THESIS SUBMITTED BY GEORGE BOND FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY.

MARCH, 1930.

TITLE OF THE THESIS:-

"THE ENDODERMIS".

Part I. "THE OCCURRENCE OF CELL DIVISION IN THE ENDODERMIS".

Part II. "THE STEM-ENDODERMIS IN THE GENUS PIPER".

Part I of Thesis:-

THE OCCURRENCE OF CELL DIVISION IN THE ENDODERMIS.

CONTENTS.

Note. This paper has been published in the Proceedings of the Royal Society of Edinburgh, Vol.L, Part 1, No.4, 1930.

THE OCCURRENCE OF CELL DIVISIONS IN THE ENDODERMIS.

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Communicated by Prof. Montagu Drummond, M.A.

So far as the author is aware, the ability displayed by endodermal cells to divide at a late stage in their history and differentiation has received little comparative treatment, but has merely been the subject of passing comment during the discussion of related subjects. In the present paper it is proposed to present a brief survey of the different types of such divisions and a discussion of points arising in the course of the survey.

Previous records of sub-division of endodermal cells concern the root almost exclusively, a fact which may be attributed to the relatively infrequent appearance of an endodermis in the stem, and also to the greater attention accorded to the endodermal relations of the root.

Nature of the Divisions.

Two types of cell division in the endodermis may be distinguished.

1. Those taking place in endodermal cells in the primary condition.

2. Those occurring with or after the inception of the tertiary stage.

This distinction depends on the characters of the parent cells. The new walls themselves show further distinguishing

features, to which reference will be made below.

The great majority of previously described examples of endodermal divisions fall into the second class, and this better known type will be described first.

A. Divisions in Tertiary Endodermal Cells.

The occurrence of these is recorded by Kroemer (1) in the roots of Ricinus communis, Comarum palustre, Vincetoxicum spp., and Helleborus niger by Van Wisselingh (2) in roots of Taraxacum officinale, Gentiana lutea, Rosa ferruginea, Potentilla Wilmottiae, and Rubus laciniatus, and by Scott (3) in the roots of Salix fragilis and Vicia Faba.

The observations of the above mentioned writers show that in these instances new walls are produced in endodermal cells which have entered upon the so-called "tertiary" stage of their development, a stage characterised by the deposition of cellulose lamellae inside the suberin lamella of the secondary endodermal cell. Kroemer (loc.cit.) considered that the divisions may occur in endodermal cells in the secondary stage, but Mylius (4) held that they belong to the tertiary rather than the secondary stage.[1] According to the observations of Mylius the new walls may appear simultaneously with the first tertiary lamellae, but never before. My own observations on the divisions in the endodermal cells of the root of Alchemilla vulgaris agree with those of Mylius.

The new walls display a radial orientation in the main.

[1] This confusion appears to be due to a slightly different use of the terms "secondary" and "tertiary" stages in endodermal development. See Kroemer(loc.cit.) p.96 et seq., Mylius(loc.cit) p.36 et seq.

writer has observed as many as seven new radial walls in the case of Alchemilla vulgaris, but Van Wisselingh (loc.cit.) cites and figures the extraordinary case of Gentiana lutea, where as many as 25 new walls may be produced in the one original cell, indicating the occurrence of 25 successive divisions. Mylius (loc. cit.) refers to unpublished observations by Bäsecke on the root and rhizome of Viola tricolor, where 20 divisions may occur in the one original cell.

Simultaneously with the appearance of the divisions in the endodermal cell an increase in its tangential dimension occurs, often of a very considerable extent (Fig. 1, A + B.). According to the author's observations an increase of 400% is common in the case of the root of Alchemilla vulgaris, while Van Wisselingh figures endodermal cells from the root of Gentiana lutea where an increase of ~~1,000%~~ 1,000 % must have occurred. As a result of this increased tangential dimension of its cells, the continuity of the endodermis is not affected by the expansion of the vascular cylinder following the formation of the secondary vascular tissues, or at least, not for some time.

The new walls are of cellulose, are massive, and show neither Caspary strip nor suberin lamella (Mylius, loc.cit., P.38, and confirmed by author in Alchemilla vulgaris. Fig. 1 & 2.)[1]. Mylius however records the formation (in Comarum palustre) of "suberin-like" material in the walls in the form

[1] Note. The lamella labelled suberin lamella in fig.2 is actually the "verkorkte mittlere Lamelle" of Kroemer, consisting of the closely fused lamellae of the primary and secondary endodermal cell, which are usually to be distinguished only after maceration.

or cutin-like" substances on the new walls in the root of Gentiana lutea. In general, however, the walls display none of the features characteristic of endodermal walls.

The suberin lamella of the stretched original endodermal cell remains intact round the sub-divided cell, so that the original cell is still clearly defined & recognisable. It will be seen that here we have a mere sub-division of the original cells of the endodermis, resulting in the production of what may be termed "multicellular endodermal units", with no formation of new endodermal cells in the real sense of the word.

Before passing on to consideration of division in primary endodermal cells, mention must be made of a point which arises in connection with the figures of Alchemilla vulgaris (Fig.1, A+B.). Mylius(loc.cit.P.36) states that in certain cases (roots of Rosa microphylla, Rosa spinosissima, Potentilla fruticosa, Rubus Idaeus) the suberin lamella of the endodermal cell in the secondary stage does not form a complete lining to the cell, but fails to develop over the Caspary strip. That is, the suberin lamella of each cell consists of two portions, an inner and an outer, with a narrow slit separating them. C. van Wisselingh(loc.cit.) confirms this for the three genera quoted by Mylius.

My own observations indicated that a similar, incomplete, suberin lamella is also present in the root-endodermis of Alchemilla vulgaris. A transverse section of this root, stained with Sudan III shows that the suberin lamella does not cover completely the radial walls of the endodermal cells, but is interrupted in the region of the Caspary strip, exactly as described by Mylius and Van Wisselingh in other rosaceous genera. (Fig.1. A+B).

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B. Divisions in Primary Endodermal cells.

The other type of division, that displayed in primary endodermal cells, appears to be much less common, no doubt because the typical endodermal cell shews the primary condition as a temporary and very early stage in its development. Instances of division in such typical cells are, however, known.

Kroemer(loc.cit.P.95) discovered divisions in endodermal cells in the primary stage in the roots of Hellebarus niger, Helianthus annuus, and Adonis vernalis. He figures a cell from the former showing a single new radial wall, which has developed a Caspary strip; he states that the new walls are usually radial, being associated with an increase in the tangential dimension of the original endodermal cell, but that tangential walls may also be formed, as for instance, in Helleborus niger and Helianthus annuus, and these tangential walls do not develop a Caspary strip. Kroemer also refers to an observation made by Van Tieghem(1871) on a similar production of tangential walls in Tagetes erecta.

Kroemer notes that the newly formed cells may proceed later to a secondary condition in a normal fashion.

Cell divisions in the primary stage are also found in endodermal cells which are abnormal in that they retain the primary condition throughout their history. The occurrence of a permanently primary endodermis will be referred to in more detail later, but at this point it should be noted that Strasburger(5) recorded the presence of such an endodermis in the root of Monstera deliciosa, and observed division in the

endodermal cells. The new walls developed Caspary strips, joining up to that of the mother cell. Van Wisselingh(loc. cit.), also, has recently described similar divisions in the root of Anacyclus pyrethrum, where again the endodermis is permanently primary. At the commencement of secondary growth in thickness of the root the endodermal cells show tangential growth and subsequently divide. The new walls are laid down in a radial direction, are thinner than the original walls, and develop a Caspary strip which is ~~more~~ less massive than that of the original wall.

The writer has recently discovered cell division of the second type in the endodermis of certain aerial stems, namely, those of species of Piper. The endodermal relations of these plants are under investigation, and it appears that the study will be productive of several observations of interest, the majority of which, however, will receive detailed consideration at a later date. For the present it may be stated that in most of the species examined an endodermis is present in the young internodes and persists throughout the later development of the internode. Considerable variation, however, is displayed with regard to the distribution of the endodermis. In some species the endodermis forms a complete cylinder enclosing the vascular system, and retains its continuity even though secondary growth in thickness of the stem proceeds to such an extent that the circumference of the stem (or of the endodermal cylinder) increases by 700%. In others this normal arrangement is not shown, for the endodermis is discontinuous, and, as seen in

transverse section, has the form of a series of arcs, each capping a bundle.[1] In a third group of species the degree of continuity of the endodermis varies during the development of the stem.

A further point germane to the present discussion is the fact that the endodermis shows a persistent primary condition throughout. In no case did the use of the appropriate reagents reveal the presence of the suberin lamella characteristic of the secondary stage. As stated above, other instances of this are known, but they are few, and a persistent primary endodermis is comparatively rare among Angiosperms. Mylius (loc.cit.), from his extensive studies of the Rosaceae, Oenotheraceae, Myrtaceae, and related orders, concluded that in roots a tertiary stage is usually reached, although in some cases a secondary condition may be persistent. In rhizomes he always found a tertiary condition, while in aerial stems the endodermis, when present, again attains in the main a tertiary stage, although a secondary stage is occasionally the final condition. Two cases of the occurrence of a persistent primary endodermis have been cited. In addition Kroemer (loc.cit.) found one in the roots of Acorus calamus, Hydrocharis Morsus-Ranae, Crinum species, and Arum italicum and other plants. Tetley (6) finds a permanent primary endodermis in the roots of several herbaceous Composites.

1. Owing to the anomalous behaviour of the secondary cambium in the stem of Piper, at no stage do the primary bundles lose their identity as separate vascular units.

In the older internodes of species of Piper a very massive Caspary strip is present, and is peculiar in that the surface view of the endodermis does not reveal the undulatory form usually associated with the strip (Fig. 3.D.)[1]. As observed by Priestley and North (7) in the case of the stem endodermis of Potamogeton perfoliatus L., treatment of surface sections of the endodermis with concentrated sulphuric acid for three minutes, or warming them in concentrated potash, results in the production of undulation in the Caspary strip (Fig. 4.A.). These undulations are presumably produced as the result of differential expansion or contraction of the cellulose wall and the Caspary strip present on it.

It was discovered that in older internodes there is an abundant production of new walls inside the endodermal cells, mainly in a radial direction, but also transversely. These walls are always produced in cells of which the tangential dimension has undergone, or is still undergoing, an appreciable increase. Shortly after their formation the new cellulose walls, both radial and transverse, develop a Caspary strip (Fig. 3, A + B.), and, as the surface view of the endodermis indicates, (Fig. 3.D.) these Caspary strips link up with those of the original walls. The new walls are clearly to be distinguished from the original ones by the general disposition of the cells, and the fact that the former are more delicate and bear a less prominent Caspary strip than the latter (Fig. 3.C.).

1. The material used was fixed and preserved in 60 % alcohol.

In addition to the above, new wall formation is occasionally to be observed in a direction other than radial or transverse. The new walls may ~~be~~ lie tangentially with regard to the original orientation of the parent endodermal cell, as indicated by the position of the Caspary strips, although, as a result of adjustments of tissues and distortion of the endodermal cells during secondary changes in the stem, these new walls may be practically radial with regard to the stem as a whole (Fig. 4 B.). They are, however, sharply distinguished from true radial walls by the fact that they do not develop a Caspary strip. Hence they fall into line with the tangential divisions recorded by Kroemer and Van Tieghem, referred to above.

Fig. 4 C. shows another arrangement of new walls occasionally encountered. The neighbouring cells of the cortex and pericycle shew a similar increase in size, accompanied by division (Fig. 3 C.), but here of course Caspary strip formation is never to be observed.

As far as my observations go this activity on the part of the endodermal cells is most marked in Piper excelsum Forst. (= Macropiper excelsum ~~Ruiz et Pav.~~ Miq.). As many as four radial walls may be produced here in the one parent cell, each one developing a Caspary strip, so that the surface view of such a sub-divided endodermal shows a complex network of Caspary strips. In this particular species the endodermis, while forming a complete ring in younger internodes, undergoes partial rupture after the inception of the secondary growth in thickness of the stem, and subsequent to this forms arcs capping

the phloem of the bundles, as seen in transverse section.[1] It is in the cells forming these arcs that the divisions are to be observed.

Cell division also occurs in Piper augustifolium, Ruiz et Pav., and in Piper decurrens C.DC., but less frequently than in Piper excelsum and to a lesser extent in each cell. In Piper augustifolium, in the stoutest internode available, rarely more than one division occurs in each original cell. This is associated with a less marked increase in tangential dimension in the endodermal cells of this species compared with that in Piper excelsum, where an increase of 500 % is common.

Here we have cell division in the endodermis resulting in the production of what may be legitimately described as new endodermal cells, the number of cells showing typical endodermal features being increased.

Significance of the Divisions.

It seems probable that in both types described this activity on the part of the endodermal cell is to be corelated with the increase in its tangential dimension occurring simultaneously. For the formation of new walls keeps pace with the increase in size of the original cell, culminating in the production of a very large number of new walls, as mentioned above. Further, in Alchemilla vulgaris the passage cells of the endodermis undergo but little increase in size and show no division.

1. The cambium produces no phloem in the interfascicular regions.

In Piper excelsum new walls are never observed in cells showing no enlargement, and conversely, division usually follows any considerable increase in size of an endodermal cell.

Two possibilities suggest themselves with regard to the actual relation between cell enlargement and division.

Kroemer (loc.cit.) considered that primary and tertiary divisions have the same significance, actually effecting that extension of the endodermal cells which is demanded by the increasing size of the stelar tissues following the initiation of the vascular cambium. That is, according to Kroemer the endodermal cell actively accommodates itself to the new dimensions of the root or shoot.

Mylius (loc.cit.), referring especially to the tertiary divisions, took a ~~more probable~~ less teleological view of the matter as a result of his observations, which showed to him that a considerable increase in the tangential size of the endodermal cell might occur without a corresponding production of new walls. Particularly was this the case in underground stems, where, despite a greater increase in the size of endodermal cells attendant on more pronounced secondary growth in thickness of the organ, division in such cells is relatively rare. These observations led Mylius to the conclusions that, rather than preceding and rendering possible an active tangential growth of the endodermal cells, the divisions followed a passive stretching of the original cells. Presumably division occurs after a cell has been stretched to a certain degree.

Mylius held it to be evident that the stout cellulose

walls fulfil a mechanical function in that they support and hold apart the stretched tangential walls of the original cell, preventing its collapse. For this reason he termed them "tertiäre stützwande." The new walls produced in primary cells in Piper and elsewhere may serve the same mechanical function, although their comparative delicacy will render them less effective in this direction than the massive "tertiäre stützwande."

Comparison and Discussion.

Thus we have two well defined types of endodermal divisions. The condition of the endodermal cell at the period of its tangential stretching obviously decides which type of division is to occur. In the great majority of cases the initiation of the tertiary stage has occurred by that time, so that division involving the production of "tertiäre stützwande" is the more common.

We have seen that while in one case the new walls develop a Caspary strip, this is not so in the other. Whatever the particular physiological factors responsible for this difference may be, it is what would be expected, assuming current views on the function of the endodermis to be correct.

Recent work has resulted in considerable physiological importance being attached to the primary endodermis on account of the presence within it, and the peculiar properties, of the Caspary network. This network is held to render very difficult the radial passage of water or of solutes through the endodermis, via the wall, as a result of the presence of derivatives of

fatty acids in the strip. It is considered (7) that the passage of water and solutes through the endodermis is placed under the control of the protoplast of the cells.. It is therefore significant that a Caspary strip develops on the new radial and transverse (~~but not tangential~~) walls in the dividing primary cell, so that the continuity of the Caspary network is not interrupted. There is no direct communication by an uninterrrupted cellulose wall between the inner and

* Neither will such direct communication arise in consequence of the absence of a Caspary strip from any new tangential wall which may develop.

~~cell possesses the suberin lamella in addition to the Caspary~~ strip. Modern workers consider that the development of this lamella cuts down the transference of water and solutes through the protoplast, rendering the secondary endodermal cell relatively very impermeable to those substances(7). In view of the presence of this lamella lining the whole of the cell[1], the absence of the Caspary strip and suberin lamella, from the new walls will not in this case interfere with the impermeability of the endodermis.

Thus in both cases the stretched parent cell is endowed with greater rigidity without the effectiveness of the endodermis as a physiological barrier being impaired.

But a difficulty arises here. For the supposed impermeability of the suberin lamella will result in the secondary endodermal cell being a "hermit cell", cut off from surrounding tissues. But we find such cells carrying on extensive metabolism

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Turning to the other type of division, here the original cell possesses the suberin lamella in addition to the Caspary strip. Modern workers consider that the development of this lamella cuts down the transference of water and solutes through the protoplast, rendering the secondary endodermal cell relatively very impermeable to those substances(7). In view of the presence of this lamella lining the whole of the cell[1], the absence of the Caspary strip and suberin lamella, from the new walls will not in this case interfere with the impermeability of the endodermis.

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leading to the production of massive cellulose walls. According to Kroemer (loc.cit. P.102) the endodermal cells of Angiospermous roots seldom contain reserve foods,[1] so that the raw materials for this activity must be absorbed from without the cell, i.e., the suberin lamella permits of an appreciable transference of material.

A further standpoint from which the subject should be considered concerns the actual deposition of the Caspary strip. What are the conditions leading to the formation of this peculiar structural feature? Whatever these are, it may obviously be stated that they are present in the young stem or root and in the old, at least in such cases as Piper, Anacyclus etc., where we find development of Caspary strips in both stages in the history of the stem or root. Previous writers have been concerned only with strip formation just behind the growing point of root or shoot, at the time when general differentiation of tissues is occurring.

Priestley and North (loc.cit.) for instance, point out that the Caspary strip appears just as phloem and xylem are beginning to differentiate, and during the latter process, organic solutes, of fatty acid nature in part, are, they suggest, liberated, and diffuse outwards to the incipient endodermal cylinder, as yet without Caspary strip. In the region of the endodermis these diffusing solutes meet the air passing inwards

1. Although Kroemer's statement is no doubt correct for most cases, the dividing tertiary cells of Alchemilla vulgaris do contain a small amount of starch.

from the cortical intercellular spaces and are oxidised, the products of oxidation being deposited on the radial and transverse walls, in the form of the Caspary strip. A comparison is drawn between the conditions prevailing here and in those resulting in the formation of suberin in the case of wounds, discussed by Priestley and Woffenden (8), and it is suggested that the two processes are similar. The association between the Caspary strip and the differentiating phloem appears to be of more importance than that with the xylem, for in the case of the root with its radially arranged vascular tissues, the endodermis develops first outside the phloem. The development of the endodermis from the innermost layer of the periblem is therefore ascribed on this theory to the proximity of this layer to the air spaces of the developing cortex. This theory affords an explanation of the fact that on any given wall, say a radial wall, the two halves of the Caspary strip are always exactly opposite to each other, an arrangement which is essential if the primary endodermis is to function as suggested above.

Upon preliminary consideration it would appear improbable that a casual oxidation and deposition could result in a very regular Caspary strip system, confined to a single row of cells; but it is the author's experience that irregularities in the position of the strip are quite commonly found. In Piper excelsum the occurrence of air spaces immediately outside the endodermis is very marked, and there is also a definite association between the developing Caspary strip and the vascular tissues.

With regard to the secondary formation of Caspary strips it should be noted that vascular tissues (now of secondary nature) are still undergoing differentiation. It is significant that the original Caspary strips themselves show a considerable enlargement during the ageing of the stem.

Summary.

1. Endodermal cells frequently show considerable increase in tangential dimension at a late stage in their existence, as a result of which the continuity of the endodermis may be maintained, despite the rapid expansion of the intra-endodermal cylinder following secondary growth.

2. This tangential increase in size of the endodermal cells is usually accompanied by their division. The divisions may actually cause the increase in size of the cells, that is, there is active growth of the cells. Or they may follow a passive stretching. In either case the new walls fulfil a mechanical function, lending added strength to the stretched walls of the original cell.

3. Two types of division are distinguishable.

(a). Those occurring in primary endodermal cells. The new walls soon develop Caspary strips, linking up with those of the parent cells.

(b). Divisions in tertiary endodermal cells. The new walls here show neither Caspary strip, nor suberin lamella.

4. It is pointed out that in neither case does the development of new walls interfere with the impermeability of the endodermis.

5. The occurrence of the divisions is considered in relation to the supposed impermeability of the suberin lamella of the secondary endodermal cell, and to the conditions resulting in the deposition of the Caspary strip.

In conclusion I wish to acknowledge the kindness shown by the Regius Keeper of the Royal Botanic Gardens, Edinburgh, in supplying material of Piper species, and to thank my colleagues of the Botany Dept., University of Glasgow, and Professor H. S. Holden, for their constant help.

Addendum.

It should be stated that in this paper the divisions occurring within the endodermis have been considered only in so far as they appear likely to affect the function and behaviour of the endodermis. From this point of view the nuclear processes associated with the divisions are not of great importance.

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LEGENDS TO FIGURES.

Fig.1. Alchemilla vulgaris L. (A).Endodermal cells as seen in transverse section of root. The cells are in the tertiary condition, show some increase in tangential dimension, & the formation of a radial dividing wall in each cell. (B).Endodermal cell from transverse section of older root, showing a very marked increase in tangential dimension,& a chambered condition as a result of the occurrence within it of four divisions.

s.l.,suberin lamella; t.l.,tertiary lamellae; n.w.,new wall; w.p.,wall of primary stage of endodermal cell; g.,gap in suberin lamella; cor.,cortex; per.,pericycle. (×1170)

Fig.2. Alchemilla vulgaris L. Endodermal cells as seen in tangential section of root. Three new radial walls are present in each cell. s.l.,suberin lamella; t.l.,tertiary lamellae; n.w.,new wall; p.c.,passage cell. (×370.)

Fig.3. Piper excelsum Forst. (A). Endodermal cell seen in transverse section of internode showing considerable secondary thickening. The cell shows greatly increased tangential dimehsion & one new radial wall has been produced, as yet without Caspary strip.(×500) (B).Endodermal cells from same section. Shows a single dividing wall with Caspary strip.(×530) (C). Endodermal cells from transverse section of older internode. A very considerable tangential stretching of the cell has occurred & three new radial walls are present, each bearing a Caspary strip.(×370) (D).Endodermal cells from tangential section of a similar internode. Walls bearing Caspary strip shown in thick black.(×370)

c.s.,Caspary strip; n.w.,new wall; cor.,cortex.

LEGENDS TO FIGURES2,(contd.)

Fig.4. Piper excelsum Forst. (A). Endodermal cells as seen in tangential section of stem, after treatment with conc. sulphuric acid for three minutes. (×370) (B). Cells from transverse section of stem, showing a displaced endodermal cell containing a new wall which is tangential with regard to the original orientation of the cell. The arrow indicates radius of stem. (×560) (C). Endodermal cell from transverse section stem, showing peculiar arrangement of the new walls. (×500)

c.s., Caspary strip; n.w., new wall; cor., cortex.

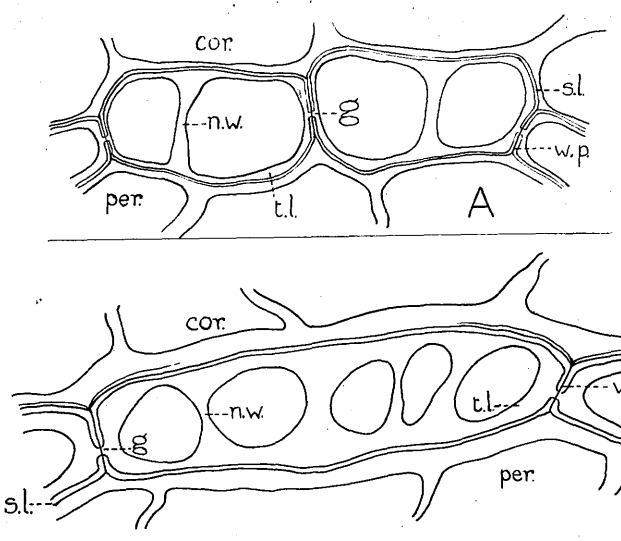

Fig. 1.

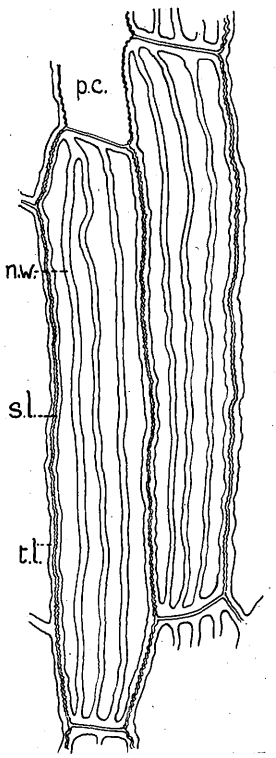

Fig. 2.

Part II of Thesis:-

THE STEM-ENDODERMIS IN THE GENUS PIPER.

CONTENTS.

1. INTRODUCTION.

The most striking feature presented by the stem of species of the genus Piper is of course the abnormal vascular structure. Medullary bundles are present throughout the genus, and there is also an anomalous type of secondary thickening. It was during an examination of these abnormalities in the vascular system that unusual features in the endodermis were detected, and brought to the notice of the author by Professor Drummond. A preliminary examination of different species of Piper suggested that a more detailed investigation would be of some value in the elucidation of the structure and function of the endodermis in general, and further that favourable material would be obtained for experimental work on the primary endodermis. The aim of this paper is then to present an account of the occurrence and the structure of the endodermis in the shoot of selected Piper species.

Reference to the literature showed that the few earlier investigators of the genus had concerned themselves mainly with the peculiarities of the vascular system. J.E.Weiss (1.) made some general observations on the occurrence of the endodermis in the genus, but no mention of the genus is made in the comprehensive monographs by Kroemer (3) and by Mylius (4) on the endodermis, or in the writings of Van Wisselingh. It would appear therefore that no detailed investigation of the endodermis in the genus has been carried out in the past.

The first part of the paper will be descriptive, discussion and theoretical considerations being reserved until later.

2. MATERIAL AND TECHNIQUE.

The material for this investigation was mainly obtained from the Royal Botanic Gardens, Edinburgh, through the kindness of the Regius Keeper, Professor Wright Smith. The following species of Piper (in the wider sense) have been investigated:-

P. angustifolium Ruiz et Pav.

P. celtidifolium ?

P. Chaba Hunter.

P. decurrens. C. DC.

P. excelsum Forst.

P. nigrum ?

P. porphyrophyllum N.E.Br.

P. tiliaefolium Schlecht.

Unfortunately the accuracy of these names is not guaranteed by the authorities of the Edinburgh Botanic Gardens, but, bearing in mind the confusion which exists in the nomenclature of the 700 species included in the genus and the difficulty of identification in the absence of flowering material, it was adjudged best to take the names as correct, and to keep permanent herbarium specimens of each species. These will serve for reference should any doubt arise as to the identity of species investigated in this work.

No authority for P. celtidifolium was given by the Edinburgh authorities, but in view of the fact that this plant was identical externally and internally with another plant bearing the name P. unguiculatum, it is very probable that

the former plant was P.celtidifolium Desf.Cat.Hort. (= P.unguiculatum Ruiz et Pav.)

The material of P.nigrum was obtained from Kew and no authority was given for the name.

In these eight species we are dealing with only a very small proportion of the total number of species in the genus Piper (in the wider sense), which is 700 according to Willis. As, however, will appear from the subsequent part of this paper, it happens that the small group of species here considered includes quite a wide range of types, and is a representative selection.

For general morphological work, material was fixed and preserved in aqueous alcohol, 50-60%. In other cases chrom-acetic acid was used as fixative.

For the particular purposes of this investigation it was usuallly essential that the successive internodes of a shoot should be kept in strict order, and to ensure this, series of small and numbered glass tubes were used, one internode being fixed and preserved in each tube.

With regard to the choice of Stain, one was required which would give the best possible definition to the Caspary Strip. Generally speaking the Caspary Strip gives positive reactions with lignin stains. Those recommended by the Marburg school (see Kroemer and Mylius, loc.cit.) are:- Phloroglucinol and Hydrochloric acid, Chlor-zinc-iodine, and Methylene Blue-glycerine. I have found Gentian-Violet-glycerine as well to be very useful, but for nearly the whole

of this work the stain used has been Ammoniacal Basic Fuchsin, on the suggestion of Dr. S. Williams. This stain is not mentioned by the principal investigators of the endodermis, but appears to me to be a very valuable one for the purpose. The reagent is made up by dropping slowly a 5% solution of Basic Fuchsin (a Rosanilin dye) in 75% alcohol into .88 Ammonia, until the originally colourless liquid takes on a straw colour. There are two methods of using the stain. The section may be immersed in a drop of the reagent on the slide and allowed to stand. As the ammonia evaporates off the lignified and suberised walls take on a vivid red colour. The cellulose walls also stain a light red, but this is washed out during the subsequent washing and dehydration with absolute alcohol. The section is cleared in clove oil and mounted in balsam. In the case of serial microtome sections, the slide is dipped into the stain for a few seconds, then withdrawn and exposed to the atmosphere for a further few seconds until the red colouration develops, and then treated as above. This is the procedure adopted in the present investigation, but an alternative method is to immerse the section in the stain, contained in a covered receptacle, for about 10 minutes, and then rinse several times in absolute alcohol, when the deep red colouration will develop in lignified and suberised cell walls. This stain has two advantages; firstly the extreme rapidity with which it can be used, for a well-stained balsam mount can be obtained within 5 minutes of cutting the section, and secondly, it is a

permanent stain. It can be used either alone or in combination, Light Green in clove oil being a suitable counter stain. In case the Basic Fuchsin is used alone, as in the present work, the degree to which the cellulose walls are stained can be accurately controlled by destaining with acetic-alcohol for a few seconds. Unless otherwise stated, all the illustrations in this paper have been made from preparations stained with Basic Fuchsin.

In the investigations of the Caspary strip and of other features presented by the walls of endodermal cells, it is very advantageous to remove cell-contents by means of a short preliminary treatment with Eau de Javelle. Especially is this the case in the investigation of the first appearance of the Caspary strip, which, while very minute, is liable to be concealed by cell contents in close contact with the walls.

Some danger, however, attends the use of Eau de Javelle in working with the Caspary strip, for the latter is liable to be changed if exposed to the oxidising action of Eau de Javelle over too long a period. According to Kroemer (loc.cit.), lignified walls in general tend to lose their characteristic properties on continued exposure to Eau de Javelle, and to assume those of pure cellulose. This, he says, constitutes a valuable distinction between lignified and suberised walls, the latter being unaffected after as long as fourteen days. The Caspary strip, as will be expected since it in all probability is lignified, is altered on relatively prolonged exposure to Eau de Javelle. Thus in some case Kroemer found that after an

exposure of an hour or more the strip no longer stained with Chlor-zinc-Iodine, although it did with phloroglucinol. Neither did the strip take up Congo Red, or Napthylene Blue. The strip, in its original condition, is of course characterised by insolubility in sulphuric acid of any strength, while the cellulose portion of the endodermal wall does dissolve in the concentrated acid. But Kroemer showed in the root of Haemanthus Lindeni after twelve hours treatment with Eau de Javelle, the radial wall in the region of the Caspary strip rapidly dissolved in 50% sulphuric acid, while the remaining cellulose portion of the radial wall was not dissolved, but merely showed swelling. Thus the action of Eau de Javelle results in the Caspary strip becoming more soluble towards sulphuric acid than is the unmodified cellulose wall in the beginning.

Mylius (loc.cit.) also refers to this matter. He finds in some cases a special sensitiveness on the part of the strip to Eau de Javelle. In the Myrtaceae - roots of Myrtus communis, Melaleuca ericifolia, Leptospermum laevigatum - after a relatively short (but not actually stated) period of exposure the strip appeared to lose its staining properties with Phloroglucinol, Chlor-Zinc-Iodine, and Methylene Blue. He concluded that the reagent removes certain constituents from the strip.

But all the authorities are agreed that in general a relatively short treatment of sections with Eau de Javelle has no evident effect on the Caspary strip, and it has been in the past a regular procedure to clear with this reagent in the

investigation of the endodermis. To make sure that the strip of Piper species is not changed by the use of Eau de Javelle, the following experiments were carried out. Thin transverse sections were cut from the second internode of a stem of P.porphyrophyllum. Good-sized Caspary strips were present, regularly deposited. The sections were immersed in fresh Eau de Javelle for varying periods, namely fifteen minutes, one hour, twelve hours, and twenty-four hours respectively, and then stained with basic fuchsin. The Caspary strip remained to all appearance unchanged in all cases, except for a slight decrease in size after the longest period. The cellulose walls showed increasing thin-ness with longer exposure.

Since in this investigation, the preliminary treatment of sections with Eau de Javelle only extended over ten minutes on the average, there is no reason to believe the strip to be in any way affected by the treatment.

The use of Eau de Javelle and of Ammoniacal Basic Fuchsin renders FIXATION by the use of the ordinary Albumen fixative impossible, for the latter will not withstand the strongly alkaline reaction of either reagent. ~~Futile~~ Unsuccessful experiments were made with the Gum Arabic and Dichromate fixative recommended by Chamberlain (Methods in Plant Histology), with Lepage Glue, etc., but at last the writer came across the Collodion-clove oil fixative used by Miss Barratt (2.) in her study of the growing point of the stem of Hippuris, where clearing was also desirable.

The actual fixative consists of a mixture of solid

Collodion and clove oil, in rough proportions of 1:1. On standing, a sticky jelly is obtained. The stretching of the ribbons is effected not on the slide to which they are ultimately to be fixed, but on a second slide lightly smeared with glycerine to obtain an equal spread of water. With a little experience this task can be carried out with accuracy and dispatch, especially if a suitable pair of broad-tipped forceps is used. A slight emulsion forms between the moisture on the ribbon and the clove oil, but is of no consequence. The ribbons thus fixed are left to dry for at least twenty-four hours, and the wax then dissolved in the usual way.[1] The complete process used in the case of serial sections was therefore:-

Stretch ribbons in water.

Transfer to slide smeared with Collodion-clove oil fixative.

Leave to dry for twenty-four hours.

Dissolve wax as usual and pass down to 25% alcohol.

Transfer to Eau de Javelle and clear for suitable period.

Wash in running water for thirty minutes.

Pass up to 75% alcohol.

Stain with Basic Fuchsin.

Destain with Acetic alcohol, if necessary, ten to twenty seconds.

Absolute alcohol, twenty seconds.

Alcohol-xylol, 1:1, five seconds.

Xylol.

Balsam.

1. By the use of this fixative permanent mounts of sections stained with Sudan III can also be obtained.

As regards the actual CUTTING, while the younger internodes cut very satisfactorily on the microtome, the older did not, owing to the presence of a high proportion of lignified tissue. This latter is mainly confined to the vascular core, and since for the study of the endodermis a section which includes the cortex and periphery of the vascular tissues is quite satisfactory, portions of peripheral tissues were cut from the thicker stems, and these microtomed relatively easily. In all cases it was found to be advantageous to soak the blocks before cutting for as long as possible in water as is recommended by Chamberlain. Hand sections were also relied upon to a considerable extent in this investigation, especially in dealing with the distribution of the endodermis.

3. GENERAL ANATOMICAL FEATURES OF THE STEM.

The medullary bundles form a very conspicuous feature of the stem throughout its development. A varying number of them are arranged in one or more rings in the parenchymatous pith, one ring in P.porphyrophyllum (Pl.I,Fig.3), P.excelsum etc., two in P.chaba and P.tiliaefolium. According to J. E. Weiss (loc.cit.) in all species of Piper examined by him the medullary bundles are foliar in nature; each leaf trace extends through at least the first internode below its point of entry to the stem as a member of the peripheral, normal, vascular ring, then passes into the medullary bundle ring and remains there for at least a further internode; finally it fuses with the medullary bundles of lower internodes. The

outer vascular ring includes larger and smaller foliar bundles, and still smaller bundles of a cauline nature. These latter are the only true cauline bundles present in the stem, and extend through the length of one internode only, and at the node fuse with nearby foliar traces or with other cauline bundles of the adjacent internode.

Pl. VIII. Fig. 32. (re-drawn from J.E.Weiss) shows the course of the vascular strands through a lower node in the stem of Chavica Roxburghii (= P.longum L.). The small cauline bundles are omitted from this diagram and presumably the vascular connections to the axillary bud were undeveloped. In the lower internode four of these cauline bundles were present in the outer ring, in addition to the ten foliar bundles. The diagram indicates how all the latter strands pass out into the leaf base at the node, and the outer ring of bundles of the upper internode is reconstructed by the passage outwards of bundles occupying a medullary position in the lower internode. Prior, however, to this outward movement of the medullary bundles, anastomoses occur between them and the medullary strands of the upper internode.

A diversion may be made at this point to consider the effect of this peculiar arrangement of the bundles on the physiology of the plant. As we have seen, the peripheral bundles are chiefly continuations of leaf traces, extend through one to two internodes only, and do not anastomose at the nodes with the peripheral bundles of other internodes. Therefore the peripheral bundles provide no continuous

vascular system up the stem. This exists only in the pith, and we are left with the conclusion that the main stream of translocation must flow through the medullary bundles, and not through the peripheral bundles. The latter appear to be of more significance in the mechanical arrangements of the stem.

This conclusion is supported by other facts. In certain semi-prostrate species - P.porphyrophyllum, P.nigrum - where great mechanical efficiency is not required, there is practically no secondary thickening or increase in size of the bundles of the outer ring in the lower internodes. Contrasted, however, with this relatively poor development of the outer bundles, the medullary bundles continue to increase in size as the result of the activity of the primary cambium present in the bundle, until they attain a relatively very massive size in older internodes, with well developed vessels and phloem(Pl.I.Fig.6., Pl.I.Fig.3.). It seems very obvious that in these examples the medullary bundles are of chief importance in translocation. In erect species the outer vascular ring is much more extensive, especially after continued secondary thickening, but the secondary vascular ring contains a relatively small proportion of typical conducting elements. For, as will be described below, a large proportion of the ring consists of living medullary ray tissue, while the bundles themselves consist mainly of fibres, with relatively few vessels (Pl.I.Fig.2).

It should be possible to test the matter in a simple

fashion, by ringing off the peripheral vascular system from a zone in a basal internode of a shoot, and observing the effect of this on the water content of the leaves above. This experiment will be carried out when suitable plants are available.

To return to the anatomy of the stem. The anomalous type of secondary thickening is important in a consideration of the endodermal relations. The cambial ring becomes completed in a normal fashion by the development of strips of cambium across the primary medullary rays. In the young stem the interfascicular cambium-mother-cells form a narrow zone of thin-walled cells, originally pro-cambial, lying between the medullary fibrous ring (see later) and the endodermis, in examples where the latter is present. In some species., e.g. P.excelsum (Pl.III. Fig.11), the zone typically comprises a single layer of cells; while in others - P.angustifolium - a double layer may be present. The occurrence of repeated tangential divisions in these cells leads to the production of a deep cambium (Pl.VI.Fig.24.). The cambium of the bundles behaves more or less normally, producing phloem on its outer side, and xylem, comprising vessels and fibres, on its inner surface. No medullary (vascular) rays are produced by the bundle-cambium in the species examined here (Pl.IV. Fig.14.). The cambium over the rays produces on its inner side a tissue composed of cells which are square in transverse section, several times as long as they are wide, and bear small circular pits on all walls. These cells become lignified very early,

but contain abundant starch grains and therefore are living. This tissue forms plates lying between the vascular bundles (Pl.I.Fig.2, Pl.I.Fig.4, Pl.IV.Fig.14.), and for them the term "primary medullary ray" is usually retained, since they resemble true primary medullary rays in position.

The interfascicular cambium displays no active tissue formation on its outer surface, and merely produces a few cells of parenchymatous nature, similar in size to the cells of the primary rays and arranged along the same radii(Pl.II. Fig.9., Pl.V.Fig.15.).

As the result of this abnormal behaviour of the interfascicular cambium, the very characteristic secondary structure of the stem arises, in which the original bundles of the primary stem do not lose their identity in a common vascular ring, as is the case in the normal stem, but are easily distinguishable throughout as vascular units, separated by the primary medullary rays (Pl.I. Fig.2).

The medullary bundles show some subsequent increase in size, as a result of the activity of the cambium of the bundles.

There remain a few other structural features worthy of note. Very beautiful arrangements of the mechanical tissues are presented by the different species. The bundles of the peripheral ring are, in the primary stem, embedded in an undulating zone of sclerenchyma, which is medullary in nature and is usually continuous (Pl.IV. Fig.13.). This fibrous ring may become ruptured as a result of subsequent dimensional changes in the stem during secondary growth, e.g. P.chaba, P.excelsum.

In the young stem the cortex is largely collenchymatous, the collenchyma forming either a complete ring in transverse section, as in P.excelsum (usually) and P.tiliaefolium, or separate strands, as in P.chaba, P.celtidifolium. If a complete ring is present it usually undergoes rupture as the stem ages and increases in diameter. Although in some species the collenchyma retains its distinctive characters throughout, in the majority a convertion of some or all of its cells into sclerenchyma is to be observed.

Pericyclic fibres may also be present capping the bundles, and may develop in the primary stem. This is the case in P.celtidifolium, and P.chaba, and in the latter the two ~~ends~~ edges of the cap are often fused with the medullary fibrous ring, so that the phloem is completely cut off from the cortex. In other cases the pericyclic fibres are not fully differentiated until after the inception of secondary growth, e.g. P.excelsum, P.angustifolium.

The above description applies mainly to the internodal region of the stem. We have at the node, as already noted, a considerable re-arrangement of vascular strands, and to facilitate this we find that at the node the medullary fibrous sheath becomes interrupted by parenchyma, and that there is no linking up of the cambium of the vascular bundles by interfascicular cambium. Thus the pith and cortex are connected by thin-walled cells. The endodermis also becomes interrupted during the outward passage of the leaf traces into the petiole.

Mention should also be made of the occurrence of Secretory cells in the stems of all the species I examined. Solereder (Syst. Anat. Dicots., translr. 1908. P.691), describes

these as approximately spherical cells, with clear or brown or yellow contents and suberised walls, and as occurring in pith, cortex, and phloem. Those in the species considered here were similar in structure and distribution, but in addition they occurred in the endodermis (see later). The nature of the contents of these cells was not definitely determined, but were not of a tannin or protein nature, and gave a positive reaction with Sudan III. Suberisation of the wall was detected in those occurring in the phloem, but not in those found elsewhere.

In addition to these secretory cells, other cells possess characteristic inclusions. Thus Calcium oxalate crystals are present in P.excelsum and P.tiliaefolium. And in P.excelsum and P.chaba a red pigment, presumably of anthocyanin nature, is present in ~~the~~ solution in cells of the cortex, pith, and even of the parenchyma associated with the vessels of the primary xylem. The cells containing this pigment do not otherwise differ in appearance from normal cells, except perhaps in the possession of fewer chloroplasts in those occurring in the cortex.

There remains one further point of interest, namely the distribution of stomata in the epidermis of these stems. In P.excelsum, P.angustifolium, P.chaba and P.tiliaefolium no stomata were to be seen in the epidermis of the stem. In P.excelsum a careful examination of serial transverse sections of the first and second internodes of a shoot, and surface sections of the epidermis of stems of all ages showed that no

stomata were present. In this species cork formation was not obvious even in the stoutest internodes, but lenticels were produced, necessarily independent of stomata, a process which of course is normal in roots and in some stems. In the remaining species stomata were present in the stem epidermis.

4. THE ENDODERMIS.

A. OCCURRENCE AND DISTRIBUTION.

J.E.Weiss (loc.cit.) paid some attention to the stem-endodermis in the genus, especially to its distribution, in which he found remarkable variation in the different species he examined. The medullary bundles were devoid of endodermis throughout. With regard to the occurrence and distribution of the endodermis associated with the peripheral vascular system, he distinguished three types:-

1. In which the endodermis formed a complete ring round the vascular cylinder, e.g. P.Bredemeyeri Jaqu, P.geniculatum Sw., P.bullatum Pth.

2. In which the endodermis was only present as a cap to the phloem or to the pericyclic fibres, e.g. P.carpunya R et Pav.

3. In which the endodermis was completely absent, e.g. P.rivinoides Kth.

He also failed to detect endodermis in the petiole and lamina.

It may at once be stated that the species examined in this investigation resembled those dealt with by Weiss in that the medullary bundles were always devoid of endodermis. As regards the appearance of endodermis in association with the outer ring of bundles, a broad distinction can be made between species in which typical endodermal cells are present, and those in which they are entirely absent. Of the eight species examined only one, P.celtidifolium, was of the latter type, and will be considered first.

In this species, a transverse section of the first internode of a shoot showed the vascular tissues moderately differentiated, but already a cap of relatively very large cells was present over each bundle, limiting the cortex internally, with no conspicuous cell contents and not possessing starch. These features are retained by the sheathing layer of cells throughout the later development of the stem (Pl.II. Fig.8.), and Caspary strips are never found.

Turning to the remaining species where endodermis is developed, a very considerable degree of variation in the distribution of the endodermis is encountered, between different species, and even between different shoots of the same plant.

In three species, however, namely, P.angustifolium, P.porphyrophyllum and P.nigrum, the endodermis shows a pretty uniform and constant distribution. In these three species, in material collected in the early part of the year (January, February, March) Caspary strip formation was obvious a little distance behind the apical bud, in the first or second internode, and took place over the bundle initially, so that at an early stage a discontinuous endodermis was present, having the form of a series of "caps" to the phloem of the bundles. But very soon strip development occurred over the primary medullary rays too, linking up the separate caps, and perfect continuity of the endodermis was established. In P.nigrum and P.porphyrophyllum this latter condition was sometimes found in the lower part of the first internode (Pl.II.Fig.10), but not until the second or third in P.angustifolium. Material

of the latter collected in July showed a similar development. A continuous endodermis was maintained throughout all later developments of the internodes in these species, so that a continuous endodermis is still present in oldest internodes despite the great increase in the dimensions of the stem which may occur as a result of continued secondary thickening. This increase is most marked in the case of P.angustifolium, an erect, shrubby species (Pl.I. Fig.4). The other two are more or less prostrate or climbing species and show little secondary growth in thickness (Pl.I. Fig.3). The above description is based on the examination of three different shoots of P.angustifolium, all from one plant, two of P.porphyrophyllum, also from one plant, and a single shoot of P.nigrum.

It is in the remaining four species that variation in the distribution of the endodermis is most marked, and a description of each species individually will indicate the range and nature of the variation.

P.excelsum will be considered first. The material of this species was obtained from two sources, namely, from a well grown plant in the Royal Botanic Gardens, Edinburgh, forming a bush six feet high, and from a cutting taken from this former plant, but grown over in the Glasgow Botanic Gardens. The results of the investigation of the former material will be dealt with first.

The first material from Edinburgh was taken in February 1928. The bush had been pruned recently and this made it impossible to obtain shoots showing more than a small number of truly consecutive internodes - i.e. internodes produced by

the continued growth of one apical bud. Only young internodes and relatively old ones were to be obtained.

Some variation in the distribution of the endodermis in the young internodes was found. The deposition of Caspary strip over the bundles, so that each bundle possessed an endodermal cap, was a very constant feature in the development of the primary stem, and was generally completed in the second internode (PL.IV. Fig.13). In the lower internodes a definite tendency towards the completion of the continuity of the endodermis was exhibited, as the result of deposition of Caspary strip over the primary medullary rays. This deposition of Caspary strip started from the ends of the bundle caps and spread tangentially across the rays. Some such deposition was generally to be seen towards the base of the second internode, and by the time of initiation of secondary vascular cambium a good proportion of rays were spanned. In two shoots, slightly before the first divisions of the cambium-mother cells, 7/17 rays were crossed, and others partly so. In a third shoot, a section showing the first cambial divisions also showed 8/16 rays spanned, and in the next lower internode, where there was naturally a more advanced cambial activity, the number of rays crossed had increased to 10/15. In a fourth shoot, a transverse section through the middle of the third internode, where a four to six cell-deep cambium was present, 8/26 rays were spanned, collected in a group to one side of the stem. Finally in another shoot, a transverse section at a rather later stage showed considerable secondary growth and an endodermis con-

-tinuous except for gaps over two rays.

To sum up; while the production of an endodermal arc to each vascular bundle occurred with great regularity, there was some variation in the subsequent deposition of Caspary strip over the interfascicular rays, and the endodermis was in a semi-continuous condition in the young secondary stem.

To turn now to the older stem material collected at the same time. Here a definitely discontinuous endodermis was found, especially in the very stoutest internodes, showing one annual ring. The diameter of these internodes had been more than doubled as a result of secondary growth in thickness. In such internodes the endodermis was practically limited to the vascular bundles, forming a cap arching over the phloem of each bundle (Pl.II. Fig.9., Pl.IV. Fig.14.).

P.excelsum was the first species to be studied in detail, and it was assumed that the endodermis would show similar developmental stages in the different internodes of one shoot, and in different shoots of one plant. On this assumption, the definitely discontinuous distribution of the endodermis in old internodes must be derived from the more or less continuous phase found in young internodes. It seemed very probable that the endodermis failed to accommodate itself to the constantly increasing diameter of the endodermal cylinder, and underwent rupture over the medullary rays, and that the initially small gaps continued to increase in magnitude until fianlly the endodermis was limited to the vascular bundles.

A great deal of time was spent looking for "stages" in

this supposed partial disintegration of the endodermis, and quite a fair case was made out. Thus in moderately thick internodes entire discontinuity was not found in the endodermis; some of the rays were spanned and intermediate stages between this and wide gaps extending right across the ray were present in the same section. When a small gap was present in the ray, the outermost cells of the radially arranged columns of cells cut off on the outer surface of the cambium were protruding into the cortex, in a manner suggesting that these cells, partly perhaps by their own expansion, and partly as a result of the increasing bulk of the tissues enclosed within the endodermal cylinder, had pushed their way through the originally complete endodermis, and had displaced some of the innermost cortical cells (Pl.V.Fig.15). That the activity of the cambium will impose a strain on the endodermis is of course obvious, especially since we have inside the cambium the ring of fibres forming as it were a rigid base upon which the cambium builds its tissues, pushing the endodermis outwards. Soon after the initiation of cambial activity it becomes evident that this is the case. Cells of the endodermis over the rays became displaced outwards, and there was a partial splitting of the radial walls of the endodermal cells along the middle lamella, both doubtless as a result of cambial pressure (Pl.V. Fig.16., Pl.V. Fig.19). At first it seemed very probable that ultimately a radial wall underwent complete splitting, and that the small gap so made increased in width as the stem thickened until finally the gap extended right across the ray. This last process is however difficult to visualise, and further a long

search failed to reveal a stage in which a complete splitting of the radial wall had obviously occurred. In fact, the experience gained in the investigation of other species leads me to the conclusion that in P.excelsum we are up against a different development of the endodermis in different shoots, and possibly in different internodes, of the same plant. The old internodes, with discontinuous endodermis, had possessed such an endodermis throughout their earlier history; while those rather younger internodes, with partial continuity, had also displayed such partial continuity throughout the whole of their development.

A further collection of P.excelsum material was made from Edinburgh in January 1930. In a first shoot, of nine internodes, the endodermis was confined to the bundles throughout. In a second, the endodermis was again confined to the bundles, but its formation did not occur outside every bundle, as was practically the case in the first shoot. Thus in the middle of the third internode, where cambium initiation was proceeding, endodermal cells were present over one third of the bundles only. In the fifth internode about half the bundles were capped, but many of the caps were scantily developed. A section through the centre of the seventh internode showed only a quarter of the total number of bundles to have any endodermal cells in association with them. In the eigth internode considerably more endodermal cells were differentiated, and in the lowermost internode, the ninth, every bundle was capped.

In a third shoot, there was again complete discontinuity

throughout, all the bundles in the lower internodes being capped, but in the fourth internode a group of four were without, and in the third a group of six, directly under the insertion of the leaf at the node above in each case. This shoot bore four lateral axillary shoots, and the distribution of the endodermis in these was followed. In these shoots, unlike the main shoot, there was appreciable formation of Caspary strips over the medullary rays, the vascular caps showing lateral extensions. This tendency was especially well marked in the lowest but one axillary shoot, where, in the third internode, several of the rays were practically crossed, and many caps showed lateral extensions.

In a fourth shoot the bundles were capped, ~~but~~ and these caps showed some lateral extension, but this tendency to continuity was not well marked.

The last shoot to be examined presented very peculiar features. Down to the base of the seventh internode the endodermis was practically entirely limited to the vascular bundles. But then in the eighth internode a very different state of affairs was discovered. A complete transverse section through this internode (diameter 5 millimetres) showed twenty five primary medullary rays to be present, and of these seven were completely spanned by endodermis, and in six others only very small gaps interrupted the continuity. In some of the latter the columns of parenchymatous cells produced on the outer side of the cambium were protruding through the gaps as in the 1928 material. Marked stretching of the endodermal cells over bundles and rays had occurred, accompanied by subdivision.

This shoot also bore lateral shoots, and the two lowest and largest were examined. In the second internode of the lower of the two, a marked approach to endodermal continuity was indicated, for 8/22 rays were completely spanned by endodermis, and others were all but crossed. In the third internode of this shoot the continuity was less marked. The upper of the two axillary shoots showed very little Caspary strip formation other than over the bundles.

In this material of P.excelsum, all obtained from one plant, we have then a very great variation displayed in the development and distribution of the endodermis, both in different shoots and in different internodes of one shoot. We can say that in perhaps the typical case the endodermis develops only in relation to the bundles of the outer ring. Yet in other cases there is a distinct approach to continuity, while at the other extreme, we have an internode in which barely half the bundles have any endodermis over them.

Cuttings from this plant were struck in Edinburgh and brought over and reared in Glasgow. Two shoots from one of these cuttings were examined when of suitable size, and here the tendency towards continuity of endodermal was very well marked, for a continuous endodermis was developed in young internodes and remained continuous throughout the later development of the internodes, even when the diameter of the latter had been doubled. In view of the great variation in the distribution of the endodermis in the original plant, it is difficult to decide whether this development of a contin-

-uous endodermis is to be related to different conditions of growth over in Glasgow, or whether it is just an extreme condition which could be found on the parent plant itself were a sufficient number of shoots examined. That the latter is probably the case is suggested by the fact that in the lateral shoots present on the two main shoots from the cuttings, only a partially continuous endodermis was present. Thus in one of these axillary shoots, in the centre of the fourth internode, 9/18 of the rays were spanned, while in the fifth internode a section through the central point showed 8/16 rays to be crossed.

So much for P.excelsum. P.chaba will now be considered. The first material of this species was collected in February 1928 from Edinburgh Botanic Gardens. The first formation of Caspary strip was observed shortly before cambium initiation, and at the time of the latter most of the smaller vascular bundles possessed a small endodermal cap. In slightly older internodes some strips were also present in relation to the larger vascular bundles, and some of the medullary rays were spanned. In the case of the larger bundles Caspary strip deposition appeared to commence at the sides of the phloem and proceed upwards over the bundle, but in the thickest internodes of this shoot complete caps over the larger bundles were never present, and a very incompletely developed endodermis was thus present here.

A second shoot was taken from a cutting of the Edinburgh plant. This shoot was characterised externally by the possession of relatively short internodes, each about five centimetres

long only, so that the development and differentiation of the shoot as a whole was cramped. In the first internode the tissues generally were incompletely differentiated, and no Caspary strips were present. There was a very sharp transition from this to the second internode, in which secondary thickening was in progress even at the top. A curious asymmetrical development of the stem had occurred in this and subsequent internodes, directly related to leaf arrangement. Other examples of this will be described later. To one side of the stem the vascular bundles were small and crowded and practically all these bundles were capped by endodermis, some of the caps being linked up. On the other side of the stem, directly under the insertion of the leaf at the node above, the bundles were much larger and there was scarcely any deposition of Caspary strips in relation to these bundles. These larger bundles represent the continuations of the larger midrib bundles of the petiole and leaf of the node above. This asymmetry was still more in evidence in lower internodes because secondary thickening had occurred more rapidly in the region of the small bundles. Thus in the middle of the third internode considerable secondary growth had occurred in connection with the smaller bundles, but practically none on the other side, and correlated with this, in the region of secondary activity the endodermis was practically continuous, while it was present over the bundles only on the other side.

In the lower internodes of this shoot formation of Caspary strips had evidently proceeded on a smaller scale.

In the middle of the fourth internode Caspary strips were present over 20/50 bundles, especially over the smaller ones, while in the fifth, Caspary strips were present outside four bundles only, and a similarly scanty development had occurred in the sixth and lowermost internode.

It must be concluded that the factors controlling Caspary strip deposition have varied during the development of this shoot, for in the first formed internodes Caspary strips were few and far between, while they were quite abundant in the second and third internodes.

In the third shoot of this species, from the original plant in Edinburgh, a rather different state of affairs was found. The internodes were longer here than above, the average length being eight centimetres, and coupled with this increased growth in length was a more gradual increase in differentiation as sections were taken at successively lower points down the shoot. No Caspary strips were present in the first internode. In the upper part of the second, the first divisions of the cambial mother cells were proceeding to one side of the stem, but there were still no Caspary strips present. At the base of this internode, however, Caspary strips had been formed outside five bundles, in a loose group. But in the third internode no Caspary strips whatever were observed, neither in the fourth. In the fifth internode, the lowermost, in a sample transverse section, only two cells were present with Caspary strips.

On the basis of this examination of three shoots of P.chaba the following conclusions may be drawn, namely, that

the degree to which the endodermis is developed varies greatly in different shoots, and in different internodes of one shoot, although all these internodes have arisen from the same apical meristem. In some cases the endodermis is absent altogether, in others is not far removed from complete continuity.

P.tiliaefolium will be considered next. This again presents puzzling features. The first material to be examined consisted of the four uppermost internodes of a shoot, and Caspary strip deposition first appeared in the third internode. A transverse section through the centre of this internode showed each vascular bundle of the outer circle to be capped by endodermis. At the base of this internode some of these caps had become linked as a result of the development of Caspary strips over some of the narrower primary rays. In the fourth internode there was a definite approach to continuity, a good proportion of the rays being spanned. Pruning had upset the sequence below this point, but in all older material collected at the same time a definitely discontinuous condition of the endodermis was evident.

In a second shoot the formation of endodermis was limited more strictly to the vascular bundles, although in the lower internodes there was some lateral extension of these endodermal caps.

In a third shoot, from a cutting grown from the above plant, here again some attempt at continuity of the endodermis was shown. For in the fourth internode 9/38 rays were crossed, although over most of the others there was no strip formation whatever. This shoot was a lateral shoot springing from a

stouter shoot base, the upper portion of the latter having been pruned off. Sectioning was continued in the internode below the point of junction of the shoot with the "stock", and again strip formation had occurred over the rays, leading to complete crossing of the rays in a few cases and incomplete crossing in others.

In a final shoot, the endodermis was confined throughout to the vascular bundles.

All this seems to suggest that in P.tiliaefolium we have a species which is essentially characterised by the possession of a discontinuous endodermis throughout - i.e. the endodermis is developed only outside the bundles - but yet the species displays a marked leaning towards endodermal continuity.

Finally we have P.decurrens. This species shows slow growth in length and the "differentiation gradient" is correspondingly steep. The internodes throughout are often relatively very stout, chiefly owing to a bulky pith, and as a result of this the diameter of the stem, and therefore of the endodermal cylinder, undergoes relatively little increase, even after prolonged activity of the secondary cambium.

There were seven internodes in the first shoot examined, and though slight variation showed itself, generally speaking a peculiar partially-continuous endodermis was present, each bundle being capped, and some of the caps linked up. In the lowest internode 11/39 rays were crossed, especially the smaller ones, and others were all but crossed. The degree of continuity was rather less in the younger internodes. A

similar partially continuous endodermis was found in a second shoot of this species. In the stoutest internode of this shoot 22/36 rays were crossed.

The above description relates only to the distribution of the endodermis through the internodes of the stem. To complete the picture some account must be given of its distribution in the nodes. These are generally swollen, due to enlarged cortex and pith. J.E.Weiss found the endodermis to be absent from the node in P.geniculatum and P.carpunya, and in addition the medullary fibrous ring and the cambium ring (in older stems) became discontinuous. It is obvious that some such structural modifications must be made to allow of the interchange of bundles between petiole, outer vascular system and medullary vascular system.

Serial sections were cut through the fifth node in a stem of P.porphyrphyllum. The medullary sheath was in a state of discontinuity in the node and was only present as an inner cap to the vascular bundles. The endodermis however was present right through the node, and only showed such local interruptions as were necessary to allow of strands passing from the outer vascular ring into the cortex and ultimately into the leaf base, or into the axillary bud(Pl.II. Fig.7.) But just above the node, at the level of the axillary bud, four gaps were present in the endodermis over medullary rays. These gaps had disappeared however at a point three millimetres above the node, and the endodermis was once more continuous. From this node two adventitious roots were springing and the sections indicated that the stèles of these roots

were sheathed in endodermis in continuity with the stem-endodermis.

Sections were also cut through the third node of the same shoot, and here the tendency towards non-development of the endodermis immediately above the node was much more stressed. In this case the endodermis was practically absent from a narrow zone level with the axillary bud, for only five bundles were capped by endodermal cells. As sectioning was continued up the second internode the endodermis soon regained its continuity, but the reconstitution was slow on the side of the axillary bud.

From the examination of these two nodes it looks as though there is a delayed strip deposition at the very base of each internode. These sections also showed that as soon as a bundle leaves the outer ring of bundles and passes into the cortex, preparatory to entering the petiole, Caspary strip formation in relation to the bundles ceases, and the latter becomes surrounded by a more or less definite starch sheath. This is the case right up the petiole into the leaf. Endodermis was not found in the petioles of any of the species investigated here.

The nodal distribution of the endodermis was also investigated in P.excelsum. Serial sections were taken through the fifth node of an axillary shoot. The endodermis in the fourth internode showed a partially continuous distribution, for in a section through the centre of this internode 9/18 of the rays were crossed by endodermal cells. The degree of continuity fell as the node was approached.

Thus at a point five millimetres above the node, only five rays were crossed, although a number of others were partly crossed. At a point very slightly below this, the endodermis was confined strictly to the vascular bundles, and as the node was still more closely approached, a gradual disappearance of the endodermis from over the bundles also ensued, accompanied by a disappearance of the secondary tissues and cambium lying between the bundles.. The fibrous medullary sheath was also gradually eliminated, and was partly replaced by inner collenchymatous caps to the bundles. At a point just above the insertion of the axillary bud, no endodermal cells whatever were present, and the pith was in parenchymatous communication with the cortex. No endodermal cells appeared through the node itself, but in a section slightly below the node, which showed the leaf trace bundles just emerging from the outer ring of bundles, here and there an odd cell bearing a Caspary strip appeared over the bundles. From this point the endodermis made a gradual reappearance accompanied by a reconstitution of the medullary fibrous ring and the interfascicular tissues. At a point 2.5 millimetres below the node, every bundle was capped, while at three millimetres below the node some of the bundle caps were linked up as a result of the development of endodermal cells over the rays. At the middle point of the fifth internode 8/16 rays were crossed. It will be seen that in this species we have a close approach to the condition which J.E.Weiss found in the nodes of P.geniculatum and P.carpunya.

These observations on the distribution of the endodermis

may now be compared with those made by Weiss. It does not appear from his account that Weiss paid much attention to the endodermis. The species he studied may have shown variation in endodermal features, or may have been relatively "fixed" types such as P.angustifolium. In any case, it is evident from the above details that the species examined here do not fall into such well-defined groups as he found. The situation is best summed up as follows:-

Endodermis absent:- P.celtidifolium.

Endodermis present and continuous:- P.angustifolium, P.nigrum, P.porphyrophyllum.

Endodermis present, but of variable distribution:- P.excelsum, P.chaba, P.tiliaefolium.

P.decurrens usually shows a condition of partial continuity.

B. Development of the Endodermis and characters of its Cells.

It is to be expected that the level at which differentiation of the endodermal cells occurs below the apex will vary, both at different seasons of the year, and in different shoots in the same season. Observations confirm this expectation.

It might be advisable to add first a note on the order of development and differentiation of the vascular system of the stem in view of the close association between the vascular tissues and the endodermis. J.E.Weiss (loc.cit.) describes the development of the different parts of the stele in species studied by him. Of the species examined in this present work the stelar development was followed in P.excelsum, and coincides with Weiss's account. The larger bundles of the outer ring differentiate first, i.e., those representing continuations of the mid-rib strands entering from the leaf inserted at the node above. The medullary traces develop next, and simultaneously or slightly later the smaller bundles of the outer ring.

The development of the endodermal layer in P.excelsum was examined first. In all shoots there was no sign of Caspary strip formation in the uppermost exposed internode. The remaining tissues of the stem were also in an early stage of differentiation at this level. The layer of cells destined to develop into the endodermis was obviously the innermost layer of the cortex, and its cells were characterised by the possession of abundant starch grains, and by their relatively large size as compared with

the small cells of the pericycle and phloem to the inside. Strip formation was first to be seen in the second internode, and generally coincided with the lignification of the medullary sheath. The first strips appeared over some of the vascular bundles, especially the smaller ones, and usually over the centre of the bundle (Pl.VIII. Fig.35.), the deposition spreading right and left from this central point until the phloem of the bundle was capped by an arc of endodermal cells. This process of strip formation shortly afterwards occurs over the larger bundles too, until all the bundles are capped. It often happens that a group of three or four bundles fail to develop Caspary strips at the same time as the remaining bundles - there is here a delayed strip deposition. Examination shows that the group of bundles concerned includes the downwards continuations of the large mid-rib traces entering from the leaf base at the node above. It often happens that although these bundles are not capped, yet on the opposite side of the stem endodermal cells are differentiating over the rays as well as over the bundles. Reference to this is made later.

The starch present in the embryonic endodermal cells does not disappear on strip formation occurring on the walls of these cells, (Pl.VIII. Fig.35.), but persists through every stage of stem development examined. Development of endodermal cells did not occur simultaneously through the whole of the second internode, for frequently strips were absent from the upper part and also from the very base of the internode. This particular species, as already mentioned, shows the occurrence of strip formation over the rays as well as over the bundles,

and this was to be observed shortly after the deposition over the bundles, or even before the latter was completed.

The development of the endodermis was also traced in P.porphyrophyllum and P.angustifolium.

Two shoots of the former were sectioned. In the first (collected in January), there were no Caspary strips in the first internode, but a perfectly continuous endodermis was present throughout the whole length of the second internode. Starch was present in the embryonic endodermal cells and subsequently in the endodermal cells. In a second shoot (collected in February), Caspary strip formation was discovered very close behind the apical bud - a transverse section five millimetres below the base of the apical bud showed each vascular bundle capped and some linking up of the caps. While at a point ten millimetres from the apical bud the endodermis was quite complete (Pl.II. Fig.10.). The presence of the endodermis in the first internode in this second shoot was associated with a more advanced differentiation of the tissues generally than in the first shoot.

In a shoot of P.angustifolium collected in February, strip formation again was present in the first internode, for a section four millimetres from the base of the apical bud showed a few strips over three bundles. Strip deposition had proceeded very rapidly from this point, and in a section taken four millimetres lower down the same internode every bundle, with the exception of a group of four, had some endodermal cells differentiated on its outer side, the majority being completely capped. At this point the proto- and meta-xylem of the bundles was well differentiated, but the

medullary sheath was still thin-walled, and the phloem was undifferentiated. As sections were taken from successively lower zones of this internode differentiation of the stem tissues generally increased (Pl.I. Fig.5), and at the base the medullary sheath was lignified, and endodermal cells had differentiated over the medullary rays, linking up some of the arcs. As will be described later, there was great irregularity in the position of the strips. The continuity of the endodermis was completed in lower internodes, in one shoot before cambium initiation occurred, while in a second shoot in the second internode cambium initiation was proceeding although a group of seven medullary rays lying to one side of the stem were still devoid of endodermis. But continuity was soon established, and in the third internode a complete endodermis was present.

The remaining species were practically identical with the above as regards the manner of the development of the endodermis.

It will have been noticed in the above account of the distribution and development of the endodermis that Caspary strip formation does not always proceed simultaneously round the whole outer vascular system. Very often there is associated with a small group of bundles a delayed strip formation, and a closer examination shows that this group is usually situated directly under the leaf inserted at the node above, so that it includes the large bundles which are continuations of the mid-rib bundles of the leaf.

The shoot of P. chaba decribed on p.27 supplies a good

example of this, as will appear from the account given there. It was not possible to say whether the formation of Caspary strips "caught up" subsequently on the backward side of the stem since in the lower internodes there was only a feeble strip-formation at all. In P.angustifolium (p.37.) a transverse section 8mms. from the apex of the first internode showed that while the great majority of bundles were capped, yet the very largest bundle & a few adjacent ones were devoid of endodermal cells. Slightly lower down the same internode all the bundles were capped.

The structure of the endodermal cells may now be considered. The most important structural feature presented by them is that they remain in the Primary condition throughout their history. Never in any species did the use of Sudan III reveal the presence of a partial or complete suberin lamella, so that a permanent primary endodermis appears to characterise the genus. Other rather isolated instances of a permanently primary endodermis are of course known, and will be referred to later.

When first differentiated the endodermal cells are approximately isodiametric in transverse section (Pl.VIII. Fig.35., Pl.VI.Fig.22), but are rather elongated, as is to be expected since strip deposition does not occur until after the internode has attained some considerable length. The walls are thin, appearing non-lamellate, and are of cellulose, the radial and transverse walls bearing the Caspary strip. The latter is initially very small. The following measurements were made from a drawing (Pl.VIII. Fig.35) of a transverse section through the middle of the second internode of a shoot of P.excelsum. The actual radial size of the strip was 2.3 μ, just one fifth of the radial wall. The strip was situated just inside the central point of the radial wall. During the ageing of the internode the strip increases in size; for example, in a transverse section (Pl.IX.Fig.38.)

from an old internode of P.excelsum the strip occupied the whole of the inner half of the radial wall, and its radial dimension was then 5.7 μ. In P.angustifolium measurements made from Pl.V. Fig.17. show that here (at the base of the first internode) the radial size of the strip was 3.4 μ, while in a stout internode (Pl.VII. Fig.28.) the radial size had increased to 4,8 μ. Other species showed similar dimensions of the Caspary strip.

Kroemer gives details of the size to which the Caspary strip may attain. In the root of Zea Mays he found the strip to measure .7 to 1.3 μ in the radial direction just after the deposition of the strip, increasing to 1.6 μ further back from the apex. In the root of Funkia Sieboldiana the size increased from 1.6 μ to 2.7 μ. These can be taken as the average dimensions of the strip generally. It will be seen that the strip in Piper species, especially in older internodes, is abnormally large.

A preliminary investigation of the appearance of the endodermis in tangential sections of the stem (when the endodermis is seen in surface view) suggested that the Caspary strip did not show the undulatory form on the radial walls of the cell by which it is often characterised especially in the root. Rimbach (γ) was able to show fairly conclusively that these undulations are usually present in the root in the normal living condition (as distinct from the sectioned condition), and are produced as a result of the permanent contraction of the root, which, as is well known, occurs after growth in length of the root has ceased, and is especially

well marked in "contractile" roots. He concluded that during this shortening the cellulose portion of the radial wall, being relatively elastic, itself contracted, while the more inelastic strip was unable to do so, and was therefore thrown into folds.[1] A similar secondary contraction does not occur in stems, and, correspondingly says Rimbach, undulations in the Caspary strip are typically absent from the stem. In cases where undulations in the Caspary strip were not present in living material, as for instance in young roots before contraction has occurred, Rimbach was able to produce undulation by plasmolysis of the endodermal cells. Presumably it is possible for similar "secondary" undulations to appear as a result of the use of inferior (from a cytological point of view) fixatives such as spirit, which usually cause some plasmolysis.

The material of Piper utilised in this investigation was all fixed in 50 to 60% spirit. In young internodes of P.angustifolium slight undulation appeared in the Caspary strip, and was not confined to the radial walls, as in the typical root, but appeared also on the transverse walls(Pl.V. Fig.18.). In older internodes of this species, there was very little undulation (Pl.VIII. Fig.34.), but it is improbable that this is to be correlated with the stretched condition of the endodermal cell in the older internodes, because the radial walls are not greatly concerned in this stretching.

1. Note. Reference to fig. 2 of the first section of this thesis shows that in the root of Alchemilla the suberin lamella is undulatory on the radial wall. It must be concluded that the contraction of the root occurs or is continued after the deposition of the suberin lamella, and that the latter, like the Caspary strip, is relatively inelastic.

some undulation was, however, present. In tangential sections through the endodermis in old internodes undulation was especially well marked in the Caspary strips on the new radial subdividing walls, and resembled that present in roots. The strips on the original radial walls sometimes showed a few very coarse undulations, or in other cases none at all. The strips on the transverse walls were practically straight.

Both lots of material of P.excelsum were fixed in the same way, and the sections were cleared and stained in a similar fashion, so that it appears that such undulations as are present in this species, and presumably in others, are present in the living condition. In that case, since no contraction in length of the stem has been demonstrated, the undulations must arise in some manner as yet unknown.

In transverse sections of a stem or a root containing primary endodermal cells the Caspary strip usually appears as a swollen, thickened part of the radial wall. It is generally stated that the strip is actually no thicker than the remaining unmodified part of the wall, and that the strip is merely a zone along which the original wall layers have undergone some chemical alteration. The fact that the strip does appear to be a swollen part of the radial wall in thansverse section is generally due to the undulatory course which the strip pursues along this wall, for a section of average thickness includes several of these undulations. Thus we read in Hosbach (9), who summarises the conclusions of earlier investigators, that "Der Streifen stellt in keinem Falle eine Membraneverdickung dar, sondern eine engbegrenzte chemische

Umwandlung der ursprünglichen Radialwand."

In Piper species however, as will appear from practically all the illustrations in this paper, the strip does appear as a definitely thickened part of the radial wall in transverse section, whether undulations are present or not. The same impression is obtained from examination of the endodermis in tangential and radial sections of the stem.

The appearance of the strip in surface view as seen in radial sections of the stem may now be considered. This was examined in greatest detail in P.angustifolium (see Pl.VII. Fig.26.,Pl.VII.Fig.29). In the older internodes of this species a fairly large strip is present, as will appear from the drawings, and is characterised in surface view by the marginal indentations which occur at frequent intervals. Closer examination shows that these indentations are due to the presence of numerous oval or circular pits in the radial walls of the endodermal cells. These pits are presumably formed before the Caspary strip, and when a pit lies in the path of the strip, the latter usually skirts round the margin of the pit, so that an indentation is produced. Sometimes a pit has lain directly in the path of the strip, and then strip formation has proceeded on either side of the pit and the latter comes to lie actually in the strip (Pl.VII.Fig.29). When a strip is narrow, as in younger internodes, the presence of a pit in the path of the strip may lead to a distinct break in the latter. Similar pits were also to be seen on the tangential walls, but not on the transverse walls, and correspondingly the margin of the strip, when the latter is seen in

surface view in a transverse section, is free from indentations (Pl.VII.Fig.28).

The Caspary strip in P.excelsum showed similar features in radial sections.

According to Kroemer, pits are rarely present on the radial walls of primary endodermal cells, and he only found them in the case of the root of Vincetoxicum officinale, and his figure is very like the figures presented in this paper. He never encountered pits in the transverse wall, although they occur commonly on the tangential walls. In later work, however, Rumpf (6) describes pits in the transverse walls of the endodermal cells in the root of Ophioglossum vulgatum, some of the pits occurring in the zone of the Caspary strip, and Mager (5) describes pits in the Caspary strip on the transverse wall in the rhizome of Psilotum triquetrum.

In addition to the larger indentations, related to the presence of pits, the whole margin of the strip has a finely corrugated nature.

As will be described in the section dealing with the accommodatory powers of the endodermis, the transverse walls of the endodermal cells often undergo considerable stretching as the stem thickens, and the Caspary strip, present upon it, must also be extensible, although it is possible to visualize an extension of the strip as a result of a process of intussusception of new strip material.

C. Accommodatory Powers of the Endodermis.

In the shrubby species of Piper the secondary vascular cambium is often very active and results in the thickness of an internode increasing very markedly, even in the first year of its existence, provided the conditions are favourable for growth. If we have, coupled with this, an endodermis which retains its continuity in the thicker internodes, then evidently that layer must possess considerable ability to accommodate itself to the changing dimensions of the stem.

P.angustifolium offers the most favourable example for studying this accommodatory power of the endodermis. Pl.I.Fig.2., and Pl.I. Fig.4. are of an internode of this species which has attained a diameter of ten millimetres after slightly more than one year's growth, as the position of the annual ring indicates. The diameter of the second internode, in which a complete endodermis is usually differentiated, of half a dozen different shoots varied between 1.5 to 2 millimetres; so that, assuming different shoots show similar dimensions at corresponding developmental stages, the stout internode mentioned above has increased its diameter by about 600%, and the diameter of the endodermal cylinder will increase to the same extent.

There are three ways in which extension of the endodermal cylinder would be made possible, viz:-

1. Extension of the individual endodermal cells in a tangential direction, either by active growth or passive stretching of the cells, and possibly accompanied by the subdivision of the cells, as described in ~~a former paper, ( )~~. the first part of this Thesis.

2. By elongation of the individual endodermal cells and the occurrence of a sliding growth between them. This would produce an increased number of cells in the endodermis in any given transverse section, i.e., an increased perimeter of the endodermis.

3. To a lesser extent by the disappearance or diminution of the undulations in the endodermal layer which are characteristic of young internodes.

There is no doubt that the first is mainly responsible for the accommodation here, with the possible collaboration of the third. That the second does not occur is indicated by the shape and disposition of the endodermal cells in surface view from older internodes. While the cells may show some longitudinal stretching at an early stage, yet later, when the stem is increasing in diameter the cells become rectangular, with the greatest dimension in the tangential direction (Pl.VIII. Fig.34., Pl.VIII. Fig.31.).

The ability of the cells to stretch in a tangential direction is very well marked. In P.angustifolium the stretching occurs chiefly in the cells over the bundles, because the bundles tend to increase in width while the rays remain of constant width or even show some diminution in width (Pl.I. Fig.2.), and also because the cambium of the bundle produces a considerable amount of phloem on its outer side, increasing the tendency to distension of the endodermal cells arching over the phloem, while the interfascicular cambium produces but little tissue on its outer surface.

In Pl.VII. Fig.28. are shown cells of typical size from over the bundles in an old internode of P.angustifolium, the internode estimated above to have undergone a diametrical increase of 600% in the course of its development. These particular cells have not undergone sub-division. The endodermal cells, when first differentiated, are approximately isodiametric in transverse section (see Pl.VI. Fig.22.) During the subsequent history of the endodermal cell the radial dimension remains practically constant, so that the length of the radial wall in Pl.VII. Fig.28. is a rough indication of the original size of the tangential walls. On this basis it is obvious that the particular cells figured have increased in tangential dimension by mearly 400%. Other cells are stretched to a greater extent, and others again barely at all. This latter may be due to variation in the extensibility of different endodermal cells, or to a restraining action on the part of adjacent cells of the pericycle and cortex to which the endodermal cell is attached.

The figures given above can obviously have an approximate value only, and the apparent discrepancy between the increase in size of the endodermal cylinder and of the individual cells of the endodermal must be due to an over-estimate of the former.

In those species where a discontinuous endodermis is present, confined chiefly to the vascular bundles, then the cells of the vascular caps show stretching and sub-division in old stems. This has already been described in P.excelsum, and is also well marked in P.tiliaefolium. Pl.VI. Fig.25. shows

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a cell from the eigth internode of a shoot of P.excelsum, and by measurements on the same basis as in P.angustifolium, it will be seen that the tangential dimension of the cell has increased by 700% approximately. Here again some cells show very little stretching.

D. Abnormal Strip Development.

While in the typical endodermis Caspary strip deposition proceeds with great regularity and precision on the radial and transverse walls of a single layer of cells, yet several instances of irregular strip formation have come before the author's notice. I have not, however, encountered elsewhere such irregularity as is to be observed in the stem of some species of Piper.

Very marked abnormal strip formation is to be seen in P.angustifolium. Here, as previously described, the endodermal cells are first differentiated over the vascular bundles, and the strips in these cells show a normal position. As development of the endodermis proceeds over the rays great irregularity in form and position of the strip becomes obvious. This is illustrated in Pl.VI.Fig.22.,Pl.V.Fig.17.,Pl.I.Fig.1.; in the former case a five-celled gap is present over the ray, while the endodermis is continuous over the ray in the second. In each case the endodermis presents a normal appearance over the bundles, but over the ray a very anomalous formation of strips is to be seen. In some cells strip deposition has occurred along the whole of a radial wall, or even continuously along two or three adjoining walls of a cell. In other cells the strips are of ordinary dimensions, but are not present on two walls only, as in the typical endodermal cell when seen in transverse section; thus in Pl.V.Fig.17. a heptagonal cell is present, and six of the walls bear Caspary strips. It is difficult to determine whether some of the strips are really centrally situated on the walls, and therefore consist of two

halves, half belonging to each of two adjoining cells, or whether the whole strip is to be related to a single cell. These abnormalities in the form and position of the Caspary strip are also present in older internodes of P.angustifolium (Pl.VI. Fig.20.), and reference to these will be made later.

A rather similar condition is found on examination of transverse sections of stems of P.excelsum and P.tiliaefolium, although in the primary stem the irregularities are not so marked. They are again confined to endodermal cells lying over the medullary rays. There is a marked tendency for the development of accessory Caspary strips on the walls separating the endodermal cells from the cambium mother cells lying immediately inside (Pl.VI. Fig.23.). The development of these strips continues after the initiation of the cambium. The same thing is to be seen in old stems of P.angustifolium (Pl.VI. Fig.20.).

So much for the characters of these abnormalities in transverse section. Their nature was further elucidated by the study of longitudinal sections, especially of older internodes of P.angustifolium. As might be imagined from consideration of the transverse views of the endodermis in this species, very peculiar and complex features are presented in longitudinal sections through some endodermal cells. Pl.VII. Fig.30.,& Pl.VII. Fig.27 illustrate typical arrangements. Strips are seen in surface view on both radial and tangential walls, and two strips may be developed on one wall. When this is the case, fusion of the two is usually to be observed at the

transverse walls, i.e. at the top and bottom of the cells. The detailed structure of the strip is much as before, with indentations, pits and corrugation. Besides these continuous strips, incomplete or discontinuous strip formation also occurs; Pl.VII. Fig.27. shows this in surface view and in section (to the right). Isolated patches of the wall have undergone modification so as to take the fuchsin stain. The longitudinal and transverse views of these cells are easily related to each other.

These abnormally-formed Caspary strips were present in all material examined of P.angustifolium. The above features also characterised longitudinal sections through endodermal cells of P.excelsum in which abnormal strips were present.

A somewhat similar but less marked irregularity in deposition of the Caspary strip is mentioned by Rumpf(6.p.19) as occurring in the roots of some ferns, including species of Ophioglossum, Osmunda regalis, and others. While Mager(5) describes them in the root of Selaginella, in the rhizome of Psilotum, and in the leaf of Isoetes(in the endodermis which develops round the lacunae of the leaf traces). Both however confine themselves to descriptions of transverse sections; and both emphasise the fact that despite the irregularities there is still no uninterrupted communication along cellulose walls from stele to cortex, so that the effectiveness of the endodermis will not be impaired (they regard the endodermis as a physiological barrier).

While these irregularities were so frequent in the foregoing species, yet others, for example, P.porphyrophyllum and

P.nigrum were practically free of them.

Another rather different type of irregular deposition of Caspary strip is presented by the older internodes of species showing a definitely discontinuous endodermis, with the latter forming caps to the vascular bundles, e.g. P.excelsum, P.tiliaefolium. As a natural outcome of the disposition of the tissues on the primary stem, it often happens that in old internodes the ends of the endodermal cap become attached to the outermost cells of the radial rows of parenchyma produced by the interfascicular cambium on its outer side. Caspary strip formation occurs on the tangential walls of the cells of these particular columns of cambial cells, and as a result, the endodermal cap is extended and the production of new phloem by the cambium of the bundle does not diminish the degree to which the endodermis surrounds the phloem (Pl.II.Fig.9)

E. Secretory cells in the Endodermis.

Mention has already been made of the occurrence of secretory cells in the pith, cortex, and phloem of the stem of Piper species. This had been recorded by previous investigators, but no mention was made by them of their occurrence actually in the endodermis. In fact the writer is not acquainted with any record of such a thing in any plant.

These cells were first noticed in the stem-endodermis of P.excelsum, but they were later found in all cases where the endodermis itself was present, and were all of one type. In P.excelsum they could be detected in the embryonic stage of the endodermis, i.e. before Caspary strip formation, and were distinguished by their relative large size, their homogeneous and somewhat granular, dense contents, and a characteristic concavity of their walls. When strip formation proceeds, a strip appears on the radial walls of these cells as in normal endodermal cells (Pl.VIII. Fig.33.).

The secretory cells are still in evidence in older stems, and indeed in P.excelsum they become more conspicuous as the stem ages, particularly in the surface view of the endodermis, because they retain their original size and shape, while the other cells of the endodermis undergo firstly a considerable longitudinal stretching, often before the Caspary strip is deposited, and subsequently a very marked tangential stretching, the latter accompanied by sub-division by radial and transverse walls (Pl.VIII. Fig.31.).

F. Production of Cambium from Endodermal Cells in P.excelsum.

The normal production in this species of a secondary vascular cambium from the narrow zone of cambium mother-cells lying between the endodermis and the medullary fibres has already been considered. It was stated that a single layer of these is present in the typical case. Sometimes there is a double layer of these, while in other cases there is not even a single continuous line of mother-cells present, so that the endodermis (when the latter is present over the ray) is in actual contact with the medullary fibres in places, apparently because one or more of the mother cells has become fibrous, as is suggested by the position of the cells. Such an arrangement is shown in Pl.III. Fig.12. In such cases, a complete interfascicular cambial layer, which as is always present in older stems, is arrived at as the result of a cell of the endodermis behaving as a cambium mother cell, and undergoing tangential division (Pl.VI. Fig.21.). Later stages are seen in Pl.VI. Fig.24. Normally the endodermal cells alternate with the cambial cells, but at a ~~b~~ an endodermal cell caps a cambial column, and the thinness of the tangential walls and the general disposition of the cells show them to have a common origin. Furthermore, these abnormal cambial columns abut directly on to the medullary fibres below. Additional strips are often developed on the upper tangential walls of these rows of cambial cells (Pl.VI. Fig.24).

This seems to indicate that here at any rate, it is the position of a cell which determines whether or not it is to function as a cambium mother cell, rather than the intrinsic nature of the cell itself.

Note on P.chaba.

An interesting tissue-accommodation here is worthy of note. In the primary stem the vascular bundle is capped on its outer side by a bulky development of pericyclic fibres, which often extend right down to the medullary sheath, so that the phloem and cambium are completely ensheathed (Pl.IX. Fig.37.). This is still the case when the interfascicular cambium arises, and obviously if a complete cambial ring is to be produced, a break through the pericyclic fibres must occur. This is actually the case; owing to increase in bulk of the phloem and cambium, and possibly to the pressure of cambial mother cells (Pl.IX. Fig.39.) from without, ruptures appear at the base of the fibrous caps. Pl.IX. Fig.40. shows the completed cambium. Once formed the gap increases as a result of the activities of the cambium.

5. Discussions and Conclusions.

One fact which emerges very clearly from the foregoing description is the definite association displayed in these plants between the endodermis and the vascular system. In every species investigated in this work, the endodermis develops initially outside the vascular bundles (of the outer ring). This in itself is a striking relation, but the anomalous vascular structure of the stem in the genus allows of a further demonstration of the association. As already described, the outer vascular ring always consists of a series of discrete strands, even after prolonged secondary thickening. Correlated with this in some species, the endodermis, subsequent to its initial development over the vascular bundles, may persist in this discontinuous condition throughout the development of the internode; so that here endodermis is formed only outside the vascular strands, and we have a further indication, this time in the mature stem, of the definite association between these two tissues.

This marked association should be considered in connection with the causal factors involved in the formation of the Caspary strip. It must be admitted that practically nothing is known of these factors. It seems to be a fair assumption, however, that a specialised structure, of relatively constant character, such as is the Caspary strip, is deposited in an essentially similar fashion wherever it occurs. Two possible methods of Caspary strip formation

suggest themselves:-

1. Deposition as the result of the activity of the protoplast of the endodermal cell. It may be that the fibrous layer of the anther, or the different types of locally-thickened xylem elements, furnish comparable cases of "localised" protoplasmic activity. It is evident that the protoplast of the endodermal cell may possess rather abnormal metabolic properties, for after the formation of the Caspary strip, whether this is a protoplasmic secretion or not, there is very often a subsequent deposition, in this case necessarily by the protoplast, of the suberin lamella, and of tertiary cellulose lamellae. The latter frequently become lignified at a later stage, or even silicified in some cases. Thus there is no obvious reason why the Caspary strip, which is believed by many investigators to be impregnated with substances closely akin to lignin and to suberin or cutin, should not be a protoplasmic secretion.

2. Formation as the result of some process practically independent of the endodermal protoplast.

During the last ten years a theory of this type has been advanced by Professor J.H.Priestley and his colleagues. Some reference to this was made in the earlier part of this thesis, but a more detailed consideration will be made now, in the light of the facts revealed by the present investigation.

The deposition of the Caspary strip in the root may be considered first, and the complexity of the subject-matter

makes it advisable to quote at some length the writers concerned. In an earlier paper by Priestley and North (12,p.120) we read: "attention must be drawn to the conditions under which the strip is invariably formed. It appears on the layer of cells around the plerome cylinder, a layer which intervenes between the plerome and the first air spaces of the cortex. Within the plerome, at the level at which the Casparian strip is appearing, xylem and phloem are just beginning to differentiate. This differentiation involves great internal alteration in the future conducting elements and undoubtedly these alterations are accompanied by the giving up of organic solutes to the sap which may be assumed to percolate through the intervening walls up to the endodermal cylinder. At the outer surface of the cylinder this sap meets the air diffusing inwards from the intercellular spaces; thus we have the conditions arising which at the surface of the plant give at one time cutin, at another suberin."

The precise origin of the substances which are subsequently deposited, in a modified form, as the Caspary strip, is not very clear. In a further passage in the same paper (p.121): "That these substances are in part of a fatty acid nature is to be expected, such fatty acids forming, according to Hansteen Cranner (Jahrb.für Wissensch.Bot.,53,pp.536-598, 1914, and Ber.der.D.Bot.Ges.,37,pp.380-391, 1919), an invariable accompaniment of the membranes of parenchymatous tissues; Czapek (Ber.der.D.Bot.Ges.,37,pp.207-216, 1919) has

shown also that they are abundant in meristem protoplasts. They would certainly be lost from the contents of the developing xylem elements. When compared with the cases of cutin and suberin formation which are better known, notably the formation of suberin at the surface of wounds (11), the presence of oxidation products of fatty acids may be anticipated in the endodermal wall,.....". In a very recent publication, (13,p.7), we read: "As already pointed out, the walls of these cells (of the stele), unlike those of the corresponding cells in the shoot apex, are as yet heavily impregnated with fatty substances and proteins;" and on p.4; "Saying that the carbohydrate mixture of the walls which intervene between the protoplasts of the root apex is impregnated with protein and fat is probably only another way of stating that when the carbohydrates were deposited at the interface between two dividing protoplasts the living protoplasm was incompletely withdrawn from the intervening region, so that the fats and proteins characteristic of the protoplasm are still found in the wall." And again on p.7, still of the same paper: "Though these substances leave the walls more slowly in the root than in the shoot, they do gradually migrate, and because of their effect on surface tension they tend to accumulate at any sufface where the liquid matrix is in contact with air. But as they thus move outward, air is diffusing inward from the intercellular spaces in the cortex, and thus the fatty substances tend to oxidize and condense in a

varnish-like strip on the radial and transverse walls of the endodermis..... inside which air spaces have not yet appeared. Thus the characteristic Casparian strip, an invariable constituent of the endodermal wall in roots of all the flowering plants, is formed."

It is apparently to be concluded, that the strip-producing substances are those present, in addition to cellulose, in the walls of meristematic cells. The theory is extended to account for the "fact" that "in stems, on the other hand, except in submerged aquatic plants and in certain cases to be considered in later papers, notably in the case of etiolated plants.....the primary endodermis with Casparian strip is never developed."(12,p.124). This statement should be compared with that made by Eames and McDaniels(Introduction to Plant Anatomy, 1925.,p.103), on the same subject: "In angiosperms it (the endodermis) occurs in the stems of a considerable percentage of herbaceous forms, probably in the majority of cases." The actual truth lies probably about mid-way between these two statements, but further investigation on this matter is needed. It is, however, obvious that any theory of Caspary strip-formation must take into consideration the occurrence of that process in a considerable number of aerial stems.

Priestley and his colleagues stress the fact that strip-formation is essentially an apical phenomenon. A careful consideration of the facts, however, indicates that the formation of the Caspary strip is only <u>initiated</u> at the apex in root and stem, and that it may be continued in regions

considerably removed from the apex. Kroemer (loc.cit.) gives a few measurements of the size of the strip. In the case of Funkia Sieboldiana the radial size of the strip in transverse section was 1.6 μ in cells 2.5 millimetres distant from the root apex, while in cells 5-6 millimetres from the apex the size of the strip had increased to 2.7 μ; Kroemer does not appear to have continued his measurements in more distal parts of the root. The occurrence of "continuous" strip-deposition in Piper spp emerges very clearly from comparison of the size of the strip in young and old internodes. Some such measurements are given on p.39 of this paper. In addition to this emlargement of the original strip, we have the deposition of new strips on any radial or transverse sub-dividing walls which may develop in a primary endodermal cell. A number of instances of this were described in the first section of this thesis, and further investigation will probably reveal others. Comparable with this is the production of new Caspary strips on the dividing walls of endodermal cells functioning as cambium mother cells, described on p.54 of this paper.

These considerations point clearly to the conclusion that the Caspary strip is formed by the endodermal protoplast, and that this ability to form strips may be retained by the protoplast for some time, perhaps permanently. The theory reviewed above makes no provision for strip-deposition in an endodermal cell some distance from the apical meristem; the association of the Caspary strip with the vascular tissues may have causal significance, but not through the mechanism

suggested by Priestley and his colleagues. There is one rather striking phenomenon, however, which does seem to demand something of the nature of Priestley's theory by way of explanation (see p.52). In the thicker internodes of P.excelsum and of P.tiliaefolium the ends of the endodermal cap may become attached to the outermost cells of the radial rows of parenchyma produced by the interfascicular cambium, and additional Caspary strips are often formed on the tangential walls of these cells, so that the bundle cap is extended. It looks in this case as though the vascular bundle, especially the phloem, were controlling in some way the orientation of the strips, such as would be the case if strip-forming substances were diffusing outwards from the phloem along different radii.

As was described in the section dealing with the distribution of the endodermis, the different species of Piper here examined can be arranged in a series in which all stages between a completely developed and a completely absent endodermis are represented.

A completely developed and continuous endodermis was always found in P.angustifolium, P.porphyrophyllum, and in P.nigrum. In P. excelsum, in some shoots a continuous endodermis was present, in others a definitely discontinuous condition prevailed, in which the endodermis was confined to the vascular bundles, while transitional stages between these two extreme types were found in still other shoots. Finally the endodermis occasionally failed to develop even over the vascular bundles.

P. tiliaefolium was a rather more stable type, and usually showed a discontinuous phase, although occasionally there was some approach to the continuous condition. P. chaba was a type displaying considerable variation in the distribution of the endodermis. While in some internodes a partial continuity obtained, yet in others the endodermis was practically absent. This species leads naturally to the ultimate condition, presented in P. celtidifolium, where the endodermis was never detected.

A consideration of these facts suggests that possibly a continuous endodermis characterised the ancestral stock of the genus, and that the above sequence, detected in extant types, actually represents the evolutionary changes by which gradual elimination of the endodermis has been effected in some sections of the genus. That is to say, starting from a continuous condition we had first the elimination of the interfascicular endodermal cells, followed by a similar non-development of the cells over the bundles. In any case it is obvious that there is irregularity in the incidence of the factors responsible for Caspary strip - deposition in some species. This does not appear to be related to any external factor, and is difficult to understand whichever theory is adopted, although variation in a protoplasmic mechanism is more feasible than in a mechanism which appears to involve non-variant factors.

Finally, note should be made of another puzzling feature, namely, that in all species , such irregularities in the position and form of the Caspary strip as do occur,

are restricted to endodermal cells lying opposite to the medullary rays. No explanation of this can be offered.

In the second place the very marked association between the vascular strands and the enodermis may have a functional significance. It suggests that the function of the endodermis, whatever it may be, is connected with the conducting channels. The possible function of the endodermis has been the subject of much conjecture in the past, for the practically universal occurrence of the layer in vascular plants, coupled with the peculiar nature of its component cells, suggests very strongly that some function of general importance is being fulfilled, comparable to the functions carried out by other tissues common to vascular plants, such as the xylem or phloem. It is not proposed to present here an account of the many different theories which are in the field; suffice it to say that no completely satisfactory theory in accord with all the facts, has yet been put forward, a state of affairs which, to some extent, is due to the formidable practical difficulties which stand in the way of the would-be experimental investigator. The experimental side of the question has been fully dealt with in a recent paper by Hosbach (9). There is, however, one aspect of the question which may with advantage be discussed here, namely, the distribution of the endodermis in plants generally, and in the individual plant.

The endodermis is found with great regularity in the

roots of all modern vascular plants, at least in the younger root, with the single exception of the Lycopodiaceae, a group of plants which, according to Mager (5) is characterised by the entire absence of endodermis in any part of the plant.

As already stated, the occurrence of endodermis in the stem, especially in that of angiosperms, has not been sufficiently investigated, but such observations as are available indicate that considerable variation exists.

The angiosperms may be considered first. It is generally stated that the endodermis is absent from woody stems in these plants, but it is a question just how far this statement is true. The presence of endodermis in woody species of Piper is a case in point. As was mentioned above, endodermis is frequently present in herbaceous stems, especially in rhizomes, and prostrate stems, and in stems of aquatic plants, e.g. Elodea, Hippuris, Callitriche, Myriophyllum, Potamogeton,etc., although as far as my knowledge goes it has not been established that this is a feature definitely associated with an aquatic habit, rather than that it happens to be a characteristic of the families to which the above-mentioned plants belong. De Bary (Comp.Anat.Phan. and Ferns.translr.1884.p.121) points out that the endodermis is usually present in stems when the vascular tissues form a relatively condensed system, that is, when some approach to the root type of vascular arrangement is present. Such a condensed vascular system is present in most of the examples mentioned above. The work of

Mylius (loc.cit.) on the Rosaceae and related families constitutes the most valuable piece of investigation available on the endodermis in aerial herbaceous stems. In annual stems the endodermis was frequently present, but in most cases only in the lower part of the stem, sometimes extending for a distance of only one centimetre up the stem, as in Spiraea Ulmaria and Potentilla alba. In other cases, e.g. Sanguisorba officinalis, Alchemilla vulgaris, Fragaria vesca, the endodermis extended considerably higher up the stem, but in all cases it was gradually replaced by parenchyma in the upper part of the stem; that is, the development of the endodermis did not occur continuously behind the apex as is the case in the root. In perennial aerial stems on the other hand Mylius found that the endodermis may develop continuously behind the apex, and thus extend through the greater part of the shoot, e.g. Potentilla fruticosa. All the species of Piper investigated here have perennial axes, and the development of the endodermis usually accords with that described by Mylius in perennial stems, i.e.,there is continuous development behind the apex, although in some species the differentiation may be incomplete, occurring outside the bundle only. In a shoot of P. chaba it was, however, evident that during the earlier growth of the stem apex no endodermal cells had differentiated , for in the lower internodes such cells were absent, while they were abundant in the younger, upper internodes. Apart from this single case the differentiation of

the endodermis appears to follow apical growth with regularity.

Turning now to the stem in Gymnosperms, it is generally stated that the endodermis is absent. (based on Eames and Mc.Daniels, Introdn. to Plant Anatomy, 1925, p.103; Plaut's paper(14) is not accessible to the author.) This recalls the fact that it is also absent from the stem of many woody Angiosperms.

The endodermis is generally present throughout in the axis of the Pteridophyta, with the exception of the Lycopodiaceae and probably of Isoetes (both according to Mager(5).).

In the case of the leaf, again no general statements can be made. There are a number of Angiosperms in which endodermis is present in the petiole and to some extent in the lamina. Mylius (loc.cit.) found foliar endodermis in a number of plants in the Rosaceae and in related families. In these cases the endodermis always extended the whole length of the petiole, but after the entry of the traces into the lamina the endodermal cells were sooner on later gradually replaced by parenchymatous cells, so that the finer veins were not ensheathed by endodremis. Mylius found an interesting relation between the occurrence of endodermis in axis and in leaf. In the plants inyestigated by him he found that a leaf possessed endodermis if it was inserted on the axis in a region where the latter itself showed an endodermis. In case the endodermis was present in the lower part only of the aerial axis, as in Sanguisorba officinalis, then the leaves inserted

on that portion of the stem possessed an endodermis, while those borne above did not. Only two exceptions to this rule were detected, namely, Spiraea Ulmaria and Callistemon semperflorens. It will be seen the Piperaceae do not conform to this rule, for endodermis was never found in petiole or lamina.

As in the case of the stem, so in the leaf it seems that a foliar endodermis is largely a family character. An example of this has just come before the author's notice. It was observed that each vein in the lamina of the leaf of Plantago maritima was enclosed in a complete endodermal sheath, composed of cells in the secondary stage, apparently throughout the whole length of the vein, (these facts are subject to confirmation). The possibility suggested itself that such a foliar endodermis might characterise halophytes generally, and be associated with the peculiar metabolism of those plants. An examination of a number of plants showed, however, that rather than a structural feature associated with a particular habitat, the foliar endodermis is a family character of the Plantagineae, and besides being present in the halophytic species, P. maritima, it is also a feature in P. lanceolata, and also in Littorella lacustris, both of which frequent habitats very different from P. maritima. A similar observation was made by Schwendener,(see Haberlandt, Phys. plant Anatomy, transln., 1914, p.373.), who found an endodermis in the leaf of all the Festuceae, both in xerophytic genera such as Festuca, as well as in the hygrophilous genus Glyceria.

The endodermis is of constant occurrence in the leaf of the Gymnosperms, and Soar (10), on the basis of a recent examination, concludes that the endodermis here serves to retard transpiration, and is a factor in promoting the successful xerophytism of the Gymnosperms.

The researches of Bäsecke (8) in the Filicales show that a foliar endodermis is present in the Leptosporangiate ferns, and that it invests the strands for practically their entire course through the lamina. In the Eusporangiate ferns the layer is generally absent. It is also absent from the leaf of the Selaginellaceae and of the Lycopodiaceae, both according to Mager (5).

The arrangement of the endodermis in the individual plant varies in the different organs. While in monostelic roots and stems the endodermis surrounds the stele as a whole, yet in the petiole and leaf the layer invests each individual bundle. There are, however, exceptions to these general rules. Thus while in the genus Piper the stem endodermis is of the "common" type, yet in the related genus Peperomia the endodermis, when present, surrounds each individual bundle of the stem, according to J.E. Weiss (loc.cit.) A still more marked case of variation in this respect is furnished by the genus Ranunculus. Haberlandt (Phys. Plant Anatomy, transln. 1914, p.374) says that in the stems of R. amplexicaulis, R. parnassifolius, and R. aconitifolius, a common endodermis is present, whereas in those of R. Lingua

and R. Flammula an endodermis of the individual type is found. A further example is provided by the genus Equisetum. The well-known work of Pfitzer (see De Bary, Comp. Anat. Phan. & Ferns, transln., 1884, p. 122.) shows that while in some species, e.g., E. limosum and E. littorale, an endodermis surrounds each bundle in the aerial stem, in E. arvense, E. sylvaticum and others there is a common endodermis limiting the whole stele, while finally in E. hiemale and E. ramosissimum there is, in addition to the outer common endodermis, also an inner one of the same type. It should be noted in passing that variation such as this complicates the question of the identity of the factors responsible for the deposition of the Caspary strip.

It is possible to arrive at certain conclusios as to the function of the endodermis on the basis of this general survey of its occurrence, and indeed, with experimental work on the subject in so unsatisfactory condition, it is probable that the former is more lucrative.

It is a striking fact that, throughout vascular plants, the endodermis, in comparable stages of its development, displays an essentially constant structure. This is strong evidence that the function of the endodermis is also essentially the same in all plants, just as it is assumed that the phloem, for example, carries out a uniform function. It is very probable that this conclusion is correct as regards the

endodermis of the root. This discussion, however, is more concerned with the endodermis of the shoot, and the problem here is more difficult. For while the endodermis is of regular occurrence in the root, in the shoot, on the other hand, at least among the Phanerogams, it appears sporadically, viewing the group as a whole. Assuming that the axis was originally (in an evolutionary sense) characterised by the possession of an endodermis, it appears that in some cases changes in the internal relations of the axis have resulted in the endodermis becoming superfluous, and its elimination has followed, while in others the layer still develops and fulfils either its original function or possibly a secondary one. Such a line of argument is quite feasible so long as the presence or absence of the shoot-endodermis characterises relatively large groups of plants, for the internal economy may vary from group to group. The position, however, becomes uncertain when variation in the occurrence of the endodermis is encountered in closely related plants, as in the genus Piper, where such variation is not only very marked between different species, but also from shoot to shoot of one plant, as was described in P. chaba and in P. excelsum. It seems highly unlikely that the internal mechanism will vary in these cases to such an extent that, while in one case the endodermis is a necessary structure, yet in another it can be dispensed with. An alternative hypothesis is that in the genus Piper,

and possibly in the shoots of all Phanerogams, the endodermis is more or less a vestigial structure, and, while in some species the factors effecting its differentiation are still in operation, in others the layer has been eliminated. This second hypothesis accords best with the facts revealed in this investigation of the genus Piper; it must not, however, be forgotten, when considering inter-specific variation, that this genus is a huge and possibly artificial one, in which considerable variation in any one character may be encountered.

A final point which deserves mention is the presence of a persistent primary endodermis in this genus. The existence of three well-defined stages in the ontogeny of an endodermal cell of the most highly differentiated type has been fully demonstrated by the Marburg school of botanists and by others. Speaking generally, such highly differentiated cells, i.e.,tertiary endodermal cells, are found only in the Angiosperms. In the Gymnosperms and the higher ferns the developmental sequence stops at the secondary stage, while in the remaining Pteridophytes (with the exception of some Selaginellas, according to Mager (5)) are characterised by a permanently primary endodermis. These facts suggest,that, besides being the first stage ontogenically, the primary stage was the primitive cell-type in the evolution of the endodermis, so that here is an example of "ontogeny repeating

phylogeny", or, to use Jeffrey's term, of the"Doctrine of Recapitulation".(Anat.Woody Plants, p.234.). It would seem therefore,that while in the majority of Angiosperms the endodermal cells proceed to the tertiary stage, yet in some cases the primitive primary condition is retained permanently. A list of plants reported to display such a persistent primary endodermis is given in the first part of this thesis, but the available observations are not as yet sufficiently extensive to determine whether this retention of a primitive feature usually characterises groups of plants, as it does in the genus Piper.

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EXPLANATION OF PLATES.

Figs. 1-10 are microphotographs by the author. The remaining figures are photographic reproductions of original camera lucida drawings. Photographs and figures are from Eau de Javelle-Basic Fuchsin preparations unless otherwise stated.

Plate I.

Fig.1. P. angustifolium. T.S. of the first internode of a shoot at a point 2 cms. from apex. Gaps are present in the endodermis over the primary rays, and irregular Caspary strips at the extremities of the bundle caps. (× 135)

Fig.2. P. angustifolium. T.S. of thick internode. (× 15)

Fig.3. P. porphyrophyllum. T.S. tenth internode of a shoot. (×23)

Fig 4. P. angustifolium. T.S. thick internode. (× 70)

Fig.5. P. angustifolium. From same section as Fig.1. (× 36)

Fig.6. P. porphyrophyllum. T.S. of second internode of shoot. (×23)

Plate II.

Fig.7. P. porphyrophyllum. T.S. through fifth node of a shoot at the level of the leaf base. Shows branch-gap in stele and absence of the medullary fibrous ring. (× 14)

Fig.8. P. celtidifolium. T.S. through stem at a point at which the secondary cambium is just arising. Shows the sheath of large cells capping the pericyclic fibres. Gentian violet preparation. (× 95)

Fig.9. P. excelsum. T.S. of moderately old internode. On the left the peripheral part of a bundle & on the right part of a ray, with interfascicular canbium above it. The end of the endodermal cap

has become attached to the outer cells of one of the radial rows of parenchyma cut off by the cambium over the rays, and two new Caspary strips have developed on the tangential walls of these cells. One of the original cells of the cap shows radial sub-divisions. (× 160)

Fig.10. P.porphyrophyllum. T.S.of a first internode 1 cm. from apex. Gentian Violet preparation.(× 33)

Plate III.

Fig.11. P.excelsum. T.S.through the base of a second internode. Showing two vascular bundles, embedded in the medullary fibrous ring, the continuous endodermis, and the single row of cambium mother cells. (×380)

Fig.12. P.excelsum. T.S.showing the production of the interfascicular cambium; the row of mother cells is interrupted. (× 500)

Plate IV.

Fig.13. P.excelsum. Diagram from a T.S.through a second internode. To show the outer series of bundles embedded in the medullary fibrous ring, the cortical ring of collenchyma, and the endodermis forming caps over all the outer bundles except over a group of four. (× 33)

Fig.14. P.excelsum. Diagram from a T.S.of a thick internode, showing the vascular bundles, separated by primary medullary rays; the cambium, particularly deep over the rays, where the outer cambial cells become parenchymatous.The dotted line represents the endodermis, over phloem & pericyclic fibres.In the cortex is a composite ring of sclerenchyma & collenchyma. The medullary fibrous ring is ruptured at one point. (× 30)

Plate V.

Fig.15. P.excelsum. An interfascicular region from a moderately old internode. Three columns of cambial cells are protuding a gap in the endodermis into the cortex. (× 334)

Fig.16. P.excelsum. T.S. of an internode in which slight secondary thickening has occurred. Showing an endodermal cell displaced outwards by a column of cambial cells, and a partial splitting of Caspary strips. (× 296)

Fig.17. P.angustifolium. T.S.base of a first internode. Showing an interfascicular region,with a bundle to right and left. The endodermis is continuous over the ray but there is considerable irregularity in the position & form of the Caspary strips. (× 566.)

Fig.18. P.angustifolium. Endodermal cells in tangential section of second internode of a shoot,showing slight undulation of the Caspary strips. (× 283)

Fig.19. P.excelsum.T.S.stem with slight seondary thickening; Interfascicular region; an endodermal cell has been pushed outwards by cambial cells. (× 296)

Plate VI.

Fig.20. P.angustifolium. T.S.old internode. Abnormal Caspary strips in endodermal cells over a medullary ray.(× 282)

Fig.21. P.excelsum. T.S.third internode of a shoot. Showing the interfascicular cambium arising; a group of four endodermal cells are acting as cambium mother cells.(× 333)

Fig.22. P.angustifolium. T.S.base of first internode. Two vascular bundles are included with the intervening ray.The endodermis is still discontinuous; abnormal Caspary strips are present.(× 574)

Fig.23. P.excelsum. T.S.primary stem. Interfascicular region with irregular Caspary strips. (× 574)

Fig.24. P.excelsum. T.S.third internode of a shoot. A several-layered interfascicular cambium is present,and to the right two endodermal cells have functioned as cambium mother cells and have produced cambial columns a and b. (× 333)

Fig.25. P.excelsum. A stretched and sub-divided endodermal cell from the eighth internode of a shoot. (× 346)

Plate VII.

Fig.26. P.angustifolium. Endodermal cells as seen in a radial section of a very stout internode. Showing Caspary strip in surface view and pits on radial walls. (× 532)

Fig.27. P.angustifolium. Endodermal cells in tangential section through same internode. Abnormal strips are present on tangential and radial walls. (× 532)

Fig.28. P.angustifolium. Endodermal cells in T.S.still of same internode. Normal Caspary strips. Pericyclic fibres lie inside the endodermis. (× 532)

Fig.29. P.angustifolium. Endodermal cells in a radial section of a younger internode. The strip is practically normal. The pits are only showing in the proximity of the strip. (× 532)

Fig.30. P.angustifolium. Endodermal cells in a radial section through same internode as in Fig.29. Irregular strips.(×532)

PlateVIII.

Fig.31. P.excelsum. Endodermal cells from tangential section of an old internode. Caspary strips in surface and in edge-on views - the single lines represent the latter.The endodermal

cells are much sub-divided. To the right of the centre is a secretory cell. (× 327)

Fig. 32. Diagrammatic representation of the course of the vascular bundles through a lower node in Chavica Roxburghii, (= Piper longum L.), redrawn from J.E.WEISS (loc.cit.). The dotted lines represent the medullary bundles and the continuous ones the bundles of the outer ring. The large dots indicate thet the bundles concerned pass out into the leaf base.

Fig. 33. P.excelsum. From T.S. of a second internode. Showing half a vascular cap of endodermal cells, including a secretory cell. (× 327)

Fig. 34. P.angustifolium. Endodermal cells in tangential section of old internode. Caspary strips in surface and end-on view. (× 287)

Fig. 35. P.excelsum. T.S. second internode of a shoot. Showing a single vascular bundle , embedded in slightly lignified medullary fibres and capped by endodermal cells with minute strips and containing starch grains. (× 575)

Plate IX.

Fig. 36. P.excelsum. T.S. moderately thick internode. Showing an interfascicular region. Three columns of cambial cells are protuding through a gap in the endodermis into the cortex. (×328)

Fig. 37. P.chaba. T.S. internode at the time of formation of the secondary cambium. The interfascicular region between two adjacent bundles is shown. The pericyclic fibrous caps(p) are continuous with the medullary fibrous ring(m). ph=pith,

xy=xylem, c=cambium mother cells. (× 277)

Fig. 38. P. excelsum. Sub-divided endodermal cell from a vascular cap in am old internode. (× 554)

Fig. 39. P. chaba. T.S. from same point in the stem as that from which Fig. 37 drawn. At a the separation of the pericyclic fibres from the medullary fibres is seen. (× 277)

Fig. 40. P. chaba. T.S. Slightly later stage in which the pericyclic cap is completely separated from the medullary fibres and the cambium is continuous. (× 277)
