The Path of the Transpiration-Current.

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With Woodcuts 2–8.

IN some recent investigations on the ascent of sap, we had occasion to reconsider the question as to whether the transpiration-current is transmitted through the lumina, or, as the supporters of the Imbibition-hypothesis maintain, through the walls of the conducting wood. Although, undoubtedly, the weight of recent experimental work on this question is in favour of the view that the sap moves in the lumina, yet as some of the methods adopted in these experiments were not, as it appeared to us, entirely free from error, we did not consider it superfluous to repeat some of the older experiments, eliminating, as far as possible, sources of error, as well as to add some new ones of our own. The present paper is occupied with an account of these experiments.

Tracing the course of the Transpiration-Current by means of Precipitates.

The first of the older experiments which we will discuss are those in which cut branches are supplied with a salt-solution,

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and after this has been drawn up by transpiration, pieces of the conducting tissues are treated with a second solution which forms a precipitate with the first. Examination is then made to ascertain whether the precipitate is confined to the lumen or to the wall. The recorded results of these experiments have been that the precipitate is confined to the lumen, and it is concluded that therefore the first solution has moved in the lumen only. It appeared to us that these experiments involve a full investigation of the question as to whether in any case it is possible for the precipitate to form in appreciable quantities in the wall ; for if it cannot, they tell us nothing as to what went on in the wall. The following experiments show the validity of this objection. Thin sections (longitudinal and transverse) of *Taxus baccata*, first soaked for some hours in a strong solution of potassium ferrocyanide or of ferric chloride, were either dried on the surfaces with filter-paper or quickly washed in water, and then treated respectively with ferric chloride or potassium ferrocyanide. On subsequent microscopic examination in no case could any more than a faint blue coloration be observed in the woody walls (perhaps in part only apparent and due to a thin film of precipitate on the cut surfaces of the section), while the lumen was choked with the precipitate. On the other hand, the cellulose walls of the bast, cortex, and medullary rays were deeply coloured, as well as the torus of the closing membrane of the bordered pits. In order to obtain denser precipitates in the lumina, the sections may be transferred several times from one of the mutually reacting solutions to the other. That the walls of the tracheides were thoroughly imbibed with the solutions there can be no doubt, seeing that the sections were very thin. Where the walls were tinted at all, the faint coloration was almost completely limited to the tertiary thickening layers, just as Strasburger has already observed in a branch of *Taxus baccata*, which stood for some days in a solution of potassium ferrocyanide, and was afterwards treated in pieces with iron sulphate[1].

[1] Leitungsbahnen, p. 628.

In order to compare the behaviour of another colloid with that of the woody cell-wall, some gelatine was dissolved in a strong solution of potassium ferrocyanide, so as to form when cool a very stiff jelly. A drop of this when melted was placed on a microscopic slip and covered with a circular cover-glass. When the gelatine had set the slip was transferred into a solution of ferric chloride, which slowly diffused through the gelatine. On microscopic examination no precipitate, if we except a faint coloration, could be detected in the gelatine. The precipitate was limited to the surface of the gelatine at the edge of the cover-glass and to the dendritic cracks formed within the gelatine by contraction under the cover-glass. In the substance of the gelatine itself $\frac{1}{16}$ Leitz failed to show precipitated particles.

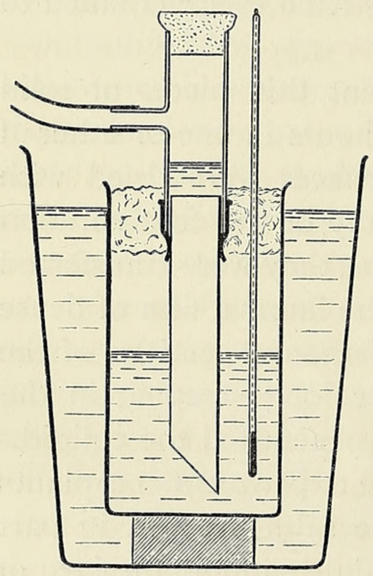
As a modification of this experiment thin pieces of solid gelatine were steeped for twenty-four hours in one or other of the salt-solutions, and after their surfaces were dried with filter-paper, so as to avoid carrying any of the first solution which was not contained in the gelatine, they were transferred into the reacting solution. While in the latter a film of dense blue precipitate formed over the surface, and sections of the gelatine showed that ultimately after long steeping in the second solution a pale blue coloration penetrated the colloid.

In view of these results we think the experiments on plants cannot be relied on to prove that the salts did not in part move in the walls, although they certainly support the idea of a ready and free passage in the lumen.

Choking the Lumen by the Introduction of Foreign Substances.

Experiments in which flagging of the branch occurs upon artificial stoppage of the lumen have been relied on as strong evidence in favour of the lumen as the water-way in the tracheal tissue. It was objected to Elfving's experiments that cocoa-butter was of too greasy a nature and might enter the wall, and gelatine was substituted by Errera and Strasburger. We confess that we would have had less apprehension of a greasy substance entering the water-saturated wall than a substance miscible in the water such as gelatine, and which, even if entering the wall in minute quantities, might be very injurious. Its extreme dilution with the material of the wall might render its detection by the microscope impossible.

We made experiments to test this last possibility. We only quote some of these. We may observe, however, that all our results agreed in showing the passage of dilute warm gelatine from cell to cell—possibly taking place in some cases only through the closing membranes of the pits, and consequently possibly altering the capability of the wall for transmitting water.



Woodcut 2.

A length of 10 cm. straight and free from side branches was cut from a branch of *Taxus baccata*, the mean diameter being 2·5 cm. This was deprived of its bark and affixed by an india-rubber ring at one end to a glass tube communicating with an air-pump; a little water in the tube covered the upper end of the wood. Woodcut 2 shows the arrangements.) On exhausting the tube, bubbles rose from the surface of the wood. These could be stopped by simply immersing the lower end in mercury. Hence it is to be concluded that continuous air-passages exist in this piece of wood which must be stopped before any tests can be made as to its permeability by gelatine. Accordingly the lower end was dipped in melted paraffin at about 70° C., the melting-point of the paraffin being 56°, and the whole length of the stick jacketed by water which was maintained at 70° for 45 minutes, a vacuum being preserved in the tube attached to its upper end during this time. Finally the stick was cooled slowly from above downwards by lowering the water-bath to allow of the contraction of the paraffin being made good by supply from below. When all was cold, the end was pared to expose lumina free from paraffin. The stick now drew up water freely, 3 or 4 cc. in 15 minutes, but let up no air, nor would it suffer mercury to pass up. The water pumped through was next tested by a solution of tannin, but remained perfectly clear. We conclude therefore that no direct air-passages remain open, and that the wood of the Yew itself will give no obscuring reaction with tannin.

Some gelatine which had been cut up into fine threads and soaked in repeatedly changed water for two days was now melted and made very dilute, so that it set weakly at 13°. At a temperature of 30° to 40° this was supplied to the lower end of the Yew, the latter as before being kept warm throughout its entire length by a water-jacket which was never raised above 40°. At the expiration of four hours the liquid within the vacuous tube had risen by about 5 cc. The experiment was then stopped, and the contents of the tube tested with tannin. There was an opalescent precipitate. Comparison with the solution below showed that much of the gelatine had been held back by the wood.

Starting the experiment a second time with the same piece of Yew it transmitted 3·5 cc. in four hours; the liquid drawn up affording this time a much denser precipitate. A final test showed the wood to be still impervious to air when a vacuum was maintained in the tube.

A similar experiment with the wood of *Pinus austriaca* gave a like result. It was observable that if the dilute gelatine was not raised some few degrees above its melting-point—i. e. till the solution almost ceases to be opalescent—its passage was much less marked; indeed in some experiments only traces were transmitted through the wood. This appears to be due to the fact that in solutions presenting an opalescent or milky appearance the gelatine is probably still in the solid or gelatinous state; the heterogeneous distribution and difference of refractive index giving rise to the milky colour. In all cases a considerable quantity of the gelatine is held back. One quantitative experiment on *Taxus* gave the percentage of gelatine in the transmitted liquid as only half that in the original solution.

In one experiment we stained the gelatine with Kleinenberg's haematoxylin. The gelatine was made of such strength as to set at about 20°, and was supplied at 40° to the wood of *Taxus baccata.* It passed out colourless into the glass tube, about 1 cc. in two hours, the length of the wood traversed being 2·5 cm., and its cross-section 2·2 sqr. cm. This wood had not been treated with paraffin, as it revealed no direct air-passages upon trial. As the haematoxylin does not stain wood, this experiment points to a mechanical separation from the gelatine owing to the passage of the latter through membranes or walls. It is possible, however, that some of the stain was taken up by the cellulose walls of the medullary rays and the tori of the pit-membranes.

Microscopical examination of branches choked with gelatine mixed with Indian ink, after the manner of Errera and Strasburger, showed that the closing membranes of the pit had exerted a straining action, accumulating Indian ink upon the one side, so that the pits were very sharply picked out as black objects. This straining action is suggestive of the passage of the medium carrying the precipitate ; and although, so far as this observation is concerned, there might have been straining of the gelatine from the water in which it was dissolved, still taken in conjunction with the other observations, we think it supports the view led to by those observations, i. e. that dilute melted gelatine can pass through the substance of the closing membranes, and if so is very probably capable of penetrating into the cell-wall, or otherwise we must suppose perforations to exist in the pit-membrane or its torus.

We decided to try the effect of using paraffin wax of low melting-point as the material for choking the lumina, comparing the effects with those of gelatine. Four similar branches of Lime, *Tilia europaea*, were cut (May 9) as far as possible under water, and put standing for twenty minutes in water at 50° C., immersed to a depth of about 20 cms. We call these A, B, C, D.

A is preserved in water at 50°.

B is transferred to melted paraffin at 50° (melting-point 48°).

C " " gelatine coloured with Indian ink at 50°.

D " " " " " haematoxylin at 50°.

Each being immersed to a depth of 20 cm. and placed in bright light, the air temperature being 16°. At the expiration of forty minutes all were transferred to water at 13°, when the end of each was thinly pared, and at 5.30 p.m. all were left finally standing in water at 13°. At 6.30 all were still fresh. At 11 a.m. on the 10th, i.e. after 15½ hours,

A was still quite fresh.

B " very much flagged.

C " less flagged than B.

D " " " " B, but more flagged than C.

All were now transferred to a strong solution of saffranine, and put in full sunshine for 1¼ hours, when they were washed and sections made for microscopical examination. So far as C and D were concerned, it is only necessary to observe that they revealed that only some of the lumina were actually stopped with gelatine. The walls of many of the gelatine-filled vessels were found stained with saffranine, which attained to 26 cm. in C, and to 5 cm. in D. The gelatine in the lumina had become stained with the saffranine.

Transverse sections of B close to the base showed all lumina choked with paraffin, while the walls between were deeply stained with the saffranine.

In polarized light with crossed nicols the appearance was very striking, the crystalline paraffin showing out strongly. Transverse sections, 2 cms. from the end, showed the large vessels still filled with paraffin. In some places neighbouring vessels apparently quite filled with paraffin had the intervening walls deeply stained; at this level, however, where the vessels were filled with paraffin the wall-staining was not quite so dark as elsewhere, but still strongly coloured. The paraffin finally attained a height of 12 cm. in one or two vessels. In no case was there any visible appearance of shrinkage of the paraffin from the wall, although in some sections, as might be expected, the action of the razor was to compress it from the cell-wall upon the one side over the section.

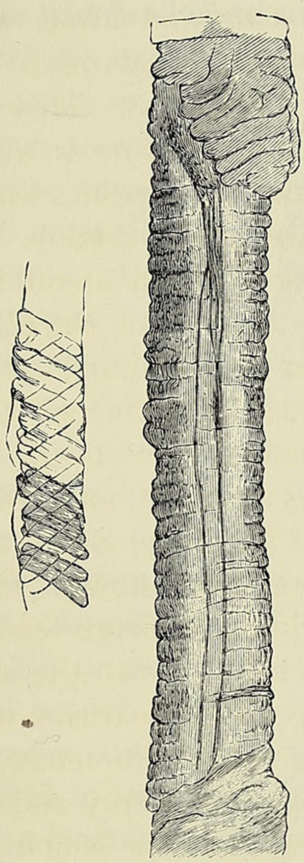
Similar experiments were made on Elm and Lime, with the added precaution of removing the paraffin or gelatine at the ends without cutting or removing any of the wood. This was effected by careful use of the razor, the object being to avoid as far as possible laying open the lumina of conduits whose terminal walls might lie upon the surface of the section. In the case of Elm and Lime again, sections taken about half a millimetre from the end showed areas over which the filling with paraffin was complete, and yet also deep staining of the intervening walls. Longitudinal sections near the end confirm this appearance; the lumina seemed quite filled. In these cases the removal of the branches from the hot paraffin was effected gradually, to secure as far as possible that solidification and shrinkage should proceed slowly from above downwards, and thus guard against shrinkage leading to the withdrawal of the paraffin out of contact with the wall. Again, the branches of Lime treated for comparison with gelatine revealed areas in the cross sections completely injected with gelatine and yet having the walls deeply stained. Thus we see that both in those experiments in which the lumina were choked with paraffin and with gelatine there was at least a feeble upward motion of the solution of saffranine in the walls. Lime-branches treated with paraffin, in some places close to the cut surface, showed the penetration of this into the protoplasm-filled cells, permeating their contents. High up, only the larger vessels were filled with paraffin.

The result of these experiments may be summed up as follows:—

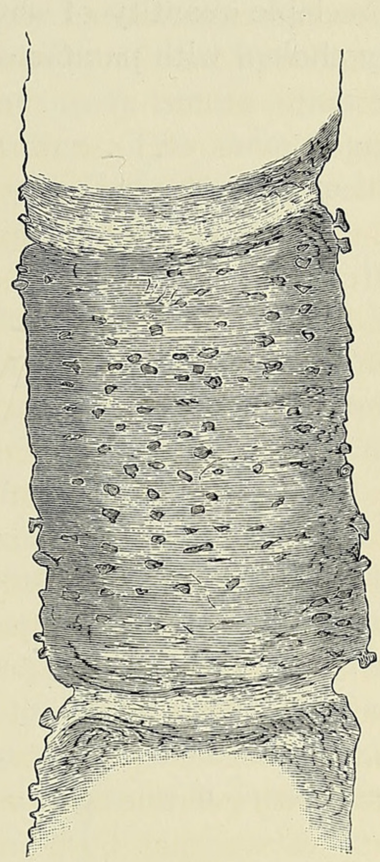
The stoppage of the lumina and the freedom of the cell-wall is preserved both by the use of gelatine and paraffin.

The flagging of the leaves appears to be the more rapid the more completely the closing of the lumina has been effected.

When the lumen is closed there is still an upward passage of liquid maintained in the wall, but which is probably much too feeble to meet the wants of the leaves.



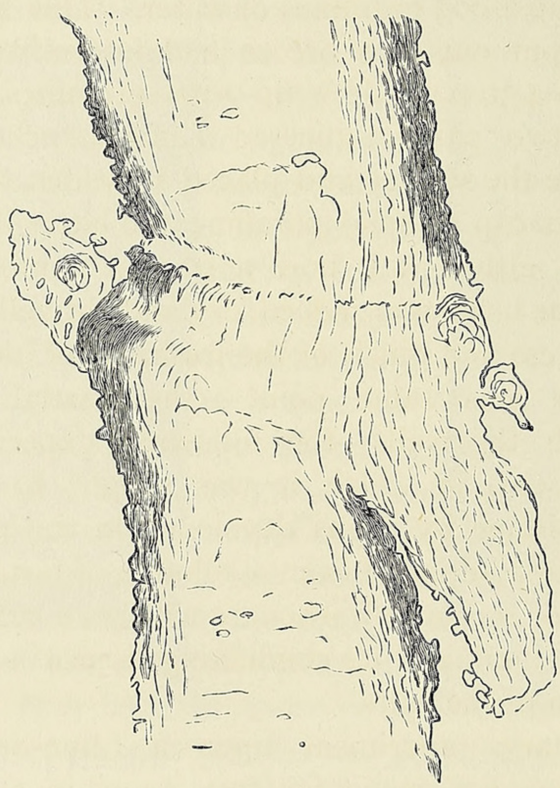
Woodcut 3.—Cast of Lime.



Woodcut 4.—Cast of Lime.

Owing probably to its extreme mobility the penetration of the paraffin is very complete in these experiments, We found it easy by its means to demonstrate the continuity of the tracheal elements forming the vessels in Lime, Sycamore and Elm. We experimented up to lengths of 35 cm. by removing with sulphuric acid the wood of branches injected as above. It is necessary to anchor the branch by a leaden weight in a deep vessel of the acid. A single night suffices in many cases to remove the wood and leave the paraffin casts of the vessels streaming upwards from below like a sheaf of fine white threads. The examination of these threads under the microscope reveals many features of interest. Woodcuts 3–5 represent portions of some of these casts.

Some further experiments were made bearing upon the ascent of water in the wall. All confirm the fact that an appreciable quantity of water ascends in branches most carefully choked with paraffin.



Woodcut 5.—Cast of Elm.

Thus while flagging will inevitably overtake a paraffined branch left furnished with the same number of leaves as it bore upon the tree, yet if the greater number of these are removed the remaining leaves will generally hold out fairly well. This experiment was tried with a control paraffined branch upon which all the leaves were left standing.

If after injection we remove part of the branch at a fork and, keeping the one part which retains the paraffined extremity in water, insert the extremity of the other through a cork into a dry vessel, the latter will flag much the more rapidly. Still more direct is the following: a paraffin-injected branch of *Tilia europaea* with nine leaves was put standing, from 4.15 p.m. May 11th till noon on the 12th, in a vessel of water which had been carefully weighed and so closely corked round the stem as to preclude possibility of loss by evaporation at its surface. In this period of nearly twenty hours the branch drew up 1·005 grammes of water. This same flagged branch, now put out into breeze and intermittent sunshine from noon till 3.30 p.m., drew up 0·161 grammes.

Again of two paraffin-injected Lime-branches, one just scraped to free the surface and placed in water, the other left closed with its cap of solid paraffin; the latter flagged much more quickly, although it bore a smaller number of leaves. In two days the latter was, indeed, dry and shrivelled, while the former had preserved much of the freshness of its leaves.

Bearing on this same point — the partial passage of water through the walls—the following experiments were next carried out, in which it was sought to replace the paraffin or gelatine by a gas developed in the plant. Thus a cut branch first supplied from a solution of tartaric acid and subsequently of sodium bicarbonate will have carbon dioxide evolved in the lumina of its conducting tissues in consequence of the reaction of the salts.

A preliminary experiment upon a Lime-branch (*Tilia europaea*) which had stood for two hours in a solution of tartaric acid and then one hour in sodium bicarbonate, before finally being transferred to pure water, showed rapid flagging of its leaves and soft shoots as the result. But as this was possibly a direct consequence of the action of the salts and not of the evolved gas, a more careful experiment was carried out upon five branches of Elm cut from the same tree with similar precautions and as far as possible of like dimensions.

A and B were placed in sodium bicarbonate solution.

C and D were placed in tartaric acid solution.

E was placed in mixed solution of tartaric acid and sodium bicarbonate which had ceased effervescing.

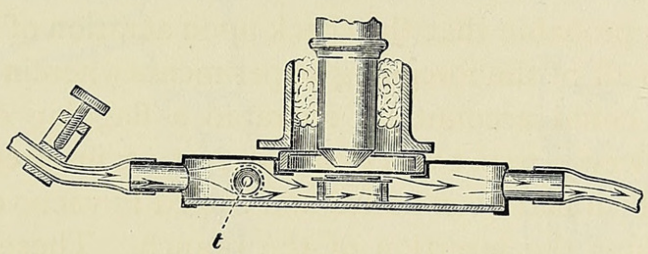
After 1½ hours A and C were interchanged in the solutions; thus, in these two only was CO_2 developed. B served as a control regarding the effects of sodium bicarbonate alone, D ditto for tartaric acid, E ditto for the effect of the mixed solution without development of gas. In five hours A and C were very much, and about equally flagged, while the rest remained fresh. Next morning, however, all were drooped, showing that prolonged treatment with any or both of these salts is injurious in any case. It was evident also that the stoppage of the lumina by the gas had greatly accelerated the flagging.

It is not probable that the check upon aeration of the tissues involved in all of the foregoing experiments wherein the lumen is choked could account for so rapid a flagging of the soft parts. However, to set this doubt at rest, we inserted branches in water which had been boiled and cooled in vacuo and coated with oil after the insertion of the branch. These, however, remained perfectly fresh, indeed they seemed in no way affected.

Choking the Lumen by means of Ice or Water Vapour.

In order to investigate this subject more fully an additional series of experiments were devised and carried out upon the passage of water through the wood of *Taxus* at very low and at very high temperatures. For it was very certain that in the one case the formation of ice, and in the other the formation of steam, would occur in the lumen before occurring in the wall, rendering the former non-conducting without the introduction of any foreign substance.

It was necessary to determine first of all the freezing-point of water in the lumen by direct microscopic observation. To effect this we used a form of cold stage which possesses certain advantages which induce us to add the accompanying figure (Woodcut 6) showing its construction. In this stage the object under examination is completely surrounded by the cooling liquid, which also flows round the bulb of the thermometer. The temperature is therefore very accurately known. The bottom of the cell is of glass; a ring screwing out upon the top serves to permit the lifting of a cover-glass acting as a water-tight window, this being luted on the edge with a little white lead. The object is luted between two cover-glasses and carried upon an open support within. It is necessary to protect the upper window from moisture precipitated from the atmosphere; this is done by the loose metal ring surrounding the object-glass and packed round with a little cotton wool. The thermometer enters by a



Woodcut 6.

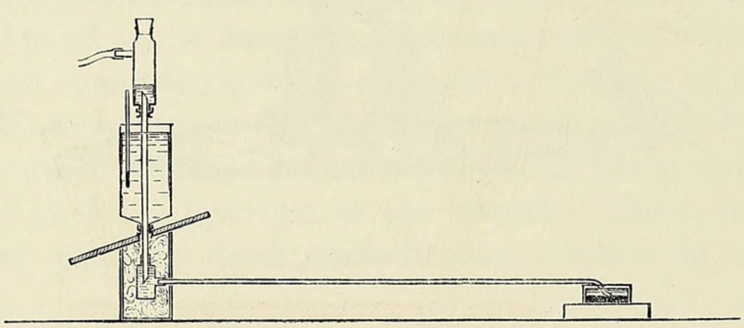
tubulure in front; its bulb appears in cross-section at *t* in the Woodcut. The regulation of the temperature is very simply effected by retarding or accelerating the current of cold liquid (brine) by means of the pinch-cock.

The section of *Taxus* is cut with as little addition of moisture as possible, so that when luted up between cover-glasses it is surrounded by air while containing water within its substance. The close proximity of the section to the upper window, some 1·5 millimeters, allows of considerable magnification.

The cold cell, after the introduction into it of the section sealed up between the cover-glasses, is placed on the stage of a microscope, and then, by the arrangement already described, the temperature is caused to fall gradually, while the water within the section is carefully observed.

The phenomena attending freezing were perfectly definite, the clear liquid in the lumina assuming the aspect of solid paraffin. In two experiments in which the reduction of temperature was effected very gradually the freezing-point was found to lie between $-10°$ and $-11°$ C. Freezing spread with great rapidity all over the field in both wide and narrow lumina. Air-bubbles present exhibited immediate reduction of volume and often distortions of shape, and it was important to observe that an exudation of sap occurred upon bare cell-walls which appearing in drops instantly turned to rough-shaped ice-crystals.

Thawing occurred at a higher temperature than freezing, no signs of melting being exhibited till $-4°$ or $-5°$ were reached. This specimen of wood was removed from a branch



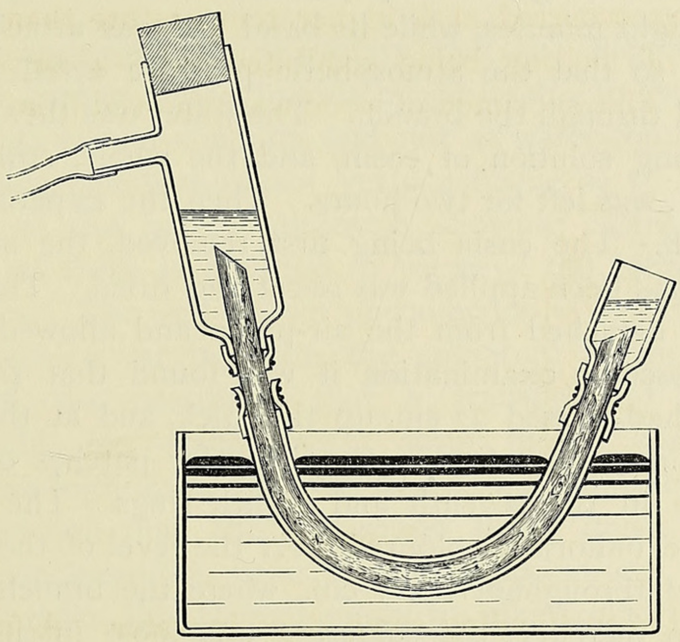
Woodcut 7.

which had been standing some days in water. A freshly cut branch of *Taxus* afforded 14·5° as the freezing-point; showing that the state of the sap appears to affect the freezing-point considerably.

Owing to the pressure-effect of the ice upon the wall visibly shown by the forcible expression of drops, there appeared some doubt whether this method would afford any result of value. However, the experiments were persisted in, and a length of 22 cm. by 6 mm. in diameter of a *Taxus*-twig, carefully washed, was attached to the apparatus shown in Woodcut 7, in which the passage of liquid through the vertically placed stick (due to a diminished air-pressure in the vessel above) is shown by the movement of mercury in the horizontal capillary tube.

The rate of transmission of water was observed while the temperature of the jacket was varied. The general results were as follows:—

In *cooling*, the current had almost ceased at $-7°$ and completely at $-11°$; in *warming*, it recommences feebly at $-5°$. It was impossible to fix upon any temperature as the actual freezing temperature in the lumina from the observations, but as all current had ceased at $-11°$, at which temperature the water in the walls was almost certainly not frozen, we must



Woodcut 8.

conclude that these observations reveal no current in the walls even of the feeblest intensity, for the method of observation is very delicate. However, the method is beset by the doubt involved in the evident ice-pressure upon the walls.

Experiments in which the wood of *Taxus* was exposed to high temperatures—above 100° C.—appear to show that coloured water can be drawn through the wood when this is at a temperature so high as 125°, and very certainly filled with water-vapour everywhere in its lumina.

Woodcut 8 shows and explains the arrangement of the experiment. The vessel into which the branch dips contains mercury heated from beneath. A glass tube surrounds the branch, the space between branch and glass being filled with mercury. To resist the tension of the vapour evolved from the surface of the wood at this temperature it was necessary to bind the stick into the tube with air-tight rubber rings overlaid with wire. The following experiment was made:—

A small branch of *Taxus baccata*, 24 cm. long, having a woody cylinder of 5–6 mm. in diameter and being composed of nine annual rings, was kept jacketed with mercury at 125°–130° for eight minutes, while its basal end was attached to an air-pump so that the atmospheric pressure acted from the distal end through the branch. The water was then replaced by a strong solution of eosin, and the whole, still kept at 125°–130°, was left for two hours. Then the experiment was broken off. The eosin being first removed, the surface to which it had been applied was re-cut and dried. The branch was then detached from the air-pump and allowed to cool. On microscopic examination it was found that the eosin-solution had passed 22 cm. up the stick, and at this height was seen in cross-section as two irregular patches occupying quadrants in the seventh and eighth rings. The walls of these were uniformly coloured. At the level of the mercury jacket and throughout the 7 cm., where the branch was immersed in mercury, the colouring was most intense in the limiting membranes. At the end where the eosin was applied the walls were scarcely coloured except those adjoining the medullary rays and immediately round the bordered pits.

The simplest interpretation of these results is that the coloured water moved in the wall while the lumen was occupied with vapour; the intenser colouration of the limiting membrane is strongly in support of this view, for it is very probable that for some distance from its surface the wall was so far choked with vapour as to impede the motion of a liquid.

These experiments then, so far as they go, are in perfect agreement with the previous set in which the lumina are choked by the introduction of foreign substances (cocoa-butter, gelatine, air, in the experiments of other authors, or by paraffin and CO_2 in our own), and show that the freedom of lumina is necessary for the rapid transmission of water, yet that a slow current may pass through the wood even when the lumina are completely blocked.

The Water transmitted in the Lumen is not in the Form of Vapour.

There appeared the possibility that the flagging of the branches having closed lumina might be due to the stoppage of them as vapour-conduits, and not as water-conduits ; that is, the experiments were not yet conclusive as to the actual function of the lumina, although showing clearly that the freedom of them is essential to preserve the turgescence of the leaves. The well-known phenomenon of the equilibrium vapour-tension varying with the curvature of the meniscus suggested the possibility that a transport of vapour of considerable importance might occur in the conduits, the meniscuses high up in the trees possessing a lower equilibrium vapour-tension than the meniscuses lower down. By successive condensations beneath and evaporations above the pit-membranes, this current might be maintained throughout the conduits unoccupied by liquid water.

This idea led to experiments in which cut branches were fed entirely upon water-vapour in the following manner :— The branch had its cut extremity fixed in a short glass vessel containing water at the bottom ; the cut surface of the wood (which is cut at a sharp angle in order to expose the larger surface) being some 5 or 7 cm. raised above the surface of the water. A side tubulure to the vessel enables a vacuum to be maintained within by means of a Sprengel pump. The vacuum was so complete that ebullition occurred upon placing the hand round the lower part of the vessel. Such experiments were made upon Elm and Lime, using control branches some of which were simply left with their cut surfaces exposed to the air, others with their ends sealed into tubes containing air but no liquid water. In no case was any result obtained going to show that the vapour-fed branch possessed any advantage over the others.