SUBJECT

THE ORIGIN OF FLESHY ROOTS PRODUCED ON APPLE GRAFTS BY THE HAIRY ROOT ORGANISM, PHYTOMONAS RHIZOGENES

By

Eugene Alfred Siegler

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

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INTRODUCTION

Apple grafts infected with the hairy-root organism, Phytomonas rhizogenes R.B.W.K. and S., develop numerous abnormal fleshy roots at the graft union. This development of roots is in striking contrast with the normally smooth well-knit graft union on which no roots are formed. The object of this investigation has been to determine, by anatomical studies, the origin and subsequent development of these pathogenic, fleshy, adventive roots and to attempt to explain the etiological relations which cause their initiation.

Apple trees are commonly propagated by grafting a stem cutting (scion element) on an understock or seedling (stock element) by placing freshly cut surfaces of these elements together in such a manner as to secure contact between the cambia of the two elements. This operation is usually performed when the plants are dormant. The grafts are then stored under conditions to insure callusing and at least partial "knitting" of the cut surfaces before they are planted. Thus it is seen that the exposure of cut surfaces and the subsequent callusing, especially so far as the scion element is concerned, is quite similar to the conditions which obtain in an ordinary stem cutting as prepared for the purpose of rooting. In the normal apple graft, roots are not produced from the scion element; roots are produced from the scion element in the graft union infected with the hairy-root organism.

Horticultural workers have been greatly interested in the factors involved in the rooting of stem cuttings in general. In the following discussion, however, it is proposed to confine the questions of callus formation and the rooting of stem cuttings to a brief resume of present-day knowledge with reference to woody plants only unless specifically stated otherwise.

REVIEW OF LITERATURE

Callus. Simon (19) defines callus as parenchymatous tissue which forms on wound surfaces after injury. Its origin undoubtedly varies between different species, although the evidence in many cases is not conclusive. Simon (19) and Eames and MacDaniels (4) and Priestley and Swingle (10) conclude that it results mostly from the proliferation of cambial cells. Knight (6) states that in plum cuttings the callus in all cases arises from the cambium. In the apple, Shippy (16) reports that the cambium is the important contributing tissue. However, the effect of polarity in callus formation should be given consideration in interpreting results. Simon (19) demonstrated not only a difference in origin but a qualitative difference between apical and basal calli in poplar cuttings. In apple grafts Sass (13) believes the cambium to be a minor factor, the primary cortex and secondary phloem being the most active in callus production. Recently Sharples and Gunnery (14) in several species have demonstrated callus resulting from the activity of medullar parenchyma and predict that its production by the ray cells will be found "to be a very common method in woody plants." Thus probably any parenchymatous cell may be able to form callus. Regardless of its origin, the quantity of callus formed is quite generally greatest at the basal end of cuttings and also at the lower end of the long sloping cut made in preparing the scion for the graft.

The processes of callusing and rooting of woody cuttings, and the processes concerned in the "knitting" of the graft union are somewhat analogous, but, as stated, differ in the fact that normally no rooting occurs on the apple cutting when the cut surface is placed in contact with a similarly made cut surface on a seedling root. However, infection with the hairy-root organism does result in root formation from the stem cutting. Therefore comparison between (a) normal rooting of stem cuttings in general, (b) normal "knitting" of the apple graft union, and (c) abnormal root production in the apple graft union is pertinent.

Priestley (9), Priestley and Swingle (10) have furnished an excellent review of the subject of apical meristem formation, especially from an anatomical standpoint, in soft-wood cuttings. Their investigations and analyses of the various phenomena associated with vegetative propagation constitute a definite advance in the manner of treatment in comparison with previous investigations of the problem in that attempts are made to relate structural changes with metabolic and physiological activities.

Callus in woody cuttings. The role of callus in the rooting of woody cuttings is somewhat questionable. However, undoubtedly it serves at least as a mechanical means of keeping the tissue from excessive drying (Priestley and Swingle (10)). This formation is dependent upon the development of suberized layers in the peripheral callus cells (Stoll (23), Priestley (9). Later there is developed a phellogen or cork cambium internal and adjacent to this suberized tissue. The normal function of this phellogen is to produce periderm.

Rooting of woody cuttings. The rooting of cuttings depends upon the further activities in the callus and upon true vascular activity, as

discussed at length by Priestley and Swingle (10). (Parenthetically it is stated that hereafter in this text the term cambium will refer to a true vascular cambium and the term phellogen will refer to the cork cambium). In the absence of pre-formed root initials, a regeneration or formation of adventive root primordia and their subsequent development in or adjacent to the callussed tissue is (Priestley and Swingle (10), Yerkes (28)) necessary to establish the cutting as a plant. Among hard-wood plants there is considerable variation, even in species and varieties, in the ability of stem cuttings to root. In the older literature, emphasis was placed on callus formation as a preliminary for rooting. Corbett (2) was one of the first to suggest that excessive callusing might inhibit rooting. Other workers (Van der Lek (26)) now agree that excessive callus formation and rooting are not necessarily compatible. This trend of thought is interesting in the light of this investigation.

Role of cambium. Cambial activity is at least necessary for the formation of root initials in cuttings (Stoll (23), Priestley and Swingle (10), and others). The endogenous origin of roots in general is well established (Strasburger (24), Eames and MacDaniels (4)). Priestley observes that not only must a meristem for root production be present but also internal conditions of nutrition be such as to favor the development of these meristems into roots. Later Priestley and Swingle (10) clearly demonstrate the initiation of root primordia in a region of vascular cambial activity (in soft-wood cuttings).

Rooting of fruit trees. Fruit trees in general are difficult to root from cuttings, although there are numerous exceptions. A species of plum (Prunus mariana) roots readily according to Knight (6). The Kieffer pear (Pyrus communis) is often propagated on its own roots in

the southern United States. The apple (Malus malus), despite numerous attempts, has proven most difficult to root and has never been successfully rooted commercially in the United States. However, Stewart (22) referring to woody cuttings in general, observes that " ... for quick propagation this large callus should be cut off." Balfour (1) states that "by paring off the surface of the callus-tumor it is possible to stimulate almost immediate root development" (in Clematis). Shaw(15) in America notes a correlation between the large number of cells constituting the cambial layer and the ability of the variety (apple) to form roots. These statements regarding cambial activity and those suggesting a mechanical alteration of the callus are significant in view of the conceptions which will be further developed in the discussion of results obtained in this investigation.

Grafting. Resort to grafting is necessary to propagate the apple. Waugh (27) emphasized the importance of the cambia of the stock and scion elements, in the formation of the graft union. Recently Sass (13) has clearly shown the various stages of callus "bridging" between the cut surfaces of the scion and stock. Further growth, he states, is dependent upon " ... an arc of cambium, continuous with the respective cambia of the stock and scion (which) is differentiated from cells of the intervening callus." After more or less successful union of cambial tissues between the scion and the stock elements growth takes place in a normal manner in that xylem and phloem elements are differentiated by normal divisions of the cambial cells.

Roots are produced on the infected graft union. In comparison with the well-knit union, free from roots, of the normal apple graft, the "hairy root" union is marked by the presence of numerous roots, abnormally fleshy in character, and their presence prevents knitting of

the cambia and the calli of the stock and scion in a normal manner. The union is mechanically weak and the resulting trees are dwarfed. No anatomical studies have been reported on this phase of the subject although Hildebrand (5) in an abstract in American Phytopathological Society 1933, states that root primordia arise in medullary ray cells in the region of the vascular cambium. Since he states that these root primordia are initiated within approximately two weeks after inoculation, it is thought that his inoculations were made at areas other than the graft union.

The pathogene. This organism was probably originally isolated by Smith and Townsend (21) and put in the general crown gall group. When the question of the identity of a type of malformation on apple grafts known as "woolly knots" was raised (Riker and Keitt (11), Muncie (8)), this organism was designated as the apple strain of crown gall (Siegler (17)). Recently Riker et al (12) have classified it and named it P. rhizogenes. Infection generally results in the formation of roots on a number of hosts.

METHODS

Apple grafts were made, using the whip and tongue method as in commercial practice. In order to avoid complicating factors, the graft union was not wrapped. The control or noninoculated grafts were stored in the same container as the inoculated grafts and all conditions of storage and growth were as uniform as possible so far as the inoculated and control grafts were concerned. Inoculation was accomplished by immersing the freshly made grafts in a water suspension of the inoculum. To facilitate examination, most of the grafts were grown on seedlings

which were planted in small pots and sunk in the soil in the greenhouse after they had callused for approximately three weeks. The graft union was covered in most cases with granulated peat moss kept just sufficiently moist to keep the scion from drying out. There was no difficulty in securing practically a 100-per cent stand by this method. In most of the experiments the variety Early Ripe was grafted on ordinary apple seedlings. In other experiments scions were grafted on clonal roots, grafts were callused in a mixture of sand and sawdust, and were field planted in the normal way. In all cases the understocks were thoroughly washed in water, dipped in bichloride of mercury solution, and washed thoroughly again in water before cutting for grafting, to insure freedom against contamination by any organisms which might be present on the understocks. This procedure quite uniformly results in the securing on the controls of comparatively well "knit" unions with a minimum of excess callus development, as shown in figure 1 A, whereas on the inoculated grafts 100-per cent infection generally results, as evidenced by subsequent formation of roots. The callus protuberances which result on the inoculated graft union are illustrated in figure 1 B. The grafts were inspected from time to time and material taken to be prepared for microscopical examination was collected at appropriate intervals. Because attention has been mainly confined to the origin of root primordia the initial origin and formation of callus has not been studied.

RESULTS

In confirmation of previous observations the formation of roots at the graft-union was invariably confined to the scion element. Further, although roots may occur at any point on the previously cut

surface of the scion it has been generally observed that they are largely confined to either the upper or lower areas of the cut surface (figure 2).

The callused graft-union generally shows a prominent lobe of callus protruding from the lower end of the scion. This most prominent lobe, among the numerous convolutions which may be present, is formed as a result of vascular cambium extension and activity which develops by growth from the cambium present at the lower end of the freshly cut surface of the scion. In all cases examined, this cambial extension bends and forms a rounded to oval-shaped area in this region (fig. 3 B). Considerable proliferation also occurs from the cortical region, especially from the phloem areas, as shown in figure 3 A at F, internal to the dark stained bast fibers. Radial growth and also callus proliferation occur from the cork phellogen in figure 4 at P.

In the case of both the inoculated and the control grafts, wood elements invariably, and most of the callus tissue are formed as a direct result of the activity, extension, and divisions of the true vascular cambium, as shown clearly in figure 3 B. No wood elements have been observed which could not be traced directly in their origin to true vascular cambium division. So-called "isolated" areas or woody "knurls", as apparently shown at W_1, W_2, figure 3 A, occur. Their presence is invariably due to the activity of an outward extension of the vascular cambium. Figure 3 B is a medium section showing the actual union of the areas W_1 with W_2 in figure 3 A at a lower level. In a tangential view, the extensions of the vascular cambium cells show typical fusiform cambial initials, as shown in figure 7. Cell division is so rapid as to give the appearance of multinucleate cells. It is emphasized at this point that this cambial layer results not from renewed activity of previously existing semi-

meristematic callus tissue but that it is an actual extension of, and is genetically connected with, the true vascular cambium. No differentiation posteriorly, as occurs in the case of the procambium at the growing point of the shoot, has been observed.

After an indefinite period of callus formation, depending upon the environmental conditions under which the grafts are stored, a suberin layer, of indefinite thickness, develops around the periphery of the callus. After planting and shortly after the buds push, the suberin layer is well developed and periderm formation proceeds in a normal manner on the control grafts, as shown in figure 8 A. In contrast with this, comparatively little normal periderm formation occurs in the infected grafts. On the contrary, callus proliferation is marked (fig. 8 B). This periderm formation is undoubtedly the result of the activity of a phellogen formed from parenchymatous callus cells several layers in from the periphery of the callus mass. It is important to note that this phellogen, as a rule, is not so strongly developed in the case of the inoculated grafts (fig. 5) in comparison with the control grafts (fig. 6).

Numerous lobes are formed, especially on the inoculated grafts, as a result of proliferation of callus cells in tissues where suberized layers and the phellogen are not well developed or have wholly or partially disappeared (figs. 9, 10) precisely as Priestley and Swingle (10) illustrate in their plate 8, figure A. In suberized areas, before any secondary proliferation occurs, cambial activity in this region has been confined to cutting off woody elements internally, and parenchymatous tissue externally, as shown in figure 3 B. When, however, phellogen activity becomes diminished and the "overflow" of proliferated callus tissue occurs, the cambial layer increases in thickness (fig. 11) and the cambial cells lose their former orientation (figs. 12, 13) and as-

sume a more or less perpendicular alignment to their previous position (figs.14, 15). Wood elements continue to be cut off internally and as a result of the orientation of the cambial cells these wood elements assume a position parallel to that of the longitudinal axis of the proliferation (fig. 16). At a point immediately anterior to these woody elements certain cambial cells, presumably, or certainly their immediate derivatives become flattened and form the meristematic, densely protoplasmic, apical cells of a root primordium (figs. 17, 18). Whether one individual cambial fusiform initial cell or several of these cells form the original intensely meristematic apical area could not be determined. Figures 17 and 18 show the earliest stages encountered and it appears that no very great number of the original cambial cells are concerned in the formation of the region of greatest activity of the growing point. This phenomenon occurs only after photosynthetic activity as a result of twig growth of approximately three inches and attendant leaf production has occurred. The usual intense activity at the growing point of course now proceeds with such extreme rapidity that the outlines of definite root primordia are clearly distinguished (figs. 19, 20).

The further growth of the root primordium with its surrounding envelope of cortical cells consisting partly of the pre-existing callus tissue, delimited usually by the suberized adjacent tissue, is shown in figures 21 to 26. The excessively "fleshy" character of these roots is due to the fact that in the vast majority of cases the primordia originate in a lobe of proliferated callus tissue which presents a macroscopic appearance shown in figure 8 B. This callus tissue is developed with the internally developing stelar tissue in its subsequent growth.

Simultaneously, spongy parenchymatous tissue is being formed from the stelar region and, due to the semi-meristematic condition of the pre-existing callus cells, there is no line of demarcation between the older parenchyma and that more recently formed. That the cortical region of the growing root consists of former callus tissue is clearly shown in figures 22, 23. Here also are shown the suberized peripheral layers of the former callus lobe. These layers form the calyptrogen. That a faintly defined layer of phellogen which has lost its meristematic properties, may also function as a calyptrogen is indicated in figure 27. The suberization quickly disappears, however, in these regions and root hairs are formed. When several primordia are emerging through a callus area the callus tissue appears to be torn mechanically resulting in a smaller bulk of callus cells being "carried along" as cortical tissue (figs. 22, 24).

Generally large cavities are observed in the rough outline of an arc anterior to the growing point (fig. 21). These cavities appear to result from dissolution of cell walls and contents. The cell walls and cellular contents disappear and the nuclei are seen to be degenerating in the formation of these cavities (figs. 28, 29).

Summary of results. The important factors, therefore, contributing to the formation of the root initials appear to be initiation, activity and development of

(1) The pre-existing dormant or semi-dormant vascular cambium

(2) The formation of a callus resulting in

(3) The suberization of cells around the periphery of the callus resulting in

(4) The formation of a cork phellogen

(5) The partial disappearance or decrease in intensity of suberization and adjacent phellogen activity in various areas

(6) The "pouring out" or proliferation of callus cells as a result of the destruction of the phellogen

(7) Reorientation of the vascular cambial cells in the regions opposite but only subsequent to the break occurring in the phellogen barrier

(8) Reorientation of the xylem elements as a result of the new orientation of the fusiform initials

(9) The direct transformation of fusiform initials into a meristem which is early recognized as a root initial

(10) The appositional development of the callus tissue with the stelar and indefinite cortical regions of the primordium results in an abnormal "fleshiness" of the adventive roots.

DISCUSSION

In the present investigation it should be noted that pathological tissues of hard-wood cuttings are concerned and therefore comparisons and discussions relating these investigations with those of other investigators may perhaps be open to question. However, it is hoped that the present studies on abnormally developed tissues may actually furnish a

partial explanation to those phenomena occurring in normal tissues. In these studies we have an accentuated condition, namely, the abnormal production of roots, and the very fact that there is an abnormality may perhaps serve to clarify rather than to confuse the more or less fundamental questions concerned. In other words, extremes rather than normal occurrences, particularly from the structural point of view, may emphasize the reactions sought sufficiently to make them evident.

Priestley (9) and Priestley and Swingle (10), as stated, have furnished an excellent anatomical and physiological background on the formation of adventive root and shoot meristems. Referring especially to adventive root initials on soft-wood stem cuttings, they are strongly inclined to the view that "any single meristematic cell is capable of forming part of an organized group which may function as any of the meristematic organizations characteristic of the plant whether phellogen cambium or root or shoot apex." However, as they clearly state, the possibilities of this differentiation "are definitely limited by its position in a complex organization." These workers stress the conception that a living cell or group of cells may resume activity to the extent of becoming meristematic because of their particular position in the organism as a whole. When a callus is formed there is not only a new environment created but new cells are produced. As a result of this new organization of tissue there is furnished an excellent means of studying the ontogeny of various tissues and correlating their mutual relations. In this discussion therefore it seems desirable to discover and follow the successive developments which initiate the formation of root primordia in the callus produced on these stem cuttings, grafted to a root.

It is recognized that cambial activity is the important factor resulting in the healing of wounds such as are made in grafting. It has been variously stated that new cambium is formed by differentiation of an inner layer of cells (Strasburger (24)) and a cambium regenerated from callus (Sitton (18)) and a cambium differentiating from spongy callus (Sass (13)). In this investigation no vascular cambium tissue was ever encountered that could not be traced genetically to the cambium previously present on the cut surface of the stem. The extension of the cambium is apparently accomplished by strand-like growth, as seen in cross section, from the pre-existing cambial cells. There was no evidence that it can be formed "de nova" or "regenerated" in the usual sense of the word, in these cuttings.

The formation of a callus at the basal end of the scion, due mainly to the activity of a vascular cambium, is in harmony with the results on Forsythia shown by Priestley and Swingle (10) in their plate 9, figure B. It is noted that there is a general conformity between the gross outline of the callus pad and the "arc" produced by the extension of the vascular cambium. The suberization of cells, generally several layers in thickness about the periphery of the callus pad, is fully discussed by Priestley and Swingle (10), and apparently results in limiting the amount of callus formed. The fact that normal periderm formation is markedly restricted in the case of grafts inoculated with the hairy-root organism indicates that these organisms act in some manner to inhibit suberin formation.

A cork phellogen is formed only after a certain amount of suberization has occurred. Its presence is never noted previous to suberization and it disappears when suberization is decreased or entirely lost. This is clearly shown in figures 9, 10, 11 where new prolifer-

ations, forming lobes in the callus, result whenever the suberin barrier disappears. As stated, this observation is in close agreement with that of Priestley and Swingle (10),as shown in their plate 8, figure A.

While it is not desired to minimize the importance of metabolic and physiological activity in connection with the initiation and growth of group meristems, it is nevertheless desired to note the fact that here we apparently have a structural explanation of the phenomenon. The reorientation of the cells of the vascular cambium as a direct result of the break or loss in intensity of the suberized layer and in the gradual disappearance of the cork phellogen, as shown in figure 12, at least furnishes definite evidence on the question of root initiation. Following Priestley and Swingle's (10) line of thought, we have the mechanical "setting" prepared for future developments which occur when the necessary metabolic equilibrium is reached. The evidence in this investigation is that the structural conditions of surrounding cells are important in making for this environment. This is exemplified by the fact that, regardless of the number of breaks in the "barrier" opposite well-developed vascular tissue, no distortion and reorientation of the cambial cells opposite these "breaks" are evident until a certain amount of photosynthetic action has been accomplished. In these experiments no indication of apical meristem formation appeared until shoot growth had reached several inches. The "formative stuffs" theory of Sachs, while perhaps inadequate (Priestley and Swingle (10)), still deserves consideration. We may require not only the position of a cell or group of cells in an organization but also proper physiological activities for the initiation of root initials.

The position of the newly formed xylem elements is changed as a natural result of cambial reorientation. Typical cambial fusiform initials in a late stage of this reorientation were shown in figure 7.

The final step prior to the initiation of the apical meristem is probably the direct transforming of one or more fusiform cambial initials or their immediate derivatives, into cells constituting a typical apical meristem. It is generally conceded that the organization of a root primordium is dependent upon and in close proximity to a vascular cambium. Priestley and Swingle (10) emphasized this fact and in this connection note that Stoll (23) originally showed that the origin of roots can usually be found to be in the neighborhood of dividing cells, which are still genetically connected with the vascular cambium. No regularly organized tissue, such as medullary ray cells (Priestley and Swingle (10)), is formed preliminary to the organization of these primordia and there is no indication of the initiation of an apical meristem prior to the reorientation of the fusiform initial cells.

The subsequent development and growth of the apical meristem is readily followed, and has been discussed. In this connection, Priestley and Swingle (10) state that the phellogen may constitute part of the root cap of the adventive root primordia in cuttings of the herbaceous Dicot, Crambe. The region which previously had been organized as a phellogen in this investigation is shown frequently to constitute a calyptrogen. Further, the semi-meristematic cells of the callus, enveloping the stelar tissues and the region of greatest meristematic activity of the growing point, constitute the bulk of the cortical regions of the advancing root primordium.

The evidence is that cambial activity is confined largely to the formation of xylem elements, largely in the form of wound wood (figs. 3, 25) (Stoll (23), Krieg (7), Sledge (20))when the outer callus cells are strongly suberized or firmly pressed against the tissues of the cut surface of the seedling (fig. 3 B at S). When suberization is partially destroyed and, as a result, the phellogen loses its identity, wholly or in part (fig. 11), cambial activity is changed in that the number of cambial "layers" greatly increase (fig. 11 at C); the cambial cells change their orientation and there is an extension of the cambium into the enlarging callus "lobe." (fig. 30). Increases in size of this callus lobe result largely from the cambial divisions of this cambium and not until sufficient suberization and phellogen activity are again manifest, does the normal vascular activity of the cambium appear. The cambial extension may curve to form an arc or a loop depending upon the degree of suberization which develops. Xylem elements are not formed when an insufficient amount of suberization occurs (fig. 31). Progressive stages in suberin formation, appearance of a phellogen, and differentiation of xylem elements are shown in figures 32 and 33. Priestley and Swingle (10) consider the possible effect of a pH gradient in determining meristem organization and activity. No attempts have been made to confirm this hypothesis but the results of this investigation suggest that cambial and phellogen activities are largely influenced by definite structural characters of adjacent tissues.

The fact that numerous breaks in the suberin "barrier" occur in the callus grafts infected with the hairy-root organism as contrasted with the comparatively few breaks in the nonpathogenic callus, indicates that the bacteria act in some manner so as to either inhibit, destroy, or render ineffective the suberin deposits. To demonstrate this point,

suitable technic would have to be developed. Breaks in the suberin "barrier" do occur in the nonpathogenic callus but the periphery of the proliferating cells soon becomes enveloped with suberized tissue. Priestley and Swingle (10) state that root primordia do not form in a region of the vascular cambium opposite such "breaks", in Crambe, as shown in their plate 8, A. In this investigation, however, the formation of root primordia has been observed, invariably, to occur in a region opposite such a break but only after a degree of suberization in the periphery of the callus has been subsequently effected. A somewhat indefinite amount of suberin and phellogen appears to be necessary to fulfill conditions necessary for the initiation of root primordia. A too highly suberized layer evidently has a retarding effect (fig. 33) while excess proliferation under conditions which inhibit suberization results merely in an extension of the vascular cambium into the callus pad (fig. 31) and not until optimum suberization occurs, does the cambial extension develop xylem elements and root initials. Early stages of root initials have never been observed unless the degree of suberization in the callus cells enveloping them was sufficient to result in immediate differentiation of the cambial cells into xylem elements.

If the assumption that bacterial action is effective in so modifying the character of the suberized layers and the phellogen as to produce, even in a mechanical manner, a condition in the neighboring cambial tissues, an explanation is offered as to why paring of the callus favors rooting, as noted by Balfour (1) and Stewart (22). A callus may be either too highly suberized (normal apple graft) or not sufficiently suberized (apple graft infected with the crown gall organism) to be in a condition favorable for initiation of primordia. Thus

the observations of Corbett (2), Knight (6), Swingle (25), and Shippy (16) that excess callus is not necessarily favorable for rooting, are in harmony with this conception and perhaps may be explained on an anatomical basis.

Zimmerman notes the effect of the pH of the medium and the effect of chemical treatments on the rooting of cuttings has been investigated by Curtis (3) and others. The latter (3) suggests that potassium permanganate favors rooting in that respiratory activity is increased by catalytically hastening oxidation. Again the suggestion is made that chemicals might be effective in either producing or altering a suberized condition as to make for optimal conditions for the formation of primordia.

The suggestive interpretations discussed above must be broadened to include the conception that metabolic and physiological processes are of signal importance in determining the development of cellular organizations. Regardless of the state of organization in the callus, root initials have never been observed to form until there is some photosynthetic activity. A certain physiological age must be attained before root primordia are initiated. Grafts kept six weeks in cool storage formed numerous callus "lobes" but no root primordia, whereas grafts of the same age but forced into growth contained primordia when a shoot growth of approximately three inches had been attained. Further, while an analogy has been made between the rooting of stem cuttings and the development of roots from the scion element of the graft union attention should be directed to the fact that adventive scion roots do not develop in an absence of a superficial contact, at least, between the calli of the stock and scion elements.

CONCLUSIONS

Infection on apple grafts with the hairy-root organism Phytomonas rhizogenes R.K.W.B. and S. results in the initiation and development of adventive root primordia which are genetically connected with the vascular cambium of the scion element.

The structural characteristics of the callus which forms on the cut surface of the scion markedly influence the internal cellular organization.

When callus suberization is optimal and metabolic and physiological processes are favorable the vascular cambial cells assume an orientation at right angles to their previous position. The longitudinal axis of the xylem elements which are subsequently differentiated, parallels the longitudinal axis of the advancing cambial initials. Root initials are formed at the anterior of this cambial strand by direct transformation of fusiform initials or their immediate derivatives into meristematic growing points.

The fleshy character of the adventive roots is due to the fact that callus parenchyma develops with the stelar and cortical regions of the primordium. The region of the callus previously organized as suberin and phellogen frequently forms the calyptrogen of the primordium.

The fact that the structural characteristics of the callus so markedly influence the internal cellular organization may aid in interpreting the various conceptions concerning the callusing and rooting of hard-wood stem cutting.

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FIG. 1. Apple grafts, variety Early Ripe. (Natural size).
A shows normal healing at the union (X).
B shows excess callusing at the union, with numerous callus "lobes." (X)

2

FIG. 2. Apple graft showing numerous fleshy roots developing at the graft union. (Natural size).

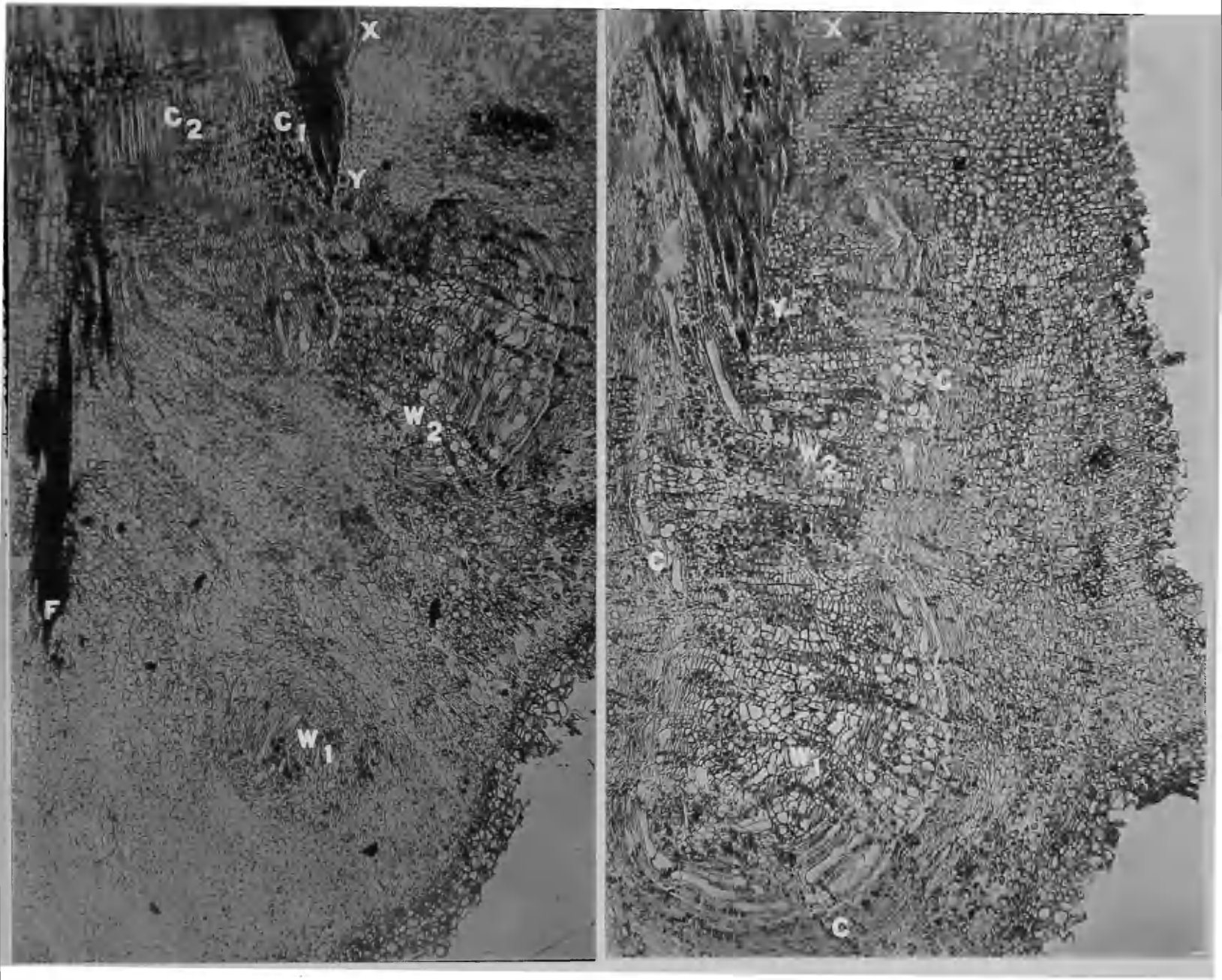
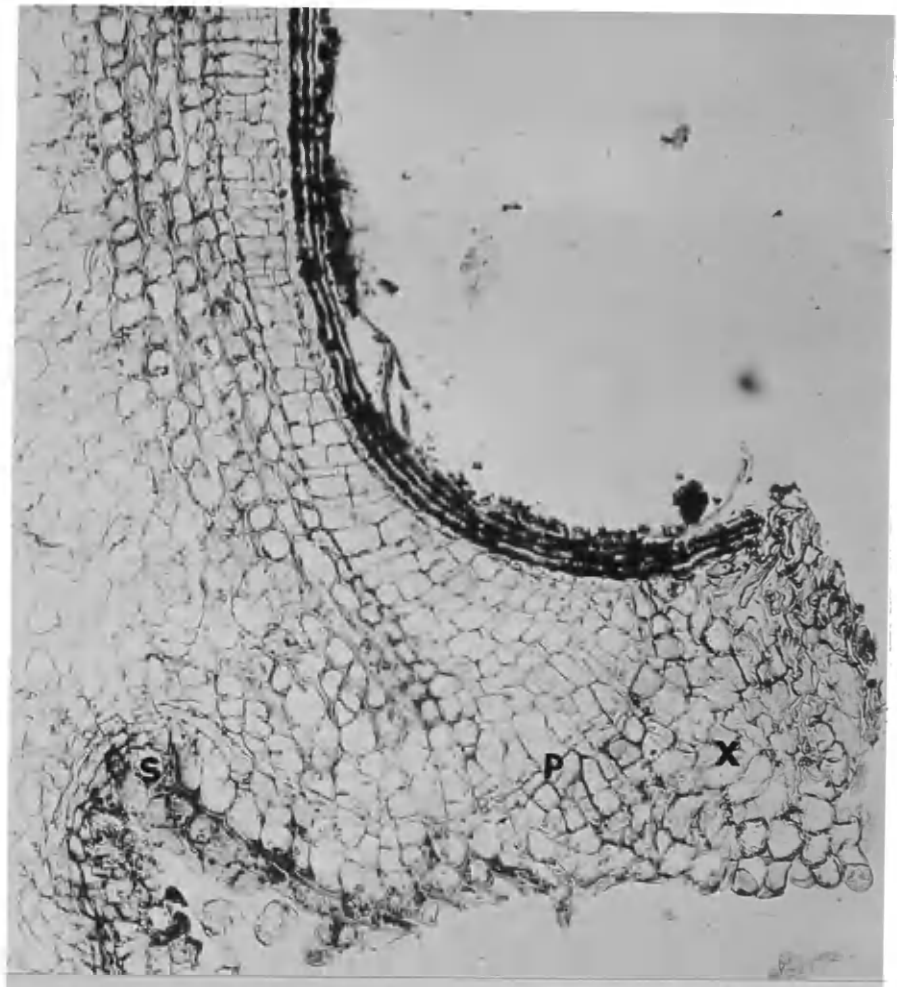


FIG. 3. Longi section from lower end of the scion. X-Y marks the slope of the cut through the xylem. (x 70)
W, in A, is clearly an outer extension of W, in B. A, F marks bast fibers. Due to radial growth the position of the cambium has progressed from C to C_1. The so-called "isolated" new wood elements are shown at W_1 and W_2.
B, median section of A, showing W_1 and W_2 connected by the cambial loop C.



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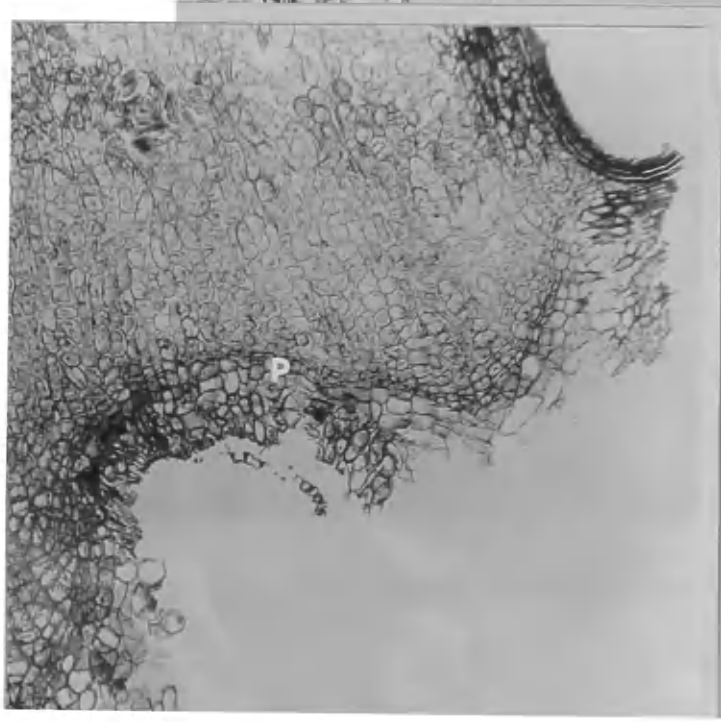

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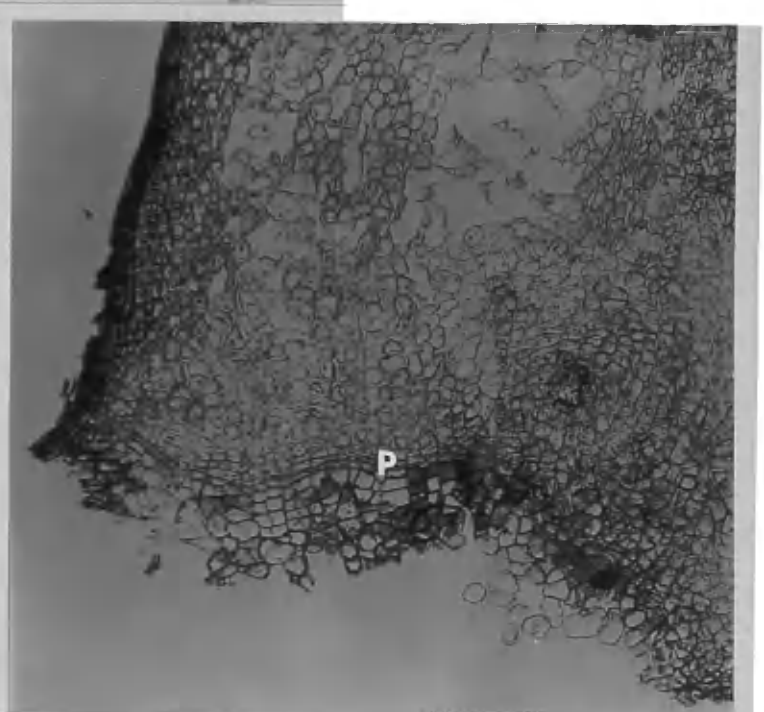

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FIG. 4. Radial growth resulting from phellogen activity is shown between the region of the older periderm and the pre-existing cortical cells. Proliferation from the phellogen at the extreme lower end of the scion is shown at X. A slightly defined phellogen is present at P and well defined beneath the suberized area at S. (x 140)

FIG. 5. Inoculated graft. Callus shows slight suberization and only slightly defined phellogen (P). (x 90)

FIG. 6. Non-inoculated graft. Callus shows more suberization and better defined phellogen (P). (x 90)

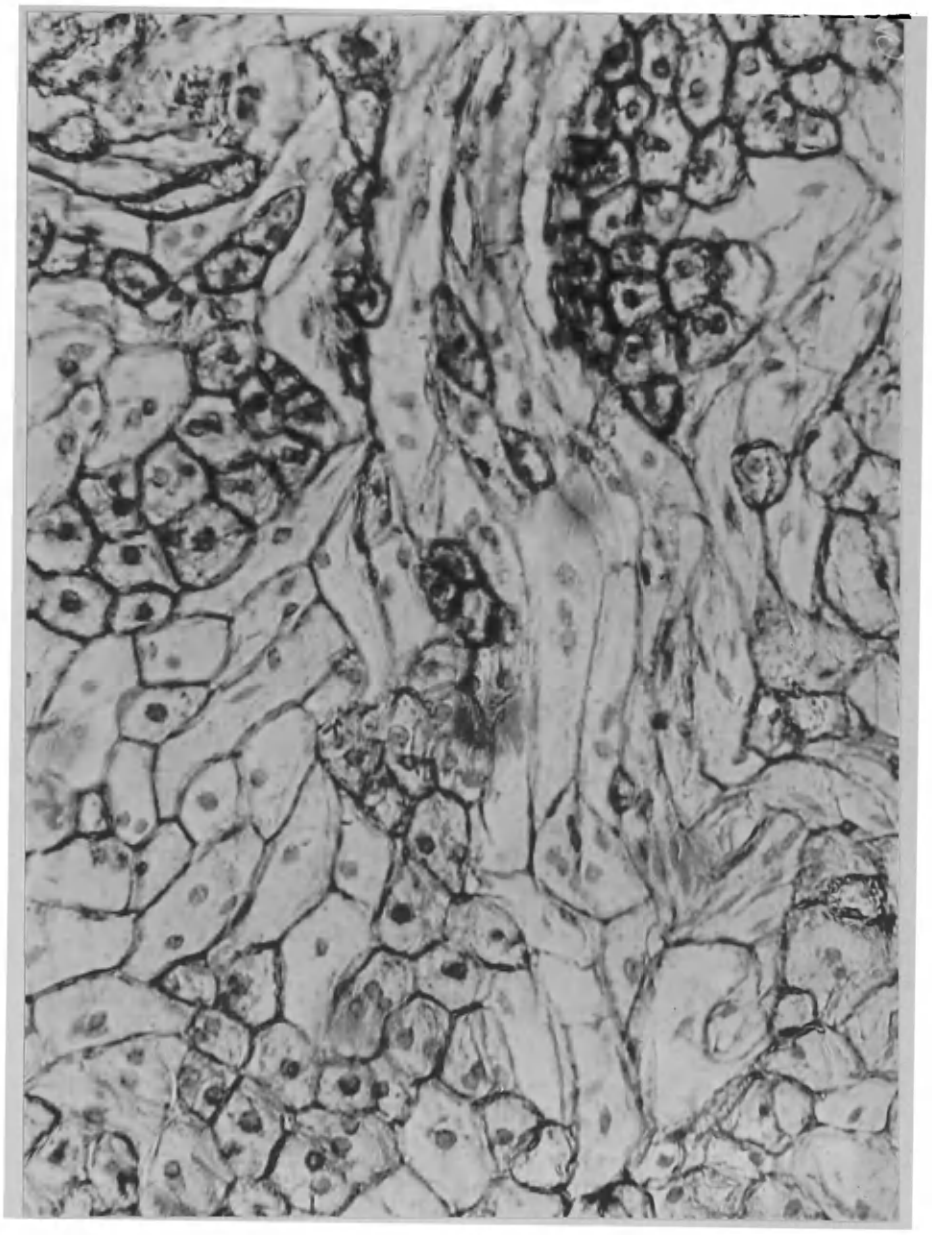

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FIG. 7. Typical fusiform cambial initials, as seen in tangential section, growing from the vascular cambium of the scion. The appearance of a multinucleate condition is due to extremely rapid cell division. (x 400)

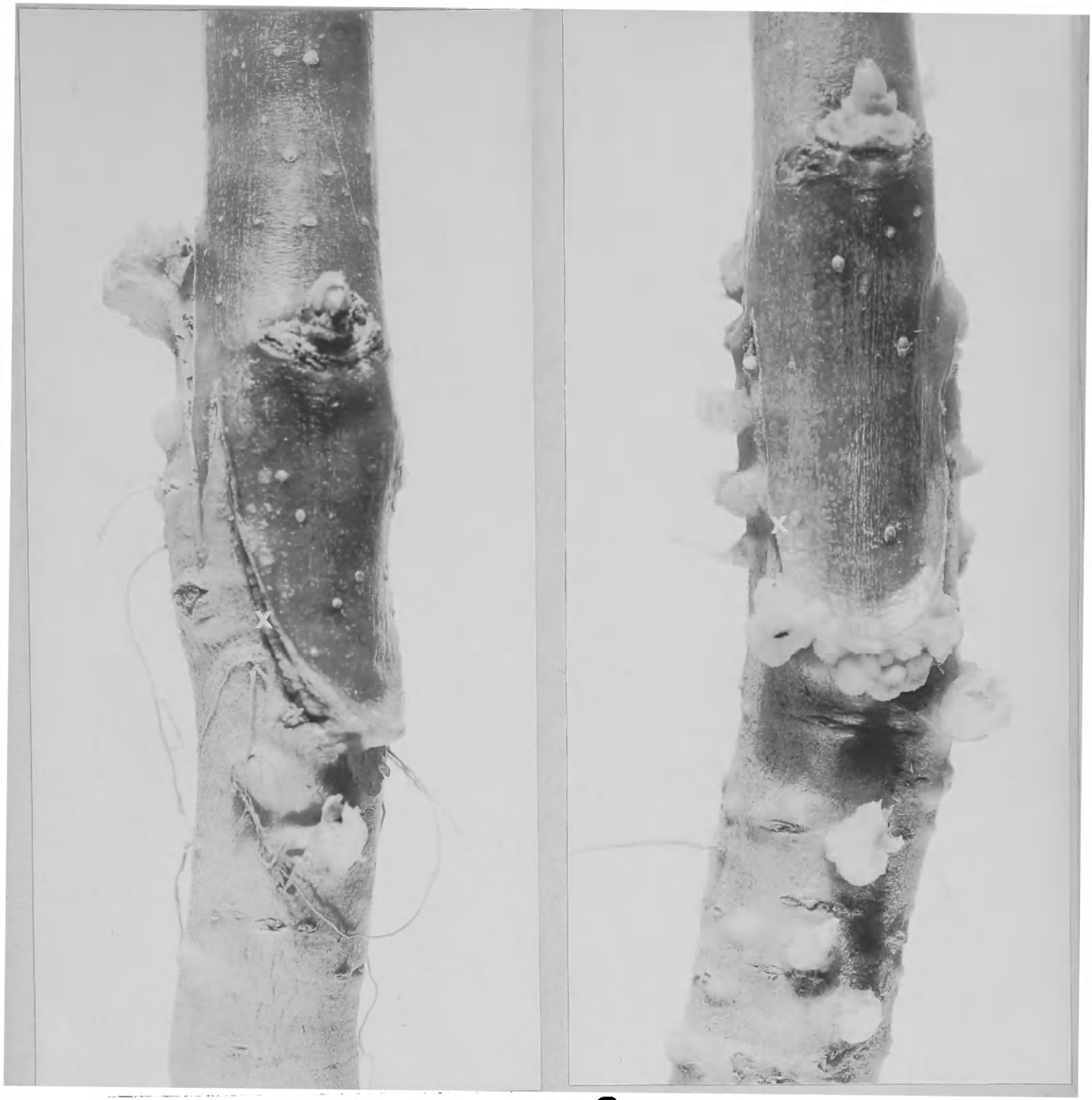

FIG. 8. Fig. 1 enlarged (x circa 4).
A. "Knitting" of union and periderm formation (X) on the non-inoculated graft.
B. Callus lobes, especially at the lower end of cut surface of the scion (X).

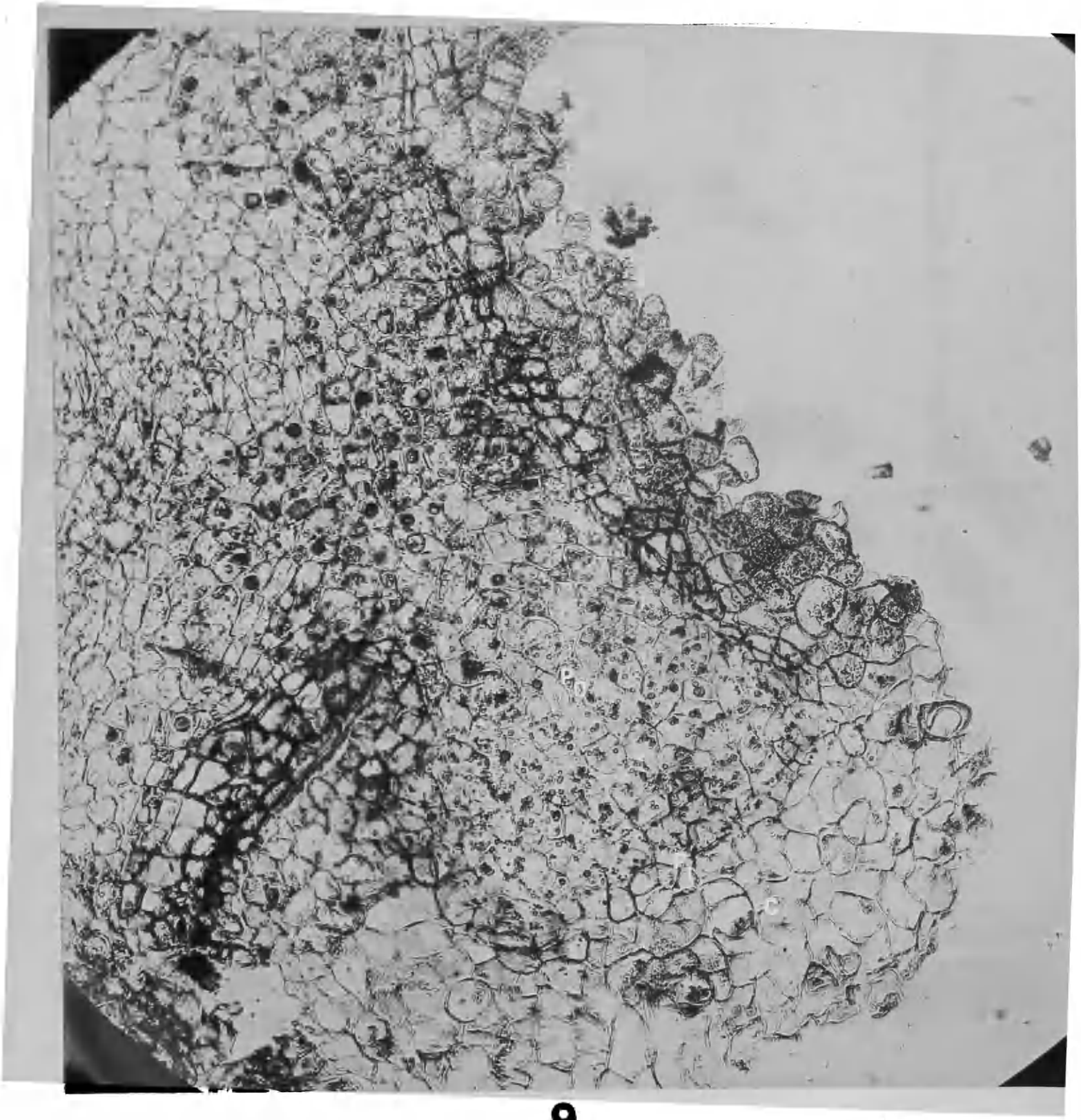

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FIG. 9. Proliferation of the callus cells (C) as a result of disappearance of suberin and the phellogen. The position of the former phellogen has been changed from P_0 to P_1, as shown by the outline of its present position (P) and the presence of fat globules in the older callus cells. (x 210)

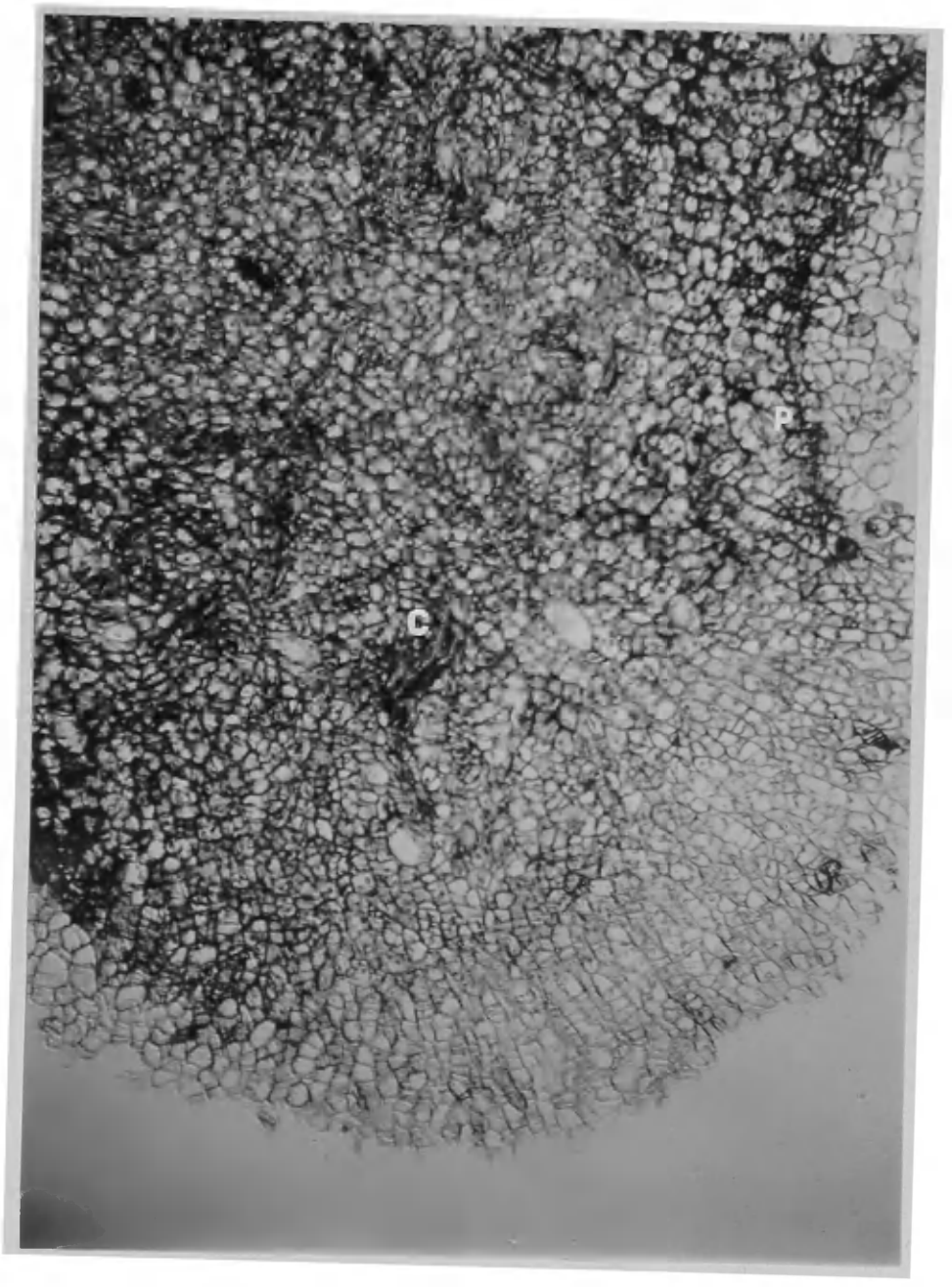

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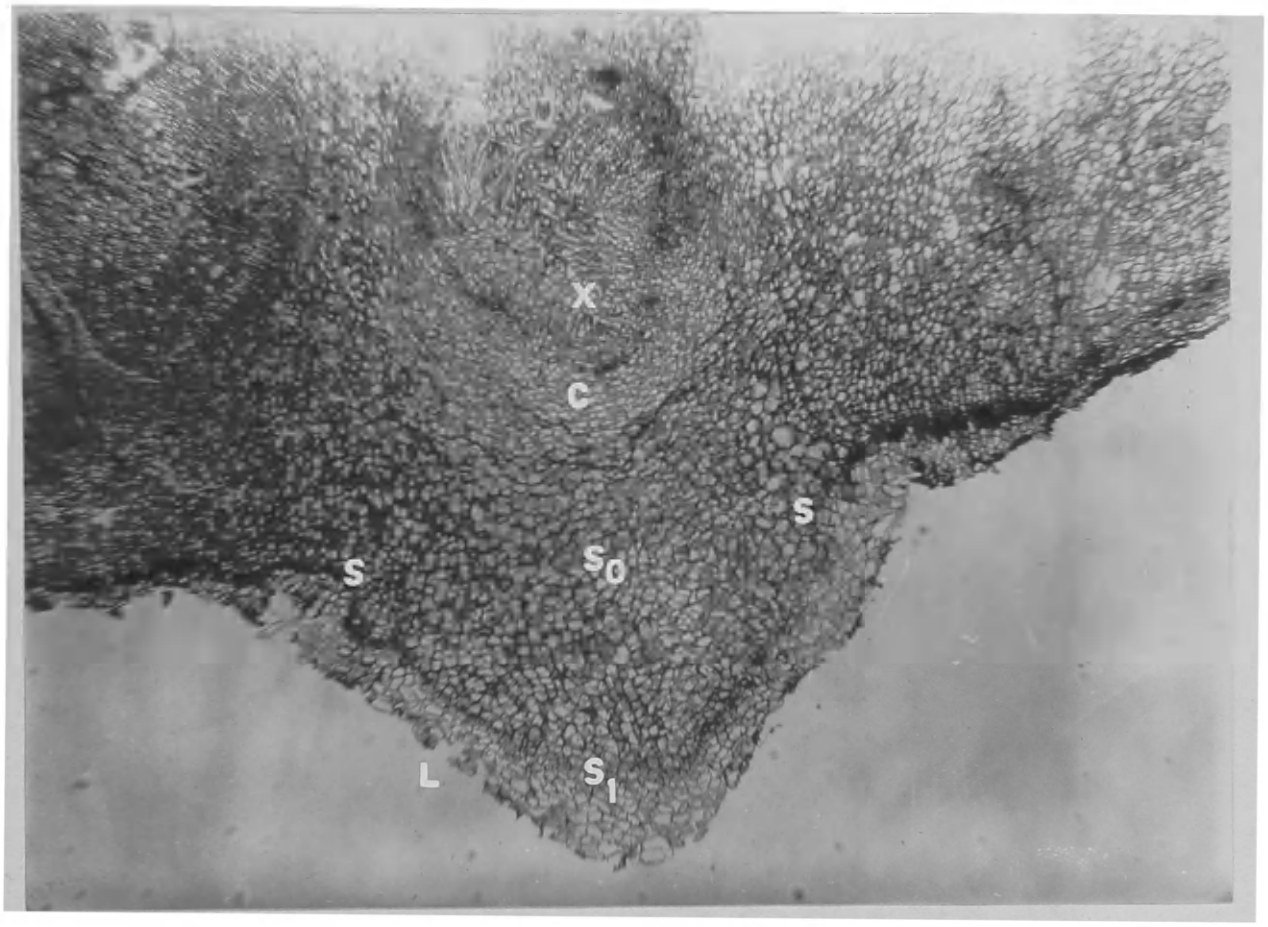
FIG. 10. Proliferation as a result of loss of suberin and disappearance of the phellogen (P). Cambial cells (C) are active in forming new callus cells. These undulating cambial strands are connected genetically with the vascular cambium of the scion. (x 90)



11

FIG. 11. A median transverse showing callus lobe (L) with attendant decrease in intensity of subserization.
The cambial layer becomes thickened at C, where previously xylem differentiation (X) was manifest as a result of the presence of suberized "barrier" which was in the position S - S_0 - S. The position of the suberized layer and phellogen has been changed from S_0 to S_1. (x 58)

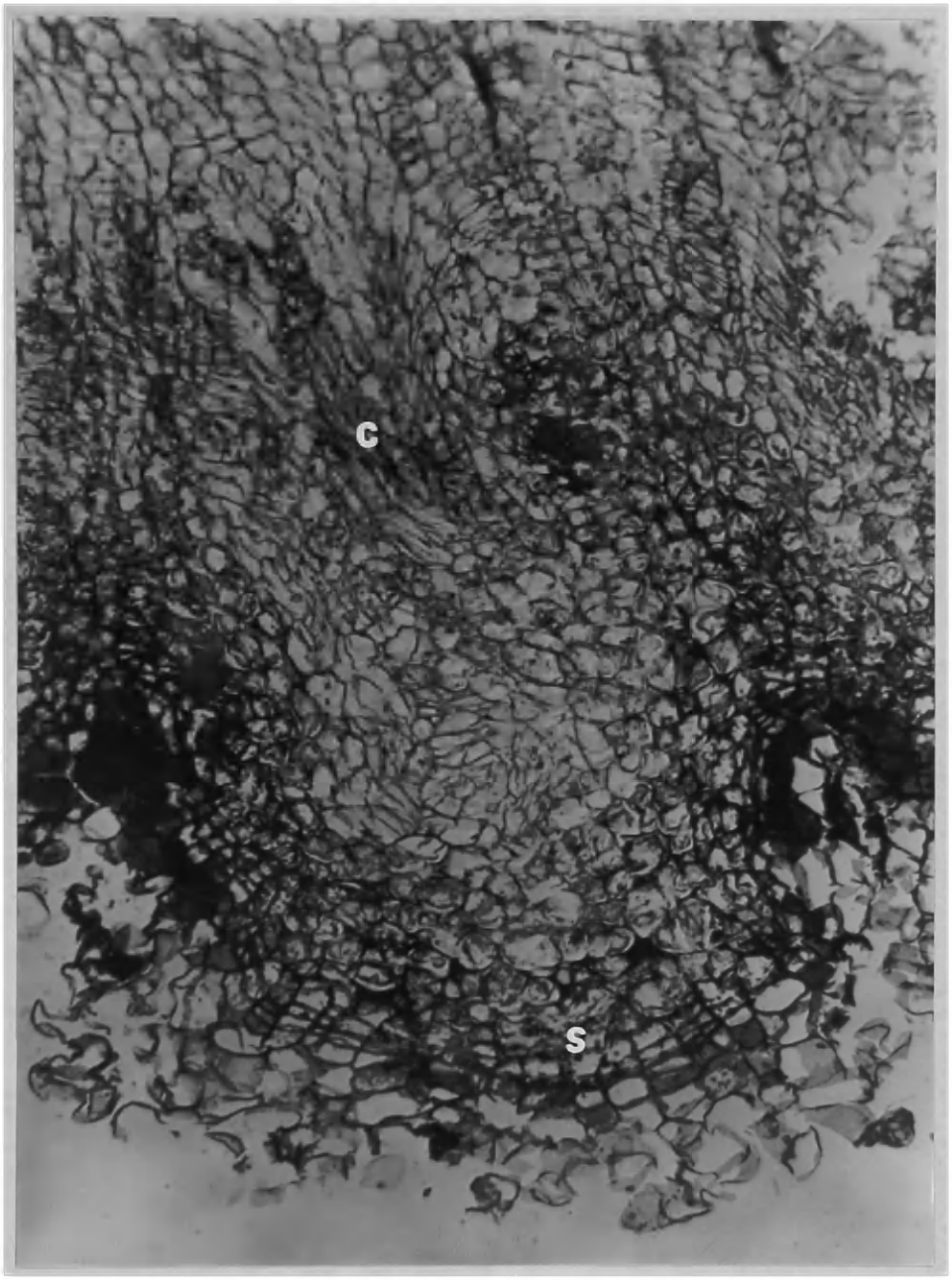


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FIG. 12. A distortion in the orientation of the cambial cells (C) opposite a newly developed break (S) in the suberin barrier. The entire area from C to S is too highly meristematic in this early stage to fulfill requirements necessary for initiation of root primordia. (x 205)

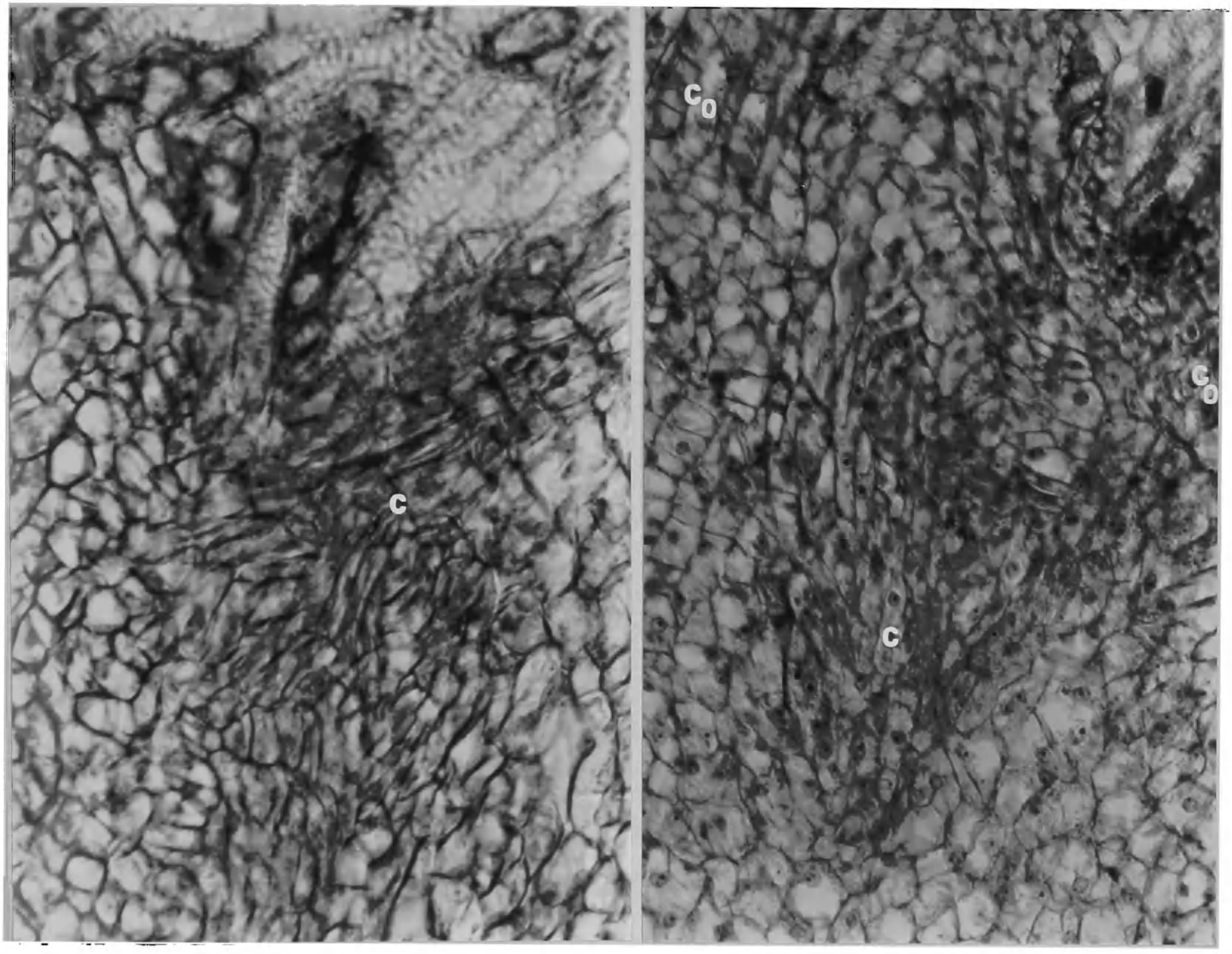

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FIG. 13. Distortion in the orientation of cambial cells (C) in an area opposite a callus lobe. (x 400)

FIG. 14. The alignment of the cambial cells at C is approximately perpendicular to the previous orientation C_o to C_o. (x 325)

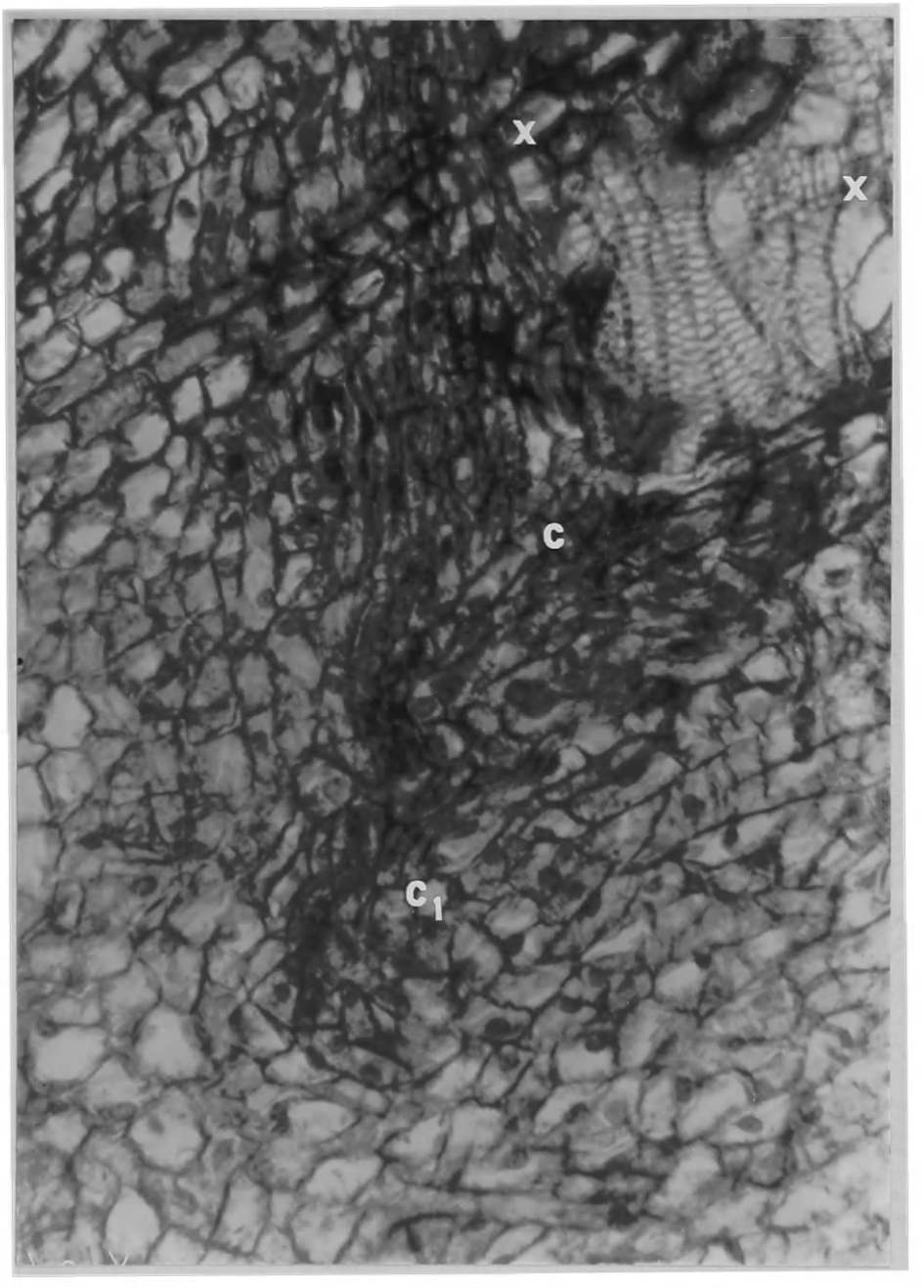

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FIG. 15. Similar to Fig. 14.
The former position of the cambium was approximately in the form of an arc at X, from which area reorientation of the cambial cells has resulted in an extension C to C. (x 400)

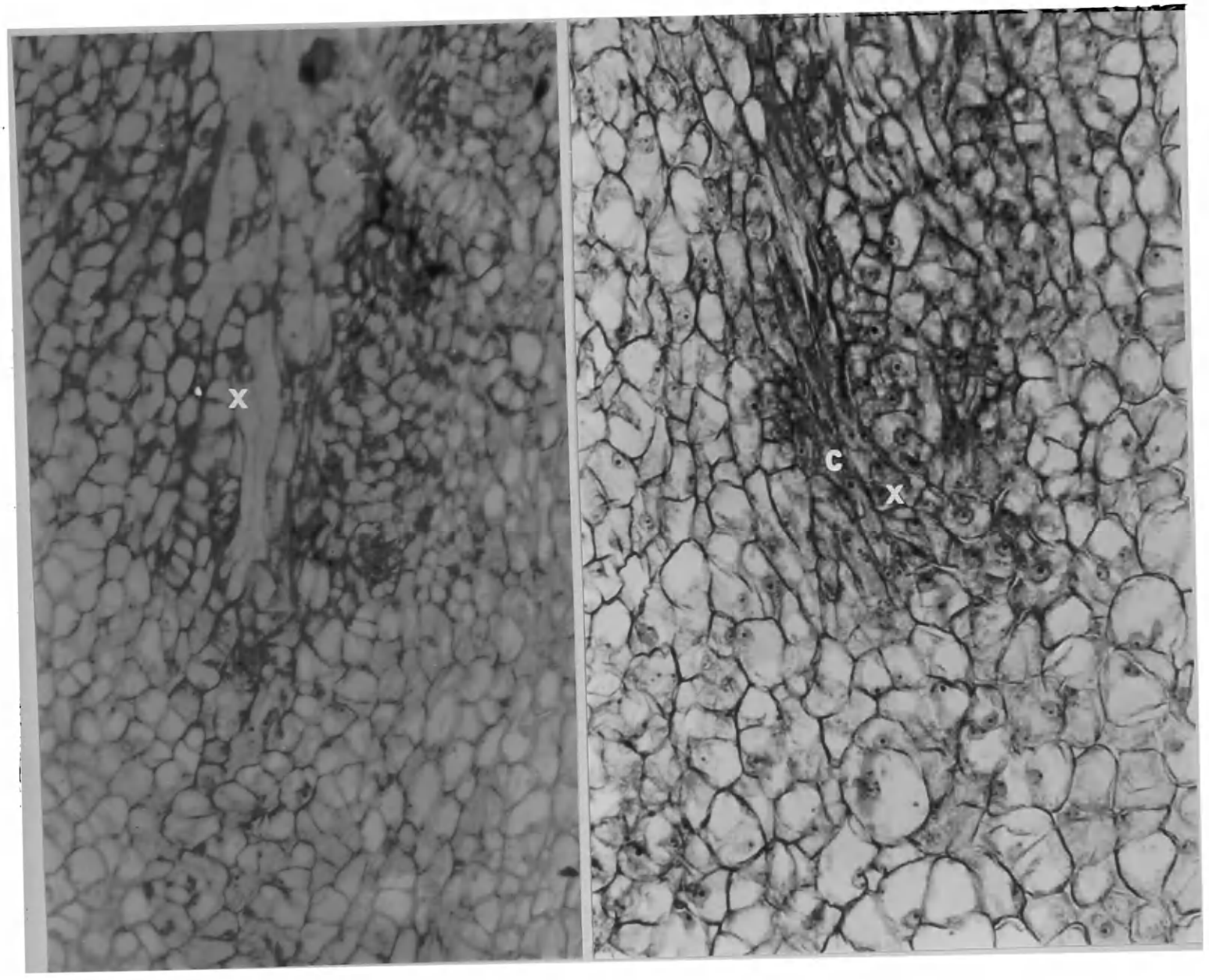

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FIG. 16. A median section of a region similar to that shown in Fig. 15. The recently differentiated xylem elements (X) are very elongated and aligned perpendicularly to the previous position of an active cambial layer (not shown). (x 280)

FIG. 17. A very early stage in the differentiation of cambial cells (C) into cells constituting the extremely meristematic area (X) of the growing point of the root primordium. (x 400)

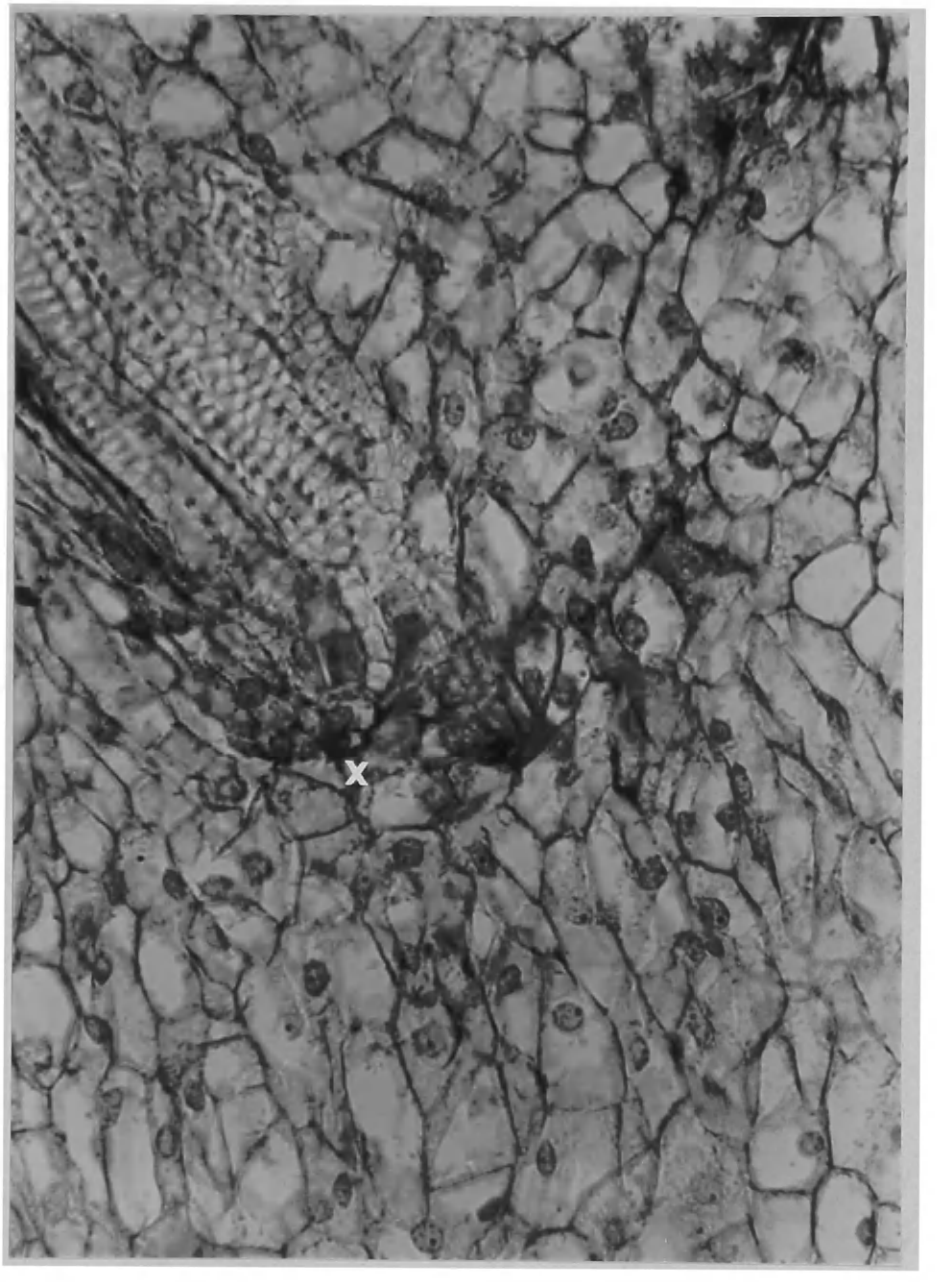

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FIG. 18. A stage of differentiation of cambial initials into the highly meristematic, densely protoplasmic growing point organization (X). Similar to Fig. 17, but a more median section. The semi-reticulate nature of the secondary thickenings of the xylem elements constituting wound wood is shown. Xylem elements are invariably (a) in close proximity to the apical initial cells, and (b) longitudinally extended. (x 650)

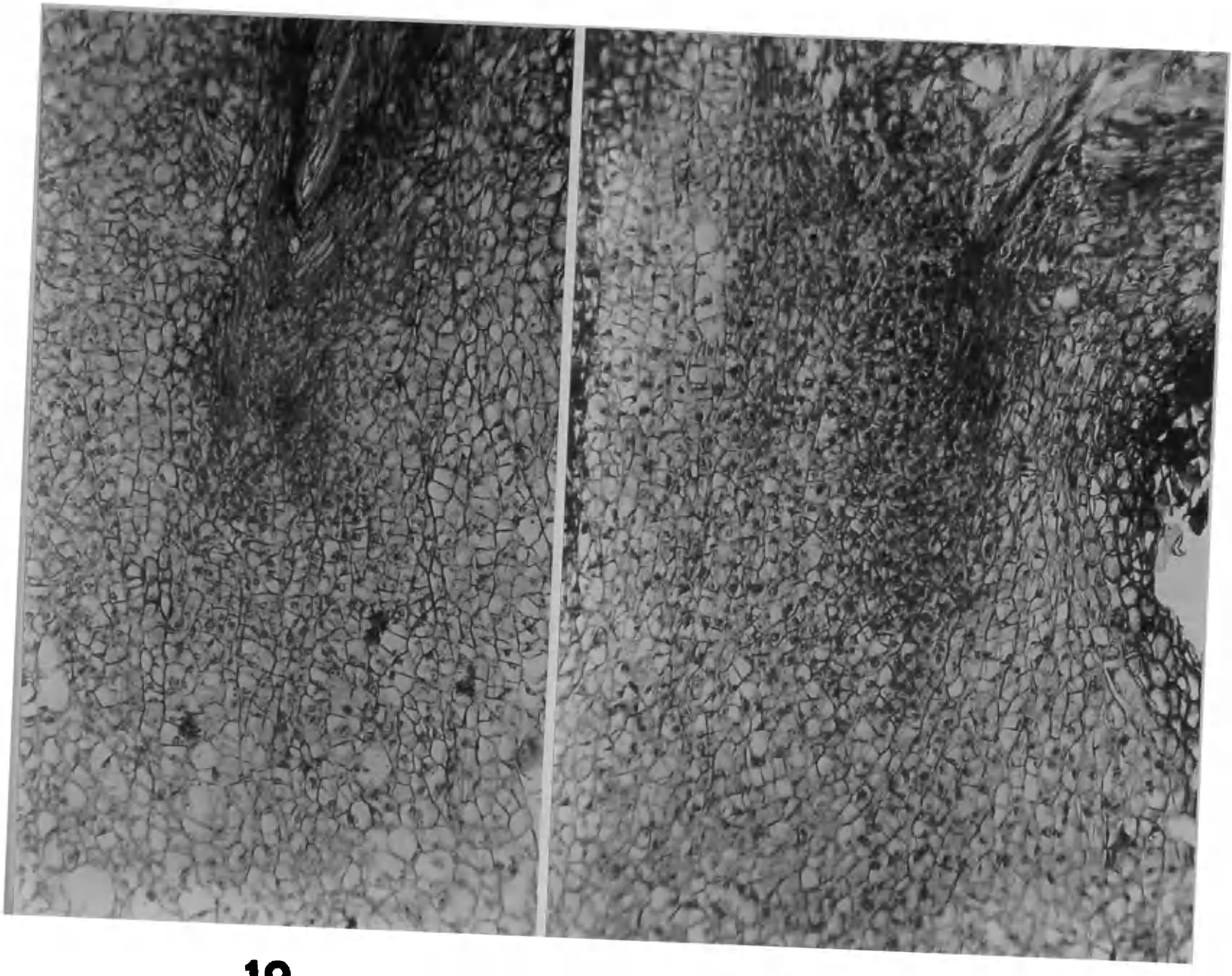

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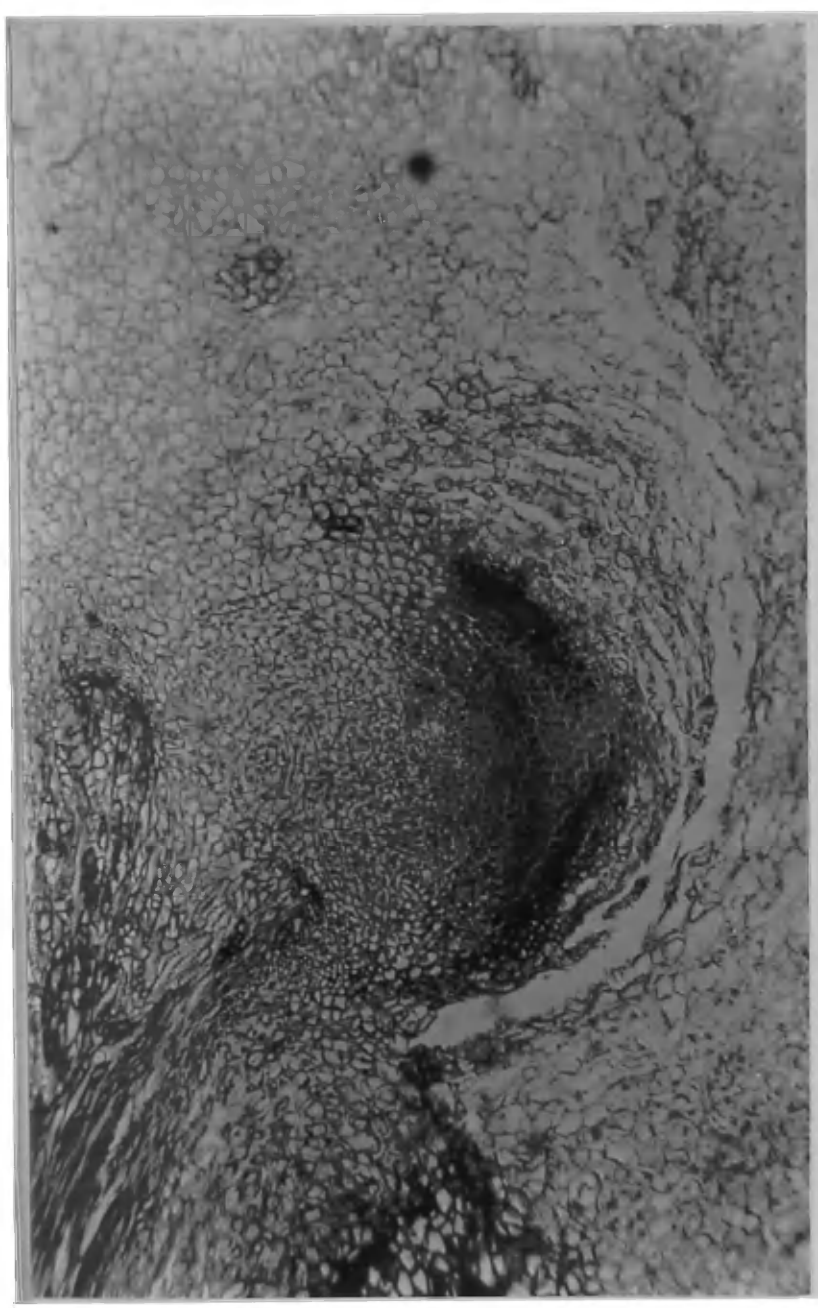
FIG. 19. Due to rapid cell division and growth the definite outlines of root primordia are noted. (x 76)

FIG. 20. Similar to Fig. 19
The suberized tissue through which callus proliferation occurred, previous to the present organization of tissues, is shown at the sides. (x 76)



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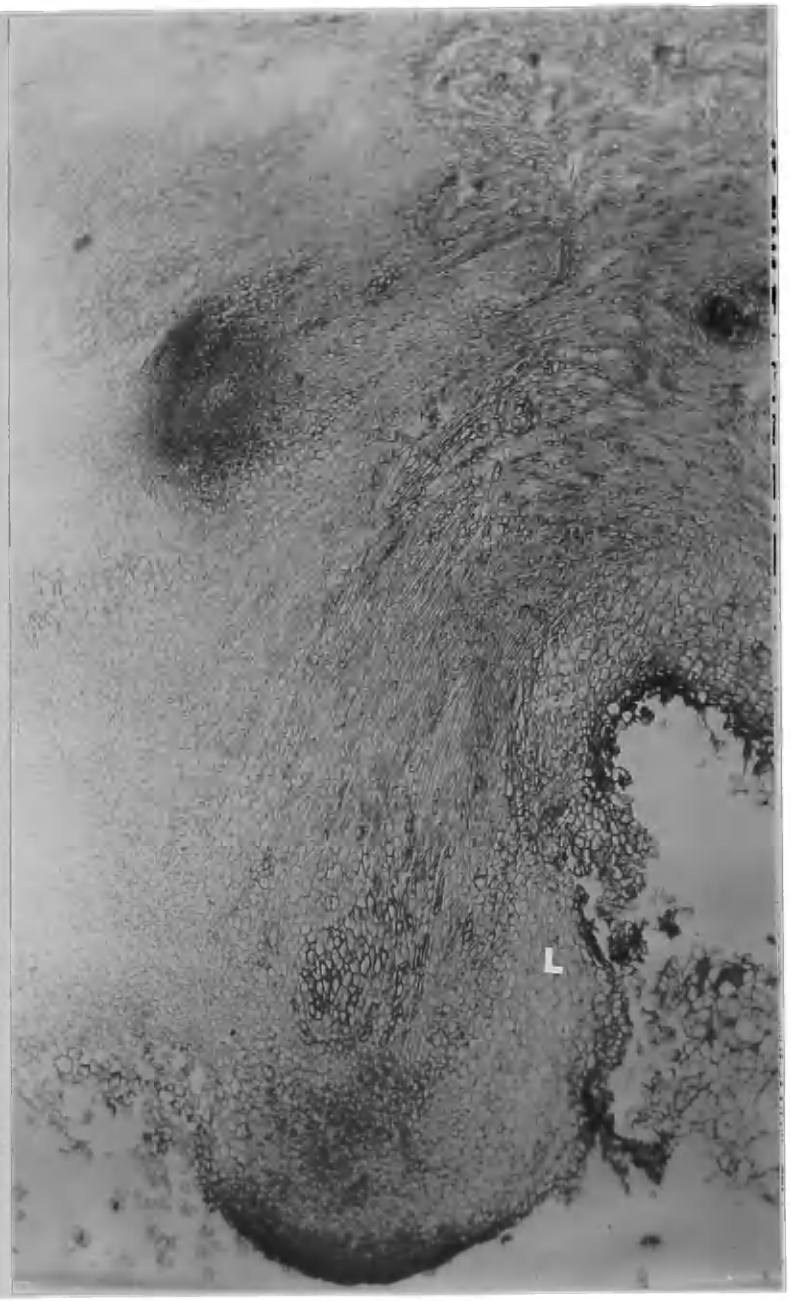
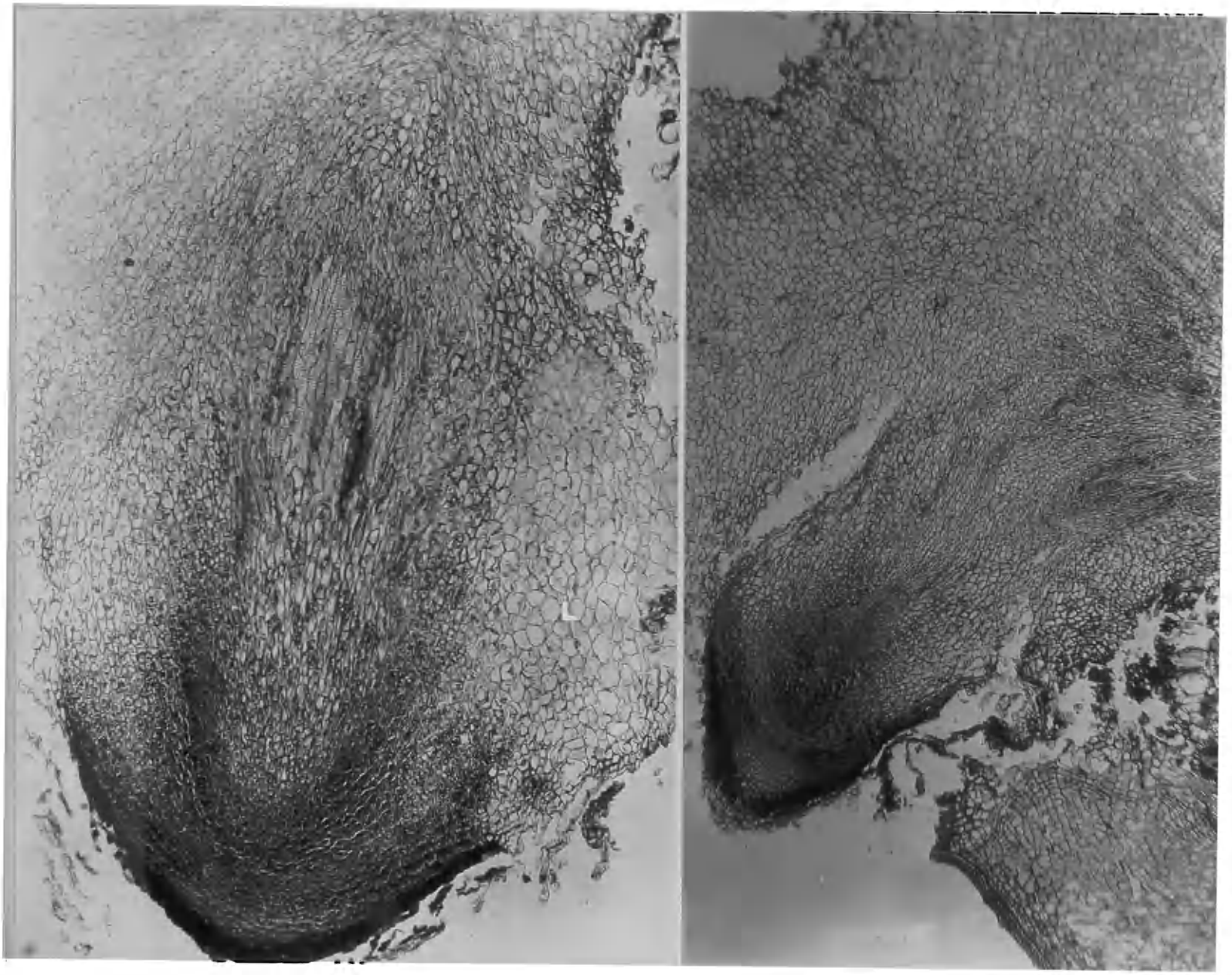


22

FIG. 21. Large cavities and breaks in the callus tissue usually appear in a position anterior to the growing point. (x 115)

FIG. 22. The cells of the callus lobe (L) constitute an indefinite portion of the cortex of the primordium. (x 62)



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24

FIG. 23. Fig. 22 at a different level. The older callus cells are shown at L. (x 62)

FIG. 24. The break in the cortical region in this case appears to be due to mechanical tearing of the tissues. (x 72)

25

FIG. 25. A transverse section showing:
X, old wood of the scion.
X_1, new xylem elements normal in structure.
C, cambial extension, from the pre-existing vascular cambium, from which are differentiated
W, xylem elements of the wound wood type.
S, highly suberized layers.
L-L, an arc connecting these two points roughly marks the outline of the callus lobe before the root primordium had reached this advanced stage. (x 35)

26

FIG. 26. A number of primordia have developed in this callus.
E, epidermal region of the scion.
The cambium has developed by extension from its original position C to form the loop C–C1. Sieve tubes are present (not shown) (x 35)

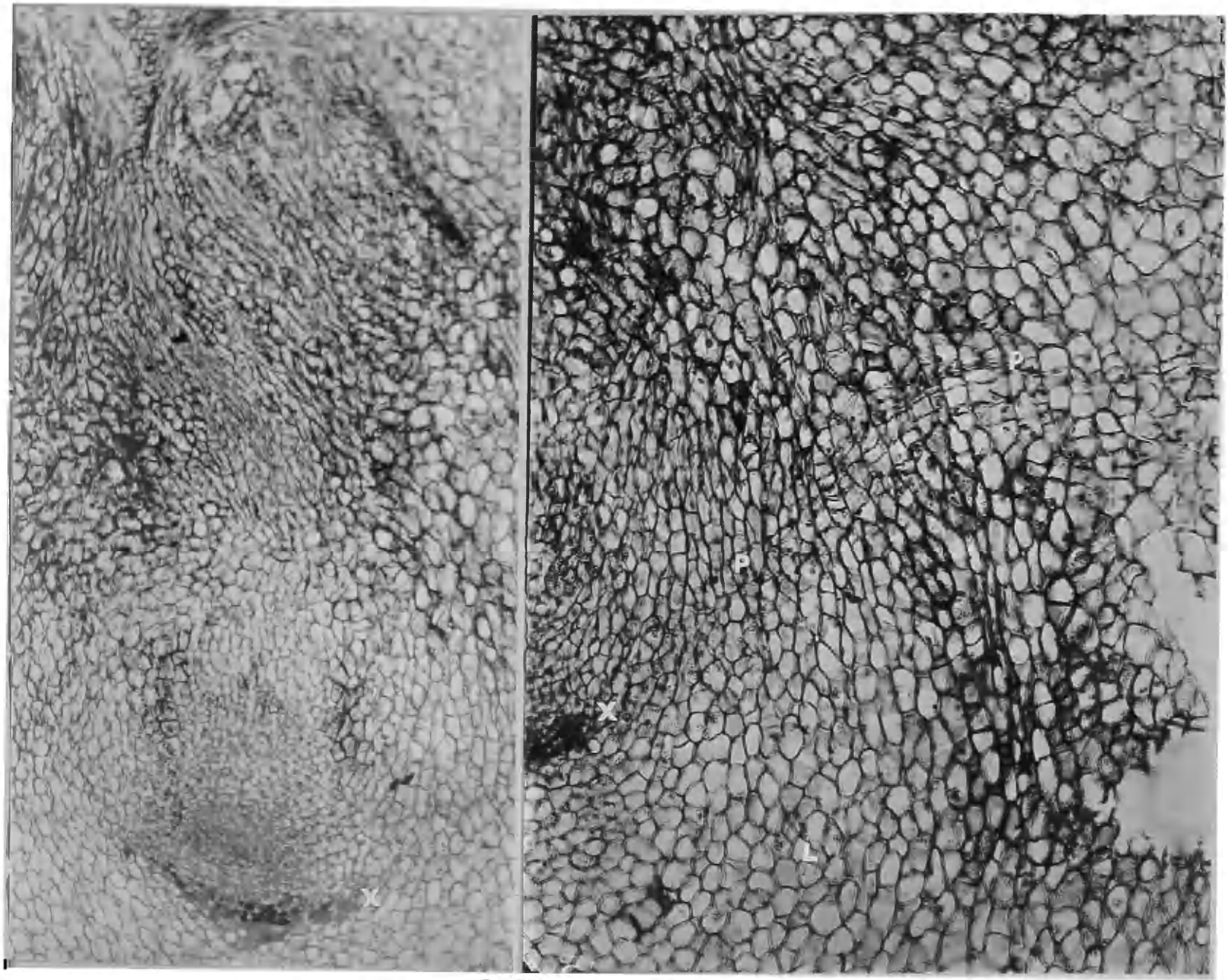
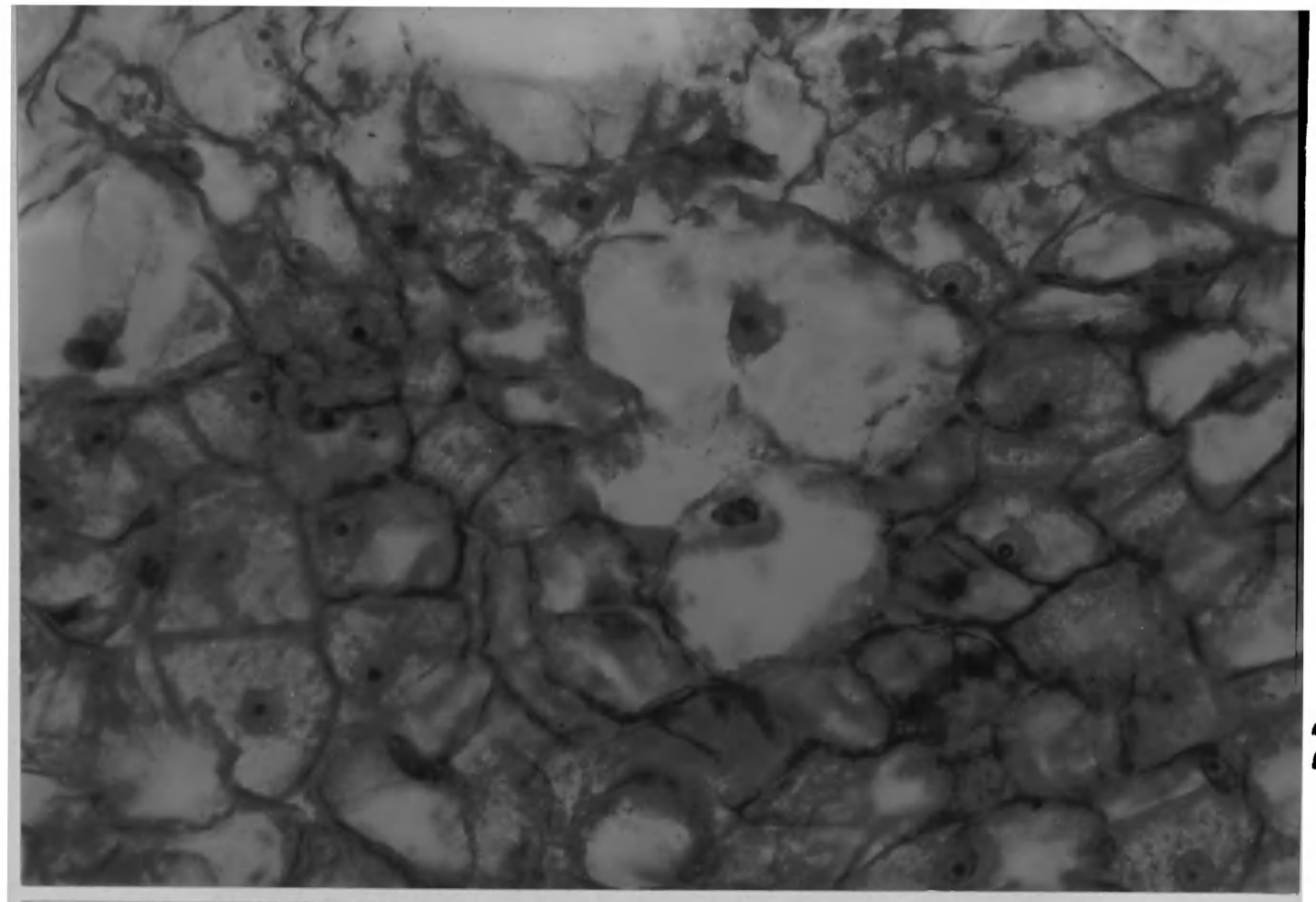


FIG. 27. The area constituting a calyptrogen (X) in A is continued in B (X) at a higher magnification to show the old phellogen layer (P), which is gradually disappearing due to a proliferation at L. (A, x 130. B, x 150).



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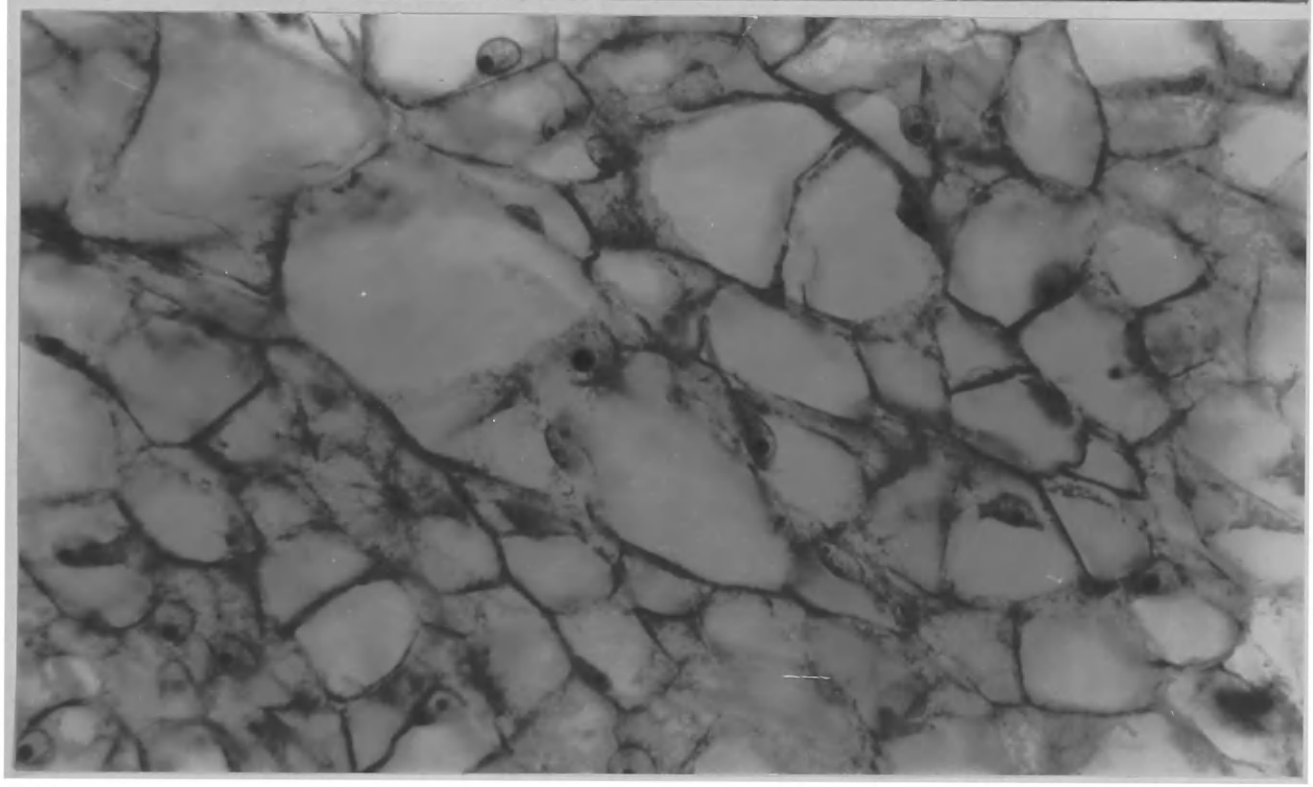

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FIG. 28. Early stage in cavity formation in a region anterior to the advancing root primordium. The cell walls are disappearing and the nucleii are degenerating. Cell contents have practically disappeared. (x 705)

FIG. 29. Similar to Fig. 28. (x 705)

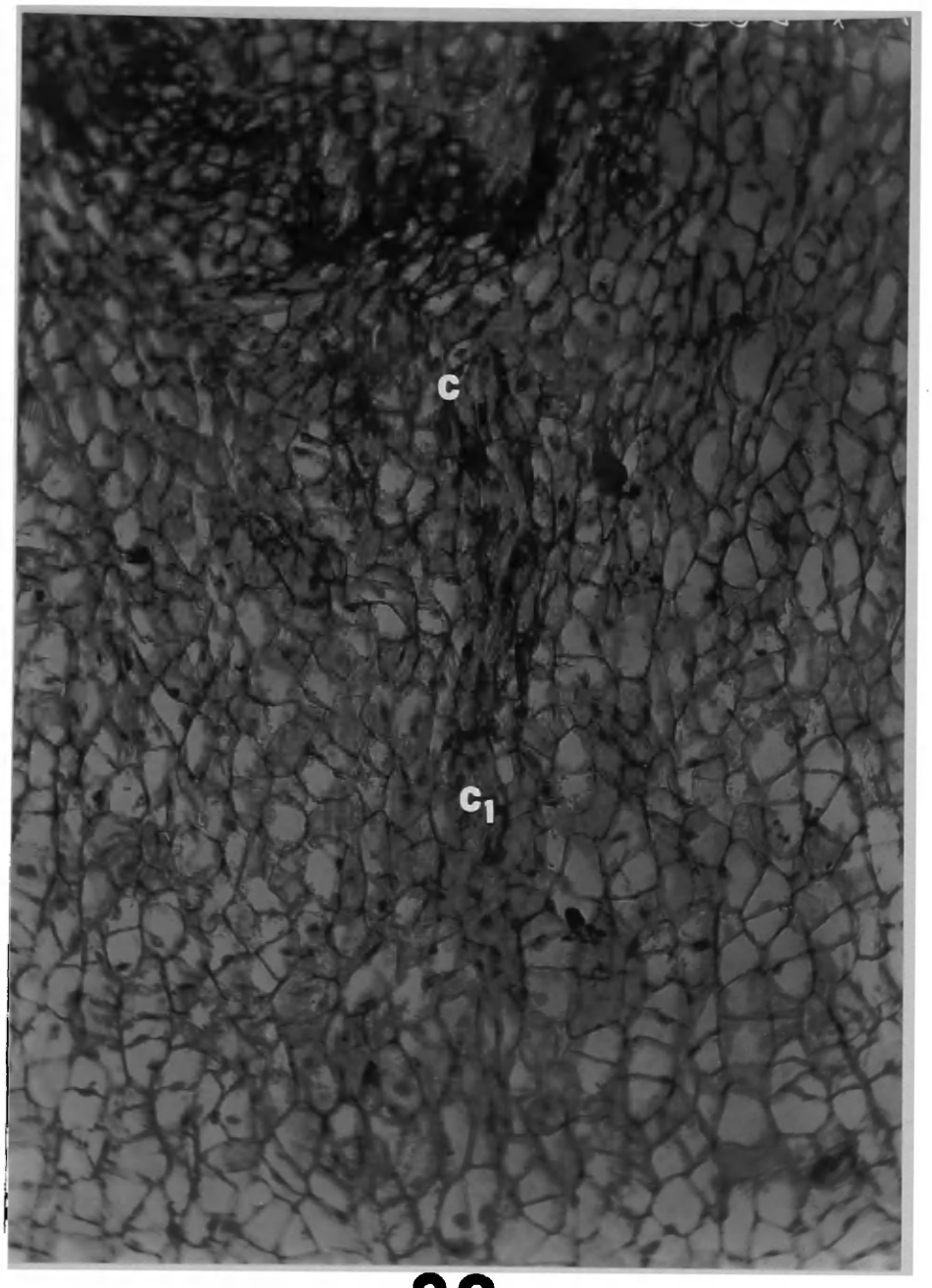

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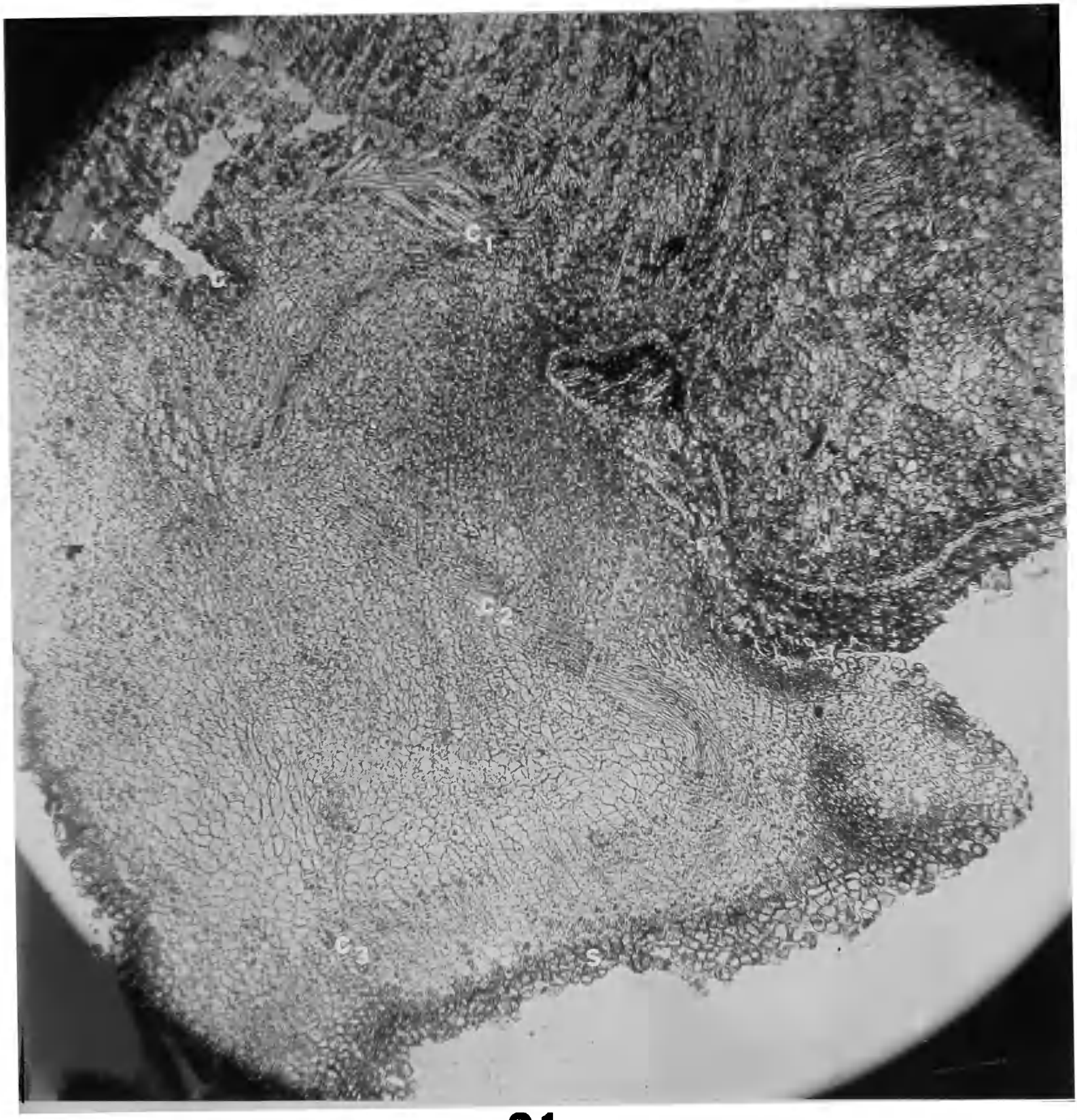
FIG. 30. Cambial extension (C to C_1) in a callus lobe. Callus cells formed by this cambium contribute to the enlargement of the "lobe." Little or no suberization occurred on the periphery of this callus proliferation and the cambial cells are therefore not differentiating into xylem elements. (x 260)



31

FIG. 31. The former cambial region was at C, adjacent to the small transverse section of old wood (X). The cambium has advanced to C_1. The lower callus lobe is of comparatively recent origin and has resulted from the activity of cambial extension progressively from C_1 - C_2 -C_3, with continual formation of callus cells internally and externally. The comparatively large amount of cells formed internally accounts for the presence of the cambial layers near the periphery. Suberization is slight at S, with no well developed phellogen. (x 90)

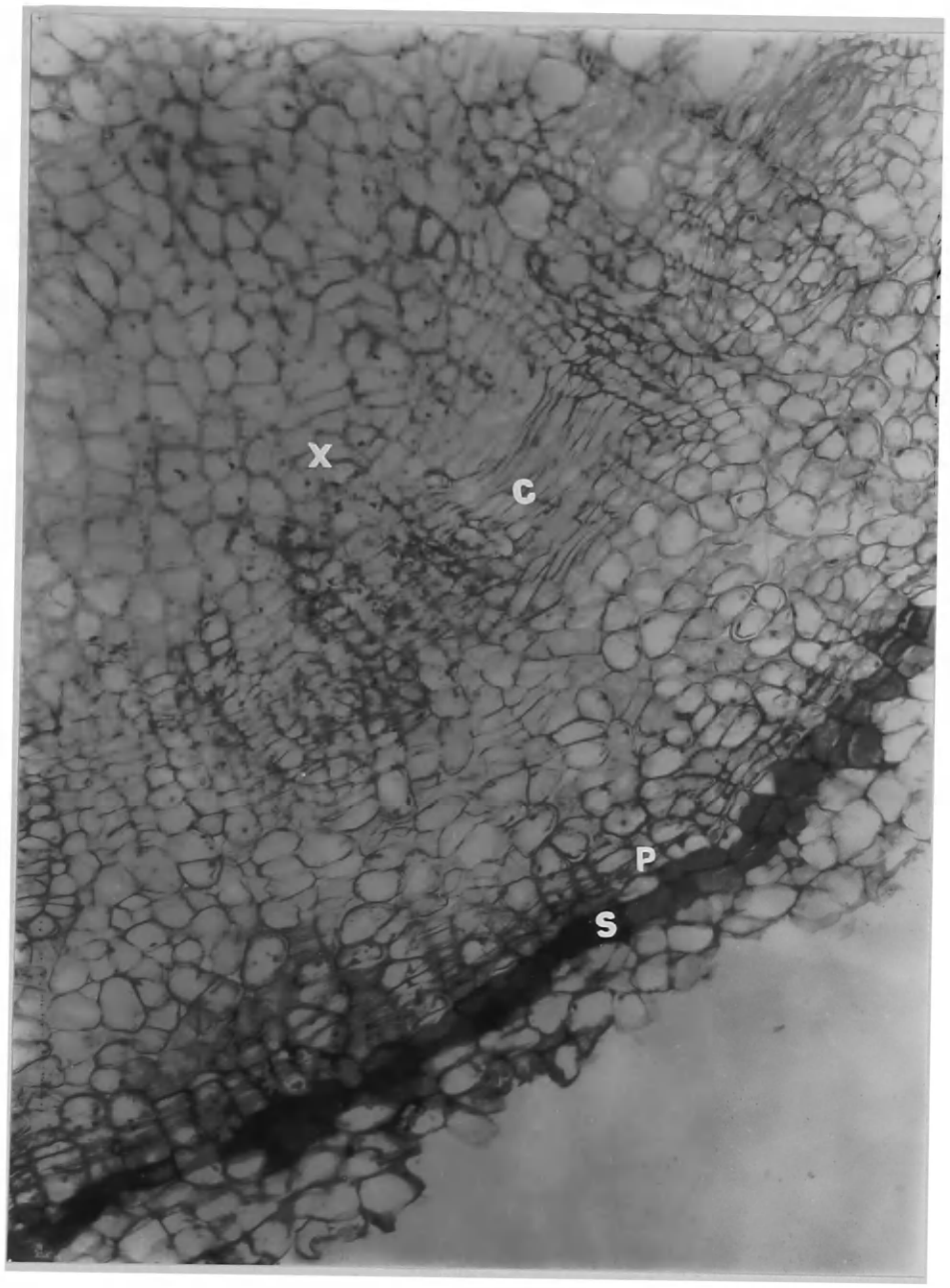


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FIG. 32. Suberization (S) confined to one or two layers only, but sufficient for development of a phellogen (P). Internally the many-layered cambium is beginning to differentiate into xylem elements (X). (x 40)

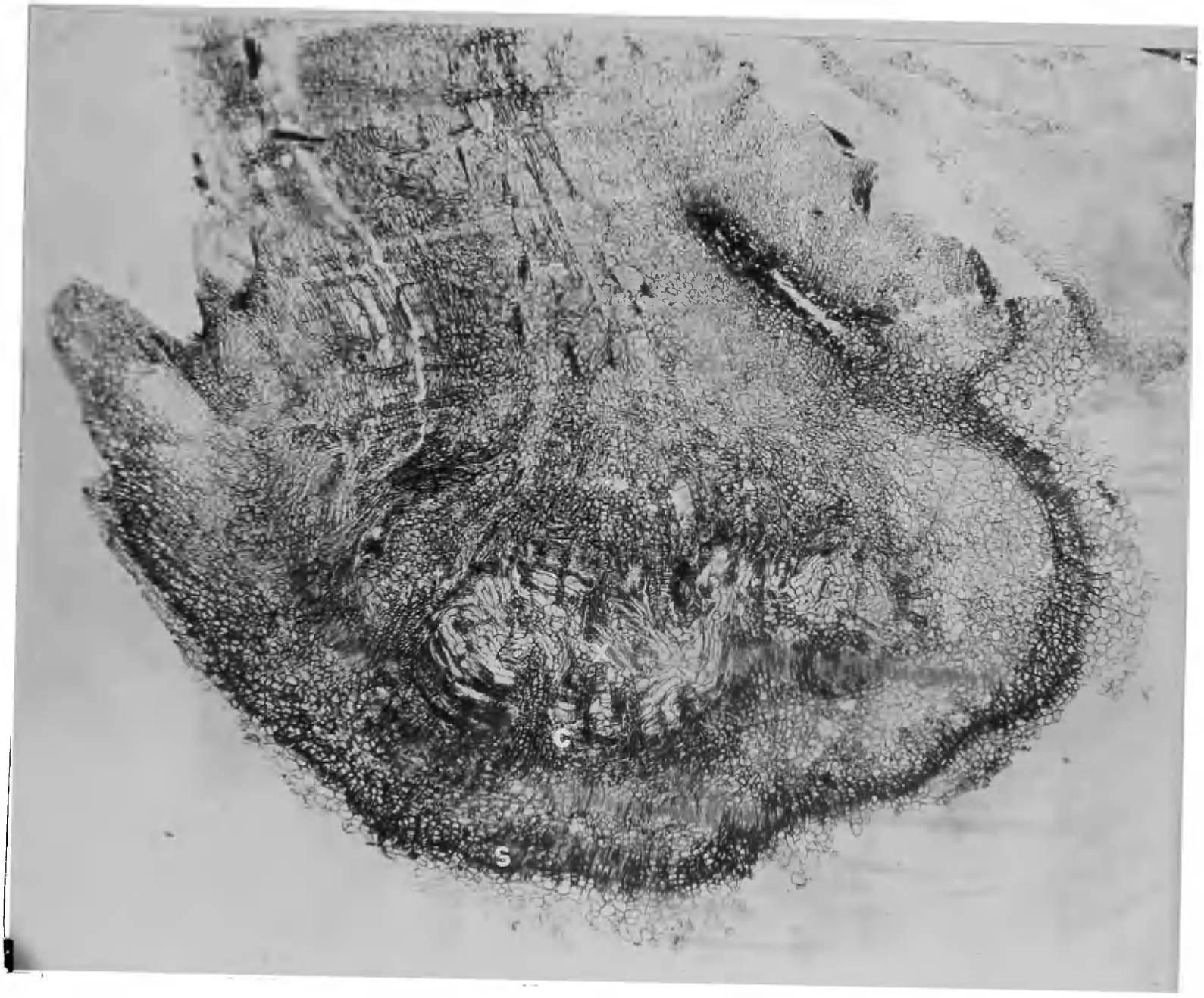

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FIG. 33. The suberized layer (S) involves several rows of cells. The cambium (C) is differentiating into xylem elements (X). This area (X) is merely an upper extension of a larger area similar to that shown in Fig. 3. The epidermis of the scion is at E. (x 60)