PHYSICAL CONDITIONS OF THE SURFACE OF THE MESOPHYLL CELL WALLS OF THE LEAF

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MOST of the extensive work carried out during the last half-century on transpiration has been mainly concerned with stomatal behaviour and the amount of water vapour passing out from the stomata during their period of opening. Very little attention seems to have been given to the nature of the outer surface of the mesophyll cell walls through which the water vapour passes, and through which at the same time active gaseous exchange takes place.

It has been stated that the surface of these walls is either moist or covered with a thin film of water from which evaporation into the intercellular cavities of the mesophyll takes place. It seemed of interest to investigate the properties of this surface more critically in the light of these suggestions.

The experimental method first employed consisted in cutting leaf strips parallel to the lateral veins. If such strips are suspended vertically with one edge in contact with water, no entry of water takes place into the capillary system, and the same result may be observed if the whole strip is immersed in water. It makes no difference whether the experiment be carried out in light or darkness, and all attempts to induce the entry of water into the capillary system proved unsuccessful.

Experiments were then performed to determine the possibility of the entry of other liquids under similar conditions. Leaf strips suspended vertically over a number of organic liquids, and with the cut end just in contact showed a ready entry of such liquids into the air space system. For this purpose benzene, chloroform, ether, petroleum ether, acetone, essential oils, olive oil and pure medicinal paraffin have been used.

In all these cases immediate entry occurs, the liquid rushing with great velocity into the capillary system of the mesophyll. In the case of benzene, chloroform, ether and petroleum ether a vertical rise of 2 cm. may be obtained in 60 sec., and the rise goes on at a progressive rate until the inflow reaches the upper edge of the strip.

The entry of essential oils, olive oil and paraffin is somewhat slower; but even with paraffin a vertical rise of 5 mm. can be observed to occur in approximately 5 min., and a more rapid rise with the lighter oils.

Direct microscopic observations of the mesophyll surface in relation to the flow of liquids seemed desirable and were carried out in the following manner. The leaf of *Ficus elastica*, by suitable manipulation, can easily be torn transversely, so that considerable areas of the mesophyll are exposed. The lower epidermis with the lower hypodermis readily splits away from the upper portion of the leaf. If the operation be carried out rapidly, no injury to the cells takes place except the individual cell ends of the spongy parenchyma next to the hypodermis. A strip as large as desired, up to 2 cm. in length and breadth, can then be cut off and placed on a slide under the microscope. In this way the whole depth of the mesophyll can be examined under a magnification of 100 or more, and an excellent view of the space system and the small lateral veins be maintained.

A drop of water placed on this surface remains suspended like a lens on the summits of the mesophyll cells and shows no disposition to spread over their surface into the capillary space system. The drop can be enlarged so that it covers the whole strip, but the intercellular spaces still remain non-infiltrated. When a drop of the organic liquids mentioned earlier is used in the same way, instant entry into the intercellular spaces occurs. All these liquids, except medicinal paraffin, have the disadvantage of being toxic and rapidly killing the cells with which they come into contact. While the phenomena to be described do not vary essentially whatever liquid is used, the microscopic observations here described relate solely to the use of pure medicinal paraffin. The use of this hydrocarbon further had the advantage of its behaviour being easily followed, while the entry of the other liquids is so rapid that it cannot be followed by the eye.

When a drop of paraffin is placed at one end of the leaf-strip with the mesophyll surface exposed, the oil immediately penetrates the capillary system. Two advancing fronts can be observed. From the edge of the drop a stream comes out which cascades down into the air space system, and this is followed by the main wave. The outflow cascading down into the air spaces is followed by the main oil wave at a distance of approximately 1 mm., and the oil thus travels the length of the exposed mesophyll in this fashion. When a drop of water is placed about the middle of the strip and oil added at one end of the strip, the oil travels along in the manner described until it comes to the water, and the initial oil wave trickles into the air space system below the water lens and continues its path on the further side.

From these observations it seems clear that while water will not flow over the surface of the mesophyll cell walls or to fill the air spaces, a number of organic liquids do so, and the phenomena can be followed by the use of a non-toxic hydrocarbon such as pure paraffin.

Experiments with Emulsions

If a small quantity of water be shaken up with a much larger amount of paraffin so that the water droplets are of the order of about 0·001 mm. in diameter, and the paraffin with water droplets be placed at one end of a strip of exposed mesophyll, the oil enters the air space system carrying some water droplets with it. It sometimes happens that a water droplet comes into contact with one of the fine veins. When this happens the water droplet appears to adhere to the surface of the vein, often sends out an arm or narrow outflow on one or two sides, loses its spherical shape, becomes flattened, diminishes in size and finally vanishes, being absorbed into the vein. When such a water droplet comes into contact with a mesophyll cell no change takes place, and, in the absence of a local current of oil sweeping it away, it remains unchanged in position.

When an emulsion of a small quantity of benzene in water is used the behaviour of the mesophyll can be observed microscopically. If leaf-strips be placed in such an emulsion and the containing tube be kept slowly agitated, the ends of the leaf-strips become infiltrated with benzene and the turbidity of the benzene-water almost disappears owing to the absorption of the larger droplets of benzene into the mesophyll air space system. If a similar emulsion be made with paraffin and water and the same conditions be observed, the ends of the leaf-strip become

oil-infiltrated. The finest droplets of oil are not absorbed, but only a slight turbidity of the emulsion remains, due to the presence of microscopic droplets.

When a leaf-strip with the mesophyll exposed is prepared and quickly placed on a slide under the microscope, and covered with a cover-glass, moisture immediately collects on the under surface of the cover-glass. It forms at first a system of microscopic polygonal strands of water, giving a minute shagreen aspect to the surface of the cover-glass when viewed under a magnification of 100. After a few minutes the system tends to coalesce. If now water is run in under the cover-glass, it immediately penetrates between the wet cover-glass surface and the mesophyll surface, but does not flow down into the air spaces of the mesophyll. If a drop of oil is placed in contact with the edge of the leaf it immediately passes into the interspace from end to end of the leaf-strip and is bounded above by the water film still adhering to the cover-glass which, owing to slight inequalities of the surface of the mesophyll, is distant 0·01–0·001 mm.

From these observations it appears clear that while the mesophyll is passing off water vapour rapidly into the air, and this can be condensed on the cover-glass surface in its immediate proximity, the cellulose walls from which the vapour proceeds cannot be wetted, nor does any condensation of water occur on this surface. Under these conditions when oil is presented to the cellulose walls, it immediately flows over them and fills the capillary space.

Infiltration of the Air Space System under Pressure with Dyes

Infiltration under a pressure gradient showed that different dyes have quite dissimilar effects. For example, a solution of Orange G filled the interspace system and coloured the whole infiltrated portion. The same treatment with Methylene Blue resulted in the whole of the dye being taken out of the solution at the point of entry while the clear water proceeds and fills the interspaces. Several preliminary trials of dyes taken at random revealed totally dissimilar effects in different cases, and it was resolved to make an extended series of experiments with a number of biological dyes.

The terms acid and basic dyes, while used frequently in dyeing processes, are sometimes misleading, and from a chemical point of view may be valueless. Every dye contains at least one group of atoms known as a chromophore which is regarded as being responsible for the colouring properties of the compounds in which it occurs. Some chromophores have a basic character and others are acid. On this basis dyes can be classified into acid and basic regardless of the solutions in which they occur. With these facts in mind, all water-soluble dyes available at the time were tested in regard to their behaviour to the surface of the walls of the mesophyll cells by infiltration under a pressure-gradient into the air space system. As a common standard a solution of 1/1,000 gm. mol. was used. The difference in behaviour between the two types of dye can be illustrated by the detailed description of the results given by a basic dye such as Janus Green and an acid dye such as Orange G. When a leaf is infiltrated with Janus Green the whole of the dye-stuff in solution is retained or absorbed on the surface of the mesophyll cells at the point of entry, while the water uncoloured by any dyestuff passes on and fills the interspaces of the mesophyll. The usual method adopted was to cut the leaf in half transversely, connect the cut petiole with a vacuum pump and place the leaf in the dye solution. In this case the whole of the dye-stuff in the solution passing in is adsorbed on the surface of the mesophyll cells within less than a millimetre from the edge of the leaf, while in less than 45 sec. the whole of the rest of the leaf becomes filled with water free from dye. Some objection may be taken to the fact that the solution passes into the space system over a front of cut and injured cells. To avoid this the whole uninjured leaf was submerged in the dye solution, the chamber exhausted. On restoration of atmospheric pressure infiltration takes place from the cut end of the petiole through the air spaces in the cortex. No infiltration takes place through the xylem strand. With this treatment exactly the same phenomena can be observed, for the whole of the dyestuff is adsorbed on the mesophyll walls in a small patch near the base of the leaf while water entirely free from dye fills the interspaces of the lamina. It thus makes no difference whether the dye solution enters the cut edges of a leaf or enters through the cortical interspaces from the petiole; immediate adsorption of the dye takes place in both cases. Microscopic examination shows that the adsorption of the dye is not due to staining of the cell contents but to an actual deposition on the outer surface of the walls. It can further be shown that the dye-stuff enters into a strong combination with the cell wall surface. If the coloured areas of the mesophyll are exposed and placed in water no portion of the adsorbed dye goes into solution; the dye remains permanently held on the surface. Immersion in acetone renders the adsorbed dye soluble and after a short time it may all go into solution and the original green colour of the mesophyll be restored. It is then clear that a dye with a basic chromophore such as Janus Green is strongly adsorbed and held on the outer surface of the cell walls.

If a dye with an acid chromophore such as Orange G be taken, an entirely different set of phenomena occurs. Infiltration results in the dye solution filling the interspaces, and the whole of the infiltrated area takes on the colour of the dye, the outer surface of the walls showing no adsorption of the dye. If now the leaf be allowed to clear itself of the water of the infiltrated solution, which it does in less than one hour in sunlight, the dyestuff is left in irregular patches on the surface of the walls. If the patches are exposed and placed in water the deposited dye immediately goes into solution, showing that it is not held or adsorbed on the surface.

The following dyes with a basic chromogen have been tested: Janus Green, Methylene Blue, Neutral Red, Safranin, Rosanalin, Methyl Violet, Crystal Violet, Methyl Green, Iodine Green, Rhodamine B. These all show the same strong adsorption of the dye by the outer surface of the mesophyll cell walls as has been described for Janus Green. Stains with an acid chromogen such as Orange G, Acid Fuchsin, Methyl Blue, Aniline Blue, Eosin, Erythrosin, all show the same features described in detail for Orange G.

Interesting features are found when the infiltration contains in solution both an acid and a basic dye. In this case the basic dye behaves as described in detail for Janus Green, while the acid dye behaves in a similar fashion to Orange G; so that the leaf is wholly coloured by the acid dye, which enters the interspaces of the mesophyll, while the basic dye is taken out of solution and adsorbed on the surface at the point of entry.

The acidity or the alkalinity of the dye solution makes no difference to the results; the adsorption or the non-adsorption appears to depend entirely on the acid or on the basic character of the chromogen group of the dye.

Action of Fatty Acids on the Wettability of the Cell Wall Surface

Numerous experiments have been carried out with the view of rendering the wall surface wettable by water.

Water containing cane sugar in extremely low concentration has been infiltrated into the air spaces and the leaf allowed to clear itself in sunlight by transpiration through the stomata. This gave no result as water did not enter the air spaces when one edge was placed in contact with water. The use of glycerine also gave no result.

The use of sodium taurocholate suggested itself as an agent for decomposing and penetrating mono-layers of protein. A leaf strip placed vertically in contact with water containing a minute proportion of sodium taurocholate (between 1/100,000 and 1/200,000) immediately showed a rise into the mesophyll spaces and the infiltration proceeded up the strip in the same manner as pure paraffin, but more slowly. After entering for some distance (10–20 mm.) the infiltration stops when a broad band of yellowish brown appears at the margin of infiltration. No further entry of water occurs beyond the band.

At first, experiments with sodium taurocholate showed a ready entry of the water, in other trials it was slight or did not occur at all. Further investigation showed that the *p*H of the solution was important. Solutions decidedly on the alkaline side were inoperative, while a *p*H of 6 and below renders the action certain.

Microscopic observations with sodium taurocholate were carried out on the mesophyll exposed by tearing in the manner described earlier.

If a glass needle be wetted with water containing a slight concentration and a droplet placed on the surface of the mesophyll, after a short interval the water globule begins to spread over the surface and eventually sinks into the lower interspaces in the same way as pure paraffin.

The above observations were carried out on a great variety of leaves. For experiments in which the lower epidermis had to be detached such leaves as *Hyocyamus muticus*, *Ficus elastica*, *Morus*, *Canna*, *Ricinus*, *Malva*, *Pancratium*, *Caryota* proved very suitable. *Hyocyamus* possesses a leaf in which nearly the whole lower epidermis can be peeled off in one operation, leaving the mesophyll cells quite uninjured below. The age of the leaf also influences the ease with which these manipulations can be carried out.

The preceding observations show: (1) that the outer surface of the mesophyll cells are highly hydrophobic; (2) that they are quickly covered by a liquid hydrocarbon; (3) on infiltration with water under a pressure gradient the liquid in the air spaces is quickly cleared when the leaf is placed in strong light, and there is no adhesion to the surface by liquid water; (4) the surface of the walls shows strong adsorption to certain dyes dissolved in water; dyes so adsorbed possess a basic chromophore; dyes having acid chromophores show no adsorption; (5) the hydrophobic nature of the surface of the cell wall is destroyed by sodium taurocholate, which has a lysic action on the protein mono-layers of blood corpuscles.

RECENT DEVELOPMENTS IN AIR PHOTOGRAPHY

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THE value of air photography for military intelligence and map revision became recognized early in 1915, and during the War of 1914–18 great progress was made in the design of aircraft cameras. Long series of overlapping pictures were obtained, stereoscopic examination of prints was commenced, and many of the types of information obtainable from suitable photographs were recognized. After that War, the use of this method in survey and map revision was studied[1], and surveys of large areas were made, especially in Canada and the United States. In some places air photographs were employed for scientific studies as I suggested in *Nature* in 1920[2], and results of importance for geologists, foresters, civil engineers and archæologists have been obtained.

The outbreak of war in 1939 found Great Britain, however, with very inadequate preparations for air photography as one of the chief sources of war intelligence. But during 1940 an organization was brought into being in which many professional men of science were enrolled, and gradually a very strong and efficient service was built up. Later a close co-operation was established with the American Army and Naval Air Services, in which ideas, equipment, materials and methods were interchanged.

The provision of photographs of the areas controlled by the enemy required the use of special aircraft, able to fly deep into enemy country at a height and speed which rendered their interception difficult. This meant the production of special types of our fastest machines, and the training of special pilots to navigate and fly them. The aircraft were generally fitted with two or more cameras, so that the maximum number of useful photographs could be obtained on every sortie. Cameras of the type in service at the outbreak of war were very largely employed, but lens makers were called upon to produce new types of lenses of long focal length for use in them. The general features of these cameras had been designed at the close of the War of 1914–18; they have automatic operation with distant control, and a magazine of film for 250 exposures. As the result of constant research and experiment, many important improvements in the design have been introduced. Later a new type, with a magazine holding 500 exposures, was brought into service, and a somewhat similar instrument designed by the Fairchild Co. in the United States was also used.

The cameras were generally mounted in the aircraft with the optical axis vertical, or slightly tilted so that by the simultaneous use of two instruments greater lateral cover could be obtained. For low-level photographs cameras were installed in an oblique position in the fuselage or under the wings; very valuable work was done with two synchronized cameras facing forwards and giving stereoscopic results. The task of obtaining pictures of specific installations from a low altitude made great demands on the skill and daring of the pilots; it was generally attended by considerable risks.

The operation of cameras at high altitudes produced some special problems, such as the effects of the intense cold on the moving parts, and the occurrence of condensation on the lenses. Special heating arrange-