WOOD CELL-WALL CRYSTALLINITY AND TRITIATED WATER AUTORADIOGRAPHY[1]

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ABSTRACT

Light microscope autoradiography gave further insight into the influence of polar solvents on wood crystal structure. Wood with and without moisture cure polyurethane coating was exposed to tritiated water vapor, and then subjected to various treatments designed to remove tritium from the wood substance. The strong retention of tritium by the wood, and its partial removal only after cyclic drying and wetting, indicated that this tritium was located on hydroxyl groups within cellulose crystalline regions. It also indicated that transformation of paracrystalline regions into crystalline regions and the reverse does occur during wetting and drying cycles.

INTRODUCTION

During the course of an investigation about the interaction of woody cell walls and a moisture-cure urethane resin, information that we felt was worth reporting about the structure of the cell wall came to light.

BACKGROUND

In wood the accessible hydroxyl groups for desorption and adsorption are located on the surface of highly ordered regions of cellulose called crystallites, and inside the lower-ordered cellulosic structure, amorphous matrix, and encrusting substances. The number of hydroxyl groups available for sorption changes with wetting and drying processes and causes the phenomenon known as sorption hysteresis (Stamm 1964). Physical properties are affected by hysteresis. This indicates that the degree of order of cellulose changes during alternate cycles of wetting and drying with a swelling solvent.

Iso-ionic exchange reactions are defined as an isotopic label being transferred from one substance to another, the process involving no chemical reactions. The occurrence of this exchange or a lack of it has been used in the study of bond character (Friedlander 1955). Exchange behavior among hydrogen and its isotopes has been reported by many early workers. King (1936) studied the sorption of deuterium oxide by cellulose and found a permanent increase in the weight of the cellulose after desorption. He concluded that two mechanisms are possible explanations, but he favors the idea that substitution by deuterium takes place. Hydrogen on highly electronegative radicals such as O-H, N-H, S-H, and halogen-H exchanges instantaneously with heavy water, but the exchange of hydrogen in C-H bonds of organic compounds is much more difficult, and occurs with water in only a few substances (Shatenshtein 1962). The possibility of carbon-linked hydrogen exchange in cellulose has been ruled out by the infrared and accessibility measurements of Lang and Mason (1960). The presence of polar groups in the molecule of an organic substance facilitates exchange with water. Exchange catalyzed by alkali is promoted by the presence of electronegative substituents in the mole-

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cule of the substance (NO_2, SO_3H, COOH, CHO, and CN) while electropositive substituents (OH, OCH_3, and NH_2) promote exchange catalyzed by acid.

Tritium in cell walls of wood that has been exposed to tritiated water could be in two forms—in bound water molecules in the cell walls or as tritium exchanged with the hydroxyl hydrogen in the cell-wall substance. Many researchers (including Lang and Mason 1960, and Sepal and Mason 1961) have indicated that all tritium remaining in purified cellulose structure, except in some hydroxyl groups, is accessible to water molecules and can be removed completely by soaking in a large quantity of normal water. Cellulose, lignin, and hemicellulose hydroxyl tritium accessible to the water would exchange with the hydrogen in the water thus removing the tritium. Bound tritiated water would be replaced by normal water, or would exchange its tritium with the normal water hydrogen. Considering that cellulose has tighter packing than any other wood substance, tritium should not remain in wood after soaking in an excess of this polar solvent except in inaccessible hydroxyl groups within cellulose structure.

The moisture-curing polyurethane coating used in this study, because of its resistance to attack by solvents and the way in which the water is bound in it, offers further insight into the type of reaction expected to take place between tritiated water and wood. During the manufacture of these resins, hydroxyl hydrogen from alcohol (usually in the form of a polyol) reacts with an isocyanate group to form a urethane linkage (only those products derived from hydroxyl groups are called urethanes). The alkalinity of nitrogen atoms in urethane linkages leads the urethane to attack another isocyanate group in the resin forming substituted allophanic acid esters. During coatings manufacture, the degree of reaction and the characteristics of the radicals on the polyol and the isocyanate determine the properties of the resin.

During cure of a water-curing polyurethane, the electron-abundant oxygen in the water molecules tends to attack isocyanate groups giving off an intermediate product, carbonic acid, which is unstable and breaks down into a primary amine and carbon dioxide. The carbon dioxide leaves the coating with the solvent, or in the case of a foam forms the bubbles. The amine formed after attack by water adds to another isocyanate group to give substituted urea, which tends to attack further an isocyanate group to yield a biuret. Isocyanates trimerize and form isocyanate urates (Lowe 1963). This reaction is simple in terms of monoisocyanate and when the radical group is small. In coatings where di-isocyanate in large molecules of polyol is involved, the self-polymerization becomes complicated and the capacity is much lower. At normal room temperature, the reaction is insignificant. Allophanate formation is improbable when the NCO-OH ratio is small (Lowe 1963). The formation of biuret generally occurs at elevated temperatures or in the presence of certain catalysts (Kaplan and Wooster 1964). The reaction of isocyanate with an amine group is faster than with water or alcohol. This is due to the high nucleophilic potential of the amine group (Bailey 1960). All six reactions may well be involved in film formation and the chemical composition of the isocyanate and ancillary materials naturally plays a vital part in determining the properties of film obtained. When tritiated water is used, the curing reaction leaves the tritium from water linked to nitrogen in the urethane polymer.

The high solvent resistance of the cured polyurethane film makes swelling of the film and thus intimate contact between a solvent and the molecules of the polymer unlikely. Thus it should be possible to use a polar solvent to swell the wood and remove the tritium therefrom, but at the same time not swell the polyurethane or remove tritium from it. This should lead to a labelled polymer both in bulk and within the wood structure, but little, if any, labelling in the wood not in contact with resin.

EXPERIMENTAL

Wood samples were taken from the sapwood of a live black spruce (*Picea mariana* (mill.) B.S.P.) for this study. This species was chosen because of its commercial importance in Eastern Canada and the comparative ease with which it can be worked in microtechniques. The samples were subdivided into small pieces, and each surface was microtomed in order to present a smooth surface for study. The final trim size of each specimen was $6 \times 6 \times 18$ mm. These specimens were dried under atmospheric pressure in an oven maintained between 105–110 C for a 4-day period. They were then transferred to a flask and heated to between 105–110 C under a vacuum of 10^{-4} torr.

Some of the samples thus dried were immersed in the polyurethane resin and placed in a vacuum system connected to a mechanical pump and evacuated until the samples were infiltrated with the resin. Others of these samples were coated on one side with a resin, and still others were untreated. All of these samples were placed in a flask coupled to a smaller diameter tube and connected to a high vacuum system. A vacuum of 10^{-4} torr was again drawn on the specimens until solvent and air entrapped in the wood was removed. Tritiated water in the tube was frozen with liquid nitrogen and a vacuum of 10^{-4} torr was drawn on this as well. The liquid nitrogen was then removed and the trapped air in the tritiated water left during the time that the water reverted to room temperature. This procedure was repeated twice, removing all trapped air in the tritiated water.

The valve connecting the glass tube with the flask containing the samples was opened and the valve connecting the high vacuum system to the reaction vessels was closed, thus isolating the reaction system and maintaining the high vacuum. The liquid nitrogen was removed from the tube containing the water, and this tube was heated to 30–40 C. The high vacuum allowed unimpeded transfer of water vapor (Yarwood 1948), and in the closed system with excess water present the vapor phase should have reached an equilibrium of 100% relative humidity. The vapor was allowed to surround the samples and react with the resin until curing was complete.

Desorption was accomplished similarly to the high vacuum drying process. During desorption, the flask containing the wood samples was maintained at 105 C by a heating element. The glass tube receiving the water was immersed in liquid nitrogen. This process was continued until all the unreacted tritiated water was recollected. The tritiated water trapped in the glass tube was saved for future use.

Control samples were prepared the same way as the others but were exposed to the atmosphere for cure rather than to tritiated water.

A further set of samples was desorbed in the same way, but was fixed in a wire frame and inserted into a sealed bottle containing tritiated water at room temperature and atmospheric pressure and allowed to cure for a short (2-hr) period. These specimens were transferred from the bottle to an oven maintained at 105–110 C immediately after the exposure period in order to drive off unreacted tritiated water vapor and complete the resin cure.

All of the samples obtained except those cured a short time were sectioned 5 μm thick across the grain using a sliding microtome. Those obtained from the reaction vessel under high vacuum were softened in distilled water before sectioning. Sections were transferred from the microtome knife to watch glasses containing distilled water.

Sections 10 μm thick were cut from the ends of the samples cured for the short period at room temperature and atmospheric pressure. Dry conditions were maintained throughout the process in order to minimize any chances of contact with traces of moisture. The sections thus obtained were directly mounted on a gelatin-coated dry slide.

The controls were sectioned by the same technique as the samples from the high vacuum reaction. Care was taken to avoid radioactive contamination during all phases of handling.

Five treatments with solvent were given to sections obtained from the wet-cut labelled samples. Solvents used were: 1. Distilled water, 2. 100% ethanol, 3. 1:1 phenol-methanol, 4. 0.1 M acetic acid, 5. 1 M acetic acid. Each treatment was continued for 48 hr. Every 12 hr, sections were removed from the solvent, air-dried, then resoaked in fresh solution. Except for the samples treated with distilled water, all samples were washed in a large quantity of distilled water before the autoradiographic study.

In order to detect the locations of the tritium in the samples, a stripping film technique, a liquid emulsion technique, and a modified liquid emulsion technique were used. Sections of all the samples were mounted on glass slides that had been cleaned by soaking in potassium dichromate. The slides were dipped in 2.5% gelatin solution containing 0.05% chrome alum to ensure adhesion. Sections from unlabelled samples were mounted on each slide as controls in detecting possible chemography. As an additional control, pieces of pre-exposed film were mounted on the sections as a standard to detect fading of the latent image. In the stripping film technique, pieces of photographic emulsion were cut and stripped from Kodak AR-10 fine grain autoradiographic stripping plates and placed on the surface of a transfer solution of controlled bromide concentration. After the emulsion became swollen, the slides bearing the sections were dipped under the film and lifted from the solution at an angle of 30 degrees. The slides were then dried in a stream of cold air and placed in a light-proof box sealed with black tape. During the exposure period the samples were maintained at 40 F.

The liquid emulsion technique involved dipping slides prepared in the same manner as for the stripping film technique into Kodak NTB-2 liquid emulsion and withdrawing from the solution. The slides were held in a vertical position for several seconds allowing the excess emulsion to drain onto a paper tissue. The back of the slide was wiped and placed face-up on a cool glass plate for drying. During exposure the same technique as for the stripping film was used.

In the modified liquid emulsion technique (originally developed by Miller, Stone and Prescott 1964), a loop of stainless steel was dipped into molten Kodak NTB-2 emulsion and withdrawn with a film of emulsion across it. This was carefully dried in a mild flow of air and placed over a dry-cut section (obtained from the specimens cured at atmospheric conditions for the short period) on its slide. The trace of moisture adsorbed on the glass surface from the atmosphere induced the thin film to adhere to the glass. A longer exposure time than in the other techniques was needed because of the lower degree of labelling in the specimens.

The slides were developed, and then viewed and photographed in a light microscope using bright field, phase contrast, and dark field illumination.

RESULTS AND DISCUSSION

In the stripping film technique, spreading of exchangeable tritium over the entire section area took place to a great extent. The results were useful but were considerably less informative than those obtained from the liquid emulsion technique, which showed a vast improvement in the spreading problem, even though it was still noticeable. The modified liquid emulsion technique eliminated the spreading effect, but increased the background because of the way the emulsion must be manipulated. The requirement of dry-cut sections for the modified liquid emulsion technique limited the thinness of the sections and thus the microscopic resolution obtainable. For the purposes of this discussion, the liquid emulsion technique yielded the most information and we will mainly confine our comments to it.

Bright field photomicrography gave good resolution of the wood section and also showed quite well where the silver grains were located in relation to the section. The dark field detected larger numbers of silver

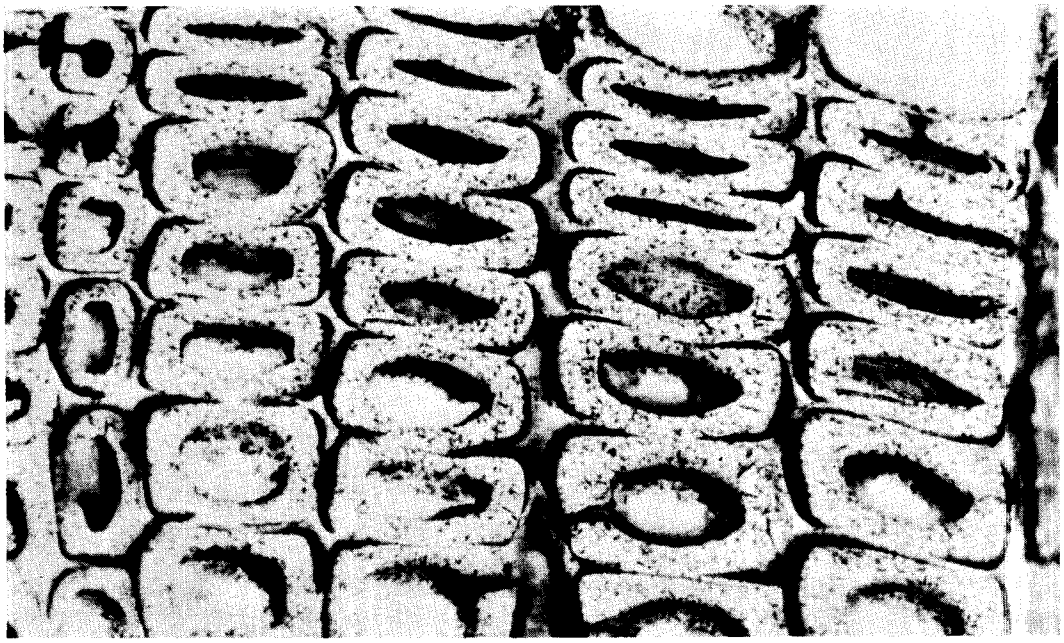

FIG. 1. Bright field autoradiograph of uncoated spruce exposed to tritiated water vapor. The middle lamella regions contain less tritium than the cell walls. 600 ×.

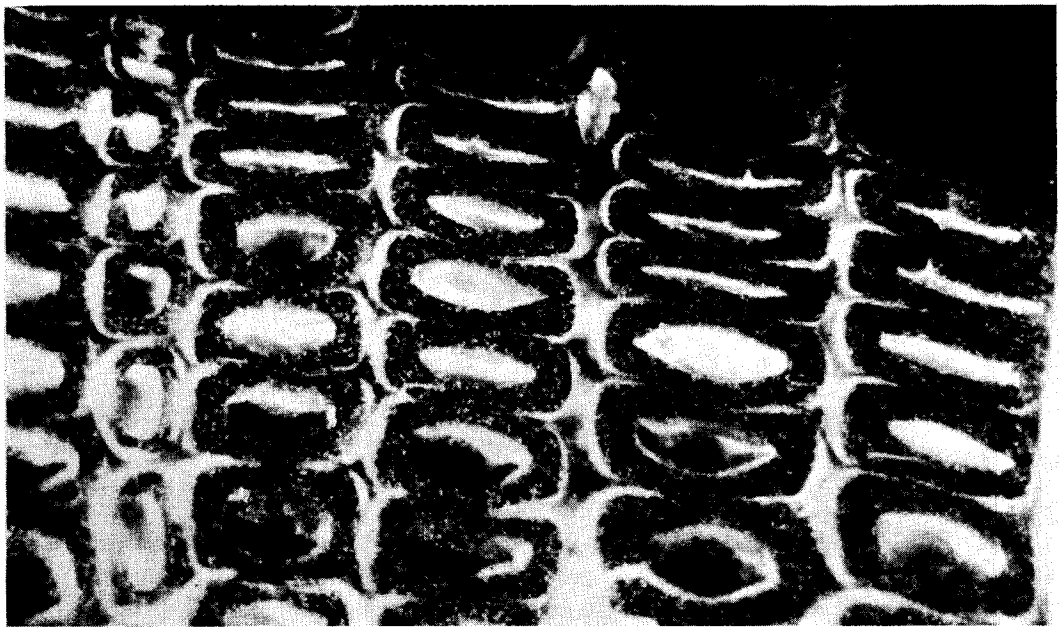

FIG. 2. Dark field autoradiograph of same area as Figure 1 showing more clearly the silver grain distribution. 600 ×.

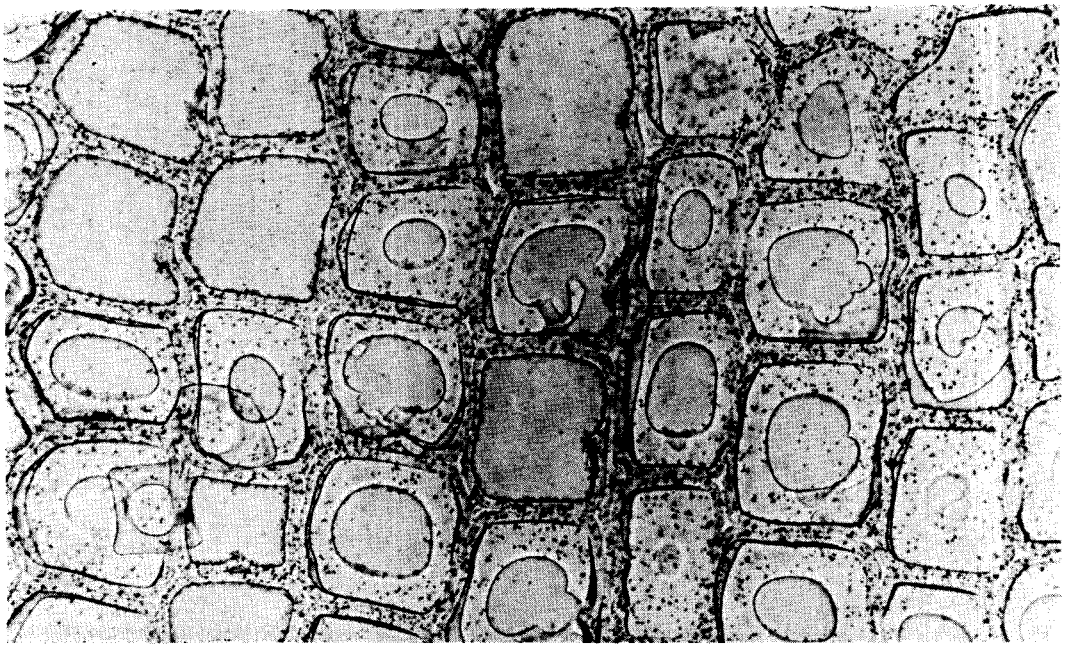

FIG. 3. Ethanol-soaked section with coating resin in some lumens. Tritium is distributed throughout resin and the wood—even wood not in contact with labelled resin. 475 ×.

grains, but gave somewhat reduced resolution in the wood. In order to obtain more information, photomicrographs of exactly the same areas were photographed using each of these systems.

Figures 1 and 2 are autoradiographs of wood that had not been in contact with polyurethane but that was exposed to tritiated water vapor and soaked in an excess of normal water. In these autoradiographs the middle lamella regions contain considerably less tritium labelling than do the secondary walls of the cells. This was noted consistently throughout the labelled wood specimens and indicated that the retention of tritium is stronger in the secondary wall than in the middle lamella region.

Figures 3, 4, 5, 6 and 7 are micrographs depicting various solvent treatments. All processing was done under the same conditions using NTB-2 liquid emulsion. Figure 3 shows that in a 48-hr, 100%-ethanol-soaked section, tritium is distributed in the cell wall and in the coating located in the cell lumens. Tritium is also present in cell walls not in contact with labelled coating. This reveals that a significant amount of tritiated water molecules did diffuse into the wood cell wall. Polarity of alcohol is less than that of water; therefore alcohol will not normally penetrate into wood structure as deeply as water. When water is present in the wood structure (causing the structure to open), alcohol should reach all the areas where the water is. All the bound water remaining in the wood structure would be removed by treatment with the pure alcohol. The tritium remaining in the cell wall as shown in Fig. 3 was apparently other than in bound water.

Figures 4 and 5 were obtained from a 48-hr, phenol-methanol (1:1) soaked section. The coating manufacturer reported that phenol-methanol is one of the most destructive solutions for the cured polyurethane film. This solvent caused swelling and intimate contact within the coating. The autoradiographs show a much higher concentration of tritium in the cell walls than in the coating indicating that the tritium in the cell wall was held more strongly than that in the coating.

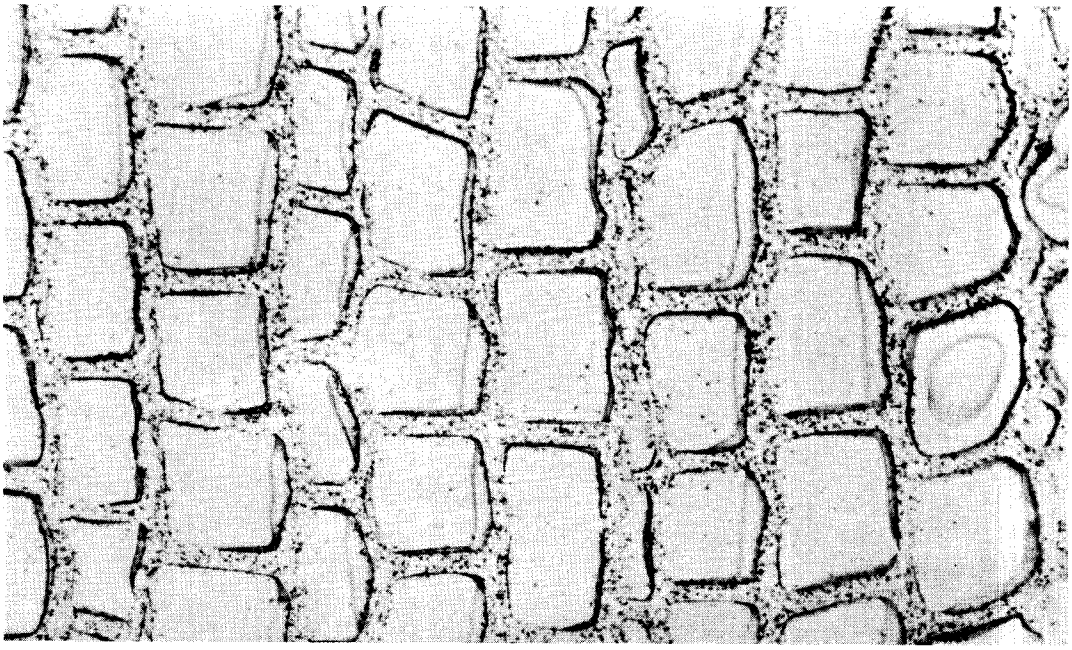

FIG. 4. Methanol-phenol soaked section showing tritium removed from resin (which fills most lumens) but remaining in cell walls. 475 ×.

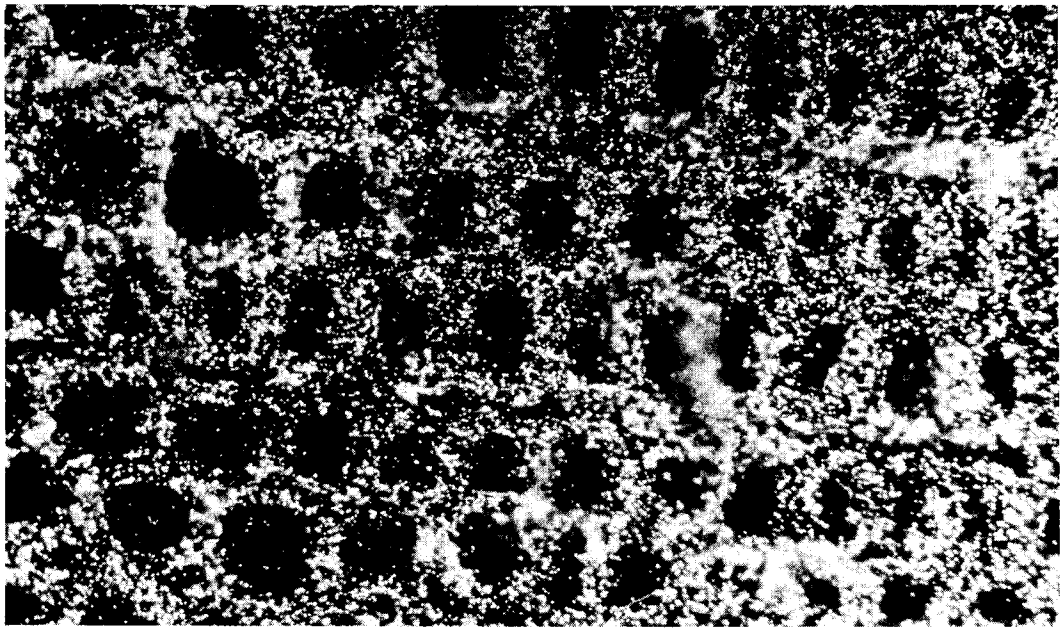

FIG. 5. Dark field autoradiograph of latewood of same section as Fig. 4. 475 ×.

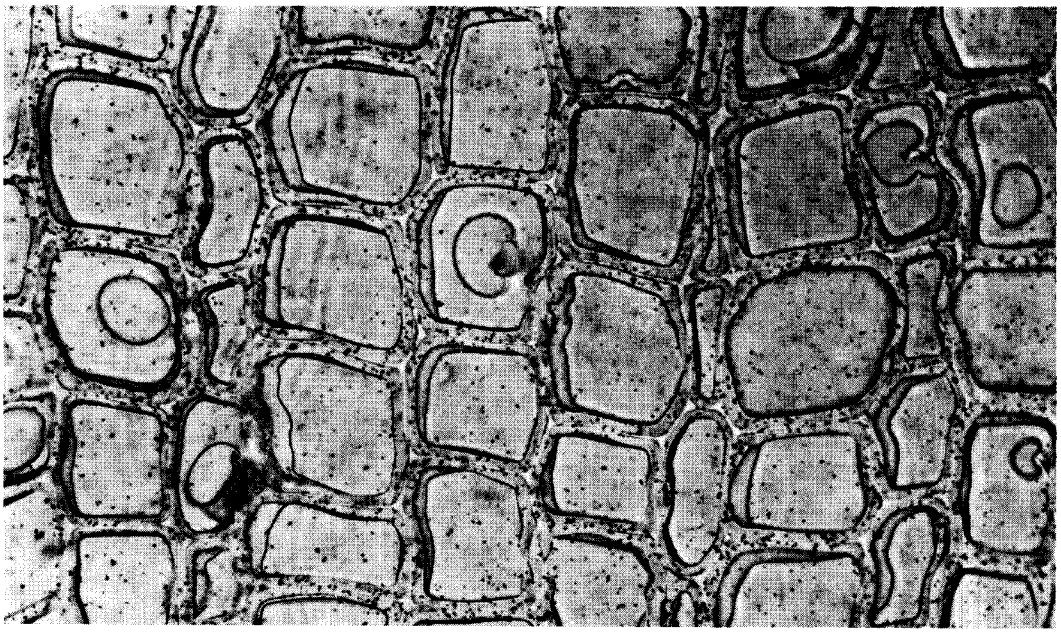

FIG. 6. Section soaked in 0.1 N acetic acid with less tritium in resin and wood than in any previous treatment. 475 ×.

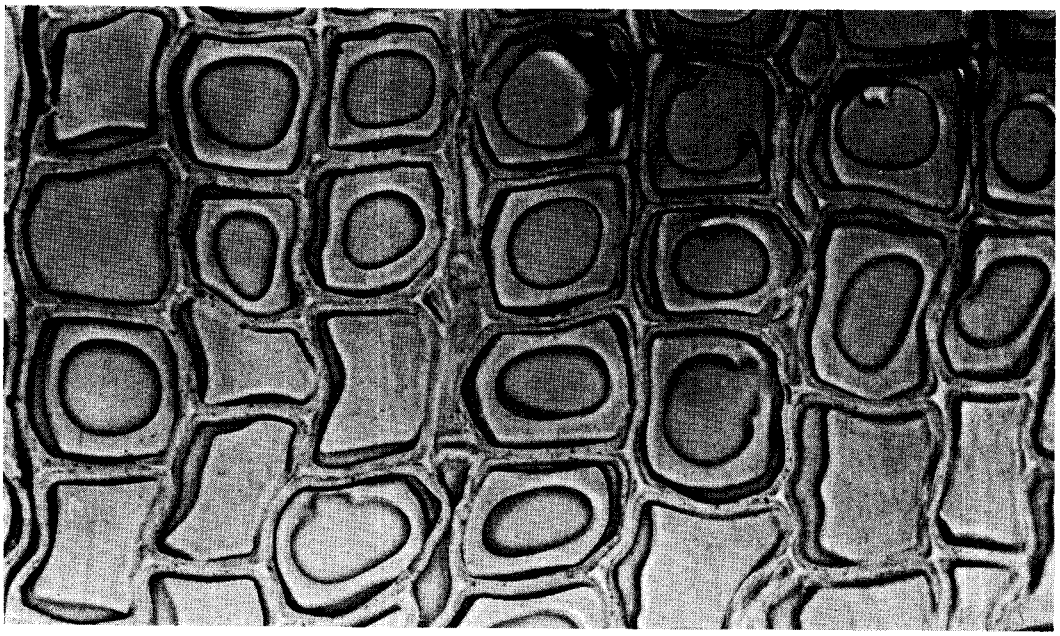

FIG. 7. Section soaked in acetic acid, 1 N, depicting nearly complete removal of tritium from wood and coating. 475 ×.

Figure 6 is an autoradiograph of a 48-hr treatment with 0.1 N acetic acid. The considerable reduction in tritium density in both the cell wall and the coating film as compared to the alcohol-treated samples is apparently caused by the catalysis of the acetic acid, which still cannot replace all the tritium in the cell wall.

Figure 7 shows the results of a higher concentration acetic acid (1 N). A very small amount of tritium can be detected in the cell walls.

Samples that were surrounded by coating and exposed to tritiated water vapor for a limited (2-hr) period showed labelling in the coating but not in the wood substance. This indicates that during this time period all of the water was taken up by the highly reactive isocyanate groups, and was not allowed to diffuse into the wood substance.

CONCLUSIONS

Tritium exchanged with the hydroxyl hydrogen in the cell-wall cellulose inaccessible regions is the probable explanation for the strong retention of the tritium in the cell walls. The probable mechanism for this is that microfibrillar crystalline regions change to paracrystalline regions and the reverse during cycles of drying and wetting. The high retention of tritium in the cell walls, even under conditions designed to exchange and remove it, can be explained on the basis that the paracrystalline regions accessible to tritiated water shift to a highly ordered state after the tritium exchange takes place. This rearrangement causes the exchanged tritium to become trapped in inaccessible crystalline regions making it difficult to displace except, perhaps, during the repeated drying and wetting cycles that again cause rearrangement, or through the use of solvents that swell and promote exchange more strongly than water.

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