Salt Balance in Mangroves [1]

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The tidal zone of tropical seas is frequently lined with great mangrove forests, the dominating species of which belong to such genera as Rhizophora, Bruguiera, Sonneratia, and Avicennia. The outstanding feature of these plants is their adaptation to growing in seawater, and even though all do well in river estuaries they seldom penetrate inland beyond the direct action of ocean tides.

A question which immediately presents itself is "How do these plants handle the salt in the seawater?" Do the roots exclude it from the transpiration stream or do the plants possess special organs for eliminating such salts as may penetrate into the sap? What balance, if any, exists between osmotic potential of seawater, roots, and leaves on one hand and hydrostatic pressure and osmotic potential of the xylem sap on the other? Our aim has been to inquire into these matters.

It has long been realized that various mangroves behave differently with respect to some of these parameters. It is thus easily ascertained that certain species accumulate salt on their leaves. In Aegialitis and Aegiceras salt crystals can be seen covering the leaves and in Avicennia and *Acanthus ilicifolia* one may easily taste the salt. In other species like Sonneratia, Rhizophora, Bruguiera, Ceriops, and Lumnitzera salt can neither be seen nor tasted (5, 17).

Various authors agree that press juices of mangrove leaves have a high osmotic potential, being more or less isotonic with seawater (5, 6, 17). Walter and Steiner, using the same species of Rhizophora, Sonneratia and Avicennia in East Africa as we worked on in Australia, found that the press juices of roots also showed similar values. They determined the transpiration rate of mangrove leaves to be about one-third of that of ordinary plants (20).

Some of the mangroves possess salt glands on the leaves, visible by naked eye as minute dimples in the surface. The histology of the glands has been described in Aegialitis by Ruhland (9) and Avicennia by Walter and Steiner (20), but no experimental studies seem to have been performed on these. However Ruhland (9) determined the amount of salt given off by the isolated leaves of statice (*Limonium latifolium*), and using leaf disks of the same species, Arisz, Camphuis, Heikens, and Van Tooren (2) found that these would secrete salt when floated on a saline solution. The secreted fluid under certain conditions became more concentrated than the medium. This process was stopped by cyanide and other respiratory poisons.

Large mangrove stands are typically rooted in deep muck which is completely anaerobic from decomposing materials. In such habitats the roots are conspicuously swollen by a spongy pneumatic tissue which communicates to the air through a multitude of lenticels located on stilt roots (Rhizophora, Bruguiera) or special pneumatophores (Avicennia, Sonneratia). The ventilatory function of these structures has been studied in detail in Avicennia and Rhizophora (4, 16, 19).

Materials

The main part of the present investigation was performed at Cape York peninsula, North Australia, on the Scripps Institution Expedition to these waters in August to September 1960. The following species were considered.

Rhizophora mucronata Lamk., *Bruguiera* prob. *exaristata* Ding Hou, *Sonneratia alba* J. Sm., *Lumnitzera littorea* (Jack.) Voigt, *Avicennia marina* (Forsk.) Vierh., *Aegiceras corniculatum* (L) Blanco, *Aegialitis annulata* R. Br.

These plants were growing within or next to a small tidal pool within the estuary of the Jardine River at the very tip of Cape York peninsula. The pool was usually inundated by high tide but could run dry at exceptionally low tides, and had a rather fluctuating salinity varying from 2.2 % to 3.6 %. Young trees or bushes from 1 to 3 meters tall were used for the most part. These species were compared with *Hibiscus tiliaceus* L, growing higher up on the sandy beach, and *Eugenia suborbicularis* Benth., found in the dry scrub forest away from the beach.

Supplementary data to these studies were obtained on *Rhizophora mangle* L, *Avicennia nitida* Jacq., and *Laguncularia racemosa* Gaertn. at Marine Laboratory, University of Miami, and at the Lerner Marine Laboratory, Bimini, Bahamas, in September 1961, and January 1962; also at La Paz, Baja Cal., July 1962.

► Salt Secretion From Leaves. The rate of salt excretion from attached leaves was determined by washing them off with distilled water at certain intervals. The wash water was titrated for chloride,

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delivering silver nitrate from a syringe burette (15) and using potassium chromate as indicator. At the end of the series, the leaves were detached from the bush and traced on paper for later area determination.

The species separated into two groups (fig 1): A, the salt-secreting species Aegialitis, Aegiceras, and Avicennia, and B, the non-secreting species which comprised the rest. When the leaves were washed every 3 hours, the secretion had a pronounced diurnal cycle with minimum activity in the night. This was particularly pronounced in Aegialitis (fig 4), less so in Aegiceras (fig 5), but did not show up clearly in Avicennia. When attached leaves of Aegialitis were enclosed in a roomy bag of aluminum foil, together with a desiccant, salt secretion almost ceased, but recovered in light. In a transparent bag there was no slowdown. A possible explanation would be a primary stomata closure in the dark, with consequent reduction of transpiration and source of salts to be excreted.

A more comprehensive study of the salt composition was made on preserved specimens at Scripps and showed that some 90 % of the chloride is matched by sodium and about 4 % by potassium, leaving the ionic ratios about the same as in seawater (table I).

Table I

Ionic Composition of Salts in Sap of Mangroves

	$\frac{Na}{Cl}$	$\frac{K}{Cl}$	$\frac{Na+K}{Cl}$
Aegialitis	94 %	1.8 %	96 %
Aegiceras	86 %	2.6 %	87 %
Avicennia	87 %	5.8 %	93 %
Seawater	85 %	1.8 %	87 %

Ammonia and total nitrogen were present in minute quantities, less than 0.1 % of the other constituents. Sodium chloride is therefore, by far, the major component of the secretion.

► Salt Glands. By a mere inspection of the leaves of Aegialitis and Aegiceras it was clear that the salt is secreted through little dimples in the leaves, corresponding to the salt glands. When exposed to the sunshine, the secreted liquid rapidly evaporates and one observes dry salt residues rather than liquid drops. However, little drops were observed to form readily under a layer of stopcock grease or oil, and this made it possible to determine the concentration of the secreted fluid. The attached leaf was turned up at the edges and charged with a pool of mineral

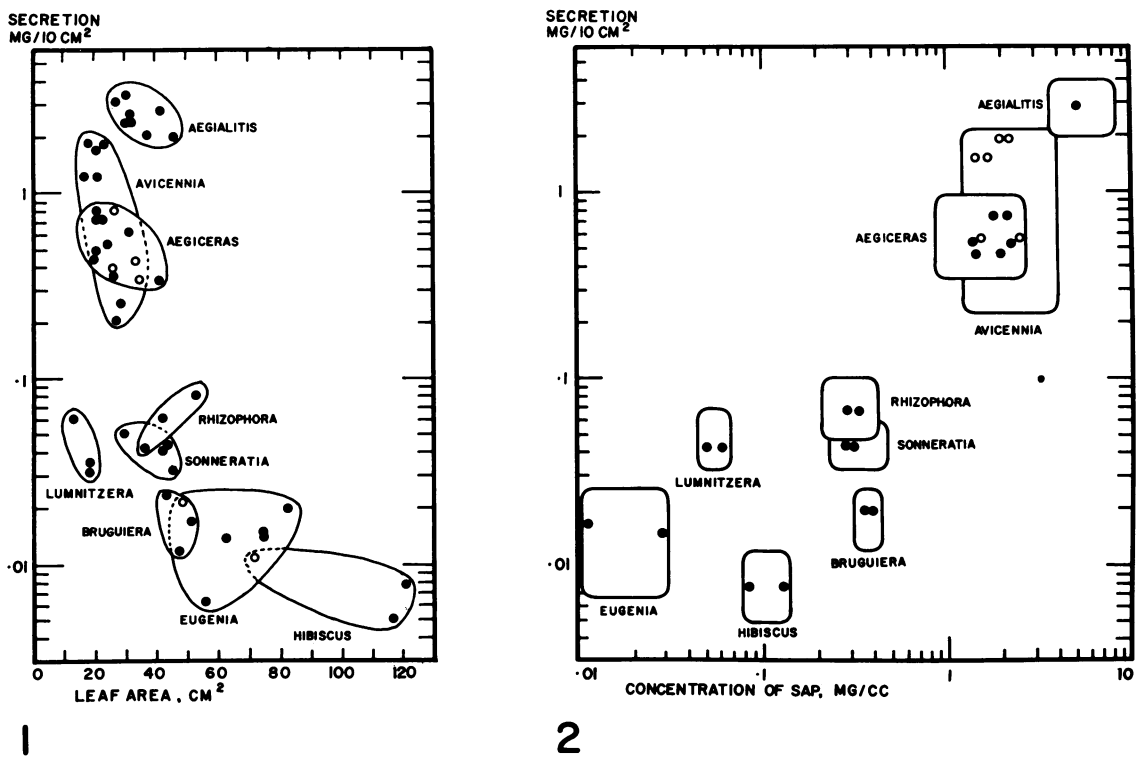


Fig. 1. Secretion of sodium chloride from the leaves of various mangroves and two control trees over 9 daylight hours (0900–1800).

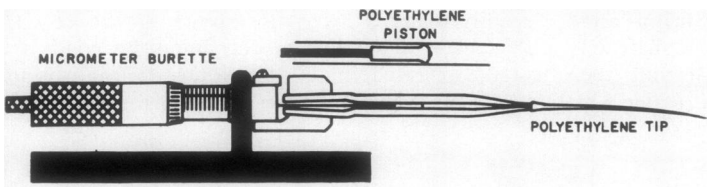
Fig. 2. Secretion of sodium chloride in various mangroves and two control trees over 9 daylight hours as related to the concentration of sodium chloride in the xylem sap. Numerous determinations of the latter were taken, the range of which is given on the width of the rectangles. Only those points are given where secretion data were obtained at the same time.

oil. Through a dissecting microscope the secretion could be observed as tiny drops under the oil (fig 6). These were made to coalesce by means of a hair-fine wire loop so that about one cubic millimeter could be drawn into the fine tip of a micrometer burette behind a bubble of air (fig 3 & 7). The volume was measured, transferred into a few drops of distilled water, and titrated for chlorides with the same burette. Checks revealed no detectable evaporation loss through the oil.

In Aegialitis the concentration of NaCl in the secreted liquid varied from 1.8 to 4.9 %, and when collected every 2 hours it revealed a marked diurnal cycle with highest value in the middle of the day (fig 8). In Aegiceras, and especially so in Avicennia, the glands were more sparse, but collection under oil was still possible. In Aegiceras the concentration averaged 2.9 % throughout the daytime and 0.9 % during the night, and one 18-hour collection from Avicennia gave 4.1 %.

► Concentration of Salts in Xylem Sap. As pointed out by Walter and Steiner (20), one would expect that plants which do not secrete salts through their leaves must carry a transpiration stream virtually void of salts. This assumption was checked by extracting sap from fresh stem sections of various mangroves, following a procedure described by Bennet, Anderssen, and Milad (3): A piece of stem, stripped of bark at the lower end, is fitted airtight into a small vacuum container, which connects to an automobile tire pump with reversed piston valve. When the handle is pulled out and fixed, short pieces are cut off from the upper end of the stem, allowing the sap to descend stepwise.

Those species which secrete salt from the leaves are the ones least able to exclude the sea salts (fig 2). But even the non-secreting mangroves may still carry some 10 to 50 times more salt in the sap than Hibiscus and Eugenia, which are in the range of common plants (7).



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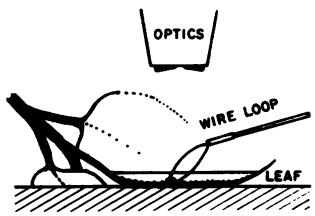

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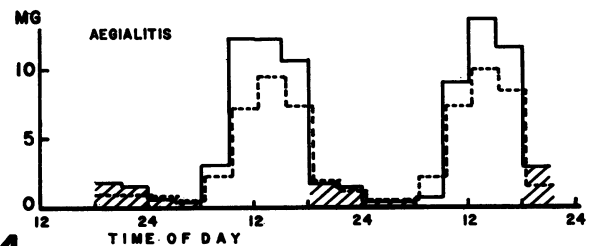

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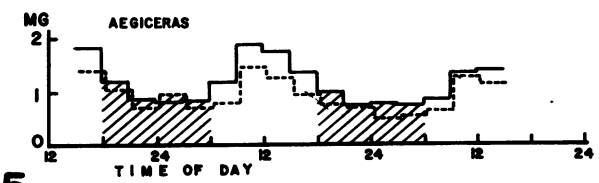

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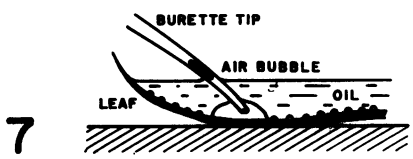

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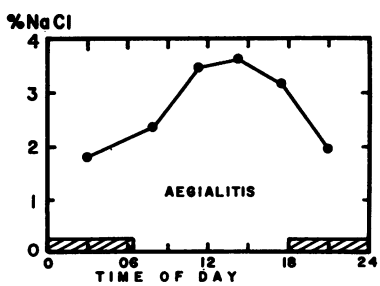

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Fig. 3. Micrometer burette used for titrating secretion of less than 1 mm³ volume. The micrometer activates a stainless steel wire furnished with a polyethylene tip which is flared at the end by heat. This flare engages tightly the bore of a precision shrunk glass tubing.

Fig. 4 & 5. Diurnal variation in the NaCl secreted from leaves of two species of mangroves, each represented by determinations in two leaves.

Fig. 6. Aegialitis leaf covered with oil for collecting secretion drops from the salt glands.

Fig. 7. Drops of secretion, brought to confluence by means of a hair-fine wire loop, are drawn into the burette tip behind an air bubble.

Fig. 8. Diurnal variation in the concentration of the secreted fluid collected under oil.

Stem samples of sap from Aegiceras, Avicennia, Sonneratia, and Rhizophora were collected at intervals from the same plant but showed no clear diurnal variation in the salt concentration, and samples taken from base, middle, and upper part of the stem revealed no concentration gradient.

► Osmotic Potential of Xylem Sap. In order to understand the processes involved in producing the near salt-free sap, one must know osmotic potential and hydrostatic pressure of this fluid. Chlorides and freezing points (15) were accordingly determined in sap specimens from a series of Atlantic mangroves, including *Avicennia nitida, Rhizophora mangle,* and *Laguncularia racemosa.* In all species the non-chloride components added at most 1 to 2 atmospheres to the total osmotic potential, which is to say that the seawater exceeds the sap at all times by close to 20 atm.

► Rate of Transpiration. In a steady state situation the amount of salt entering the roots equals that excreted by the leaf glands, plus whatever salt may be transferred to tissues. The latter fraction must be very small compared to the salt secretion in species like Aegialitis, Avicennia, and Aegiceras. The salt is transported by the sap flow, and one may, therefore, calculate the transpiration rates from the rate of salt secretion and the concentration of salt in the xylem sap. The average daytime values came out as follows: Aegialitis 5 mg/dm²/minute, Aegiceras 2.5 and Avicennia 6.5. In the non-secreting species the relative salt loss from the sap into tissues may be appreciable and would give too-low transpiration estimates; but this potential error would be counteracted by contamination of the sap with salts from non-conducting severed tissues of the xylem. With these reservations, the figures are: Rhizophora 2.5, Sonneratia 1.5, Lumnitzera 6.5, Hibiscus 6.5, and Eugenia 7.5 mg/dm²/minute. All of these values are low compared to the bulk of data published for other plants, including halophytes, which range from some 10 to 55 mg/dm²/minute (18). The commonly used technique depends upon measuring the weight loss of a freshly detached leaf, with a concomitant disturbance of the normal hydrostatic balance. It would seem that our figures for the transpiration rates in the salt-secreting group of mangroves should be rather reliable.

► Hydrostatic Pressure in Sap of Mangroves. True to classical concepts, one might predict that the hydrostatic sap pressure in mangroves would permanently linger around −20 atm, namely, in order to balance the osmotic potential of similar magnitude in seawater, roots, and leaves. The salt separation would then be explained essentially as an ultrafiltration in the roots, powered by a 20-atm transpiration pull. It would, therefore, be of pivotal interest to be able to measure negative sap pressure, but this, we must painfully admit, is still beyond the wits of man. The cause is not totally lost, however, for there are various ways of detecting strong negative pressures, even when they cannot be accurately measured.

Three different approaches have been used, any one capable of indicating negative pressure, namely, A: the closed burette technique (11, 12), B: the delta pressure technique (14), and C: Renner's potometer technique (8).

► I. With the *closed burette technique* (fig 9) one determines the lowest pressure against which the xylem can absorb water; if absorption continues in spite of vacuum, the sap pressure is negative. A disk of bark is carefully removed by means of a cork bore and the exposed surface dried off and lightly greased. A brass button with 5 mm bore and "O" ring passes through a hole in a hose clamp and is strapped tightly onto the xylem. The bore is filled with water, and after test for tightness the xylem is scooped out shallowly with a razor-sharp, specially-

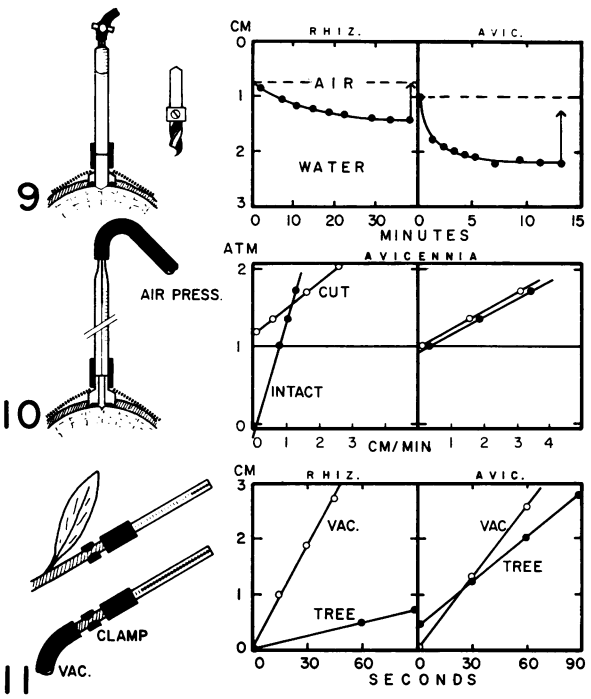

Fig. 9. Closed burette technique for estimating sap pressure. Specially ground drill is shown. Arrows in both panels indicate the shrinking of the gas volume when water was admitted to the burette. The original air volume is between the broken line and the upper frame (vol 0). The curve denotes the position of the meniscus at various times.

Fig. 10. The delta pressure technique for estimating stem pressure. Known air pressures are supplied to the microburette from a pressure tubing. The panels show the effect of added pressure upon the absorption rate in the intact stem (*filled circles*), and in the cut-off stem (*open circles*). Ambient pressure is 1 atm. Each panel shows measurements in one plant. Filtration rates are relative and are given as cm/minute on burette of approximately one millimeter bore.

Fig. 11. Renner's technique for estimating sap pressure. In both cases the vacuum drew liquid through the compressed xylem faster than did the tree.

ground drill which passes through the water. Shavings are flushed out and the burette connected. Air bubbles are dislodged by prolonged and forceful evacuation with a 100 cc syringe; if air leaks in steadily through the xylem another site must be found. The rate of water absorption is determined, whereupon the burette is closed, including a known air volume, and the rate of water absorption is again observed. If it comes to a stop, the gas volume is read before and after admitting water, and the pressure is calculated from the shrinking of the gas. One finally checks that full absorption is resumed when the burette is opened.

A series of measurements, performed on sunny days, gave the following results: 6 *Rhizophora mangle* 0.4 to 0.6 atm, 6 *Avicennia nitida* 0.4 to 0.7 atm, 4 *Laguncularia racemosa* 0.5 to 0.7 atm. In several additional cases practically no water was taken up unless pressure was added, and the sap pressure must hence have been close to ambient (fig 10).

In contrast to these results, one should realize that when this technique is applied to plants with substantial negative pressure, such as may develop in the grape or rattan vine, the picture is very different. One may thus fill the burette completely before stoppering it and the plant will nevertheless absorb the water practically as fast as if the burette were open; in healthy vines the water simply mass-cavitates (boils) and no air, or only traces, leaks from the xylem (11, 12, 13).

► II. With the *delta pressure technique* (fig 10) one determines how sensitive is the absorption rate of water through a xylem cut to changes in the burette pressure. Instrumentation is similar to I, but the cut is kept very small in order to avoid flooding and backpressure in the xylem. Absorption rates are read on a micro burette. Healthy stems frequently yield only traces of gas and one usually gets a linear relation between absorption rate and delta pressure, i.e., like in a simple filtration system. When the rate is plotted on the abscissa and the sap pressure on the ordinate, we assume that the extrapolated intercept reflects the approximate sap pressure. When, as a control, a short section bearing the burette is sawed off, a value close to ambient is obtained (fig 10).

The result will be seen in figures 10 and 12. In most cases the pressure extrapolated to a fraction of one atmosphere, but modest negative pressures were not uncommon. In figure 10 (*right*), we see a strong indication of near ambient pressure in that particular bush. The method appears theoretically sound provided no air spaces are cut open. Cavitation may occur if negative pressure obtains, but stays confined to the severed elements and floods immediately when the cutting edge is withdrawn.

► III. In Renner's *potometer technique* (fig 11) a capillary burette is connected to an attached twig and the water absorption rate is reduced by compressing the xylem with a screw clamp. The twig is detached and one notes how fast a moist vacuum can draw water through the resistance. Assuming simple filtration, the sap pressure can be calculated from the ratio of the flow rates (8).

In Rhizophora water absorption was very slow, and was reduced to one-half by the clamp, but vacuum pulled water through eight times faster than did the tree. In Avicennia the unrestricted flow was very rapid and was slowed down to 1-10 by the clamp; nevertheless, vacuum pulled the water through faster than did the tree. Two samples of each species gave similar results. Also this technique indicated that these plants pulled with a pressure differential of less than one atmosphere.

The main objection which can be raised against these techniques is their vulnerability to a gas phase, and we shall, therefore, briefly discuss this possibility. When a transpiring stem is cut off in air, the sap recedes until stopped by the pit membranes of the first cross-walls. Every active transport element which has been severed thus becomes completely filled with air. Even so, it holds for all common plants that drinking resumes when the stem is promptly put into water. The bypass around this gross embolism takes place through flooded tracheids or other perivascular micro elements which were not severed. If normal flow is restored, it goes at the cost of a considerable pressure drop across the inactivated vessel sections (11, 14).

If, similarly, we make a dry cut into the xylem of our transpiring mangroves, air is drawn into every severed active tracheary compartment. When this cut is inundated and vacuum extracted, as described, air bubbles escape; and when normal pressure is admitted, water enters the xylem, leaving approximately 5 to 10 % of the length of each active

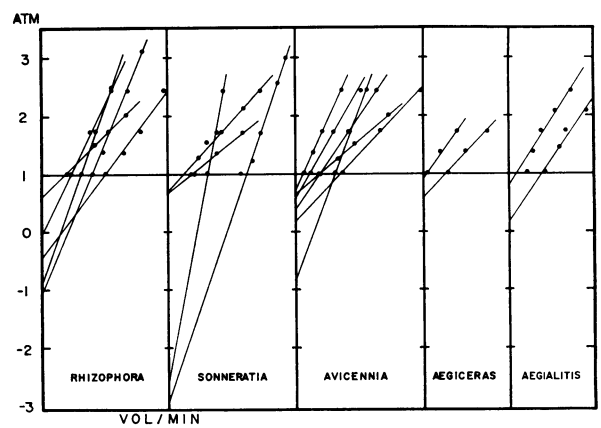

Fig. 12. The delta pressure technique applied to the stems of various mangroves. Ambient pressure is 1 atm. The water absorption rate at various added pressures extrapolates to the stem pressure at the ordinate intercept. Rate units on the abscissa have only relative significance and vary from one experiment to the next, but range from 5 to 20 mm^3/minute.

channel occupied by a bubble. Nevertheless, water is always steadily taken in after this treatment, which is to say that also in mangroves there is a ready bypass around an embolism of considerable size[3]. When the cut is properly executed under water no air enters, but the disturbance might conceivably cavitate the sap if high negative pressure obtained. The vapor locks would instantly collapse, however, and whatever bubble might be left would be trifling even at half an atmosphere's pressure; and yet, half an atmosphere stops the flow. Clearly, this is very difficult to reconcile with the idea of negative sap pressure.

A possible source of error would be if gas from a leak would blanket off the cut. Air leaks cannot be derived from active xylem channels if these are to operate at −20 atm, but only from other structures. Xylem leaks are common and vary from a few insignificant micro-bubbles to a steady gush, but it has always been possible to select sites which yield an insignificant amount of gas upon repeated evacuations. With a large and shallow cut and wide bore throughout, most bubbles will rise freely through the vertical burette, and it is easy to prevent gas collection on the cut by steady and sharp tapping of the horizontal stem.

Yet, convincing as these arguments might seem, they must be tempered by other observations that seem conflicting. Thus, when a healthy young stem of *Avicennia nitida* was cut off and the rooted stump was connected with a moist evacuated gallon jug and left overnight, no sap was yielded in spite of a final pressure reading close to vapor tension. Similarly, cut twigs attached to the bushes did not yield sap by vacuum. Possibly the sap pressure was slightly negative in these cases, or perhaps gas was yielded much easier than sap. We did not observe bleeding from an isolated root kept in seawater. Further studies are clearly called for before one may claim a full understanding of the hydrostatic situation in mangroves. But presently it does not seem possible to us that negative pressures of such colossal magnitude as −20 atm could consistently be concealed by three independent techniques.

► Sap Concentration in Relation to Aeration of Roots. In earlier investigations on Atlantic mangroves, it was shown that when high tide covers the lenticels on the stilt roots in Rhizophora or the pneumatophores in Avicennia, the oxygen tension drops in the root system and the gas pressure falls. When the tide recedes, air is aspirated through the lenticels and the oxygen tension rises. If the lenticels are clogged with grease, the oxygen tension falls from some 18 to 12 % down to near zero in a few days (16). It was, therefore, natural to postulate that oxidative processes might assist in the salt separation. In order to test this the pneumatophores of two Sonneratia bushes were cut off and the cut surfaces greased, so as to shut the root system off from air. Similarly, the stilt roots of a Rhizophora were greased. The oxygen tension was determined by drawing gas samples from a hypodermic needle implanted in the roots under the mud. In the Sonneratia plants, which grew on a sandy tide flat, the oxygen tension did not drop below 14 %, but in the Rhizophora growing in deep mud the oxygen fell from 18 % to 4 % in 2 days. In neither case did the salt concentration increase in the stem sap. Possibly, the anoxia was not severe enough to break down the mechanism of salt exclusion.

Discussion

In the present material of mangroves, one may distinguish between two categories, namely, those which excrete salt through the leaves and those which do not. Both groups are rooted in a substrate which is closely isotonic with the seawater. The non-secreting species have a xylem sap which is almost salt-free; and even in the salt-secreting species, the osmotic potential of the sap is mostly below 2 atm. Evidence so far indicates that the hydrostatic pressure of the stem sap under full transpiration, although occasionally a few atmospheres negative, is usually positive but below atmospheric. We may, hence, conclude that it would be premature to treat the steady state separation of freshwater from the sea by the roots in terms of a simple equivalence between hydrostatic pressure and osmotic forces in a semipermeable system, for this would require a permanent sap pressure of at least −20 atm, which is not indicated by present evidence. One is led, therefore, to consider the possibility of active transport. The fact that press juices from roots and leaves are more or less isotonic with seawater gives little help one way or another; but lack of osmotic gradient along the stem shows the rather obvious; namely, that at least here the sap moves by mass flow, rather than by osmosis.

Unbalance between osmotic potential and hydrostatic pressure is commonplace in animals, and certainly occurs in plants. For instance, both marine and freshwater fish have an osmotic potential in the blood of about ten atmospheres, but do not solve their osmotic problem by adjusting the blood pressures to −10 and +10 atmospheres pressure, respectively. The milk pressure in coconuts is another case where such relations do not apply (10), and the salt glands on mangrove leaves, secreting brine under oil, belong here also.

Mangrove roots are well ventilated through pneumatic tissues, and an aerobic energy source is, therefore, readily available for an active transport. One might visualize a system steadily taking in seawater by a moderate transpiration pull. Active transport would eliminate the salts fast enough to satisfy the

[3] The perivascular xylem of Avicennia and, in particular, Rhizophora consists of fibers rather than tracheids, but the vessel walls in both species are densely studded with pits, suggesting free water passage into the perivascular tissue. In Avicennia, perivascular fine channels are conspicuous; in Rhizophora not.

transpiration flow, allowing for an inevitable diffusion loss of water at the separation site. The only other system capable of operating on a moderate hydrostatic pressure difference appears to be one involving active secretion of water.

The salt-secreting species all contain a small amount of salt in the xylem sap, which is eliminated by the salt glands on the leaves. The excreta are some 10 to 20 times more concentrated than the sap and may exceed that of the seawater. The secretory process is not driven by the evaporation, for it also takes place under a layer of oil. It also proceeds for some time in detached leaves where the hydrostatic pressure of the sap is kept near ambient.

Interesting questions are: What salinity gradients do these cells operate against? Is the concentration accomplished in one spectacular step from nearly salt-free sap to double seawater? Are the glands situated at the end of a local concentration gradient within the leaves, such as possibly indicated by the high salinity of the crushjuices? Would such a gradient possibly be subtended by a (xylem-phloem) counter-current exchange system such as commonly found in animals, e.g., in the kidneys or swim-bladder where large concentration gradients are maintained? Another facet which invites comparison with animals is the fact that the salt glands regularly become covered by sodium chloride crystals on sunny days. It would appear that the glandular cells are capable of full activity, even though in direct contact with a saturated brine. There is hardly any parallel to this to be found in animal excretory systems. In our sweat glands, for instance, the secreting cells are separated from the drying secreta through a long spiraling duct. In light of the paradoxical situation in the mangroves, one might postulate that the function of these striking ducts in man may be to provide the active cells with a protective diffusion gradient.

Summary

A study has been made of various parameters of the salt balance in several species of mangroves. Some species, like Aegialitis and Avicennia, eliminate large quantities of salts through special glands on the leaves, a property which other species such as Rhizophora and Sonneratia do not possess. The salt concentration in the excreted fluid is often higher than that of seawater and has a marked diurnal cycle in concentration as well as quantity, both with a maximum in the daytime. The xylem sap in the salt-secreting species carries about 0.2 % to 0.5 % sodium chloride, a concentration which exceeds that of non-secreting species by some 10 times, and that of ordinary land plants by about 100 times. The osmotic potential of the sap of the mangroves is at most a few atmospheres. The sap pressure has been studied by three different approaches, which indicate that the pressure is usually below ambient, but that it seldom becomes negative and then only by a few atmospheres. It would, therefore, seem premature to postulate that the separation of fresh water from the seawater is a simple ultrafiltration, for this would demand a permanent sap pressure of −20 atm or less. The root system of mangroves is ventilated by air, and it seems more likely that the separation involves a case of active transport.

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Glycolic Acid Oxidase Formation in Greening Leaves [1, 2, 3]

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The amount of glycolic acid oxidase in green tissue is much greater on a protein nitrogen or weight basis than in tissue without chlorophyll (8, 10, 13, 15). The active enzyme cannot be isolated from roots and tubers (10, 13), but it can be detected in small amounts from etiolated tissues. During greening of the plant tissue in the light the activity of the oxidase increases immensely. Increased enzyme activity has been found in etiolated tissue kept in the dark upon feeding an excess of glycolate to the intact leaves. When glycolate was added to a cell-free extract from etiolated leaves, the enzyme activity increased greatly after 18 hours of incubation at 2 C (15).

An initial explanation for these phenomena was based upon substrate activation of the enzyme and the assumption that glycolate was not present in roots or etiolated tissue (15). For green plants it was known that large amounts of glycolate were produced by photosynthesis (11). However, the presence of some glycolate has since been reported in both roots and etiolated tissue (5, 6, 7). Thus a substrate activation hypothesis seems unsatisfactory unless the possibility of compartmentalization within the cell is invoked. In this paper we have reinvestigated the previous observations on the activation of glycolic acid oxidase. A substantial amount of proenzyme for glycolic acid oxidase has been found in etiolated plants, but in amounts insufficient to account for all the active enzyme in the corresponding green tissue. Since the cofactor for this enzyme is FMN (16), the level of FMN and FAD in etiolated green plants was also measured. Preliminary studies were made on conditions for holoenzyme formation.

Materials & Methods

Etiolated wheat *Triticum vulgare L,* var. Thatcher, was grown in sand with or without nutrient in a totally dark room at about 21 C for 9 to 10 days at which time the plants were about five inches tall. The leaves were ground in a cold mortar immediately

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