PHYSIOLOGICAL STUDIES IN PLANT ANATOMY

V. CAUSAL FACTORS IN CORK FORMATION

By J. H. PRIESTLEY and LETTICE M. WOFFENDEN

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Introduction

We propose to attempt in this paper a reconsideration of the phenomena connected with cork formation under the various conditions in which it is known to occur within the plant. The attempt will involve the brief re-statement of the results of many other investigators, but this repetition seems worth while as it is hoped to show that in every case, the formation of cork follows from certain causally connected phenomena, a sequence of causation which so far as we are aware has not been traced previously.

We have been led to examine cork formation from this point of view as the result of other work which was proceeding in the laboratory, and which began with a study of the mechanism of sap exudation in the phenomenon of root pressure (19). A wider extension of the investigation has shown that sap pressures may arise within the vascular strands as the result of the activity of parenchymatous tissues in root, stem or leaf (21). At the same time other observations (23, 24) have directed attention to the importance of this sap pressure in initiating and sustaining the activity of meristematic tissues. Such tissues are always parenchymatous tissues irrigated by the sap from the vascular strand. This sap supply is in many cases controlled and contained by the limiting cylinder of the endodermis (Priestley and North (22)), and in such cases the meristematic activity of parenchymatous tissues external to the endodermis may be strictly limited.

When we apply these considerations to the case of cork formation we are dealing with the origin of a tissue which arises from the

meristematic activity of parenchyma occupying very different positions in different plants. An analysis of these occurrences of the phellogen is attempted in the succeeding pages and it will be found that in every case good reason can be assigned for the assumption that the phellogen activity had to be preceded by a slow accumulation of sap either from the exudation activity of adjacent parenchyma or more usually from the adjacent vascular strands. The accumulation of the sap can in the majority of cases be traced to a previous blocking of a parenchymatous surface, occurring in close proximity to the region where the phellogen subsequently arises. This blocking, frequently due to deposition of suberin or cutin upon surfaces exposed to air, has often been reported but so far as we are aware its causal connection with the subsequent meristem activity has not previously been emphasised. Our approach to the subject from this point of view has led us to pay special attention to the chronological order in which the various processes associated in cork formation occur.

We are hopeful that the simple causal sequence thus suggested in connection with the process of cork formation may prove of value in interpreting the results of practical experience in such processes as pruning, grafting and the cutting of potato sets before planting. We shall be the more gratified if that hope proves well grounded because the prosecution of the work was only rendered possible through a grant made to the University of Leeds by the Ministry of Agriculture.

In the exposition of our special standpoint in reference to cork formation, it is a simplification to begin with the study of wound cork. Having established by reference to other investigators and by our own further experiments our case for a causal sequence in the case of wound cork formation, a brief discussion will show how the same general chain of causation may apparently be traced in the formation of cork in the cushion left upon leaf-fall and in the normal production of cork upon a perennial stem.

I. The Formation of Wound Cork

In one case of great practical importance, the tubers of *Solanum tuberosum* L., many statements are available concerning the processes following upon wounding, and these agree as to the main features. Appel(1) makes it very clear that if the cut surface of the potato is left at room temperature in a moist atmosphere, the first stage in healing is the deposition of an impermeable brown deposit

along walls and intercellular spaces, in an unbroken layer just below the cut surface. Appel emphasises the fact that this impermeable deposit is of great practical significance, because at an average temperature it forms within twelve hours, whilst the periderm formation, the product of a meristem, will only arise within 48 hours and will not be a very effective barrier until somewhat later. The pathogenic organism *Bacillus phytophthorus* (Appel) can penetrate deeply into a cut surface within a few hours: as, however, it fails to penetrate if sown upon the cut surface after twelve hours, it is clear that its entry is prevented by this preliminary brown deposit.

This brown deposit is considered by Appel to be suberin and he confirms the statements of earlier investigators, Kny(14) and Olufsen(18), that the formation of suberin is definitely connected with access of oxygen. Thus he explains its inadequate formation in potato tubers placed after cutting in warm dry air, in that the cut surface then dries rapidly and the entry of air is prevented by the hard dry surface. Suberisation in such cases therefore occurs at irregular depths in small patches around intercellular spaces and not in a continuous layer. As the hard outer layer often cracks irregularly and in any case softens and permits the entry of fungi and bacteria when the tuber is placed in moist earth, the potato sets are very subject to disease when planted. The grafting experiments of Kabus(13) when cut tubers of potato were pressed closely together gave further evidence that the preliminary suberisation was connected with air, as if these cut surfaces made good contact, subsequent staining with Sudan III showed patches of suberin developed only at places where the two surfaces had imprisoned an air bubble.

The access of air to the cut surface may be prevented in various ways; in Kabus' experiments the potatoes were cut under previously boiled water, the cut surfaces pressed together and then bound together by some material to prevent subsequent access of air. Under these conditions, even potatoes from which all buds had been removed would slowly develop cellular outgrowths, of the nature of intumescences, which interpenetrated the two opposed surfaces and finally bound them together, without hindrance from suberisation and without the intervention of a meristem. Similarly both Olufsen(18) and Kabus found that if a piece of potato were removed by a cork borer, then pressed back into the hole and the ring-shaped cut in the outer surface sealed, no suberisation occurred at the cut surface, but the spaces created by the injury were bridged by proliferating cells without the intervention of a meristem. Kabus cut

out a cylinder of the "Magnum bonum" potato, 1·5 cm. in diameter, and placed this in moist air until the exposed cut surface of the cylinder was covered with periderm. He then cut the cylinder across transversely and joined the cut ends together under the protection of a rubber band. He found that the cylinder would grow together again without the activity of a meristem, provided that one of the cut surfaces included at least one tracheid from a vascular strand.

These experiments show that if suberisation does not occur meristem formation may not follow. On the other hand a meristem may form without any previous suberisation of the cut surface. Thus Appel's observation on the surface of tubers dried in the sun showed that very little suberisation occurred here, but such rapidly dried potatoes usually show a slight meristem formation beneath the hard dry surface although it is somewhat irregular. A simple experiment which may easily be performed will also show meristem formation without any suberisation. If the cut surface is immediately covered with melted paraffin wax and left a few days, examination will show regular meristem formation without a trace of previous suberisation. These cases of meristem formation are significant. In every case the essential antecedent to meristem formation is the blocking of the cut surface. When the surface is kept moist and suberisation prevented as in the experiments of Olufsen and Kabus described above, then the cut surface is not blocked and meristem formation does not occur. It will be found also that if the cut surfaces are left under boiled water for a week or two, suberisation will not occur and meristem formation does not follow. This experiment and the comparison with the behaviour of cut surfaces under paraffin wax have been proved sufficiently reliable to use as class experiments.

Meristem formation in these experiments with potato tubers may thus be attributed to the blocking of the cut surface. This blocking then appears to react upon the tissues within, through the accumulation in the walls and intercellular spaces of a sap which provokes the activity of a meristem. In this connection attention may be drawn to the following further facts. Appel in his experiments shows that in the cut potatoes the vascular bundles show a different staining reaction with ammoniacal gentian violet to considerable depths below the cut, a reaction connected by him with the presence of secreted substances in the vascular strand. As a result of the cut, moist air has entered the tissue and probably caused increased turgor followed by rapid excretion. The cells at the base of the bud probably contribute readily to the secretion, hence Kabus finds that

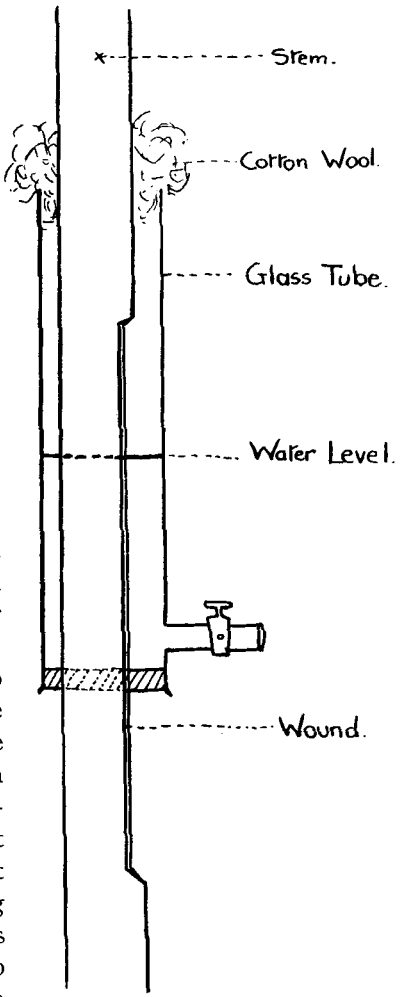
portions of tubers bearing buds most readily grow together by intumescence-like outgrowths. The production of this excretion probably explains Olufsen's observation that the larger the wound the moister it must be kept to enable it to heal, also the prompter rate of healing shown by new potatoes when compared with last year's tubers of the same variety. The use of such an excretion in the necessary cell proliferation may also explain Kabus' observation, cited above, that the two halves of the "Magnum bonum" cylinder only reunited if a tracheid appeared at one cut surface. Haberlandt (7, 8, 9) has also emphasised the dependence of cork formation upon a supply of substances from the vascular strand though he traces these substances to the phloem elements.

The case for the necessity of such an accumulation of sap to initiate the meristem activity is much strengthened if we extend our observations from the case of the potato tuber to the more general phenomena of wound healing. Here again the same two processes may be traced, first the closure of the wounded surface and secondly the appearance of the meristem. As the available data are less complete we will quote chiefly from our own experiments. The first closure of the wound again appears to be due to a deposit of suberin or some allied substance. Kabus found that this substance did not stain with Sudan III: in our experiments the brown deposit resisted concentrated sulphuric acid, went brown with iodine reagents, and frequently stained with Sudan III. We probably have to do, however, with a mixture of substances, in part produced by chemical decomposition in the dying cells at the cut surfaces, in part deposits from the excreted sap which evaporates at the exposed surface.

The following experiments explain themselves, in the light of the previous discussion. On March 21st, the epidermis was scraped off a long stretch of the stem of a begonia growing in a greenhouse. The exposed surface was partly covered with melted paraffin wax. On April 25th the two surfaces showed marked differences, the waxed surface being very much less brown. On staining sections with Sudan III the outermost layer of collenchyma only was slightly suberised under the wax, and the five to six layers of cells below this, formed by the phellogen, were quite free from suberin. At the unwaxed surface more layers of cells bore a deposit of suberin, fewer cells had been formed by the phellogen but the outer two or three layers of periderm were strongly suberised. The slight suberisation under the wax may probably be accounted for by the gradual

splitting of the wax surface. It seems evident that with complete exclusion of air no suberisation would occur.

On March 7th cut surfaces of a begonia stem were similarly covered with gelatin. Examined on March 23rd, suberisation had occurred to a considerable extent though perhaps not so freely as at the completely exposed surface. But a gelatin film could not be expected to keep out the air in the same manner as a film of paraffin wax. Meristem formation had begun in both cases. Stems covered with gelatin in this manner on March 7th were re-examined on May 23rd. In this case there was clearly both a larger amount of suberisation and a more active meristem present on the freely exposed wound surface than on the one covered with gelatin.



On March 7th an exposed cut surface was partly covered with distilled water in the manner shown in the diagram (Text-fig.). The water was renewed daily to avoid the development of disease as far as possible. The cut surface was examined on March 19th and more suberisation had occurred on the surface of the cut above the water, only a slight indication of suberisation being obtained in the superficial cells covered by the water. As no meristem had yet formed, the experiment was repeated, in this case the cut surface extended below the base of the tube as well as above the water in the tube. The experiment was started on March 18th and stopped on April 6th. The cut surface both above and below the water showed much suberin deposited and the presence of a meristem. In the region under

water there was slight suberisation of the outer layer of collenchyma but no trace of a meristem.

The absence of a meristem suggested to us incomplete blocking of the exposed surface in contact with the water. This might be due to the products which accumulated at the surface in the air, diffusing under the experimental conditions into the water. In each experiment the water changed each day was collected and kept. At the end of the experiment it was evaporated down and the residue in the evaporating dish extracted with boiling chloroform. The chloroform extract, filtered and evaporated, left a little amorphous waxy deposit which stained yellow brown with iodine. Treated with iodine and dilute sulphuric acid, part of the substance gave a reddish reaction very reminiscent of phellonic acid (see Priestley (20) for an account of the organic acids present in suberin). After saponification with boiling alcoholic potash, the residue from evaporation of the water was again evaporated to dryness and taken up with boiling chloroform. A much larger quantity of the residue now went into solution and this substance after evaporating off the chloroform again gave the phellonic acid reaction. As however the residue in part dissolved in ether, in which phellonic acid is insoluble, it cannot be entirely composed of phellonic acid, and the ether soluble constituent also gave a reddish violet reaction with iodine and sulphuric acid. These reactions suggest then that there had diffused from the injured surface into the water substances which, like the suberogenic acids, were soluble in fatty solvents, and which also were partly present as condensation products, insoluble in fatty solvents without previous saponification. At the surface exposed in air the accumulation of these substances may well be responsible for the stronger and more widely distributed suberin reaction.

In these experiments with begonia we obtain evidence again that periderm formation involves two processes, first a suberisation which seals the injured surface, secondly a meristem formation which follows upon the accumulation of sap at the injured surface. If a plant is taken which unlike begonia has a secondary endodermis in the stem, this will completely retain the sap pressure within it (Priestley and North (22)). A superficial wound should then be followed by suberisation without subsequent meristem formation. Experiment shows that facts are in accordance with this expectation. The stem of *Camellia japonica* L. has a strongly developed secondary endodermis, in which all walls are suberised. The young stem has a clear green cortical tissue outside the endodermis. Superficial cuts

were made in this green stem on March 11th, and subsequent examination on April 6th and April 18th showed suberisation of the exposed cells without any phellogen appearing. On the other hand deep cuts or pinpricks penetrating the endodermis were followed in the same time, not only by superficial suberisation but by phellogen production and activity beneath the injured endodermis.

There are certain problems left in the literature which we think can be cleared up in the light of the above facts.

Holden (12) has studied wound reaction in the Filicinean petiole, practically confining his attention to Leptosporangiate ferns. These ferns develop at an early stage Kroemer's (15) "Secondary" type of endodermis with complete suberisation of at least one tangential as well as the radial wall (Bäsecke (3)). It is therefore significant that whilst in all cases closure of the wound by processes of the nature of suberisation or wound gum formation was observed, meristem formation was only observed in wounds made at a region where the endodermis would only be partially differentiated or in Kroemer's primary stage. To avoid recurrence to the case of the Filicineae, it may be pointed out here that natural meristems, analogous to periderm formation, are only reported from the Eusporangiate Marattiaceae (Massart (16), *loc. cit.* p. 27) in which the endodermis in the petiole is missing and in the stem is only in the "primary" stage.

Wächter (29) has drawn attention to the fact that cuts on the cortical surfaces of the stem of *Hippuris vulgaris* L. are followed by suberisation and secretion of wound gum at the exposed surface, but that traces of meristem are never seen. As the stem of Hippuris, like that of most submerged aquatic plants, has a well-marked endodermis, this case is exactly parallel to the wound reaction described above for Camellia. This incapacity to form periderm seems general amongst aquatic Phanerogams (see Massart, *loc. cit.* p. 47). The one exception known to us, *Polygonum amphibium* L., is the one submerged aquatic stem in which a superficial cork layer is naturally present and in which cork is formed to heal cortical injuries: it is also the one submerged stem in which no trace of either a primary or secondary endodermis has been found.

Massart also draws attention (*loc. cit.* p. 50) to the incapacity of the cortical cells of roots to form wound cork. He discusses it in relation to the age of the tissues, but the real difference between the behaviour of the young cortical cells of the root of *Vicia Faba* L. and the shoot of *Sambucus nigra* L., contrasted by him, probably lies

in the fact that the endodermal barrier in the young root prevents the accumulation of sap essential to the phellogen (see p. 256).

Schneider-Orelli(27) has made some observations on apple and pear fruits which also admit of explanation from this point of view. The young fruits react to wounds by forming cork tissue, the ripe fruits do not. But this is not merely a question of age because the young fruits removed from the tree no longer form cork when wounded. The conclusion is natural that the cork formation depends upon the sap pressure still active in the young fruit upon the tree and no longer operative when the fruit is removed from the tree or has ripened.

Finally a reconsideration of the phenomena of wound healing in the case of leaves shows that they fall readily into line from this point of view. Blackman and Matthaei(4) have given a general account of the wound reaction in leaves. It may be recalled that all the various modifications of the process described show the following features in common. First a solid mass of tissue is formed at the cut surface by outgrowths from adjacent mesophyll cells, this proceeding *pari-passu* with the dying of the cut cells and some of their neighbours and the suberisation of the walls at this region. Within this solid mass of tissue, which seals off the internal intercellular spaces from the external air, there now develops a meristem in some cases but not in all. Under conditions of considerable moisture this meristem layer may act as an absciss layer and exfoliate tissues bordering on the injury; under drier conditions it forms periderm.

From our present standpoint these phenomena make a natural sequence. The only point we wish to emphasise is that under certain conditions, and in some plants, meristem formation follows the occlusion of the air spaces and sealing of the cut surface. This took place in Blackman and Matthaei's experiments, even in leafy branches cut off from the tree, but kept with their bases in water and their leaves in a moist atmosphere. Under these conditions (Priestley and Armstead(21)) it is quite possible for sap pressure to be developed within the tissue after the closure of the cut base of the branch by suberisation. The subsequent formation of callus, and, in the case of Oleander leaves, of crops of adventitious roots, is evidence that such sap pressure had indeed developed and therefore meristem formation was possible.

To obtain further evidence we have carried out experiments with injured leaves of *Prunus Laurocerasus* L., and *Camellia japonica* L., in which the cut leaves have been supplied with water, either by

standing them in water or applying it under increased or reduced pressure by means of a mercury column. The pressures were small, averaging about ten centimetres of mercury. The differences were very striking; under even this small reduced pressure these leaves were soon drooping and dry and even the young leaves ceased to grow. These leaves had to be left out of the comparison, but the leaves under increased pressure compared with those standing in water showed striking differences within the short period April 29th to May 12th. Under pressure a sharp brown edge had formed around the cut in one day, whilst without the pressure the brown edge formed much more slowly. By May 12th the cuts in the leaves under pressure could be distinguished with the naked eye by the thin translucent line, due to the formation of a meristem bordering the clearly defined brown margin on all sides. In the leaves not under pressure the brown margin was wider and lighter and there was still no meristem. In young leaves under pressure meristem formation was clearly visible in sections by May 10th. The interesting exfoliation patterns described by Blackman and Matthaei in the leaves of *Prunus Laurocerasus* kept in a moist atmosphere, seem to receive considerable elucidation if it is considered that the formation of the meristem, functioning here as an absciss layer, depends upon a sap pressure, the incidence of which throughout the leaf is determined by the venation system.

II. Leaf Fall

The formation of periderm at the leaf scar requires only brief consideration for which the necessary data are provided by Tison's(28) very comprehensive memoir on the subject. Reference to this paper will show that at the time the leaf falls, as the result of various types of activity in an absciss layer, the cushion of cells at the surface of the scar has already undergone, or immediately undergoes, a process of lignification and suberisation, the lignification being specially strong in the deeper seated layers. When the tissues of the leaf scar are blocked in this manner, the vascular strands being blocked by tylosis or the deposit of wound gum or other substance within the vessels and tracheids, then the formation of periderm may occur within the cushion, below the suberised and lignified surface layers. In no case does the meristem arise before the tissues of the scar are sealed by the previous suberisation. On the contrary in many cases no phellogen appears until the sap rises in the following spring; in the relatively few cases where the meristem is formed in

the same season as the leaf falls, Tison's data suggests that the suberised cap of tissue is formed unusually early so that the leaf scar is blocked before the sap pressure has ceased to be a significant internal factor with the advent of the autumn.

One small point of difficulty arises from a consideration of Tison's observations upon those compound leaves in which absciss layers are formed not only at the base of the main petiole but also at the base of the leaflets. In two cases, *Juglans regia* L. and *Pyrus aucuparia* L., he describes a periderm, very slight in character, as forming at the base of the leaflet scars, in the tissue of the petiole *after* its fall from the stem. This phenomenon was unexpected, but it is a point to which it is hoped to direct further attention as opportunity offers. So far, in the fallen petioles collected in this country, no trace of such a periderm has been found, but in *Fraxinus excelsior* L. a cork meristem has been found at the base of leaflets which have fallen before the main petiole. Probably closer examination will show that all cork layers at the base of leaflets have arisen whilst the meristem was still able to receive supplies from a petiole still in communication with the stem.

III. Normal Cork and Lenticel Formation

In the light of the previous discussion of wound cork and of the periderm arising below a leaf-scar the problem of normal cork formation upon stem and root may be visualised from an unusual angle. In any species the position of the cork phellogen seems quite definite but in different species this meristem arises in very different positions: it may be nearly superficial or even arise in the epidermis or it may be as deep seated as the pericycle. This question of the position in which the periderm arises obviously requires analysis to see whether it admits of correlation with a supply of sap from the vascular tissue and a blocked surface external to the phellogen which provides for an accumulation of this sap.

In the root the phellogen may occur in one of two alternative positions, either just within the endodermis (the usual position —de Bary(2), *loc. cit.* p. 553) or just within the exodermis. A very extensive analysis of cases will be required before any generalisation can be advanced with confidence to cover all cases of periderm formation in the root, but the following tentative conclusions have been found to cover all cases so far studied. When the growing root is young, the endodermis is in the primary stage (Priestley and North(22)) with fatty substances deposited in the radial and trans-

verse walls and with the protoplast firmly attached right across the cell to this girdle, the Casparian strip. As this fat impregnated layer is very impermeable, and at this stage the protoplasts are also relatively impermeable the endodermis provides a very complete barrier to the outward leakage of organic solutes. If a cork layer is now laid down the phellogen becomes active within the endodermis and the cork is therefore pericyclic in origin.

As the root grows older, the protoplasts of the endodermis become more permeable, but this alteration is usually associated with an accumulation of fatty substances all over their surface and the consequent formation of a suberin lamella. The cells of the endodermis have then passed into the secondary stage (Priestley and North (22)). If all the endodermal cells pass over into this stage then the endodermis has become a still more effective barrier, preventing the diffusion of inorganic salts as well as organic solutes. Within such a secondary endodermis a cork layer frequently arises in the pericycle, but no case has ever been seen of a phellogen active in the cortex outside such an endodermis.

On the other hand the fat deposits sometimes fail to accumulate in certain passage cells which therefore remain uncoated with a suberin lamella. The protoplasts of these cells are now readily permeable to organic solutes, as may be shown by the ease with which they admit the entry of organic dyes, and such an endodermis permits the formation of a cork phellogen external to it in the cortex. When such a phellogen arises, it always appears just below the exodermis which may be expected to act as the necessary blocking surface causing the accumulation of the sap flowing from the vascular tissues. An example of such a cortical cork layer arising outside an incomplete secondary endodermis and beneath a sclerenchymatous exodermal region is provided by the aerial root of *Philodendron erubescens*. *Monstera deliciosa* Liebm. provides an example of an aerial root which as it grows older never produces a secondary endodermis. As the protoplasts of the primary endodermis become more permeable, cork appears below the exodermis. In a root of Monstera, 20 feet long, obtained from the Cambridge Botanic Garden, a primary endodermis appeared within a few (8) inches of the tip; 27 inches from the tip a cork phellogen was visible and two or three layers of cells had been formed by its activity; at the base of the root six to eight layers of cork cells were present and a thick band of sclerenchyma had also formed outside the endodermis, but this endodermis itself remained primary.

One further case deserves mention. Mylius(17) has described a polyderm formation which is practically a type of multiple secondary endodermis. Within the original secondary endodermis a phellogen becomes active and cuts off two or three rows of cells. One of these rows then becomes converted into a secondary endodermis by the deposit of a suberin lamella upon every cell, the meristem then becomes active within this and the process may be repeated many times. A magnificent example of such a polyderm is provided by the raspberry, *Rubus Idaeus* L.

In the stem, Douliot(6) shows that the cork may be superficial, pericyclic or intermediate in origin. In view of the very numerous cases to be examined a causal explanation of the position taken by the phellogen can only be very tentative in nature. The following observations seem, however, to be relevant. In all the cases of pericyclic cork so far examined, with one exception, the stems have been found to possess a complete secondary endodermis within which the phellogen arises. Examples are provided by most dicotyledons growing upon British peat (Priestley and Hinchliff(25)), by *Escallonia rubra, Saxifraga rotundifolia* and *Camellia japonica*. Many underground rhizomes, as for instance several species of Geum, possess a secondary endodermis, within which a cork layer arises, the activity of this phellogen often leading later to the exfoliation of the cortex. All these cases are obviously in line with the general hypothesis put forward in this paper.

The exception is provided by species of Ribes in which no functional endodermal barrier can be found outside the deep seated phellogen. But experiment in which dyes such as acid green were forced under pressure into these stems showed that these dyes failed to leak into the outer cortex, save in the region of the nodes, even before any cork barrier prevented their diffusion. Further examination led to the conclusion that the superficial layers of the stem of these plants were really petiolar in nature (compare Saunders(26)) and save at the nodes were still delimited from the inner regions of the stem by the presence of a cuticle which could be traced by careful staining with Sudan III. The phellogen in this plant is not pericyclic in origin and its apparently deep seated origin is due to the presence of this envelope of petiolar tissue upon the stem.

The case of stems without a functional endodermis now requires consideration. In these stems it is a very suggestive fact, well established by the researches of Devaux(5), that periderm formation

begins at the lenticel, before the phellogen forms a continuous layer encircling the cortex. Devaux's extensive investigations provide other data of considerable interest, notably that the number of lenticels developed per internode suggest that lenticel formation is in direct relation to the vigour of growth of the shoot. As a result of his experimental analysis of the external and internal conditions leading to the formation of what he terms the primary lenticels upon a shoot, Devaux arrives at the conception that the formation of the lenticel depends upon an adequate supply of internal moisture, the "hydrose" of the tissue. As he also develops the idea that the continued functioning of the lenticel consists of an alternate closing, owing to extensive suberisation, and opening owing to the internal meristematic activity forcing the tissue through the hermetically sealed surface, an alternation of "cicatrisation" due to dryness of external and internal conditions and of proliferation due to excess of internal moisture, it is obvious that in one respect Devaux is very close to the point of view developed in this paper.

He departs from it widely however in that he sees the cicatrisation of the lenticel beginning with the proliferation of the subepidermal cells lining the stomatal chamber. He notes an early cutinisation of the surface of these cells lining the intercellular spaces bordering on the stoma (Devaux, *loc. cit.* pp. 81 and 97) but he regards this as a secondary feature. We should anticipate on the other hand that in this blocking of the surface cells bordering upon the stomatal chamber we have the causal feature preceding the sap accumulation, which in its turn brings about proliferation and the meristematic activity which results in the formation of the lenticel.

Attention was therefore specially directed to this point, and upon examining sections of young internodes of the stems of cork-forming plants clear evidence was obtained of the early cutinisation of the exposed surfaces of the cells bordering upon the stomatal chamber. In *Pyrus torminalis* Ehrb., *Acer pseudoplatanus* L. the first internode, in *Sambucus nigra* the second and in *Syringa vulgaris* L. the third, showed the clear red stain of a fatty deposit all round the surface of the stomatal cavity when sections were examined in Sudan III.

It seems that when the endodermis is not functional the positive pressure of sap within the vascular strand will be effective in irrigating the tissues right up to the subepidermal layer bordering upon the stoma which is probably thus supplied with water and solutes upon its free surface. As evaporation proceeds from this surface the

solute will be left deposited along the surface exposed to the air and thus a continuous deposit, very similar apparently in its nature to the original cuticle, appears upon the exposed surface. The presence of fatty substances in this layer is not surprising in view of the fact that Priestley and Armstead(21) found an ether soluble fatty substance present in the sap rising from the vine root, whilst Hansteen-Cranner(10, 11) has shown that fatty substances, both water soluble and water insoluble in nature, seem to form an invariable constituent of the walls in parenchymatous tissues. If these deposits at the wall surface are to be associated with such a drive of sap from the vascular strands, then the sub-stomatal cavity may be free from them where the stem endodermis is functional. Examination of the stomata upon the green cortical region of the stem of *Camellia japonica* certainly failed to show any signs of such deposits, stainable in Sudan III, the contrast in comparison with the stomatal cavities of the plants previously mentioned being very striking.

Douliot(6), in his extensive study of the distribution of the periderm in Dicotyledonous stems, draws attention to the marked effect of light upon the formation of periderm in the stem. In certain cases, *Prunus spinosa* L., *Acer oblongus* and *Virgilia lutea*, no periderm at all had formed upon the shady side of the stem when active periderm formation had occurred upon the sunny side. Some unpublished work by Miss R. Rea, M.Sc., in this laboratory, has drawn attention to the rôle played by sunlight in causing the rapid condensation of phellonic acid, one of the suberogenic acids (see Priestley(20)), but we are inclined to attribute this distribution of cork under lateral illumination to the more rapid evaporation from stomata in the sun and thus the earlier blocking of the substomatal apertures.

The facts and observations quoted above lead to the conclusion that the natural formation of cork at the surface of the stem follows as the result of the same general causal factors, previous blocking of sap flow, either at the endodermis or at the sub-stomatal region, followed by accumulation of sap to be followed again by the appearance of a meristematic phellogen.

SUMMARY

1. Cork formation has been studied from a generalised standpoint arising out of a study of the effect of the sap contained within the vascular strand upon the meristematic activity of the tissue supplied by these strands.

2. Wound cork formation, the scar left at leaf fall and natural cork formation all show the following causal sequence: first, the blocking of a parenchymatous surface, usually by a deposit of suberin or cutin formed in presence of air; secondly, the accumulation of sap at the parenchymatous surface thus blocked; and thirdly, the consequent development and activity of a phellogen amidst this parenchyma.

3. The pericyclic origin of a periderm must be attributed to the presence of a functional endodermal barrier when the phellogen is formed.

4. Special cases, such as the general absence of periderm formation in the cortex of roots, in the axes of aquatic plants, and in leptosporangiate ferns, provide additional evidence for the correctness of the causal sequence stated above.

5. In the case of cork formation in leaves experimental evidence is supplied to show that the meristem formation depends upon a sap pressure within the tissue.

6. Further experimental evidence is also supplied, confirming previous statements to the effect that the preliminary suberisation depends primarily upon air.

7. It is further shown that if the parenchymatous surfaces are artificially blocked by experimental methods, a subsequent sap pressure may produce meristematic activity as a result of which tissues like normal periderm may be formed, save that the walls are not suberised.

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