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Sainte-Marie G. A paraffin embedding technique for studies employing immunofluorescence. *J. Histochem. Cytochem.* **10**:250-6, 1962.
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Years after the technique of immunofluorescence was developed, the procedure still inconveniently required frozen tissue sections. This paper reports why and how a method was developed to replace such preparations by sections of fixed and paraffin-embedded tissues. [The *SCI*® indicates that this paper has been cited in over 880 publications since 1962.]

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Before entering the laboratory of Albert H. Coons at Harvard Medical School as a postgraduate student, I had investigated plasmocyte formation in lymph nodes. I was fascinated by the variable and little-understood morphology of these organs and wanted to study their functioning during immune responses. The technique of immunofluorescence, developed by Coons about two decades earlier,[1] seemed most useful for such a study.

Initially, the technique of immunofluorescence employed sections of frozen tissues or of tissues dried and paraffin-embedded following freezing. This technique was used because conventional chemical fixation and the histological procedure for treatment of tissues inactivated antibodies and most antigens. However, I soon realized that cutting frozen lymph nodes was impractical for my morphological studies, which required abundant undamaged tissue sections. Moreover, macromolecules diffused from their original locations and were thus diluted in the process of tissue freezing. Fixation of these substances *"in situ"* and their concentration in cells shrunk by histological procedure were desirable.

I reasoned that if antibodies could be precipitated *in vitro* with cold alcohol and still remain active, the same should be possible in tissues. The problem, however, was that it was thought that antibodies would be rendered inactive as alcohol concentration reached 70 percent in tissues. To minimize the dreaded effect of alcohol and other reagents, the ratio of volume reagent:tissue was kept high, and tissues were agitated to accelerate fluid exchange. Moreover, the cold reagents were frequently renewed and the duration of each step of the histological procedure was minimized. Immunofluorescence observations obtained with tissues fixed and treated under different conditions (concentration or nature of reagents, size of tissue samples, duration of steps, temperature, and so on) were compared extensively to observations with frozen tissues. After fixation of tissue antibodies proved successful, trials were repeated with antigens. Then colleagues B.K. Watson and D.G. Scott kindly tested the new technique in their own work. My investigation was moreover facilitated by the work of J.H. Peters, H.O. McDevitt, and J.G. Harter,[2] who simultaneously developed a method to purify fluorescent conjugates.

If my paper is much cited, it is probably because the use of fixed instead of frozen tissues usually improves immunofluorescence observations. Paraffin sections also make it much easier to apply immunofluorescence and derived techniques to a wide range of studies.

1. **Coons A H, Creech H J, Jones R N & Berliner G.** The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. *J. Immunology* **45**:159-70. 1942. (Cited 410 times since 1955.)
2. **Peters J H, McDevitt H O, Pollard L W, Harter J G & Coons A H.** Purification of fluorescent conjugates by column chromatography. *Fed. Proc.* **20**:17. 1961.