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This Week's Citation Classic®

Bessey O A, Lowry O H & Brock M J. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum.
J. Biol. Chem. **164**:321-9, 1946.
[Div. Nutrition and Physiology, Public Health Res. Institute of the City of New York, NY]

A method for serum alkaline phosphatase is described that depends simply on liberation, from a colorless reagent, of nitrophenol, which is highly colored in alkali but colorless in acid. Consequently, any blank for nonspecific serum absorption can be obtained by a shift in pH. [The *SCI*® indicates that this paper has been cited in over 1,850 publications since 1955.]

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Otto Bessey died in February 1984 at the age of 79. I am writing this in his place as a tribute to a truly great investigator and fine human being.

In 1941, a few months before Pearl Harbor, Otto was appointed as the first employee of the Public Health Research Institute of New York City. He had an excellent record in nutrition and was a pioneer in regard to biochemical consequences of malnutrition. He invited me to join him in the Division of Nutrition and Physiology, which he was to head. I was ignorant in nutrition but somewhat of a microanalytical nut, which Otto hoped would be useful.

After Pearl Harbor, we decided we could best contribute to the war effort by developing a battery of tests for appraising nutritional status in large populations. All blood assays were to be designed for samples obtained by finger stick. One was to be for alkaline phosphatase, a sensitive indicator of vitamin D deficiency. The Fiske and SubbaRow phosphate method had been useful for phosphatase assays but seemed unnecessarily complicated. King and Delory had studied a series of aromatic phosphates that changed color upon phosphate cleavage.[1] From their list, we chose p-nitrophenyl phosphate, partly because it was available from Eastman. This new reagent filled our requirements exactly. A 5μl volume of serum was added to 50μl of reagent and the absorption was measured directly 30 minutes later, after stopping the reaction with 0.5 ml of NaOH. Any (small) correction from nonspecific serum components was determined by a second reading after acidifying the sample.

The method was developed in essentially final form in the spring of 1944, and we used it extensively in nutrition surveys in New York City and State and then in Newfoundland "outports" with a peripatetic laboratory and Paul Zamecnik as a coerced co-analyst.

Before publishing, we fortunately discovered that Ohmori[2] and Fujita[3] had used p-nitrophenyl phosphate for the same purpose and clearly had priority. Nevertheless, we published anyway, because our procedure was simpler, required less plasma, and the *Journal of Biological Chemistry* had a much wider circulation in those days than the *Journal of Biochemistry* (Japan) or *Enzymologia*.

I expect the success of the method has been due to its obvious simplicity, the early availability of the substrate in stable form, the clinical relevance of serum phosphatases, and the general research interest in a wide spectrum of tissue phosphatases.

***Postscript*: After the war, Eastman stopped making p-nitrophenyl phosphate. Coming back from a Federation meeting, I mentioned this to Dan Broida, who was just cranking up Sigma, and suggested he might like to make it instead. He obliged (and made a more stable product) and used to say that this was a factor in his really getting involved in manufacturing top-notch biochemicals. Perhaps a little acorn for his oak?**

1. **King E J & Delory G E.** The rates of enzymic hydrolysis of phosphoric esters. *Biochemical J.* 33:1185-90, 1939.
2. **Ohmori Y.** Über die Phosphomonoesterase. *Enzymologia* 4:217-31, 1937.
3. **Fujita H.** Über die Mikrodestimmung der Blut-Phosphatase. *J. Biochem. Tokyo* 30:69-87, 1939.

