

## This Week's Citation Classic

Kirk J T O. Studies on the dependence of chlorophyll synthesis on protein synthesis in *Euglena gracilis*, together with a nomogram for determination of chlorophyll concentration. *Planta* 78:200-7, 1968. [Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth]

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**This paper, which includes a nomogram for chlorophyll determination, argues that the dependence of pigment synthesis on protein synthesis is not due to the lability of the enzyme used to make the chlorophyll precursor, d-aminolae-vulinic acid, but to inhibition of formation of chlorophyll-carrier protein. [The SCI® indicates that this paper has been cited over 120 times since 1968.]**

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"Antibiotics whose primary site of action is the inhibition of protein synthesis on 70 S or 80 S ribosomes are usually also potent inhibitors of chlorophyll formation in higher plants and algae. While there is general agreement that chlorophyll formation is highly dependent on continued protein synthesis, two opposing interpretations of the basis of this dependence have been put forward. According to one school, the enzyme required for synthesis of the chlorophyll precursor, d-aminolae-vulinic acid (ALA) undergoes turnover and must be continually resynthesized if chlorophyll formation is to continue. The other view, which I had proposed in 1962,<sup>1</sup> held that chlorophyll formation could only continue while there was carrier protein available to accept it, and that prevention of formation of the carrier protein was the reason for the inhibition of chlorophyll synthesis. The present paper was one more skirmish in this ongoing controversy. It described work I had carried out while a lecturer in the biochemistry department of the University of Wales,

Aberystwyth, showing that the inhibitory effect of antibiotics on chlorophyll synthesis in *Euglena* was not overcome when ALA was supplied, thus making it very unlikely that the inhibition of chlorophyll synthesis was primarily due to turnover of the ALA synthesizing enzyme.

"The relationship between protein synthesis and chloroplast pigment synthesis has been quite a popular field of research over the years, and many of the citations of this paper have been by people working in that area. There is, however, no doubt in my mind that the paper has in most cases been cited because of the nomogram for chlorophyll *a* and *b* concentration that I included within it. Chlorophyll concentration is one of the measurements that plant biochemists and physiologists most frequently have to make, and they normally do so by substituting the absorbance values at 663 and 645 nm, measured on an acetone extract, into appropriate simultaneous equations. I found the necessity to continually do this arithmetic rather tedious. It occurred to me that it should be possible to construct a nomogram by means of which the concentrations of both chlorophylls, and their sum, could be read off directly by simply laying a ruler between the appropriate points on the 663 and 645 nm absorbance scales. After some trial and error I succeeded in devising one. Initially this was only for my own use, but it proved such a time saver that I decided to publish it. I sent it off, just as a method on its own, to the *Biochemical journal*, but they refused to publish it without, as far as I can recall, specifying why. Accordingly, when the time came to write up my chlorophyll synthesis paper for *Planta*, I decided to include the nomogram as well. The extent to which it has been cited since shows that it has proved useful to many other people and vindicates my decision to persevere with its publication."

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1. Kirk J T O. Effect of streptomycin on [<sup>14</sup>C] leucine incorporation in *Euglena*. *Biochim. Biophys. Acta* 56:139-51, 1962.