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The Advantages Of Vestopal W. As An Embedding Material For Biological And Medical Electron Microscopy

Robert R. Cardell Jr.

THE ADVANTAGES OF VESTOPAL W. AS AN EMBEDDING MATERIAL FOR BIOLOGICAL AND MEDICAL ELECTRON MICROSCOPY

ROBERT R. CARDELL, JR.*

INTRODUCTION

The rapid growth of biological and medical applications of electron microscopy has been possible through significant advancements in sectioning techniques. Sections less than 500 A thick are required for study with the electron microscope, and thus some means of producing such ultrathin sections was the primary requirement in the application of this instrument to biological material. Porter and Blum[1] were among the pioneers in the development of an ultramicrotome, and currently there are several types of ultramicrotomes available for ultrathin sectioning.[2]

The second necessity for ultrathin sectioning is a sufficiently sharp cutting edge to slice the material. Sjostrand[3] developed a technique for further sharpening razor blades to the desired sharpness; Latta and Hartman[4] discovered that glass could be broken in a particular way to produce a cutting edge sharp and perfect enough for cutting ultrathin sections; Fernanez-Moran[5] experimented with and perfected the use of polished diamond chips as cutting surfaces. The electron microscopist, therefore, now has a number of cutting edges that will produce ultrathin sections of the desired thickness.

In order to produce ultrathin sections of biological material, it is necessary to embed the tissue in a medium harder than the paraffin used in tissue preparation for light microscopy. The use of the polymer, methacrylate, as described by Newman *et al*,[6] was a major step in the development of biological and medical electron microscopy. Methacrylate is used in most electron microscopy laboratories today, but this is far from the perfect embedding medium. Since fine cellular structure is not ideally preserved by this plastic, the search for a substitute for methacrylate continues. Maale and Birch-Andersen[7] have used epoxy resins and Kellenburger *et al*[8] have experimented with certain polyesters. Lack of contrast in epoxy-embedded tissue necessitates the use of additional "electron stains" in order to obtain high resolution electronphotomicrographs. The polyesters offer more contrast than the epoxy resins but less contrast than methacrylate. In 1958 Ryter and Kellenburger[9] described the use of polyester Vestopal W. for their work with ultrathin sections of bacteria.

In our laboratory we have been experimenting with Vestopal W. as an embedding medium and it is the purpose of this report to compare the results of Vestopal-embedded material to methacrylate-embedded material. Human skin* and salamander pituitaries are used to illustrate the advantages of Vestopal over methacrylate.

MATERIALS & METHODS

The procedure for both the skin and the pituitaries was similar therefore they will be described together.

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*Edsel B. Ford Institute for Medical Research, Department of Physics.
*The skin specimen was obtained by Dr. Hu, Department of Dermatology, and a more detailed report on the ultrastructure of the skin will be reported in a later publication.

For methacrylate embedding, the tissue was fixed for two hours in buffered 1% osmium tetraoxide (pH.7.4), dehydrated in a graded series of alcohol, and infiltrated with 3:1 butyl to methyl methacrylate. The specimen was placed in gelatin capsules containing catalized methacrylate (2% Luperco CDB) and allowed to polymerize in an oven at 65°C for twenty-four hours. After the methacrylate had hardened the gelatin capsule was removed and the specimen trimmed in the shape of a truncated pyramid. Ultra-thin sections were cut on a Porter-Blum ultramicrotome or on a LKB ultratome using either glass knives[4] or a polished diamond chip.[5] The sections were placed on #100 Athene grids which were previously coated with a thin film of parlodian and carbon. The sections were viewed with a modified RCA electron microscope and electronphotomicrographs were taken of selected area.

The procedure for the Vestopal W.-embedding is similar to the methacrylate-embedding procedure except that acetone is used for the dehydrating agent, since vestopal is not miscible with alcohol. (It is necessary to dry the acetone over copper sulfate to insure that all the water is removed from the acetone, for if water remains

LIST OF ABBREVIATIONS

pcn	Parenchyma Cell Nucleus
ps	Perisinusoidal Space
sg	Secretory Granule
gg	Gonadotrophic Granule
m	Mitochondria
ga	Golgi Apparatus
psc	Perisinusoidal Cell Cytoplasm
f	Fibroblast
cf	Collagen Fibers
mg	Melanin Granules
bcn	Basal Cell Nucleus
bm	Basement Membrane

FIGURE 1

Salamander pituitary gland embedded in methacrylate. This is a section through the perisinusoidal space showing two parenchyma cells bordering this space. Note the large intercelilular spaces (between arrows) and the discontinuous cell membranes. The secretary granules (sg) and the gonadotrophic globules (gg) of the parenchyma cells are shown. The reader should compare this electron micrograph with figure 2 and 3. (12,000X)

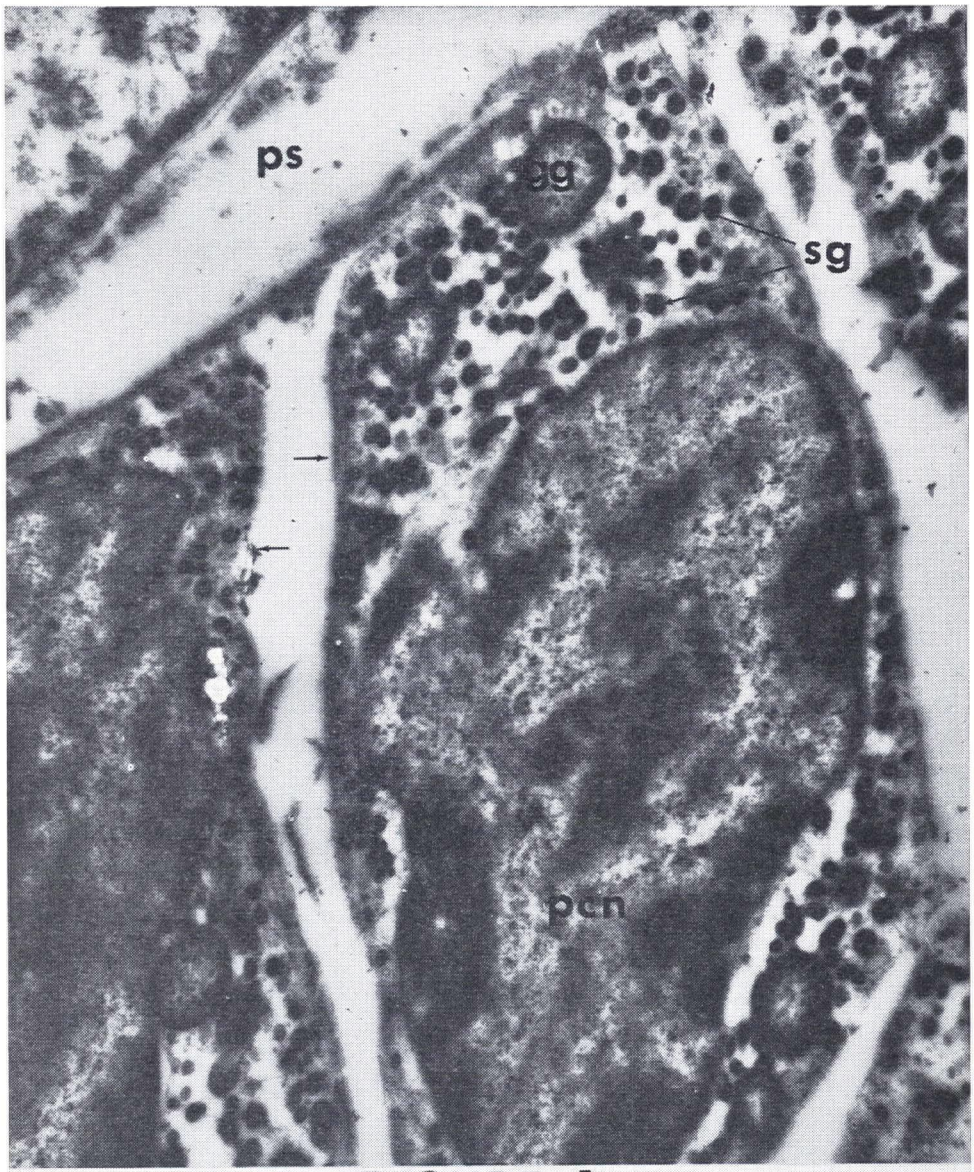

FIGURE 1

in the tissue, bubbles are formed during the embedding procedure.) Vestopal W. is much more viscous than methacrylate and requires a longer time for the infiltration process. The author infiltrates the tissue over a twenty to twenty-four hour period. The tissue is then placed in gelatin capsules and placed in an oven for twenty-four hours. The preparation of the ultrathin sections is similar to that described above for methacrylate-embedded specimens.

RESULTS & DISCUSSIONS

In a study of the ultrastructure of the pituitary gland of the salamander *(Triturus viridescens)*[10,11] the author was interested in the ultrastructure of the cell types of the *pars distalis,* and in the relationship of each cell to each other and to the circulatory system of the pituitary. Obviously it is imperative in a study of this nature that the embedded material under study be consistent from one preparation to another. With methacrylate-embedded specimens one could not be confident that such consistency always was obtained.

FIGURE 2

Salamander pituitary gland embedded in Vestopal W. This is a section through the perisinusoidal cytoplasm with several different parenchyma cells bordering the perisinusoidal cytoplasm (psc). Compare the continuous cell membranes of this preparation to the broken cell membranes of the parenchyma cells in figure 1. Also note the well preserved mitochondria (m) and the lack of large intercellular spaces. (12,000X)

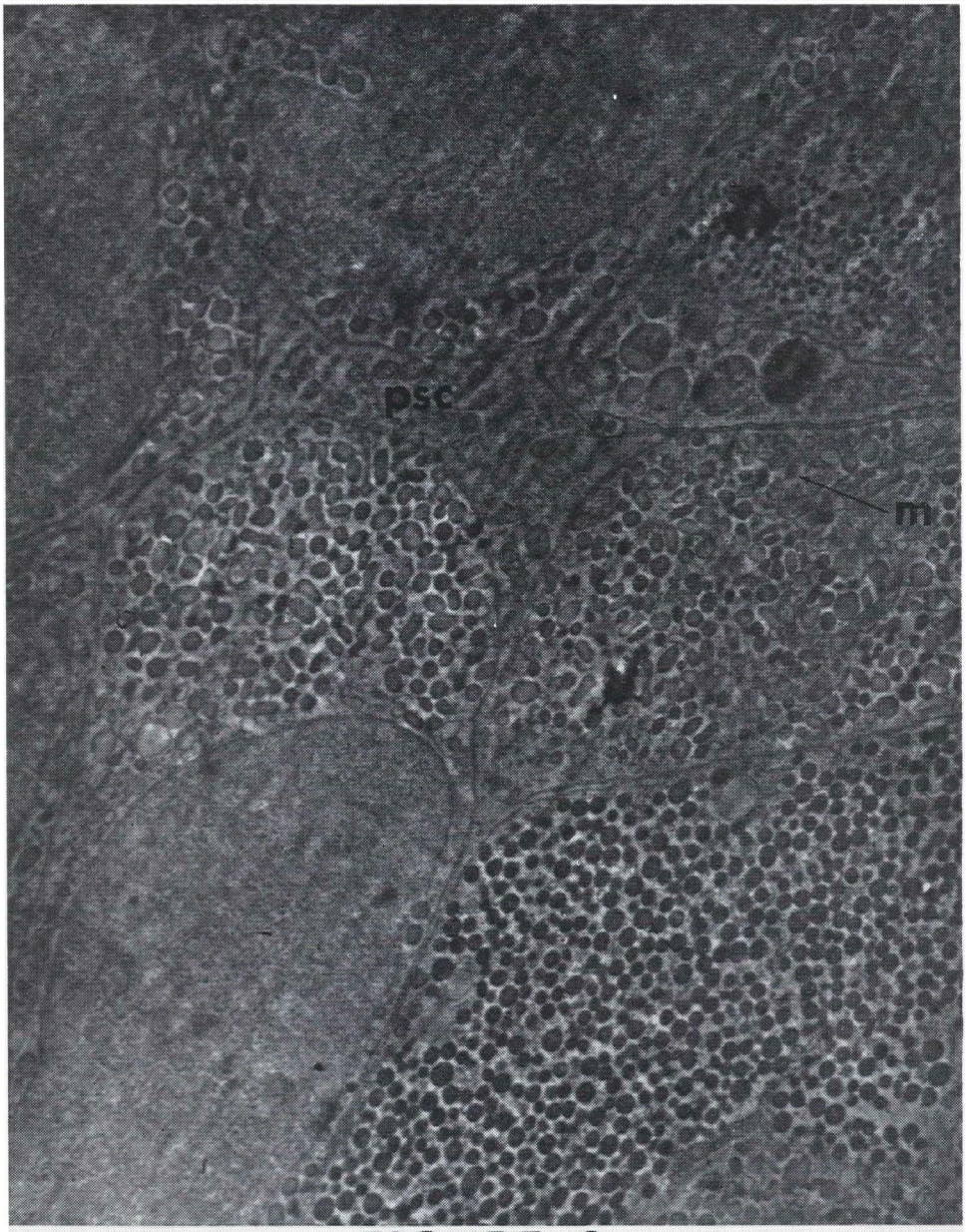

FIGURE 2

To illustrate, figure 1 is an electron micrograph of a section through the perisinusoidal space with three parenchyma cells bordering it. This material was embedded in methacrylate and as one studies this preparation it is obvious that considerable amount of cell shrinkage has occurred. Notice the space between the cells and the broken cell membrane. The fine structure within the cell gives the appearance of "poorly fixed" material. The author attempted to improve the quality of the preservation of the material by altering the fixation procedure in various ways, but significantly improved results were not obtained consistently. Therefore, attention was given to the embedding material. It was soon apparent that by changing no procedure in the preparation other than substituting acetone for alcohol and Vestopal W. for methacrylate, the preservation of the fine structure of the cells could be improved greatly, and cell shrinkage eliminated. Consistently good preparations were thus obtained enabling the study of the ultrastructure of the cells and the cell relationships, without the artifacts characteristic of methacrylate-embeddings. To illustrate this difference, note figure 2 which is a section through the perisinusoidal cell with four parenchyma cells bordering the cytoplasm. The reader's attention is directed to the complete lack of cell shrinkage as evident by the cell membrane being separated from

FIGURE 3

A higher magnification of parenchyma cells bordering perisinusoidal cytoplasm (psc). Note the improved (compare with figure 1) preservation of the cytoplasmic structures as shown by the endoplasmic reticulum, cristae of the mitochondria (m) and the Golgi apparatus (ga). The small intercellular space is shown between the arrows. (31,000X)

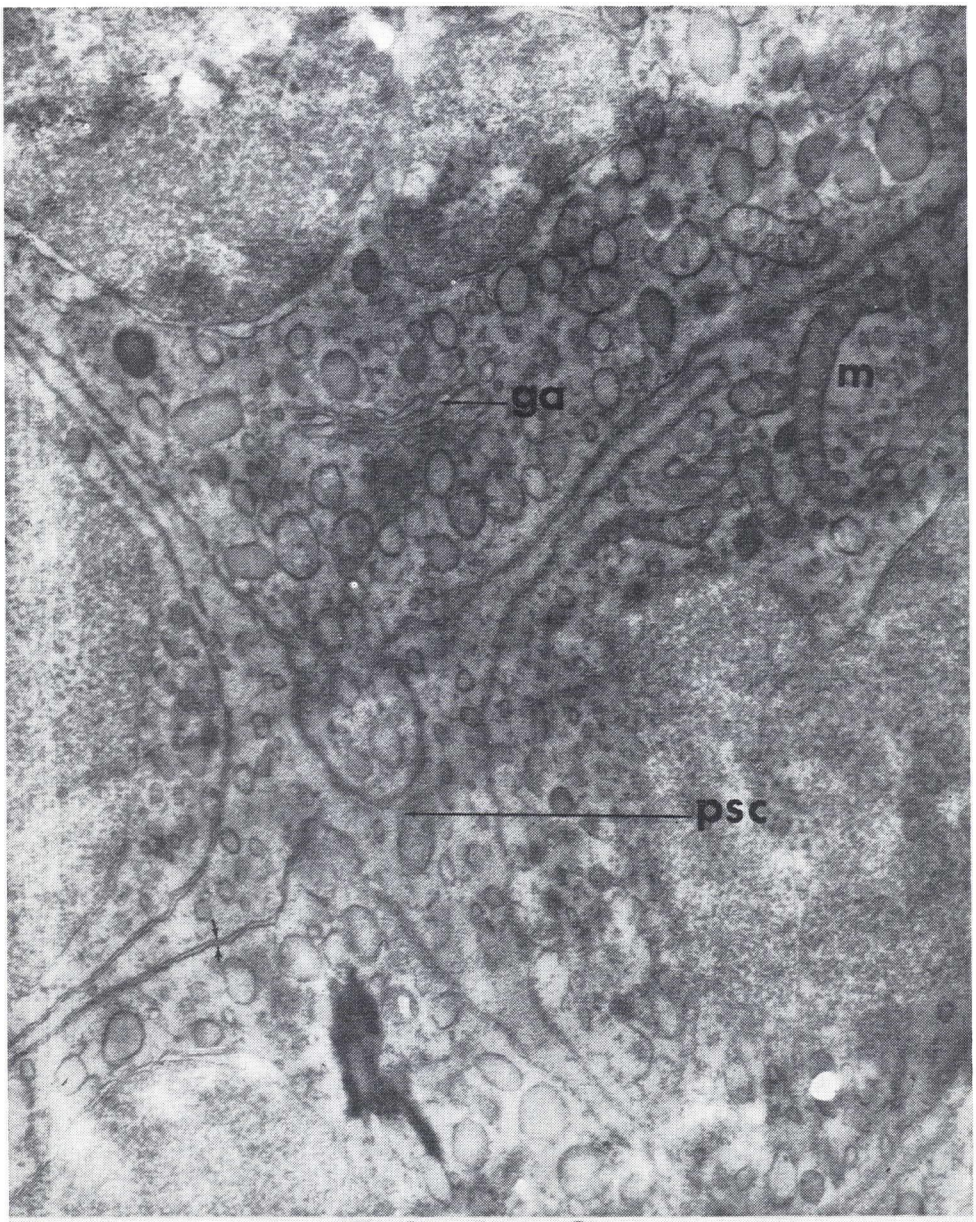

FIGURE 3

each other by a small distance (.03μ); and also note the continuity of the cell membrane. Within the cell the mitochondria are well preserved showing the characteristic organization of mitochondria, and the Golgi apparatus is well defined.

Human skin presents particular problems to the electromicroscopist in that the ultrastructure is difficult to preserve and good sections are not easily obtained. In a study of normal human skin it was found that Vestopal W.-embedded specimens showed the cellular fine structure much better preserved; clearly defined, continuous cell membranes; unbroken, conspicuous membranes (Fig. 3); and well-resolved collagen fibers of the dermis (Fig. 4). This is in sharp contrast to the methacrylate-embedded specimens in which it is very difficult to study these structures. It should be pointed out in the study of the skin that it is necessary to add phosphotungstic acid as an additional "electron stain" to improve the contrast of the specimen.

Vestopal W. is not restricted to the examples illustrated above but has been used by the author for thyroid, lung, human pituitary tumor, dog pituitary and crayfish testis tissue with essentially similar results as described above.

FIGURE 4

Human skin embedded in Vestopal W. The basement membrane is clearly shown (follow the series of arrows) and it is unbroken. The basal cell of the epidermis contains melamin granules (mg) and the cells do not appear to be shrunken. Note the well preserved collagen fibers (cf) and the fibroblasts (f) of the dermis. (12,000X)

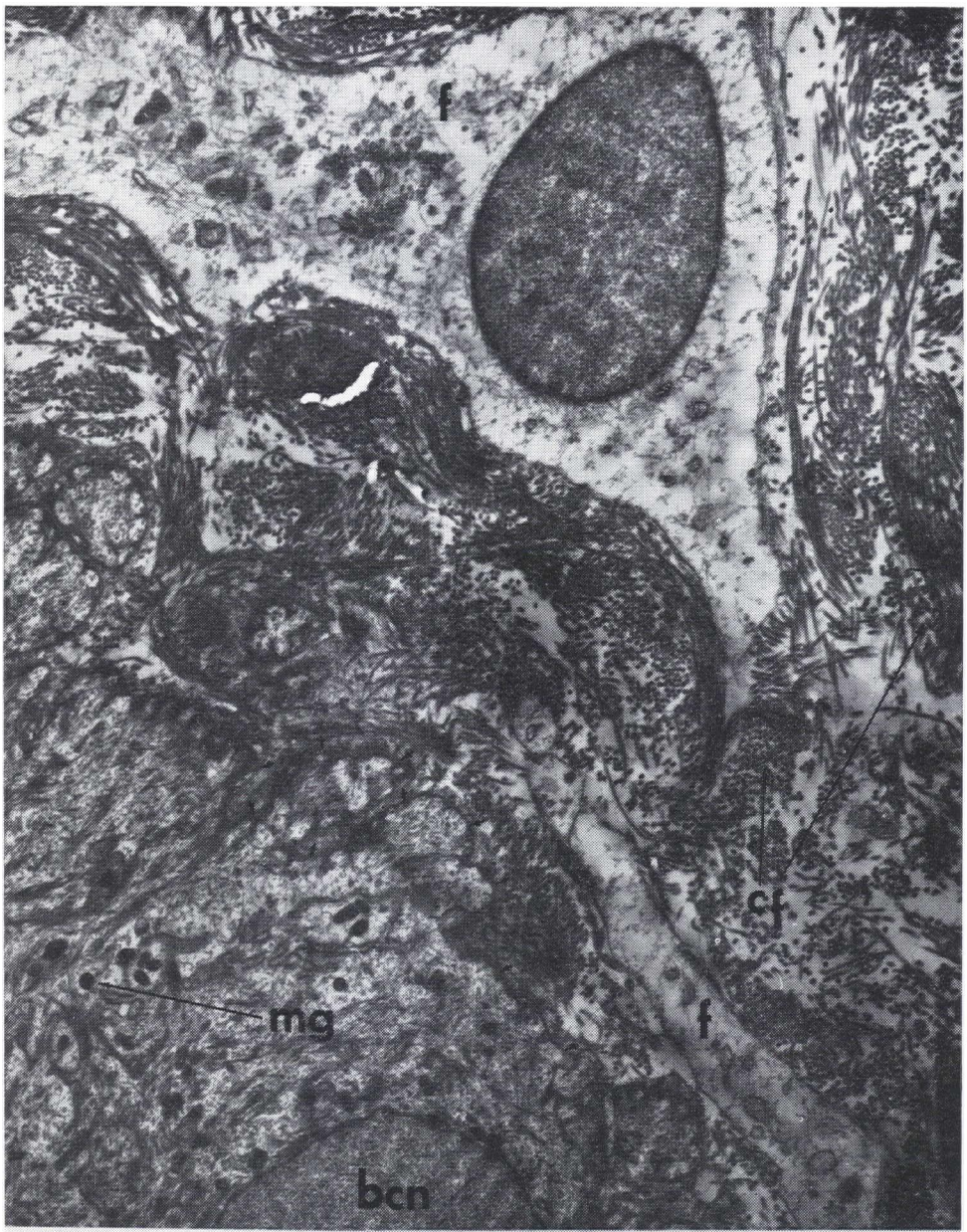

FIGURE 4

Finally, a technical advantage of using Vestopal W. is that the sections may be cut thicker than with methacrylate without sacrificing good resolution. Other than the advantage of having to strive for extremely thin sections, there is also the advantage of being able to produce sections with a greater area in each section. For example, with methacrylate it is extremely difficult to cut sections much greater than 0.5^2 mm. but with Vestopal it is possible to cut sections 1.0^2 mm. and still produce pictures with high resolution. This increases the area of tissue available for study four times per section and is of considerable value when one wishes to study several different areas in a given tissue.

It should be pointed out, however, that vestopal W. is more difficult to section. While this is annoying during the sectioning process, it is the author's opinion that the improved results are well worth the extra work during the sectioning procedure.

FIGURE 5

A section through the dermis of human skin embedded in Vestopal W. Note the regular packing of the collagen fibers. (12,000X)

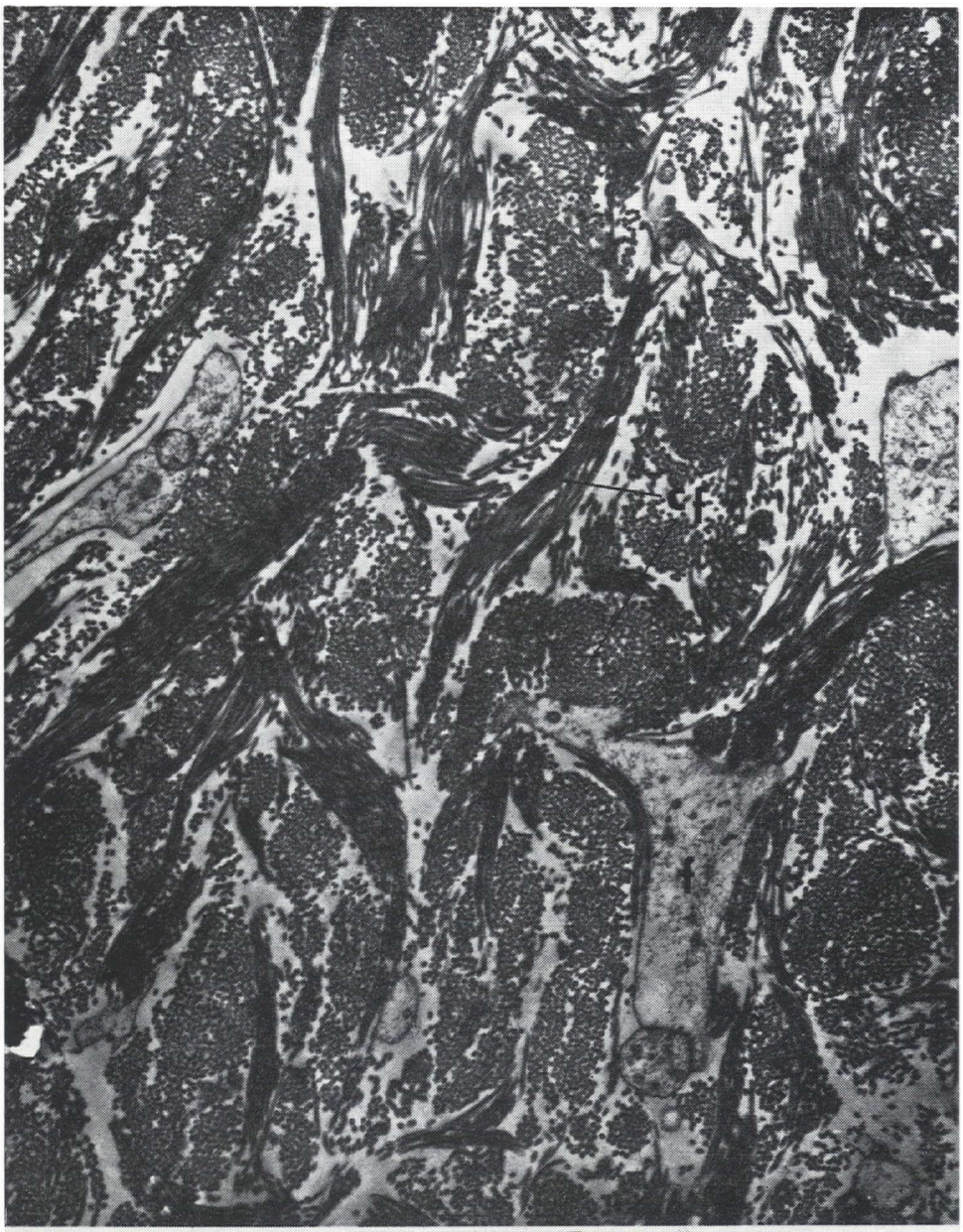

FIGURE 5

SUMMARY

Vestopal W. has been compared to methacrylate as an embedding material for electron microscopy. Evidence is presented which shows that Vestopal W.-embedded specimens have less cell shrinkage, better preservation of fine structure within the cells, the membrane structure and cytoplasmic inclusions are better preserved and in general the results with Vestopal W. are more consistent from preparation to preparation than those with methacrylate. It is noted that sections of methacrylate-embedded specimens possess much more inherent contrast than Vestopal embedded material.

REFERENCES

1. Porter, K. R., and Blum, J.: Study in microtomy for electron microscopy, Anat. Rec. 117:685, 1953.

2. Gettner, M. E., and Ornstein, L.: Microtomy, In, Oster, G., and Pollister, A. W. eds.: Physical Techniques in Biological Research, New York, Academic Press, 1956, v. 3 pp. 627-686.

3. Sjostrand, F. S.: A new microtome for ultrathin sectioning for high resolution electron microscopy, Experientia 9:114, 1953.

4. Latta, H., and Hartmann, J. F.: Use of a glass edge in thin sectioning for electron microscopy, Proc. Soc. Exper. Biol. & Med. 74:436, 1950.

5. Fernández-Morán, H.: Applications of a diamond knife for ultrathin sectioning to the study of the fine structure of biological tissues and metals, J. Biophys. & Biochem. Cytol. 2:29 (Suppl.) 1956.

6. Newman, S. B., Borysko, E., and Swerdlow, M.: Uutra-microtomy by a new method, J. Research Nat'l. Bur. Stand. 43:183, 1949.

7. Maale, O., and Birch-Andersen, A.: On the organization of the 'nuclear material' in *Salmonella* typhimurium, Symposium Soc. Gen. Microbiol. 6:261, 1956.

8. Kellenberger, E., Schwab, W., and Ryter, A.: L'utilisation d'un copolymére du groupe des polyesters comme material d'inclusion en ultramicrotomie, Experientia 12:421, 1956.

9. Ryter, A., and Kellenberger, E.: L'inclusion au polyester pour l'ultramicrotomie, J. Ultrastruct. Research 2:200, 1958.

10. Cardell, R. R., Jr.: Observations on the structure and ultrastructure of the pituitary gland of the salamander (*triturus viridescens*), ASB Bulletin 8:27, 1961.

11. Cardell, R. R., Jr.: Observations on the ultrastructure of the *Pars distalis* of the pituitary gland of the salamander (triturus viridescens), J. Applied Physics (To be published).