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# This Week's Citation Classic

**Duncombe W G.** The colorimetric micro-determination of long-chain fatty acids. *Biochem. J.* **88**:7-10, 1963.
[Wellcome Research labs, Beckenham, Kent, England]

**Long-chain fatty acids dissolved in chloroform will form chloroform-soluble copper soaps. The reaction of these with a chromogenic reagent for copper is the basis for the determination described. [The *SCI*® indicates that this paper has been cited over 705 times since 1963.]**

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"The start of this work was the need for a sensitive quantitative analysis for radioactive long-chain fatty acids eluted from paper chromatograms, so that their specific activities could be determined. I found a reference to a method in which the copper soaps of such acids were analysed by spectroscopy of their solutions in chloroform, making use of their blue colour.[1] However, the sensitivity was not adequate for my purpose, so thoughts about increasing it naturally followed, and it needed no great intellectual feat to wonder whether using one of the chromogenic reagents for copper would have the desired effect. Further search in the literature failed to show that anyone had done this, so I pursued the idea and developed a sensitive and reproducible method for the analysis of solutions of fatty acids.

"Ironically, the application for which the method was devised proved to be unsatisfactory. Extracts from paper gave high and unreproducible blanks, which could not be made acceptable by pre-washing the paper and purifying the solvents. However the basic method seemed to be worth publishing, and soon reports started appearing in the literature in which it was being used in a variety of applications in which the concentration of long-chain fatty acids extracted from biological materials was required.

"A further development led to increased use. Non-esterified fatty acids in blood plasma had previously been determined by titration. An adaptation[2] of the colorimetric method now permitted this determination to be carried out directly on plasma. Although not entirely specific under these conditions, comparison trials showed that the colorimetric method gave results comparable with those obtained by titration, and it began to be adopted for clinical and other investigations in which changes in fatty acid levels are more important than absolute values.

"It is of course well known that the frequency of citation of a paper is no criterion of its usefulness or significance. Happily this does not seem to be the case with the paper in question, since citations mostly occur when the method or some variation on it is actually being used. Improvements and modifications are indeed suggested from time to time, but perhaps this too is a reflection of the basic value of the method."

1. **Iwajamn Y.** New colorimetric determination of higher fatty acids. *Yakugaku Zasshi* **79**:552-4, *Chem. Abstr.* **93**:14819, 1959.
2. **Duncombe W G.** The colorimetric micro-determination of non-esterified fatty acids in plasma. *Clin. Chim. Acta* **9**:122-5, 1964.