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Preservation of Mitochondria in Fat Solvents by Uranium Nitrate.

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Attention has already been called to the fact[1] that Cajal's uranium-silver method, devised primarily to demonstrate the Golgi apparatus, occasionally stains mitochondria. We are not aware of any critical test on record made to determine whether or not this method, without the part of silver-nitrate impregnation, is adaptable to the demonstration of mitochondria. In the course of our studies on the effects of heavy metallic salts upon cell structures, we have had occasion to use Cajal's "uranium-formalin fluid" as a general fixative. Various tissues of frog, white mouse, and guinea-pig were fixed in this fluid for 10 hours. After a quick wash in distilled water, the pieces were dehydrated and embedded in paraffin, and sections were stained with fuchsin and methyl green. In all cases, it was found that the mitochondria had been well fixed and excellently differentiated but mostly in cells near the surface of the block, the deeper-lying ones being less affected. This was evidently due to the poor penetration of the fixative. An effort was made therefore to improve this effect by adding to the fixing fluid a small quantity of glacial acetic acid. The results were so satisfactory that this method seems to be in certain respects better than others so far available.

Further, we experimented on a mixture of this "uranium-formalin fluid" with fat solvents such as alcohol of 80% to 100% strength, ether and chloroform in various proportions with a view to testing their solubility upon mitochondria. It was surprising to find that in tissues, notably pancreas, liver, kidney, gastric and intestinal glands, the mitochondria remain either in long filaments or short rods, distributed abundantly in the superficial as well as in the deeper-lying cells. This observation shows that fat solvents such as acetic acid, alcohol, etc., when combined with uranium-formalin solution, do not dissolve the mitochondria. It seems probable that the mitochondria are rendered insoluble in fat solvents by the uranium salt, perhaps just as in the case of Zenker's fluid plus acetic acid where the bichromate may have oxidized the mitochondria so that they are no longer soluble in acid.[2]

[1] Da Fano, C., *Med. Sci.*, 1924, ii, 319.

[2] Young, R. T., *Anat. Rec.*, 1928, xl, 351.