THE EFFECTS OF HIGH HYDROSTATIC PRESSURES ON A SUCTORIAN

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INTRODUCTION

High pressure tends to encourage changes which result in a decrease of volume. It may affect the packing and configuration of molecules, with resulting changes in the properties of the materials concerned, and it may influence the rate and equilibrium of chemical reactions if a volume change is involved. Clearly the effects of pressure on a living cell may be very complicated.

At biological temperatures water is compressed by about 5% at 1000 atm. (about 15,000 lb./sq.in.), and its viscosity is increased by about 5%. Lipoid components of protoplasm no doubt undergo changes in the same direction, and might also solidify. Myosin gels solate progressively with increasing pressure in experiments ranging up to 8000 lb./sq.in. (544 atm.) (Marsland & Brown, 1942), and the same appears to be true of the plasmagel of living cells (Brown, 1934; Brown & Marsland, 1936). The rounding up of Amoeba (Marsland & Brown, 1936), and the disappearance of cleavage furrows in dividing sea-urchin eggs (Marsland, 1938) at these pressures, are attributed to the solation of the cortical gel. Moderately high pressure also increases the tension exerted by contracting muscle (Brown, 1934, 1936). The effects of high pressure on proteins no doubt play an important part in these phenomena. Egg albumen is coagulated at 5000–7000 atm. (Bridgman, 1914), and various enzymes are reversibly or irreversibly inactivated at comparable pressures (Curl & Jansen, 1950a, b). It is therefore possible that less drastic effects on molecular structure occur at lower pressures. Under certain experimental conditions and particularly at rather high temperatures, catalysis by various enzymes is accelerated by pressures of around 4000–7000 lb./sq.in. (272–476 atm.) (Johnson, Brown & Marsland, 1942a; Eyring, Johnson & Gensler, 1946; Werbin & McLaren, 1951). It has been suggested that high pressure prevents the unfolding of proteins from the globular state and so opposes denaturation. Narcosis by certain drugs is counteracted by high pressure, presumably because of some interaction between drug and enzyme (Johnson, Brown & Marsland, 1942b). It is not clear from all this work to what extent in living cells high pressure acts merely by reason of changes in volume of enzyme and substrate during activation and reaction, and to what extent it actually alters the structure of enzymes or substrate and so brings these into greater or less steric conformity, in accordance with Goldacre’s (1952) model. Although work has so far been centred on the effects of pressure on enzyme systems,
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there are other interesting fields open for investigation, including ionization and the configuration of molecules at interfaces. The biophysical chemistry of moderately high pressures is still in a very early state.

On the biological side the need for more detailed observations on a wider variety of material has led to a new design of pressure vessel for use with the microscope. With this an initial investigation has been made of the effects of pressures ranging up to 15,000 lb./sq.in., or just over 1000 atm., on the fresh-water suctorian *Discophrya piriformis* Guilcher (1947).* This animal feeds on holotrich ciliates, the cytoplasm of which it appears to suck up through its tentacles. During the process of feeding the body surface expands, and there is evidence to suggest that this expansion is an active process which serves to lower the internal pressure of the organism (Kitching, 1952a). It will be shown in this paper that hydrostatic pressures of over 2000 lb./sq.in. cause an expansion of the pellicle, and sometimes of the protoplasmic surface as well, perhaps similar to the expansion which occurs during feeding. At rather higher pressures results are obtained which provide a basis for comparison with amoebae and dividing sea-urchin eggs. The course of recovery after release of pressure throws interesting light on the relation of protoplasm and pellicle.

METHODS

*Discophrya* was cultured in Bristol tap water in covered glass dishes, and was fed periodically with *Paramecium*. In some cases silk threads were floated on the surface of the water as a convenient substrate.

The pressure vessel, designed by Mr G. A. Shephard, Alkali Division, Imperial Chemical Industries Ltd., is shown in Text-fig. 1. It consists of a 'high tensile' steel box with two glass windows set in steel disks which are held in position by means of annular holding screws. The upper disk with its window is removed when material is to be mounted in the pressure vessel. (Each window disk actually consists of two components. Details of the window assembly are also shown in Text-fig. 1. The neoprene washer protects the window from stress due to the holding screw. The two components of the window disk are only separated when it is necessary to fit a new glass. Each holding screw is slotted to receive a key with which it can be tightened or undone, but this is not shown in Text-fig. 1.) The pressure vessel is connected by heavy-walled steel tubing to a hydraulic pump fitted with a pressure gauge.

The pressure vessel is held in a vice, and is used in conjunction with a suitably modified microscope. A Watson 'Holos' objective (x 10, 25 mm.) has been used in this work, together with an inclined extension tube fitted with a screw micrometer eyepiece having a magnification of x 25. Photographs were taken with a Leica microscope camera with a x 20 eyepiece. On the advice of Dr C. R. Burch, a plano-convex lens specially made by him, with its centre of curvature coinciding

* This species is closely related to *Podophrya collmi* Root (1915), and was called *Podophrya sp.* in my earlier papers (Kitching, 1951, 1952a, b). It reproduces by evaginating a single internally produced embryo, and therefore in accordance with Guilcher's (1950) classification must be called *Discophrya*.
with the position of the organism under observation, was placed in oil-immersion contact with the outer surface of the upper window, to correct for spherical aberration. The 'Holos' objective (N.A. = 0.30) will have a maximum numerical aperture of 0.45 when used in conjunction with this oil-immersion correcting lens. The cone of light diverging from the organism in the hanging drop is restricted by the geometry of the window of the pressure vessel, which has a maximum numerical aperture of 0.58. The maximum numerical aperture for the condenser, illuminating the organism through the lower window and through the cavity of the pressure vessel (containing medicinal paraffin) is about 0.40. In practice the window glass sometimes developed slight internal cracks, and the resolution suffered somewhat as a result. The photographs (Pls. 3–5) show that it was adequate for the purpose. The centre of the field was used, and chromatic errors were not obtrusive.

The pressure vessel was filled with medicinal paraffin ('mineral oil'). Experimental material was mounted in medium from its own culture in a hanging drop on...
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The inner side of the upper window. After a preliminary examination on the stage of an ordinary microscope, the window in its disk was set up in the pressure vessel. The Discophrya chosen for observation lay with its long axis in one optical plane.

Discophrya was observed before compression, during exposure to one or several different hydrostatic pressures, and finally after release of pressure. Pressures of from 1000 to 15,000 lb./sq.in. (68-1020 atm.) were used, and the periods of application ranged from 5 min. to 8 hr. The pressure was raised in from 1 to 3 sec., and released in less than that time. Sometimes Discophrya which had survived exposure to high pressure was left in the pressure vessel for several days after the pressure had been released. It remained alive and apparently healthy; there were no signs of deterioration due to confinement in the pressure vessel.

Text-fig. 2. Diagrammatic tracings from photographs of a newly settled D. piriformis. The pressure was raised to 3000 lb./sq.in. for 73 min., and then to 9000 lb./sq.in. for 72 min. See the text for a fuller account.

RESULTS

Effects of high pressure on the general appearance

For convenience in description an account will first be given of the single experiment on a newly settled Discophrya illustrated in Text-fig. 2. This figure consists of diagrammatic tracings of a few of the photographs taken during this experiment. About 20 sec. after application of 3000 lb./sq.in., a fold or crease became visible in the body surface (Text-fig. 2, c), and it deepened during the next minute (d, e). The protoplasm also became separated by a narrow space from the overlying pellicle. After a period of 73 min. at 3000 lb./sq.in. the pressure was raised to 9000 lb./sq.in.
for 72 min. The protoplasm gradually became further separated from the pellicle, and eventually appeared to occupy a smaller volume (Text-fig. 2, k) than originally. Most of the tentacles disappeared, and there was no detectable activity of the contractile vacuole at this pressure. On release of pressure the meganucleus became immediately visible (l), and the protoplasm gradually filled up the pellicle (m). The contractile vacuole first became visible about 25 min. after the release of pressure, and was active from then onwards. The few remaining short tentacles disappeared, the body surface became somewhat irregular, the meganucleus faded (p), and after many hours, during which there was a further reorganization of the shape of the body, tentacles reappeared (q, r).

The following summary is based on thirty-two experiments in which photographs were taken at frequent intervals as a record of changes in the shape of the body, and on a number of other experiments without photographs. Sometimes several organisms were kept under observation in a single experiment. In most experiments pressure was applied for 10–60 min., but in some for a much longer time. 1000 lb./sq.in. (68 atm.). No effects on the shape or size of the body were observed with the pressure maintained for 60 min. (in eight individuals) or less.

2000 lb./sq.in. (136 atm.). Creasing appeared some 15 sec. to 1 min. after the application of pressure and usually attained its maximum within about 2 min. It might occur anywhere on the surface of the body and in any direction. The creasing subsequently became reduced, and (in six individuals out of nine) the body surface became completely smooth and rounded again within an hour, even though the pressure was maintained. In some cases, as a stage in the disappearance of the wrinkles, the pellicle became minutely corrugated (see Kitching, 1954, fig. 1). The tentacles remained normal in appearance. No further changes occurred on release of the pressure.

3000 lb./sq.in. (204 atm.). The body became creased as at 2000 lb./sq.in., but the creasing persisted as long as the pressure was maintained. After about 10–15 min. the protoplasm in some cases became partly separated from the pellicle. The pellicle appeared from a comparison of photographs to be larger than it had been before the application of pressure, but this question will be considered again later (p. 61). In the shorter experiments the tentacles appeared unaffected, and this is true also of a Discophrya which was maintained at 3000 lb./sq.in. for 3 hr., the body being heavily wrinkled. In another case after 8 hr. at this pressure the tentacles were reduced in number and abnormally short. On release of pressure the protoplasm spread back to the pellicle within a few minutes, and the normal appearance of the organism became restored during the course of 12 hr. or less.

4000–9000 lb./sq.in. (272–612 atm.). The higher the pressure, the more rapid was the separation of protoplasm from pellicle. On release of pressure, recovery took several hours. In the experiment illustrated in Pl. 4 the volume appears to have been distinctly smaller after recovery than that before treatment.

10,000 lb./sq.in. (680 atm.). The organism responded within a second of the application of pressure by a separation of the protoplasm from the pellicle. The protoplasm became rounded within a pellicle which appeared bigger than before
The effects of high hydrostatic pressures on a suctorian (Pl. 5, C2-4, D2 and D4). In a few cases the protoplasm appeared shrunk (Pl. 5, series D; see also Text-fig. 5). The tentacles appeared slightly irregular and a few became obviously bent. On release of pressure after half an hour at 10,000 lb./sq.in. (five experiments) the protoplasm spread within about a minute and made contact with most of the pellicle, following the creases and wrinkles of the latter (Pl. 5, C5 and D3). The protoplasm appeared to occupy the pellicle more fully within the next few minutes, as though swelling. The tentacles became still more bent and irregular, faded away, and apparently disintegrated. Sometimes the meganucleus, normally scarcely visible, became conspicuous on release of pressure (Pl. 5, C5), but later faded. During the course of the following few hours, the perimeter of the organism, as seen in optical section, grew progressively shorter. During this process the pellicle lost its deep folds, became minutely wrinkled (Pl. 5, C7) and finally within a day or two became smooth. However, in several cases, in a region where the protoplasm had failed to make contact with the pellicle, a small wrinkle of pellicle persisted for many hours (Pl. 4, B9). New tentacles appeared as the body surface became smooth again (Pl. 5, C9). Discophrya which had been exposed to 10,000 lb./sq.in. for half an hour gave rise to flourishing cultures.

12,000-15,000 lb./sq.in. (816-1020 atm.). The effects on the general appearance were the same as those at 10,000 lb./sq.in. There was usually an immediate rounding up of the protoplasm within the pellicle. The meganucleus usually became visible on release of pressure. The organisms eventually recovered their normal appearance provided that exposure to high pressure had not lasted too long. A few organisms survived a pressure of 15,000 lb./sq.in. for 7 min. The contractile vacuole resumed activity, and the body partly recovered its shape in about 3 days. However, the number of tentacles was small, and the general appearance was abnormal. Other individuals swelled up soon after release of pressure and were obviously dead.

Interpretation of creasing

Creasing of the body surface might be due either to a decrease in body volume or to an increase in the area of the body surface. It is not possible to determine changes in volume from the photographs, because the rotational symmetry of the organism is lost with creasing. The general impression obtained from watching the process of creasing is that the organism shrinks in volume. This is strongly suggested by Pl. 4, as well as by some of the superimposed tracings of organisms before treatment and after rounding up of the protoplasm at high pressure (Text-fig. 5). However, it cannot be definitely asserted that creasing at relatively low pressure is accompanied by a loss of volume, because an increase in area equivalent to only a small increase in radius could cause a considerable localized creasing without change in body volume.

Comparisons were made of the outline of Discophrya before and after creasing, and the length of the perimeter of the pellicle was measured in each case. Examples in which the protoplasm had not yet substantially separated from the pellicle are given in Text-fig. 3. The perimeter always increased with creasing, and the increase presumably affected the protoplasmic surface as well as the pellicle. For
seven organisms subjected to 10,000 lb./sq.in. for half an hour or longer, so that the protoplasm became partially rounded up within the pellicle, the average increase in perimeter (measured along the pellicle) was 11% with extremes of 4 and 16%.

**Text-fig. 3.** The creasing of *D. piriformis* at high hydrostatic pressure. Tracings from photographs obtained in twelve experiments on different individuals. In each case the continuous lines represent the outline before treatment, and the broken line the outline at high pressure as stated below. The change in length of the perimeter is also given for each case as a percentage of the original length. The protoplasmic surface, in all these cases, was still adhering closely to the pellicle.

- a, 1 min. at 4250 lb./sq.in.; +5% (or 12% along fold).
- b, 15 sec. at 4250 lb./sq.in.; +6%.
- c, 18 sec. at 6100 lb./sq.in.; +4%.
- d, 4 min. at 5000 lb./sq.in.; +10%.
- e, 13 min. at 5000 lb./sq.in.; +9%.
- f, 13 min. at 5000 lb./sq.in.; +17%.
- g, 3 hr. at 3000 lb./sq.in.; +7%.
- h, 3 min. at 6000 lb./sq.in.; +3%.
- i, 2½ min. at 4000 lb./sq.in.; +2%.
- j, 5 min. at 4000 lb./sq.in.; +4%.
- k, 8 min. at 3500 lb./sq.in.; +14% and +17%.
- l, 12 min. at 15,000 lb./sq.in.; +8%.

**Text-fig. 4.** Shrinkage of *D. piriformis* by exosmosis. Tracings from photographs of six individuals caused to shrink slightly (upper row) and then more drastically (lower row) by treatment for about 20 min. in 0-1 M sucrose, followed by about 30 min. in 0-2 M sucrose. In the upper row the animals are shown before treatment (continuous line) and after treatment with 0-1 M sucrose (broken line); in the lower row the same animals are shown before any treatment (continuous line) and after treatment with 0-2 M sucrose.

In contrast to this, organisms caused to wrinkle by exosmosis in a 0-1 M solution of sucrose always showed a decrease in length of the perimeter, the average for seven cases being 3-6% with extremes of 0-4 and 6% (Text-fig. 4). In a number of
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cases the increase in perimeter resulting from high pressure was very obvious, the most extreme being that of the newly settled organism shown in PI 3, in which the increase in length of the perimeter amounted to 28%. The protoplasmic surface remained adhering to the pellicle in this experiment, and must have increased in area to the same extent. Other examples are illustrated in PI. 4, series B (increase in perimeter 15%) and PI. 5, series C (increase 12%).

Text-fig. 5. The rounding up of the protoplasm of D. piriformis at high hydrostatic pressure. Tracings from photographs obtained in six experiments on different individuals. In each case the left-hand drawing shows the outline of the organism before treatment; the centre drawing shows the outline of the organism and of the protoplasm within the pellicle at high pressure, as stated below; and the right-hand drawing shows the outline of the protoplasm (without pellicle) before treatment (continuous line) and at high pressure (broken line). The treatments were as follows: a, 30 min. at 10,000 lb./sq.in. b, 7 hr. at 6000 lb./sq.in. c, 30 min. at 10,000 lb./sq.in. d, 32 min. at 10,000 lb./sq.in. e, 73 min. at 3000 lb./sq.in. followed by 69 min. at 9000 lb./sq.in. f, 73 min. at 3000 lb./sq.in. followed by 72 min. at 9000 lb./sq.in.

Further experiments on creasing

In a few experiments organisms were subjected to pressures normally sufficient to produce creasing, but the pressure was released before creasing occurred. In two cases a pressure of 5000 lb./sq.in. was applied for 5 sec., and in two others 3000 lb./sq.in. for 10 sec. No creasing developed either during the application of pressure or afterwards.

In another experiment a Discophrya was subjected for brief periods (3 sec.) to pressures of 5000–6000 lb./sq.in., alternating with longer periods (6–12 min.) without applied pressure. Some wrinkling developed during the exposure to pressure, but not as much as would be expected if the pressure were maintained. During the
interval without applied pressure the wrinkling gradually faded, and eventually disappeared entirely. Pressure was applied and released in this way four times with the same result.

**DISCUSSION**

Pressure causes an expansion of the pellicle of *Discophrya piriformis*. This process is not immediate, but takes a minute or more, at any rate at the lower pressures. It is not known what causes this expansion, but it seems likely that it depends on some effect of pressure on the underlying protoplasm. The expanded pellicle does not return immediately to its original condition after release of the pressure, but undergoes a slow process of reorganization over a period of hours; contact with the surface of the protoplasm seems to be necessary for this purpose, and possibly surface enzymes play a part. It seems possible that expansion of the pellicle as a result of pressure is of the same nature as expansion during feeding. It is of the same order of magnitude in both cases. Perhaps pressure merely sets in motion a process normal to the life of the organism.

The protoplasmic surface appears also to expand in many cases in which the pressure is not too high. It is not known whether it is drawn out by the expanding pellicle, permitted by the expanding pellicle to exercise an innate tendency to spread, or caused to expand by a direct influence of pressure upon it. The second alternative is perhaps the simplest, and receives some slight support from the observation that protoplasm which has been caused to recede from the pellicle or round up at high pressure usually spreads and makes contact with the pellicle within a few minutes after release of the pressure. A capacity of the protoplasmic surface to expand might provide a basis for suction.

Prolonged exposure to high pressure usually causes a recession of the protoplasm from the pellicle, and at rather high pressures the protoplasm may become partly rounded up. This may be comparable with the rounding up of amoebae (Marsland & Brown, 1936), and the retrogression of cleavage furrows in sea-urchin eggs (Marsland, 1938) at high pressure, and in terms of current theories of the arrangement of cell proteins (Goldacre & Lorch, 1950; Mitchison, 1952), may be ascribed to a folding of protein molecules to a contracted and possibly even globular state. The paradox of expansion at moderate pressure and contraction at higher pressures may be explained in various ways, as for instance by supposing that moderate pressures expand the pellicle and allow the protoplasmic surface to expand, but that higher pressures affect the protoplasmic surface directly and cause it to contract.

The whole discussion of the effects of high pressure on *Discophrya* is complicated by the fact that certain of the records suggest a decrease in body volume, but there is considerable variation in this matter. The change in volume due to the compressibility of water would not be noticeable and cannot explain these cases. There must be a loss of material from the organism. Water might for some unknown reason pass out through the tentacles, or perhaps pressure might produce a redistribution of water between the organism and environment owing to the volume change of concentration or dilution of cell solutes. It is not possible to say whether
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a decrease in volume contributes significantly to creasing at the lower pressures, but the fact that creasing is fully effective at pressures too low to cause a serious decrease in the rate of output of the contractile vacuole indirectly but strongly suggests that there is no loss of water from the hyaloplasm.

It would be imprudent to speculate any more on the effects of high pressure on Discophrya until a comparison can be made with other materials.

SUMMARY

1. The suctorian Discophrya piriformis Guilcher has been subjected to pressures ranging from 1000 to 15,000 lb./sq.in. (68-1020 atm.).

2. Pressures of 2000 lb./sq.in. and over cause a creasing of the body surface. Except at the higher pressures this occurs some seconds after the application of pressure.

3. Creasing is accompanied by an expansion of the pellicle. At the lower pressures, there is also an expansion of the protoplasmic surface. A comparison may be made with the expansion of the body surface which occurs during feeding.

4. In many cases, and particularly at the higher pressures, the protoplasm later separates from the expanded pellicle. With prolonged treatment it sometimes rounds up, and there is evidence of a loss of volume of the protoplasm.

5. On release of pressure the protoplasm spreads back to the pellicle, usually within a few minutes. The wrinkled and expanded pellicle is then slowly re-organized to its normal shape and size, over a period of many hours.

I am greatly indebted to the Alkali Division of Imperial Chemical Industries Ltd., through the courtesy of which I received much help from Mr G. A. Shephard, Mr H. J. Welbergen and Mr T. A. Wych. Mr Shephard designed the pressure vessel, Mr Welbergen made the glass windows for it after a series of trials, and Mr Wych made the metal parts for the windows and tested the windows at pressure. Imperial Chemical Industries Ltd. (Alkali Division) provided the high tensile steel and other components for the pressure vessel.

I am grateful to Dr J. M. Burch for polishing many windows, to Mr H. Banwell for making the pressure vessel, to Mr M. Gillet for modifying a microscope for use with it, and to Mr E. Livingstone for culturing Discophrya.

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REFERENCES


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EXPLANATION OF PLATES

PLATE 3

Series A. An experiment in which D. piriformis was subjected to 2700 lb./sq.in. for 184 min. This series illustrates to an exceptional degree the expansion of the body surface and pellicle at high pressure, as well as the slow recovery of the normal appearance after release of pressure. During reorganization of the pellicle small folds remained, and are visible on the left side in A8 and A9. The contractile vacuole is visible in A3 and A4, and in some of the other photographs.

PLATE 4

Series B. An experiment in which D. piriformis was subjected to 3000 lb./sq.in. for 27 min., followed by 9000 lb./sq.in. for 7 min. There was no creasing to 10 sec. (B3) after application of 3000 lb./sq.in., and it was far advanced after 3 min. at this pressure (B3). The protoplasm receded from the expanded pellicle and appears to have decreased in volume (B4). A short exposure to 9000 lb./sq.in. produced little further change (B5 and B6). On release of pressure the protoplasm made contact with the overlying pellicle (B7 and B8), and the normal body shape was restored, with shrinkage of the pellicle. However, a small fold remained where the protoplasm and pellicle were not in contact (B9). The body was slightly smaller than before the experiment (B9 and B1).
Before treatment

2700 lb./sq.in. for 184 min.

After release of pressure

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Series C. An experiment in which a D. piriformis was subjected to 10,000 lb./sq.in. for 31 min. The protoplasm rounded up within an expanded pellicle (C3), and some of the tentacles became bent and irregular (C4). On release of pressure, the surface of the protoplasm re-expanded under the pellicle (C5) and the latter became thrown into folds which were everywhere in contact with protoplasm (C6). The protoplasmic surface and pellicle were slowly reorganized to their normal shape and smoothness (C7-9). The old tentacles disappeared (C6) and new ones were formed (C9). The contractile vacuole is visible in C1 and in some of the other photographs. The meganucleus is just visible in C5, occupying one-third of the width of the organism.

Series D. An experiment in which D. piriformis was subjected to 10,000 lb./sq.in. for 10 min., released to atmospheric pressure for 13 min. and then recompressed to 10,000 lb./sq.in. D1, before treatment; D2, after 10 min. at 10,000 lb./sq.in.; D3, 1 min. after release of pressure; D4, 5 min. after recompression to 10,000 lb./sq.in. The pellicle is slightly folded and appears slightly larger, and the protoplasm is rounded within it, in D2. The protoplasm has spread within the pellicle and has made contact everywhere with it in D3. The protoplasm has rounded up again in D4.