

**Classic Articles**

"[THE COLORIMETRIC DETERMINATION OF PHOSPHORUS](#)" Fiske, et al., 66: 375–400.

"[PHOSPHOCREATINE](#)" Fiske, et al., 81: 629–679.

The Determination of Phosphorus and the Discovery of Phosphocreatine and ATP: the Work of Fiske and SubbaRow

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The Colorimetric Determination of Phosphorus (Fiske, C. H., and SubbaRow, Y. (1925) *J. Biol. Chem.* 66, [375–400](#))

Phosphocreatine (Fiske, C. H., and SubbaRow, Y. (1929) *J. Biol. Chem.* 81, [629–679](#))

Cyrus Hartwell Fiske (1890–1978) received an A.B. degree from the University of Minnesota in 1910 and an M.D. degree from Harvard in 1914. From 1914–1917 he spent two years of study in biochemistry at Harvard Medical School followed by two years at Western Reserve School of Medicine. He then joined the faculty at Harvard as an Assistant Professor and rose through the ranks to Professor in 1937 before retiring in 1957.

Yellagapada SubbaRow (1896–1948) came to Harvard in 1923 to study tropical medicine after completing his medical training in India. After receiving a diploma from the Harvard School of Tropical Medicine, he became interested in biochemistry and started working with Fiske. He received his Ph.D. in 1930 in part for the work described in these Classic papers. With the support of Otto Folin, Chairman of Biological Chemistry and the author of a previous *Journal of Biological Chemistry* (JBC) Classic (1), he was appointed to a junior faculty position. In 1940, he moved to Lederle Laboratories where he worked until his death in 1948.

Fiske and SubbaRow, the two names are nearly inseparable, made many discoveries together, but the most notable and lasting resulted from their work on the metabolism of muscle tissue leading to the discovery of both phosphocreatine and ATP (2–4). Neither discovery, however, would have been possible without the simple, accurate phosphate determination method they developed.

The colorimetric determination of phosphorus described in the first paper of this JBC Classic set is one of the most highly cited papers in the history of biochemistry and certainly the one for which Fiske and SubbaRow are most remembered. Citation information from ISI indicates that this paper is the fourth most highly cited JBC paper of all time with over 19,000 citations as of March 2001. This a large underestimate because ISI only started to monitor citations in 1945, and this paper was published in 1925 so there are 20 years of uncaptured citations. (For reference, the most highly cited JBC paper is the method for protein determination developed by Oliver Lowry and co-workers, which was published in 1951, after ISI started collecting citation data, with about 250,000 citations. The Lowry paper and the Folin-Ciocalteu paper, which was the basis of the Lowry method, will be reprinted in a future installment of JBC Classics.) As with many methodological studies, this one built on earlier work, particularly the work by Doisy, the author of previous JBC Classics (5). It attempted to resolve shortcomings in three areas, stability of the color reaction, sensitivity, and interfering substances. The original assays for phosphorus relied on the reduction

of phosphomolybdic acid by hydroquinone to form a blue color. This reduction was slow and influenced by various interfering substances. Somewhat by chance, they tried 1-amino-2-naphthol-6-sulfonic acid as the reducing agent with great improvement in results. Although the assay was developed initially for use with urine and blood samples, it was quickly adapted for many other kinds of samples and for use in measuring, not only inorganic phosphorus but organic phosphorus as well.

The inquiry into phosphate compounds of muscle filtrates may well have been initiated because of the work of Otto Folin, chairman of their department, on nitrogenous compounds in blood and urine including creatine. Fiske and SubbaRow were able to demonstrate that much of the phosphate in muscle filtrates was not inorganic phosphate, as had been believed, but a compound that was equimolar creatine and phosphate. They also were able to show that phosphocreatine was hydrolyzed during muscle contraction and resynthesized during the recovery period (2, 3). This JBC Classic, although not their first report of phosphocreatine, presents the most thorough characterization of both its chemical and physiological properties. This paper also provides an interesting glimpse into the competition involved in the discovery of both phosphocreatine and ATP between Fiske and SubbaRow and Karl Lohmann, a member of Meyerhof's group in Germany. On page 630 of the phosphocreatine JBC Classic reprinted here, there is the following passage related to the method for determining phosphorous: "Lohmann and Jendrassik, in Meyerhof's laboratory, finding also that color develops slowly in the case of muscle filtrates, and assuming the presence of some interfering substance to be responsible, adopted the use of heat (as suggested by us earlier) under the guise of a new modification of the method. Since Lohmann and Jendrassik's interpretation of the delayed reaction is not correct, their criticisms of our technique are altogether pointless."

The competition between the two groups continued over the discovery of ATP. Fiske and SubbaRow published their isolation and characterization of "adenosinetriphosphate" in 1929 (4). Also in 1929, Lohmann published his identification of "adenylpyrophosphate" (6).

Fiske provided a supportive research/training environment. One of his Ph.D. students, George H. Hitchings, had come to Harvard Medical School as a Teaching Fellow in Biological Chemistry intending to work with Otto Folin. Folin sent him to Fiske for the first year, as was his practice, and Hitchings was caught up in the work of Fiske and SubbaRow and decided to complete his thesis work in this laboratory. His project was to develop analytic methods for the determination of purine bases for physiological studies. This work provided the foundation for a career developing cancer chemotherapies based on purine and pyrimidine analogs. Hitchings received the Nobel Prize in Physiology or Medicine, along with James W. Black and Gertrude B. Elion in 1988.¹

Footnotes

¹ We thank Eugene Kennedy, Professor Emeritus of Biological Chemistry at Harvard Medical School, for providing much of the biographical information for Cyrus H. Fiske. Professor Kennedy was Chairman of the Department of Biological Chemistry during the later stages of Professor Fiske's career.

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2. Fiske C. H., SubbaRow Y. (1927) *Science* 65:401. [FREE Full Text](#)
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5. JBC Classic: Volar C D, Thayer S, and Doisy F A (1930) *J Biol Chem* 87: 357-371; Thayer S A, Levin I, and Doisy F A (1931) *J Biol Chem* 91: 655-665; MacCormacdale D W, Cheney I C, Binkley S R, Holcomb W F, McKee R W, Thayer S A, and Doisy F A (1930) *J Biol Chem*, 131, 357-370 (<http://www.jbc.org/cgi/content/full/277/28/e17>).
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