Brief Notes

On the Nature of the Deoxyribonucleic Acid-Methyl Green Reaction.* BY HERBERT S. ROSENKRANZ‡ AND AARON BENDICH. *(From the Sloan-Kettering Division of Cornell University Medical College, New York.)*§

Methyl green has frequently been used for histochemical and *in vitro* demonstration of deoxyribonucleic acid (DNA). It has been reported that this color or staining reaction requires the presence of highly polymeric DNA (1) and that its molecular size influences the extent of the reaction. Thus, as the DNA is broken down by deoxyribonuclease (DNase), there is a corresponding decrease in the binding of the dye (1, 2). The reaction is greatly decreased when applied to DNA which has been broken down by ultraviolet (3) and by x-irradiation (4), by acidification (3), or by heating (1). It has been deduced (5–8) that such treatments cause not only a diminution in length of the twin-helical chain (9) (*i.e.* degradation) but also a separation of the twin strands (denaturation). It was therefore of interest to determine which of the structural or macromolecular features of the DNA are responsible for the staining reaction and whether degradation or denaturation (or both) lead to the diminished affinity for the dye. Such a study is feasible as it has been shown that the sonic treatment of DNA leads to extensive degradation unaccompanied by denaturation (5, 10–12).

A solution (0.4 mg. per ml. of 0.2 M NaCl) of DNA, prepared from calf thymus by the method of Schwander and Signer (13), was subjected to sonic vibrations (9 kc. sonic oscillator, Raytheon Mfg. Co., model S102A) and aliquots withdrawn at different exposure times. This permitted the preparation of a graded series of DNA samples varying in weight average sedimentation coefficient from 21.3 to 5.5 *S*. This corresponds to a range of molecular weights from 6.7×10^6 to 1.7×10^5 as calculated using the formula of Doty, McGill, and Rice (11). Upon acidification to pH 2.7 and alkalinization to pH *ca.* 13, the samples exhibited the hyperchromic shifts expected (14, 15) of undenatured DNA. A detailed description of the DNA samples will be given elsewhere.

Identical aliquots of the variously sonicated and unsonicated samples were mixed with methyl green according to the method of Kurnick (16). After standing at 37° for 18 hours, the optical density at 640 mμ (Beckman DU spectrophotometer) was read against the appropriate blank. All of the samples exhibited the same optical density and this indicated an unaltered behavior towards methyl green.

These results suggest that as long as the double helical structure of DNA is intact, the binding of methyl green by the DNA remains the same regardless of chain length within the range explored. When this structure is destroyed, as in the denaturation which is accomplished by heating the unsonicated as well as the sonicated samples at 100°C. for 15 minutes or digesting them with DNase, the DNA exhibits a greatly reduced affinity for the dye.

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BIBLIOGRAPHY

1. Kurnick, N. B., *J. Am. Chem. Soc.*, 1954, **76,** 417, 4040.
2. Kurnick, N. B., *J. Gen. Physiol.*, 1950, **33,** 243; *Arch. Biochem.*, 1950, **29,** 41.
3. Devreux, S., Johannson, M., and Errera, M., *Bull. soc. chim.*, 1951, **33,** 800.
4. Harrington, N. J., and Koza, R. W., *Biol. Bull.*, 1951, **101,** 138.
5. Doty, P., *J. Cell. and Comp. Physiol.*, 1957, **49,** suppl. 1, 27.
6. Overend, W. C., Peacocke, A. R., and Stacey, M., *Tr. Faraday Soc.*, 1954, **50,** 305.

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7. Cox, R. A., and Peacocke, A. R., *J. Polymer Sc.*, 1957, **23,** 765.
8. Errera, M., *Biochim. et Biophysica, Acta*, 1952, **8,** 31, 115.
9. Watson, J. D. and Crick, F. H. C., *Nature*, 1953, **171,** 737.
10. Hall, C. E. and Litt, M., *J. Biophysic. et Biochem. Cytol.*, 1958, **4,** 1.
11. Doty, P., McGill, B. B., and Rice, S. A., *Proc. Nat. Acad. Sc.*, 1958, **44,** 432.
12. Rosoff, M., personal communication.
13. Schwander, H. and Signer, R., *Helv. Chim. Acta*, 1950, **33,** 1521.
14. Cavalieri, L. F., Rosoff, M. and Rosenberg, B. H., *J. Am. Chem. Soc.*, 1956, **78,** 5239.
15. Hotchkiss, R. D., *in* Methods in Enzymology, (S. P. Colowick and N. O. Kaplan, editors), New York, Academic Press, 1957, **3,** 708.
16. Kurnick, N. B., *Arch. Biochem. and Biophysics*, 1953, **43,** 97.

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