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This Week's Citation Classic

Lowry O H & Lopez J A. The determination of inorganic phosphate in the presence of labile phosphate esters. *J. Biol. Chem.* **162**:421-8, 1946.
[Div. Nutrition and Physiol., Public Health Res. Inst., New York, NY]

Earlier colorimetric methods for inorganic phosphate, based on phosphomolybdate reduction, caused rapid cleavage of labile organic phosphates such as phosphocreatine or acetyl phosphate. This problem was solved by using a more powerful reducing agent, ascorbic acid, at a much less acid pH. [The *SCI*® indicates that this paper has been cited over 970 times since 1961.]

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"Herman Kalckar was stranded in the US during World War II and was hired by Otto Bessey to work with us in the newly formed Public Health Research Institute of New York City. There Kalckar discovered that hepatic nucleosidase, thought to be a simple hydrolase, was actually a phosphorylase,[1] and that one product, ribose-1-P, is very unstable under conditions for P_i assay by the standard Fiske and Subbarow[2] method. This made it impractical to measure the enzyme by P_i appearance or disappearance. I had been fiddling with the analytical conditions of the P_i assay (to try to increase sensitivity) and bet Herman that I (meaning of course Jeanne Lopez) could easily find conditions that would overcome this problem. Jeanne and I won (I forget what the bet was) but it took several months and a notebook full of experiments.

"Most early P_i methods depended on the fact that the phosphomolybdate complex is much more readily reduced (to give a blue color) than is molybdate itself. Bell and Doisy[3] were probably the first to use this principle to design a practical biological method, but the Fiske and Subbarow procedure became better known (and certainly would have been a Citation Classic in those days –and perhaps still is?). I knew both Fiske and Subbarow quite well, and Fiske had told me (between long pauses to puff on his pipe) some of the ways this exceedingly complex and versatile system can be manipulated.

"Four variables affect the reduction of molybdate and phosphomolybdate: pH, temperature, molybdate concentration, and the reducing agent. The first three affect hydrolysis of most organic phosphates. We started by tampering with pH, using the reducing agent of Fiske and Subbarow (sulfite with aminonaphtholsulfonic acid catalyst). When the pH was raised from that recommended (0.65) to the range between 0.8 and 1.8, molybdate itself was reduced. Above pH 3.2, phosphomolybdate was only slowly reduced. This left a pH window between 1.8 and 3.2 that could have been used with some gain in organic phosphate stability. However, by substituting ascorbic acid as the reducing agent, the useful window was shifted even further, to pH 3.5-4.5, and permitted the use of a lower molybdate concentration. (Ascorbic acid had been employed earlier by Ammon and Hinsberg,[4] but only in strong acid.) These changes increased stability 15-fold for P-creatine, and over 100-fold for ribose-1-P and acetyl-P and made it easy to measure P_i in their presence.

"The generous number of citations of this paper merely reflects the tremendous importance of phosphate transactions in all manner of metabolic systems. However, this paper may be cited much less in the future. This is because of the discovery that phosphomolybdate gives 20 or 30 times more color when complexed with malachite green than when it is reduced.[5] Moreover, the reaction is nearly instantaneous. This probably means that even though strongly acid conditions are required, with a little more tinkering the absorption can be read in the presence of most phosphate esters before disturbing hydrolysis has occurred."

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1. **Kalckar H M.** Enzymatic synthesis of a nucleoside. *J. Biol. Chem.* **158**:723-4, 1945.
2. **Fiske C H & Subbarow Y.** The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**:375-400, 1925. [The *SCI*® indicates that this paper has been cited over 10.300 times since 1961.]
3. **Bell R D & Doisy E A**. Rapid colorimetric methods for the determination of phosphorus in urine and blood. *J. Biol. Chem.* **44**:55-67, 1920.
4. **Ammon R & Hinsberg K.** Colorimetrische Phosphor- und Arsensaurebestimmung mit Ascorbinsaure. *Z Physiol. Chem.* **239**:207-16, 1936.
5. **Itaya K & Ui M**. A new micromethod for the colorimetric determination of organic phosphate. *Clin. Chim. Acta* **14**:361-6, 1966.