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THE BUOYANCY OF THE CUTTLEFISH, *SEPIA OFFICINALIS* (L.)

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(Plate I and Text-figs 1–12)

Cuttlefish are amongst the most remarkable of all animals, and we can well understand why Paul Bert (1867) was inspired to undertake a general study of *Sepia officinalis* L. This he vowed not to leave until the physiology of *Sepia* was as well understood as that of the frog. Bert did little work on the cuttlebone; he analysed the gas contained in it and expressed the opinion that this gas would vary according to circumstances, in the same way as the gas in the swim bladder of fish, then the subject of the beautiful experiments of Armand Moreau (1876). The lack of interest of physiologists in the cuttlebone, one of the most common objects on our sea shore, is perhaps excusable, for the cuttlebone is dead. In this and the following three papers we show that, although dead, the cuttlebone is not unchanging and that the cuttlefish can use it as a variable buoyancy tank. This extraordinary animal can change its density quickly and does so in response to changes in light intensity. Finally we show that liquid is probably moved in and out of the cuttlebone by an osmotic mechanism, and not by changes in the internal gas pressure which is the method used in the swimbladder.

The cuttlebone has a special interest in that it is closely related not only to the shells of the living *Nautilus* and *Spirula*, but also to the shells of the fossil Nautiloidea, Ammonoidea and Belemnoidea (see Naef, 1923; Morton, 1958). These animals dominated the Palaeozoic and Mesozoic seas and their evolution from a crawling to a free-swimming life was probably determined by the use of the shell as a buoyancy device. We hope in the light of new knowledge of the cuttlebone to see more clearly how these important animals lived. The chambers of the cuttlebone contain liquid as well as gas, and the cuttlefish changes its density and posture by varying the amounts of liquid which the chambers of the bone contain. If the fossil cephalopods could also have done this their behaviour must have been very different from that postulated on the usual assumption that their chambered shells were completely filled with gas.

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MATERIAL AND METHODS

Cuttlefish, *Sepia officinalis* (L.), were obtained from the trawling grounds off Plymouth, mostly in depths between 25 and 40 fathoms. The animals were brought to the Laboratory daily and were studied ashore. In March 1959 a special cruise was made aboard R.V. 'Sarsia' to study cuttlefish immediately they were brought aboard. These are referred to as freshly caught animals.

Animals were not kept longer than two weeks before being used for experiment.

Cuttlebones were dissected from *Sepia* anaesthetized with $\frac{1}{2}\%$ urethane in sea water. Sandoz MS 222 was tried but, although preferable in some respects, it has the disadvantage that the cuttlefish usually discharges its ink, sometimes to such a degree that the ink sac is almost emptied.

Weighings of less than 10 g were made on accurate torsion balances. Material weighed at sea was stored in sealed polythene bags in closed jars, and reweighed a few hours later ashore.

The volume and density of a cuttlebone were found by weighing it first in air and then under sea water together with enough lead to make it sink. Now, if w is the weight of the bone in air, y is the weight of the bone and the lead under sea water, and z is the weight of the lead under sea water; then the negative weight of the cuttlebone in sea water, u, equals $z-y$. According to the principle of Archimedes the loss in weight of the cuttlebone on putting it under sea water, i.e. $w+u$, is equal to the weight of the sea water displaced. If the weights are measured in grams then the volume, v, of the cuttlebone is $(w+u)/1{\cdot}026$ ml., where 1·026 is the density of the sea water.[1] The density d, of the cuttlebone is equal to w/v.

Other methods are given in the various sections below.

RESULTS

DIFFERENCES IN BUOYANCY BETWEEN DIFFERENT CUTTLEFISH

Cuttlefish kept in aquaria appear to differ greatly in buoyancy. Two groups of animals were chosen: in one group the animals appeared to be very buoyant, these we shall call 'floating' animals; in the second group the cuttlefish could rest on the bottom of their tank with no apparent effort, these we shall call 'sinking' animals. The cuttlefish were anaesthetized and then weighed both in air and under sea water. Floating animals were less dense than sea water and their weight in sea water was found by weighing them together with a known weight of lead. The cuttlebones were now dissected from the cuttlefish and their volumes, densities, and weights under

[1] During the course of the experiments the density of the sea water in the reservoirs of the laboratory varied a little from the value of 1·026 given above. The small differences in volume and density which these variations would give have been neglected in the calculations of this communication.

sea water were found. Finally the cuttlefish without their cuttlebones were weighed under sea water. Table 1 gives the weights in sea water of the intact *Sepia*, its cuttlebone, and the animal without its cuttlebone, as percentages of the weight of the whole *Sepia* in air. It can be seen that over 80% of the difference in density between the 'floating' and 'sinking' cuttlefish is attributable to differences between their cuttlebones. This is probably an underestimate since, as was later found, cuttlebones of low density take up sea water quicker than cuttlebones of high density and thus will be more changed during the measurements under sea water.

TABLE 1. WEIGHTS IN SEA WATER OF DIFFERENT PARTS OF CUTTLEFISH AS PERCENTAGES OF THE WEIGHT OF THE WHOLE ANIMAL IN AIR

	Whole animal (%)	Mean (%)	Bone only (%)	Mean (%)	Animal minus cuttlebone (%)	Mean (%)
'Floating' animals	−0·98 −0·93 −0·71	−0·87	−4·50 −4·81 −4·28	−4·53	+4·40 +3·88 +3·57	+3·95
'Sinking' animals	+0·35 +0·38 +0·54	+0·42	−3·69 −3·56 −3·19	−3·48	+4·04 +4·03 +3·73	+3·93
Difference between means	—	1·29	—	1·05	—	0·02

Cuttlefish which had just been trawled from about 70 m were studied aboard ship. It was found that their cuttlebones exhibited a much smaller range of densities than those caught in the same way but studied after keeping in an aquarium ashore. The freshly caught animals had cuttlebones whose densities fell within the range 0·57–0·65 (mean = 0·62, S.D. = 0·02, N = 17), whilst those studied ashore had bones whose densities varied between 0·48 and 0·71 (mean = 0·60, S.D. = 0·06, N = 38). It seemed, therefore, that the cuttlebone was not an unchanging organ but that a cuttlefish could vary the density of its cuttlebone. It is shown later that quite quick changes in cuttlebone density can occur in the living healthy cuttlefish (Denton & Gilpin-Brown, 1961*a*).

THE STRUCTURE OF CUTTLEBONE

The standard description of the cuttlebone is that of Appellöf (1893), and the following account is based on his work supplemented a little by our own observations. In a large adult the bone consists of about 100 lamellae of calcified chitin placed one above the other and held about 0·6 mm apart by numerous pillars. The spaces between the lamellae form chambers which are themselves divided by about six thin membranes running parallel to the lamellae. The chambers are sealed laterally and anteriorly by the thick calcified layer forming the curved dorsal surface of the cuttlebone. They are

laid down successively, the newer ones below the older, as the animal grows. Sections through the cuttlebone are shown in Text-fig. 8 and Pl. 1. In the region labelled *xy* the posterior margins of the lamellae turn over to close the chambers. The surface so formed has a special importance in the physiology of the cuttlebone. In *Sepia* it represents the active part of the siphuncle which in *Nautilus* and *Spirula* is a closed tube passing through the chambers of the shell. We shall refer to this surface as the 'siphuncular' surface.

THE INDEPENDENCE OF THE CHAMBERS

A chamber of the cuttlebone, with its numerous pillars and thin horizontally dividing membranes, appears to be a very complicated structure. The following experiment shows it to be functionally very simple. A needle was pushed from the ventral surface about half way through a cuttlebone thus puncturing about half of its chambers. This half-punctured bone was now placed under sea water containing *Sepia* ink in a vacuum desiccator and gas was pumped out of the desiccator with an efficient vacuum pump. When bubbles no longer came from the hole in the cuttlebone atmospheric pressure was restored and this pushed inky liquid into the space from which the gas had just been extracted. The cuttlebone was then removed from the ink, dried gently and sawn up. It was found that the ink had only penetrated into those chambers which had been punctured, but that these chambers were completely filled with ink (Pl. 1). It is clear that the chambers of the cuttlebone are independent of one another but that within any one chamber gases and liquids are fairly free to move.

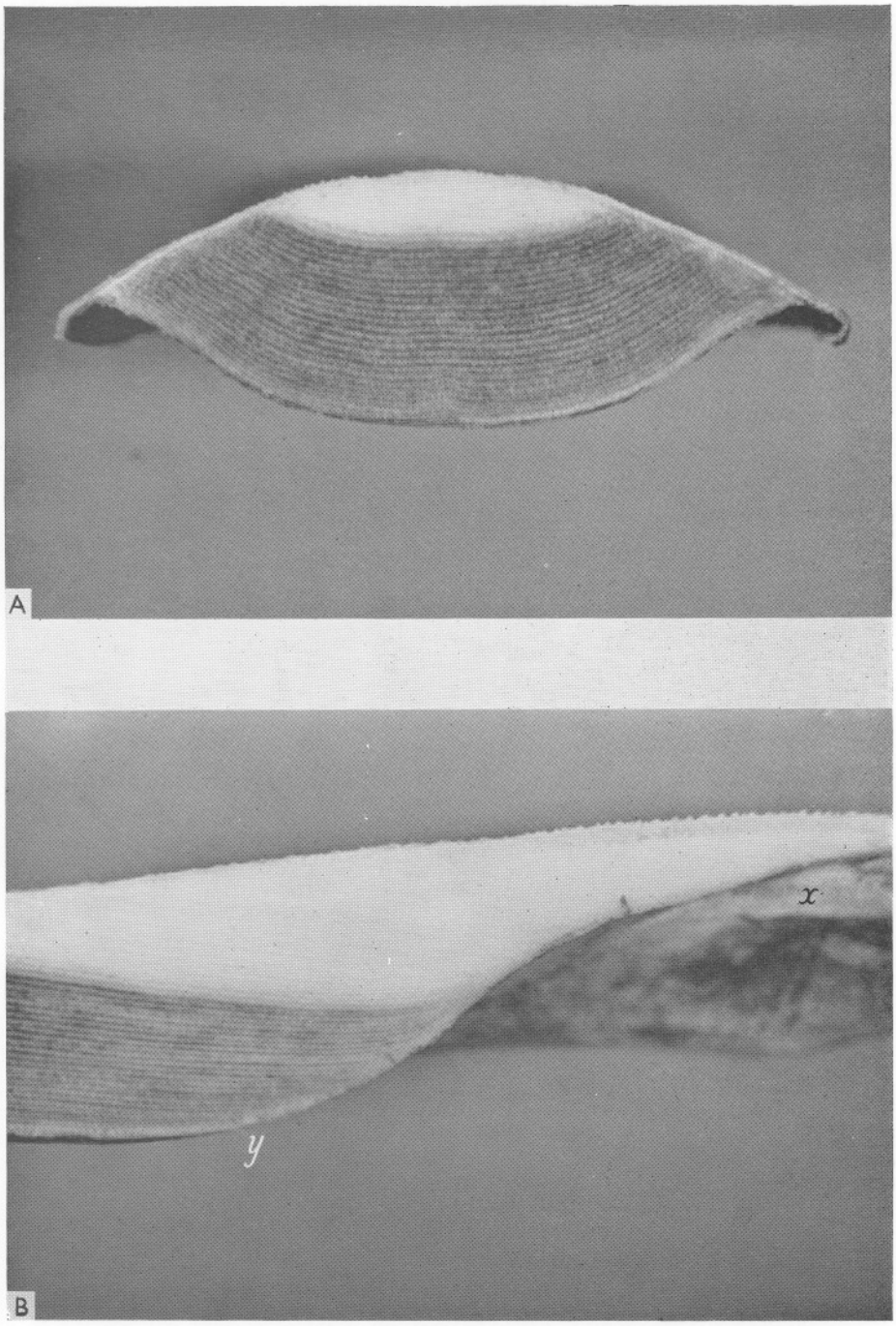
THE PROPORTIONS OF GAS, LIQUID, AND SOLID IN A CUTTLEBONE

The weight, density, and volume of a freshly dissected cuttlebone were found. The cuttlebone was then pierced several times with a needle so that all its chambers were punctured. It was now placed in sea water under vacuum. When no more bubbles came from the holes in the cuttlebone atmospheric pressure was restored. Several hours later, when the spaces in the cuttlebone were completely filled with sea water, the cuttlebone was taken out of the water, dried gently on a cloth and weighed in air. Finally the bone was dried in an oven at 110° C to constant weight in air.

EXPLANATION OF PLATE I

Sepia officinalis (L.). A. Transverse section of the cuttlebone. B. Longitudinal section of the cuttlebone, showing the siphuncular wall *x*–*y*. *x* is posterior to *y*. A number of chambers were punctured from the ventral side of the cuttlebone, and gas was removed through the hole by vacuum. The pressure was then brought back to atmospheric, and inky water filled the chambers from which gas had been removed.

Dorsal



(*Facing p.* 322)

We shall now give a numerical example of the calculation used to derive the proportions of gas, liquid, and solid spaces in the bone.

Initial weight of bone in air	30·5 g
Initial density of bone	0·68
Initial volume of bone	45·1 ml.
Weight of bone full of sea water	55·1 g
Final dry weight of cuttlebone	17·5 g
Therefore, water lost on drying was	37·6 g

If we assume that the liquid originally in the cuttlebone had the same composition as sea water, then the cuttlebone would have contained 1·3 g of salt before drying at 110° C. The final liquid content of the bone (i.e. before drying) is thus equal to the water lost on drying plus this calculated weight of salt:

$$37{\cdot}6 + 1{\cdot}3 = 38{\cdot}9 \text{ g.}$$

Its density would have been approximately 1·026, so that its volume would have been

$$\frac{38{\cdot}9}{1{\cdot}026} = 38 \text{ ml.}$$

But this is a volume equal to that of both the liquid and the gas space which the cuttlebone initially contained and we can therefore write

$$\frac{\text{Volume of liquid and gas spaces}}{\text{Volume of bone}} = \frac{38}{45{\cdot}1} \text{ or } 84{\cdot}1\%$$

The volume of the dry matter of the cuttlebone is therefore

$$100 - 84{\cdot}1 = 15{\cdot}9\%,$$

and the weight of this dry matter is the final dry weight minus the weight of salt

$$17{\cdot}5 - 1{\cdot}3 = 16{\cdot}2 \text{ g.}$$

Now the weight of liquid which the bone initially contained is equal to the initial weight of the bone minus the weight of the dry matter

$$30{\cdot}5 - 16{\cdot}2 = 14{\cdot}3 \text{ g.}$$

If the volume of this liquid is obtained by dividing its weight by 1·026, the volume of the initial gas space can be found by difference.

To summarize, we give the approximate initial constitution of the bone per 100 ml. of bone:

	Weight (g)	Volume (ml.)
Dry matter	36	16
Liquid	32	31
Gas space	Trivial	53

The density of the dry matter is

$$\frac{36}{15{\cdot}9} = 2{\cdot}26.$$

This value is intermediate between the densities of chitin and aragonite, the substances of which the cuttlebone is largely constructed (Richards, 1951; Clarke & Wheeler, 1922; Nicol, 1960). A second cuttlebone which was studied in the same way gave results almost identical with those given above (the density of the dry matter was found to be 2·24). Many of the other experiments described later required the determination of water, dry matter and gas, and the results of some of these experiments are shown in Text-fig. 7.

The proportion of the animal which is cuttlebone is almost constant and varies little with the sex and age of the animal. It may be that baby cuttlefish and ripe females form exceptions to this rule, but neither of these two groups has been studied.

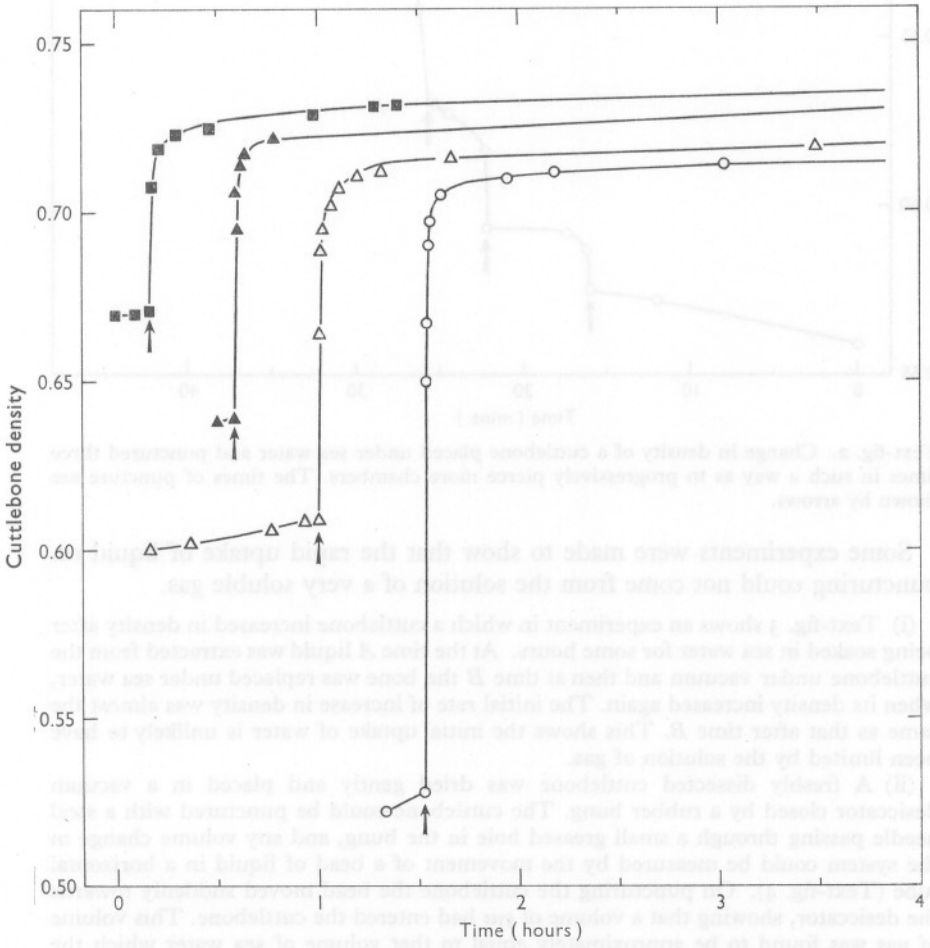
THE PRESSURE OF GAS IN THE CUTTLEBONE

When the cuttlefish is at depth in the sea, unless the hydrostatic pressure were opposed by some other force, the cuttlebone would fill with water. In the swim bladder of a fish, gas is secreted into or reabsorbed from the swimbladder and the volume of the swimbladder is held approximately constant at 5% of the fish's total volume. The pressure of gas within the swimbladder always equals the external hydrostatic pressure of the sea (Moreau, 1876; Harden Jones & Marshall, 1953). We may then ask first whether this system which works so well in the fish is also used in *Sepia*. On this hypothesis, in an animal living at 70 m the cuttlebone would contain gas at a pressure equal to the external pressure, i.e. 8 atm (7 atm for the sea and 1 for the air above it). We should expect that if an animal were freshly trawled from this depth, either that gas bubbles would be found in the tissues around the cuttlebone, or that on puncturing a stream of bubbles would come from the hole. In fact gas is never found in the tissues around the cuttlebone and, on puncturing under sea water, no stream of bubbles comes from the hole but instead the weight of the cuttlebone suddenly increases as water enters the bone. The gas within the cuttlebone, far then from being at 8 atm pressure, appears to be at a pressure less than atmospheric.

Ashore, freshly dissected cuttlebones of varying density were placed under sea water and punctured in several places so as to pierce all the chambers. Before and after puncturing such a cuttlebone it was weighed under sea water together with enough lead to make it sink. The results of this experiment are shown in Text-fig. 1 where cuttlebone density is plotted against time. It can be seen that on puncturing there is a very rapid increase in density as sea

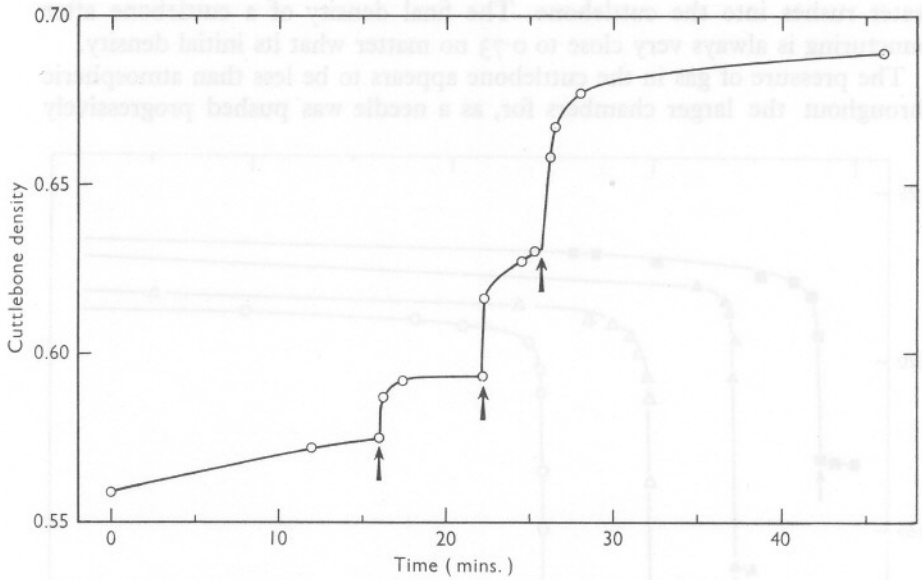
water rushes into the cuttlebone. The final density of a cuttlebone after puncturing is always very close to 0·73 no matter what its initial density.

The pressure of gas in the cuttlebone appears to be less than atmospheric throughout the larger chambers for, as a needle was pushed progressively



Text-fig. 1. Changes in density of cuttlebones of various initial densities punctured under sea water. The times of puncture are shown by the arrows. For clarity the curves are arbitrarily displaced along the abscissa.

farther into the cuttlebone, more water was taken up with each increase in the number of chambers punctured. Text-fig. 2 shows an experiment in which the larger chambers of a cuttlebone were punctured in three steps. With each increase in the number of chambers punctured there is an increase in the density of the bone.



Text-fig. 2. Change in density of a cuttlebone placed under sea water and punctured three times in such a way as to progressively pierce more chambers. The times of puncture are shown by arrows.

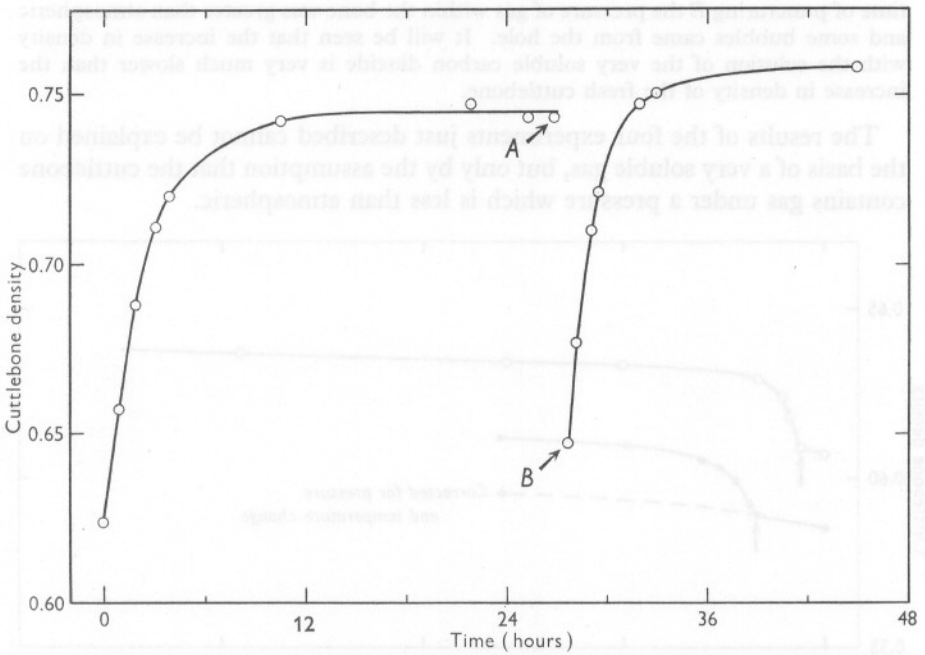
Some experiments were made to show that the rapid uptake of liquid on puncturing could not come from the solution of a very soluble gas.

(i) Text-fig. 3 shows an experiment in which a cuttlebone increased in density after being soaked in sea water for some hours. At the time *A* liquid was extracted from the cuttlebone under vacuum and then at time *B* the bone was replaced under sea water, when its density increased again. The initial rate of increase in density was almost the same as that after time *B*. This shows the initial uptake of water is unlikely to have been limited by the solution of gas.

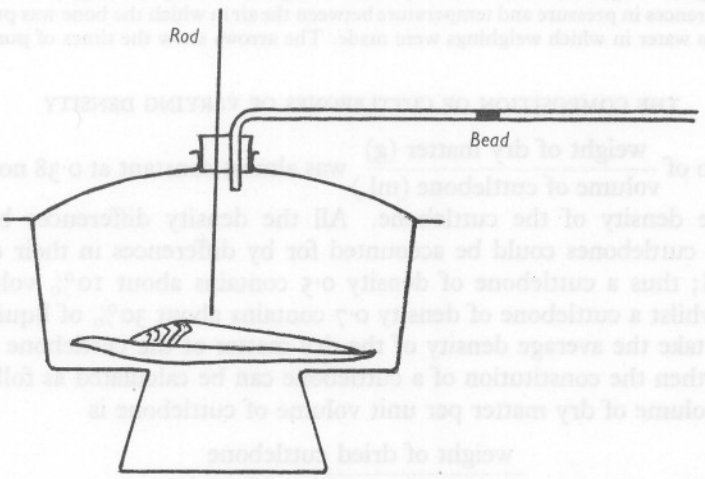
(ii) A freshly dissected cuttlebone was dried gently and placed in a vacuum desiccator closed by a rubber bung. The cuttlebone could be punctured with a steel needle passing through a small greased hole in the bung, and any volume change in the system could be measured by the movement of a bead of liquid in a horizontal tube (Text-fig. 4). On puncturing the cuttlebone the bead moved suddenly towards the desiccator, showing that a volume of *gas* had entered the cuttlebone. This volume of gas was found to be approximately equal to that volume of sea water which the cuttlebone would have taken up if it had been punctured under sea water.

(iii) As a variant on experiment (ii), cuttlebones were weighed under sea water, then punctured in air and immediately returned to sea water and re-weighed. The results of two such experiments are shown in Text-fig. 5. It can be seen that only a small uptake of sea water took place and this could be mostly accounted for by the differences between the temperature and pressure of the sea water and the air in which the cuttlebone was punctured.

(iv) A cuttlebone was soaked in sea water saturated with carbon dioxide so that the gas spaces of the cuttlebone were filled with carbon dioxide. Text-fig. 6 compares the puncturing under sea water of this cuttlebone (*B*) with a fresh cuttlebone (*A*). At the



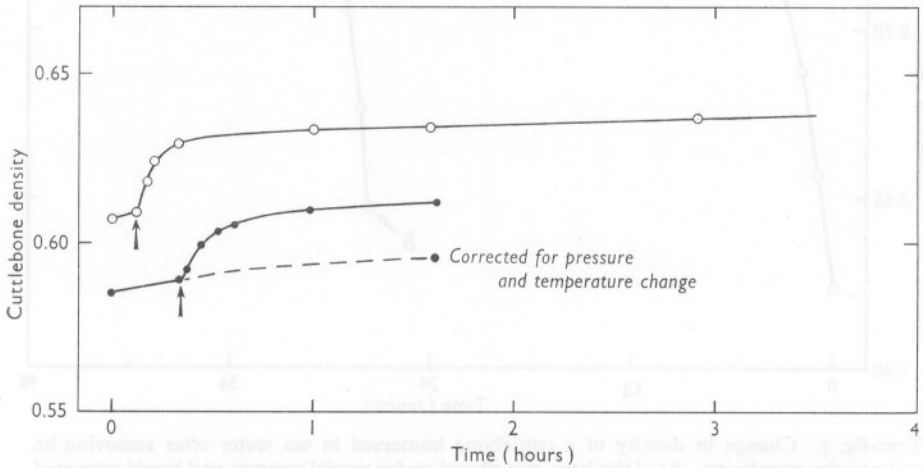
Text-fig. 3. Change in density of a cuttlebone immersed in sea water after removing its siphuncular membrane. At *A* the bone was placed under partial vacuum and liquid extracted through the siphuncular surface. At *B* atmospheric pressure was restored, the bone then took up water again at approximately the same rate as at the beginning of the experiment.



Text-fig. 4. Apparatus used to measure the uptake of air when a cuttlebone is punctured in air. The rod, which passes through a small greased hole in the rubber bung, is used to puncture the cuttlebone. The uptake of air is measured by the subsequent movement of the bead of liquid.

time of puncturing *B* the pressure of gas within the bone was greater than atmospheric and some bubbles came from the hole. It will be seen that the increase in density with the solution of the very soluble carbon dioxide is very much slower than the increase in density of the fresh cuttlebone.

The results of the four experiments just described cannot be explained on the basis of a very soluble gas, but only by the assumption that the cuttlebone contains gas under a pressure which is less than atmospheric.



Text-fig. 5. Change in density of the cuttlebone. The cuttlebones were weighed under sea water. After the second weighing they were taken into the air, dried and then punctured in several places so as to pierce all their chambers. They were then replaced in sea water and their change in weight followed. The very small changes in density can largely be accounted for by differences in pressure and temperature between the air in which the bone was punctured and the sea water in which weighings were made. The arrows show the times of puncturing.

THE COMPOSITION OF CUTTLEBONES OF VARYING DENSITY

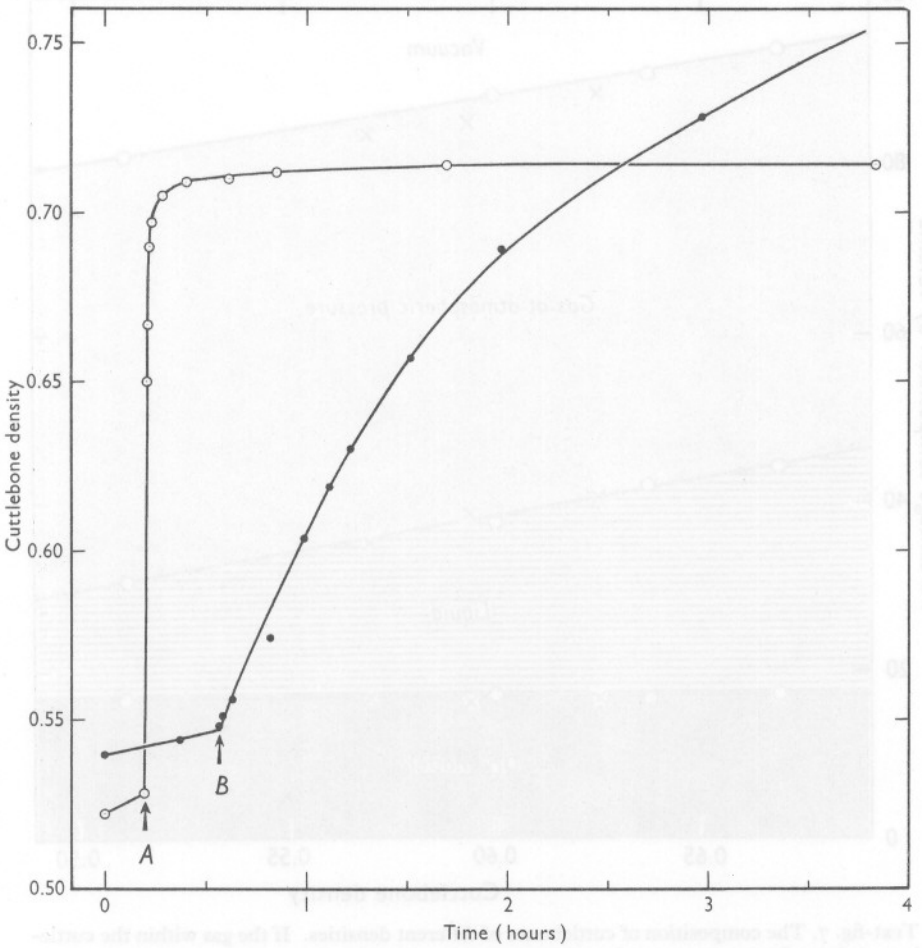
The ratio of $\frac{\text{weight of dry matter (g)}}{\text{volume of cuttlebone (ml.)}}$ was almost constant at 0·38 no matter what the density of the cuttlebone. All the density differences between different cuttlebones could be accounted for by differences in their content of liquid; thus a cuttlebone of density 0·5 contains about 10% volume of liquid, whilst a cuttlebone of density 0·7 contains about 30% of liquid.

If we take the average density of the dry matter of the cuttlebone as 2·25 (p. 324) then the constitution of a cuttlebone can be calculated as follows[1]:

The volume of dry matter per unit volume of cuttlebone is

$$\frac{\text{weight of dried cuttlebone}}{2{\cdot}25 \times \text{volume of cuttlebone}}.$$

[1] Small corrections were made for the salt content of the dried bones but, for the sake of simplicity, these are not described here.



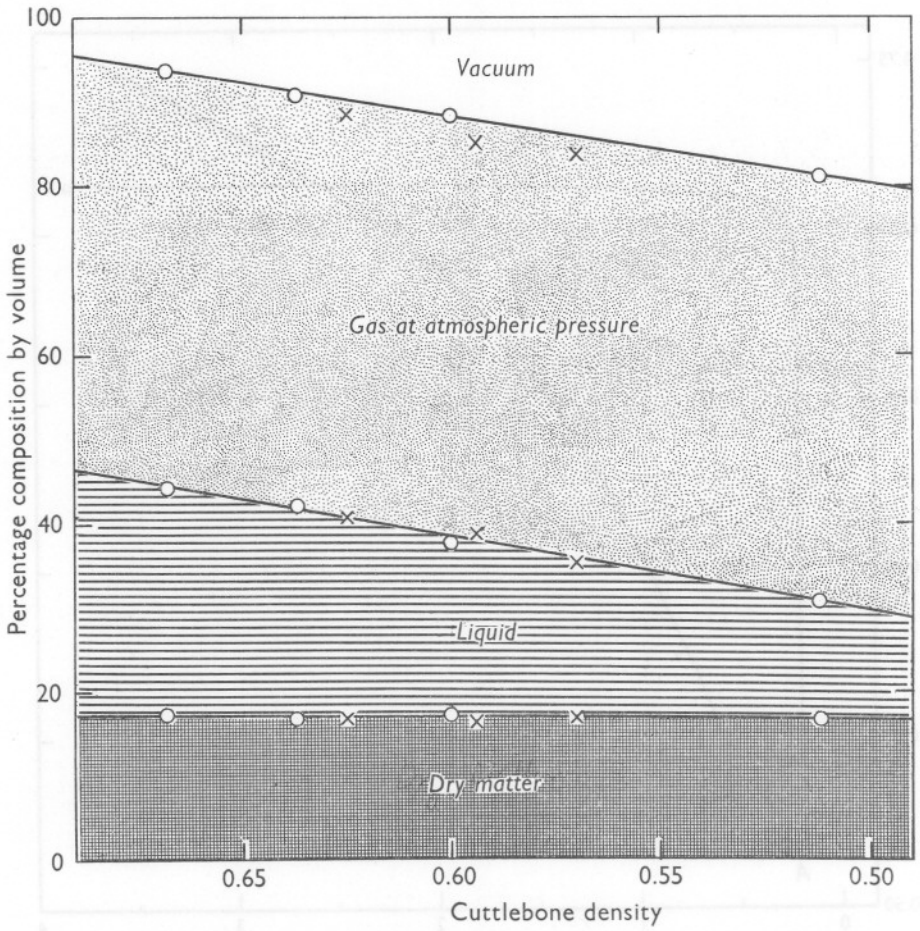
Text-fig. 6. Changes in density of cuttlebone: *A*, freshly dissected and punctured under sea water; *B*, soaked for many hours in sea water saturated with carbon dioxide, and then punctured under sea water. The arrows mark the times of puncture.

The volume of liquid per unit volume of cuttlebone is

$$\frac{\text{Initial weight of cuttlebone in air} - \text{weight of dried cuttlebone}}{\text{volume of cuttlebone}}.$$

The volume, Vg, of the gas space which the cuttlebone initially contained is

Volume of cuttlebone − (volume of dry matter + volume of liquid initially present).



Text-fig. 7. The composition of cuttlebones of different densities. If the gas within the cuttlebone is brought to a pressure of one atmosphere its volume and hence mass remains constant no matter what the density of the bone. The gas is normally at less than atmospheric pressure and fills both the volume indicated by the stippled area and also the clear space above this area. Variations in density are brought about by changing the liquid content of the bone. ○, Bones punctured under sea water (see Text-fig. 1). ×, Bones from which the siphuncular membrane was removed and which were then soaked in sea water (see Text-fig. 11).

The volume of the gas, when reduced to atmospheric pressure (at ambient temperature) is

$$Vg - \text{volume of liquid which enters on puncturing.}$$

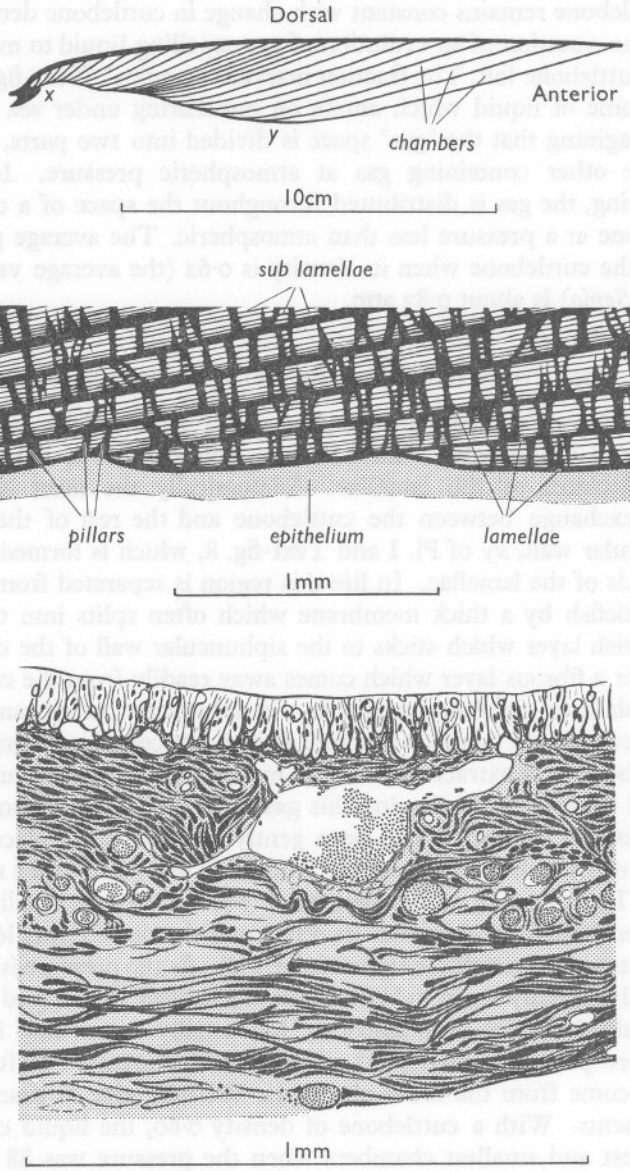
The results obtained on a number of cuttlebones are shown in Text-fig. 7. It can be seen that for all the cuttlebones the volume of gas reduced to atmospheric pressure remains almost constant. Thus the *mass* of gas within

the cuttlebone remains constant with change in cuttlebone density, and there can be no question of an evolution of gas expelling liquid to make the density of the cuttlebone fall. The fraction marked 'vacuum' (Text-fig. 7) represents the volume of liquid which enters on puncturing under sea water. We are here imagining that the 'gas' space is divided into two parts, one a vacuum and the other containing gas at atmospheric pressure. In fact, before puncturing, the gas is distributed throughout the space of a chamber of the cuttlebone at a pressure less than atmospheric. The average pressure of gas within the cuttlebone when its density is 0·62 (the average value for freshly caught *Sepia*) is about 0·83 atm.

THE SITE OF GAIN AND LOSS OF LIQUID IN THE CUTTLEBONE

The cuttlebone's dorsal surface is covered with a thick layer of calcified chitin, and the anterior ventral surface runs parallel to the many layers of calcified chitin of the lamellae. Anatomically the most likely place for liquid exchange between the cuttlebone and the rest of the animal is the siphuncular wall, *xy* of Pl. I and Text-fig. 8, which is formed by the turned over ends of the lamellae. In life this region is separated from the viscera of the cuttlefish by a thick membrane which often splits into two layers, one a yellowish layer which sticks to the siphuncular wall of the cuttlebone, and the other a fibrous layer which comes away readily from the cuttlebone. The yellowish layer we shall describe as the siphuncular membrane.

The cuttlebone contains gas and, if it is not completely impermeable, we should be able to extract some liquid merely by placing the cuttlebone under reduced pressure and allowing this gas to expand. Cuttlebones from which the yellowish membrane had been gently removed were placed ventral side uppermost in a vacuum desiccator connected to a pump and a mercury manometer. The pressure in the desiccator was reduced in small steps and the cuttlebone carefully observed. It was found that liquid does leave the cuttlebone, but only through the siphuncular wall. As the pressure is reduced drops of liquid suddenly begin to 'sweat' out of this wall. The liquid does not come out of all chambers simultaneously but usually comes first from the older and more posterior chambers; only when the pressure is further reduced does it come from the other chambers. We may give as examples two such experiments. With a cuttlebone of density 0·60, the liquid came first from the oldest and smallest chambers when the pressure was 88 mm Hg below atmospheric pressure, and from all the chambers except the four newest, when the pressure was 180 mm Hg below atmospheric pressure. The average pressure of gas predicted from Text-fig. 7 for a cuttlebone of this density is 156 mm Hg below atmospheric. In a similar experiment on a cuttlebone of density 0·56 some liquid was observed to come from the oldest chambers at 155 mm Hg below atmospheric pressure and from all chambers except the



Text-fig. 8. Structure of the cuttlebone and the siphuncular membrane. A. Diagrammatic longitudinal section through a cuttlebone from an adult animal which would have about 100 chambers. The siphuncular surface is marked *xy*. B. Detailed longitudinal section showing the siphuncular surface of a few chambers. (Simplified from a 50 μ celloidin section of decalcified bone; stain, acid fuchsin.) C. Transverse section through a part of the shell-sac lying against the siphuncular surface (*xy*) of the cuttlebone. The thin siphuncular membrane is uppermost and below this can be seen a large vein (also shown in the reconstruction of Fig. 10) whose tributaries communicate with numerous spaces just below the membrane. Many of these smaller vessels contain fixed blood (heavy stipple).

newest four at 264 mm Hg below atmospheric. The average pressure of gas predicted was 190 mm Hg below atmospheric pressure. This experiment shows that the pressure of gas within the cuttlebone is probably not uniform but differs between the different chambers. This conclusion was confirmed later by direct pressure measurements (Denton & Gilpin-Brown, 1961*b*).

The average reduction of pressure which will make liquid leave the cuttlebone is always approximately equal to the pressure difference between the cuttlebone gas (predicted from the experiments on the puncturing of cuttlebones) and atmospheric pressure. This means that surface forces (capillary attractions) do not play an important role by hindering the flow of liquid in and out of the cuttlebone. Although the pores through which liquid is moved are probably very small, the chitinous material of which the cuttlebone is partly formed is not very 'wettable'. If the pen from *Loligo*, which is made of very similar material to the chitin of the cuttlebone, is placed under sea water in a vacuum desiccator and the external pressure is reduced, gas bubbles form much more readily on the pen than on the glass walls of the desiccator and the pen periodically rises to the surface and discharges its bubbles just as does a grape in champagne (Bragg, 1925).

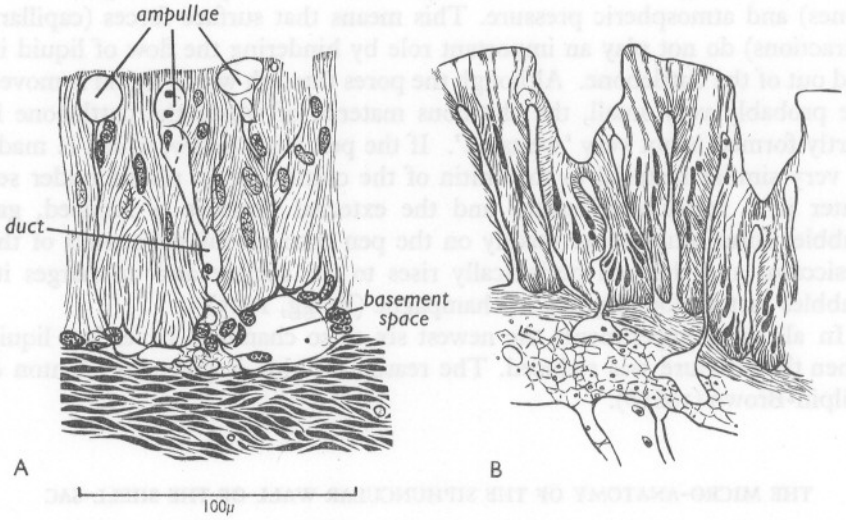
In all these experiments the newest six or so chambers never lost liquid when the pressure was reduced. The reason for this is given by Denton & Gilpin-Brown (1961*b*).

THE MICRO-ANATOMY OF THE SIPHUNCULAR WALL OF THE SHELL-SAC

The experiments described show that liquid exchange can occur across the siphuncular surface of the cuttlebone. This surface is covered by a specialized membrane, and a few square centimetres of this membrane were fixed in formalin, embedded, and sectioned at 16μ normal to the siphuncular surface. A modification of Masson's trichrome stain was tried but haematoxylin followed by Van Geison's connective tissue stain was found to be more useful. In the siphuncular region the wall of the shell-sac is very fibrous and contains numerous arteries and veins (Text-fig. 8C). The siphuncular membrane lies above a thick bed of connective tissue and is supported by a meshwork of thin connective tissue fibres at its base. Some of these fibres penetrate almost to the free surface which, in life, is applied to the siphuncular surface of the cuttlebone, (Text-fig. 9A). The cellular constituents are supported in cups of connective tissue fibres and sections made tangential to the membrane's surface show that these cups are roughly circular in cross-section.[1]

[1] According to Appellöf when a chamber is completed the epithelium which has secreted it begins to secrete the next chamber except at the siphuncular end where the epithelium is infiltrated with connective tissue from the layers lying below and the secreting cells degenerate.

The most distinctive features of the membrane are the numerous ampullae (Fig. 9A) which can be seen in the membrane, close to its free surface. These ampullae are connected by very fine ducts lying between the cups of connective tissue and leading to spaces in the basement connective tissue which in turn communicate with the veins. From a series of sections a reconstruction was made (using enlarged photographs of each section to ensure precise orientation) of all the ampullae, ducts, spaces, and veins which

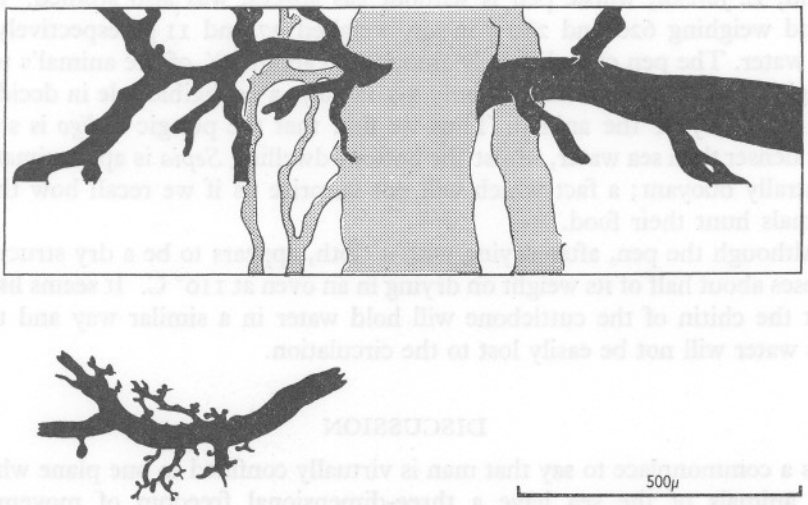


Text-fig. 9. A. *Sepia officinalis*. Camera lucida drawing of a section of the siphuncular membrane showing a duct joining an ampulla with one of the spaces in the basem ent connective tissue. B. *Nautilus*. Detail of the siphuncular membrane for comparison with that of *Sepia*. Redrawn to be of approximately the same scale as A from a lithograph by Willey (1902).

were definitely inter-communicating in an area of just over half a square millimetre. The reconstruction is shown in Text-fig. 10. In the same figure, and to the same scale, this reconstruction is compared with the picture obtained by an injection of the shell-sac vessels with *Sepia* ink. The two are very similar, though the injection brings out the finer detail which could not be reliably resolved in the reconstruction. There is little doubt that the ampullae of the siphuncular membrane communicate through their ducts, with a series of spaces in the basement connective tissue; these in turn communicate with the underlying veins. Some arterioles were traced as far as the siphuncular membrane where they became much finer than the ducts, but no connexion with the ampullae could be found. Injection of the arterial system confirmed this, for the injected vessels ended in very small capillaries at the membrane.

The micro-anatomy of this region shows then a system of vessels which

can bring liquid from the circulation into close communication with the liquid within the cuttlebone. Although communicating with the blood system these ampullae and their ducts are probably not a functional part of the circulation, for fixed blood was never seen in the ampullae and their ducts even although it was present in very much smaller blood vessels in the underlying connective tissue (Text-fig. 8c).



Text-fig. 10. Above, a reconstruction of all the vessels found to communicate with the ampullae in the siphuncular membrane (here above the plane of the drawing). The basement spaces and the ducts leaving them are shown in black whilst the veins in the underlying connective tissue are shown stippled. Below, to the same scale, the appearance of similar ducts and spaces following an injection of veins with *Sepia* ink.

RESULTS WITH OTHER CEPHALOPODS

Sepia elegans

Measurements were made on two freshly caught specimens of *S. elegans* which were in very good condition. One animal was 0·38% denser than sea water, the other 0·38% less dense than sea water. The initial densities of their cuttlebones were 0·47 and 0·42 and the ratios of cuttlebone volume to *Sepia* volume were 8·4 and 7·1% respectively. On puncturing under sea water both bones took up sea water and attained the final density of 0·63. Although the bone of *S. elegans* is a little less dense than that of *S. officinalis* (it is perhaps a little less strongly constructed) these figures show the essential similarity between the cuttlebones of the two species.

An examination in the British Museum of Natural History of a collection of cuttlebones from many different species of cuttlefish showed that they differed appreciably in the ratio of siphuncular surface area to the total surface area of the cuttlebone. We should expect that the species with cuttlebones

with relatively large siphuncular surfaces are those which can change their density most quickly.

Loligo forbesi

As we have seen, the cuttlefish can vary the density of its cuttlebone but the whole animal is nevertheless always fairly neutrally buoyant. The common squid, *L. forbesi*, whose pen is without gas spaces, was also studied. Two squid weighing 620 and 280 g in air, weighed 27 and 11 g respectively in sea water. The pen of *Loligo* only amounts to about $\frac{1}{2}\%$ of the animal's total weight and since its density is only 1·2 it plays a negligible role in deciding the buoyancy of the animal. Thus we find that the pelagic *Loligo* is some 4% denser than sea water, whilst the bottom-dwelling *Sepia* is approximately neutrally buoyant; a fact which will not surprise us if we recall how these animals hunt their food.

Although the pen, after drying with a cloth, appears to be a dry structure it loses about half of its weight on drying in an oven at 110° C. It seems likely that the chitin of the cuttlebone will hold water in a similar way and that this water will not be easily lost to the circulation.

DISCUSSION

It is a commonplace to say that man is virtually confined to one plane whilst the animals of the sea have a three-dimensional freedom of movement because the sea takes away their weight. Unless an animal is very nearly neutrally buoyant this freedom is, however, very incomplete. A weight in sea water of only a few per cent of the weight in air can only be held up in the yielding medium of the sea by vigorous and continuous effort. If we compare the restless activity of *Loligo* with the effortless hovering of *Sepia* which, as it stalks its prey, can poise in the water to shoot out its tentacles, we may see clearly how the whole behaviour of an animal is determined by its weight in water.

The cuttlebone represents a particularly elegant solution to the buoyancy problem. It is true that it occupies about 9·3% of the animal's volume, compared with the 5% of the gas-filled swimbladder of a fish, but it serves *Sepia* as a skeleton as well as a buoyancy tank. The cuttlebone, which is on the upper side of the animal, seems to be well placed and the anaesthetized cuttlefish lies in its natural swimming position. In man-made machines for travelling in mid water, such as the submarine and the bathyscaphe, changes in buoyancy can be used to give vertical movements in the sea. We have shown that the cuttlefish can change the density of its cuttlebone and we shall see later (Denton & Gilpin-Brown, 1961*a*) that the cuttlefish can use its cuttlebone as a variable buoyancy tank to enable it to become more or less dense than sea water.

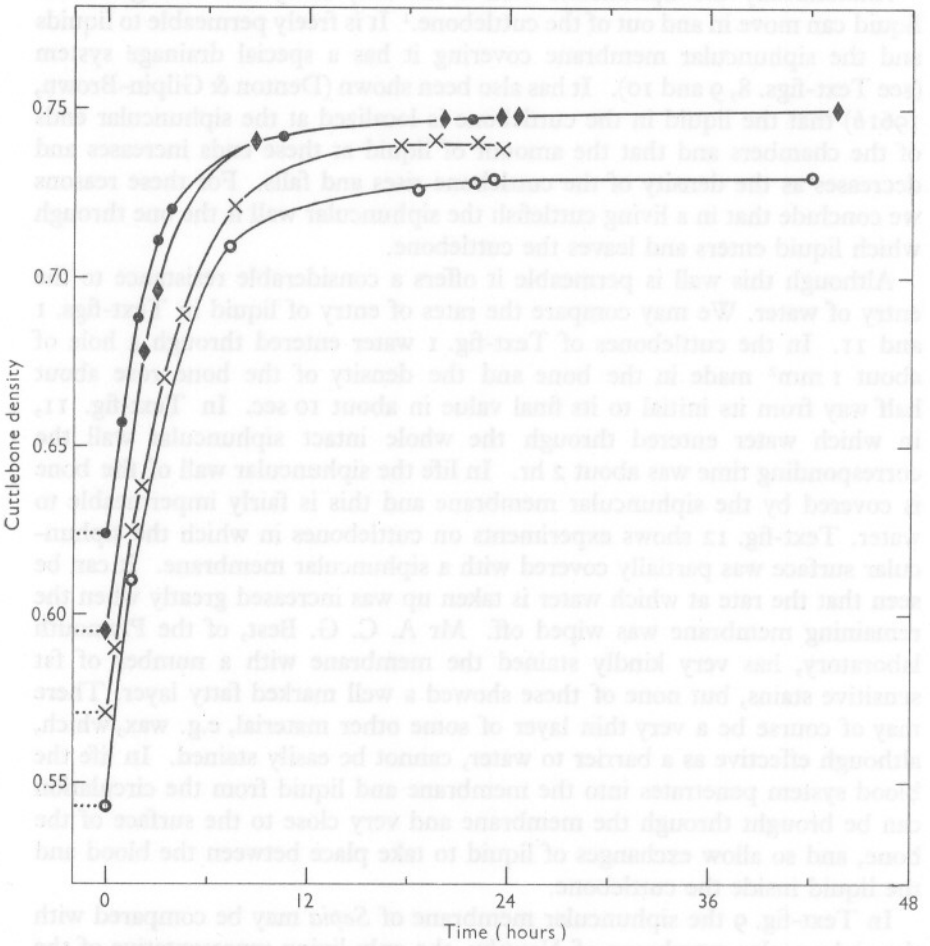
Anatomically the siphuncular wall is the only likely one through which liquid can move in and out of the cuttlebone.[1] It is freely permeable to liquids and the siphuncular membrane covering it has a special drainage system (see Text-figs. 8, 9 and 10). It has also been shown (Denton & Gilpin-Brown, 1961*b*) that the liquid in the cuttlebone is localized at the siphuncular ends of the chambers and that the amount of liquid at these ends increases and decreases as the density of the cuttlebone rises and falls. For these reasons we conclude that in a living cuttlefish the siphuncular wall is the one through which liquid enters and leaves the cuttlebone.

Although this wall is permeable it offers a considerable resistance to the entry of water. We may compare the rates of entry of liquid in Text-figs. 1 and 11. In the cuttlebones of Text-fig. 1 water entered through a hole of about 1 mm^2 made in the bone and the density of the bone rose about half way from its initial to its final value in about 10 sec. In Text-fig. 11, in which water entered through the whole intact siphuncular wall the corresponding time was about 2 hr. In life the siphuncular wall of the bone is covered by the siphuncular membrane and this is fairly impermeable to water. Text-fig. 12 shows experiments on cuttlebones in which the siphuncular surface was partially covered with a siphuncular membrane. It can be seen that the rate at which water is taken up was increased greatly when the remaining membrane was wiped off. Mr A. C. G. Best, of the Plymouth laboratory, has very kindly stained the membrane with a number of fat sensitive stains, but none of these showed a well marked fatty layer. There may of course be a very thin layer of some other material, e.g. wax, which, although effective as a barrier to water, cannot be easily stained. In life the blood system penetrates into the membrane and liquid from the circulation can be brought through the membrane and very close to the surface of the bone, and so allow exchanges of liquid to take place between the blood and the liquid inside the cuttlebone.

In Text-fig. 9 the siphuncular membrane of *Sepia* may be compared with the siphuncular membrane of *Nautilus*, the only living representative of the very numerous fossil Nautiloidea (Willey, 1902). Although the gross anatomy of *Nautilus* is very different from that of *Sepia* (in *Nautilus* the siphuncle lies within a tube passing through the chambers of the shell) their siphuncular membranes are very much alike. We find the same unusual epithelial structure with numerous ampullae close to the bone connecting by small ducts to an anastomosing system of vessels below the membrane which in turn communicate with the siphuncular vein.[2] This great similarity suggests that

[1] Dr H. Mutvei (personal communication) tells us that this wall, unlike the others, contains no calcareous material. It has the same mineralogical constitution as the fragile parts of the siphuncular tube of *Nautilus*.

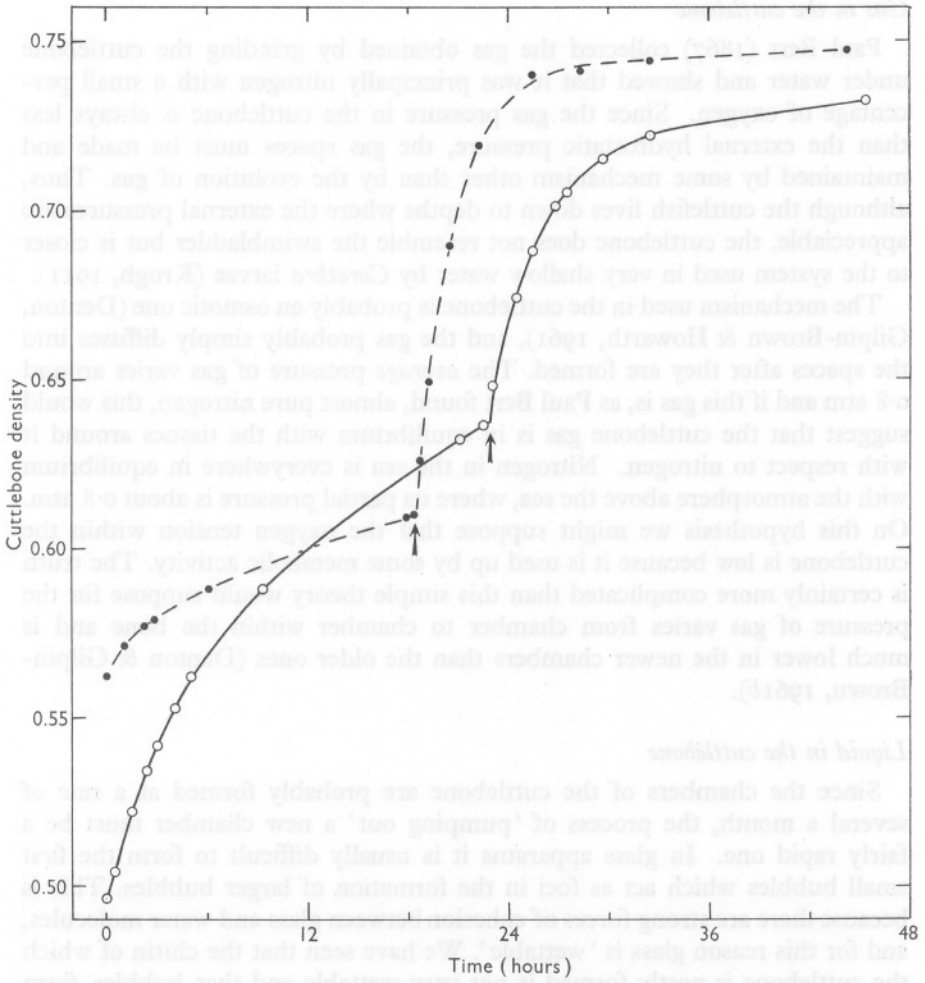
[2] Willey uses a nomenclature different from that adopted in this paper. He describes the ampullae as epithelial spaces and the spaces below the membrane as a trabecular meshwork.



Text-fig. 11. Changes in density of cuttlebones of various initial densities after removal of their siphuncular membranes and soaking in sea water. It will be noted that the ordinate indicates much longer time than that of Text-fig. 1.

the mechanism for moving liquid described here and in the following communications for *Sepia* is also used by *Nautilus*, and hence probably it was also used by the fossil cephalopods.

In the experiments on dissected bones the pressure difference which pushed water into the cuttlebone was that between atmospheric pressure and the pressure of gas within the bone, that is a pressure difference of about 0·2 atm. In life at the bottom of the sea this pressure difference may be one of several atmospheres so that even if the circulation to the cuttlebone were occluded, the membrane (about 1 mm thick) separating the liquid of the visceral cavities



Text-fig. 12. Changes in density of cuttlebones freshly dissected and placed under sea water. The siphuncular membranes of the two cuttlebones were initially only partially intact. On rubbing the residual membranes off at the times indicated by arrows the rate of uptake of water increased sharply.

from the cuttlebone would have to be very impermeable to keep liquid out of the cuttlebone. In this respect the cuttlefish differs from *Nautilus* and *Spirula* where the occlusion of the siphuncular circulation would give a long length over which diffusion would have to take place. If the tissues in the siphuncle could sustain both dehydration and anoxia this diffusion barrier could hold water out of the chambers for a long time. The relations between the cuttlefish and *Nautilus* and *Spirula* are discussed by Denton (1961).

Gas in the cuttlebone

Paul Bert (1867) collected the gas obtained by grinding the cuttlebone under water and showed that it was principally nitrogen with a small percentage of oxygen. Since the gas pressure in the cuttlebone is always less than the external hydrostatic pressure, the gas spaces must be made and maintained by some mechanism other than by the evolution of gas. Thus, although the cuttlefish lives down to depths where the external pressures are appreciable, the cuttlebone does not resemble the swimbladder but is closer to the system used in very shallow water by *Corethra* larvae (Krogh, 1911).

The mechanism used in the cuttlebone is probably an osmotic one (Denton, Gilpin-Brown & Howarth, 1961), and the gas probably simply diffuses into the spaces after they are formed. The *average* pressure of gas varies around 0·8 atm and if this gas is, as Paul Bert found, almost pure nitrogen, this would suggest that the cuttlebone gas is in equilibrium with the tissues around it with respect to nitrogen. Nitrogen in the sea is everywhere in equilibrium with the atmosphere above the sea, where its partial pressure is about 0·8 atm. On this hypothesis we might suppose that the oxygen tension within the cuttlebone is low because it is used up by some metabolic activity. The truth is certainly more complicated than this simple theory would suppose for the pressure of gas varies from chamber to chamber within the bone and is much lower in the newer chambers than the older ones (Denton & Gilpin-Brown, 1961*b*).

Liquid in the cuttlebone

Since the chambers of the cuttlebone are probably formed at a rate of several a month, the process of 'pumping out' a new chamber must be a fairly rapid one. In glass apparatus it is usually difficult to form the first small bubbles which act as foci in the formation of larger bubbles. This is because there are strong forces of cohesion between glass and water molecules, and for this reason glass is 'wettable'. We have seen that the chitin of which the cuttlebone is partly formed is not very wettable and that bubbles form very readily on it. There will not therefore be any special difficulty in forming the first small bubble in a newly completed chamber particularly since large forces, i.e. greater than several atmospheres, must be available to form gas spaces in the cuttlebone when the cuttlefish is at the bottom of the sea.

As we have seen some of the liquid which the cuttlebone contains can be extracted by placing the bone under reduced pressure, the gas inside the cuttlebone then expands and pushes liquid through the siphuncular surface of the bone. This fraction of cuttlebone liquid must be readily available for extraction in the intact animal. Since the average density of cuttlebones in freshly caught animals is 0·62 and densities as low as 0·5 have been found, the cuttlefish can extract more than half the liquid which its cuttlebone

usually contains. The extraction of liquid must be an active process since the pressure gradient is against the loss of liquid.

In brief, just as the submarine commander varies the buoyancy of his craft by increasing or decreasing the water content of its buoyancy tanks, so the cuttlefish varies its density by increasing or decreasing the mass of liquid which the cuttlebone contains. To become less dense the cuttlefish extracts liquid from its cuttlebone thus increasing its gas space; to become denser the cuttlefish lets (or pumps) liquid into the cuttlebone, thus decreasing its gas space.

We wish to thank Mr A. C. G. Best for kindly making the histological preparations, Captain C. A. Hoodless and the crew of R.V. 'Sarsia' for their help, and Mr R. G. Maddock for excellent technical assistance.

SUMMARY

The excess weight in sea water of the living tissues of *Sepia officinalis* (L.) is approximately balanced by the cuttlebone, which accounts for about 9·3% of the animal's volume. The density of cuttlebone varies around 0·6. The cuttlefish without its cuttlebone would be about 4% denser than sea water.

The chambers of the cuttlebone are independent of one another but liquids and gases are free to move within any one chamber.

Animals caught and studied fresh aboard ship exhibited a much less wide range of cuttlebone densities than those kept in an aquarium.

Specimens kept in aquaria vary greatly in buoyancy. These variations result from changes in density of the cuttlebone.

Cuttlebones differ not in the weight of dry matter per unit volume, which is always close to 38%, but in the amount of liquid they contain. A cuttlebone of density 0·7 contains about 30% liquid whereas a cuttlebone of density 0·5 contains about 10% liquid. The remainder of the cuttlebone contains gas, but this gas is at less than atmospheric pressure. The pressure of gas varies around 0·8 atmosphere. Within the duration of the experiments described here, the mass of gas per unit volume of bone remained almost constant whatever the bone's density. The pressure of gas is lower the less dense the cuttlebone. There can be no question of an evolution of gas expelling liquid from a bone when it becomes lighter. The constancy of the mass of gas within the cuttlebone is explained in terms of the slowness of diffusion of gases into the bone.

Liquid is added and removed from the cuttlebone through its siphuncular wall. The reduction of external pressure below atmospheric which is required to extract liquid from the cuttlebone is approximately equal to the difference between atmospheric pressure and the gas pressure inside the bone. Surface

forces do not therefore play an important role in the mechanism of the cuttlebone. The yellowish coloured siphuncular membrane covering the siphuncular wall of the cuttlebone acts as a barrier to the penetration of liquid. Serial histological sections show that it contains numerous ampullae close to the bone, draining into the veins. A very similar system of vessels has been found in the epithelium which lines the siphuncle of *Nautilus*.

The cuttlebone of *Sepia elegans* has physical characteristics similar to those of *S. officinalis*. In *Loligo forbesi* the pen has a volume of only 0·5% of that of the animal and its density 1·2 is greater than that of sea water. The whole animal, including its pen, is approximately 4% denser than sea water.

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