Letter to the Editor

Contrast Staining with Reduced Osmium Complexes


On the one hand, I was glad to see the revival of the use of ferrocyanide as an additive to OsO$_4$ post-fixation in LR White resin procedures. On the other hand, I was disappointed when I noticed that (in a historical sense) the authors were referring to Karnovsky's short 1971 abstract (2) and not to my more informative 1968 abstract (3), in which the use of reduced osmium was introduced, or to later works by White et al. (4) or Hayat (5). This aspect alone would not justify a letter to the editor. However, in the Discussion (1) (p. 1292, line 18 from the bottom) incorrect statements are present that might have escaped the referee's attention: "The mechanism of fixation by OsO$_4$ is not fully understood. Its powerful oxidizing effect may be responsible for the fixation of macromolecules (reference to Pearse). We speculate that the effects of osmic acid [sic!] on decreasing cytochemical reactivity are minimized by reduction with potassium ferrocyanide. Therefore, reactivity with moderately adequate fine structure is retained." This is followed by two references (1984 and 1993) about the use of reduced osmium for improving membrane contrast.

I will not exclude the possibility that the first line of the quotation may have been truncated in the process of typing and printing, and that it originally might have read, "is not fully understood by us," or "is not available to us," because original papers of earlier dates are parked along the electronic highway in library search systems, as, for example, in our library system, in which papers dated earlier than 1984 are no longer accessible electronically and papers one decade before that point require special hand searching.

To counteract the impact of this discussion and to contribute some information in a positive way, I give below a short summary of "scale" information about this aspect of reduced osmium tetroxide in papers from our laboratory between 1968 and 1984 excluding, in an unfriendly way, those from other authors active in that period:

1. After the initial introduction in 1968, the selectivity of Os(VIII)O$_4$ plus complex cyanides such as K$_4$Fe(III)(CN)$_6$ or K$_4$Fe(II)(CN)$_6$ for glycogen and membranes was given and illustrated in 1973 (6).
2. The use of reduced oxalatovan Os(VIII)O$_4$ and hexavalent osmium oxide compounds (Os(VI)O$_3$) in combination with ferrocyanide was explained and illustrated in (7).
3. The tissue-deposited complex formed between hexavalent osmium plus ferro- and ruthenium cyanide and the oxidation/reduction balance (requested by the authors) were explained and illustrated in 1975 (8).
4. The ligands in the tissue involved in the reduction with the osmium(VI) and iron(III) complexes were published in 1976 (9).
5. By X-ray microanalysis, the presence and atomic composition of both ferrocyanide- and ruthenium cyanide-reduced osmium compounds was determined both in non-aldheyde-fixed (10) and aldehyde-fixed glycogen (11) in 1980 and 1981.
6. The reduction of Os(VIII)O$_4$ by azale complexes was shown in 1984 (12) for animal and plant tissues (13). In another paper (12), the valence of the various osmium compounds in the tissue (glycogen) was also determined.
7. The reaction of osmate with sugar moieties was explained in 1984 paper by the late Prof. Riemersma and colleagues (14), who also participated in the very early discussions with Korn about membrane contrast.

I know that Tamaki and Yamashina (1) realize that presently active scientists are standing on the (knowledge) shoulders of those earlier active in the field, and that searching for that old stuff is difficult despite the availability of electronic systems. In general, however, I do hope that sentences like the ones I am opposing here will be completely banned from published reports, because such remarks can harm those who have devoted part of their scientific life to finding the truth as carefully as possible.

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Letter to the Editor

Contrast Staining with Reduced Osmium Complexes: Authors’ Response

We thank Dr. Bruijn for his detailed information about articles concerning osmium complexes. However, we could not help feeling a slight embarrassment at his comments on our recent Technical Note (1). His contributions analyzing the chemical natures of osmium complexes are quite respectable, but we believe there are some differences between the points he questions and our intentions. He appears to oppose only the first part of the paragraph in the Discussion. Reading the remainder of the paragraph carefully should make it quite obvious that our real intention was to discuss the relationship between post-fixation and “preservation of the cytochemical reactivity.” Therefore, the true intent of the sentence criticized by Dr. Bruijn was that “the mechanism of fixation of cytochemical reactivity with OsO₄ is not fully understood.”

Many investigators have employed ferrocyanide-reduced osmium as a useful tool for contrasting cytoplasmic elements, unit membranes in general, membranes in particular, and glycogen particles (4). Most of the articles, including those cited by Dr. Bruijn in his Letter, attempted to identify the mechanism of fixation or the staining effects on cell components. However, there remain a large number of undetermined aspects concerning the effects of post-fixation on the detection of cellular macromolecules by cytochemical reactions. We also employed reduced osmium to improve the morphological background on ultra-thin sections of a hydrophilic resin and, “to our surprise,” excellent preservation of cytochemical reactivities was obtained. As shown in our Note, a number of procedures have been designed to improve the balance of reactivity and morphology on sections of hydrophilic resins (5–7), but discussion about the relationships between the procedures and the improved results was still insufficient. We also conducted quantitative evaluations to compare our procedure with conventional methods. The results indicated that our procedure greatly improved morphological background while preserving cytochemical reactivity, although the mechanism of preservation remained to be elucidated. To establish a refined method of electron microscopic cytochemistry, cytochemists always seek better preservation of reactivity on an excellent structural background.

We are also grateful to Dr. Bruijn for noting our lack of precision in descriptions such as “osmic acid.”

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