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Examples of the Use of Active Transport of Salts and Water to Give Buoyancy in the Sea

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Examples of the use of active transport of salts and water to give buoyancy in the sea

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[Plates 34 and 35]

(1) To give themselves buoyancy several families of squid and crustaceans accumulate large amounts of NH_4^+ ions in special compartments within their bodies. This is often in high concentration, approximately 0.5 mol/l, and very acid; sometimes two-thirds of the body weight consists of such strong ammoniacal solutions. Possible mechanisms for the accumulation of NH_4^+ are discussed.

(2) Cephalopods using chambered shells for buoyancy once dominated the seas, they included the nautiloids, ammonites and belemnites. Three types of such shells can still be found in living *Sepia*, *Spirula* and *Nautilus*. They differ greatly in morphology but all function in the same way. While being formed a chamber is full of a liquid isosmotic with sea water, later this liquid is pumped out against the hydrostatic pressure of the sea. It is shown that gases play no role in this pumping of salts and water and an account of our knowledge of the processes involved in the pumping is given.

Neutral buoyancy could obviously be of great advantage to a mid-water marine animal for it would not have to work to stay at one level in the sea. An active control of buoyancy would be even more useful. Submarines and bathyscaphes are made to have densities very close to that of sea water and so can remain at a chosen depth with little effort and they also use changes in density to move from one depth to another. It is not surprising, therefore, to find very ingenious buoyancy mechanisms in mid-water animals. It does, however, seem curious, at first sight, that some of the most effective buoyancy mechanisms are possessed by animals which live on the bottom of the sea, e.g. cuttlefish, deep sea sharks and conger eels. But these animals spend much of their lives just off the bottom of the sea hunting their prey and clearly they can do this much more quietly and efficiently if they are neutrally buoyant.

Often a considerable fraction of an animal is devoted to giving it lift and here we shall describe two striking buoyancy mechanisms which depend on the movement of ions and of water. In one animals accumulate extraordinary amounts of ammonium in high concentration, in the other watery fluids are pumped against appreciable hydrostatic pressures.

The use of ammonium

In Krogh's famous book on *Osmotic regulation of aquatic animals* his first example of an animal with an internal environment different from the medium in which it lives is the protozoan *Noctiluca miliaris*. This animal, which is only about 0.5 mm across, is sometimes found in enormous numbers at the surface of the sea. It attracts attention because it luminesces very brightly. As early as 1892 Goethard & Heinsius (cited by Krogh 1939) showed that *Noctiluca* contains a good deal of ammonia and that it swells when placed in a solution hyposmotic to sea water. They deduced that this animal contains a solution isosmotic with sea water but floats because this solution contains a high concentration of ammonium ions. Krogh writes 'The significance of this old paper can scarcely be overestimated. The experiments show conclusively, (1) that the cell wall is water permeable, (2) that the cell will rapidly come into osmotic

equilibrium with the surrounding solutions down to the point where osmotic uptake of water causes disruption of the wall, and finally, (3) that the ionic composition of the sap is entirely different from that of the surrounding water, a characteristic of living cells which is quite general, but has only in recent years become generally appreciated.'

The explanation of the buoyancy of *Noctiluca* given by Goethard & Heinsius has not always been accepted. Thus Gross & Zeuthen (1948) thought it more likely that *Noctiluca* gained buoyancy merely by excluding the heavier divalent ions of sea water and Iida & Iwata (1943) were unable to find much ammonium in *Noctiluca*. It is, however, difficult to put any other interpretation on Goethard & Heinsius's results other than that which they gave and recently Dr K. J. Singarajah (private communication) has found ammonium to be present in *Noctiluca* in concentrations which would give appreciable lift.

The use of high concentrations of ammonium ions for buyoancy is now well established in a number of other larger animals which can be studied more easily than *Noctiluca*. These are squid of the family Cranchidae (Denton, Shaw & Gilpin-Brown 1958; Denton, Gilpin-Brown & Shaw 1969), squid of the families Histioteuthidae, Chiroteuthidae and Octopoteuthidae (Clarke, Denton & Gilpin-Brown 1969) and certain oceanic crustaceans (E. J. Denton, P. F. Foxton & J. B. Gilpin-Brown, unpublished work). In this paper we shall discuss only experiments on the four families of squid. In these animals neutral buoyancy was always attributable to the low density of certain body fluids. Since these fluids always had freezing-point depressions quite close to that of sea water simple exclusion of salt would not account for their low densities and these could only have been achieved by the accumulation of some solute, or solutes, of large partial molar volume. Analyses showed that this solute was always ammonium but that the distribution of this ion within the body was very different in different species.

The cranchid squid have enormous coelomic cavities full of liquid. This liquid typically accounts for about two-thirds of an animal's total weight, it has a relative density of about 1.010 (sea water is about 1.027), its principal ions are ammonium, sodium and chloride in concentrations of around 470, 85 and 630 mmol/l respectively and it has a pH of about 5. The coelomic cavity of such a squid is not compartmentalized and the ammoniacal fluid which it contains can all be drained through a single cut in the wall of the coelom. We do not know where the coelomic liquid, and its contained ammonia, comes from. In other cephalopods, e.g. *Sepia*, *Loligo* and *Octopus*, ammonia is secreted into the renal sacs probably by the very conspicuous kidney tissue which envelops the large veins, particularly the afferent branchials. In one cranchid squid, *Helicocranchia*, whose anatomy was studied by Denton *et al.* (1969), these vessels were found to carry no obvious kidney tissue, and Chun (1910) noted that there was none in *Cranchia scabra*. It seems very unlikely that the ammonia found in the coelomic cavity of the cranchid could have been first secreted into the renal sacs and then passed through the very narrow reno-pericardial canal. The ammonia is presumably secreted directly into the coelom by some of the structures which lie within it.

Squid of the families Chiroteuthidae, Octopoteuthidae and Histioteuthidae are also approximately neutrally buoyant. It is not possible, as in the cranchid squid, to drain off the ammoniacal liquid which they contain, for this liquid is not confined to a single chamber but spread in tissues around their bodies. Now if the only components denser than sea water in a marine animal were protein and amino acids and the only component giving lift a body fluid isosmotic with sea water but in which sodium ions were replaced by ammonium ions, then the animal would be neutrally buoyant if the ratio of ammonium nitrogen to total protein and

amino acid nitrogen was about two to three. We can, therefore, by analysing whole animals decide whether or not the accumulation of ammonium is a principal buoyancy mechanism. For a specimen of *Octopoteuthis danae* this ratio was found to be 0.69 while for a specimen of *Calliteuthis reversa* it was 1.3. Clearly animals like *Calliteuthis* and *Octopoteuthis* derive the lift giving them neutral buoyancy mostly from the ammonium ions which they contain. The *average* concentrations of ammonium in these specimens of *Calliteuthis* and *Octopoteuthis* were 360 and 260 mmol/l. These are extremely high values for ammonium but they are, nevertheless, lower than those obtained for liquid extruded from the buoyant parts, e.g. the arms and the mantle of other specimens. The buoyant tissues contained very large amounts of vacuolar tissue and their buoyancy could be quantitatively explained if these vacuolar tissues alone contained ammonium at the concentrations found in the extruded liquids. The vacuolar tissue is not completely separated from the other tissues in a special compartment and small muscles and nerves run through this tissue with no obvious protection from the high ammonium concentrations surrounding them. It does seem likely, however, that the nerves in such squid are protected from high concentrations of ammonium, for larger nerve trunks from such animals, e.g. the axial nerves of the arms of *Calliteuthis*, could not conduct impulses when the ammonium concentration in the medium in which they were bathed was raised to high values (E. J. Denton, J. B. Gilpin-Brown & P. G. Wright, unpublished work). The ammonium concentrations in the blood of some of these animals were also measured and found to be only a few millimoles/litre.

It is not known how this vacuolar tissue is formed, how the ammonium is secreted or how the high concentrations of ammonium are maintained. We can be certain, however, that the amount of ammonium which these squid contain is very large in relation to their total metabolism for if such a squid had a gross growth efficiency of 33 % (a value found by Corner, Cowey & Marshall (1967) for *Calanus*), then even if ammonia were the only end product of protein metabolism it would have had to retain about 40 % of all the ammonia which it produced throughout the whole of its life in order to maintain neutral buoyancy.

The acidity of these buoyant fluids may account for their retention in special compartments when once secreted. Small unionized molecules often penetrate biological membranes rather readily as compared with ionized salts. Now consider the simple situation in which a strongly ammoniacal fluid in one compartment (e.g. the blood) is separated from another fluid in a second compartment (e.g. the coelom in the cranchid squid) by a wall impermeable to NH_4^+ but through which unionized ammonia can pass. In both compartments the reactions

$$NH_4^+ + OH' \rightleftharpoons NH_4OH, \tag{1}$$

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \tag{2}$$

will rapidly approach an equilibrium in which relatively very little ammonia is present as NH_4OH and where for reaction (2)

$$\frac{[NH_4^+]}{[NH_3]\,[H^+]} = \text{a constant } K_1. \tag{3}$$

The diffusion of unionized ammonia across the separating wall will tend to make its concentration the same in both the coelom and the second compartment. At equilibrium

$$\frac{[NH_4^+]\text{ (coelom)}}{[NH_4^+]\text{ (second compartment)}} = \frac{[H^+]\text{ (coelom)}}{[H^+]\text{ (second compartment)}} = K_2, \tag{4}$$

where K_2 is the factor by which the ammonium and hydrogen are concentrated.

Now the ammoniacal liquids of these animals have a pH around 5 and contain approximately 500 mmol/l ammonia (including both ionized and unionized forms). The ammonium concentrations found in the bloods of such animals were ones of a few millimoles per litre and the pH values of the blood of squid are reported as being between 7.0 and 7.9 (Nicol 1960; Potts 1965). It appears therefore that equation (4) could fit reasonably well. The above argument does not, of course, tell us how the high concentration of 'ammonia' is achieved. One possibility suggested by Jacobs (1940), to explain the accumulation of ammonia in animals, is that an acid fluid is actively secreted into a space and that this traps unionized ammonia molecules diffusing in from the blood stream converting them into relatively impermeant ammonium ions. This would not be a surprising mechanism since, for example, the mammalian stomach and kidney can secrete solutions which are more acidic than the coelomic fluids of the cranchid squid. Potts & Parry (1964) have suggested a similar mechanism to explain the concentration of ammonia in the renal sacs of *Sepia* and this fits most of Potts's observations on ammonia excretion of *Octopus dofleini*. In *O. dofleini* Potts found that much of the ammonia arises in the kidney, perhaps by the deamination of glutamine. Another possibility to explain the results on these ammoniacal squid is, of course, that ammonium chloride is secreted and that the acidity arises secondarily.

Pumping liquid against hydrostatic pressures

The buoyancy mechanism discussed above is found in cephalopods whose shell is absent or reduced to a thin transparent pen or gladius. The very evolution of the cephalopods was, however, probably determined by a particular solution to the problem of buoyancy which involved the use of a buoyant shell. The remains of the shells of nautiloids, ammonites and belemnites are among the best known and most beautiful of fossils and three types of such shells can still be found among living cephalopods. These three belong to the pearly *Nautilus*, to the cuttlefish *Sepia* and to the oceanic mid-water squid *Spirula*. These shells look very different from one another but there are very important similarities in fine structure and in the way in which they function.

The shell of *Nautilus* is robust and coiled. It has a large chamber in which the animal lives and it seems, apart from its coiling, to be very like the nautiloid's orthocone. The shell of *Spirula* (figure 1) is also coiled but it is small and fragile and is enclosed within the animal while the shell of *Sepia* (figure 2, plate 34), though also totally surrounded by the animal's tissue, is an uncoiled structure. The chambers of the *Nautilus* shell are large, up to about 20 ml in volume, and they are bounded by strong dividing septa which can be 15 mm apart. The largest chambers in the cuttlebone, on the other hand, have a volume of only about 2 ml and the dividing septa are about 0.7 mm apart. In the cuttlebone each chamber is subdivided by about six thin partitions parallel to the main walls and these are held apart by very numerous irregular pillars. In *Nautilus* and *Spirula* the siphuncle is a long thin tube which runs through all the chambers of the shell, while in *Sepia*, the siphuncle is almost flat. In studying the physiology of these shells the siphuncle has a special importance for it is the only part of the shell which is permeable to liquids and exchanges of substances between the insides of the chambers and the animal's tissues can only take place through its walls. The siphuncular epithelia which lie against the siphuncular walls of the shells of *Nautilus* and *Sepia* are like each other (figure 3, plate 35).

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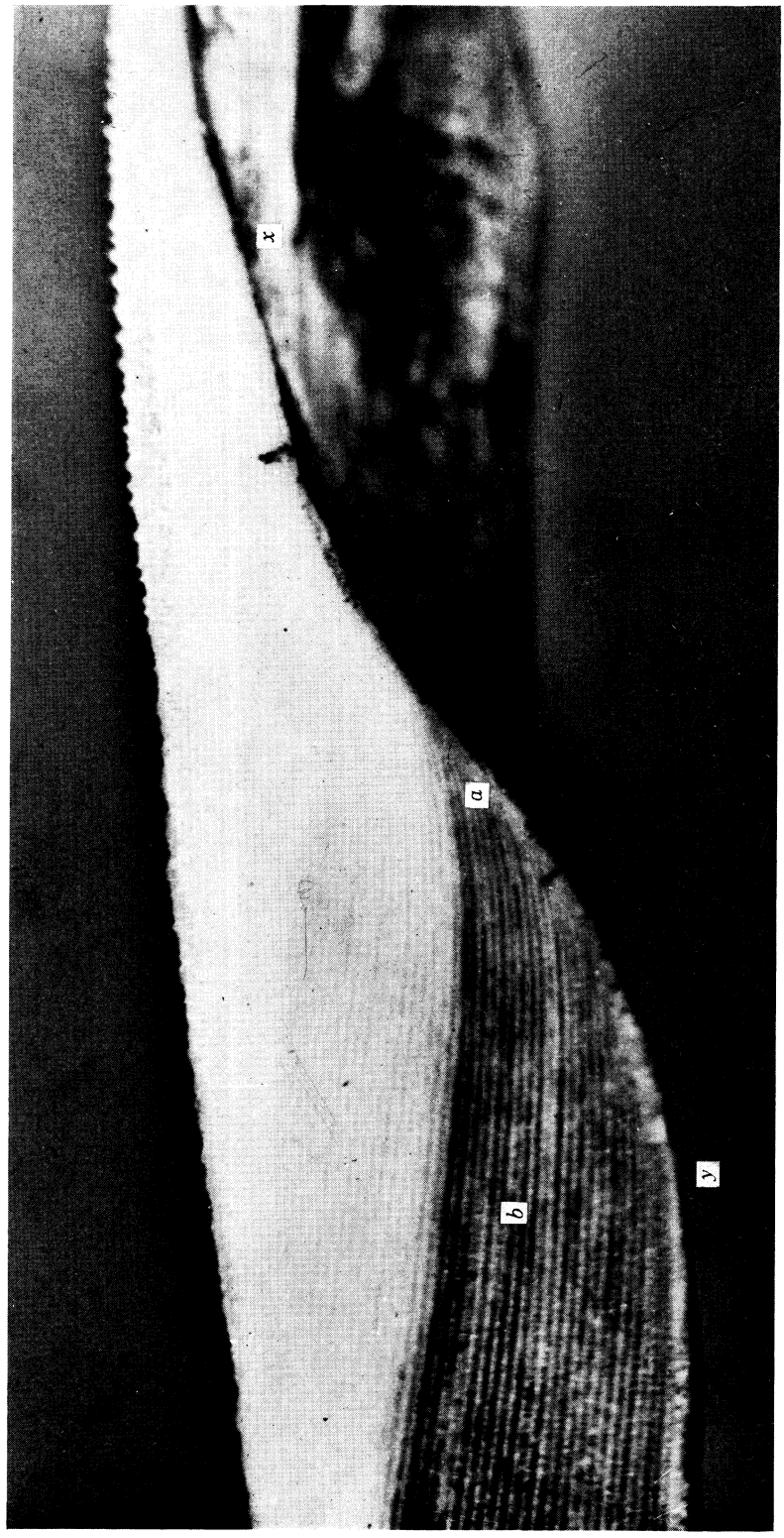

FIGURE 2. *Sepia officianalis*. A transverse section through the posterior part of a cuttlebone. The lower chambers have been filled with ink and their dividing walls which are, on the left-hand side of the picture, about 0.7 mm apart can be seen. The chambers narrow as they go posteriorly, i.e. to the right. The region *xy* is covered with an epithelium which can pump liquid (largely a solution of sodium chloride) into or out of the cuttlebone. This siphuncular epithelium resembles in many ways that shown in figure 3 for *Nautilus*. If the animal has recently been subjected to a change in pressure the liquid close to this epithelium (e.g. at *a*) differs in concentration from that deeper inside the cuttlebone (e.g. at *b*).

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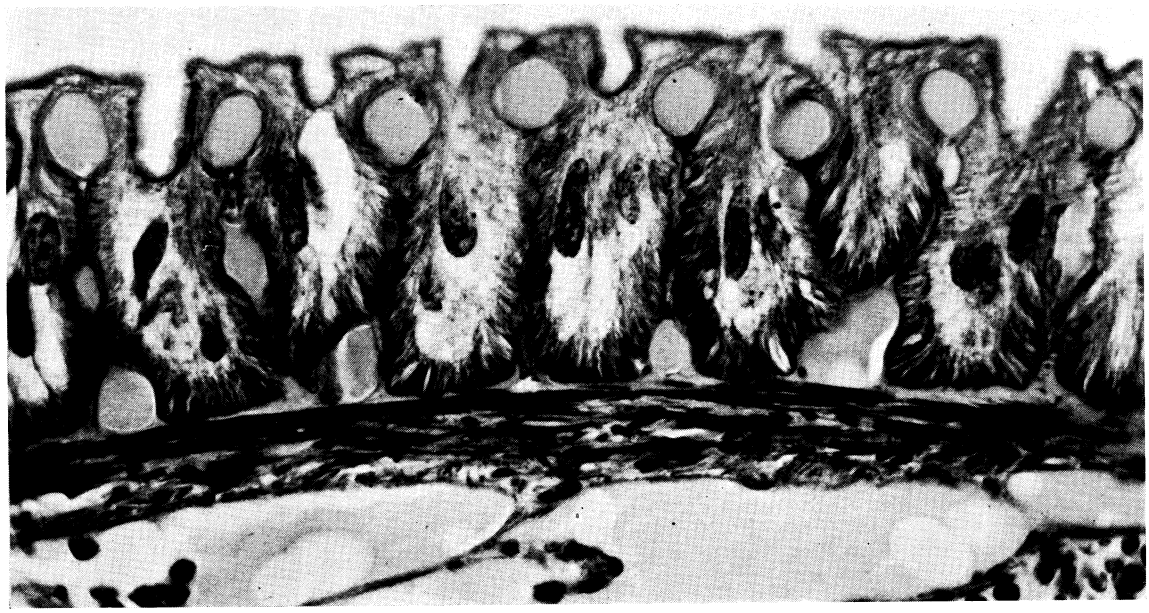

FIGURE 3. *Nautilus*. Photograph of transverse section of the siphuncular epithelium. In life the brush borders, here upwards, are applied to the siphuncular tube which separates the living tissue from the interior of a chamber. This particular epithelium was from siphuncle passing through a chamber from which most of the liquid had been withdrawn but from which further liquid would still be taken. The section was cut at 12 μm and stained in Heidenhain's haematoxylin. The epithelial cells are about 100 μm long.

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Sepia

The only cephalopods with chambered shells which are readily accessible are the various species of cuttlefish and of these animals *Sepia officinalis* is the one which has been studied most (Denton & Gilpin-Brown 1961*a*, *b*, *c*). The chambers of *Sepia's* shell, or cuttlebone, do not intercommunicate and they are only permeable to liquids at their posterior ends where they are very thin. The cuttlefish can change its buoyancy and can be induced to do so by changing the illumination to which it is subjected. It does this by increasing or diminishing the amount of liquid which the cuttlebone contains and so reducing or increasing the cuttlebone's gas

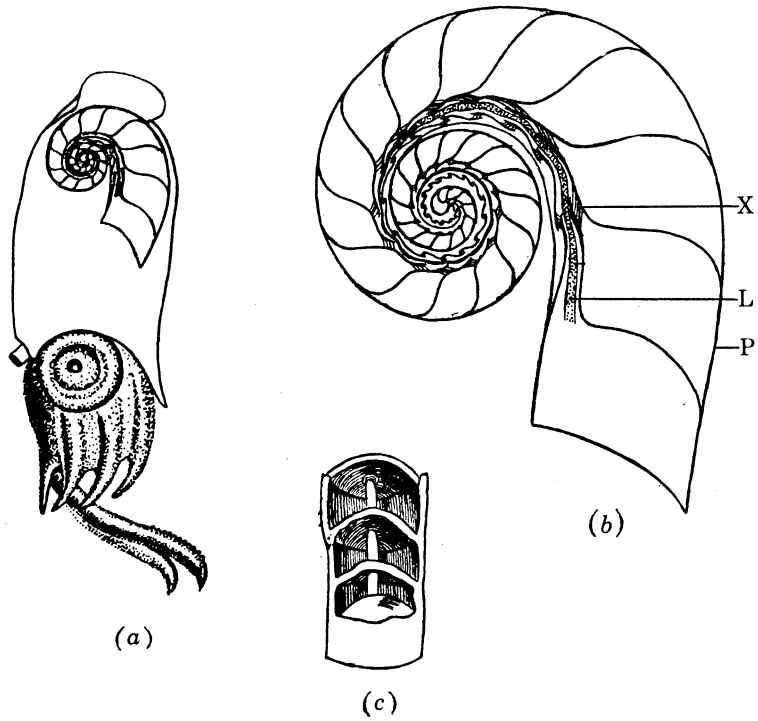

FIGURE 1 *Spirula spirula*. (*a*) A diagram showing the animal in its natural swimming position (and approximately its natural size). The shell, which is internal, is shown as are two small stern fins. (*b*) The shell. The hatched part (X) represents the region through which liquids must move. The stippled part (L) is the living part of the siphuncle. The continuous lines (P) the pearly parts of the shell which are impermeable to liquids. (*c*) Some chambers of the shell opened to show the position of the siphuncle. Diagram taken from Denton (1961).

space. The gas within the chambers of a cuttlebone is largely nitrogen. In chambers which have been formed for some time, the gas pressure is only about 0.8 atm† yet in a newly formed chamber the gas pressure is very much lower than this. The pressure of gas within the chambers is independent of the depth at which the animal lives, and does not match the pressure of the sea to which the animal is subjected.

The cuttlefish is not a very deep-living animal; off Plymouth it is most frequently taken at 30 to 80 m and it is thought to go occasionally to about 100 m and it will therefore be commonly exposed to pressures of around 8 atm and occasionally to pressures of 11 atm. If a dissected cuttlebone is placed under a pressure of less than 0.8 atm some of the liquid which it contains

† 1 atm ≈ 100 kPa.

comes out of the siphuncular ends of the chambers and samples of this liquid can be obtained under liquid paraffin for analysis. By measuring the freezing points of liquids obtained in this way, Denton, Gilpin-Brown & Howarth (1961) showed that liquid obtained from animals soon after they have been caught and brought to the surface was hyposmotic to sea water and that it was principally a solution of sodium chloride. These observations suggested that simple osmotic forces between this liquid and the blood (the latter is almost isosmotic with sea water (Robertson 1949, 1953)) might play a role in holding liquid out of the cuttlebone when the animal is at depth. It was found, for example, that in animals taken where the depth of water was 70 m the osmotic difference between blood and cuttlebone liquid could balance at least 5 atm of the 7 atm difference in hydrostatic pressure between the inside and the outside of the cuttlebone at this depth.

Samples of liquid for freezing-point determinations can also be obtained from within the cuttlebone by cutting the cuttlebone into two sagittally, placing a half under liquid paraffin and taking samples directly from within the chambers. Both methods of sampling show that if the animal has been subjected to pressure changes in the period before the sampling, there are always differences in concentration of salts between the liquid close to the siphuncular wall and that deeper inside the shell (see figure 2, plate 34). Changes in concentration of cuttlebone liquid must always arise by exchanges in salt or water across the siphuncular membrane and concentration changes deeper within the bone must depend on the diffusion of salts either towards or away from the region close to the membrane.

When an animal caught at about 70 m was soon afterwards placed under a hydrostatic pressure equal to that found at this depth and kept at this pressure for a day or so, only very small differences in concentration were found between samples taken from various positions within the bone. The liquid within the chambers was everywhere markedly hyposmotic to the animal's blood and the difference in concentration between the liquid within the chambers and the blood was approximately that which would, if placed across a suitable semipermeable membrane, give an osmotic pressure of approximately 7 atm, i.e. that required to match the pressure of the sea at 70 m depth. When animals caught at about 70 m depth were kept in shallow water for about 2 weeks the liquid within the cuttlebone was everywhere close to being isosmotic with sea water and the animal's blood. One animal caught at 70 m was placed in a pressure tank at 13 atm above atmospheric pressure. After 20 h there were still differences between the liquid close to the siphuncular membrane and that deep inside the cuttlebone. The liquid a few millimetres from the siphuncular membrane was more dilute than that deeper within the bone and of a concentration which would, against the animal's blood, give an osmotic pressure of about 11 atm. This is almost but not quite enough to balance a hydrostatic pressure to which the animal had been exposed. The liquid immediately adjacent to the siphuncular epithelium must, however, have been a little more dilute than this and closer to that required for the osmotic pressure between it and blood to balance the external pressure to which the animal had been subjected. These unpublished observations by E. J. Denton and J. B. Gilpin-Brown on *Sepia* suggest that water movements in and out of the cuttlebone are decided mainly by the relative magnitudes of the hydrostatic pressure of the sea and an osmotic force between the blood and the liquid immediately internal to the siphuncular epithelium. Although such an osmotic force could prevent water being pushed into the cuttlebone and could, if sufficiently high, extract water from the cuttlebone it could not balance the crushing effect of the sea's pressure and the bone has to be mechanically strong. The cuttlebone is,

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however, quite strong enough to withstand the pressures found at the depths at which the animal lives for it only implodes when subjected to pressures greater than about 24 atm.

The observed differences in concentration within the liquid of a given chamber with distance from the siphuncular membrane can be accounted for by the slowness of diffusion of salts within the liquid inside the cuttlebone. If, at a given time, the movement of liquid into or out of the cuttlebone depends only on a difference in concentration *immediately* across the siphuncular membrane then the fact that the liquid deep inside the cuttlebone only changes slowly in concentration might well be advantageous to the cuttlefish. If, for example, a cuttlefish moves from one depth to another a small immediate exchange of salt or water across the siphuncular membrane can re-establish a balance between osmotic and hydrostatic pressures and, even if no pumping of salts or water takes place, the buoyancy of the cuttlefish will hardly change. The liquid deeper inside the cuttlebone will, over a period of hours, preserve a concentration close to that suitable for its original depth and if the cuttlefish returns to this depth then the osmotic work which will have to be done to adapt to these changes in depth will be very small indeed.

In the summer, when the sea around our coast is warm, cuttlefish grow very quickly and lay down two to three new chambers to their cuttlebone every week. While building, these chambers are full of a liquid isosmotic with the animal's blood. When they are completed, and can withstand the external pressure of the sea, the liquid which they contain is completely pumped out leaving behind a space containing gas under very low pressure. This pumping must be a relatively quick process since it is rare to find a chamber only partially emptied but there is no reason to postulate a different pumping mechanism from that used later to conserve buoyancy.

Spirula

Spirula lives very much deeper in the sea than the cuttlefish. Clarke (1970) has recently made a careful analysis of *Spirula* caught in horizontally towed opening and closing nets in the ocean off Fuertaventura (Canary Islands). He found that the great majority of the *Spirula* population performs a vertical diurnal migration. During daylight the entire population is found deeper than 550 m the greatest concentration being around 600 to 700 m with occasional animals down to almost 1000 m. During darkness most of the population lives *shallower* than 300 m but Clarke caught none in the depth range 0 to 100 m. It seems that the very young animals may not migrate immediately after hatching but remain deep in the sea even at night-time.

It was easy to show very great similarities in the functioning of the shells of *Spirula* and *Sepia* (Denton, Gilpin-Brown & Howarth 1967). No matter at what depth a *Spirula* had been caught, the pressure of gas within its chambers was always less than atmospheric. In the older chambers it was about 0.8 atm, in the newly formed ones much less than this and the shell of Spirula was sufficiently strong to withstand the pressures to which the animals are subjected in life for their shells only implode when subjected to high pressures corresponding to depths of around 1700 m (range 1300 to 2300 m) (Denton & Gilpin-Brown 1971).

Although *Spirula* is a much smaller animal than *Sepia* it has proved possible, because of differences in anatomy, to study the formation and evacuation of a new chamber in much more detail.

On figure 4 we show the sequence of events leading to the formation of a new chamber. The cylindrical side walls (x) are built first and within the space so formed, a clear liquid replaces the tissues. This liquid is isosmotic with sea water. A septum completes the new chamber and

divides off a liquid filled space from the animal's tissues. We can estimate how recently a chamber, like that marked A in figure 4 has been formed by finding the length x of the cylindrical walls as a fraction of y, the length of chamber A. We can then correlate the results of freezing-point determinations on the liquid in A with the stage of formation of the chamber. Using this method of analysis it has been shown that when the chamber is just completed the liquid which it contains is isosmotic with sea water but that the depression of freezing point then falls to very low values (from about -1.9 °C to about -0.4 °C) before the first tiny bubble of gas space appears in the chamber. It is evident that the concentration of salts within a chamber falls to

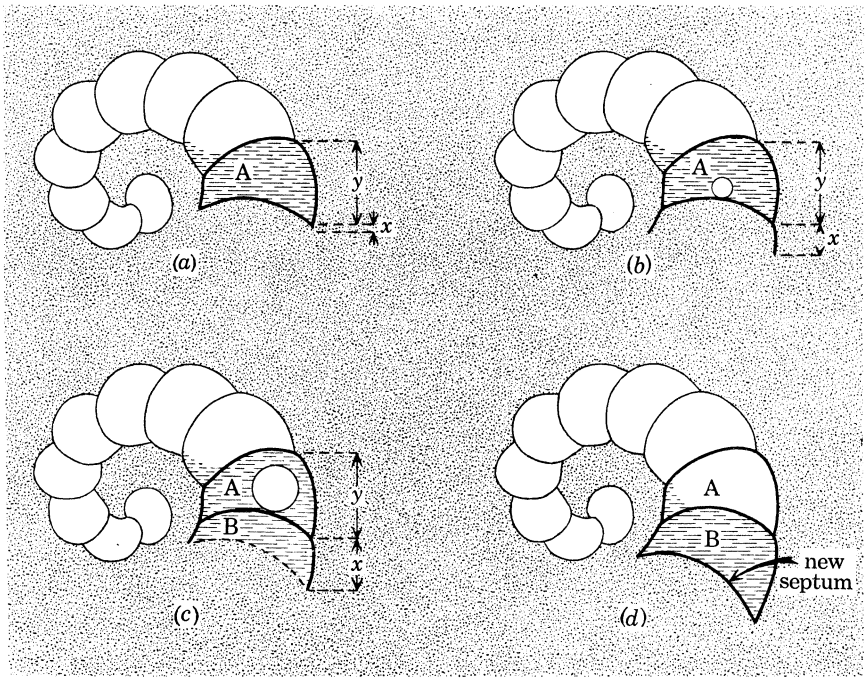

FIGURE 4. Diagram showing stages in the formation of a new chamber of a *Spirula* shell. (*a*) The cylindrical side walls of the next chamber to be formed are added as a continuation of the walls of chamber A. At this stage chamber A is still full of liquid isosmotic with sea water (Δ_t about -1.9 °C). (*b*) A small bubble of gas (under very low pressure) appears in A, but before this happens solutes have been removed from the liquid which it contains and it is now markedly hyposmotic to sea water (Δ_t about -0.4 °C). (*c*) More liquid has left A and that remaining is still very hyposmotic to sea water. A clear liquid isosmotic with sea water has been secreted by the tissues (stippled) into the space B; this space is not yet cut off from the animal by a septum. (*d*) A septum has been built sealing off the chamber B which (like A in (*a*)) now contains a liquid isosmotic with sea water. The shell is completely embedded in the animal's tissues. The distances x and y referred to in the text are shown. The walls of chambers A and B have been emphasized.

a very low value (about one-fifth that of sea water and the animal's blood) before water begins to leave the chamber. On puncturing a newly formed chamber which contained a gas bubble under liquid, the bubble almost completely collapsed as liquid rushed into the chamber: in life these chambers must, therefore, have contained gas under very low pressure.

As *Spirula* grows older, and the number of chambers of its shell increases, the smaller and earlier formed chambers refill with liquid and this liquid gradually changes from being hyposmotic to sea water to being almost isosmotic to sea water. This observation, like those described above on the formation of a new chamber, suggests a strong link between concentration difference between the blood and the liquid within a chamber and the pumping, or holding, of liquid out of the chamber.

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Nautilus

The pearly *Nautilus* has a special interest for its shell is so like those of the fossil nautiloids and ammonites that it is impossible not to believe that we can apply results obtained on *Nautilus* to them. *Nautilus* is known to come into relatively shallow water at night but the greatest depth at which it lives is not certain. Since the pressures of gas in the chambers of its shell are always subatmospheric and the shell implodes at pressures corresponding to depths of about 600 m, we may suppose by analogy with *Sepia* (shell implodes at about 240 m; generally found at less than 100 m) and *Spirula* (shell implodes at about 1700 m; generally found (in the daytime) between 600 and 700 m) that it does not usually descend below about 300 m. The shell of *Nautilus* appears at first sight to be very different from that of *Sepia* and *Spirula*. The animal lives inside its shell while the shells of *Sepia* and *Spirula* are surrounded by the tissues of these animals. The general features of the physiology of the shell are, however, like those of *Spirula* and *Sepia*. The gas pressures within the chambers of the shell are subatmospheric and individual gases have the partial pressures predicted by the hypothesis that they have merely diffused into spaces within the chambers and have not been actively secreted into these chambers. The gas pressures found in newly formed chambers are very low and the liquids found within the chambers are markedly hyposmotic to the animal's blood (Denton & Gilpin-Brown, 1966).

Siphuncular membranes

The tissue which pumps liquid out of the chambers of the shells of these cephalopods is evidently the siphuncular membrane which covers the only permeable surfaces of their shells. The structure of this membrane has been studied in *Nautilus* and *Sepia*.

In *Nautilus*, the living strand of the siphuncle is a vascular appendix of the visceral portion of the mantle (Willey 1902). In the centre lies the siphuncular vein (the haemocoel) surrounded by a loose meshwork of trabeculae whose spaces communicate with each other and the central vein. To one side of the strand is the siphuncular artery which lies within a connective tissue sheath. The siphuncular epithelium surrounds these structures and lines the non-living part of the siphuncular tube, which is the only part of the shell of *Nautilus* permeable to liquids. This epithelium clearly has two functions in succession. It first secretes the non-living calcareous and chitinous parts of the siphuncular tube and later, when this is done, pumps salts and liquid out of the chamber which it serves. The change in function is matched by a change in structure. Figure 3 shows a siphuncular membrane at a time when it is pumping liquid from its chamber. It has a number of striking features some of which have been figured by Haller (1895) and Willey (1902). The basement membrane is thrown into very many regular folds whose outer ends reach almost to the surface of the epithelium. Typically this epithelium is about 90 μm high and the folds which occur at about 40 μm intervals reach within 5 to 10 μm of the surface. The tops of these folds are expanded and form a regular series of longitudinal ducts within the siphuncular epithelium. At intervals the folds in the basement membrane open out so that the longitudinal ducts in the epithelium are in communication with the spaces beneath it and these in turn are connected with the various spaces of the haemocoel. Between the folds of the basement membrane there are tall cells with elongated nuclei. When these cells are in contact with the basement membrane, i.e. at their bases and around their longitudinal ducts, they stain deeply and appear, under the optical microscope, fibrous. Electron microscope studies by Dr V. C. Barber (personal communication) and E. J. Denton, J. B. Gilpin-Brown & Jane Whish (unpublished

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work) show that this appearance is given by the numerous mitochondria which the cells contain. The outer surface of these epithelial cells, which in life is applied to the inner side of the non-living siphuncular tube, has a conspicuous brush border about 2.5 μm thick. The epithelia has then the features which Keynes (1969) lists as common ones for secretory epithelia, i.e. the cells are closely joined at one surface of the epithelium, there is a great expansion of this surface by numerous microvilli, the membrane on the other surface is folded to form canaliculi, and there are numerous mitochondria in the cells.

Pumping mechanisms

It does seem probable that the mechanism of pumping salts and water is basically the same in all these shells and that the movement of water follows that of salts. We have seen that when the cuttlefish *Sepia* is at some given depth the hydrostatic pressure of the sea is approximately balanced by an osmotic force between the blood and the liquid immediately inside the cuttlebone and beneath the siphunucular epithelium. In *Sepia* it would only be necessary to pump either sodium or chloride against a concentration gradient of 2:1 to allow this animal to pump salts and water out of the cuttlebone down to depths of close to the maximum depth at which this animal lives.

A similar balance between hydrostatic and simple osmotic pressures could not exist for *Spirula* at all the depths at which it lives. The maximum observed difference in osmolarity between the liquid within a chamber and sea water, although large, could only produce pressures of about 20 atm yet Clarke has shown that *Spirula* probably spends only a small fraction of its life at depths where the pressure is less than this. If *Spirula* relied on simple osmosis to pump liquids out of the chambers of its shell it could only do this at less than about 200 m depth and at other depths it would have to stop the diffusion of liquid from the siphuncle into the chambers by some impermeable membrane whilst maintaining the siphuncular circulation. It is found, however, that the shells of intact animals either dead or anaesthetized do not fill with liquid when suddenly exposed to high hydrostatic pressures so it is not possible to rule out this hypothesis. Another possibility is that localized high concentrations of solutes could be built up in a 'pumping' epithelium by a mechanism similar to that proposed by Diamond & Bossart (1967, 1968) and used to extract liquids from the chambers of the shell by simple osmosis. No matter what the pumping mechanism, in the course of 'pumping out' a new chamber the blood returning along the siphuncle to the main body of the animal must at first be hyperosmotic and later hyposmotic to sea water.

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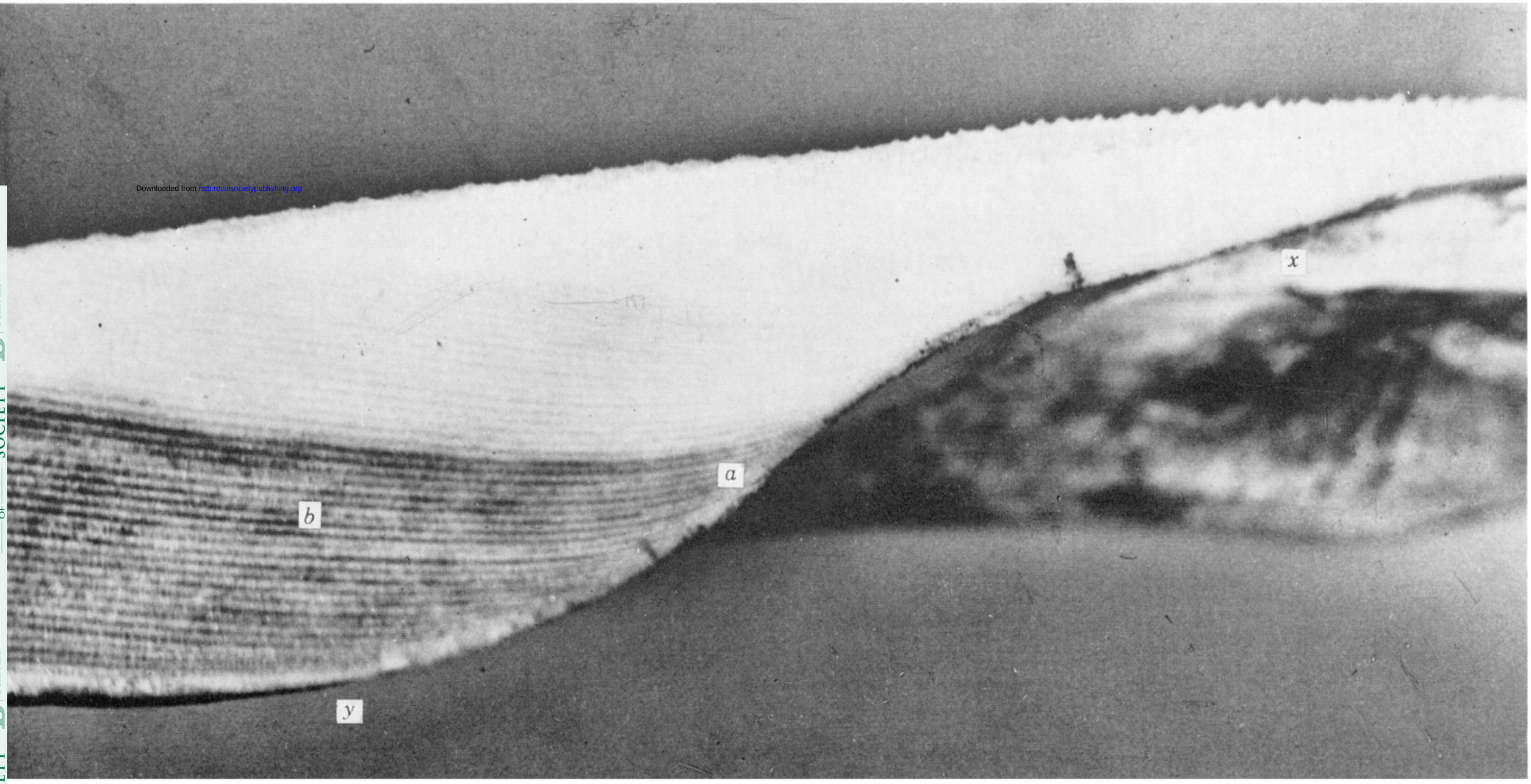
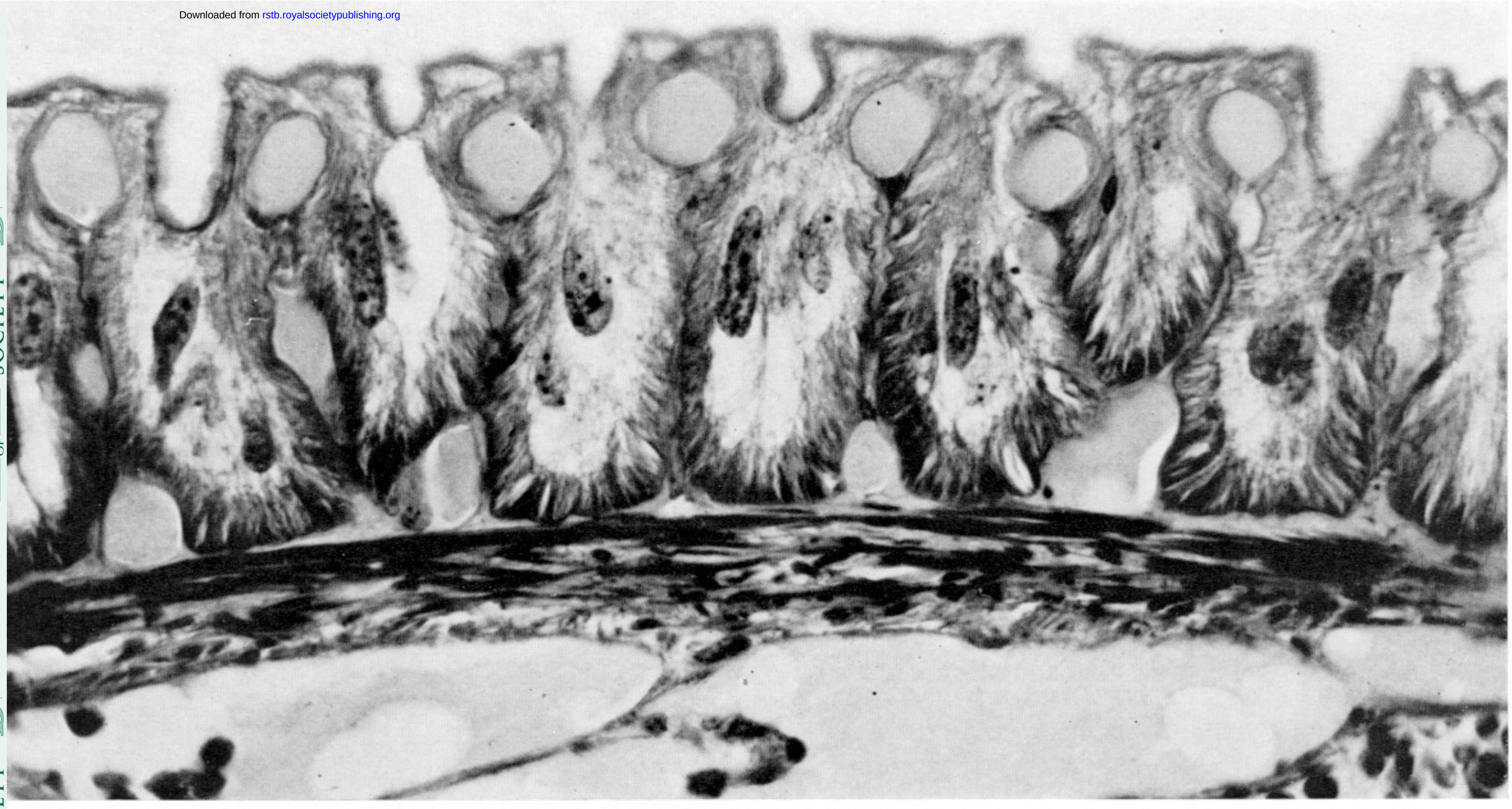


FIGURE 2. *Sepia officianalis*. A transverse section through the posterior part of a cuttlebone. The lower chambers have been filled with ink and their dividing walls which are, on the left-hand side of the picture, about 0.7 mm apart can be seen. The chambers narrow as they go posteriorly, i.e. to the right. The region *xy* is covered with an epithelium which can pump liquid (largely a solution of sodium chloride) into or out of the cuttlebone. This siphuncular epithelium resembles in many ways that shown in figure 3 for *Nautilus*. If the animal has recently been subjected to a change in pressure the liquid close to this epithelium (e.g. at *a*) differs in concentration from that deeper inside the cuttlebone (e.g. at *b*).



GURE 3. *Nautilus*. Photograph of transverse section of the siphuncular epithelium. In life the brush borders, here upwards, are applied to the siphuncular tube which separates the living tissue from the interior of a chamber. This particular epithelium was from siphuncle passing through a chamber from which most of the liquid had been withdrawn but from which further liquid would still be taken. The section was cut at 12 μm and stained in Heidenhain's haematoxylin. The epithelial cells are about 100 μm long.