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Citation Classics

Chen P S, Toribara T Y & Warner H. Microdetermination of phosphorus. *Analyt.Chemistry* **28**:1756-58, 1956.

This paper presents a method for phosphorus determination sufficiently sensitive to dispense with microtechniques and special glassware and apparatus. The procedure also possesses the advantages of stability, constancy, and linearity. An ascorbic acid method for the reduction of phosphomolybdate is applied to the determination of phosphorus in whole blood, plasma, serum, and urine with a sensitivity about 8 times greater than that of the aminonaphtholsulfonic acid method. [The *SCI*® indicates that this paper was cited 1,984 times in the period 1961-1975.]

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"This method was worked out in early 1954 immediately after the writing had been completed on my PhD thesis in pharmacology at the University of Rochester. Entitled 'Studies on the Renal Excretion of Calcium,' the thesis describes calcium excretion by the dog as a function of its physico-chemical state in the serum (i.e., ultrafilterability) and as influenced by the serum level of various ions (calcium itself, strontium, phosphate) and of various pharmacologic agents (complexing agents, metabolic inhibitors, etc). The dependent relationship of serum calcium and inorganic phosphate was an important variable in these studies. During my thesis research, I used the well-known Fiske and Subbarow colorimetric method to determine inorganic phosphate in blood serum and urine.

"As a graduate student, I was greatly influenced and aided by Dr Taft Toribara, an analytical chemist who had trained under the renowned H.H. Willard at the University of Michigan. Taft's wise counsel on such problems as ultrafiltration and calcium analysis contributed greatly to my own research style.

"By the end of 1953, my thesis was written and submitted to my thesis advisor, Dr. William Neuman; and other members of my graduate committee for review prior to the final oral examination With some leisure time on my hands, and a happy feeling of having completed an arduous period of training, I happily perused a wide range of publications in the medical school library. Early in 1954, I read the publication by Lowry et al.1 describing their microanalytical studies of a variety of constituents of brain tissue. Their article revived my interest in the analytical problems relating to phosphate determination in which I had a continued interest.... On the basis of a number of experiments run in the spring of 1954, it appeared that ascorbic acid reduction of the phosphomolybate complex would not only result in a blue color with much greater molar absorbancy than the product produced by aminonaphtholsulfonic acid in the Fiske-Subbarow method, but offered a number of other advantages as well...

"During the summer of 1954, Huber Warner, who had just finished his freshman year at Williams College, came to work as a summer employee in Dr. Toribara's laboratory. He was assigned the task of comparing the new ascorbic acid method with the Fiske-Subbarow method. Thus by the end of the summer of 1954, the basic characteristics of the procedure had been worked out... The paper was.finally written and submitted to *Analytical Chemistry* about the time that I left the University of Rochester to assume a position at the National Heart Institute, National Institutes of Health.

"Over the years, I have received a number of personal testimonials from friends and colleagues who had found the method useful in determining phosphorus in a wide variety of biological samples. I believe the basic reaction has proven itself over the course of time to be a most reliable and useful technique and the authors are most gratified at the kindly reception which it has received from a broad spectrum of users."

1. **Lowry O H, Roberts N R, Leiner K Y, Wu M L & Farr A L**. The quantitative histochemistry of brain. I. Chemical methods. *J. Biol.Chem* **207**:1-17, 1954.