CC/NUMBER 2
JANUARY 14, 1980

# This Week's Citation Classic

**Woessner J F, Jr.** The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch. Biochem. Biophys.* **93**:440-7, 1961. [Labs. Biochem., Howard Hughes Med. Inst., and Dept. Biochem., Univ. Miami Sch. Med.. Miami, FL]

**This paper presents an improvement on the method of Stegemann[1] for the determination of hydroxyproline and an extension of that method for the determination of hydroxyproline in samples containing as little as one part of hydroxyproline in 4,000 parts of other amino acids. [The *SCI*® indicates that this paper has been cited over 645 times since 1961.]**

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July 19. 1979

"Collagen is one of the most widely distributed proteins in the animal kingdom. It is found in most tissues and is intimately involved in many important disease processes including atherosclerosis, arthritis, cirrhosis, and tumor invasion. It is not surprising, therefore, that there is great interest in the measurement of hydroxyproline, an amino acid which is found almost exclusively in collagen and which provides a direct measure of collagen content. There have been at least five other published methods that, in my estimation, could be considered 'Citation Classics'.[1,5]

"My interest in hydroxyproline measurement began in the early fifties during my doctoral work at the Massachusetts Institute of Technology involving the measurement of small amounts of collagen produced in tissue culture. The best method at that time was barely adequate for the purpose.[4] It was based on oxidation of hydroxyproline with hydrogen peroxide, followed by coupling of the resultant pyrrole to dimethylaminobenzaldehyde. The color development was variable due to difficulty in removing the last traces of peroxide. High levels of amino acids tended to depress color formation, while other compounds such as tyrosine gave extraneous color Fortunately, these errors tended to balance one another.

"In 1958 a classic method was published by Stegemann.[1] Almost all current methods of hydroxyproline determination trace their roots to this work. Stegemann introduced the use of chloramine -T as an oxidizing agent in place of peroxide. Unfortunately, this paper did not enjoy the popularity it deserved because it was written in German. Part of my contribution was to present the method in an English version. At the same time, I was able to increase the intensity and stability of the final color. More important was the modification of the method so that hydroxyproline could be measured in the presence of large amounts of other amino acids. Recalling earlier problems caused by peroxide, I turned this around and used peroxide to destroy the final chromagen. The remaining color was taken as a blank attributable to interfering substances.

"At that time, and continuing to the present, it has been my practice to have one or two high school students in the laboratory in the afternoons and summers to become acquainted with scientific research. When I had written the method in such a form that the current students were able to carry through the assay successfully on their own, it was deemed suitable for publication.

"In 1976, while preparing a chapter on hydroxyproline determination, I found that over 60 methods and modifications had appeared in the literature since 1961, with a current rate of 5-7 per year.[6] Although several of these offer advantages over my original method, none has improved on the determination of hydroxyproline in the presence of large amounts of other amino acids. This feature continues to be important for the assay of small amounts of hydroxyproline in serum, culture media, and tissues of low collagen content. However, the continuing outpouring of methods gives eloquent testimony to the fact that the ideal hydroxyproline assay method has yet to be published."

1. **Stegemann H.** Mikrobestimmung von Hydroxyprolin mit Chloramin-T and p-Dimehylamino-benzaldehyd. (Microdetermination of hydroxyproline with chloramine-T and p-dimethlamino-benzaldehyde.) *Hoppe-seylers Z. Physiol. Chem* **311**:41-5, 1958.
2. **Bergman I & Lovley R.** Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal. Chem.* **35**:1961-5,1963
3. **Prockop D J & Udenfriend S.** A specific method for the analysis of hydroxyproline in tissues and urine. *Anal. Biochem.* **1**:228-39, 1960.
4. **Neuman R E & Logan M A.** The determination of hydroxyproline. *J Biol Chem* **184**:299-306,1950.
5. **Juva K & Prockop D J.** Modified procedure for the assay of $H^3$- or $C^{14}$- labeled hvdroxyproline. *Anal. Biochem.* **15**:77-83, 1966.
6. **Woessner J F, Jr.** Determination of hydroxyproline in connective tissues. *The methodology of connective tissues research.* (Hall D A. ed.) Oxford. England: Johnson-Bruvvers Ltd. 1976, p. 235-45.