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Negative Pressure in the Interstitial Fluid of Animals

Fluid tensions are spectacular in plants; in animals they are elusively small, but just as vital.

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When a piece of bark is peeled off a transpiring tree and a cut is made in the xylem, no sap runs out; in fact, a drop of water placed on the cut is drawn in, for the sap is under negative pressure. This negative pressure may be enormous in mangroves and desert plants (−40 to −60 atmospheres) (*1*), and even in trees of the flooded forest of the Amazon, which we studied during the 1967 expedition of the research vessel *Alpha Helix*, the pressure was negative by several atmospheres. [By negative hydrostatic pressure (= tension) we simply mean pressure below 1 atmosphere, ambient.]

Similarly, when the subcutaneous tissue of an animal is exposed without bleeding, fluid does not seep out. Yet animal tissues in general are readily pervious to water and microsolutes. It appears that here, also, the pressure of the interstitial fluid must be below ambient pressure. Attempts made, with needle punctures, to measure this pressure have failed, mainly because of the small pickup area of the probe and the high resistance to flow in the tissue. Finally, Guyton (*2*) succeeded in measuring this pressure, by implanting a perforated capsule, about 8 millimeters wide, in the tissue. After the wound had healed for several weeks, the fluid pressure in the capsule could be measured, by means of needle puncture and a suitable recording method. This technique was tested in a variety of subcutaneous and other locations—for example, the retroperitoneal region—and normally gave a slight negative pressure. When venous flow in a limb was interrupted through application of a tourniquet, development of edema (positive pressure) could be followed. When the colloid concentration (and the concomitant osmotic pressure) in the blood was raised, the interstitial-fluid pressure became more negative (that is, tension rose); when the colloid concentration was lowered, the pressure became less negative, as one would have predicted. Attempts to measure pressure of pleural and peritoneal fluid failed, because of inflammation.

We have found that the negative pressure in animal tissues can be measured easily and rapidly by means of a capillary tube fitted with a cotton wick to increase the pickup area. After this technique became available we added some studies on negative pressure in animals to our studies of sap tensions in plants, during the expedition of the *Alpha Helix* to the drowned forest of the Amazon.

Measurement of negative tissue-fluid pressures in animals. The negative fluid pressure in a piece of filter paper suspended as shown in Fig. 1, *A*, can be measured manometrically with a capillary; a wick is used to establish continuity of the liquid. The net force on the meniscus in the capillary is balanced by the manometer. In Fig. 1, under conditions of no evaporation, T is the fluid tension in the wick, or the hydrostatic lift from the wick to the free surface; C is the capillarity of the glass tube; H is the hydrostatic height of the meniscus above the wick, and M is the manometer reading. The fluid tension, T, in the wick is accordingly $T = M + C - H$. In animals, the wick enlarges the area of contact with tissues and thereby greatly accelerates attainment of pressure equilibrium. In addition, greater negative pressures (as much as −0.5 atmosphere greater) can be measured with this device than can be measured with a simple capillary tube.

If the weight (*W* in Fig. 1) compresses the wick and paper but still allows flow into the capillary, the same tension will be measured, because it is the fluid pressure and not the matrix pressure that is being measured. In the absence of external forces, fluid and matrix pressure are equal, and opposite in sign.

In this technique, a loose cotton wick is pulled into the end of a Teflon tubing (1.5 millimeters or less in diameter) by means of a monofilament loop (Fig. 1, *B*). The wick end is boiled in 0.9-percent saline. Inserted into the other end of the Teflon tubing is a glass tube, *C* (previously cleaned with chromic acid), having a known capillary pull of about 4 cm-H_2O. The assembly is filled with saline to a given mark (the vertical line in Fig. 1, *C*). For easy insertion and minimum discomfort, the probe is fitted within a hypodermic needle which is inserted and then pulled back, leaving only the Teflon tubing with wick under the skin. Two sutures in the animal's skin secure the probe, one at the point of insertion, the other farther out. In a successful measurement there is an immediate response

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Table 1. Negative interstitial-fluid pressure (see also Figs. 2–11).

Animal	Pressure (cm-H_2O)	
	Subcutaneous	Peritoneal
Sloth (*Bradypus tridactylus*), normal	−10 to −15	−2 to − 6
Bittern (*Botaurus pinnatus*), normal	−12 to −18	−2 to − 3
Parrot (*Amazona* sp.), normal	−15	
Land turtle (*Testudo tabulata*), dehydrated		− 7
Land turtle (*Testudo tabulata*), killed by sun	−16	−16
Water turtle (*Podocnemys unifilis*), normal		− 3
Water turtle (*Podocnemys unifilis*), dehydrated		−11
Sea turtle (*Caretta caretta*), normal	− 1	
Crocodile (*Caiman niger*), normal	− 7 to −15	
Green lizard (*Ameiva* sp.), normal	− 3 to − 4	
Iguana (*Iguana iguana*), normal	−10	− 4 to − 5
Surinam toad (*Pipa americana*), measured in toe web	− 2	

of the meniscus when the skin above the wick is lightly touched. There was no osmotic effect evident when the wick was moved from saline to blood. Two manometers are used, one with water containing a little detergent, the other with mercury for a higher pressure range.

What is being measured? As Guyton found (*2*), we see (Table 1 and Figs. 2–9) that the interstitial fluid normally has negative pressure of −1 to −20 cm-H_2O, the magnitude depending upon the site of the probe and the species of animal.

As shown in Fig. 2, simultaneous measurements were made on an iguana with two subcutaneous probes, one on each side of the back. The two curves ran closely parallel for more than an hour. When the animal was disturbed, both showed an acute transient lowering of the pressure. This effect of activity was common in all the animals tested (Fig. 3). It is likely that the probe distorts somewhat the measurements of pressure changes induced by movements.

Both the interstitial-fluid pressure and the peritoneal-fluid pressure are negative by only a few centimeters of H_2O (Table 1), whereas the total osmotic pressure in blood is some 1000 times greater. Our measuring system seems to respond only to hydrostatic pressures, not to osmotic pressures, since replacing the isotonic NaCl (0.15*M*) in the probe with distilled water did not change the pressure measured in the boa constrictor (Fig. 8). This is to be expected, since channels in the wick are too large to impede the flow of NaCl relative to the flow of water. This is to say that the flow into and out of the wick and capillary is hydrodynamic rather than diffusive. Proteins are also too small to be impeded by the wick. In addition, the fluid in the wick is undoubtedly largely replaced by interstitial fluid during the course of most experiments.

Inflammatory reaction leading to edema is a danger, but in our test animals it has not been a problem. After each experiment the probe is pulled out carefully to make sure that there has been no bleeding.

Experiments on dehydration. In the experiment of Fig. 4, a toad was dehydrated in air for several hours after insertion of probes in the abdominal cavity and under the lumbar skin. The curve for interstitial-fluid pressure showed a short plateau, then a rapid decline to −10 cm-H_2O, and the animal died. The peritoneal-fluid tension was also −10 cm-H_2O; the weight loss was 25 percent. More rapid evaporation in other toads resulted in excessive drying of the skin, and the subcutaneous-fluid tension might exceed 100 cm-H_2O. This was especially so if the wick was caught in a fold of skin. A peritoneal-fluid pressure of −10 to −15 cm-H_2O was always lethal. These findings, which have bearing on the status of the human epidermis, are discussed below.

Experiments on edema. In the experiment of Fig. 5, the venous circulation in the leg of a toad was obstructed by application of a loose tourniquet. The tissue-fluid pressure rose from negative to positive in 90 minutes. In the experiment of Fig. 6, edema in a crocodile subsided following removal of a tourniquet that had been left on overnight. The edema fluid seeping out around the cannula gave a strong protein reaction. The drift seen in the peritoneal-fluid pressure could have been caused by abdominal-wall pressure or by peristalsis.

Snakes on a tilt table. It has been shown in tall trees that the sap pressure in the xylem displays accurately the hydrostatic gradient when transpiration is low. Thus, if a twig shot down from the top of the tree shows a pressure of −30 atmospheres, another from 50 meters lower shows pressure of −25 atmospheres (*1*). With this in mind, we performed some experiments with large snakes (3 meters long) on a tilt table. Two species were available—anaconda (*Eunectes murinus*), a water

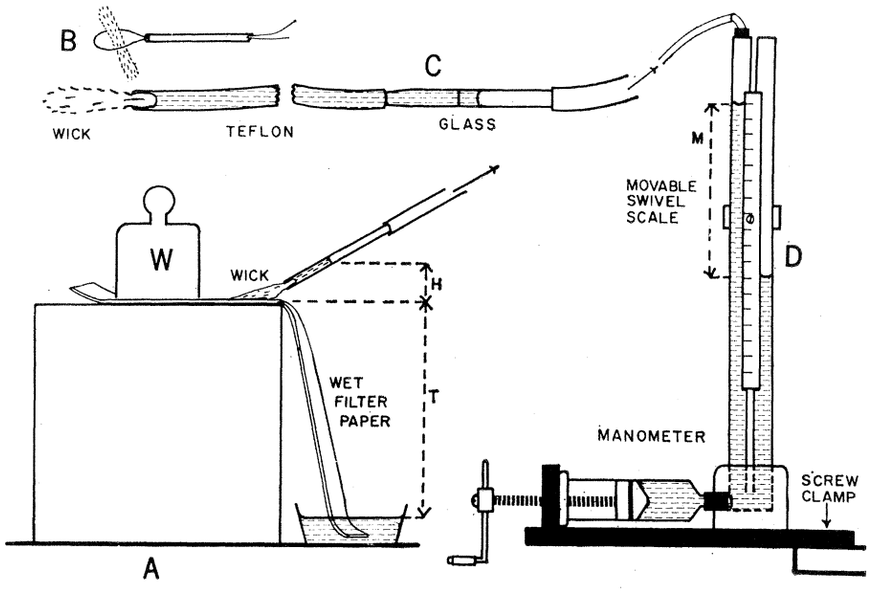

Fig. 1. (*A*) Schematic diagram of the technique for measuring negative pressure by means of wet filter paper. The wick capillary is connected with the manometer (*D*), which balances the meniscus. The tension is calculated from the equation $T = M + C - H$ (see text). (*B*) Threading a wick into the microtubing; (*C*) wick assembly with measuring capillary; (*D*) plastic manometer; (*W*) weight.

snake, and boa constrictor (*Constrictor constrictor*), a tree-climber. One would predict that hydrostatic compensation, if any, would be superior in the boa.

The snakes were secured in a form-fitting, aluminum-clad trough which could be tilted 45 degrees either way. One probe was placed under the skin in the neck and another was placed near the tail. The tilt produced a hydrostatic gradient of 100 cm-H_2O between the two wicks. Measurements were made with the snake in horizontal, head-up, and head-down positions. The probe meniscus was always kept level with the wick.

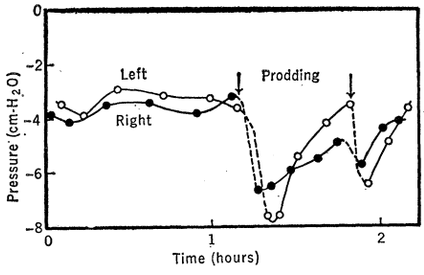

Fig. 2. Subcutaneous interstitial-fluid pressures in an iguana. Separate probes ("right" and "left") were inserted on each side of the spine, and measurements with the two were made simultaneously. (Arrows) Times when the animal was prodded; (broken line) fluid pressures when the animal was moving.

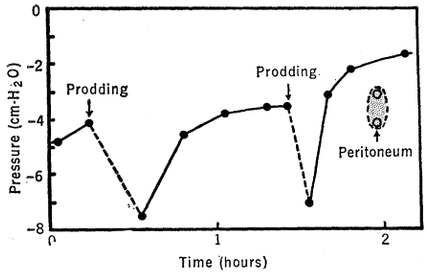

Fig. 3. Interstitial-fluid pressures in the pygmy anteater (*Cyclopes didactylus*). One subcutaneous probe was inserted in the thigh; the other, in the abdominal cavity.

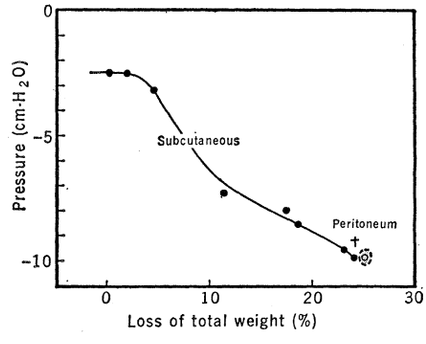

Fig. 4. Interstitial-fluid pressure in a toad (*Bufo marinus*) subjected to dehydration. One subcutaneous probe was inserted under the lumbar skin; the other, in the abdominal cavity.

Figure 7 shows the results for one anaconda; a second gave similar results. When the head was tilted up, the tissue-fluid pressure, as measured by the probe in the neck, increased; when the snake was returned to a horizontal position the pressure decreased; when the head was tilted down, the pressure decreased further; when the snake was again returned to the horizontal position there was a positive surge in pressure. In the tail, all compensatory surges, as recorded by the second probe, went in the opposite direction. Either end of the snake, therefore, reacted to a tilt by a transitory overcompensation.

Compared to this crisp homeostatic reaction of the anaconda, the response of the boa was a surprise (Fig. 8). At the head end the tissue-fluid pressure was kept well within bounds, but at the tail end it was not. Indeed, with the tail low, the pressure had positive values. With the tail up, there was a surge of 10 cm-H_2O in negative pressure. Another specimen gave a similar response.

We see, therefore, that the anaconda is equipped with a sophisticated hydrostatic control system which obviously it does not need in the water, whereas the tree-climbing boa is burdened with a tail prone to postural edema. This points to a mixup in design, or is the anaconda simply a newer model?

A probe inserted into the peritoneal cavity of a boa showed a prompt and regular response of pressure to tilting, such as one would expect in such a freely floating, quasi-fluid system (Fig. 9). The anaconda was not tested in this respect.

Tension-volume curves in animal tissues. When water is withdrawn from a fully hydrated leaf, (i) the sap pressure drops rapidly as turgor disappears. Then follows (ii) a slow drop, which accelerates as the osmotic pressure increases. Finally, when about 50 percent of the cell water is gone, there is (iii) a precipitous drop in the sap pressure and the leaf yields no more liquid. We interpret this to mean that the structural elements of the cells are being packed together and thus offering a mechanical hindrance to water loss. These events stood out clearly in leaves of the drowned forest of the Amazon, and we were interested to see whether analogous events could be traced in animal tissues.

Figure 10A shows a tension-volume curve from thigh muscle of a toad. The muscle, about 2 centimeters long and spindle-shaped, was threaded lengthwise with a wick and tied around the end of a capillary tube, in the manner shown in Fig. 10B. The muscle dried slowly; changes in its tension and weight were followed. There was an initial slow drop in pressure analogous to that found in phase ii in leaves. When the water content had reached 68 percent, the pressure plunged steeply (phase iii), indicating packing of the cell constituents.

In the thigh muscle of the Surinam toad (Fig. 10B) we observed a variation from this response. When the water content of the muscle had reached

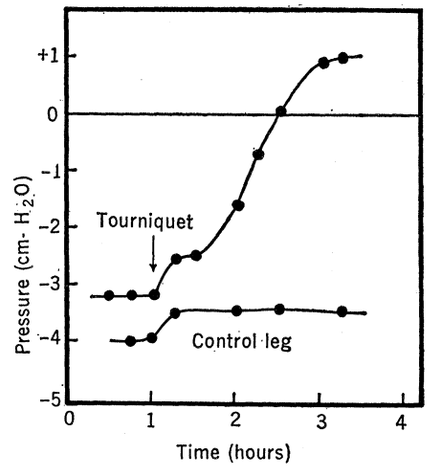

Fig. 5. Edema produced in a toad (*Bufo marinus*) by venous occlusion. The probe was subcutaneous, inserted in the hind leg.

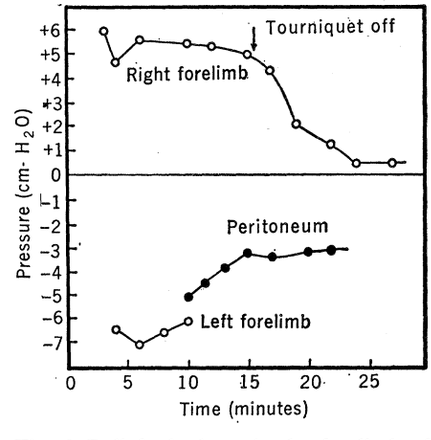

Fig. 6. Relief of edema in the forelimb of a crocodile (*Caiman latirostris*) upon removal of a tourniquet. There were three subcutaneous probes, one in the upper part of each forelimb and one in the abdominal cavity.

71 percent, the pressure plunged, indicating packing of the cell constituents. When the muscle was manipulated for the next weighing, it shortened and the pressure rose. The wick protruded and was trimmed off. Again there was a steep drop in pressure; this was followed by a second shortening of the muscle and rise in pressure, and then by a final, steep drop. This suggests a stepwise rearrangement in the packing of the cell constituents.

Figure 11A shows similar measurements from the electric organ of an eel. A cylinder, 4 millimeters thick, of this gelatinous organ was excised with a cork borer and threaded through with a wick. Dehydration of the material to water content of 78 percent led to only a slight drop in tissue-fluid pressure. Further dehydration led to an abrupt drop in pressure. In an adjacent muscle (Fig. 11B) there was a sudden drop in tissue-fluid pressure when the moisture content reached 77 percent.

Discussion

We have described a technique for rapid and easy measurement of interstitial-fluid pressure in animals. We find, as Guyton did, that this pressure is normally negative, and in general we agree with his concepts (*2*). We have extended the wick technique to the study of tissues and have added experiments illustrating the speed of response of interstitial fluid to various stresses.

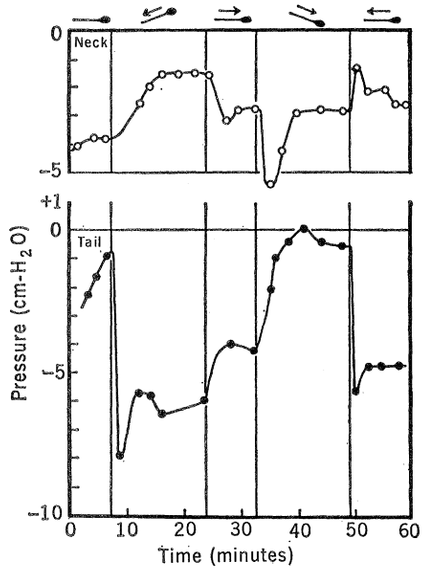

Fig. 7. Hydrostatic compensation of interstitial-fluid pressure in the neck and tail of an anaconda (*Eunectes murinus*), 3.5 meters long, on a tilt table. The probes were subcutaneous. The position of the snake is indicated by small diagrams at the top of the figure. The arrows indicate direction of gravity displacement.

Table 2. Colloidal osmotic pressure in blood.

Animal	Pressure (cm-H_2O)
Toad (*Bufo marinus*)	29.6
Toad (*Pipa americana*)	32.8
Lizard (*Iguana iguana*)	29.6
Sea turtle (*Caretta caretta*)	26.4
Crocodile (*Caiman niger*)	31.2
Snake (*Constrictor constrictor*)	33.1

Rate of pressure changes. The slow changes in pressure associated with edema are readily explainable in terms of an ultrafiltration from the capillaries, but the quick response to muscular motions and to postural changes offers some problems.

We usually find an abrupt transient lowering of the interstitial-fluid pressure as a result of sudden muscular movements, a lowering which indicates that tissue space is dilated or that fluid is absorbed. The tilting experiments showed compensatory changes in the interstitial-fluid pressure, which opposed a gravity displacement indicated by the arrows in Figs. 7 and 8. One might imagine a rise to be caused by a primary vasodilation which, by simple displacement of space, would increase the tissue-fluid pressure. One could argue that ultrafiltration is also a factor, for when we elevate our arm there is an increase in the blood volume in our fingers, due to the pulse. The argument is weakened, however, by the fact that this effect is dominated by a decrease in total volume because of venous drainage (*4*). Possibly this drainage was small in our snakes.

Level of negative pressure. When the snakes of our experiments were in a tail-down position on the tilt table, the tail was more than a meter lower than the head. One would expect edema to develop, in view of the fact that the colloidal osmotic pull by the blood is rarely more than 25 to 35 cm-H_2O (compare the data of Table 2). In mammals such an event is countered by a mechanism—the valvular pump—which permits uphill transport of blood and lymph without creating back pressure in the drainage area.

Consider a 10-centimeter segment of a vertical vein with a one-way valve in each end for upward flow. Let an external force flatten the segment so that it nearly empties; when relaxed, the segment collapses, at which time pressure at the lower valve is zero and pressure at the upper valve is −10 cm-H_2O. A small vein draining into the segment through its own valve has ample pressure (10 to 15 cm-H_2O) to fill the segment. It is irrelevant how high the blood is later lifted by external muscle action, for the total blood column is kept divided into a series of low-pressure segments. This is part of the edema-preventing mechanism in our legs.

There are difficulties when we consider the lymphatic system, which yields fluid at positive pressure from a source which has normally negative pressure. One possibility is that the first valvular segments of the lymph vessels are filled only during surges of positive pressure. If they are also filled during times of negative pressure, the segments must have an active or elastic distension which can draw in fluid more negative in pressure than the fluid in the interstices (*3*). Regardless of the way in which they are filled, from that point on the lymph will be transported by means of valvular pumping similar to that described above for the veins.

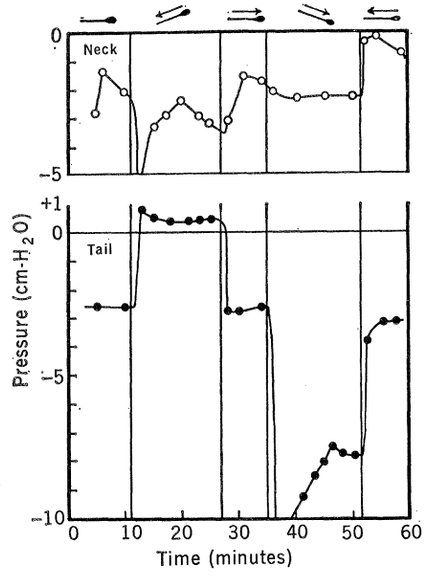

Fig. 8. Hydrostatic compensation of interstitial-fluid pressure in the neck and tail of a boa constrictor on a tilt table. The probes were subcutaneous. The position of the snake is indicated by small diagrams at the top of the figure. The arrows indicate direction of gravity displacement.

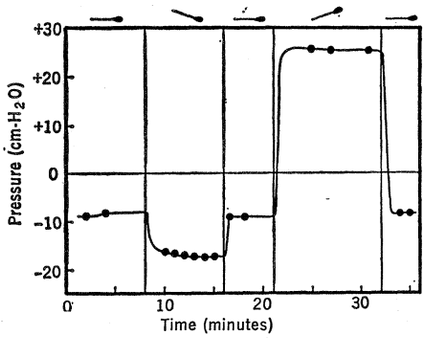

Fig. 9. Interstitial-fluid pressure in the peritoneal cavity of a boa constrictor in response to tilting on a tilt table.

The similarity in magnitude between the subcutaneous-fluid tension and the peritoneal-fluid tension suggests that the two have the same origin: the interstitial fluid simply permeates the peritoneal membrane and keeps it wet. This could well be true for all serosa spaces (such as pleural, pericardial, and testicular space) and also for synovial fluid in joints. The pressure at which the lung collapses when the pleura is opened amounts to only -5 to -10 cm-H_2O, and therefore cannot normally pull fluid out from the pleural interstitial spaces.

Puckering of the skin of our fingers. When one's hand is kept immersed in freshwater, the surface layer puckers. This layer also puckers when the hand is enclosed in a rubber glove, which provides an isosmotic atmosphere, and even when it is kept immersed in sea-

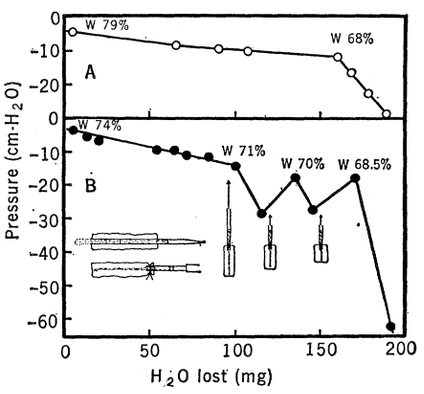

Fig. 10. (A) Interstitial-fluid pressure in the dehydrating excised muscle of a toad (*Bufo marinus*). (B) Interstitial-fluid pressure in the dehydrating excised muscle of a Surinam toad (*Pipa americana*), showing the stepwise compaction of the muscle, with attendant relief of tension. (Inset) Diagram showing the technique for threading a muscle with a wick in the fused measuring capillary.

water. This indicates that the dead outer-cell layer of the skin (the stratum corneum) is pervious to solutes, and that puckering is a simple swelling of the matrix. The normal, nonpuckered state is the result of evaporation which maintains a steep tension gradient within a high-resistance surface layer of the skin. The evaporation maintains a considerable compression of the outer layers of the skin, for when the humidity over a saturated gel is lowered by only 10 percent—that is, from 100 to 90 percent—evaporation will ultimately result in compression of the gel matrix by 130 atmospheres!

Negative pressure in plant and animal tissues. It is interesting to compare the buildup of tension in plant tissues and in animal tissues when these are progressively dehydrated. We have already described the three phases in leaves: (i) loss of turgor; (ii) no turgor; and (iii) cell compaction (negative turgor).

In animal tissues there is no good analog for turgor; rather, we start with phase ii. At a given dehydration we recognize a sharp drop in pressure (Figs. 10 and 11), which we interpret as the packing of structural elements. The stepwise change in the configuration of the *Pipa americana* muscle (Fig. 10B), with an attendant release of tension, supports this view. The fact that packing in the leaves occurs with a relatively high dry weight simply reflects their stiff and heavy cellulose walls (Fig. 12).

Some trees in the drowned forest of the Amazon, when under a glaring sun, had a xylem sap tension so high that the leaf cells must occasionally experience negative turgor without harm. The toads of our studies could withstand a peritoneal-fluid tension down to -10 cm-H_2O; this was about the negative pressure at which the muscle cells started to show packing. We have seen, also, that the skin of a toad locally would stand very considerable compaction. Indeed, this seems to be the natural state of the dead surface layers of our own skin.

One could cite examples of living materials which tolerate enormous dehydration, such as seeds, insect eggs, resting spores, mosses, lichens, and algae. Dehydration to the point of matrix compression may be a defense against further water loss, for with the further loss of water the organism has the greatest power to gain this water

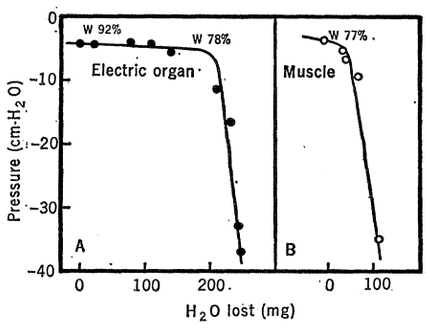

Fig. 11. Interstitial-fluid pressure in the dehydrating electric organ and muscle of the electric eel (*Electrophorus electricus*).

back. At the same time, this degree of dehydration is a protection against excessive solute concentrations; leaves double their solute concentration only before they start packing.

In a comparison of plants with animals, it is the enormous difference in the tensions under which plants and animals operate that is particularly striking. In the drowned forest, turgor is lost at pressure of -5 to -10 atmospheres, and in many desert plants and mangroves it is lost at -40 to -60 atmospheres. The collapse or packing occurs at negative pressures nearly twice these figures. In animal tissues, analogous events take place at tensions which are less by four orders of magnitude; what in plants is measured in atmospheres, in animal tissues is measured in centimeters of H_2O (Fig. 12). This difference results from the enormous differences in the permeability of the membranes involved (the endothelium in animals, the plasmalemma in plants) and in the structural strength of the tissues.

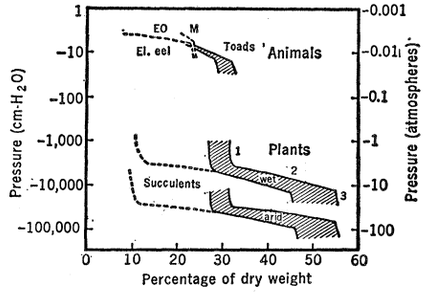

Fig. 12. Tension buildup in the interstitial fluid of animal and plant tissues as a function of dehydration. Dry weight is given as a percentage of total weight. (*EO*) Electric organ in eel; (*M*) muscle; ("wet plants") drowned-forest trees; ("arid plants") desert plants and mangrove; (1) turgor; (2) no turgor; (3) negative turgor (packing).

Summary

A simple and painless microtechnique for measuring interstitial-fluid pressure is described. We agree with Guyton that this pressure is normally negative. Dehydration and edema were studied in various animals by means of subcutaneous and peritoneal probes, and the hydrostatic compensation against tilting was studied in large snakes. Fluid pressure was followed in dehydrating muscles and electric organs; the measurements show an abrupt increase in tension when the water content reaches 70 to 80 percent. This increase is attributed to packing of the structural elements. These measurements were made as a sequel to similar studies of negative pressure in the drowned forest of the Amazon. They demonstrate that the parameters in the two systems are the same, but that the negative pressures in plants are some 10^4 times greater than those in animals.

References and Notes

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5. The investigation discussed was performed aboard the research vessel *Alpha Helix*, on the Brazilian-American Amazon Expedition, 1967, and was made possible by continuing support from the National Science Foundation (grant GB-5196). It was supported also by research grant GM-10521 from the U.S. Department of Health, Education, and Welfare and by the Smithsonian Institution. We are deeply indebted to our Brazilian colleagues and to the Brazilian officials who made this expedition possible: Dr. Antonio M. Couceiro of the Brazilian Research Council; Dr. Paulo Sawaya of the University of São Paulo; Captain Paulo da Silva and Commander Manoel Pérez of the Brazilian Navy; Their Excellencies Governor Arthur Reis and, later, Governor Danilo Areosa of Amazonas; Dr. Djalma da Cunha Batista of the Instituto Nacional de Pesquisas da Amazonia; and Drs. Jose Conduru, J. Murça Pires, and Domiciano Dias of the Instituto de Pesquisas Experimentaçao Agropecuarias do Norte. Most of the animals used were secured by Mr. H. W. Schwartz of Manaus; the anacondas were generously lent us by Professor Paulo B. Cavalcante of the Museu Goeldi in Belém, where the anaconda experiments were performed. We are indebted to James Faughn and Robert Haines, captains of the *Alpha Helix*, and to Mr. Howard Davis for management of the expedition.

Facilitated Proton Transfer in Enzyme Catalysis

It may have a crucial role in determining the efficiency and specificity of enzymes.

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A question frequently asked concerning enzyme action is, When a substrate molecule is bound at the active site of an enzyme, is the susceptible bond already distorted or under strain so that it is rendered more reactive? Recent x-ray data on the lysozyme-tri-*N*-acetylglucosamine complex suggest that when a larger substrate is bound to this enzyme there may be considerable distortion in the susceptible section of the substrate molecule (*1*). On the other hand, infrared studies show that the CO_2 molecule bound at the active site of carbonic anhydrase is definitely not distorted or under appreciable strain (*2*). These observations show that, although the "strain theory" (*3*) might be applicable in some cases, it cannot be the general explanation of enzyme catalysis. There is also the alternative theory of enzyme action based on the activation entropy effect, according to which enzyme catalysis is only a special case of general acid-base catalysis, having the particular advantage that the activation step does not involve a large decrease in entropy since the responsible acid and base groups are already nearby. While this activation entropy effect is undoubtedly an important factor, it is generally believed that enzymes must have additional characteristics which enable them to carry out their remarkable function. In this article I suggest that facilitated proton transfer along rigidly held hydrogen bonds (*4*) may play a crucial role in determining the efficiency and specificity of many enzymes. For clarity, let us examine these possibilities by considering selected examples.

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Carbonic Anhydrase

Carbonic anhydrase contains one firmly bound zinc ion per enzyme molecule. It catalyzes the hydration of CO_2 to HCO_3^- and the reverse dehydration of HCO_3^-. Accurate difference infrared spectrometry shows that CO_2 bound at the active site of carbonic anhydrase exhibits an infrared absorption peak at wave number 2341 cm^{-1}, due to the asymmetric stretching of this linear molecule. Since this wave number is very close to the corresponding values for dissolved CO_2 (2343.5 cm^{-1} for CO_2 dissolved in water, 2340 cm^{-1} for CO_2 dissolved in methanol, and 2336 cm^{-1} for CO_2 dissolved in benzene) it has been concluded that the CO_2 at the active site is neither coordinated to the Zn(II) nor appreciably distorted, but is loosely bound to a hydrophobic surface or cavity of the protein, as in clathrate compounds (*2*). The infrared studies also show that the inhibitor azide ion is coordinated to the Zn(II) of the enzyme, and that the binding of a single azide ion at this Zn(II) prevents the binding of CO_2 at the specific CO_2 site mentioned above. Since the binding of inhibitors has not been observed to lead to gross conformational change in this enzyme (*5*), it was concluded that the specific CO_2 site must adjoin the Zn(II) so that the ligand azide can protrude at least partly into that site to interfere sterically with the binding of CO_2.

Nitrate and bicarbonate were found, in these infrared studies, to displace both the azide from the Zn(II) and the CO_2 from its specific binding site. But