Thin Sections. II. A Simple Method for Reducing Compression Artifacts. By Peter G. Satir and Lee D. Peachey. (*From The Rockefeller Institute for Medical Research.*)*

One of the artifacts produced during the cutting of thin sections for electron microscopy is a shortening of the section in the direction of knife travel. Normally, a thin section cut with a glass knife is shortened 30 to 50 per cent of its expected length[1] (1). Floating the sections on a dilute solvent (10 per cent acetone is commonly used) at room temperature does very little to expand the compressed section. Higher concentrations of solvent in the fluid filling the collecting trough are more effective, but unfortunately, the concentrations necessary for removing the shortening artifact can also remove a large enough portion of the embedding material to affect seriously the electron microscopic appearance of the sections. One of us (1) has previously discussed the problem of compression of thin sections and has described a method of considerably reducing the artifact by softening the floating sections with heat. A serious and admitted disadvantage of this method is the production of thermal changes in the microtome which interfere with further sectioning. The present paper describes a simple method for spreading sections which does not have this disadvantage.

We have found that treatment of the floating sections with solvent *vapor* is very effective in spreading the sections while producing no noticeable removal of embedding material. The mechanism of the action is apparently a softening of the sections by the solvent, followed by a spreading under the influence of surface tension forces. Since the sections are not in direct contact with solvent in a liquid phase, there is no solvent extraction of the section.

A convenient method consists of dipping an instrument, such as a needle, a glass rod, or a fine camel's hair brush into xylene and slowly bringing the instrument *close to*, but *not in contact with*, the sections. The effect is very rapid and dramatic; it consists of an expansion of the ribbon parallel to the direction of cutting. Most of the compression artifact is eliminated, the section length becoming 85 to 95 per cent of the expected length. If the solvent is brought too close, the sections expand in both length and width to dimensions that are larger than those of the face of the block. The effect is probably due to excessive concentration of solvent vapor near the section. The sections shrink back when the solvent is withdrawn, but there is no advantage to overexpansion, and it is possible that permanent damage may be done to the fine structure of the material in the sections. Therefore, care should be taken not to go beyond the point necessary to produce the initial and rapid expansion of the sections.

Some measurements of changes in section length are recorded in Text-fig. 1. All curves in Text-fig. 1, except curve *D*, represent experiments in which the collecting trough was filled with 10 per cent acetone in water. In the experiment represented by curve *D*, the collecting trough was filled with xylene-saturated water. The curves show that these concentrations of solvent in the trough are relatively ineffective in removing compression. In each case, an equilibrium length is reached in a few minutes which represents a removal of only about one-fourth of the compression artifact. Treatment with solvent vapor, however, quickly removes most of the remaining artifact, as shown by the immediate increase in section length seen in Text-fig. 1 at points where vapor treatment is indicated.

Curves *A* and *B* in Text-fig. 1 are representative of the variation in the amount of compression that can be removed by vapor treatment. This is presumably due to variation in the amount and form of wrinkling of the section which, in turn, depends on such factors as the thickness of the section and the quality of the knife. A comparison of these curves with curve *C* illustrates that there is no advantage in waiting before treating the sections. Treatment immediately after cutting is as effective as treatment after waiting for several minutes. It can also be seen from the curves that the combined action of the solvent in solution and vapor treatment is not better than that shown by vapor treatment alone. Therefore, solvent vapor treatment can be used alone to spread sections, and the

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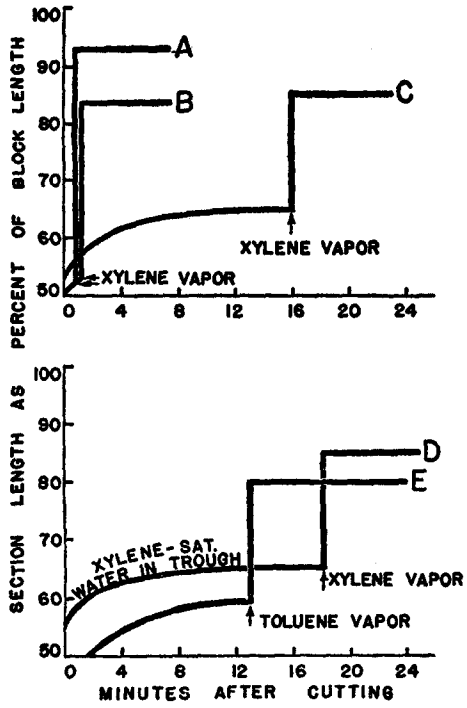
[1] This length refers to the length the section would have if there were no shortening, and equals the vertical dimension of the block face.



TEXT-FIG. 1. Plot of section length as a function of time for solvent treatment in various forms. 10 per cent acetone in water was used in the collecting trough for all curves except curve *D*, for which the trough was filled with xylene-saturated water. The arrows indicate points at which treatment with solvent vapor was applied. Measurements of section lengths were made with a reticule in the eyepiece of the viewing microscope, as described by one of us (1).

treatment can be applied as soon after cutting the sections as is convenient.[2]

We considered the possibility that the superiority of this method was not due to the use of vapor, as opposed to liquid solvent in the trough, but perhaps just to the choice of xylene over other solvents. Curve *D* in Text-fig. 1 shows, however, that the saturation of the water in the trough with xylene is no more effective in removing compression than 10 per cent acetone, and curve *E* shows that toluene vapor is as effective as xylene vapor. Thus we can conclude that the important factor is the use of solvent *vapor*, the choice of solvent being less important. It does seem desirable, however, from a practical standpoint, to use a solvent that has a relatively high vapor density and a relatively low vapor pressure. A vapor of low density will not descend upon the sections, and a solvent with high vapor pressure may evaporate before the instrument can be brought near the sections.[3]

The effect of solvent-vapor spreading on the electron microscopic appearance of tissue sections is demonstrated in Figs. 1 and 2. Fig. 1 shows one of a pair of adjacent serial sections which was picked up before solvent-vapor spreading. Fig. 2 is the other member of the pair which was treated with xylene vapor, as described above. The direction of cutting is vertical, as indicated by a knife mark at the left margin of each figure. The nuclei in the untreated section appear oval with their major axes not quite perpendicular to the direction of compression, which is parallel to the knife mark. This indicates that these nuclei were originally somewhat oval, since a compressed circular profile would have its major axis perpendicular to the compression direction. The section shown in Fig. 2, expanded to about 90 per cent of the length of the block face when treated with xylene vapor. The nuclei appear more rounded, and their major axes have rotated farther from the line perpendicular to the compression direction. Their shape in this section more nearly represents their original shape in the block, the remaining ellipticity being due to a combination of residual compression and the original shape of the nuclei. Except for a general change in shape, there is no difference in morphology of the cellular components between the treated and untreated sections. The solvent vapor appears not to contribute any damage to the tissue section.

The advantage of this method of spreading sections lies in the fact that the section is not brought into contact with solvent in a liquid phase, and thus solvent extraction of the embedding material

[2] It does seem valuable, however, to include a small amount of solvent in the trough as a bacteriocide, since bacterial contamination of the electron microscope grids was found when distilled water which had been stored in a small bottle for some time was used to float sections.

[3] Since the completion of this work, Sotelo (2) has described a flattening method using chloroform vapor. No quantitative data or micrographs are given. The difficulty due to the high vapor pressure of chloroform is overcome by using a relatively large quantity of the solvent.

is avoided. The method is simple, rapid, and in no way damages the section. It restores the shape and spatial relationships of tissue components to more nearly what they were in the block, producing a thinner and more uniform section, and allowing a truer and more accurate interpretation of the micrographs obtained.

Bibliography

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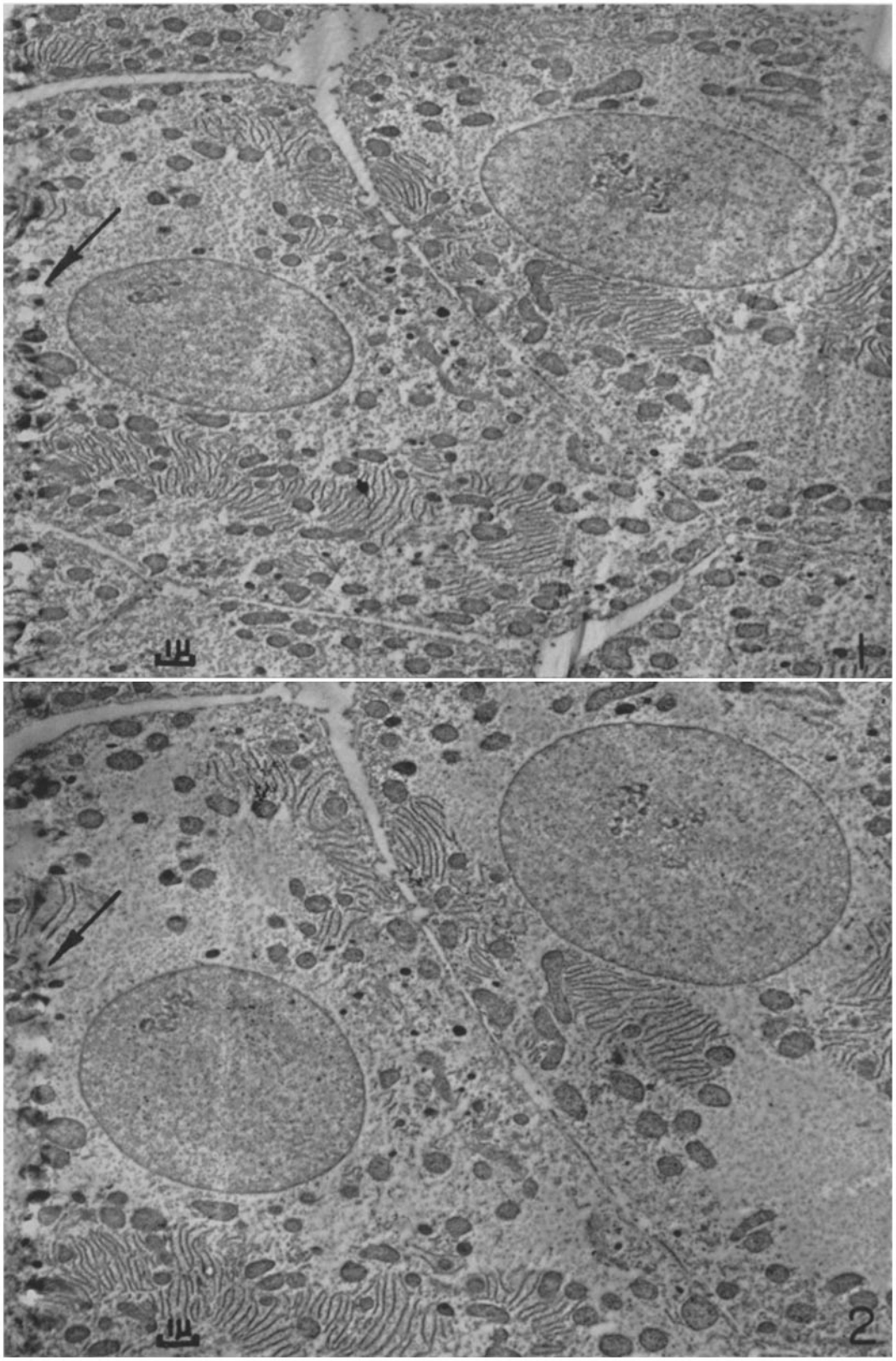
2. Sotelo, J. R., Technical improvements in specimen preparation for electron microscopy, *Exp. Cell Research*, 1957, **13**, 599.

EXPLANATION OF PLATE 193

FIGS. 1 and 2. Electron micrographs of adjacent serial sections of parenchymal cells of mouse liver. A knife mark at the extreme left of each figure (arrows), indicates the direction of cutting. The diagonal light lines are thin areas in the carbon support film. Tissue was fixed in 1 per cent osmium tetroxide in acetate veronal buffer at pH 7.5 plus 4.8 gm. sucrose per 100 ml. final volume. After fixation for 90 minutes, the tissue was embedded in *n*-butyl methacrylate plus 10 per cent methyl methacrylate. Sections were cut on a Sorvall model of the Porter-Blum microtome. Section thickness was 70 mμ, as judged by interference color (1). RCA EMU-2 electron microscope. × 5200.

FIG. 1. Section picked up after a few minutes on 10 per cent acetone in water. The two nuclear profiles shown indicate clearly the magnitude of the compression, although some of the ellipticity is due to the original ovalness of the nuclei, as shown by the fact that the major axes of the ellipses are not perpendicular to the cutting direction.

FIG. 2. Same field from next serial section, treated with xylene vapor. A general expansion in a vertical direction is obvious. Subcellular components, such as nuclei, mitochondria, endoplasmic reticulum, etc., are correspondingly expanded, and show no detectable damage due to the treatment.



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