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**Studies on Utilization of *Sasa*-bamboos
as Forest Resources**

**4. Pulping of Young Culms and Histological Change of Cell
Structure of the Culms in Growing Process***

By

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ササの資源化に関する研究
第4報 幼稈のパルプ化と生長過程における稈の組織の変化

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Abstract

When young *Sasa*-bamboo culm was boiled with 1 to 2% KOH solution, the pulps suitable for folk art paper were easily obtained, because the fibers were composed of thin wall, different from those composed of sclerenchymatous fibers from the adult culm. In relation to the utilization of the young culm, histological change of vascular bundle, bundle sheath, and parenchyma cells constituting ground tissue in the culm in growing process was investigated in detail by the observation with SEM, hitherto obscured and inferred by optical microscopic method. The vascular bundle in the sprout observed with SEM for the first time, showed a simple structure consisting of proto-xylem and -phloem surrounded with thin-wall fiber cells of bundle sheath before thickenings.

Keywords: *Sasa kurilensis*, Young culm pulp, Scanning electron microscopy, Vascular bundle, Ground tissue.

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Introduction

There are a few reports^{3,10,25)} on the observation of *Sasa* culm tissue, as described in Part 1²⁶⁾. Concerning adult *Sasa*-bamboo culms, the histological structure has been hitherto considerably understood owing to the similarity to that of bamboo species^{1,7,9,11~13,16~24)} and their observation using a scanning electron microscope (SEM)^{9,11,18,24)}. Though the authors examined the main histological structure of a gigantic *Sasa*-bamboo (*Sasa kurilensis*) by microscopic method, they have now made the observation of the vascular bundle etc. in the young culm with SEM, easily producible of the pulp like the features of Japanese paper.²⁷⁾

From the results of these investigation, the authors could understand the change of the culms in growing process and obtain some new knowledge in confirmation of the points not to have been perfectly comprehended by conventional microscopic method. In present paper, the histological structures of the young culms which have not been ever studied and the changes of the structure in both growing and maturing processes are reported by the observation with SEM, namely, the characteristics of protoxylem and phloem, the occurrence of reticulate vessels in metaxylem in the vascular bundle seen in the young culm, and thickening of the cell walls forming polylamellate structure¹⁶⁾ in fiber and parenchyma cells.

Since the young culm composed of various cells of very thin wall is in the course of the differentiation, and has a lignin content as low as about a half of that in the adult one,^{14,27)} like the young bamboo species,^{2,8)} it can be easily pulped by boiling with some 1% sodium or potassium hydroxide solution. The observation of the obtained fibers was also microscopically carried out so as to grasp the features of the immature fibers, in relation to the properties of the pulps from the young culms, being different from those of the mature culm fibers composed of sclerenchymatous fibers built up on maturity.

The authors expect very much that the utilization of the *Sasa*-bamboos, now unexploited resources, is promoted through the production of the folk art paper using the young culms.

The authors thank Professor K. FUKAZAWA and Associate Professor J. OHTANI of Laboratory of Wood Physics, Hokkaido University, for the use of the scanning electron microscope.

1. Materials

The materials used in the study are gigantic *Sasa*-bamboos (*Sasa kurilensis*) which were collected from Compartment 413 of Uryu Experiment Forest, Hokkaido University, in the middles of June and July, 1984, and the beginning of August, 1985. The stand is a sparse forest composed of a few broadleaved trees, under which only the *Sasa*-bamboos grow densely, estimated at an enormous growing stock of 131 tons per hectare (Fig. 1).



Fig. 1. *Sasa kurilensis*-growing circumstances in Compartment 413, Uryu Exp. Forest, Hokkaido University.

In the *Sasa*-bamboo growing land, their edible sprouts began to shoot to the length of 10 to 20 cm from the middle of July, including the underground buds. Furthermore, the harvest carried out in the middle of July was favorable for the collection of the young *Sasa*-bamboos in various growing stages. The longest culm harvested was about 2.5 meters long, which would be 3.5 meters long at the completion of growth.

While a part of the young culms was used for pulping, the rests were stored in a refrigerator so as to avoid decay. An example of the young culms photographed during drying is shown in Fig. 2.

The samples for the observations are as follows:

No. 1; The bud in the underground. The size is 1.5 cm long and about 1 cm in diameter at butt. Since the color is white and the culm sheath is not formed (Fig. 3), the bud is supposed to maintain on the whole the features built up in the autumn of the previous year.

No. 2; The less than 1-year-old culm of 115 cm in length and 12 mm in diameter at butt in the course of growth. The samples are the parts of the first and fifth

internode above the ground. The portion of the culm coming out from the sheath by elongation of the internodes is green in color. These culms are soft and pliant due to the early stage of lignification, which can be easily defibrated by boiling with a dilute alkaline solution.

No. 3; The 2-year-old (over a full year old) culm of 340 cm in length and 17 mm in diameter at butt, elapsing for over 1 year till the harvest. The sample is the part of the second internode. Though this culm is also immature, and shrunk by drying at 105°C as described in Part 1²⁵⁾, the chemical composition is nearly the

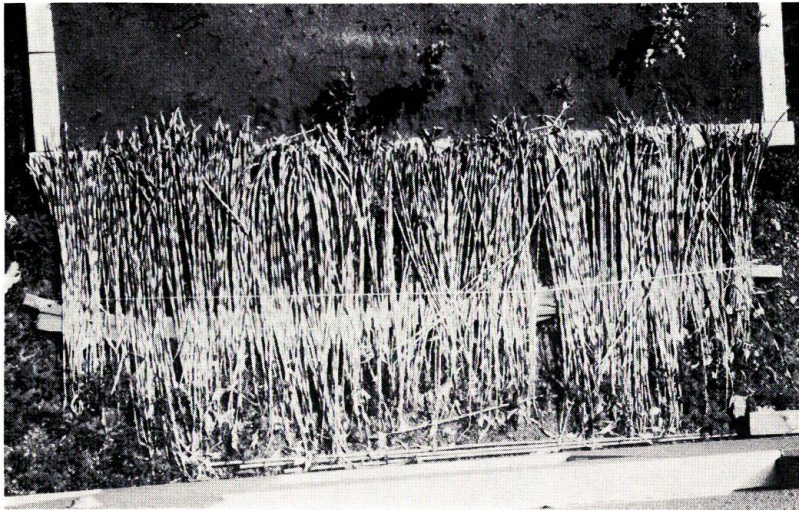


Fig. 2. Young culms of *Sasa kurilensis* during seasoning.

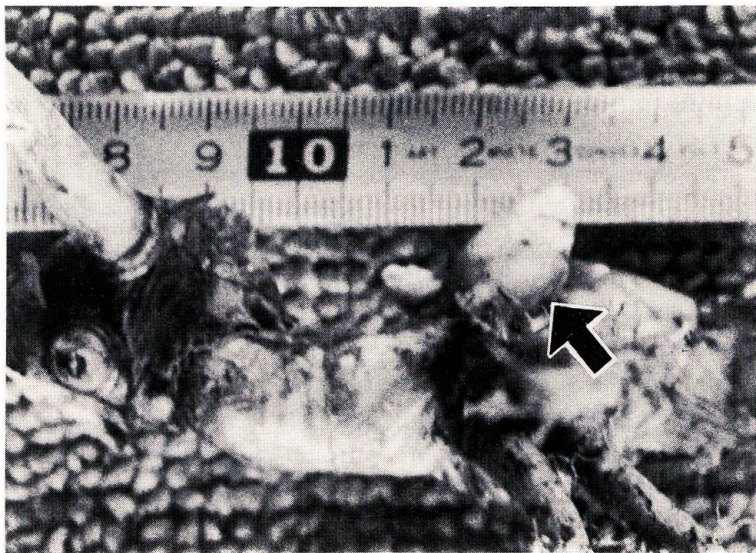


Fig. 3. Underground bud before elongation.

same as that of the adult one, having about 20% of lignin at the completion of lignification.

No. 4; The 4-year-old (judged from the number of branching) culm of 350 cm in length and 19 mm in diameter at butt. The sample observed is the part of the second internode. The culm sheaths have perfectly come off but their marks are left. The color is somewhat yellowish.

The samples of Nos. 1 and 2 were fixed immediately with FAA (the solution mixed with 60 parts of ethyl alcohol, 30 of water, 5 of commercial formalin, and 5 of acetic acid by volume) at the place where the samples were collected.

2. Methods

The young culms of low lignin contents were pulped by boiling with 1 to 2% potassium hydroxide solution in consideration of the disposal of the waste liquor, instead of sodium hydroxide used in the previous study²⁷⁾. Though in the previous study the air-dried chips were used, the moisture content of which is lowered at 7% during the storage in a refrigerator for 6 months, in this study the culms of different moisture including 90% of green condition and several drying stages were used for the pulping, separated into 3 portions, upper, middle and lower, varying in the degree of lignification, after removal of the nodes. The pulps obtained were chemically analyzed and handsheets were made according to Tappi Procedure. The strengths of the pulpsheets were also examined, while the fibers and the sheets were microscopically observed.

Meanwhile, all the samples fixed with FAA were dehydrated through a grade series of ethyl alcohol, replaced with isoamylacetic acid, and dried under critical temperature with liquid carbon dioxide. The specimens from Nos. 1 and 2 observed with SEM were made by cutting with a razor blade, while the specimens from Nos. 3 and 4 were planed with a sliding microtome using the blade, after cut with a conventional cutter, and embedded with an instant adhesive (cyanoacrylic resin called "Aron alpha"). Especially the specimen for the observation of sclerenchymatous fibers composed of polyamellate structure was planed with a glass knife on LKB Ultratome 8800. After the specimens embedded with "Aron alpha" were treated with acetone for removal of the embedding agent and dried, they were coated with gold using an Ion Coator and observed with SEM (JSM-2) at 15 kV.

3. Results and Discussion

1) Pulping of young culms

It is no exaggeration to say that all the studies on *Sasa* pulps have been hitherto carried out using the adult culms. Since a large mill providing the facilities of high pressure and high temperature is requisite for the production of the pulps from the adult culms, the utilization of the *Sasa*-bamboos has not been nearly developed, together with high harvesting cost. It is desired to obtain easily pulps from them with conventional tools and chemicals. The authors have tried to

prepare the pulps from the young culms of low lignin content (Table 1) harvested before perfect lignification using dilute alkaline solution, which dissolves a large quantity of lignin even in the adult culms,⁴⁾ and have shown the good results by boiling the chips of the young ones of less than 2 m long with 1 to 2% potassium hydroxide solution.

While the results of the various properties will be reported in detail in the next paper, in this paper morphological properties of the fibers and the handsheets are mainly described by the optical and electron microscopic observation, as mentioned below. The fibers in the pulps thus prepared are composed of thin cell wall and are left in the combined form (bundle sheath). Accordingly, they have characteristics similar to bast fiber used for Japanese paper. These easily defibrated fibers are soft and pliant, as shown in Fig. 4, different from the sclerenchymatous fibers from the adult culms (Fig. 5). The observation of the pulpsheets shows the features similar to a beaten pulp of strong fiber-to-fiber bond in spite of no beating and no fibrillation (Fig. 6). The high strength of the sheet seems to

Table 1. Chemical composition of *Sasa* culms and pulp from the young culm (%)

Sample	Ash	Extractives		Lignin	Holocel- lulose	Pentosan	Metyl pentosan
		1% NaOH	Alcohol- benzene				
Less than 1-yr-old culm	5.0	43.2	1.7	11.6	66.5	24.5	1.9
” culm pulp	—	0	0.6	2.4	93.5	36.0	4.0
2-yr-old culm	1.5	32.9	4.9	21.6	67.0	—	—
4-yr-old culm	1.5	32.9	6.8	21.1	63.8	—	—



Fig. 4. Fibers from young culm of *Sasa kurilensis*. ($\times 40$)

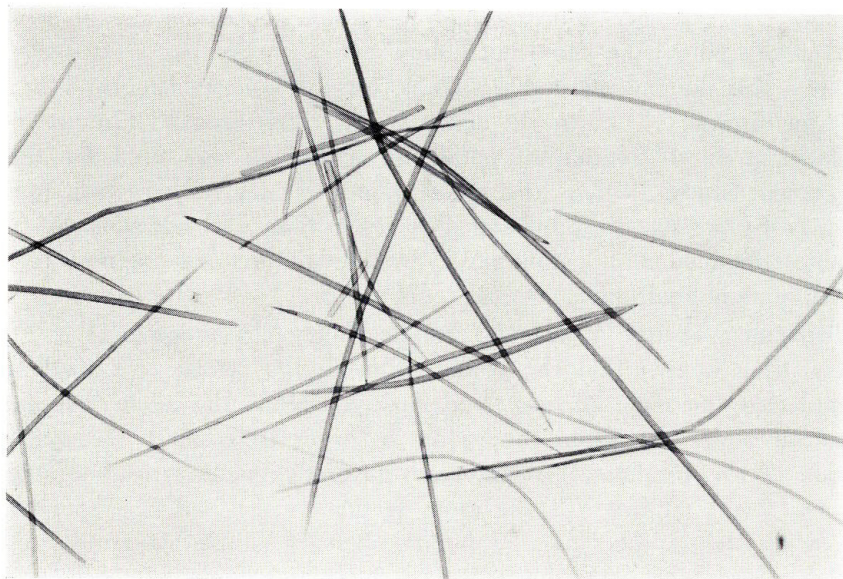


Fig. 5. Fibers from adult culm of *Sasa kurilensis*. ($\times 40$)



Fig. 6. Surface of pulp sheet (unbeaten) from young culm. ($\times 380$)

account for this pliability and the high pentosan content, showing a match for alkali or sulfate pulp from the adult culms.^{5,6,26)}

Though this pulping method is suitable for the household industry in mountain villages, the disposal of the waste liquor would cause trouble. In order to solve the problem, a dilute solution of potassium hydroxide was used for the pulping in the present study. When the waste liquor is neutralized with hydrochloric acid, it may be possible to be changed to a fertilizer. In addition, the properties of the pulps obtained with potassium hydroxide solution were almost the same as those with sodium hydroxide.

2) Histological change of vascular bundle in growing process

It has been known that the young culms in the course of growth are easily pulped and that the pulps have a good quality,²⁷⁾ while the adult ones are difficult to be pulped under the same condition. In order to confirm the difference the observation of the histological structures was firstly begun from the stage of the underground bud. Figure 7 is the cross section of the bud corresponding to the initial growing stage. The vascular bundle shows a simple structure consisting of protoxylem vessels and protophloem (sieve tubes and companion cells), which are surrounded with a large number of very thin wall fiber cells (immature bundle sheath) before wall thickening. In this stage, the metaxylem has not been seen

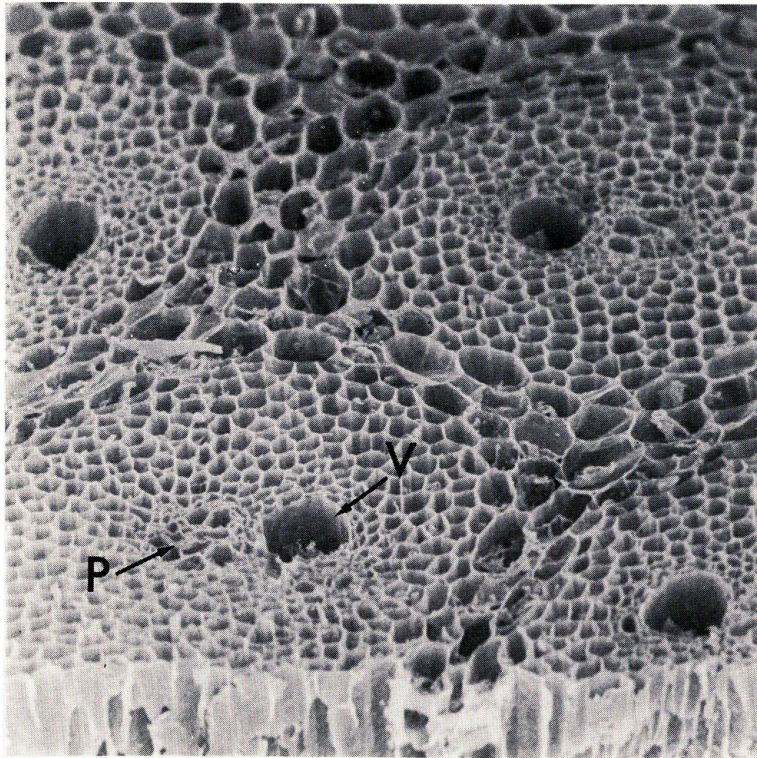


Fig. 7. Vascular bundle in the bud.
V; Vessel in protoxylem P; Protophloem ($\times 230$)

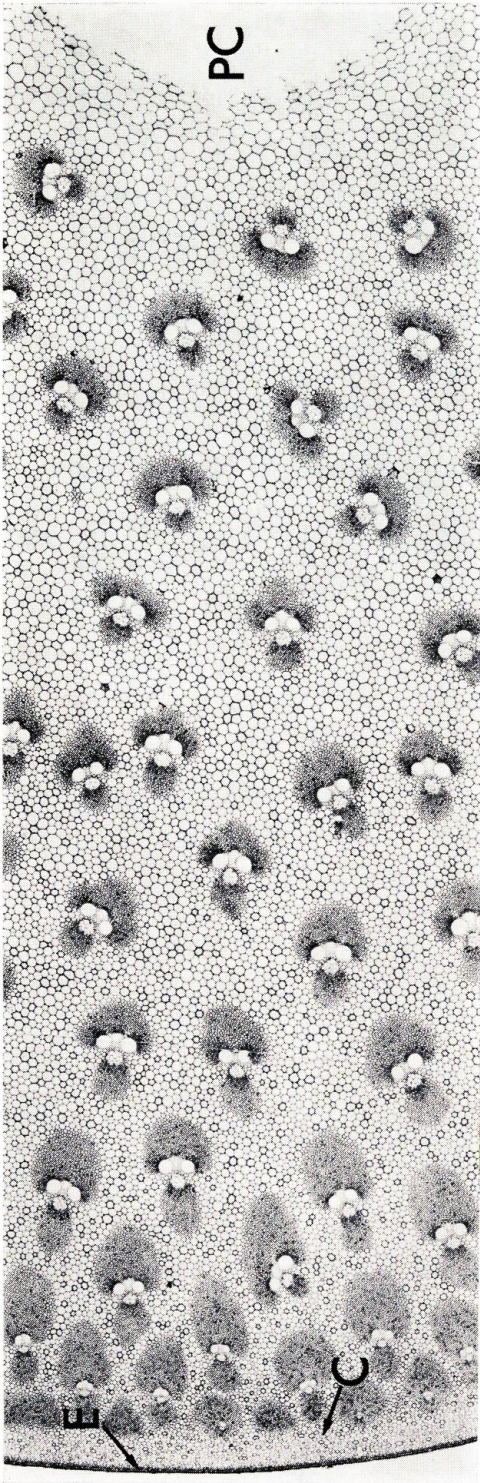


Fig. 8. Cross section of first internode of the young culm (less than 1-year-old culm).

E; Epidermis C; Cortex PC; Pith cavity (×20)

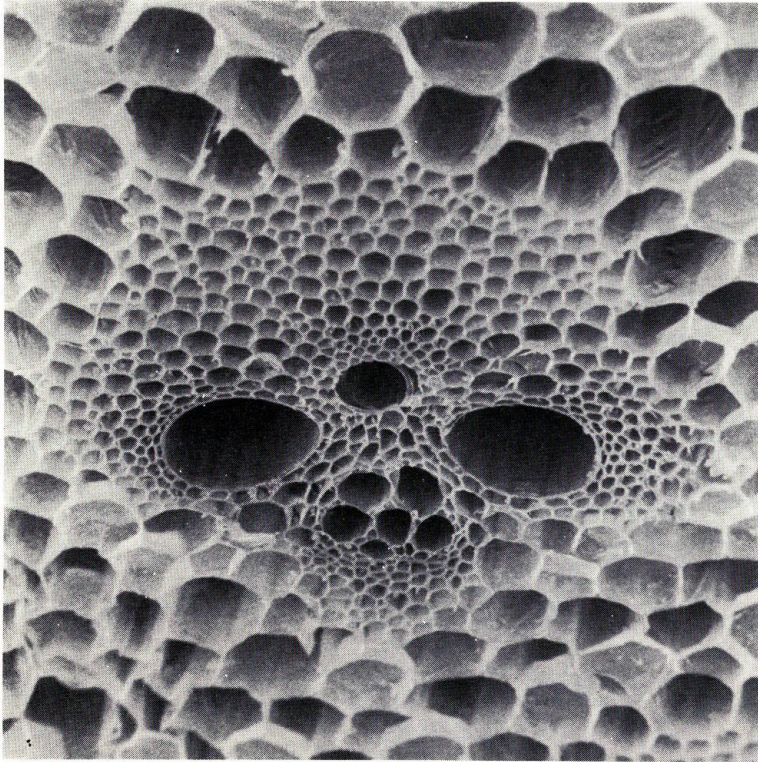


Fig. 9. Vascular bundle in fifth internode of the young culm. ($\times 300$)

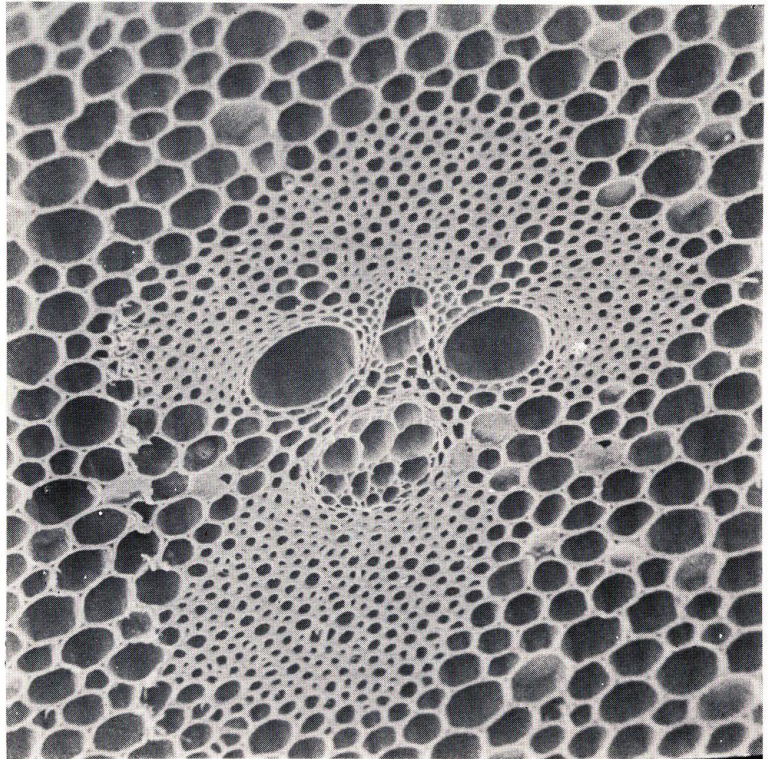


Fig. 10. Vascular bundle of second internode of the 2-year-old culm. ($\times 230$)

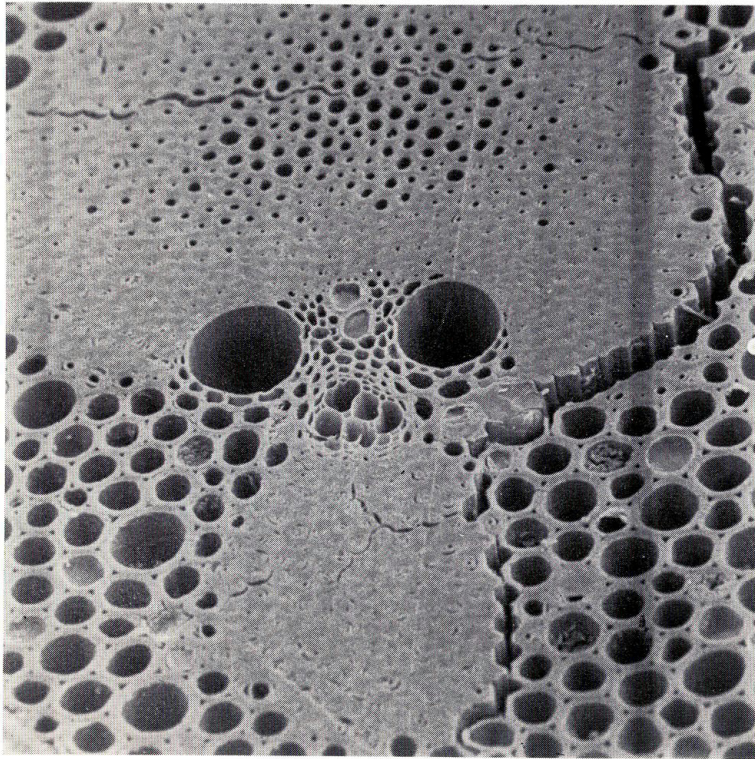


Fig. 11. Vascular bundle of second internode of the 4-year-old culm. ($\times 230$)

but the time is not clearly confirmed when the occurrence of reticulate vessels exactly begins, due to no microscopic observation of the sprout just coming out on the ground. When the bud grows to the sprout of a certain length by differentiation, a pair of reticulate vessels has already occurred in the metaxylem of the vascular bundle, putting the protoxylem vessels in the center. The features of it is just like a human face skeleton, i. e. a pair of reticulate vessels and phloem corresponding with eye-sockets and a nosehole, respectively. In the outer part, the vascular bundles reveal peculiar vessels of small diameter surrounded with bundle sheath composed of fiber cells (Fig. 8), while those scattered from the center to the inner parts in the ground tissue reveal really the skeleton-like features, the shape of which is not changed by elapse of age, as shown in the less than 1-year-old (No. 2, Fig. 9), the 2-year-old (No. 3, Fig. 10), and the 4-year-old culm (No. 4, Fig. 11). The protoxylem consists of a large annular vessel and several small spiral vessels lying between the phloem and the annular vessel, which gradually expands its diameter and seems to shift into the intercellular space, breaking the primary wall.

2-1) Vessels

Large-size annular vessel in the protoxylem has initially a septum (Fig. 12) which changes to ring-like annulus with development of the vessel, and the annulus

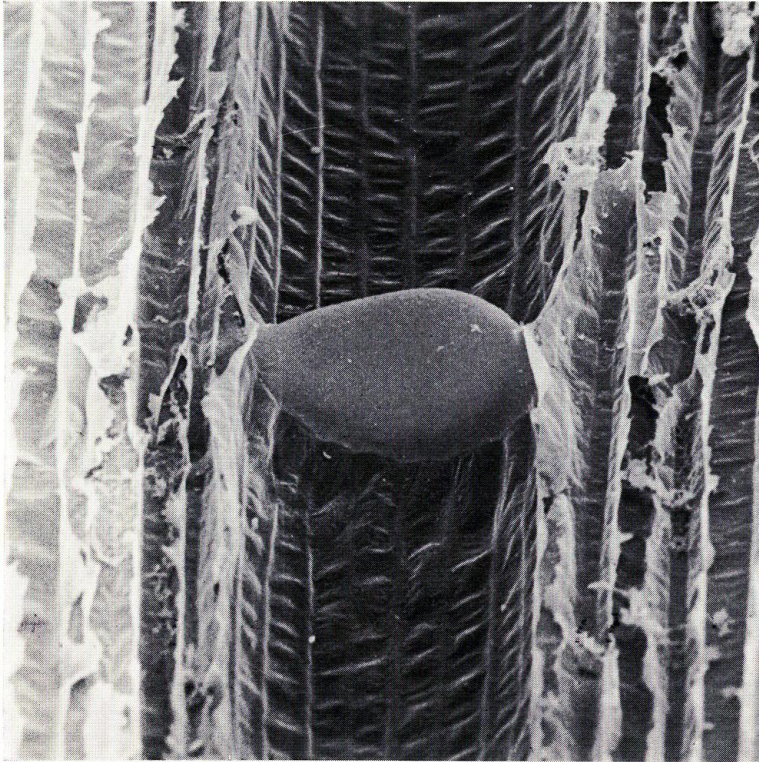


Fig. 12. Septum of annular vessel. ($\times 770$)

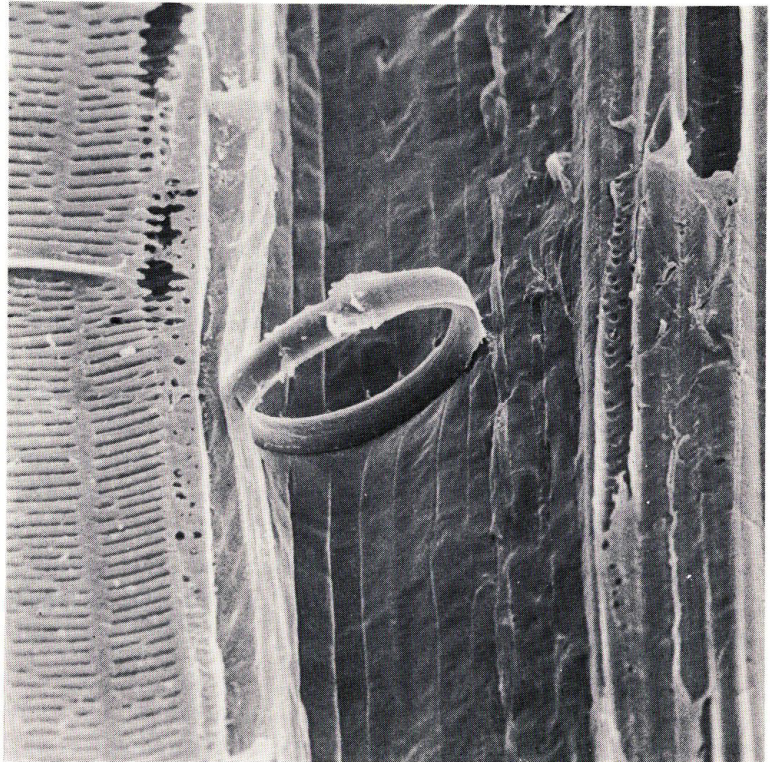


Fig. 13. Annulus of annular vessel in protoxylem. ($\times 770$)

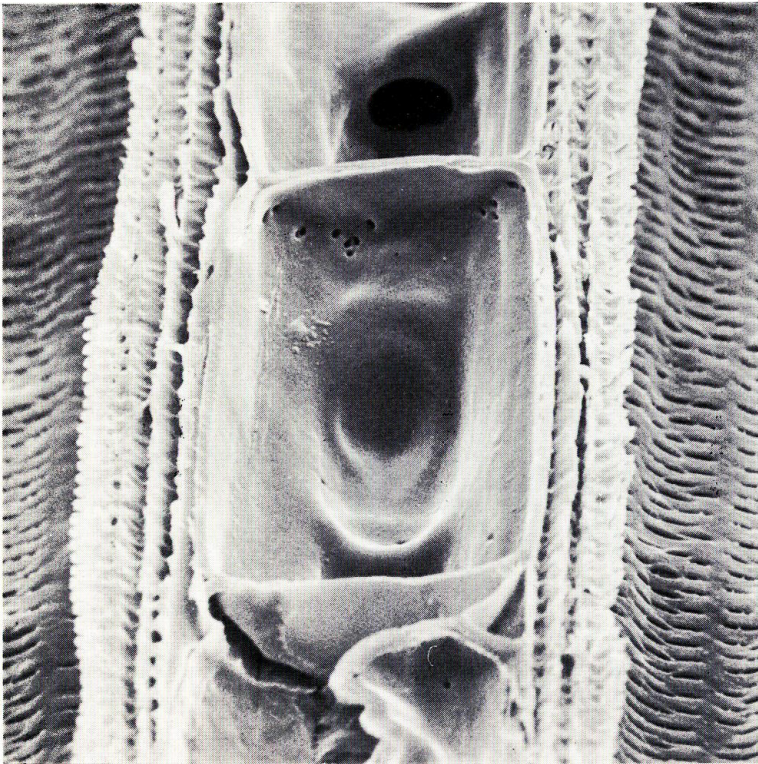


Fig. 14. Thick wall tyloses developed in intercellular space. ($\times 770$)

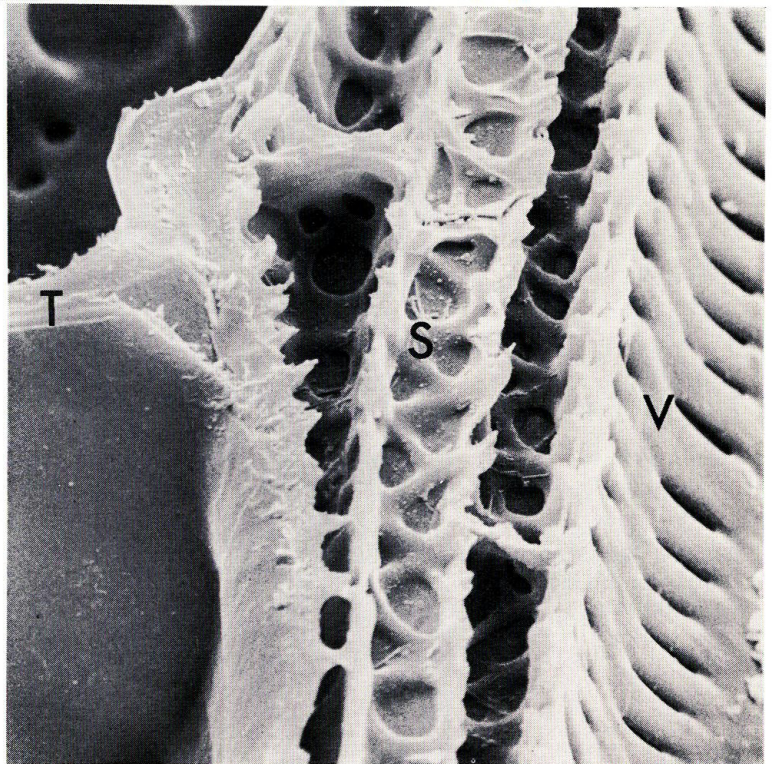


Fig. 15. Vertical section of vascular bundle.
V; Reticulate vessel
S; Spiral vessel
T; Tyloses ($\times 2300$)

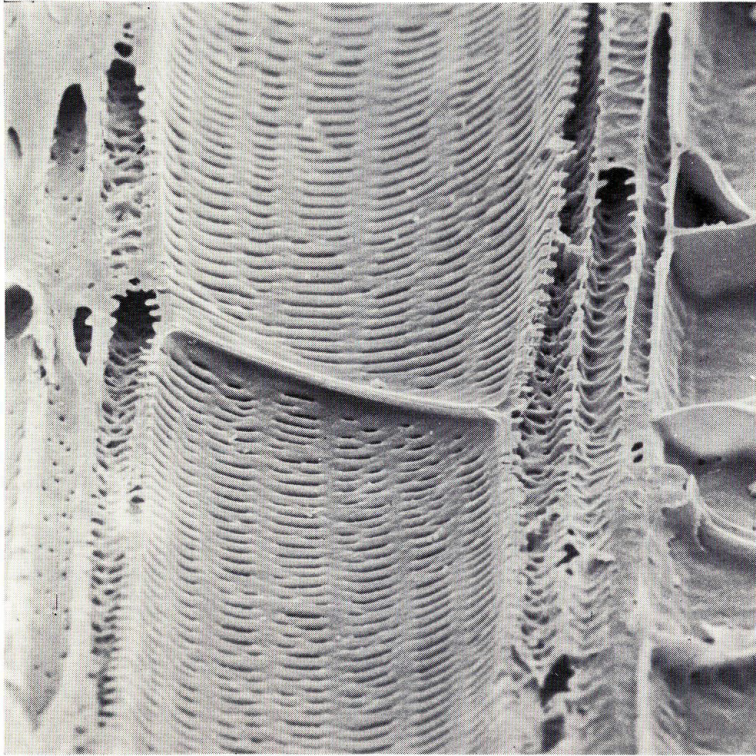


Fig. 16. Reticulate vessel in tangential section. ($\times 770$)

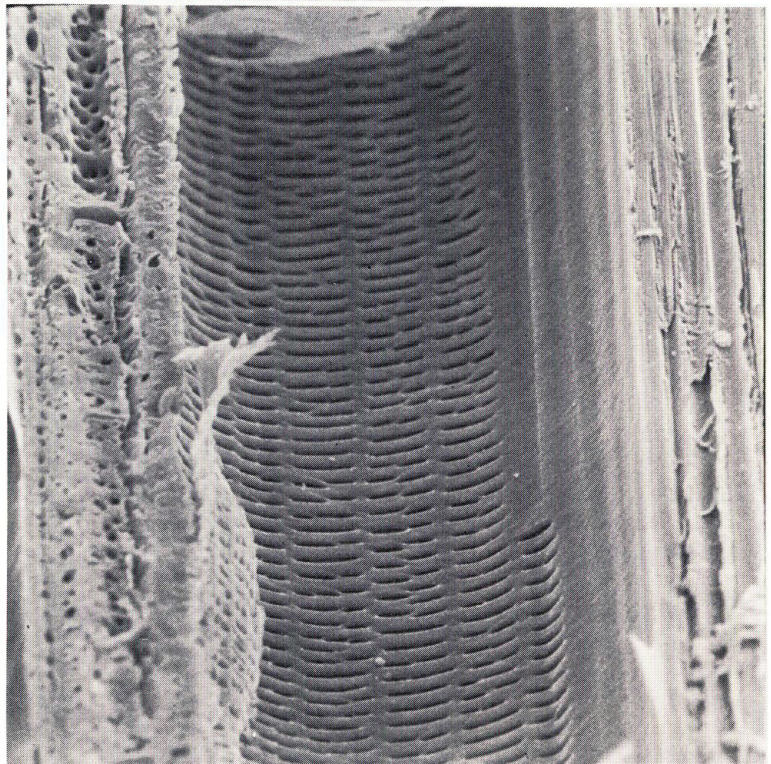


Fig. 17. Reticulate vessel partially lacking in pits. ($\times 770$)

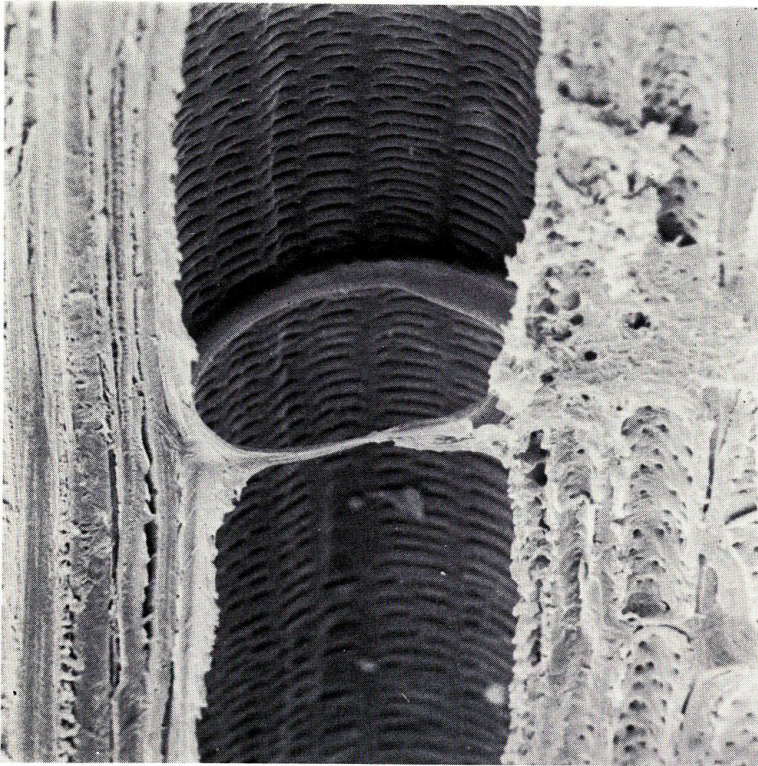


Fig. 18. Simple perforation plate usually observed in reticulate vessel. ($\times 770$)

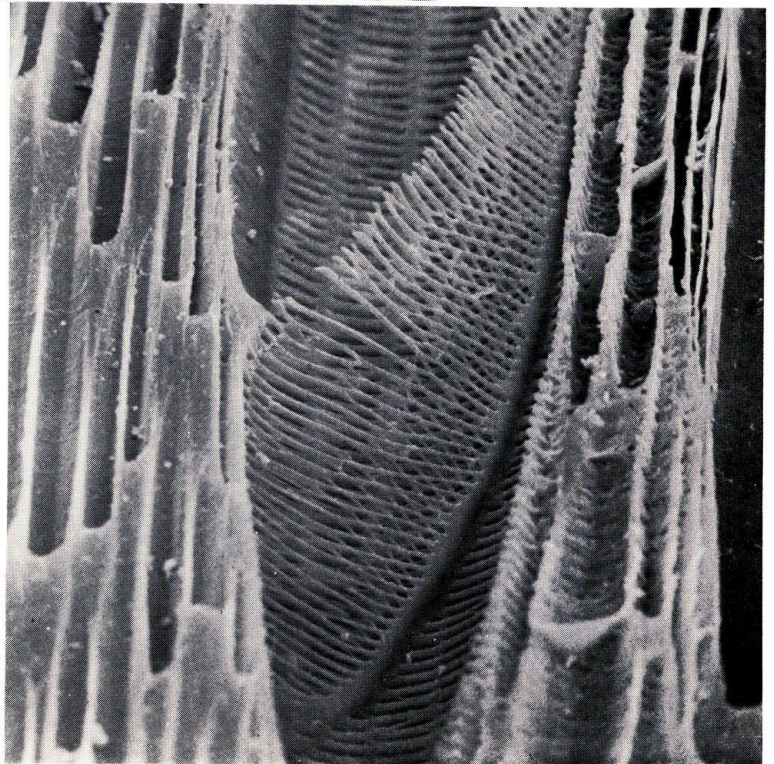


Fig. 19. Scalariform perforation plate rarely observed in reticulate vessel. ($\times 770$)

is separated with expansion in the diameter of the vessel (Fig. 13). Namely, only the annuli in the annular vessel show the thickening of secondary wall, while its primary wall is broken by growth. Accordingly, the intercellular space is developed around the place, and the space is often filled with thick wall tyloses derived from parenchyma cells (Fig. 14).

Small-size spiral vessels in the protoxylem exist in the center of the vascular bundle surrounded with the annular vessel, phloem and reticulate vessels. The spiral vessels thicken their wall by spiral formation of the secondary wall with growth as shown in Fig. 15.

Meanwhile, the reticulate vessels in the metaxylem are large except those of the vascular bundle lying near the epidermis, and show the secondary thickenings. The vessels have scalariform pits (Fig. 16), but are sometimes lacking in them seemingly when the vessels connect with fiber cells (Fig. 17), as reported in Part 1.²⁵⁾ It is observed that the perforation plate in the reticulate vessels consists nearly of simple perforation (Fig. 18), and scarcely of scalariform perforation plate (Fig. 19), the location of which can not be exactly determined.

2-2) Phloem

The cross section of the phloem from No. 2 specimen is shown in Fig. 20.

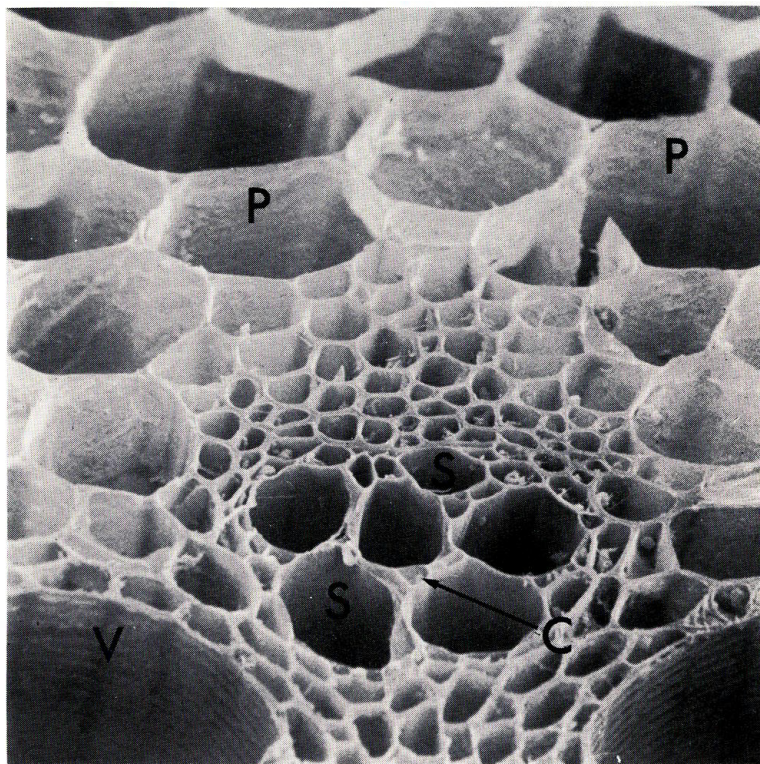


Fig. 20. Phloem of fifth internode of the young culm.
S; Sieve tube C; Companion cell P; Parenchyma cell
V; Vessel in metaxylem ($\times 770$)

The cells of large diameter seen in the center are sieve tubes (S) in the metaphloem, while the small cells seen among the sieve tubes are companion cells (C). The protophloem which ought to exist upon the metaphloem can not be distinguished. Though the protophloem is usually collapsed by the growth of the metaphloem, the trace of it is not also recognized in this photograph. Apart from the phloem, the photograph shows comparatively small cells surrounding the phloem, which are fiber cells constituting the bundle sheath, but the thickness of their wall is very thin in this growing stage. In addition, large cells seen in the upper part in Fig. 20 are parenchyma cells (P), constituting the ground tissue which do not similarly start the thickenings of the wall. Since the phloem is not lignified naturally even on maturity,²⁵⁾ the change of the phloem in growing process was not observed.

3) Bundle sheath

The bundle sheath is composed of fiber cells. The cell walls of the fibers from the less than 1-year-old culm are thin, composed of 1 or 2 layers which are gradually increased through differentiation (Fig. 21). Those from the 2-year-old culm and 4-year-old one are composed of 3 to 4 (Fig. 22) and 7 to 8 layers (Fig. 23), respectively. The thickenings of the wall seems to progress at the rate of 2 or 3 layers per year into the lumen till the completion of the cell wall thickening, corresponding probably to the age of 4 years old.

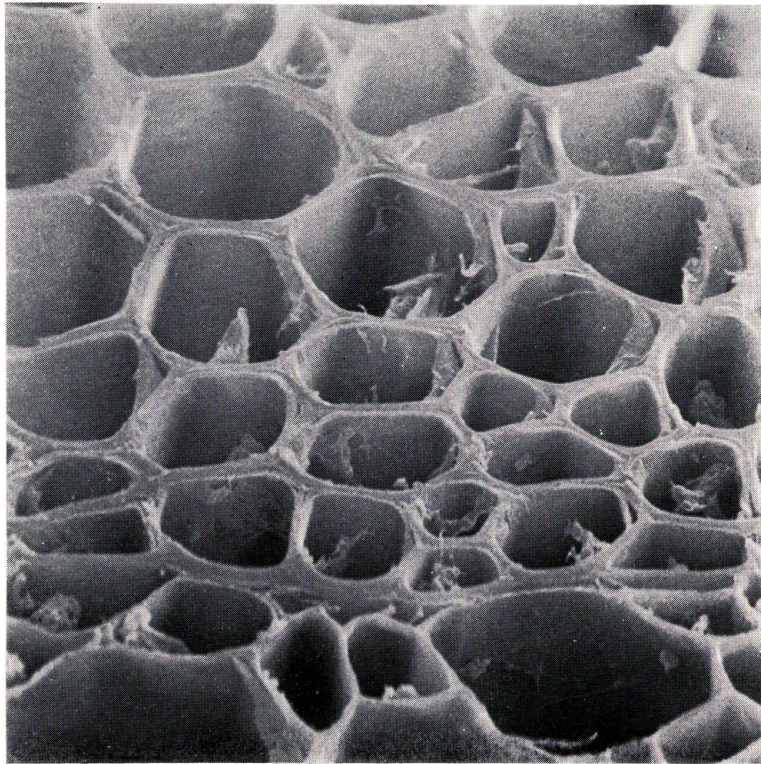


Fig. 21. Fiber cells from the young culm. ($\times 2300$)

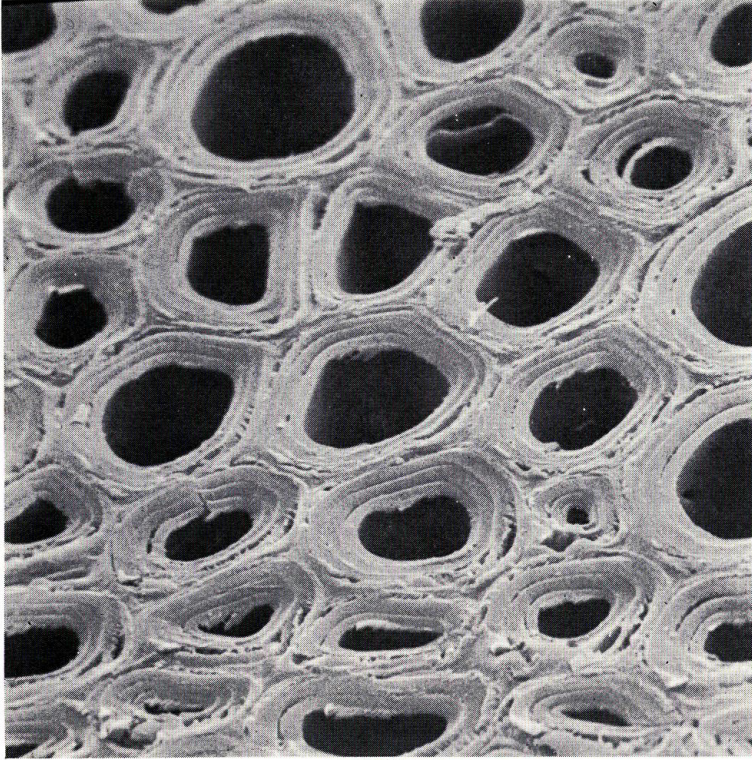


Fig. 22. Fiber cells from the 2-year-old culm. ($\times 2300$)

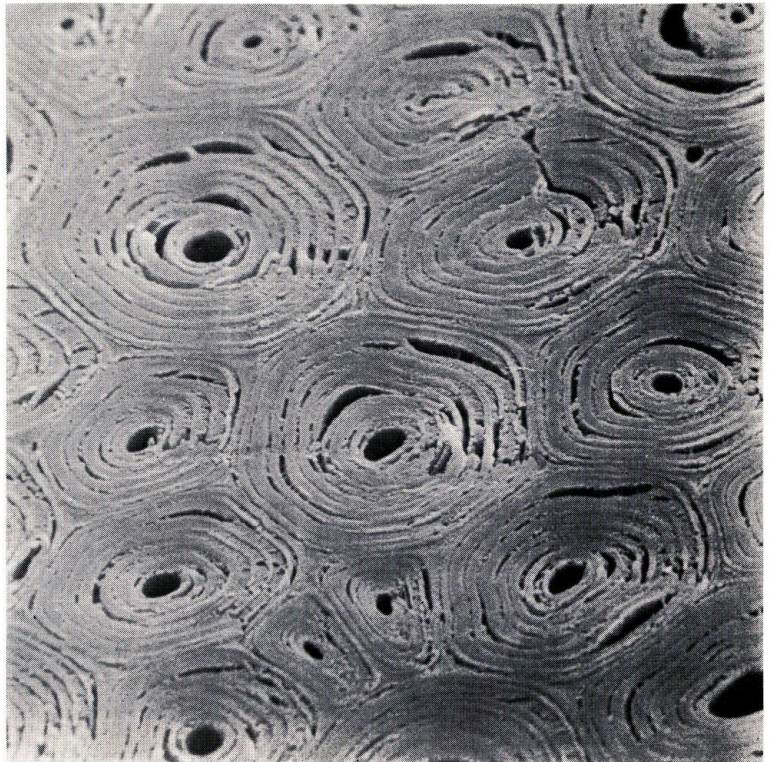


Fig. 23. Fiber cells from the 4-year-old cell. ($\times 2300$)

Since the fibers from the adult culm are sclerenchymatous fibers, individual fiber obtained by defibration looks like a needle (Fig. 5), while the fibers from the young culm are pliant in spite of no beating (Fig. 4) and are framed with thin wall. This is the reason why the paper made from the young culm show a high strength and characteristics like Japanese paper. Owing that the hardness of the embedding agent is relatively soft, the glassy-planed surface was not obtained as shown in the photographs. However, these make rather the wall structure distinct through clear appearance due to different microfibrill angles in each layer,²¹⁾ on which the fibers from bamboo culms are reported to be composed mutually of thick and thin lamella structures.²⁰⁾ The layer structure has been observed with an optical microscope,²⁵⁾ but it may be necessary to be more minutely observed with a transmission electron microscope (TEM).

The SEM observations show that the simple pits seen in the fibers have a large number of long pit canal in the 4-year-old culm (No. 4 specimen, Fig. 24). The difference is not so distinct between the fiber near the epidermis and that constituting the bundle sheath.

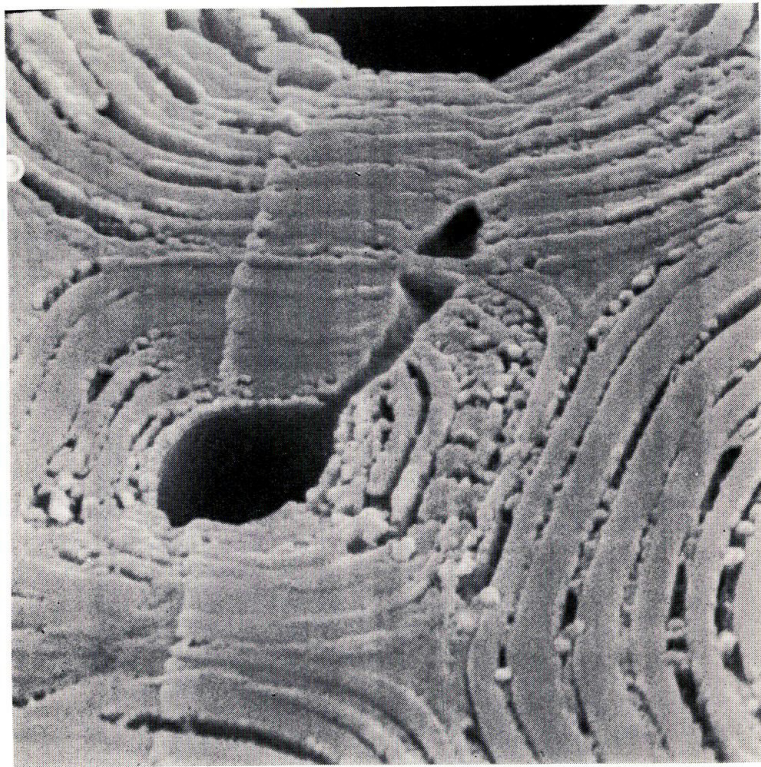


Fig. 24. Simple pit forming long pit canal. ($\times 7700$)

4) Ground tissue

Small-size parenchyma cells constituting the ground tissue distribute around the epidermis of the culm, while large-size ones distribute in the inner part (Fig.

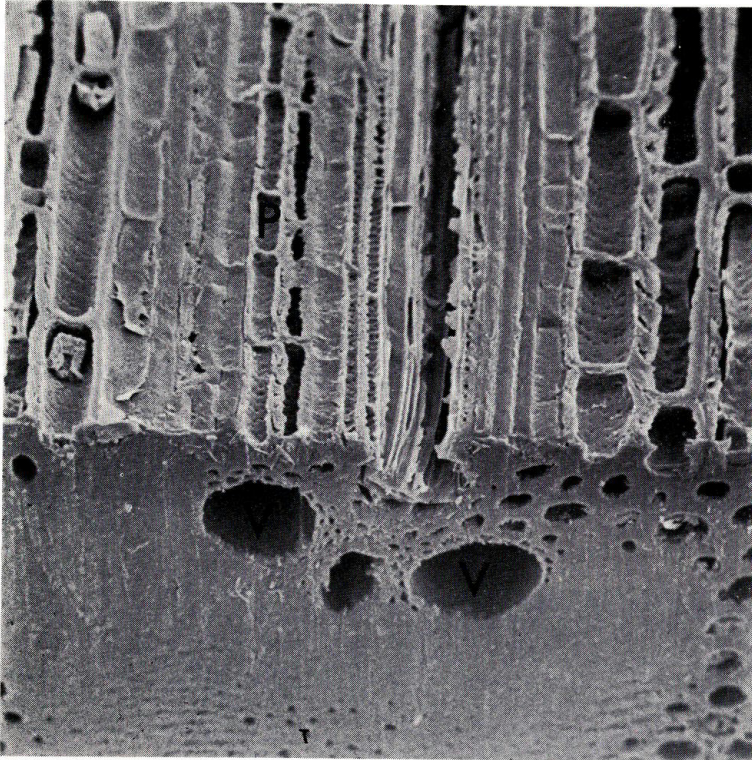


Fig. 25. Small-size parenchyma cells (P) near vascular bundle.
V; Reticulate vessel
($\times 230$)

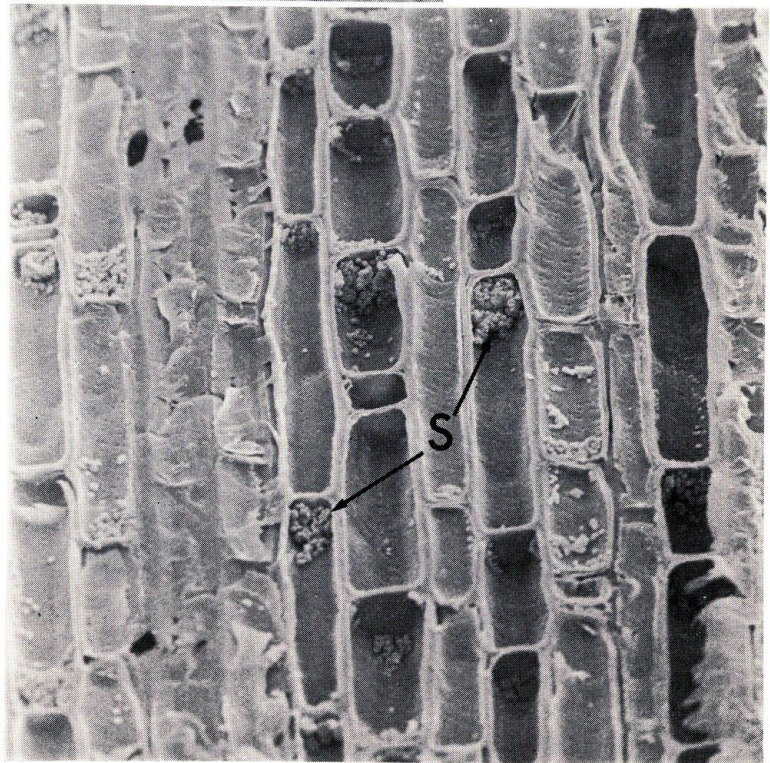


Fig. 26. Large-size parenchyma cells constituting ground tissue.
S; Starch grains ($\times 230$)

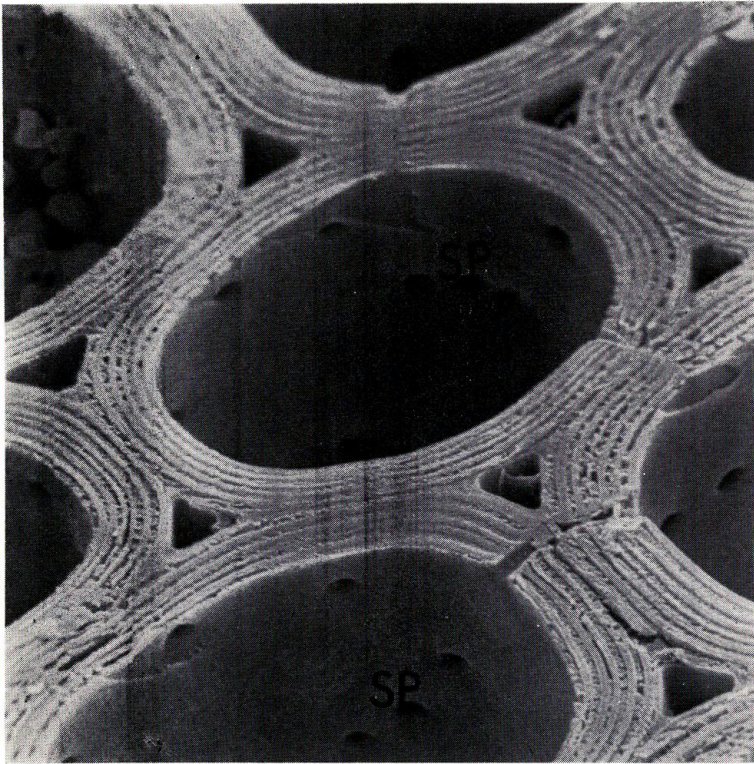


Fig. 27. Large-size parenchyma cells showing polylamellate structure in the 4-year-old culm. SP; Simple pit. ($\times 2300$)

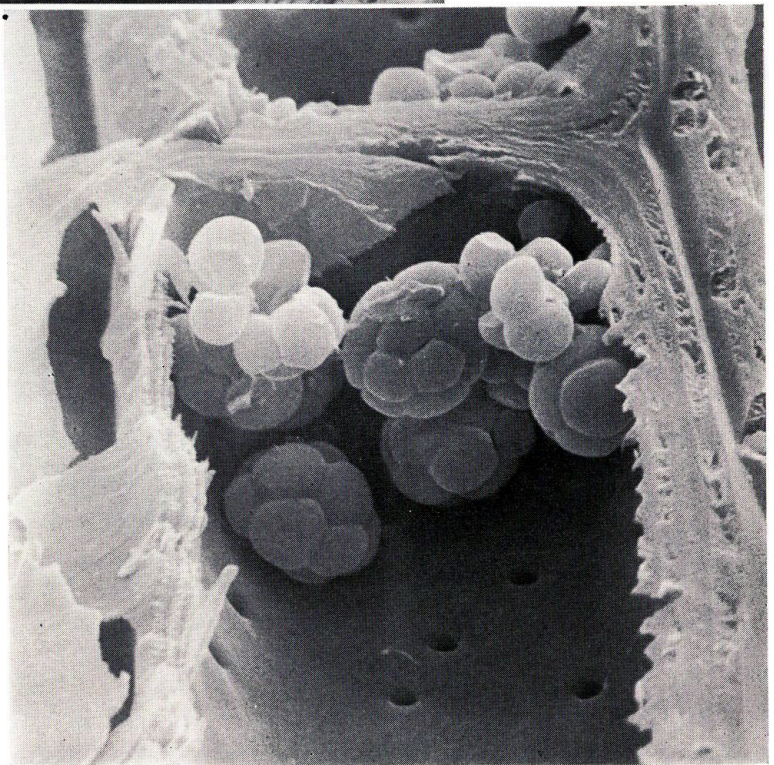


Fig. 28. "Soccer ball"-like starch lumps rarely observed in large-size parenchyma cells. ($\times 2300$)

8). A number of simple pits are also observed in the parenchyma cells both near the vascular bundle (Fig. 25) and in the ground tissue (Fig. 26). The cell wall is thickened, increasing the layer with aging, as shown in the parenchyma cells composed of 7 layers or so from the 4-year-old culm (Fig. 27). Meanwhile, lots of starch grain confirmed by iodine reaction²⁵⁾ are observed in the parenchyma cells constituting the ground tissue, but not in the small sized ones near the vascular bundle (Fig. 25). The large starch lumps ("soccer ball"-like shape as reported by ISHIDA and OHTANI¹⁰⁾) could be rarely observed (Fig. 28). Concerning the "soccer ball", it is not obvious whether that is formed by gathering of single grains, or reversely the stored ball-like starch is decomposed into the single grains. Small rectangular parenchyma cells which are composed of thin walls, seem among the large-sized ones, are not lignified as confirmed by double staining²⁵⁾ and by the observation with SEM (Fig. 29). The fact has been already reported in the case of bamboo species.²²⁾



Fig. 29. Small-size rectangular parenchyma cells (arrow) observed among large-size parenchyma cells. ($\times 770$)

Conclusion

The *Sasa*-bamboo culms have been chemically investigated for the utilization as forest resources, and the properties of the culms have been also gradually elu-

cidate through the studies on the pulping. However, the histological study of the culms has been delayed. In part 1 of this study, the authors studied the cell structure with an optical microscope. Based on the facts that the lignin in the *Sasa*-bamboo culms has a characteristic much soluble with a dilute solution of sodium hydroxide and that the young culms in the course of lignification have a low lignin content, the chips of the young culms were boiled with 1% of sodium hydroxide solution and pulps were easily produced. The resultant pulps showed good properties because of the thin wall fibers. Such pulps could be prepared also under the same condition with potassium hydroxide solution, the waste liquor of which might be changed to a fertilizer. The features of the histological structure in the young culms could be also backed up from the properties of the pulps.

In this study, a considerable part of the cell structure was elucidated with SEM, hitherto obscured and inferred from the optical microscopic observation. Above all, the change of annular and spiral vessels in the protoxylem could be observed in detail in the growing process, and the occurrence of polylamellate structure could be grasped in the fiber and parenchyma cells with increasing 2 or 3 per year into the lumen.

It is necessary for the utilization of the *Sasa*-bamboos to be elucidated more minutely on the histological structures of the culms using both the optical and layers electron microscopes.

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要 約

チシマザサの開葉の始まる前後の幼稈を1~2% KOHで煮沸したところ、NaOHの場合と同様、容易にパルプ化でき、薄壁繊維のため未叩解でも強い紙葉を作ることができた。またパルプ中には維管束鞘部分が結束繊維状に多数混入しているため、和紙に似た民芸的にも興味のもてる面白い風合いの手漉紙が得られた。このようにササ稈は伸長・成熟のいろいろな段階でその性質に適合した利用方法が考えられるので、それぞれの段階における維管束や基本組織の状態を主として走査電子顕微鏡を用いて観察し、光学顕微鏡では観察困難な部分の補完と新知見を得た。

1. 幼稈パルプは薄壁で柔軟性があるため (Fig. 4), 成熟した稈の厚壁の硬質繊維パルプ

(Fig. 5) とは明らかに異なっている。また幼稈パルプから作った紙葉の表面 (Fig. 6) は、パルプが未叩解のため、フィブリル化はみられないが、繊維間結合の強い紙葉であることが伺われる。

2. 廃液は塩酸で中和すると KCl が本来ササの中に多量に存在するカリウムと合わさってカリ肥料として利用できるため、廃液の処分が容易である。

3. 地中にある伸長前のタケノコの維管束 (Fig. 7) は原生道管と原生師部の2種類が、薄壁で壁形成途中の繊維細胞群に囲まれた単純な形である。

4. タケノコが伸長して稈がある程度の長さになると、維管束の中に原生道管を挟んで1対の後生道管が発達し、道管が眼窩、師管部が鼻窩に相当する人間の頭骨前面のような形となる (Fig. 9~11)。

5. 大型の原生道管すなわち環紋道管は形成当初隔壁をもっている (Fig. 12)。この隔壁は道管が発達するにつれて環紋となる可能性があり、さらに環紋は道管の直径が拡大すると片側の壁から引き離された状態となると考えられる (Fig. 13)。

6. 環紋道管の環紋部以外は二次壁肥厚を示さず、その表面生長により一次壁は破れ、その周辺に細胞間隙が発達する。この間隙は周囲の柔細胞から発達した厚壁チロースで閉塞される (Fig. 14)。

7. 小型の原生道管、すなわち、らせん紋道管は環紋道管と師部および後生道管に囲まれた中央部のみに存在する。これらにはらせん状に二次壁形成がおこり、らせん肥厚が認められる (Fig. 15)。

8. 当年生の繊維壁は1~2層の薄壁であるが (Fig. 21)、2年生 (満1年) になると3~4層 (Fig. 22) さらに4年生になると7~8層となる (Fig. 23)。したがって1年間に内腔側に2~3層ずつ形成されるものと考えられ、4年生 (満3年) 以後では内腔がほとんど埋めつくされ、それ以上層は増加しないものと思われる。

9. SEMによると壁の層構造は光学顕微鏡によるよりはっきり観察できるが、厚い層と薄い層が交互に存在するのか、薄い層状に見える部分は厚い層の接着部が切削によって剝離して層状に見えるのかは判断しにくい。それを明らかにするためには TEM による観察などが必要であろう。

10. 柔細胞の壁にも繊維細胞と同じような多層構造がみられる (Fig. 27)。

11. 柔細胞の中には多量の澱粉粒があり、まれにサッカーボール状のものがみられる (Fig. 28)。