

Stegemann H. Mikrobestimmung von Hydroxyprolin mit Chloramin-T und *p*-Dimethylaminobenzaldehyd. (Microdetermination of hydroxyproline with chloramine-T and *p*-dimethylamino-benzaldehyde.)
Hoppe-Seylers Z. Physiol. Chem. 311:41-5, 1958.
[Med. Forschungsanstalt Max-Planck-Gesellschaft. Silikose-Labor. Göttingen. Fed. Rep. Germany]

This paper introduced a hydroxyproline determination after chloramine-T-oxidation in a buffer. It prompted a series of papers along this line, since less interference by other amino acids and easier decomposition of the oxidant (compared to H₂O₂ used earlier) was observed with this method. [The SCJ® indicates that this paper has been cited in over 745 publications since 1958.]

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As a PhD student in the Kaiser-Wilhelm-Institut für Biochemie in Tübingen (the French Zone of postwar Germany kept this name longer; the American and British Zones changed it to Max-Planck-Institut), I was attracted by protein chemistry in 1946, and have been ever since. In Göttingen in 1952, again in an MPI (Medizinische Forschungsanstalt), I worked with a most lovable "oldtimer," Karl Thomas, who had introduced the idea of calories into life science and had now turned from nutritional problems to occupational diseases.¹

In order to determine the cause of collagen formation in the lung due to inhalation of quartz and coal, minerals were isolated from lung tissue by a neutral formamide proteolysis. Residual tissue was most reliably detected by hydroxyproline determination. The excellent method originated by Neuman and Logan² is disturbed by tyro-

sine. Since it is based upon oxidation by hydrogen peroxide in alkaline medium, the precipitating Ca⁺⁺ interfered in samples of mineral-containing tissue. We looked for a handy, easily destroyable oxidant without color formation by tyrosine and found chloramine-T. The pyrrole/aldehyde reaction was done in HClO₄ instead of H₂SO₄, to avoid precipitates with calcium salts. We searched for a compatible medium for the tricky oxidation and introduced complexing buffers. Phosphate, borate, and citrate worked quite well, but in the last days of 1957, the buffers in our lab were all used up. Since the institute was usually closed between mid-December and early January, no technical help was available, and I was too lazy to mix a new buffer. However, there was enough left of an acetate/citrate buffer of pH 5, which we used for our Stein and Moore columns (by the way, we introduced the first one-column separation for all amino acids³). As it turned out, this was the buffer of choice for several reasons. The triad of chloramine-T, buffer, and usually perchloric acid has remained a characteristic of hydroxyproline determinations. The original paper was published in German. English versions with minor changes appeared: Prockop and Udenfriend,⁴ Stegemann and Stalder,⁵ and so on. Woessner, who mentioned this in his *Citation Classic*,⁶ employed a modification of this technique to be used when other amino acids are in large excess.⁷

The *Science Citation Index*® counts 745 citations to the original paper. The method, still applied after 26 years, is not due to an ingenious discovery, but rather to a convenient oxidant and a fitting buffer. Popularity today is mainly based on the great interest hydroxyproline is receiving in medical, food, and plant science.

1. Thomas K. Fifty years of biochemistry in Germany. *Annu. Rev. Biochem.* 23:1-16, 1954.
2. Neuman R E & Logan M A. The determination of hydroxyproline. *J. Biol. Chem.* 184:299-306, 1950. (Cited 920 times since 1955.)
3. Stegemann H. Die Gesetzmäßigkeiten der Aminosäuretrennung an Austauschersäulen bei kontinuierlicher Elution. *Hoppe-Seylers Z. Physiol. Chem.* 319:87-101, 1960.
4. Prockop D J & Udenfriend S. A specific method for the analysis of hydroxyproline in tissues and urine. *Anal. Biochem.* 1:228-39, 1960. (Cited 950 times.)
5. Stegemann H & Stalder K H. Determination of hydroxyproline. *Clin. Chim. Acta* 18:267-73, 1967. (Cited 340 times.)
6. Woessner J F. Citation Classic. Commentary on *Arch. Biochem. Biophys.* 93:440-7, 1961. *Current Contents/Life Sciences* 23(2):10, 14 January 1980.
7. -----, The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. *Arch. Biochem. Biophys.* 93:440-7, 1961. (Cited 950 times.)