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This Week's Citation Classic

Goodwin T W & Morton R A. The spectrophotometric determination of tyrosine and tryptophan in proteins. *Biochemical J.* **40**:628-32, 1946. [Johnston Labs., Dept. Biochemistry, Univ. Liverpool, England]

The availability for the first time of a photoelectric spectrophotometer allowed the accurate determination of tyrosine and tryptophan in solutions containing mixtures of the two. The method was adapted to assay these amino acids in unhydrolysed protein solutions. [The *SCI*® indicates that this paper has been cited over 1,175 times since 1961.]

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"It is somewhat ironic that after I have spent over 30 years investigating carotenoid and sterol biochemistry, I should receive an accolade of a *Citation Classic* for the very first paper I published in the *Biochemical Journal* on a topic remote from terpenoids. A moment's thought, however, reveals that this selection is purely an alphabetical accident. The work reported in this paper began when I was a research student and it was conceived and directed by my supervisor, the late R A. Morton. It was completed during the war in spare time during the tenure of a research assistantship in Morton's laboratory. Morton can be considered one of the true founders of biochemical spectroscopy and, like me, never returned to amino acids after this investigation.

"The early spectrometric apparatus used to analyse binary mixtures of substances with overlapping absorption spectra had neither the sensitivity nor accuracy required for satisfactory results. The appearance of the Beckman photoelectric spectrophotometer changed this situation overnight and the availability to us of one of the first of these machines to be produced allowed us to develop an accurate method of tryptophan and tyrosine analysis. The knowledge that tyrosine and tryptophan are the only amino acids in proteins which significantly absorbed ultraviolet light above 220 nm and that their spectra were essentially unaltered in peptide linkage (only later did more sophisticated methods reveal small but significant differences related to the micro-environment of these amino acids in proteins) allowed us to propose a reasonably accurate method of assay after a means of correcting for scattered light caused by the opalescence of protein solutions had been worked out.

"It is interesting to record why Morton's laboratory was the first academic department in the UK to obtain a photoelectric spectrophotometer (Shell Research had the .first). Morton, because of his expertise in vitamins and spectroscopy, had been asked by the British Ministry of Food to control a programme of vitaminization of margarine and other products, and to take part in a large collaborative investigation on determining the vitamin A requirement of humans. I was the research assistant employed on these projects. After much discussion it was agreed that in order to forward this work, Morton should be provided with the Beckman spectrophotometer under the Lend-Lease agreement between the US and UK governments.

"The main reason why the method became popular and is still quoted is that it is simple; it can be carried out directly on a solution of a protein and no hydrolysis is necessary, a particularly important advantage when the instability of tryptophan to alkaline hydrolysis is remembered; furthermore, no derivatization was necessary. The second main reason at the time the paper was published was the relatively small amount of protein required for an assay –25 mg! This requirement would certainly be a major disadvantage today.

"The method has survived into the present time probably because of the correctness of its basis, its simplicity, and the fact that with modern instrumentation it can be scaled down to use acceptable amounts of protein. Further information on the subject has been reported by T.T. Herskovits."[1]

1. **Herskovits T T.** Difference spectroscopy. *Methods Enzymol.* **11**:748-75, 1967.