ing test. However, the technique is objective, quantitative and sensitive at a serum dilution of 1:25, so that the dissociated rat submandibular model is a valuable research tool for studying the mechanism of the secretory response induced by CF sera and other secretagogues.

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Perception of constant hydrostatic pressure. A physiological basis for the vertical stratification of marine habitats

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Summary. This paper reports an experimental demonstration of the ability of sessile marine animals (*Balanus balanus* L., Crustacea, Cirripedia) to perceive constant hydrostatic pressure. The results suggest the presence in *Balanus* of a previously unknown sensory system with a tonic response. Such a system may also be present in freely moving animals, but be masked by their locomotor activity.

Experiments to test and to record the effects of light-dark cycles on marine animals have frequently been performed. Experiments to study the effects of high-frequency pressure cycles on marine animals have not yet been done, owing to a lack of appropriate methods[2]. In effect, apparatus must be devised whose operation is equivalent to raising an animal quickly to the simulated surface and just as quickly replacing it under water at a changed pressure. The problem has been the requirement that when the animal changes its depth in the water, all the environmental parameters apart from pressure must remain constant; for instance flowing water is necessary for constant conditions to be ensured, because in standing water the oxygen in the vicinity of the animal is rapidly consumed.

Experiments to record the effects of constant hydrostatic pressure are best performed with an animal of sessile habit, because the relatively small pressure changes associated with the ascents and descents of a freely moving animal in the experimental chamber might mask behaviour reflecting detection of the average pressure. The animals used in these experiments were sessile barnacles (*Balanus balanus* L., Crustacea, Cirripedia).

3 methods have been developed to test experimentally the effects of constant hydrostatic pressure over appreciable periods of time.

1. A method for recording the activity of sessile animals at different simulated depths (figure 1). Because the principle of the seesaw, previously used for recording locomotor activity of freely moving animals, is unsuitable for recording the activity of sessile animals, the principle of the swing - not previously used for activity recording - was modified for the requirements of the experiments. 2. A method for ensuring constant experimental conditions at different simulated depth (figure 2). 3. A method for adjusting and maintaining hydrostatic pressure in a physiological experiment (figure 3).

A single apparatus fulfilling the requirements listed has been devised.[1]

The motor activity of the barnacles, the beating of the cirri, is performed with the regularity of clockwork. Each change in stimulus intensity is followed by an interruption of cirrus beating (figure 4, b). Occasional pauses also occur while experimental conditions are at a constant level. It is the frequency and duration of these pauses, rather than a change in the basic beat rate, that determines the average beat rate characteristic of a particular stimulus.

During each experiment the animals were subjected to an alternation, at regular intervals, between surface pressure (p_1) and the pressure at a depth of 20 m underwater (p_2). During such a series of stimuli (p_1p_2 60:60 min, 30:30 min, 15:15 min, 10:10 min, 5:5 min) an equal total time was spent at each pressure (number of measurements see figure 4, a).

In the present experiments the experimental conditions other than pressure are held constant at different simulated depths. When the beat rate is monitored quantitatively over the listed periods of time (p_1p_2 60:60 - 5:5 min) a difference in the beat rate between the simulated surface and 20-m-depth conditions persists. There is a significant ($p < 0.05$) difference in the beat rate even when the pressures are maintained for as long as 60 min (figure 4, a).

Because the difference in the amount of activity per unit time persists, the results can be interpreted as follows. If it is the case that the observed differences in pressure behaviour indicate differences in metabolic adaptation, the metabolic rates during a 60-min period of stimulation must increase while stimulus duration decreases (60 min:5 min $\triangleq$ 12:1) under otherwise constant conditions. No metabolic system is known which increases metabolic rates only when stimulus duration is decreased. It is unlikely that the differences in beat rate indicate differences in metabolic adaptation (a metabolic model to understand diurnal vertical migration depending on transmitted light has been described previously[5-7]. Light behaviour in sessile animals has been interpreted as a metamorphosis of larval swimming activity[8-10,13-16]). Measurable effects of hydrostatic pressure on the metabolic rate of marine animals have been found only with the simulation of great depth (1000–9000 m)[17,18].

The theoretical difficulty in understanding the result lies in the fact that all known pressure-sensitive receptors are more-or-less rapidly adapting; their excitation falls to zero after a certain period of time. Previous observations of the motor activity of freely moving animals[3,4,19,20] have revealed only a brief change following slight pressure changes, with a rapid return to normal even when the new pressure is maintained (phasic response). A maintained response to pressure as recorded must be mediated by mechanisms of unknown nature, and therefore hard to approach experimentally. However, the presence of such mechanisms must be inferred from the observed behaviour of an animal: a behavioural activity maintained at a level which is a function of the maintained stimulus intensity implies that information about the constant stimulus is available.

It follows, then, that the physiological mechanism responsible for the change in cirrus beat rate is non-adapting. The results suggest the presence in *Balanus* of a previously unknown sensory system with a tonic response.

In the search for experimental confirmation of the ability to detect constant hydrostatic pressure one encounters fundamental theoretical and practical problems. Practical problems are solved by using the methods 1–3. To look at the theoretical problems let us consider the properties of hydrostatic pressure.

Hydrostatic pressure acts as a variable stimulus when an animal changes its depth under water (category 1, below), whereas at a given depth it is constant (category 2). The effects known to be brought about by variable pressure interacting with light have been summarized in several publications[19,20]. In evaluating the differences between variable and constant hydrostatic pressure, 2 observations should be kept in mind: – there is a fundamental distinction, from the point of view of the detector, between directional and non-directional stimuli, and – the transformation of the pressure-stimulus into excitation occurs differently in animals having gas-filled cavities within their bodies and in those lacking such structures.

Consideration of these principles may lead to the following classification.

1. Variable hydrostatic pressure can act directionally (a) or non-directionally (b). a) Stagnation pressure – that which is built up in front of a moved object or a marine animal during locomotion – is effectively directional, in that the pressure gradient produced causes displacement or asymmetrical distortion of particular parts of the body. The action of a wave upon a stationary body is similar. b) Hydraulic compression and decompression during upward and downward movements through water are effectively nondirectional, in that the pressure is exerted uniformly over the entire body. In marine animals with gas bladders compression reduces the size of the bladder and decompression enlarges it. These nondirectional changes are transformed into bending or shear stimuli, which act on mechanoreceptors within the body to produce excitation. Marine animals without gas bladders cannot be sufficiently deformed by hydraulic compression and decompression, because the compressibility of the body fluids and the surrounding water is very slight (the increase in density at a depth of 1000 m is about 0.5%). Thus the body weight and volume of these animals remain approximately constant under pressure changes of the speed and magnitude ordi-

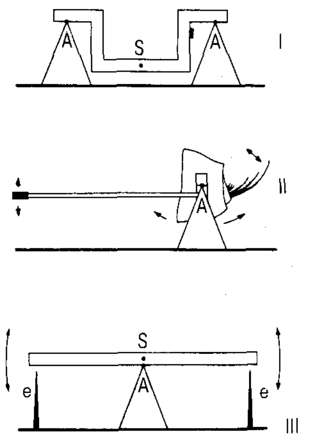

Fig. 1. The principle of the swing (apparatus diagrammed from the front in I and from the side in II) compared with the principle of the seesaw (III). A support points; S centers of gravity; e electrical contacts. I. S lies below A (stable equilibrium). S of the swing on which the animal rides can be raised as high as the support points A. At the transition from a stable equilibrium (S below A) to a labile equilibrium (S above A) the expenditure of force required to rotate the system (< 0.5°) is least. Under these conditions the feeble force of the sessile animal (approximately 10 erg · sec^{-1}; Crisp and Southward[21]) suffices to move the swing. II. The end of a pointer rigidly fixed to the swing interrupts a beam of light at each cirrus beat; the pulses are amplified, recorded and counted. III. In the horizontal plank of a seesaw (ordinarily realized as a disk) S lies above A (labile equilibrium). As a rule the apparatus is constructed so that locomotor activity of an experimental animal causes opening or closure of electrical contacts (e) located below the edge of a disk. The more numerous the contacts around the circumference, the more precisely are the animal's changes of location recorded.

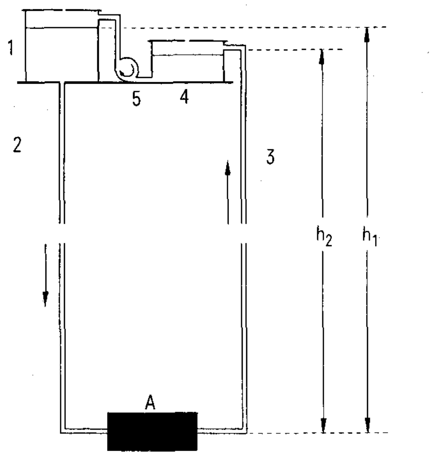

Fig. 2. The principle underlying the method by which experimental conditions other than pressure are held constant at different simulated depths. 1, water reservoir; 2, descending conduit of arbitrary length; A, pressure chamber containing the experimental animals; 3, ascending conduit of arbitrary length; 4, collecting tank for returned water; 5, rotary pumps to move the returned water to heigh h_1; h_1: hydrostatic pressure, to which the animals are exposed in the experiments, (h_1–h_2): pressure difference between the inflowing and outflowing water, which equals the driving force for water flow. The rate of flow depends on this pressure difference which compensates different lengths and diameters of 2 and 3. A 10-m-column of sea-water (with salinity 35‰, at 20 °C) ≘ 1004,965 mbar. Conversion factors: 10^{-6} bar = 1 μbar = 0.1 N · m^{-2} = 1 dyn · cm^{-2})[22]. If another set of 1, 4 and 5 is placed at a lower level than h_1 and h_2 and the two systems of circulating water are connected with A, then the simulated depths can be changed by alternating operation of valves in the two systems (see figure 3). For a critique of currently available methods of pressure production see[2].

narily encountered in the biotope[3]. Other stimuli, which are present during both active and passive vertical movements through the water, are also used to select the optimal depth. Among these is the flow of water relative to the animal, which can displace appendages. These stimuli are distinct from the simultaneously occurring pressure changes[19].

2. Constant hydrostatic pressure is always nondirectional. The vertical stratification of marine habitats has been known since the expeditions of Challenger I (1872-76) and various aspects have often been described[2]. This stratification is characterized by species specificity - individual species remain throughout their lives in the depth optimal for them. This fact implies a problem which must be formulated precisely: Pressure changes from one level to another, to which an organism is not yet adapted, are known to be physiologically effective until adaptation has finished; then excitation falls to zero. A constant pressure, to which an organism has become adapted, does not act as a stimulus to the known receptor mechanisms. Nevertheless, it can be assumed that a capacity to detect constant pressure is involved in establishing the vertical arrangement of marine habitats (differences in the intensity of transmitted light are useful only in the euphotic zone, for active correction of depth following passive drift).

For the progress of science, it usually makes little difference which comes first, the data or the hypothesis. By analysing some problems of marine biology and by using appropriate methods, some data have been collected, and are presented in this paper. They demonstrate the ability of marine animals to detect constant hydrostatic pressure. The mechanism by which constant hydrostatic pressure is transduced physiologically is still obscure. Such a mechanism may also be present in freely-moving animals, but be masked by their locomotor activity. Probably the vertical stratification of marine habitats depends on hydrostatic pressure, as the horizontal stratification of the surface area between the poles depends on temperature. Further experiments ought to be done.

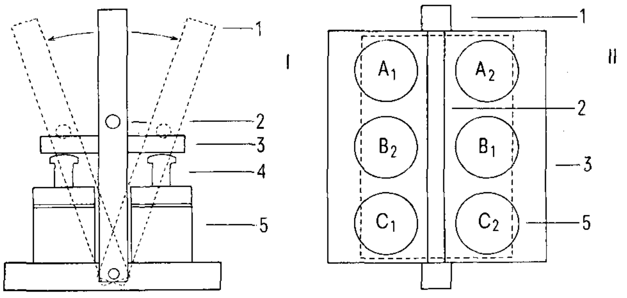

Fig. 3. The principle of the method by which hydrostatic pressure is adjusted in physiological experiments. Diagram of the alternating operation of the valves in 2 systems of circulating water under pressure, seen from side (I) and above (II). 1, lever; 2, depressing pin; 3, depressed plate; 4, valve heads; 5, bodies of valves (inlets and outlets not drawn). I. When the lever (1) is moved, the pin (2) pushes the plate (3) down on 1 side. This pressure on the head of the valves (4) cuts off the passage of water through the valves. II. Switching diagram for the valves controlling 2 systems of high-pressure water circulation. Each of the 2 sets of valves (A_1, B_1, C_1) and (A_2, B_2, C_2) belongs to 1 circulation system. A_1 and A_2 regulate inflow and C_1 and C_2 outflow of the water. Because the organic content of standing water can change, the water prevented from circulation through the experimental aquaria must be made to flow along another path: alternately closing the valve series (A_1, B_2, C_1) and (A_2, B_1, C_2) a 'short circuit' pathway is opened whenever the main path of circulation is blocked, and vice versa (B_1 or B_2).

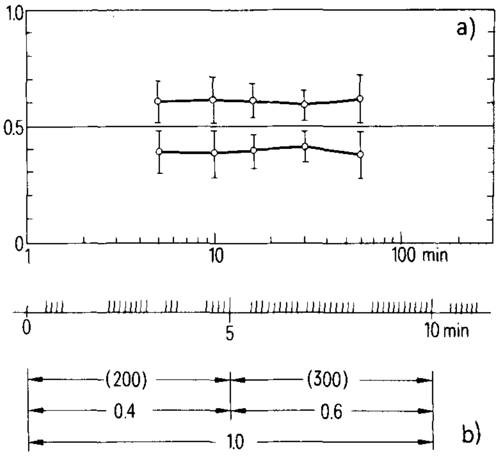

Fig. 4. The cirrus beat rate at 2 different pressures. *a* Horizontal lines: the relative amount of activity (ordinate, cf. b) per unit time (abscissa) under simulated surface (p_1; 0-0.5) and 20-m-depth (p_2; 0.5-1) conditions in 5 animals; vertical lines: SD (the horizontal lines differ significantly at the level $p < 0.05$). Number of measurements per data point: $n = 120 \cdot 5$ (p_1p_2 5:5 min), $n = 80 \cdot 5$ (p_1p_2 10:10 min), $n = 384 \cdot 5$ (p_1p_2 15:15 min), $n = 192 \cdot 5$ (p_1p_2 30:30 min), $n = 120 \cdot 5$ (p_1p_2 60:60 min). The activity must be expressed in relative terms (ordinate) because during long experiments the absolute rate of activity can increase or decrease by a factor of 10 (i.e. 300-3000 cirrus beats per h)[9]. The animals are more active at depth (0.5-1) than at the surface (0-0.5); only in rare individuals is this situation reversed. The difference in relative activity is maintained regardless of the time span over which the animals are kept under constant pressure (5-60 min; abscissa). *b* This is a diagram of one of $(120 \cdot 5) = 600$ records from which the graph in a (5 min; abscizza) is constructed. The fully automated recording device used in methods 1-3 produces a mark on the paper strip of an event recorder for each cirrus beat of an animal. Vertical lines. Cirrus beats (proportion 1:10). In this case the alternation between p_1 and p_2 occurred every 5 min. At the surface (0-5 min; p_1) the rate of activity is less (200 cirrus beats per 5 min) than at a simulated depth of 20 m (5-10 min; p_2; 300 cirrus beats per 5 min). The relative activities are thus 0.4 and 0.6 (total 1.0) as in a.

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