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Bartlett G R. Phosphorus assay in column chromatography.
J. Biol. Chem. 234:466-8, 1959.
[Scripps Clinic and Research Foundation, La Jolla, CA]

A modification of the Fiske and SubbaRow method[1] for the analysis of phosphorus is described that greatly increases the method's sensitivity and reliability and is of special value for multiple total phosphorus determinations on eluates from chromatographic columns. [The *SCI*® indicates that this paper has been cited in over 6,650 publications since 1959.]

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After working in the 1940s on various aspects of carbohydrate metabolism in different tissues from rat kidney to yeast to higher plants, first with E.S.G. Barron at the University of Chicago and then with Eaton MacKay at the Scripps Clinic in La Jolla, I decided to concentrate on the red blood cell. The mature human erythrocyte was already known at the time to have an exclusively anaerobic metabolism, with glucose being converted almost quantitatively to lactate and with expected participation of pyridine and adenine nucleotides. Importantly, there were indications that this metabolism was essential for the survival of the cell in the circulation and during blood-bank storage. It seemed to me that the relatively simple and easily accessible red cell might prove a good model for studies of metabolic controls in normal and deficient cells. I felt that one good approach would be to isolate as many of the intermediate metabolites as possible for quantitative assay and for measurement of their turnover after incubation of the red cell with ^{14}C-labeled glucose and ^{32}P-inorganic phosphate using miscellaneous experimental probes to alter the metabolism.

During World War II, there was a revolutionary development in chromatographic techniques because of the availability of new organic polymers to which were attached positive or negative groups. These so-called ion-exchange resins have proved to be extremely useful for the separation and quantitative recovery of a large variety of natural products. An early use was the separation of purine and pyrimidine bases and their nucleoside and nucleotide derivatives, notably by Waldo Cohn and colleagues at Oak Ridge.[2] The isolation of these compounds was facilitated greatly by the fact that all had high absorption in the ultraviolet.

I started to explore the use of ion-exchange column (now called liquid) chromatography for the isolation of the intermediate metabolites of the red cell, and it soon became apparent that the study would be accelerated greatly by having a method of high sensitivity for assay of organic phosphorus suitable for multiple assays of column eluate fractions. We were able to develop a modification of the Fiske and SubbaRow procedure with a several-fold increase in sensitivity and much greater color stability, which was published, along with its use for ion-exchange liquid chromatographic analysis of red cell metabolites, in the March 1959 issue of the *Journal of Biological Chemistry*.

I moved to the Laboratory for Comparative Biochemistry in 1962, where I have continued to use this phosphorus assay method extensively in a series of studies on the metabolism of fresh and stored human red cells[3,4] and in studies on phosphate compounds in red cells of birds, reptiles, and fishes.[5]

In visits to other laboratories around the world, I am always surprised and pleased to find many still using our phosphorus method.

1. **Fiske C H & SubbaRow Y.** The colorimetric determination of phosphorus. *J. Biol. Chem.* 66:375-400, 1925. (Cited 13,925 times since 1955.)
2. **Cohn W E.** The separation of nucleic acid derivatives by chromatography of ion-exchange columns. (Chargaff E & Davidson J N, eds.) *The nucleic acids*. New York: Academic Press, 1955. Vol. 1. p. 211-41.
3. **Bartlett G R.** Phosphorus compounds in the human erythrocyte. *Biochim. Biophys. Acta* 156:221-30, 1968. (Cited 65 times.)
4. ----------. Red cell metabolism: review highlighting changes during storage. (Greenwalt T J & Jamieson G A, eds.). *The red cell in vitro*. New York: Grune & Stratton, 1974. p. 5-29.
5. ----------. Phosphorus compounds in vertebrate red blood cells. *Amer. Zool.* 20:103-14, 1980.

