A METHOD FOR COMPLETE EXTRACTION OF EMBEDDING MATERIAL FROM TISSUE SECTIONS FOR THE ELECTRON MICROSCOPE*, **

by

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INTRODUCTION

FULLAM AND GESSLER[1] reported on a study of embedding materials which might be removed from tissue sections by sublimation, and in this work recognized the necessity for and difficulty of removal of embedding material without distortion.

An obvious method for removal of embedding material consists in extraction with a suitable solvent. Surface tension effects during drying and possibly during the dissolving introduced serious distortion as pointed out by HILLIER[2].

Partial sublimation in the microscope of paraffin-celloidon and of butyl methacrylate has been noted[3]. Under ordinary conditions, however, clearing is incomplete. In this laboratory near dirt particles on butyl methacrylate embedded sections, it has been observed that there is often complete clearing on exposure to the electron beam with little or no distortion. It was thought that heat developed by the particle due to electron absorption was responsible for the extraction. Exposure of sections to an incandescent tungsten filament in vacuo removed the embedding material adequately[4]. However, this often introduced distortion presumably due to the boiling of the plastic. The presence of distortion in many sections cleared by simple heating and its absence in sections cleared by the electron beam near dirt particles suggested a difference in the mechanism of clearing.

Since incomplete extraction occurred on exposure to operating beam intensities in the microscope, it appeared that gradual changes took place in the plastic during exposure to the beam which prevented all of it from being extracted. Accordingly, much higher beam intensities were tried. Removal of the condenser aperture from the microscope (RCA EMU-2A) increased the available beam intensity by about 100 times. Exposure of sections to the highly intense beam resulted in almost instantaneous removal of embedding material. Little or no distortion could be detected on comparison with unextracted sections (Figs. 1 and 2).

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** The material in this paper appears in University of Rochester Atomic Energy Project Report, UR-205.

References p. 354.

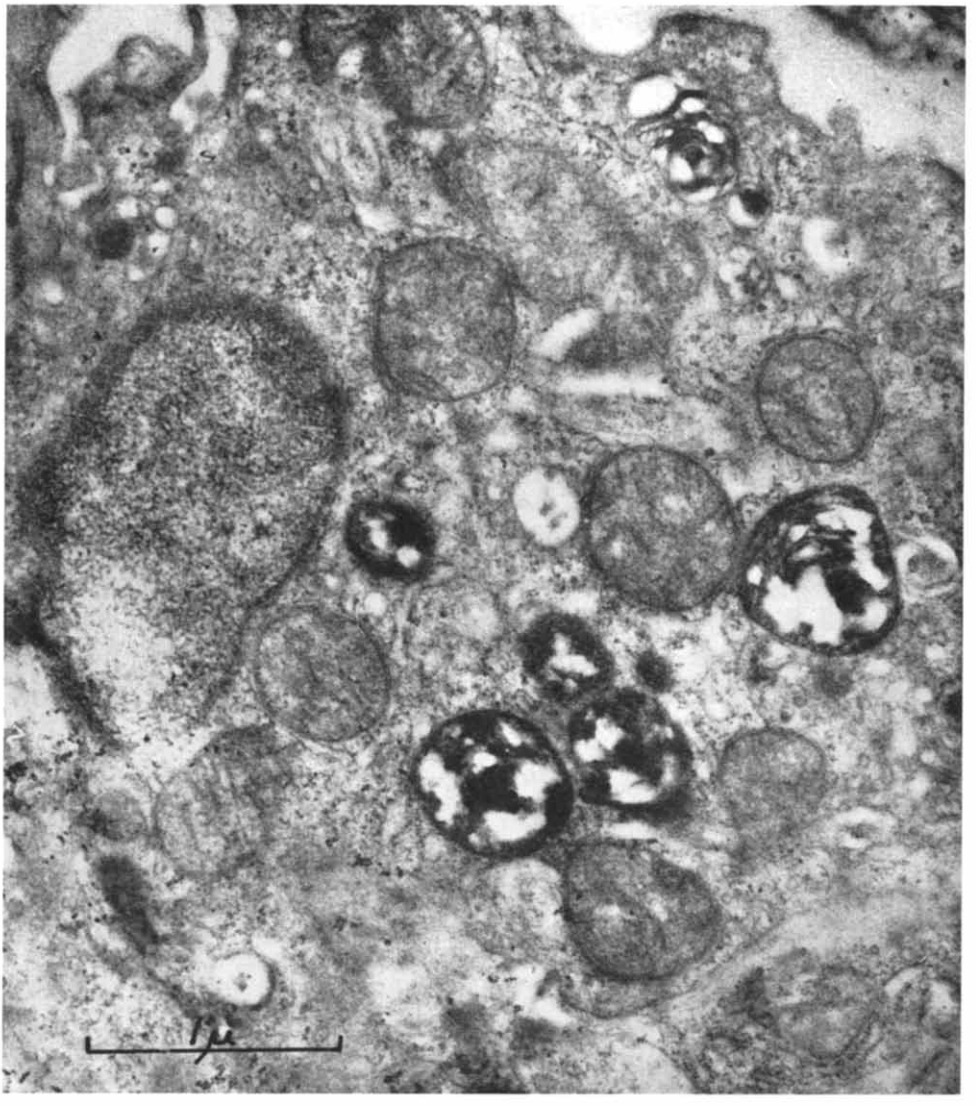

Fig. 1. Rat lung. This is a fairly thin section. The embedding material has been partially removed by electron bombardment at normal operating intensities.

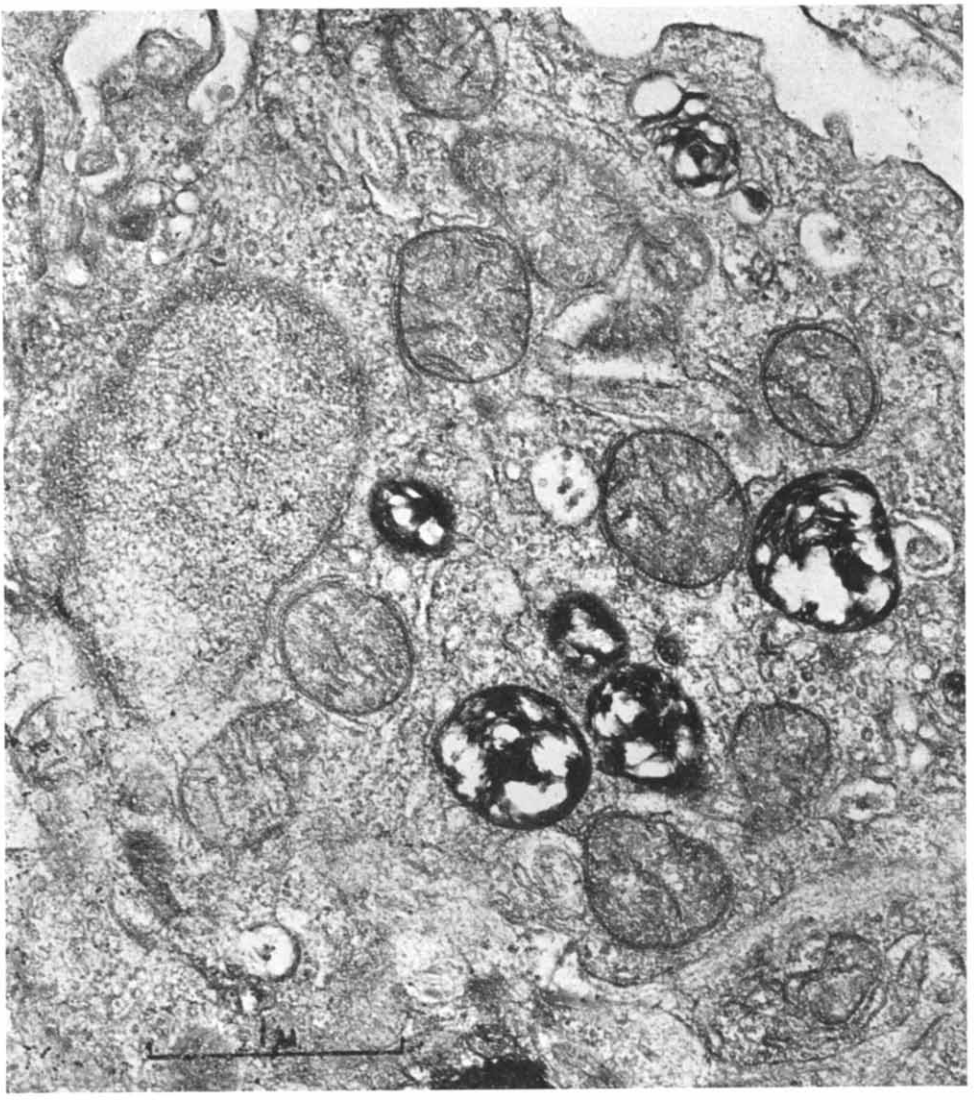

Fig. 2. Same field as shown in Fig. 1 after exposure to high-intensity electron bombardment. The embedding material has been completely removed. There is much greater contrast between small details and the substrate than in Fig. 1 although the photographic treatment was the same in both cases. Some details may be seen in this view which are very difficult to detect in Fig. 1.

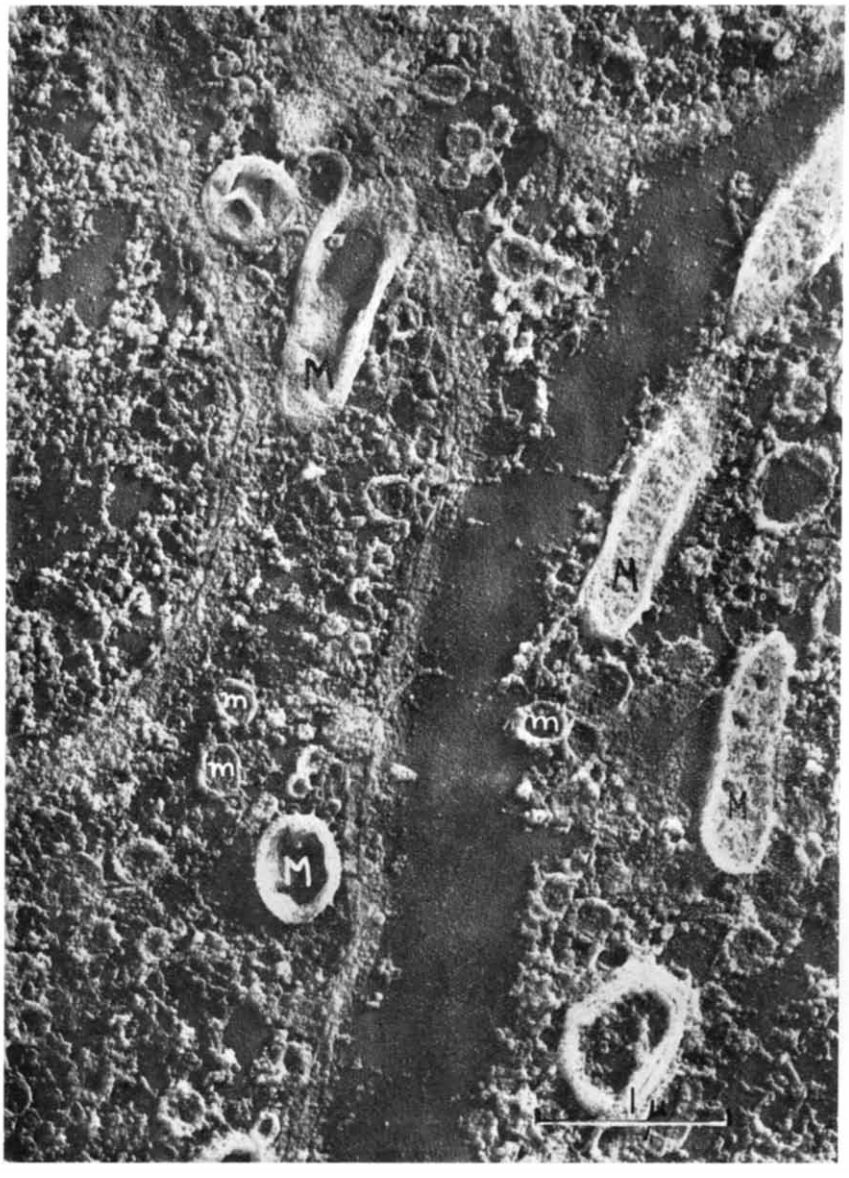

Fig. 3. Male rat germinal epithelium. Uranium shadow-cast at an angle of 5:1 after removal of embedding material by electron bombardment. A portion of a young resting spermatid lies on the left and on the right is a portion of the Sertoli cytoplasm. Details of the spermatid cell wall and the nuclear wall are clearly visible. Relatively greater shrinkage of microsomes (m) over that of the mitochondria (M) in a direction normal to the section is apparent.

METHODS

To carry out this type of extraction the condenser aperture is removed*. The specimen is placed in the usual position. The condenser current is set at maximum before turning on the beam. This is necessary because reflections of the beam apparently from the objective pole-piece surfaces make it difficult to control the illumination on the specimen if cross-over is below the specimen. With the microscope in operation an area is selected for clearing. The condenser current is reduced at such a rate that the image of the filament is brought into focus in about one-half of a second. Before this focus is reached the section should clear. The condenser current is immediately returned to maximum.

Clearing occurs at lower beam intensity in the middle of a square and is most satisfactory with 100 mesh rather than 200 mesh specimen grids. Silica-coated colloidon substrates are used to provide adequate strength for the extraction process. A certain amount of trial is necessary to find the minimum amount of silica required. Experience has shown that thick sections are much more apt to tear than thin ones.

RESULTS

Figs 1 and 2 show views of a section before and after electron extraction. It is apparent that minute details barely visible in the unextracted section are easily seen in the same section after extraction.

An important advantage of complete electron extraction over partial electron extraction lies in the fact that the section may be effectively shadow-cast. Fig. 3 shows a view of the male rat germinal epithelium after shadowing. It is apparent from this picture that shrinkage normal to the substrate has occurred since some structures which originally extended to the limits of the section cast shorter shadows than others. These include the nuclear wall, the cell wall, and the microsomes. The mitochondria are about three times as high as the microsomes. Thus we are offered the possibility of estimating relative water contents of tissue components on the basis of their relative shrinkage on electron extraction.

It has been found that many sections which would otherwise be too thick to be used prove satisfactory when the embedding material is removed. However, where the smallest details are of interest thin sections must be used since overlapping structures will mask each other in thick areas even though the resolution is good optically speaking.

Electron extraction has been regularly used in this laboratory for more than a year with satisfactory results. The only distortion which has been noted appears in the case where rod-shaped bodies are sectioned transversely and where the section thickness is large in comparison to the rod diameter. Such objects often fall over on removing the embedding material. This has been observed in cross-section of sperm tails which, in the case of the rat, contain ten longitudinal fibrils.

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* The microscope should be monitored for excessive X-ray production. Our microscope produced about 80 mr per hour at 4 inches from the viewing chamber with the beam at cross-over when the condenser aperture was removed.

References p. 354.

SUMMARY

A method for complete extraction of embedding material from butyl methacrylate embedded sections for the electron microscope is described. The extraction is carried out in the microscope by electron bombardment at high beam intensities made possible by removing the condenser aperture. Little or no distortion due to the method of extraction is observed. Extraction is not the result of simple heating but may be due to a depolymerization resulting from electron bombardment.

Shrinkage normal to the section occurs and is greater in some tissue components than in others. It is suggested that this observation may make possible an estimate of the relative water contents of different tissue components.

RÉSUMÉ

L'auteur décrit une méthode qui permet d'extraire complètement la matière d'inclusion de coupes enrobées de méthacrylate de butyl destinées au microscope électronique. L'extraction se fait dans le microscope en bombardant la coupe par un faisceau d'électrons à haute intensité rendue possible, par suppression de l'aperture du condensateur. Cette méthode d'extraction produit peu ou pas de distorsion. L'extraction n'est pas due à un simple chauffage: elle pourrait être causée par une dépolymérisation due au bombardement électronique.

Une contraction a lieu perpendiculairement à la coupe, contraction qui est plus importante pour certains constituants des tissus que pour d'autres. L'auteur suggère l'idée que cette observation pourrait rendre possible une évaluation des teneurs relatives en eau des différents constituants cellulaires.

ZUSAMMENFASSUNG

Es wird eine Methode zur vollständigen Extraktion des Einbettmaterials aus in Methacrylsäurebutylester für das Elektronenmikroskop eingebetteten Schnitten beschrieben. Die Extraktion wird im Mikroskop ausgeführt durch ein Elektronen-bombardement bei hoher Intensität, welche durch Entfernung der Kondenserapertur erreicht wird. Es wurden wenig oder keine Zerstörungen beobachtet, die auf die Extraktion zurückzuführen sind. Die Extraktion ist nicht das Ergebnis einer einfachen Erhitzung: sie ist vielleicht auf eine durch das Elektronenbombardement verursachte Depolymerisation zurückzuführen.

Es tritt eine Schrumpfung senkrecht zum Schnitt ein, die in einigen Gewebeteilen grösser ist als in anderen. Es wird vermutet, dass diese Beobachtung eine Bestimmung des relativen Wassergehalts verschiedener Gewebekomponenten ermöglicht.

REFERENCES

1 E. FULLAM AND A. GESSLER, *Rev. Sci. Inst.*, 17 (1946) 23.
2 J. HILLIER, Paper delivered before the 8th Annual Meeting of the Electron Microscope Society of America. Detroit, September 1950.
3 J. HILLIER, S. MUDD AND A. SMITH, Paper delivered before the 8th Annual Meeting of the Electron Microscope Society of America. Detroit, September 1950.
4 M. WATSON, *Biochim. Biophys. Acta*, 8 (1952) 369.

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