MECHANISMS OF FOLIAR PENETRATION OF SOLUTIONS[1]

By Wolfgang Franke

Institut für Landwirtschaftliche Botanik der Universität Bonn, Germany

From a classical point of view green leaves appear to be organs whose sole purpose is the production of organic materials by photosynthesis. While gases like CO_2 and O_2, both necessary for and arising from assimilation and respiration, are exchanged by stomata and intercellular spaces, water and mineral salts are supplied by the roots. However, during the last few decades it has become more and more evident that absorption of inorganic and organic materials can also take place through the surfaces of leaves. This is rather surprising at first sight, since these surfaces are covered by the cuticle, which has been considered impenetrable. The same is true for excretion. Aside from gases and water vapour, it was thought that other materials, which are no longer useful to the plant or which are surplus, could not leave the assimilating organs but must be stored within the vacuoles or in special tissues. It has been shown, however, that some export of substances takes place from roots and even more from leaves which may sometimes be important for the plant itself and perhaps for plant communities.

There are numerous reports on foliar spraying, foliar absorption and foliar leaching, many of which have already been discussed in former reviews in this series (8, 69, 107). Since then, the number of substances applied to leaves has grown enormously; more and more growth regulators, pesticides and nutrients are used for theoretical and practical purposes. The results of such work have been reviewed primarily in relation to applied problems during the last few years (11–13, 17, 36, 44–46, 108, 109).

While practical aspects were emphasized, the location of the sites of foliar absorption and excretion and the nature of the underlying mechanisms have more recently been investigated. Since substances to be absorbed or excreted by leaves must pass through a cell wall covered by a cuticle, the process of penetration may be somewhat different from that of absorption by root cells, which do not possess an outer layer structurally comparable to that of the leaves. The purpose of this review is to present current views, to reveal gaps in the existing knowledge and to reflect on both. No attempt has been made to refer to the whole body of information available at present. The amount of published work has made it necessary to select only literature directly relevant to the matters considered below. Further literature is cited in reviews referred to above.

To understand the mechanisms of foliar penetration it is necessary to find out what the pathways are through which penetrating solutions pass

[1] The survey of literature pertaining to this review was concluded in July 1966.

from the surface of leaves to the protoplasts of epidermal cells and vice versa. These pathways must be sought generally at three sites: in the cuticle, in the cellulose wall and in the plasma membrane. Information on the pathways will first be discussed in this order. The question of mechanisms will be considered later.

PATHWAYS OF UPTAKE AND EXCRETION

The Role of the Cuticle

The problem of pores.—Roots and root hairs as absorbing organs are characterized by cell walls easily penetrable via interfibrillar spaces which belong to the so-called free space. In contrast to roots, the outer walls of the epidermal cells of leaves and stems are covered by a more or less thick cuticle which, in addition, may be interspersed and superimposed by wax extrusions. The lipid character of these wax and cutin layers can form an obstacle which prevents the penetration of hydrophilic substances. Although the existence of extremely thin cuticle on the outer walls of root cells has also been suggested (90, 91), convincing electron microscope studies to support the possibility have yet to be reported.

Up to the present time the obstacle to penetration caused by the cuticle in leaves has been considered by many authors as so serious that uptake of hydrophilic compounds was considered to occur only through stomatal pores. This deduction seemed to be confirmed by the unquestionable observation that the uptake mediated by the lower leaf surfaces is almost always greater than by the upper surfaces which either have no stomata or many fewer (6, 8, 18, 35, 50, 76, 96, 107). However, the passage through stomatal pores has only the effect that the solutions enter cavities such as stomatal chambers and intercellular spaces but not the cells themselves. Since the outer walls of cells lining these cavities are also covered by an "internal cuticle" (30, 44, 88, 98), the problem of penetration is merely shifted from the outer to the inner surfaces of the leaves. Of course such stomatal penetration would be of some advantage because the absorbing surface is enlarged, the internal cuticle within the cavities may be thinner and more easily penetrable, and because the risk of spray solution drying down on the surface is reduced (14).

Decisive, however, is the fact that stomatal chambers and intercellular spaces are filled with gases, and that the surface tension of the applied solutions and the hydrophobic nature of the cuticle lining the stomatal pores normally do not permit the passage of aqueous solutions. By using detergents this passage may, of course, be experimentally induced (14, 16, 18). Thus, under natural conditions absorption of solutes must take its regular course by the penetration of cuticles. Indeed, many experiments have also shown that absorption through upper leaf blades, which are totally lacking in guard cells, occurs to a considerable extent (8, 15, 35, 50, 107).

The question arises as to whether there are pores in the cuticle serving as pathways of absorption and excretion. Efforts to demonstrate such pores remained, however, unsuccessful. According to many investigations by

electron microscopy the cuticle always appears as a uniform continuous, and poreless membrane (98). Only locally thinner spots and sometimes breaks, fissures, or punctures made by insects (4, 50, 89) may be observed. However, fissures as studied by Bauer (4) merely affected the outer cuticular surface and did not totally traverse the cuticle. Only in epidermal cells of *Trifolium repens* and *Brassica oleracea* have true perforations been found, through which wax is said to be extruded (39). In special cases such as glandular hairs of *Mentha piperita* (2)and trichome hydathodes of *Cicer arietinum* (82), single pores perforate the cuticle through which volatile oils or droplets of aqueous solutions may be released. But there is no indication of a general occurrence of such openings.

Orgell (67) has stated that the cuticle may be characterized by an imbricate arrangement of lipoid platelets cemented together by hydrophilic pectinaceous materials. Thus an "intercuticular penetration" (107) should be possible for aqueous solutions because pectin layers underlying the cuticle (Fig. 1) and extending to the middle lamellas of the tissues should provide a hydrophilic pathway towards the small vascular bundles positioned close to the epidermal tissue of the leaves (71, 72). But there is no direct proof of such a pathway. Perhaps observations made by Yamada (110) can be considered as evidence of an imbricate arrangement of cutin platelets. By isolating the cuticle of some plant organs by enzymatic decomposition of pectinaceous materials he obtained in some species many minute thin fragments. However, the cuticle seems mostly to be a uniform membrane.

Therefore, as a rule, in foliar absorption substances to be absorbed have, in contrast to the situation in roots, first to penetrate a lipid-like layer. This means that an "intracuticular penetration" (107) without pathways through distinct pores has to be performed before solutions can enter the cellulose walls.

Structure and physico-chemical nature of the cuticle.—The covering of epidermal cells on the outer surface, commonly named cuticle, is built up as a rule of several layers. Apart from a possible wax extrusion, the outermost layer in a young leaf consists of cutin in a tight lamellar order. In mature leaves, fine wax films are inserted between the cutin lamellas (Fig. 1) (98). This layer alone shall be denoted as the cuticle proper according to Sitte & Rennier (97). The layers beneath, often several in number, shall be named cuticular layers. Their behaviour under the polarisation microscope is different from that of the cuticle proper and they contain, besides cutin and intrusions of wax in the form of periclinal platelets, hemisubstances, formerly called hemicellulose, i.e., polyuronides and glycans (98). Towards the cellulose wall, but still outside of it, some single cellulose fibrils can often be seen (Fig. 1). These are not connected to a network as in the true cellulose walls, which underly the cuticular layers (30, 32, 79, 97, 98). The occurrence of one or more thin layers of pectin (polyuronides) has repeatedly been reported. These are arranged between the cuticle proper and the true cellulose wall (7, 30, 72, 79, 89).

The chemical structure of cutin has not yet been fully elucidated. It is

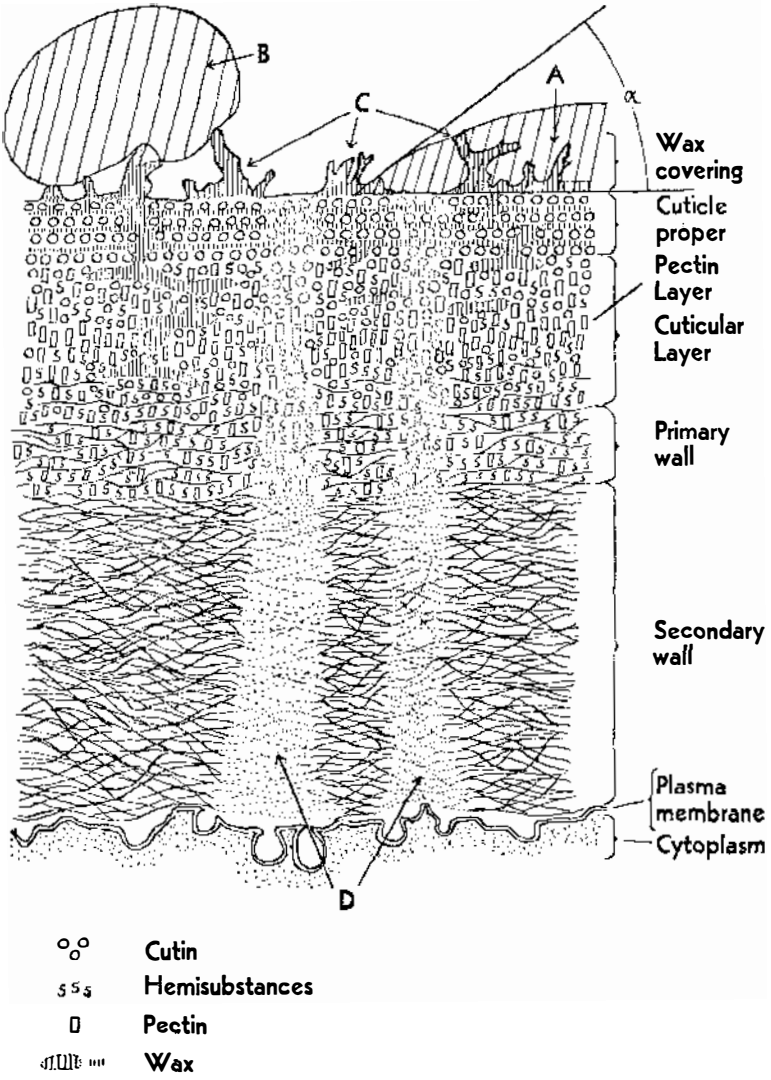

FIG. 1. Simplified scheme of the outer wall of an epidermal cell. A = water droplet with detergent, B = without detergent. C = wax rodlets. D = ectodesmata as non-plasmatic structures. α = contact angle. [With reference to van Overbeek (69), Fig. 1 modified].

assumed that this material is a polymerisation and condensation product of C_{18}-hydroxy fatty acids which are synthesized in the protoplasm and penetrate the wall as procutin in the form of droplets (7, 31). Extruding on the surface these precursors are oxidized and polymerised forming a spongelike frame of submicroscopic dimensions. Recently Heinen & V.D. Brand (40) obtained evidence that three enzymes—stearic acid oxidase, oleic acid oxidase, and lipoxidase—are involved in the synthesis of cutin. The activity of these enzymes in leaves of *Gasteria verrucosa* could be demonstrated as well as the presence of saturated and unsaturated C_{18} fatty acids. The authors have proposed a model in which cutin is built up of so-called basal units of a few hydroxy-C_{18} fatty acids combined by ester bonds and peroxide bridges. These basal units polymerise to macromolecules which may then form a three-dimensional network by further linkages. Because of the distances between the hydroxy fatty acid chains within the cutin units, as well as between the macromolecules themselves, intermolecular spaces exist as in the structure of cellulose. The magnitude and size of them is not yet known. Presumably these spaces are large enough for the passage of small molecules. But there must also be broader interspaces, since larger organic molecules such as dyestuffs, streptomycin, and other antibiotics are able to penetrate the cuticles of leaves (37, 50, 52, 78). If, as in mature leaves, wax films alternate with cutin lamellas, these films should inhibit the penetration of aqueous solutions because of the apolar lipophilic properties of wax. In this connection Sitte (98) notes that these lipid molecules are in constant thermodynamic motion, perhaps oscillating permanently around a medium position. Thus a dynamically changing ultraporosity of the wax films will result which may also render possible the penetration of larger hydrophilic molecules. The observation that the penetration of substances such as phenylmercuric acetate through isolated cuticles increases with increasing temperature lends some support to this idea (96).

Penetration is affected not only by intermolecular spaces but also by intermolecular forces and charges of polar groups within the macromolecules of cutin. Cutin contains both hydrophilic (–OH and –COOH) and lipophilic groups (–CH_2- and –CH_3). Thus cutin should be a hemihydrophilic substance (30). This property has been confirmed by the observation that pure cuticular waxes and other completely apolar substances cannot be stained at all by Sudan dyes as had been formerly assumed (97). Staining of the cuticle with Sudan III or IV, therefore, does not indicate an exceptionally lipoid character but just the hemihydrophilic nature of cutin.

Because of the presence of hydrophilic groups, hydration and imbibition of the cuticle proper and of the cuticular layers become explicable. Consequently the penetration of water and water-soluble substances through these layers to the outer or inner surfaces of the cuticle is facilitated. These relations form the basis of foliar absorption, foliar excretion, cuticular transpiration, and leaching.

The fact that the cuticle is negatively charged as a whole has the effect that these charges are at first neutralized by cations. Therefore the additional application of cations such as Ca^{++}, Mn^{++} and others, which bring about

dehydration, may increase the entry of larger cationic molecules such as streptomycin (36); cations such as Ca^{++} and Mn^{++} are able to penetrate more quickly because of their smaller ion radius, thus facilitating the passage of larger cationic molecules.

Since the polymerisation of cutin is dependent upon oxygen, macromolecules directly adjacent to the surrounding air are said to be more completely polymerised than those located farther inside. Furthermore, extrusions of wax on the surface of the cuticle have the effect that more lipophilic groups are accumulated outside rather than inside the cuticle. All this results in a gradient of polarity from the exterior of the cuticle, which is more or less apolar, to the interior of the cuticular layers and of the cellulose wall, which exhibits high polarity (12).

The possibility has been repeatedly discussed that water-soluble polar compounds may follow a special aqueous route through the cuticle, while apolar lipid-soluble substances may use a lipid path. This two-way hypothesis, however, still lacks adequate experimental support (17), although it seems attractive in so far as on the one hand wax extrudes through the cuticle and, on the other, water with water-soluble compounds, leachable by rain and dew, are excreted at the same time. The possibility of special channels for extrusion of cutinaceous material within the cuticular membrane has recently been discussed (42, 43). Yamada et al. (115) have found that radioactive inorganic ions and urea penetrating isolated cuticular membranes are bound in part to pointlike areas in the cuticle, especially along the anticlinal walls and stomatal pores of guard cells. This points to special paths of penetration even within the cuticle, since otherwise the whole surface would be expected to be uniformly interspersed with bound ions.

The Relations in the Cell Wall

Structure and physico-chemical nature.—The outer cellulose wall of epidermal cells is not separated from the cuticle by a sharp boundary. There is a state of transition produced by the cuticular layers. This is shown by the observation that single microfibrils of cellulose are sometimes embedded in cutin and hemisubstances of the cuticular layer while the same hemisubstances form the ground substance (matrix) of the adjacent primary wall (Fig. 1). This and the secondary wall are the two main layers of the cellulose wall. The secondary wall again consists of several layers which differ in their textures.

A fresh primary wall contains only about 10 per cent cellulose, while a dry wall contains 30 per cent (32). The cellulose is in the form of microfibrils loosely embedded in the matrix (Fig. 1). In the secondary wall, which contains 60 to 94 per cent cellulose and only a little matrix, the microfibrils are closely packed and interwoven into a network of a different ultratexture. Between the microfibrils and between the elementary fibrils, which are the subunits of microfibrils (32), exist interspaces. The size of the intermicellar spaces between the elementary fibrils is about 10 Å. They should be penetrable, therefore, by small molecules such as water and halogen ions. The interfibrillar spaces between the microfibrils, however, reach a diameter of up

to 100 Å through which larger molecules should pass freely if the spaces are not filled with non-cellulosic material.

The hydrophilic properties of the walls are based on the hydroxy content of cellulose and hemisubstances which facilitate the passage of water-soluble substances. *In vivo*, as a rule, most submicroscopic spaces may be filled with imbibed water. Therefore, the penetration of lipophilic substances may be more difficult in the cell wall than in the cuticle. Pectin and the hemisubstances of the matrix possess free carboxy groups which contribute to polarity.

Although, according to the structure described, one could visualize that penetration should occur through all the interspaces of the cuticle and of the cellulose wall, large scale transport appears to be limited to special pathways separated from one another. Evidence of this will be discussed in the following paragraph.

The problem of ectodesmata.—The detection and study of ectodesmata (19, 20, 23, 53, 80, 84, 85, 87, 93) have led to a new outlook with regard to the penetrability of leaves and stems. Ectodesmata are fine structures in the outer walls of epidermal cells. By use of a special method (53, 80) they have become demonstrable with the light microscope. Initially they were considered homologous to plasmodesmata, which perforate the inner walls of tissues and connect neighbouring protoplasts. Because of their similar shape and their localization within the outer walls they were at first named "outerwall plasmodesmata" but were later defined as ectodesmata. Since they extend from the cuticle, which they do not perforate, through the wall to the lumina of epidermal cells, they seem to provide an almost direct connection of the protoplasts with the surrounding outside medium of leaves. Therefore, ectodesmata were supposed to be involved in foliar penetration. Indeed, it was demonstrated that they are directly related to foliar absorption and excretion (19, 20, 21, 28, 86). In isolated epidermal tissue of bulb scales of onion, convincing evidence was given that the distribution of droplets, which were excreted through the outer walls after embedding the tissue in paraffin oil, coincided with that of ectodesmata (22). Rows of ectodesmata along the anticlinal walls and other patterns could be observed in the same position as the droplets. Furthermore, after radioactive solutions had been applied to epidermal tissues of spinach and *Viola tricolor* leaves, autoradiograms of the epidermis showed accumulations of silver grains only where ectodesmata in the walls could be demonstrated in larger numbers (24). This is especially true of guard cells (25). In studies on cuticular transpiration of tritiated water, it has been observed in autoradiograms that the bulk of black spots in the stripping film appeared on top of the edges of stomatal pores and above the guard cells, while the film above the pores themselves was free of silver grains (55–57). In these cases, too, the distribution of ectodesmata coincides with the sites of excretion of tritiated water (29). These results strongly suggest that ectodesmata are pathways of foliar penetration. This may also be true if penetration through stomatal pores is induced by detergents and if the substances to be absorbed have entered the stomatal chambers and the intercellular spaces because ectodesmata have

been demonstrated in the walls lining these spaces (28, 53). In xeromorphic leaves, however, a very thick astomatous cuticle prevents foliar absorption (96). The fact that in these cases ectodesmata cannot at all be demonstrated (53) again gives evidence of a connection between foliar penetration and the occurrence of ectodesmata, negative though it may be. Recently it has been suggested that even virus particles, the magnitude of which goes far beyond that of organic compounds hitherto studied in absorption experiments, may use ectodesmata as paths for entry into cells (9, 10).

To avoid misunderstandings it must be pointed out that ectodesmata, which cannot be seen in untreated tissues, must be very fine submicroscopic structures. Special treatment using the mercuric chloride method (53, 80) is needed to make them coarser and render them visible with the light microscope (23, 27). Although it was thought that ectodesmata should be homologous with plasmodesmata, direct proof of protoplasm in ectodesmata is lacking (27). Plasmodesmata have been shown to be cytoplasmic tubuli surrounded by plasmalemma and containing endoplasmic reticulum. Such structures, however, have never been found in the outer walls up till now. Rather, the structure of ectodesmata as seen in the electron microscope (80, 87) appears to be very fine strings which may or may not be related to interfibrillar spaces within the cellulose walls.

In a critical study of the ectodesmatal structure, therefore, it has been proposed (27) that ectodesmata are not proper plasmatic threads within the wall but merely interfibrillar spaces or groups of them (Fig. 1) of a well defined structure containing liquid excretion products of the epidermal protoplasts. Among the excretion products there appear to be reducing substances which with mercuric chloride form a precipitate. This precipitation reaction forms the basis of a staining technique with pyoctanin used to demonstrate ectodesmata (26, 28, 85). According to the hypothesis, the substances excreted by the protoplast will be transported through the wall towards the cuticle by the cuticular transpiration stream. The quantity and the distribution of the reducing substances capable of reacting with mercuric chloride within and along the supposed interfibrillar spaces determine then the ectodesmatal forms which appear to traverse the wall either totally or partially. In the latter case, they protrude from the cuticle only up to the middle of the wall or not quite as far (27). These so-called shortened ectodesmata, which are often to be seen, can be explained on the basis of this hypothesis. If they were extensions of the protoplasts into the wall, they should be connected to the protoplast rather than adhere to the cuticle.

Ectodesmata are predominantly found in special sites such as along the anticlinal walls, in some hairs, in the basal cells of hairs, or in epidermal cells surrounding hairs. They also appear accumulated above, beneath, and on both sides of the veins. Most interesting is the abundance of ectodesmata in guard cells where they frequently show a typical distribution such as along the edges of stomatal pores and along the rear walls of the guard cells.

The characteristic distribution of ectodesmata may be explained on the grounds that either interfibrillar spaces in these sites are a little bit broader in diameter because of mechanical factors such as pressure or tension (27), or

that at these sites, excretion of the reducing substances occurs within the protoplasts perhaps by a process which is the reverse of pinocytosis (83, 94, 95). Further studies are needed to answer these questions.

Sites of absorption and excretion.—Previous studies on the absorption of staining materials by leaves have shown that the entry of dyestuffs is often limited to special areas of the leaves where absorption proceeds at least more rapidly than through neighbouring areas. Thus, trichomes and hairs are often particularly active in absorption. Frequently they also function as glandular hairs in the excretion of volatile oils or mucilages and seem to be especially appropriate as portals for the entry and emergence of water or solutions (16, 44). Sometimes the hair cells themselves absorb the stains, or the basal cells of hairs or those epidermal cells surrounding them take up most of the dye. The particularly rich supply of ectodesmata in these cells may be closely related to this function (19, 21, 53, 80, 93). In other cases foliar absorption occurs only or predominantly in epidermal cells above, beneath, or on both sides of the veins. These places, as already mentioned, contain numerous ectodesmata in the outer walls (19, 21, 28).

Where anticlinal walls reach the surface, slight depressions are to be observed (54). Here droplets of rain, dew, or sprays easily adhere to the leaf blade. And, again, ectodesmata crowd in tight rows along these anticlinal walls (19, 21, 22,28). Into this pattern fits the recent observation that also in isolated cuticles the binding sites of radioactive ions and urea penetrating these membranes are lined up along the anticlinal walls (115). The exit of water vapour by cuticular transpiration and the excretion of droplets by onion bulb scales (22, 33) are also mediated predominantly by these anticlinal walls (75).

Finally the guard cells are sites of favoured exchange of aqueous substances, not through the stomatal pores which function in gas exchange only, but through the guard cells themselves. This has not only been deduced from numerous investigations (6, 16, 48, 62, 63, 76), but has also been demonstrated in studies on the absorption of radioactive solutions by the surface of leaves. When the epidermis is covered with stripping film, silver grains appear predominantly on top of the guard cells, but not above the open stomatal pores (24, 25). The frequency and density of silver grains was especially high above the edges of the guard cells along the stomatal pores. The same sites have been shown to be favoured places of exhalation of tritiated water vapour (55–57), of binding sites of radioactive ions in isolated cuticles (115), and of a large abundance of ectodesmata (19–21, 24, 25, 28, 29, 53, 80, 93). The additional ability of guard cells and, if present, of accessory cells, for peristomatal transpiration and water absorption (92) and many other activities (55, 58, 70), makes it evident that guard cells are special places for foliar penetration. Thus it is also understandable why the lower side of leaf blades with their higher percentage of guard cells are more effective in foliar absorption than the upper leaf blades. Not the number of stomatal pores but the greater abundance of ectodesmata together with high functional activity in the guard cells may be the true cause of this effect.

From the statements above it may be concluded that foliar absorption as

well as foliar excretion and cuticular transpiration do not occur in all areas of leaves with equal intensity and, even more important, that penetration does not seem to proceed through the total plane surface but appears to be localized mainly in special sites which very probably correspond to the ectodesmata.

The Plasma Membrane

On reaching the surface of the protoplasts the substances coming from the outside have to overcome another barrier, the plasmalemma. This protoplasmic membrane, which does not contain identifiable pores, is probably of a bimolecular film of closely packed globular lipoprotein molecules (34). Between areas of smaller globular particles areas of larger ones may be present thus effecting the aspect of a submicroscopic mosaic (32). The interspaces between the molecules are so narrow, up to 4 Å, that only water molecules (about 2 Å) may enter the cytoplasm behind the plasma membrane without much difficulty. The membrane, therefore, is called semipermeable. For other compounds, then, the permeability may be determined by the lipoid-filter theory (98). The penetration through this plasma membrane, however, is connected with the mechanism and is even less completely understood than that through the cuticle or the cellulose wall and will be considered in more detail later.

MECHANISMS OF FOLIAR PENETRATION

In the Cuticle

As shown above, the cuticle appears principally to be penetrable via intermolecular spaces. The frequency of fissures, breaks, and punctures is certainly not so great that these openings allow a mass flow (79), although they may sometimes facilitate penetration (50). In order to study the penetrability and the mechanism of cuticular penetration, several recent investigations have been performed with isolated cuticles (5, 15, 37, 41, 52, 65, 68, 79, 96, 99, 110–113).

To isolate cuticles, they may be torn off and cleaned mechanically, but this is certainly the most precarious method (59). Pectinaceous substances underlying the cuticle proper can be dissolved by boiling in ammonium oxalate and H_2O_2 or oxalic acid (5, 37, 73, 74). However, with this method the walls of epidermal cells sometimes remain united to the cuticle and the physico-chemical structure of the cuticle may be altered by the boiling. In a more biological approach, tissues are treated with combinations of enzymes such as cellulase, hemicellulase, and pectinase (15, 67, 110, 112). This seems to result in the least alteration of the nature of the cuticle, although it should not be overlooked that only the cuticle proper will be preserved, while other cuticular layers may be destroyed because of the content of pectin. In different species the membranes remaining after treatment may be distinguished by their specific nature or by seasonal dissimilarities. Thus, the results of penetration studies performed with them may not always be generalized to all species (110).

The commonly employed method of studying penetration is to place

isolated membranes, which previously have been examined for fissures, breaks or punctures, between two chambers, one containing a solution of the penetrating substance such as inorganic or organic ions, amino acids, sugars, antibiotics, growth regulators or inhibitors, and the other pure water (37, 96, 110, 112, 113). As a control, synthetic dialyzing membranes are sometimes used instead of the cuticular membrane. The passage of ions can be measured by several means, such as by the determination of conductivity in the pure water chamber. However, radioactive labeling of the solutions appears to be the best method since exact determinations are possible in association with paper chromatography and autoradiography.

From such studies the penetrability of cuticular membranes is confirmed. Stomatous cuticles sometimes exhibit greater permeability than astomatous cuticles. Studies with the electron microscope indicate that in these cases the stomatal openings are not true perforations but only invaginations of the membrane (37, 113). The internal cuticle of the stomatal chambers seems to be glued to the main cuticle of the epidermal cells. But because these internal cuticles are thinner than the main cuticle, greater penetration may result. Of course the extent of penetration may be influenced by the thickness of the cuticle (96), by wax depositions, wax intrusions, or by other factors such as aging (50, 110). Penetrability as a whole, however, is not decisively influenced by these factors apart from extreme thickness. From the kinetics of the appearance of penetrated substances in the pure water chamber it appears that the movement of ions and organic compounds occurs by diffusion (15, 65, 110). The experimental results fit a mathematical equation derived from theoretical considerations of diffusion (110). With different applied compounds 1.5 to 3.3 per cent was found to pass through isolated apricot leaf cuticles during 48 hr at 25° C.Penetration was directly proportional to concentration, and increased with increasing temperature and with the higher lipophilic character of the applied compounds (15). With isolated tomato fruit and onion leaf cuticles, 0.2 to 2 per cent of applied cations and anions penetrated the membranes, but about 80 per cent passed through synthetic dialyzing membranes (112). Similar percentages have been found with radioactive leucine and several antibiotics using isolated apple leaf cuticles (52). These results show that it may not be simple diffusion. Kamimura & Goodman (52) even discuss the possibility that isolated cuticles may have lost their permeability. This may be possible with the chemical method of isolation. Certainly, chemical and physical characteristics of the isolated cuticles may influence diffusion and lower it to a considerable extent. Thus, more cations penetrate over time than anions. The presence of charge sites within the cutin causes, at first, ion binding in isolated cuticles (111–113, 115) According to such studies, the binding of cations is indeed far greater (up to 1000 times) than that of anions. It depends on the type of ion, concentration, time of exposure, and on the species of cuticle. If the cuticles are immersed in a solution for 5 min and then washed for 5 min in deionized water, from the remaining bound ions left in the membrane, the anions are exchanged 100 per cent and the cations only 70 per cent. Thus cuticular membranes exhibit ion exchange properties. The negative charges must pre-

dominate, resulting in a weaker exchange capacity for cations and a reduced penetration of anions in comparison with cations. Cations which at first are bound to negative charges may later dissociate and be displaced by other cations. Following the concentration gradient they will move towards the weaker concentration and thus penetrate the cuticle more rapidly through intermolecular spaces of cutin than anions which are hampered by the negative charges. Of course this inward movement of cations must be compensated by an outward movement of cations of other species if the anionic counterparts show little penetration.

It is surprising that the penetration of cuticular membranes from inside to outside is quantitatively different from that in the reverse direction. This unequal ratio of permeability was first reported for astomatous cuticles of *Hedera helix* leaves (79) in which more water penetrated inward, i.e., towards the protoplast, than outward. The same result has been found with inorganic ions and undissociated organic compounds in astomatous and stomatous cuticles, but not in synthetic dialyzing membranes (111–113). An opposite observation has been reported for apple leaf cuticles (37, 52). In this case, the cuticles were isolated by chemical means and their structure may have been altered by the boiling, thus causing the reversed behaviour.

More important appears to be the observation that the ion binding capacity, after 5 min of contact with the ion solution, is, contrary to the mode of penetration, greater in the outward direction than in the inward direction. Organic compounds, however, are bound in equal quantities from both sides (112, 113). From the extent of ion binding it may be inferred that the binding sites are localized chiefly in the inner side of the cuticle adjoining the cellulose wall. This is consistent with the hypothesis of Crafts & Foy (12) according to which a gradient of polarity exists with more polar groups in the inner side of the cuticle than in the outer. The question arises whether the favoured inward penetration of ions is to be explained on the grounds that the ions have to pass first through the outer cutin lamellas, which contain less sites of charges, and then have to occupy the charged sites in the inner lamellas while penetrating outward the inverse order would be expected. This difference, however, should last only for a short time, since penetration should be equal in both directions if a steady state is reached. But this does not seem to be the case. The question also remains as to why the inward penetration of organic compounds through cuticles should be favoured, while the retention of these compounds is nearly the same on the outer and inner surfaces of the cuticles. Evidently further research is necessary to solve this problem.

While diffusion is to be assumed as the main driving force for both ions and also many organic compounds, urea penetrates the cuticular membrane with a velocity higher than what one would expect from simple diffusion (113, 114). The kinetics of urea penetration are markedly different from those of other substances. The extent of penetration exceeds that of ions by 10 to 20 fold and is independent of the concentration. This increased permeability for urea also favours foliar absorption of ions which are applied together with urea, such as iron and phosphate, in bean plants, citrus, and pineapple (64,

66, 109, 114). A similar increased foliar absorption of leucine or streptomycin by apple leaves, if fed together with sucrose (51), is contrary to findings with bean leaves which were provided with phosphates and sugars at the same time; in the latter the uptake of phosphates was reduced (66, 110). The examination of this effect of urea and sugar on ion penetration in isolated cuticles has now shown that urea indeed increases the permeability of the cuticular membrane not only for itself but also for phosphate, Rb^+, and Cl^- ions applied at the same time. The penetration of phosphate, Sr^{++}, and SO_4^{--} ions, however, is neither favoured nor reduced by the addition of glucose or sucrose, although both sugars themselves penetrate the cuticle (110, 113, 114). Possibly sugar may favour the uptake of leucine indirectly in that it serves as a source of energy in the active uptake of leucine and other compounds into the protoplast (51).

Thus urea proves to be a promoter of permeability, which is also true for some derivatives of urea and some other compounds of increasing lipid character (66, 110). From these findings and from the observation that urea and methyl urea can elute an ether-soluble portion of pulverized tomato fruit cuticle (110), Yamada et al. (113) conclude that the effect of urea on the cuticular permeability is based upon a loosening of the membrane structure by changing ester, ether, and diether bonds between the macromolecules of cutin. However, it should be remembered that the loosening of bonds like these requires enzymatic catalysts. Nevertheless, since the ultraporosity will apparently be increased for ions entering together with urea molecules, there must be some decisive alteration in the structure of cutin which has still to be examined.

With regard to the penetration of lipophilic substances through the cuticular membranes, a process of solution by means of lipophilic components of cutin and waxes may take place.

Finally, a remarkable although still inexplicable observation must be mentioned. Biological regulators and inhibitors of respiration and other enzymatic processes have been reported to influence the ion binding capacity in isolated cuticles and also the penetration of the cuticles. Thus the binding of Ca^{++} ions is said to be increased with freshly prepared, unboiled tomato fruit cuticles by kinetin and KCN, but decreased by DNP (111). This effect was lacking in boiled cuticles and normal dialyzing membranes. However, the procedures used for isolating and storing the membranes rule out the presence of much, if any, enzymatic activity within them. The binding process seems to be of a physico-chemical nature and must obviously be studied further.

In summarizing, it may be stated that isolated cuticular membranes are permeable to both organic and inorganic ions and undissociated molecules. The penetration of ions is determined by the kind of charge, adsorbability, and ion radius. The mechanism of penetration is a physical process of diffusion, which in isolated membranes may sometimes proceed slower than in intact tissues. Urea seems to penetrate by a process of facilitated diffusion, while lipophilic substances may penetrate by a process of solution. Their

penetration is determined by the solubility, partition, and molecular size. In intact tissues it is to be assumed that the same physical processes take place during penetration.

In the Cell Wall

After having penetrated the cuticle and the cuticular layers, substances to be absorbed enter the cellulose layers of the wall, as do materials to be leached which have been excreted by the protoplast. Although the interfibrillar spaces between the cellulose fibrils appear large enough to be penetrated by free diffusion, it has been pointed out that wall penetration apparently occurs in special epidermal areas and through separated pathways, such as ectodesmata. Therefore, the question again arises as to their structure. If these ectodesmata were extensions of the protoplast into the cellulose wall, i.e., plasmatic threads, their surface would be coated with a plasma membrane, the plasmalemma. The penetrating substances, immediately after having passed the cuticle, would then have to overcome this barrier. Such absorption would correspond to the process of incorporation into the living protoplast. However, in ultrathin sections of the outer wall of the epidermis no membranes coating ectodesmata have been found so far. Only fine strings are to be seen which are so narrow that membranes like the plasmalemma, with a diameter of about 70 to 100 Å, could not fit within them, much less the double membrane forming a tubule of at least 200 Å. Normally, however, plasmodesmata possess a width of about 600 Å. It therefore appears more likely that ectodesmata are not plasmatic structures, but rather special interfibrillar spaces constituting pathways for the transport of substances via diffusion.

Because cutin and waxes or precursors of them must pass through the cellulose wall when the cuticle is formed and wax is extruded, at least during cell growth and some time later, some interfibrillar spaces should be filled with these lipophilic substances (7, 31, 42, 43). These pathways may then facilitate the penetration of lipophilic substances to be absorbed through the cellulose wall, while hydrophilic solutes may follow other spaces, which are filled with water or aqueous solutions, supported by the hydrophilic groups of the cellulose molecules. However, more information on these processes is needed. Clearly the mechanism of penetration through the wall must be diffusion, i.e., a physical process.

In the Plasma Membrane

Foliar absorption.—Incorporation of penetrating substances into the protoplasts is the decisive step of absorption and occurs along the surface of the plasma membrane or at particular sites on it. This surface *in vivo* is certainly not smooth, but irregularly protruding and invaginating, thus forming a large extension of surface, the shape of which may change dynamically. As stated above, the plasma membrane offers no openings large enough for physical penetration with the exception of water molecules (34). Thus, the molecules which have penetrated the wall can at first only be adsorbed to the surface of the plasma membrane. Then they will be incorporated by

energy-requiring processes. These may consist in an enlargement of the interspaces between the lipoprotein molecules (change of permeability), or in a removal by single globular molecules of the membrane (34) which carry the adsorbed substances into the cytoplasm beneath the membrane (Carrier hypothesis), or by pinocytosis, i.e., invagination of the membrane and formation of a vesicle, which is tied off and moved into the cytoplasm. These and other concepts have been discussed with regard to absorption by roots and root hairs, although no conclusive proof of the special mechanism has yet been given. The assumption that related active processes occur also in leaves seems to be justified.

The energy required for active absorption can be derived from respiratory metabolism, or, as in green leaves, from photosynthetic processes. In the first case oxygen is necessary, and it is known that lack of oxygen reduces the rate of foliar absorption, while aeration increases it, e.g., with whole bean leaves the uptake of phosphate was increased by 52 per cent (46). If the energy is provided by photosynthetic anabolism, then light and CO_2 should increase the rate of uptake. That light quality and intensity improves the rate of absorption of different substances has been shown by numerous investigations (46, 47, 51, 62, 76, 77). However, it is not at all certain whether light energy favours absorption directly or indirectly by increased photosynthesis. The addition of $NaHCO_3$ increases the uptake of Rb^+ if it is applied in light (47). Since energy mostly is thought to be delivered in the form of ATP, addition of this compound should increase the uptake if it is absorbed readily at all. This has been stated for the uptake of Rb^+ by cells enzymically isolated from tobacco leaves, but not for the uptake of phosphate (47). Instead of oxygen, intermediates of respiratory metabolism, and instead of light and CO_2, high energy products of photosynthesis, have been applied to leaves together with substances to be absorbed. Thus, feeding with succinate increases Rb^+ uptake in dark and light (47), and feeding with sucrose stimulates the absorption of leucine by apple leaves in the dark (51), while the uptake of phosphate by bean leaves was decreased by sucrose in light and dark (64, 66).

In respiration and photosynthesis many enzymes function as catalysts. Therefore the active absorption dependent upon these metabolic processes should be reduced or even stopped if enzyme inhibitors are used. This has also been shown in many investigations with leaves (Table 1). Since chloramphenicol, ribonuclease, and fluorouracil, which interfere with the synthesis of protein and RNA, also lower the uptake of ions, it has been suggested that protein synthesis is closely correlated to active absorption in leaves as it is in roots (100) and that carriers of proteinaceous character may play an important role in uptake (Table 1) (45, 47, 49). However, uptake of phosphate was increased by both ribonuclease and 5-fluorouracil (45).

Under normal circumstances uptake of ions constitutes an accumulation against a concentration gradient in leaves (45) as in roots. The complex process of absorption is not yet completely understood. Many factors such as pH (76), the concentration of substances applied (1, 36), the sort of ions or compounds used (15), and the effect of other substances such as growth regu-

TABLE I

INFLUENCE OF METABOLIC INHIBITORS UPON FOLIAR UPTAKE (PERCENTAGE INHIBITION)

Inhibitor	Concentration	Bean leaves			Isolated cells of tobacco leaves		Apple leaves	
		Rb^+	PO_4^{---}	Sucrose	Rb^+	PO_4^{---}	Leucine	Streptomycin
KCN	$10^{-3}M$	—	—	100[d]	—	—	—	—
DNP	$10^{-4}M$	37[a]	42[a]	—	33[b]	44[b]	—	—
	$2{,}4 \times 10^{-5}M$	—	—	55[d]	—	—	—	—
	10^{-4} to $10^{-7}M$	—	—	—	—	—	50[e]	50[e]
Arsenite	$10^{-3}M$	—	—	—	—	—	50[e]	50[e]
Iodoacetate	$10^{-5}M$	—	—	—	—	—	50[e]	50[e]
NaN_3	$10^{-3}M$	—	—	100[d]	79[b]	44[b]	—	—
5-Fluorouracil	$5 \times 10^{-3}M$	25[a]	—	—	—	—	—	—
Ribonuclease	100 ppm	33[a]	—	—	—	—	—	—
D-Chloramphenicol	100 ppm	—	39[a]	—	—	—	—	—
	250 ppm	20[a]	—	—	83[c]	—	—	—
	1000 ppm	—	50[a]	—	—	—	—	—
L-Chloramphenicol	250 ppm	—	—	—	64[c]	—	—	—

a, see (45); b, see (47); c, see (51); d see (106); e see (49).

lators (36, 38, 76), may influence the rate of foliar absorption. But the results of the numerous experiments suggest that the proper process of incorporation of substances into the protoplasts of the epidermis is metabolic and as such a physiological one. This is further supported by the observation that the temperature coefficient (Q_{10}) is 2 to 3 (Table 2), while in a physical process like diffusion the Q_{10} is only about 1 to 1.5.

From the details described above it appears that the overall process of foliar absorption takes place in three stages. In the first stage, substances supplied to the surface of leaves penetrate the cuticle and the cellulose wall via limited or free diffusion. In the second stage, these substances, having penetrated the free space, are adsorbed to the surface of the plasma membrane by some form of binding, while in the third stage, the adsorbed substances are taken up into the cytoplasm in a process requiring metabolically derived energy. Indeed, kinetic studies show the presence of several phases in the uptake of solutes by leaves. These phases may be different in duration, intensity, and dependence upon the species, the sort of substance to be absorbed, upon secretion, and other factors (1, 106). That substances during the first and second stage merely fill up the free space and are only adsorbed but not taken up, may be seen from the fact that they totally or, to a large extent, can be washed out again. From the curves of such studies it can be deduced that at first a more or less linear shape characterizes an initial process, which corresponds to the first two stages of cell wall penetration and adsorption. A less rapid uptake afterwards corresponds to the physiological process of absorption as an active uptake.

Foliar excretion.—In foliar absorption as in foliar excretion, penetration of the cell wall and of the cuticle occurs, but in the opposite direction. That substances can be excreted through leaf surfaces and especially through

TABLE II

TEMPERATURE COEFFICIENT (Q_{10}) OF FOLIAR ABSORPTION

	Rb^+	PO_4^{---}	2, 4-D	Streptomycin
Bean leaves	1, 55[b]	1, 82[b]	2, 3[c]	2, 0[a]
Apple leaves	—	—	—	2, 4[a]
Tobacco leaves[e]	1, 74[d]	3, 16[d]	—	—

a, see (35); b, see (45); c, see (76); d, see (47); e, isolated cells.

guard cells has been shown convincingly by the use of radioisotopes and other methods (3, 29, 33, 56, 57, 102–104). At first the substances to be excreted must penetrate the plasma membrane probably often by a process involving energy. Practically nothing is known about the mechanism. Excretion can be performed in connection with a carrier system, by a transitory change of permeability of the plasma membrane (116), or by an ion exchange with ions of carbonic acid which are formed by the dissolving of CO_2 of the air in the water of the cuticle surface (61). Another mechanism may be a reverse of pinocytosis, i.e., by forming vesicles in the cytoplasm and by the opening of these vesicles, which contain the substance to be excreted, towards the cell wall as has been suggested for wall materials in root-hairs (94) and in rhizoids (95) or for volatile oils in *Typhonium* (83). Here a wide gap in our knowledge has to be filled. That excretion is connected with metabolic processes of respiration has been shown for the mucilage of *Drosophyllum* glands, since secretion of this mucilage is reduced to a large extent by inhibitors such as KCN, NaF, NaN_3 and DNP (81). On the other hand the intensity of leaching of inorganic ions like Ca^{++} and Rb^+ is not influenced by DNP. Leaching in itself is a diffusion process and results from a concentration gradient from the free space inside the wall to water droplets or films deposited by irrigation, rain, or dew on the cuticle surface. However, excretion always forms the basis of leaching and, in this case, the excretion of Ca^{++} and Rb^+ ions, followed by leaching, seems to be a passive process as a whole (61).

Outside the plasma membrane the excreted substances may follow the same routes in the wall as in foliar absorption, i.e., moving by diffusion in interfibrillar spaces (e.g., ectodesmata) sometimes supported by the water stream which provides cuticular transpiration (61). Among the numerous inorganic and organic materials which evidently can be released by the protoplasts (104), there must also be those reducing substances which migrate towards the cuticle and render possible the demonstrability of ectodesmata (27). If a secretion pressure effects protrusion of mucilage through the wall, or if a concentration gradient causes movement of excretion products towards the cuticle, these products will finally emerge on the outer surface of the cuticle, forming droplets (22, 33), since apparently the excretion does not occur through the plane surface but is limited to well defined pathways. If the surrounding air is dry, aqueous solutions will immediately dry but the nonvolatile substances may be leached later by rainfall or heavy dew and thus

get into the soil or on leaves of neighbouring plants, which may absorb them again (60, 61, 101, 105).

TRANSLOCATION

Following incorporation into the protoplasts of epidermal cells a translocation of the absorbed materials to other areas of the leaves or to other plant parts takes place regularly. Although this translocation, which is often thought to occur via plasmodesmata (symplast), shall not be dealt with here, a special feature of some epidermal tissues will be mentioned. Several authors have observed that in some species the cuticle appears to surround the epidermal cells completely, thus forming an inner cuticle between the epidermal and subepidermal cells, such as in tomato fruits, apple leaves and leaves of *Prosopis juliflora* (37, 44, 111). These inner cuticles ought also to be considered as a barrier to the translocation of substances to neighbouring cells, if they are not perforated by plasmodesmata. In this connection it is of interest that by using the method for the demonstration of ectodesmata in outer walls, in many instances similar structures have been found in the inner walls of epidermal cells (19, 21). Although they occasionally appear somewhat deformed, they remind us of plasmodesmata with regard to their localization and because they connect neighbouring cells. However, since plasmodesmata cannot normally be made visible by this method (27, 53), the question arises as to whether these plasmodesma-like structures in the inner walls should not be the same as ectodesmata in the outer walls (27). If ectodesmata are nonplasmatic special interfibrillar spaces and occur also in the inner walls, they could provide pathways for penetration in connection with translocation. This means that in this case translocation does not occur only by the symplast in plasmatic strands but also by a penetration through nonplasmatic pathways. This possibility has still to be examined.

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