CRYSTALLINITY AND ULTRASTRUCTURE OF AMMONIATED WOOD PART II. ULTRASTRUCTURE

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ABSTRACT

Cell-wall layering, pits, and lumen surfaces of loblolly pine wood were examined in the electron microscope for changes due to ammonia treatment. Both normal and compression wood cell walls were crimped circumferentially after ammoniation as evidenced by deformations in the S3 and/or S1 layers. Such crimping would imply a cell-wall consolidation due to shrinkage in the S2, and the overall phenomenon was probably responsible for the increase in X-ray crystallinity of the same material. Other ultrastructural changes included definite pit aspiration and the deposition of an incrustant-like substance onto both pit structure and lumen surfaces. This incrustant was probably some residual wood extractive or other wall constituent partially solubilized by condensed ammonia in the cell.

INTRODUCTION

The first part of this paper dealt with the crystallinity of ammoniated loblolly pine wood, discussing the information and implications from wide-angle X-ray diffraction data. Although no change in crystallite width occurred as a result of ammonia treatment, the degree of crystallinity increased in both normal and compression wood. The exact cause of this increase was undetermined but conjectured to arise from a consolidation of the intra cell-wall structure. Electron microscopic evidence that does imply such a phenomenon is presented in this report.

Previous study of the ultrastructure of ammoniated wood appears limited to the description of microcompression failures (slip planes). These occur across the cell walls of wood as it is flexed in the plasticized state (Pentoney 1966). The present research was directed at describing more completely other ultrastructural changes, if any, that occur in ammonia-plasticized wood.

METHODS

The source of experimental material and the sampling scheme are described in part I of this paper. Suffice it to say that samples for electron microscopy were as closely matched as possible to those used for X-ray diffraction and identically treated.

Cell-wall layering was studied from ultrathin sections (800–1200 A) cut with a diamond knife. Specimens were embedded for sectioning in a 4:1 mixture of butyl:methyl methacrylate. Sections were picked up on 100-mesh copper grids coated with a celloidin-carbon substrate. The methacrylate was removed with chloroform, and the sections were shadowed with germanium before being examined in an RCA electron microscope.

Surface detail was examined from direct carbon replicas of the face of cleaved radial chips (Côté, Koran, and Day 1964). Some material was solvent-exchange dried (Thomas and Nicholas 1966) in order that pits could be studied in their unaspirated state prior to treatment.

RESULTS AND DISCUSSION

Cell-wall layering

Fig. 1 shows the normal cell-wall layering of loblolly pine in the untreated state. Typical here are the relatively thin S1 and S3 lamellae of the secondary wall separated by the thick and longitudinally oriented S2. The latter constitutes the major portion of both the earlywood (*EW*) and latewood (*LW*) cell wall.

An untreated compression wood tracheid is shown in Fig. 2. Evident here are the helical wall checks extending from the lu-

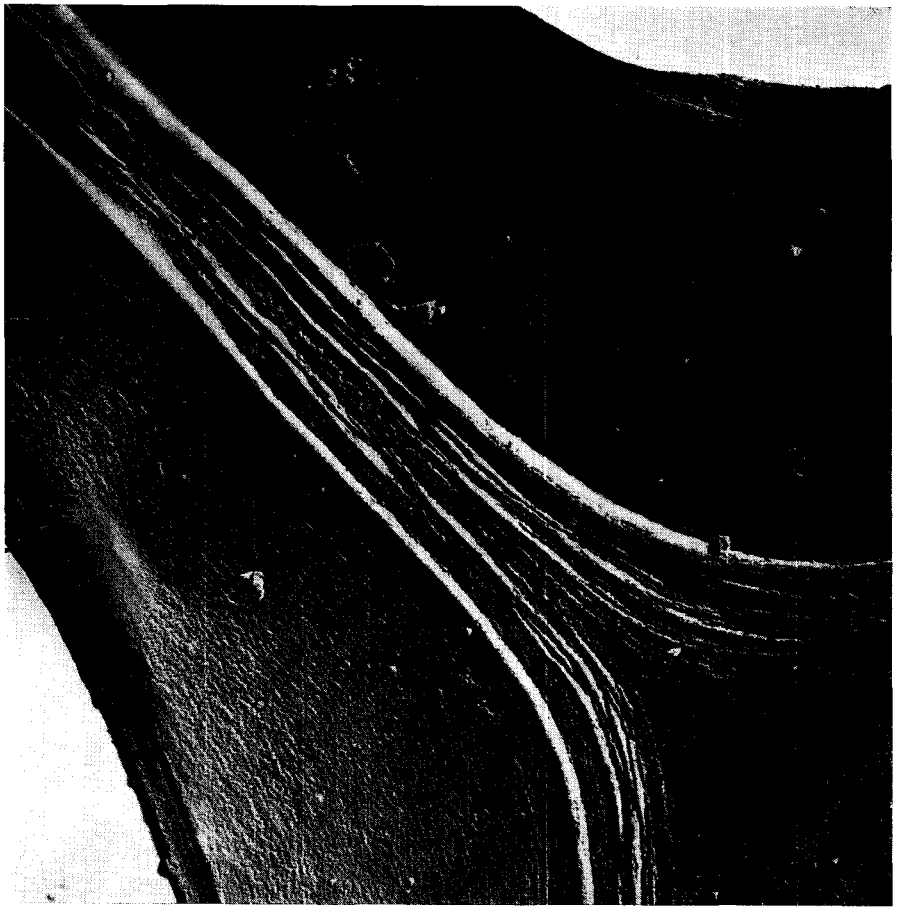

FIG. 1. Transverse section of normal loblolly pine earlywood. 13900×

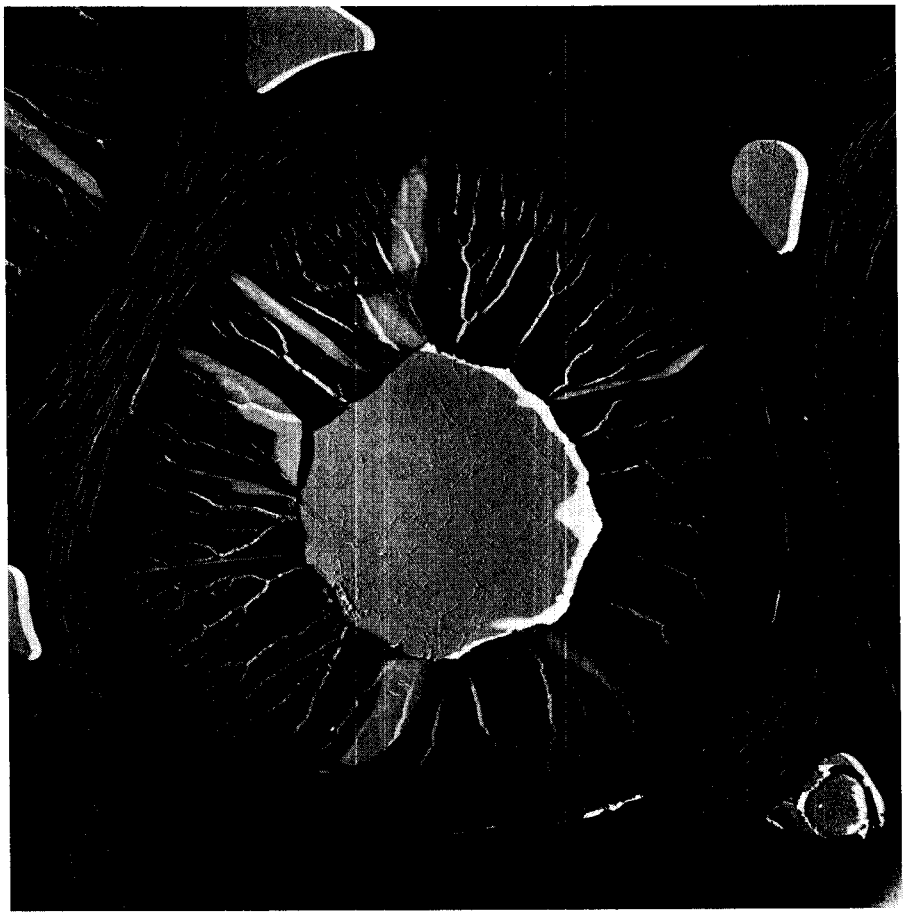

FIG. 2. Transverse section of loblolly pine compression wood. 4950×

FIG. 3. Transverse section of ammoniated earlywood. Note crimpings in S3 layer. 10750×

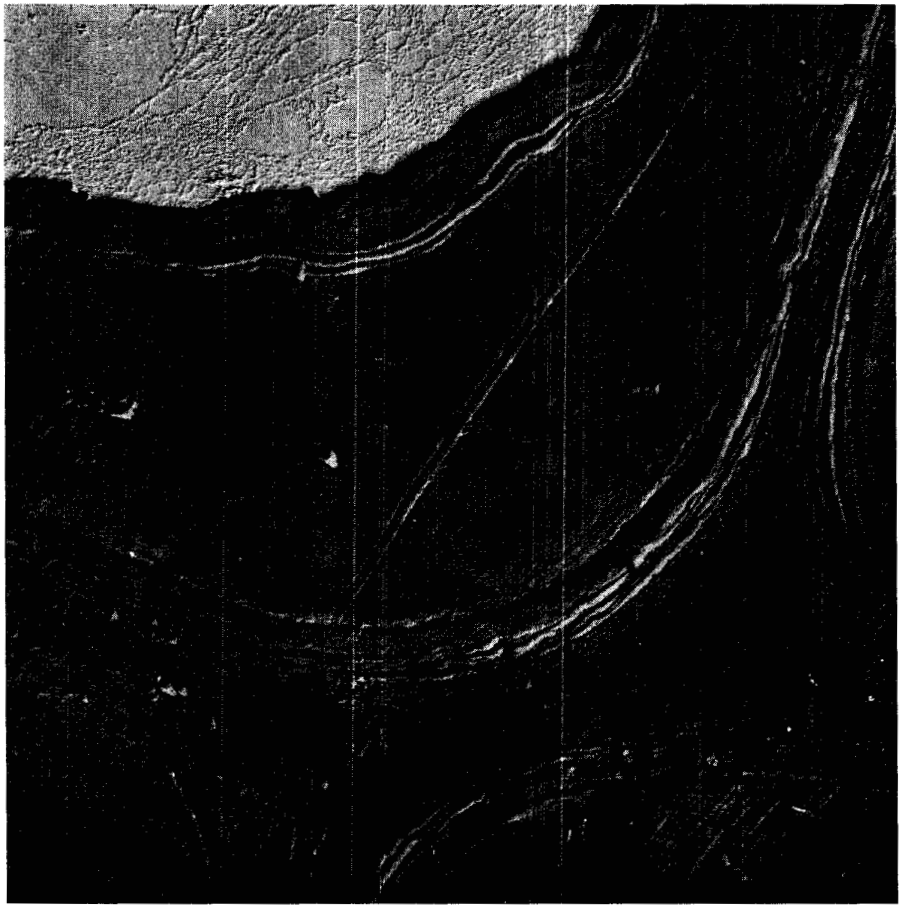

FIG. 4. Transverse section of ammoniated earlywood. Note crimpings in S1 and S3. 11800×

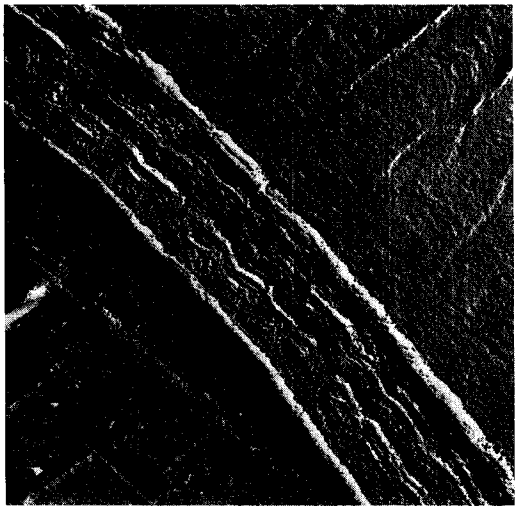

FIG. 5. Transverse section of ammoniated compression wood. Wall crimpings are restricted to S1 region. 10600×

FIG. 6. Exploded cell walls of ray parenchyma in ammoniated compression wood. Transverse section. 4820×

men side of the S2 outward. Small as well as larger checks branch in the outer S2 before reaching the S1. The absence of an S3 layer and presence of intercellular spaces are also apparent.

After ammonia treatment cross sections of normal *EW* and *LW* revealed a pronounced crimping deformation in the S1 and S3 layers with no obvious effects in the S2 (Figs. 3 and 4). *CW* cells exhibited a less radical deformation but still showed crimping in the S1 layer (Fig. 5). It would appear that both normal and *CW* tracheids were subjected to some external compressive force which caused them to crimp circumferentially. However, consideration of the possible intra cell-wall effects of ammonia and individual cell shrinkage and swelling implies that perhaps the driving force emanated partially, if not wholly, from within the cell wall itself.

Both swelling and shrinkage of the wood cell wall is controlled to a large extent by that of the S2 portion, and changes in this layer probably tend to obscure those exhibited by the S1, S3, or primary wall. If micro- and/or elementary fibrils within the S2 were drawn into more intimate association laterally by ammoniation and drying, the adjacent S1 and/or S3 wrappings, the majority of their orientations at a steep angle to the S2, could also consolidate; but, as a result of the similar but overriding action of the S2, these latter wrappings would also tend to be crimped in their axial direction.

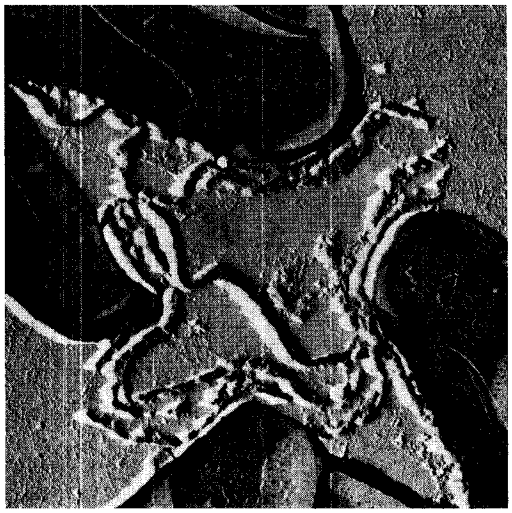

FIG. 7. Exploded membrane of a bordered pit in ammoniated earlywood. Transverse section. 6050×

Fig. 8. Unaspirated pit membrane in untreated earlywood. 10750× (Figs. 8–14 are direct carbon replicas preshadowed with chromium.)

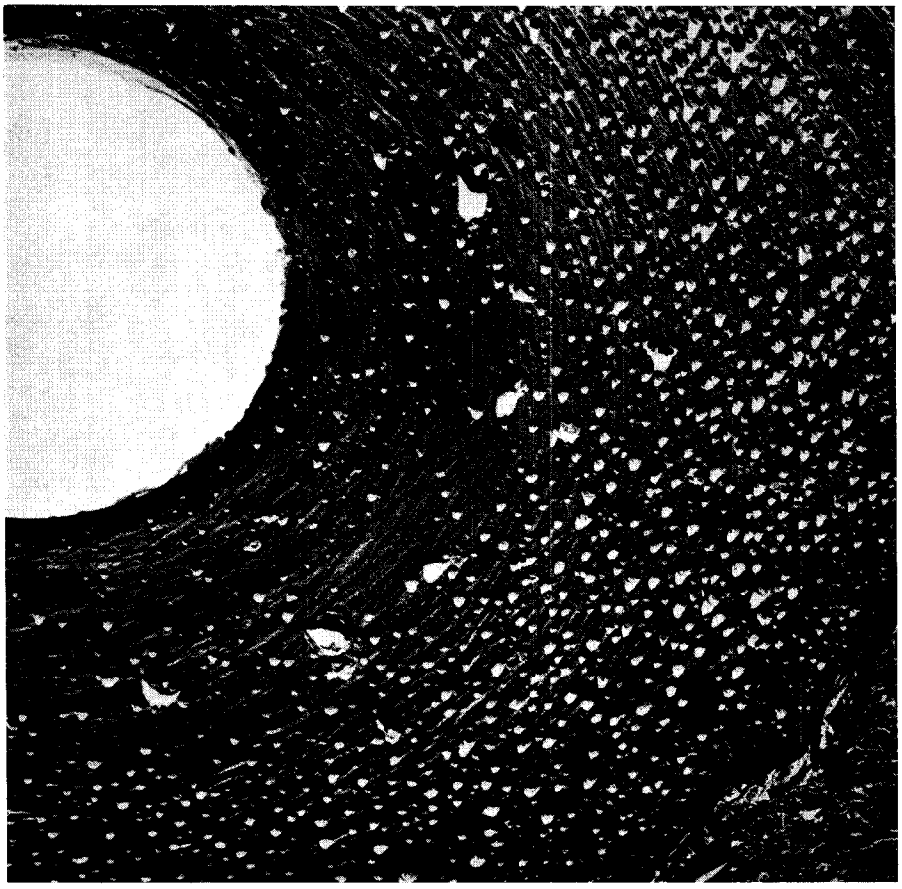

FIG. 9. Appearance of pit border prior to ammonia treatment. 10750×

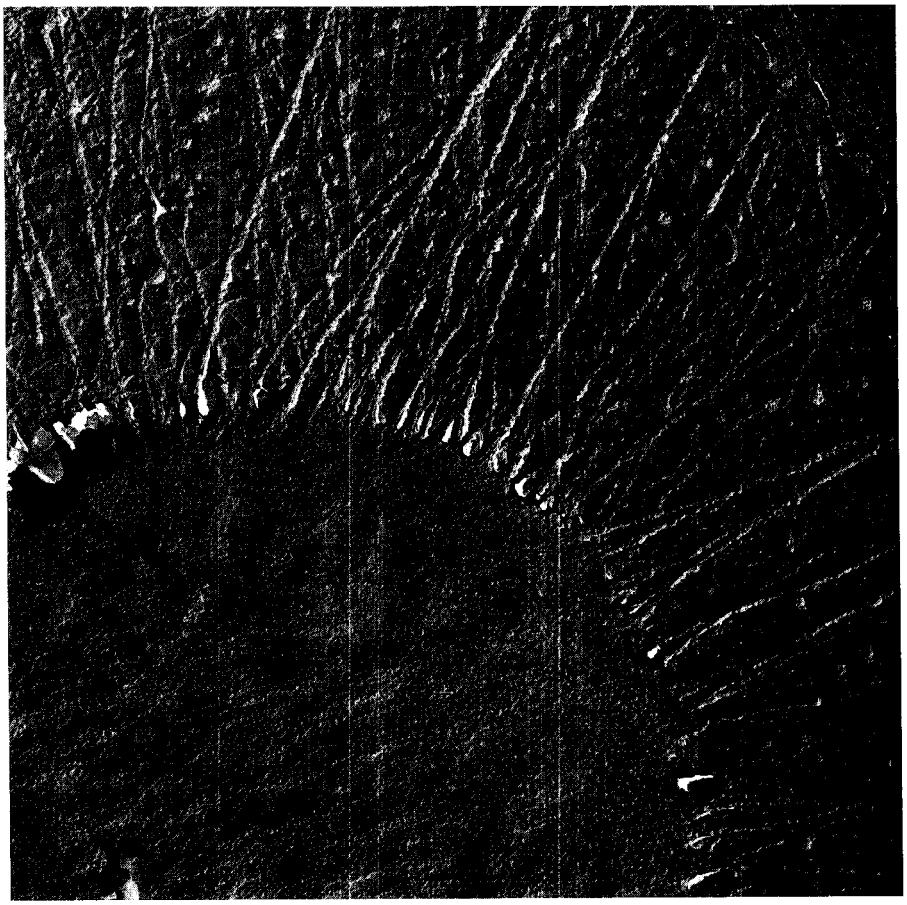

FIG. 10. Aspirated and incrusted pit structure in ammoniated earlywood. 10300×

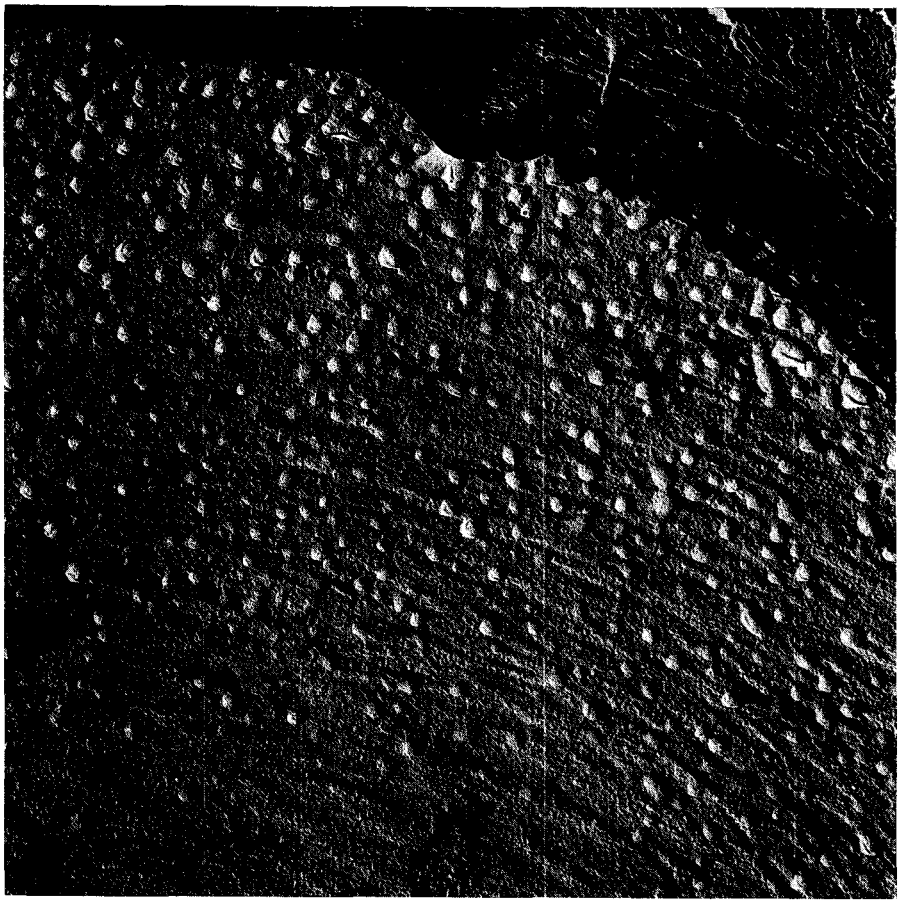

FIG. 11. Incrustation on pit border after ammonia treatment. 18900×

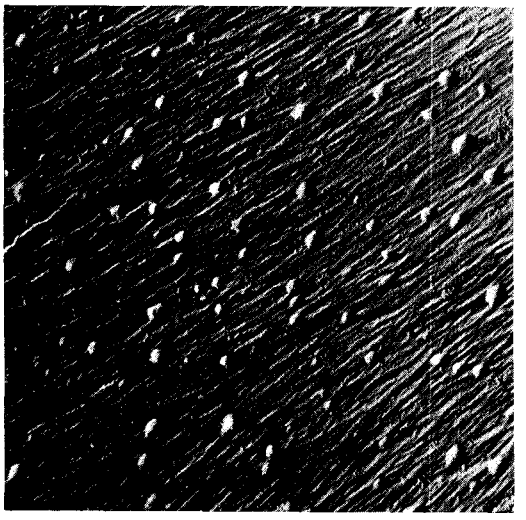

FIG. 12. Lumen surface of untreated normal wood showing warty layer and S3. 6050×

Pollisco (1969) proposed a series of cell-wall changes that probably describe the way in which a wood cell responds to swelling with ammonia and then drying. During the initial stage of treatment, the S3 layer is the first region of the cell wall to swell and be plasticized. Since the outer cell-wall layers are still dry, this initial swelling is primarily into the lumen area. This action should account for some of the S3 crimpings observed in the present study. In the next stage, the entire wall becomes plasticized and swelling is directed outwardly. During drying subsequent to ammoniation, the S3 is again the first wall region to undergo changes. It begins to dry and shrinks toward the lumen with subsequent set while the outer wall is still plasticized. Further drying causes the outer wall layers to shrink toward the lumen, and retraction of the wall around this drier core proceeds until the overall cell cross section, as well as the lumen, is smaller than it was originally. This latter action probably accounts for the S1 crimping observed in the present study.

In *CW* the S3 layer was lacking; therefore there was even less resistance applied against any action by the S2. Only the S1 could offer any opposition, and it appeared crimped in most cells.

Primary-wall structures

Most ray parenchyma cell walls and all bordered and half-bordered pit membranes appeared to be of primary wall construction. Untreated, these structures remained intact after methacrylate embedment and sectioning. However, after ammoniation and the same embedding procedure, they all exhibited an explosion artifact usually characteristic of very tenuous structures (Pease 1964) (Figs. 6 and 7). The distinct difference in susceptibility of these structures to this phenomenon before and after treatment implies some definite effect of ammonia on the intra cell-wall coherency. The exact nature of this effect was undetermined.

Surface detail

Pitting.—Through solvent-exchange drying most intertracheid and tracheid-ray tracheid pits could be studied in their unaspirated condition (Fig. 8). Such pits revealed minute structural detail, but even in pits that were fully aspirated, most of the pit structure was easily resolvable. When pit membranes were removed during specimen preparation, the warty layer was seen to line the overarching pit borders (Fig. 9). Warts were very distinct here along with circularly-oriented microfibrils composing the interior cell-wall layer.

Figure 10 depicts a typical bordered pit following ammonia treatment and drying. Normal wood that had been solvent-exchange-dried revealed no pit membranes that maintained the untreated, unaspirated position. This would suggest that although gaseous ammonia was used for treatment, there was liquid ammonia present in the cell lumen or pit chamber as specimens were dried. Such condensation was not unexpected but did seem to be confirmed by pit aspiration upon drying.

Closer examination of the aforementioned pits, their adpressed membranes, and the open pit border of Fig. 11 reveals what appears to be some type of incrustant deposition that obscures structural detail. The pits resemble those characteristically incrusted with heartwood extractives, although mate-

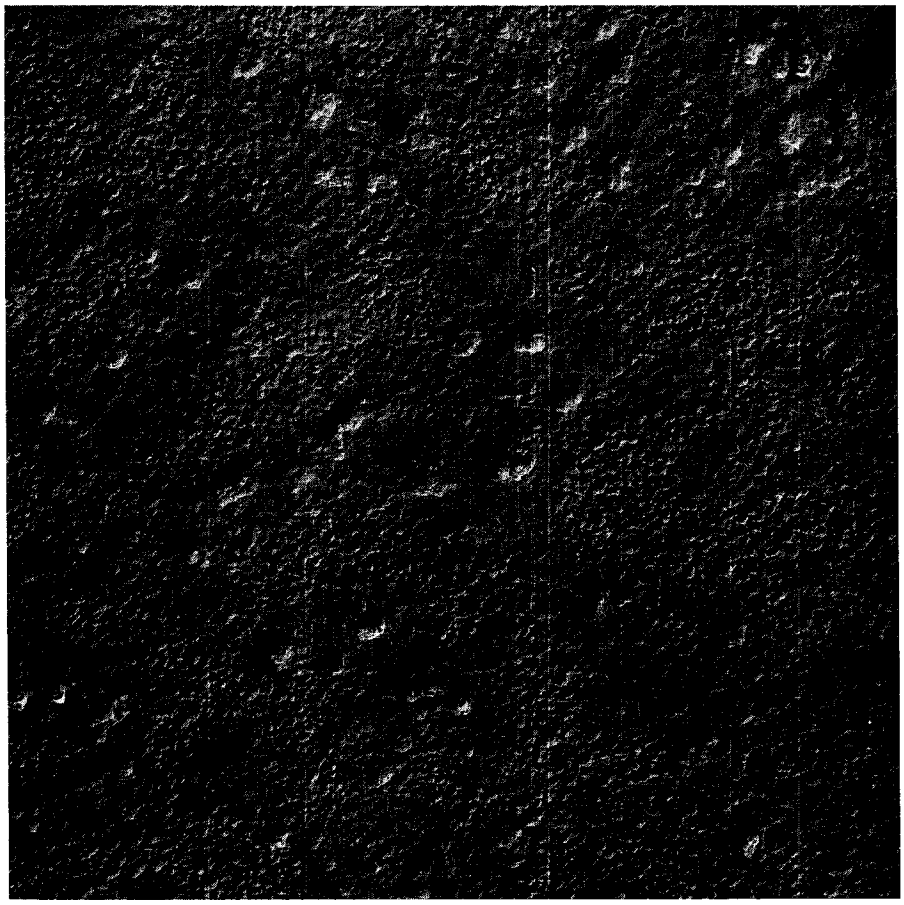

FIG. 13. High magnification of lumen area which was heavily incrusted due to ammonia treatment. 18900×

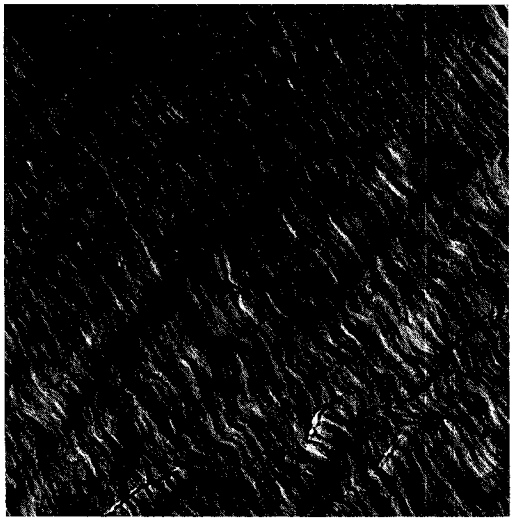

FIG. 14. Crimped lumen surface of ammoniated normal wood. Note that crimp folds are nearly perpendicular to the S3 orientation. 10600×

rial came from exhaustively extracted sapwood. Still, perhaps the ammonia was able to leach out some residual cell-wall extractive or other amorphous substance while it was present in the cell as a condensate.

Cell Lumens.—Untreated lumen surfaces exhibited the characteristic warty layer of southern yellow pine (Côté and Day 1969), although it was sometimes sparse. The S3 microfibrils are plainly visible underneath (Fig. 12). After ammoniation the lumen surfaces, as with the pits, appeared to be coated with a granular, amorphous-like substance (Fig. 13). Perhaps some low molecular weight lignin, hemicellulose, or reaction byproduct of either was solvated during treatment and left to coat the pits and cell lumen upon evaporation of the ammonia.

Crimping of the S3 layer in ammoniated wood is evident from the direct carbon replica seen in Fig. 14. The warty layer is sparse here and the crimp lines are easily seen. They follow a direction that is at a steep inclination to the S3 orientation. This direction is probably that of the S2 layer, the crimping most likely due, at least in part, to shrinkage in the latter.

CONCLUSIONS

Electron microscopic evidence has been presented to show that there is a tendency for the wood cell wall of longitudinal tracheids to compact circumferentially as a result of ammoniation and redrying. If such a compaction does actually occur, it most likely involves the entire cell wall. However, that resulting from shrinkage of the S2 layer is probably the most effective. The increase in crystallinity observed on the same material probably also stems from the cell-wall compaction.

Other investigators (Smith 1969) have suggested that consolidation of the wood cell wall (primarily the S2) is influential in determining mechanical properties of ammoniated wood, gaseous ammonia causing an increase in wood tensile strength, increase in stiffness, and a brash failure in bending tests. X-ray and electron microscopic evidence from the present study also points to a cell-wall consolidation.

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