Experiments on the Fixation of Lipids by Osmium Tetroxide

By J. T. Y. CHOU

(*From the Cytological Laboratory, Department of Zoology, University Museum, Oxford*)

Summary

The black material seen in paraffin preparations of tissues fixed with osmium tetroxide is not merely reduced osmium. The lipid is still present in combination with osmium. Osmicated lipid globules are insoluble in chloroform or benzene. They regain their solubility in lipid solvents when the blackness caused by osmium tetroxide is bleached away.

Introduction

IN paraffin sections of tissues fixed with osmium tetroxide, the lipid-sites are often black. The purpose of the present investigation was to find whether the lipids are still present in these lipid-sites or whether they have been dissolved away by the antemedium before the tissue was embedded in the paraffin wax, leaving only a black residue.

Materials and Methods

The skin of the mouse and the liver of the newt were used for this investigation.

The skin, shaved with a razor-blade, and the liver were cut into small pieces and fixed for 24 h either in Flemming's fluid or in 1% osmium tetroxide.

A standardized process of dehydration was used throughout. After the tissue had been washed for 24 h in running water, it was left for $\frac{1}{2}$ h in each of the following grades of ethanol: 50%, 70%, 80%, 90%, 95%, and absolute. Tissue was first left in a mixture of equal volumes of absolute ethanol and antemedium for $\frac{1}{2}$ h and then in the antemedium alone for the same period (the fluid was changed once). The antemedia used were chloroform, benzene, toluene, and xylene. After being soaked in one of these antemedia for $\frac{1}{2}$ h, the tissue was transferred to melted paraffin wax (m.p. 56° C) and left for 1 h (with one change of wax).

Sections of the skin of the mouse were cut at 15 μ and those of the liver of the newt at 8 μ. All sections were dewaxed in the fluids previously used as antemedia.

The sections were examined under the microscope while still in water and then bleached in 3% hydrogen peroxide solution. The period of bleaching varied; but after bleaching, the sections were always examined under the microscope to make sure that the blackness had been removed. The sections were then coloured for 5 min in a saturated solution of Sudan black B in 70% ethanol. After coloration, the sections were rinsed for 5 sec in 70% ethanol

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and 1 min in 50% ethanol; they were washed in distilled water and mounted in Farrants's medium.

To study the solubility of osmicated lipid globules in lipid solvents other than the antemedia already mentioned, acetone and pyridine were also used. The skin of the mouse was fixed in Flemming's fluid, dehydrated, and passed through benzene into paraffin. Sections were dewaxed in benzene and transferred to cold acetone at room temperature (about 20° C) for 24 h; or, alternatively, sections were dewaxed in benzene and treated in boiling acetone for the same period. The sections were brought to water and bleached by the method described above; they were then coloured with Sudan black.

Pyridine was used in a similar way. After dewaxing, sections were transferred to pyridine either at room temperature for 24 h or at 60° C for 19 h. They were then coloured with Sudan black.

Results

The lipids of both kinds of cell were blackened by fixation in osmium tetroxide solution. When the sections were left in the antemedia for 5 min to

Table 1

The reaction between the different antemedia and lipid globules

Period of dewaxing at room temp. (20° C) (min)	*Antemedium and dewaxing agent*			
	Xylene	*Toluene*	*Chloroform*	*Benzene*
5	+	+	+	+
40	O	O	+	+

\+ indicates coloration by Sudan black.
O indicates that no sudanophil material was present.

remove the wax, the lipid-sites were still black. If, however, the sections were left in xylene or toluene for 40 min instead of 5 min, the lipid-sites were colourless, and usually appeared empty. They remained black, however, if benzene or chloroform was used for the same period.

When sections that had been 5 min in any of the antemedia were brought down to water and bleached, the lipid could easily be coloured by Sudan black. If sections were left for 40 min in the antemedia, the ones treated with xylene or toluene were not coloured by Sudan black (see table 1). This showed that there was no lipid in them. However, those that had been treated in benzene or chloroform for 40 min showed a positive result.

The lipid globules in the skin of the mouse sometimes showed a network-like structure, though they were black all through before the bleaching reagent was used; sometimes, however, these globules were homogeneously coloured by Sudan black. The lipid globules of the liver of the newt sometimes showed as a thick, black cortex, but in most cases they were homogeneously blue-black with Sudan black.

Sections were bleached with hydrogen peroxide, taken up to the same antemedia in which they had been dewaxed, left there for 5 min, and then brought down to 70% ethanol; eventually they were coloured in the usual way with Sudan black. The result was in all cases negative. This showed that the lipid-sites no longer contained lipids. The same results were obtained whether the antemedium was xylene, benzene, toluene, or chloroform.

From the above experiments the conclusion can be drawn that so long as the lipid-sites were still black, they contained lipid. The black substance must be a compound of lipid with osmium. This compound is insoluble in benzene or chloroform, but dissolves slowly in xylene or toluene; lipid is set free by bleaching.

When sections that had been dewaxed in benzene were treated with cold or boiling acetone, the osmicated lipid globules usually remained black all through, and could be blackened by Sudan black after bleaching. Sometimes, however, a certain amount of solution by acetone occurred, and the Sudan black was then distributed in the form of a network.

In sections treated with cold pyridine and bleached, the sudanophil material was in the form of a network. It is evident that partial solution by cold pyridine had occurred. After treatment with pyridine at 60° C, no osmicated lipids remained in the lipid-sites.

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