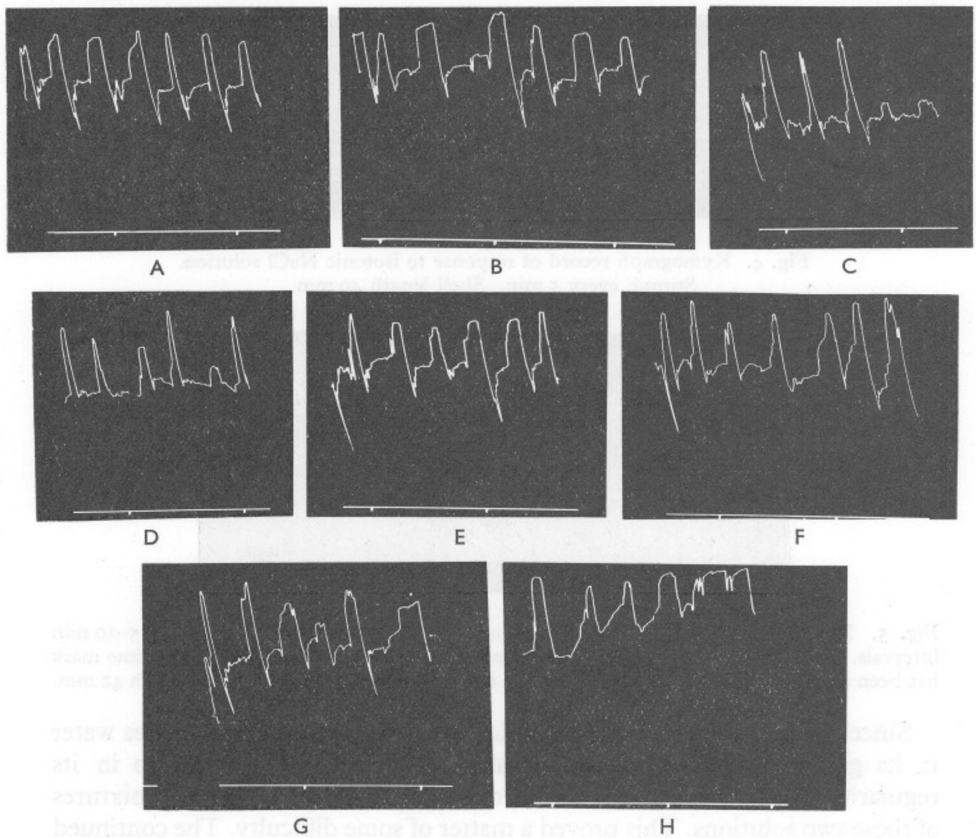

Fig. 6. Kymograph record of short sequences of responses to solutions of varying calcium content. For explanation, see text. Stimuli every 5 min. Time mark every 15 min. Shell length 40 mm.

constant for each upward movement, as did the amount of the subsequent downward movements. Individual peaks were narrow, indicating rapidity of movement, and had clear-cut points (Fig. 6A). Response to an artificial sea water showed a similar pattern, but with the peaks two or three times as wide, indicating a corresponding slowness of movement (Fig. 6B). The pattern of response to isotonic sodium chloride solution resembled the two foregoing patterns in certain respects, but was markedly irregular in the heights attained at each upward movement (Fig. 6C).

When isotonic sodium and calcium chloride mixtures were used, that containing 1·5% calcium chloride resulted in a pattern similar to that induced by sodium chloride alone (Fig. 6D). Responses to mixed solutions containing 2·5 and 3% calcium chloride more closely resembled the responses to sea water (Fig. 6E and F). The solutions which contained 5 and 20% calcium chloride induced patterns of movement closer to that elicited by stimulation with isotonic calcium chloride solution alone, namely slowing of all movements and reduction of the downward component with successive stimuli, though the heights attained with the upward movement remained regular (Fig. 6G and H). The trace shown in Fig. 6G is more irregular in the heights attained than was usual for limpets stimulated in this manner; as it was actually the last test conducted in this experiment the pattern may have been modified by fatigue.

By calculation, the mixture containing 2·5% calcium chloride solution, considered to give the best approximation to the pattern of movement characteristic of sea water, contained 0·36 g/l. Ca″, while the artificial sea water should contain 0·40 g/l. Ca″. The limpets might therefore be adapted to a slightly lower concentration of calcium ion than is normally present in sea water. To check this hypothesis the calcium content of the artificial sea water sample used and of inshore water at the time the experiments were conducted (mid-June, 1958) were determined by the method of Kirk & Moberg (1933). The artificial sea water was found to contain 0·41 g/l. Ca″, while two samples of sea water contained 0·37 and 0·38 g/l. Ca″ (inclusive of strontium). These values differ from those for the open sea (Harvey, 1955), but explanation of the discrepancy is beyond the scope of this paper.

Response to magnesium and potassium chloride

Magnesium chloride solution, isotonic with sea water, induced an upward movement similar to that given to sodium and calcium chlorides, but marked by its own specific characters (Fig. 7). The heights attained in response to successive stimuli were quite regular and the movements themselves reasonably smooth and fairly rapid. For a while at least the amount of each downward movement was also stable. However, each upward movement was succeeded by a period in which the limpet remained extended and almost motionless and the onset of contraction was often much delayed, though rapid enough when once it started. As the experiment proceeded these characteristics became ever more pronounced until after about the tenth stimulus a superficial narcosis ensued. In this condition the limpet would respond, albeit slowly, to direct mechanical stimulation of the foot, but it no longer reacted to fresh water, reduction of light intensity or jarring of the bench or apparatus.

In contrast, isotonic potassium chloride solution dropped upon a slightly expanded limpet induced an immediate and complete contraction, followed by a rapid sequence of abrupt, jerky, up-and-down movements, after which the majority of limpets tested in this manner climbed out of the polythene cup and so terminated the experiment.

The effect of absence of magnesium and potassium ions was tested by use of artificial sea waters in which their chlorides were replaced by an osmotically equivalent amount of sodium chloride. These replacements did not have any effect upon the size of the responses as compared with those given to natural and the complete, artificial sea water.

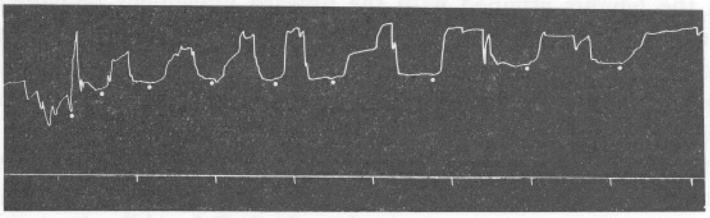

Fig. 7. Kymograph record of response to isotonic $MgCl_2$ solution. Stimuli at approximately 10 min intervals. Time mark every 15 min. Shell length 41 mm.

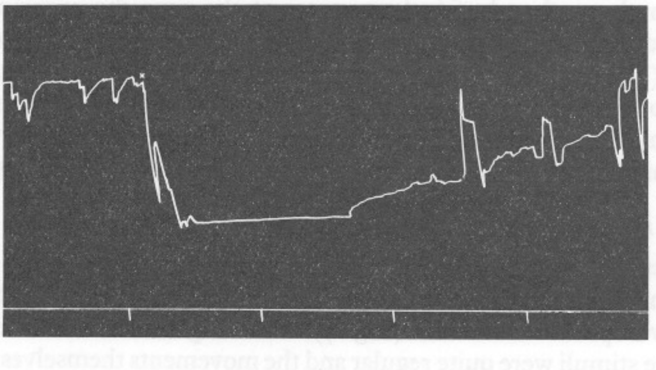

Fig. 8. First part of kymograph record of response to isotonic Na_2SO_4 solution. Stimulus given at *x*. Time mark every 5 min. Shell length 37 mm.

Response to sodium carbonate and sulphate

Isotonic solutions of these two salts induced downward movements. With sodium carbonate the limpet contracted as far as possible upon stimulation, then began a series of fairly rapid up-and-down movements which gradually died away into the normal steady state with its periodic small contractions. With sodium sulphate solution as stimulus the initial contraction was followed by a prolonged period of inactivity (Fig. 8), succeeded by a series of rapid up-and-down movements which might continue for two or more hours before the normal pattern of the quiescent animal was restored.

DISCUSSION

The experiments here described have confirmed the earlier results and show that the responses of *Patella vulgata* to natural and diluted sea water cannot be attributed to recognition of variation in either pH or osmotic pressure of the media. Further, the occurrence of similar reactions when a simple artificial sea water is used as stimulus shows that the responses are not due to the influence of minor ions since, apart from contaminating traces of arsenic and iron in the reagents used, these would be absent. Upward movements have been obtained with simple chloride solutions (except with potassium chloride), but not with sodium sulphate or carbonate, and it follows that the reactions may most easily be attributed to sensitivity to the concentration of chloride ions. Calcium ions apparently exert a stabilizing influence when in optimum concentration and the behaviour of the animal soon indicates whether the amount of calcium present is above or below that of the inshore water to which it is accustomed.

The response of *P. vulgata* to dilution of the external medium differs in its nature from that found in other animals so far investigated. The estuarine lamellibranch *Scrobicularia plana* (da Costa) exhibits behavioural changes when placed in sea water diluted with distilled water, but is unaffected when dilution is secured by means of 0·9 M sucrose solution (Freeman & Rigler, 1957). Loss of activity is shown by the copepod *Tigriopus fulvus* (Fischer) when subjected to salinities of 90‰ and upwards, recovering when the medium is once again diluted (Ranade, 1957), but it is not clear whether the reaction denotes sensitivity to osmotic pressure or to ionic concentration.

The effects of various ions upon *P. vulgata* show interesting similarities to those found for animals belonging to other groups. Thus the branchiopod *Artemia salina* (L.), placed in sodium chloride solution isotonic with sea water survives for several days, but in other solutions the survival time is greatly reduced, the animal becoming moribund in similar solutions of magnesium and calcium chlorides (6–9 h), potassium chloride (30 min) and sodium bicarbonate (5 min) (Croghan, 1958). The importance of calcium ions in resistance to reduced salinity has been demonstrated (Pantin, 1931) for the turbellarian *Procerodes ulvae* (Oersted).

The ecological importance of these reactions in *Patella* can be in little doubt, for they provide a means whereby the feeding period of the limpet may be prolonged beyond the time during which the animal is covered by the tide. In the first place, they enable *P. vulgata* to take advantage of spray cast by breaking waves or carried by an onshore wind; but more than this, the sensitivity to chloride ions and tolerance to a lowered concentration provide a mechanism which would allow the limpet to utilize those periods when the rocks are damp through high humidity or light rain, while ensuring that the animals do not leave their scars when heavy rain temporarily washes the rocks and vegetation free of salt. It is known that *Patella* spp. do browse upon damp rocks during periods of exposure (Orton, 1929; Stevenson, cited by Thorpe, 1956), especially at night, and this has also been observed at St Andrews. Browsing during the period of exposure is least important to those animals which inhabit the lower tide levels, most important to those near the upper limits of the shore. The increased responsiveness to splash and tolerance to diminished salt content of the water shown by limpets near high water mark (Arnold, 1957) can be readily interpreted as an adaptation to adverse environmental conditions which, together with increased radula length (Brian & Owen, 1952), enables these animals to maintain the minimum feeding period necessary for their growth and reproduction.

SUMMARY

Further experiments on the response of *Patella vulgata* to sea water and to the major ionic constituents of sea water are described. These responses cannot be attributed to recognition of variation in pH, osmotic pressure or minor ions. Instead they can be related to chloride ion concentration, modulated by the presence of calcium ions. The calcium ion concentration appears quite critical and the nature of the response given by the limpet alters when this is varied. It is suggested that these responses provide a means by which animals living on the higher portions of the shore can take advantage of spray or of periods of light rain or high humidity to prolong the feeding time into the periods of exposure by the tide.

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