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# Citations Classics

**Burton, Kenneth.** A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochemical Journal* **62**:315-22, 1956.

**The color reaction of Dische (1930) between diphenylamine and deoxyribonucleic acid (DNA) has been studied and modified. The principal modifications are to add acetaldehyde and to perform the reaction for several hours at 30°C instead of for 3-10 min. at 100°C. Using this modified reaction, the author studied the conditions for the quantitative extraction of DNA from bacteria. [The *SCI*® indicates that this paper was cited 5,037 times in the period 1961-1975.]**

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"Of course, I am pleased that one of my papers has been cited so often and am less pleased that most of my other papers have collected very few citations. On reflection, it seems clear that my 1956 paper has not affected the development of biochemistry, despite its frequent citation. If it had not been written, other available methods would have been used instead. It appeared at a time when it was becoming popular to measure DNA. A simple and specific method for measuring, say, arabinose would have been less fashionable but probably of greater value because of the lack of a simple alternative for the measurement of this sugar.

"The title of my paper reflected the fact that the diphenylamine colour reaction of Zaccharias Dische was already a well-used method of estimating DNA and was fairly satisfactory. It was only by chance that I modified it. One evening, I had not enough time to complete the colour development by heating at 100°C and so I left various unheated mixtures of DNA and reagents on the bench overnight. I was astonished next morning to find beautiful intense blue colours with low 'blanks' and straightaway I substituted overnight incubation at 30°C for the development at the higher temperature. (I was not then aware that Patterson had done much the same in 1948. Soon after, I returned from Chicago to England and started work in Oxford. It was an awful moment to find that only very faint colours were formed at 30°C.

"While a student at Cambridge, I had been privileged to hear Cowland Hopkins tell of the discovery of tryptophan and how this had started with studying a temperamental colour reaction. Hopkins and Cole had discovered that the glacial acetic acid needed prior exposure to strong sunlight or– better still–the addition of a small quantity of a suitable aldehyde. The acetic acid I had used in Chicago had indeed been stored in strong sunlight not to be found in Oxford-especially in the winter. Eventually, I selected acetaldehyde as an additional component of the reagent mixture, so obtaining reproducible intense colours on development at 30°. The acetaldehyde probably initiates a chain reaction and many other substances (e.g. hydrogen peroxide) can be used instead.

"The fact that DNA gave a colour that was ostensibly due to deoxyribose on incubation at such a low temperature led me to study whether free deoxyribose was produced and how the backbone chain of the DNA was split. Although I found that free deoxyribose could not be an intermediate, I discovered the diphenylamine-formic acid degradation of DNA which produced pyrimidine tracts quantitatively. This has been of limited value in DNA sequence work (e.g. by Southern in the analysis of satellite DNA) but it has been totally eclipsed by the methods now being so brilliantly developed and exploited by Sanger and his colleagues."