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Mizuhira V & Futaesaku Y. New fixation method for biological membranes using tannic acids. *Acta Histochem. Cytochem.* 5:233-6, 1972.
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For good fixation of soluble polypeptides and proteins in tissue, tannic acid (TA) was added to ordinary aldehyde fixative as a prefixative, then postfixing was done with osmium tetroxide or other heavy metal salts. This method requires coexistence of the aldehyde group and TA and control of the pH range and TA concentrations. The sensitivity is very high—almost a hundred times higher than that for the aldehyde group—and the minimal concentration against protein is 1 mg per ml (0.1 percent). [The *SCI*® indicates that this paper has been cited in more than 150 publications, making it the most-cited article published in this journal.]

Fixation Method for Proteins Using Tannic Acid

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In 1969, while in Nashville, Tennessee, as a visiting professor at Vanderbilt University, I chanced upon a basket of beautiful persimmon fruit in a supermarket. This was an unexpected surprise because the persimmon is not common in the US. At home, I bit into one and, to my surprise, lost consciousness due to the astringency of the fruit. When I came to my senses about 30 minutes later, I found cloudy yogurt-like sediments in the saliva that had dripped from my mouth. In a flash, I realized that what I was seeing was a clue to the development of a new fixation method for peptides and proteins using tannic acid (TA).

After examining various works using TA, my colleagues and I found that it could be very useful for studying many kinds of peptides and proteins. However, there were several essential factors, such as the required coexistence of the aldehyde group and TA, control of pH, the number of composing amino acids, and the type of peptide. The sensitivity for soluble peptides is 100 percent of the recovery rate, with up to 0.1 to 0.3 percent TA against the radioactive isotope-labeled peptides.[1,3] If there was no aldehyde group, the material precipitated with TA could be recovered with an alcohol or alkali.[1-3] TA-osmium-protein complex displayed high electron opacity as well as good electrical conductance, showing that TA is also very useful for increasing electrical conductance and electron contrast for SEM observation of biological materials.[4]

Small molecular peptides composed of less than 10 amino acids could not be fixed with TA. Neutral, acidic, glyco-, phospho-, and metallc-proteins (peptides) could form insoluble precipitates with TA in different pH ranges.[1,3] Elastic fibers also could be fixed and stained for light and electron microscopy.

The method is also very useful for examining the ultrastructures of cross-sectioned microtubules. The 13 (A-tubule) and 11 (B-tubule) globular subunit structure can be directly and very clearly observed.[2,4,5]

Recently, we improved a microwave irradiation technique for examining biological samples using TA (0.1 percent) mixed with a fixative.[6] We also have obtained excellent ultrastructure views of many tissues and cells, using TA fixation for ultrastructural, autoradiographic, and X-ray microanalyzing methods.

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