Lipid Retention During Electron Microscope Preparation of *Penicillium chrysogenum*

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Introduction

For accurate visualization by electron microscopy of membranes and other structures with high lipid content, it is essential that a large percentage of lipid be retained during the fixation and dehydration process. It is also necessary to know the amount and distribution of labeled lipid lost in solvents during cell preparation.

It has been determined that lipids can best be preserved by using osmium-gluteraldehyde fixation process (3). Using this procedure Ainsworth reported a loss of only 0.7% to 7.2% total lipids in rat liver. In autoradiographic studies with hamster tissue, Dermer (1) found that a lipid loss of about 16% during preparation with osmium did not prevent localized radioactivity in specific intracellular structures. Stein and Stein (8) have shown that less than 20% ^{14}C-labeled total lipid was lost if the time for each alcohol and propylene oxide dehydration step was reduced.

These and other studies suggest that the degree of lipid retention depends upon the kind of intracellular lipid, the type of tissue and the technique in preparation (4-7).

Methods and Procedures

Young and aging submerged cultures of *Penicillium chrysogenum,* Q 176, were incubated with 1-^{14}C-palmitic acid according to procedures in our laboratory (6, 9). After 2, 10, 60, and 120 minutes incubation with labeled media, 20 ml samples of cultures were removed, filtered, washed with distilled water and subsequently processed for electron microscopy.

Mycelial cells were prepared for EM according to the procedure given in Table I. At each step of the fixation and dehydration schedule 0.1 ml of the washings was collected and the radioactivity determined by liquid scintillation spectrometry (Beckman ambient temperature liquid scintillation counter, Model LS-133). The amount of label incorporated into the mycelia in 20 ml samples of culture was determined by collecting other similar samples. The mycelia were filtered, washed, resuspended in 20 ml of 100% methanol and sonified for 2 min. The radioactivity of 0.5 ml of the sonicate was determined.

After the mycelia were prepared for electron microscopy, and just prior to embedding, total lipids were extracted from the treated mycelia and also extracted from similar samples of untreated mycelia (2). The lipids were separated into major lipid classes by thin layer chromatography using plates coated with silica gel G impregnated with 0.02% Rhodamine G solution using two solvent systems. (Solvent system I: n-hexane:ether:glacial acetic

TABLE I *Fixing, Dehydration and Embedding Procedure*

Reagent		
3% gluteraldehyde	2 hr	(1X)
cacodylate buffer, pH 7.2, 0.1 M	30 min	(3X)
cacodylate buffer, pH 7.2, 0.1 M	overnight	(or hold)
phosphate buffer, pH 7.3, 0.1 M	10 min	
phosphate buffer, pH 7.3, 0.1 M	overnight	
1% OsO_4	2 hr	
ddH_2O	1 hr	
50% ethanol	2 min	(1X)
70% ethanol	2 min	(1X)
95% ethanol	5 min	(2X)
100% ethanol	5 min	(3X)
propylene oxide:resin; 1:1	overnight	infiltration
embedding resin	12 hr, 35 C	
	12 hr, 45 C	
	12 hr, 60 C	

acid:absolute methanol, 90:2:2:3, v:v:v:v) and Solvent system II; n-hexane;ether, 94:6, v:v). Bands were compared with standards (phospholipids, diglycerides, free fatty acids, triglycerides, chloesterol ester, and epoxy) scraped into scintillation vials and radioactivity was determined.

Results and Discussion

When the total amount of label extracted from the mycelia into the solvents was compared with that in untreated mycelia it was found that 6 to 7% of the total lipid was lost during electron microscope preparation (Table II). Although as much as 50% of these lipids were extracted in the alcohol changes, the propylene oxide dehydration extracted the largest amount of cell lipids of any single step. Aging cells tended to lose more lipids in alcohols. The large amount of lipids extracted from young cells by gluteraldehyde was not observed in aging cells (Fig. 1).

TABLE II *Extraction of Labeled Lipids by Solvents in EM Preparation*

	Young (pH 7.4)	Aging (pH 3.4)
Mycelia[A]	15,883 dpm[B]	41,200 dpm
Washes from mycelia	1,172 dpm	2,489 dpm
Percent loss	7.3%	6.0%

[A]From 20 ml of culture.
[B]Decompositions per minute.

Thin layer chromatographic analysis of lipid components of untreated mycelia revealed that the free fatty acid fraction represented the largest portion of lipid in aging cells. The amount recovered in young cells was negligible (Fig. 2). Aging cells not only accumulated lipids as pools of free fatty acids but distribution of label indicated that aging cells incorporated large amounts of the

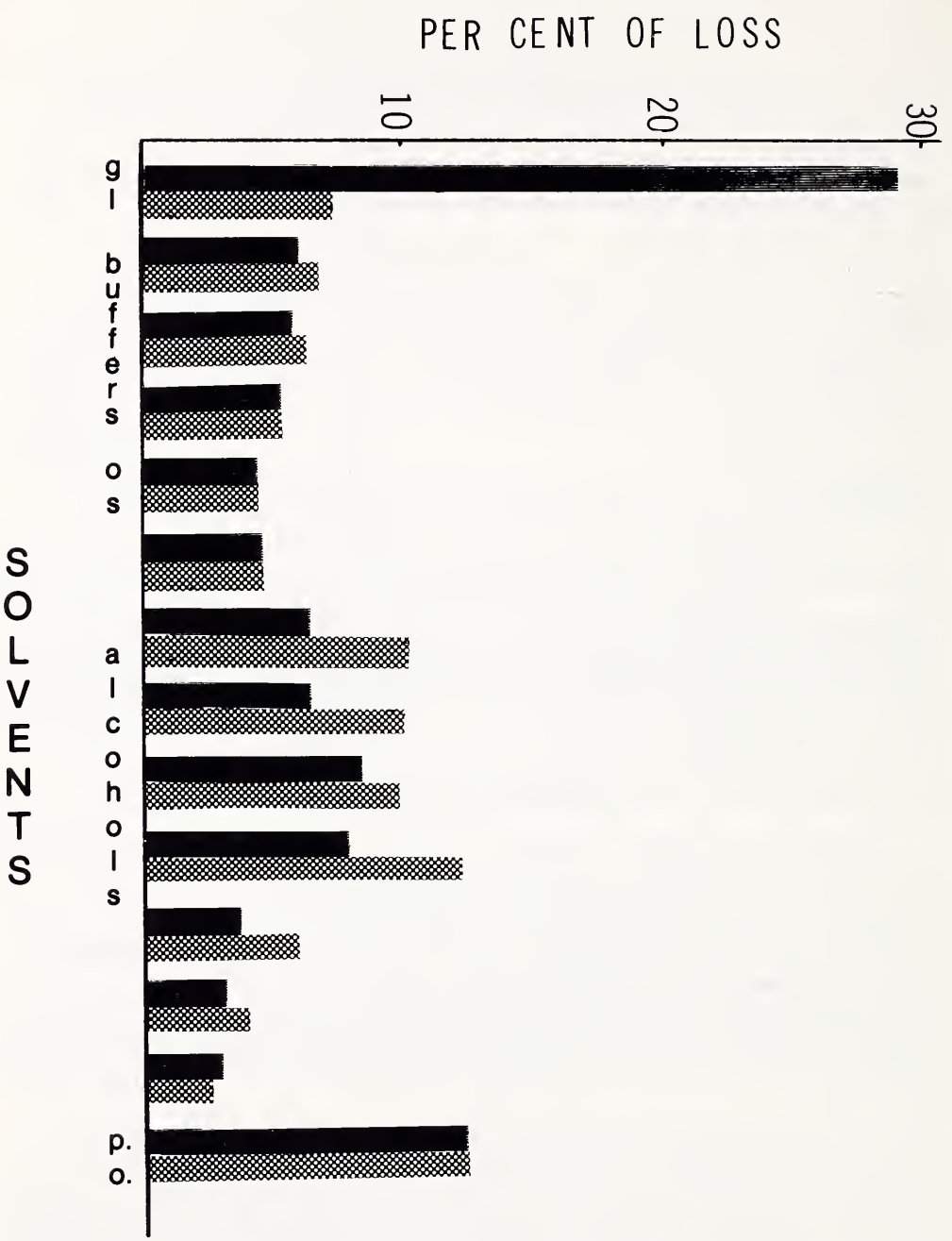

FIGURE 1. *Extraction of Labeled Lipids by Solvents in Electron Microscopy Preparation. gl—gluteraldehyde, os—osmium tetroxide, p.o.—propylene oxide.* *Young,* *Aging.*

labeled palmitic acid into free fatty acids also. In untreated cells, a maximum of 74% of the label was recovered in the free fatty acid fraction after 2 minutes. At all other incubation times approximately 70% of the label was recovered from the free fatty acid fraction. Smaller amounts of other lipid components were present; phospholipids comprised about 20% at all time points. In aging cells treated for electron microscopy, more than half of the incorporated label was extracted in the dehydration procedure. Large amounts of the free fatty acids

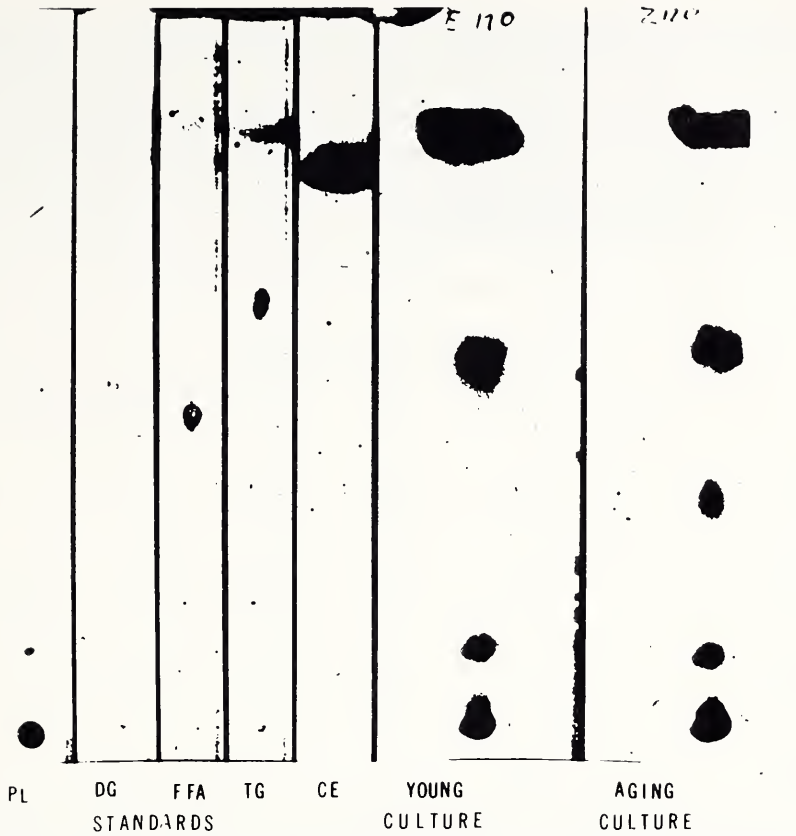

FIGURE 2. *Distribution of Lipids from Young and Aging Cultures of* ***Penicillium chrysogenum****. PL—phospholipids, DG—digylcerides, FFA—free fatty acids, TG—triglycerides, CE—cholesterol esters.*

were lost; the percentage dropped from 70% to only 15% after EM preparation. Phospholipids were retained in aging cells; all other lipid components remained approximately the same (Table III).

TABLE III *Effect of EM PREP on Distribution of Label in Lipid Classes (Aging Cultures)*

Time[A]	Percent Label Incorporated Into: PL	DG	FFA	TG	CE	EP
2 min						
before[B]	15	6	74	4	1	
after	33	14	13	13	13	14
10 min						
before	20	8	64	6	2	
after	45	12	11	11	10	11
60 min						
before	19	5	68	5	3	
after	43	11	12	10	11	13
120 min						
before	21	4	72	3	-	
after	40	11	12	12	13	12

[A]Time after addition of label
[B]Before and after EM PREP

Young cells, unlike aging cells, incorporated label primarily into the phospholipids with smaller amounts in other lipid components. Young cultures prepared for electron microscopy retained most of the labeled phospholipids throughout the solvent treatments. The percent of phospholipids in the distribution dropped from 70% to about 50% in treated cells. In young cells about 15% of the label was found in the epoxy resin, indicating little label loss in the other lipid components (Table IV).

TABLE IV *Effect of EM PREP on Distribution of Label in Lipid Classes (Young Cultures)*

Time[A]	Percent Label Incorporated Into: PL	DG	FFA	TG	CE	EP
2 min						
before	59	10	13	12	6	
after	42	10	14	11	11	12
10 min						
before	55	9	15	15	6	
after	34	11	14	12	13	16
60 min						
before	73	4	11	11	1	
after	34	11	15	13	14	13
120 min						
before	80	3	15	2	-	
after	67	6	8	6	6	7

[A]Time after addition of label

Conclusions

Retention of total lipids appeared adequate for electron microscope autoradiography in both young and aging cells of *Penicillium chrysogenum.* However, unbound lipids, such as free fatty acids, were highly soluble in the solvents and tended to be lost from the cells during electron microscope preparation. Distribution of labeled lipids was altered considerably in the aging cells because of their high free fatty acid content. These changes in distribution should be taken into consideration in autoradiographic analysis of cells characterized by large lipid reserves.

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