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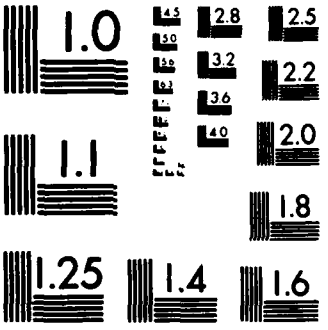

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AFRRI SCIENTIFIC REPORT

AFRRI SR83-23

Karnovsky-osmium as a primary fixative for electron microscopy

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Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

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A combined Karnovsky-osmium tetroxide primary fixative is proposed for transmission electron microscopy. This fixative, hypothetically, seems to overcome the drawbacks of sequential fixation techniques. Evaluation of this combined fixative reveals very acceptable fixation of hepatocytes, lymphocytes, and fibroblasts, as well as various organelles within these cells.

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G. W. Bailey, Ed., *Proceedings of the 41st Annual Meeting of the Electron Microscopy Society of America*

KARNOVSKY/OSMIUM AS A PRIMARY FIXATIVE FOR ELECTRON MICROSCOPY.

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In general, most researchers who use electron microscopy as a research tool use sequential glutaraldehyde-osmium tetroxide as the preferred method for fixing cells or tissues. There are certain problems inherent with this technique. Glutaraldehyde serves to cross-link proteins and increase cell membrane permeability,[5] thus making the vehicle vitally important.[7] But the membrane mobility following the aldehyde fixation is perhaps involved in the artifactual formation of membrane blisters and seriously affects interpretation of membrane structure.[1] Osmium was believed originally to be involved primarily with fixation of the lipid moiety.[7] Recent evaluation of its chemistry reveals much more: it fixes and stains via the mechanism of hydrogen bonding with proteins, nucleic acids, and "the aliphatic side chains of some cellular lipids."[6] To overcome the drawbacks of using sequential fixation, it was suggested[6] that combined primary fixation, using glutaraldehyde/osmium, would incorporate the advantages of each. "Shrinkage effects and chromatin clumping produced by glutaraldehyde are presumably antagonized by osmium."[6] Positive results[2] were obtained by using this procedure in the fixation of human lymphocytes, monocytes, and neutrophils. It was found that there was a great increase in fixation reproducibility, increased ease of classification between lymphocytes and monocytes, sharp membrane definition and preservation of granules and vesicles, as well as the observation of a cell-surface coating not seen using other fixatives. There was a complaint that neutrophil glycogen was poorly defined. It has been found, however, that glutaraldehyde/osmium-fixed cells are not as well stabilized as those fixed by glutaraldehyde alone.[3] A combined-aldehyde fixative was introduced by Karnovsky. "...formaldehyde penetrates faster than the glutaraldehyde and temporarily stabilizes structures which are subsequently more permanently stabilized by glutaraldehyde."[3]

In an effort to find a more effective and reproducible primary fixative, I have experimented with Karnovsky/osmium. The fixative was prepared by mixing full-strength Karnovsky and 2% osmium tetroxide (1:1), each in 0.15 M Na Cacodylate, pH=7.3. Tissues were initially diced in the Karnovsky fixative and then placed into the combined fixative. The cell colonies were placed directly into the combined fixative.[8] Fixation took place 1 hour at 4°C. Following wash with 0.3 M Na Cacoldylate, the specimens were post-fixed in 2% osmium tetroxide, 2 hours, at 4°C. Dehydration took place via ETOH and propylene oxide; embedment was in epon/araldite.

The results of the combined Karnovsky/osmium primary fixation are seen in photomicrographs 1 through 4. 1 and 2 (guinea pig liver hepatocyte) illustrate the high quality of smooth endoplasmic reticulum (S), peroxisome (P), mitochondria (M), and rough endoplasmic reticulum (RER) fixation. 3 is representative of in vitro fixation of thymus-derived lymphocyte cells showing mitochondria, Golgi (G), and vacuolar structures (V). 4 is representative of a mouse fibroblast cell with its large Golgi and extensive RER.

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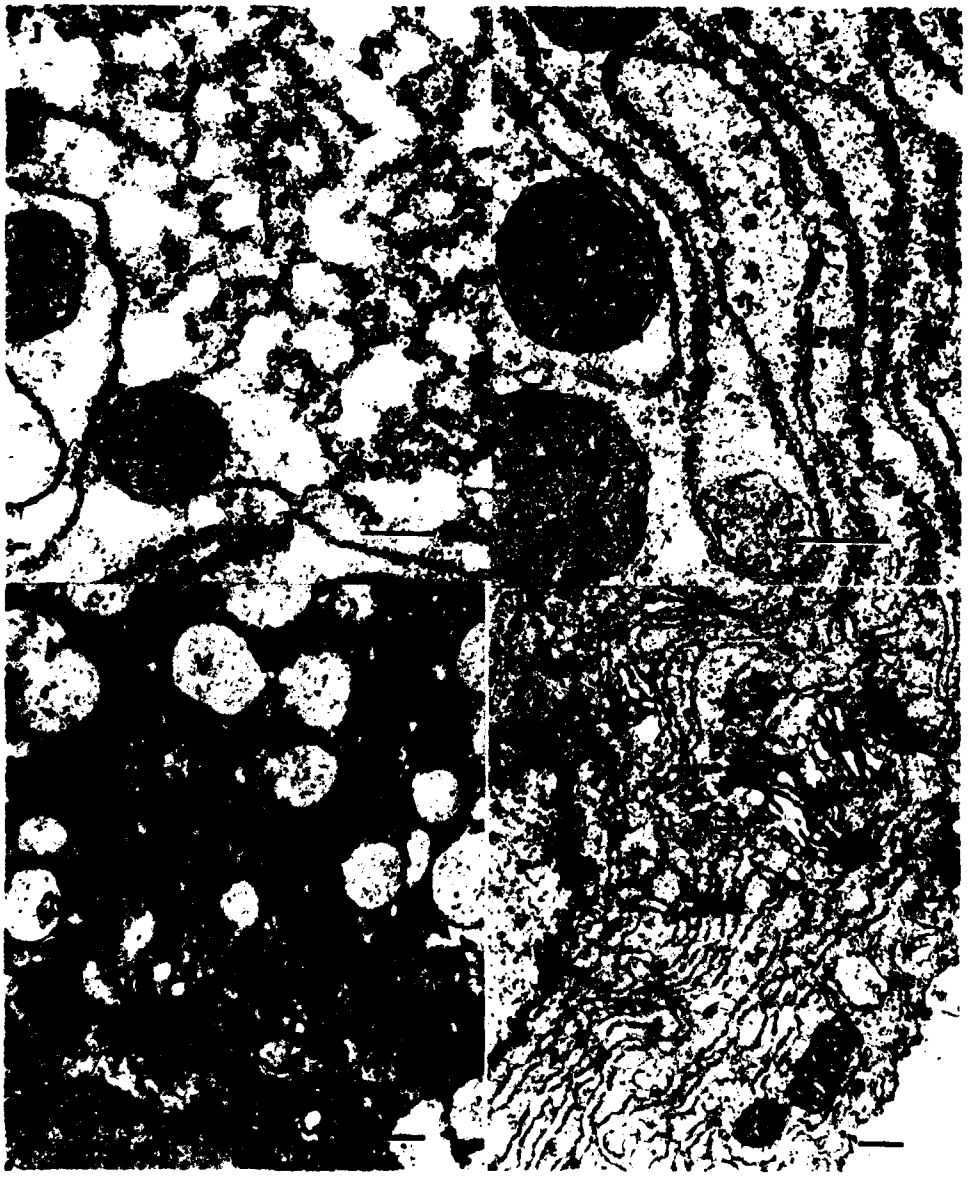
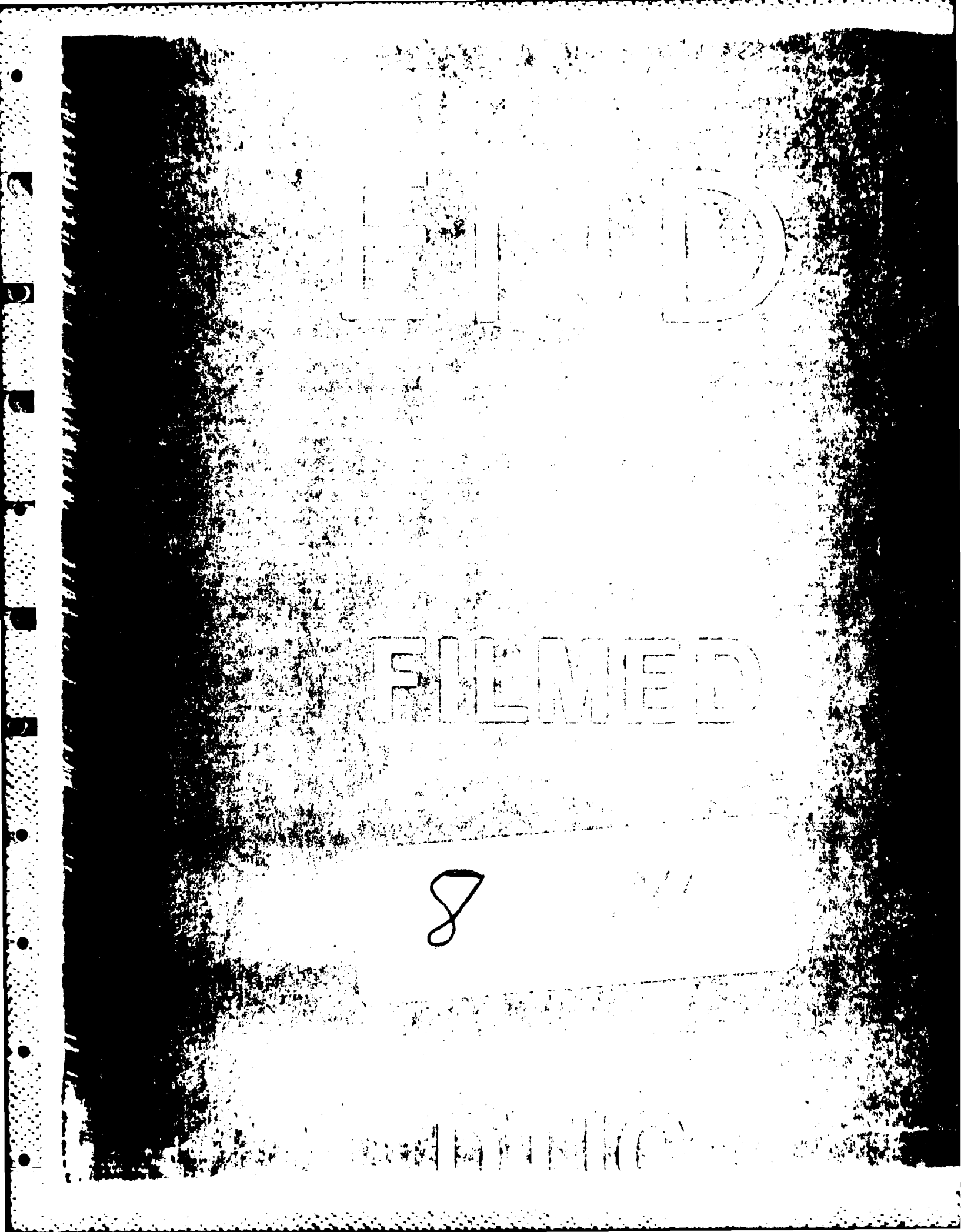
9. I wish to express my appreciation to Dr. T. J. MacVittie for the cell colonies and Dr. G. D. Ledney for the mouse tissue.

NOTE: All markers represent 0.3 micrometers.

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