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This Week's Citation Classic

Waddell W J. A simple ultraviolet spectrophotoraetric method for the determination of protein. *J. Lab. Clin. Med.* **48**:311-14, 1956. [Dept. Pharmacology, Univ. North Carolina School of Medicine, Chapel Hill, NC]

The method utilizes the ultraviolet absorbancy of the peptide bond instead of that of aromatic amino acids. This permitted greater sensitivity, accuracy, and specificity than was possible with methods previously described. [The *SCI*® indicates that this paper has been cited over 700 times since 1961.]

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"Younger biologists might well wonder why the National Institute of Neurological Diseases and Blindness supported the apparently isolated development of a quantitative method for protein in solution. The National Institutes of Health (NIH) grant which supported the work in the mid-1950s actually was for the study of the chemical and physical disposition of antiepileptic agents. However, at that time investigators were freer to use their judgment to follow valuable leads during the progress of the work even if these leads were not at all a part of the original application.

"We decided to investigate the effect of acid-base alterations in dogs on the disposition of the anticonvulsants which are weak acids. Infusion of sodium bicarbonate intravenously promoted redistribution of phenobarbital from the brain to blood and hastened the renal excretion of this drug.[1]

"The infusion of sodium bicarbonate intravenously in dogs also produced hemolysis.[2] The question that arose was whether the membrane of these cells was contracting or whether the cell was increasing in volume. It was thought that this could be measured from the dilution of a protein solution in which the red cells were suspended. The protein methods available did not satisfy the investigator. This protein method, which is now a *Citation Classic*, was devised to answer the red cell question and then published as a general method since it had advantages of greater simplicity, rapidity, sensitivity, accuracy, and specificity than other methods. It is for this reason that the method is widely used.

"In addition, this research project clarified the principle by which weak acids and bases distribute among compartments of different pH values; this resulted in a method for determination of intracellular pH values.[3] A metabolic product (DMO) of one of the anticonvulsants appeared to be almost ideal for intracellular pH measurements and is now widely used for that purpose.

"The current wisdom of granting agencies in requiring tightly planned, detailed, and highly focused applications which are carefully followed by the investigator might answer some questions. In my opinion, however, it is unfortunate that funding is a thing of the past for investigations that are broad but that acquire original, unanticipated, and useful knowledge."

1. **Waddell W J & Butler T C.** The distribution and excretion of phenobarbital. *J Clin. Invest.* **36**:1217-26, 1957.
2. **Waddell W J.** Lysis of dog erythrocytes in mildly alkaline isotnnic media. *Amer. J. Physiol.* **186**:339-42, 1956.
3. **Waddell W I & Butler T C.** Calculation of intracellular pH from the distribution of 5,5-dimethyl-2,4-oxazolidinedione (DMO). Application to skeletal muscle of the dog. *J. Clin. Invest.* **38**:720-9, 1959