The Fixative Action of Uranyl Acetate in Electron Microscopy

Uranyl acetate has been widely used by many electron microscopists as a contrasting agent since its introduction for that purpose by STRUGGER[1]. In this initial work uranyl acetate was used on the blocks prior to dehydration and embedding. It was later recommended for the same purpose by WATSON[2] who, instead, used it to stain ultrathin sections. Since then, uranyl acetate has usually been used to stain preparations already embedded and sectioned, either alone or in combination with alcaline lead.

During their important work on the fixation of bacterial nucleus, RYTER and KELLENBERGER[3] found that uranyl acetate, when used before the dehydration, as done by STRUGGER, had a stabilizing effect on DNA fibrils, preventing their aggregation during the posterior steps in the specimen preparation for electron microscopy. Since then, uranyl acetate treatment of blocks became of common use among bacterial electron microscopists. During studies on the influence of some factors involved in the RYTER-KELLENBERGER-fixation on the preservation of the membranes of some Gram-positive bacteria, we found that uranyl acetate treatment was necessary to have well fixed mesosomes in all the *Bacillus* strains studied[4]. This effect was considered as a fixative one and chemical studies were initiated to clarify its mechanism. These preliminary studies showed that the amount of phospholipid phosphorus extracted during the dehydration of *Bacillus subtilis* fixed by OsO4 and treated with uranyl acetate was 11 times less than that extracted from bacteria of the same culture not treated with uranyl acetate. Meanwhile, uranyl acetate started to be used by several electron microscopists before dehydration on higher cells with good results. These results have usually been considered as due to the contrasting effect of uranyl acetate. Similar results have been found with many different tissues in our laboratory; in a comparative work[5] we studied the influence of the treatment with uranyl acetate done before the dehydration on the ultrastructure of rabbit retina cells. In this study we found a marked improvement in the preservation of several structures including cell membranes. This effect was considered as due to a fixative rather than contrasting action of uranyl acetate and was related with the effect described in *Bacillus* cells. The chemical studies done with *B. subtilis* were then extended to animal cells. Here we shall present a brief report on the results of that study.

The brains of several Sprague-Dawley rats were cut into blocks about 1 cm^3 which were randomized. Several samples with the same weight were treated in the following ways: (1) fixed with Palade's OsO4 for 2 h and dehydrated in increasing concentrations of ethanol (30, 50, 75 and 90%, 15 min each, and 100%, 2 changes of 30 min each). (2) As in (1) but with a treatment with uranyl acetate (0.5% in acetate-veronal buffer) for 2 h at room temperature after the OsO4 fixation and before the dehydration. (3) Directly dehydrated in ethanol as in (1) and (2) without any fixation. (4) Treated with uranyl acetate as in (2) without fixation with OsO4, and then dehydrated as described. (5) Extracted with chloroform-methanol (2/1, v/v) as control. The amount of phospholipid phosphorus lost from brain tissue in the OsO4, uranyl acetate and ethanol was estimated by the Fiske-Subbarow-method after extraction with chloroform-methanol (2/1, v/v) and compared with the amount extracted in (5). The results are presented in the Table. Samples from the preparations (1) and (2) were processed for electron microscopy after embedding in Luft's Epon and viewed in a Siemens Elmiskop I A. As can be seen in the Table, the treatment with uranyl acetate significantly reduces the amount of phospholipid phosphorus extracted by the ethanol (0.16% of the total extracted directly with chloroform-methanol in comparison with 18.01%). The Table also shows that the treatment with uranyl acetate directly applied to the brain tissue without previous fixation with OsO4 reduces by about 3 times the amount of phospholipid phosphorus extracted by the ethanol. In the same experiment, the amount of protein extracted during dehydration was found to be 4 times less when the treatment with uranyl acetate was employed.

These results support the view, deduced from morphological observations, that uranyl acetate has a fixative action when used before the dehydration of the specimens to be embedded and sectioned for electron microscopy[6,7].

Loss of phospholipid phosphorus during preparation for electron microscopy of rat brain

Treatment		Phospholipidic phosphorus	
		Amount extracted μg	% of total
1	OsO4	2.21	0.17
	Ethanol	237.30	18.01
2	OsO4	2.21	0.17
	Uranyl acetate	0.79	0.06
	Ethanol	2.06	0.16
3	Ethanol	836.60	63.51
4	Uranyl acetate	2.10	0.16
	Ethanol	290.80	22.08
5	Chloroform-methanol	1317.30	100.00

Mean values from 3 experiments using the same amount of tissue.

Resumen. La utilización del acetato de uranilo después del osmio durante la fijación, reduce la cantidad de fosfolípidos extraídos durante la deshidratación de los tejidos, lo que demuestra sus propiedades de fijador químico.

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6 After this report had been accepted for publication, a paper by J. A. TERZAKIS was published (J. Ultrastructure Res. *22*, 168, 1968) presenting morphological evidence for a fixative action of uranyl acetate when used before dehydration on the membranes of *Plasmodium gallinaceum*.
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