This Week's Citation Classic

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Marchalonis J J. An enzymic method for the trace iodination of immunoglobulins and other proteins. *Biochemical J.* **113**:299-305, 1969.
[Walter and Eliza Hall Inst. Med. Res., Parkville. Victoria, Australia]

A simple, gentle, and reproducible method for the trace iodination of immunoglobulins and other serum proteins by a system consisting of purified lactoperoxidase, hydrogen peroxide, and radioiodide is described. [The *SCI®* indicates that this paper has been cited in over 830 publications since 1969.]

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"After obtaining my PhD in biochemistry with CM. Edelman, I went to the laboratory of G.J.V. Nossal at the Walter and Eliza Hall Institute as a postdoctoral fellow to learn cellular immunology. At the time I joined the laboratory, Nossal and G.L. Ada were carrying out a series of studies using radioiodinated protein antigens designed to ascertain the distribution of antigen in lymphoid tissues during various stages of immunization.[1] I happened to subject labeled antigen preparations to polyacrylamide gel electrophoresis and found evidence that the proteins had been severely denatured during the process of radioactive labeling which employed the oxidizing agent chloramine-T. This observation gave impetus to the search for a method which would allow the radioiodination of proteins under conditions which did not cause noticeable denaturation. Initially, I planned to use a mince of thyroid because thyroid peroxidases catalyze the covalent binding of iodide to the phenol ring of tyrosine. However, it was my good fortune to discuss this problem with a group of graduate students at a pub close to the University of Melbourne whereupon I learned that G.R. Jago and D.McC. Hogg, a graduate student in his laboratory, had purified the enzyme lactoperoxidase from milk. It could be anticipated that lactoperoxidase would act in a fashion very similar to the original enzyme system I had chosen. The availability of the purified peroxidase was an obvious advantage over any system which would involve a tissue mince. I obtained the lactoperoxidase and carried out a straightforward series of experiments designed to show that, first, radioactive iodide could be covalently bound to proteins using this enzyme activated by peroxide, and, then, that the labeled proteins retained their normal electrophoretic characteristics and that the label was covalently bound to tyrosyl residues.

"Why has this paper become so highly cited? In the first place, the timing of its appearance was auspicious because many workers in various fields were becoming involved in the use and application of radioimmunoassay technology, and it was clear to them that the then currently used major labeling approaches were not adequate for a number of reasons. The use of lactoperoxidase-catalyzed iodination provided a simple and gentle means to label material for radioimmunoassay, tissue localization, and biochemical studies. This was the first paper in what was to become a burgeoning technology where various adaptations and modifications were made to the basic scheme allowing high-level uptake of iodide[2] and tailoring of the conditions for various proteins. The second reason for broad citation of this paper is that the lactoperoxidase-catalyzed radioiodination approach proved to be a general means of labeling exposed tyrosyl residues in proteins on the outer surface of plasma membranes of living cells.[3-5] This paper started initially as an exercise in 'problem solving' focusing upon the issue of externally labeling proteins in the absence of denaturation. The solution to the problem turned out to have general applicability to many biochemical systems as well as to be the first step in the identification of surface proteins of living cells."

1. **Nossal G J V & Ada G L**. *Antigens, lymphoid cells and the immune response.* New York: Academic Press, 1971. 324 p.
2. **Thorell J I & Johansson B G**. Enzymatic iodination of polypeptides with ^{125}I to high specific activities. *Biochim. Biophys. Acta* **251**:363-9, 1971.
3. **Phillops D R & Morrison M.** Exposed protein on the intact human erythrocyte. *Biochemistry* **10**:1766-71, 1971.
4. **Baur S, Vitetta E S, Sherr C J, Schenkein I & Uhr J W.** Isolation of heavy and light chains of imraunoglobulin from the surfaces of lymphoid cells. *J. Immunology* **106**:1133-5, 1971.
5. **Marckalonis J J, Cone R E & Santer V.** Enzymic iodination: a probe for accessible surface proteins of normal and neoplastic lymphocytes. *Biochemical J.* **124**:921-7, 1971.