this reason the kinetic method of Brown and Fletcher cannot be used to determine accurately the composition of the mixture. The compositions of binary mixtures were determined by comparing the amount of chloride ion produced when 0.2 *M* solutions were solvolyzed in 80% ethanol at 50° for 48 hours with the amount of chloride ion resulting from each of the pure isomers under the same conditions. The chloride ion concentration was determined as described above. Analysis of these mixtures was much less accurate than for the binary mixtures described in the preceding sections. In order to test the accuracy of the method synthetic mixtures of the approximate composition obtained from the radical additions were analyzed and in each case the value obtained was within 3% of the correct value for the amount of *trans* isomer.

MADISON 6, WISCONSIN

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Studies on the Structure of Nucleic Acids. IX. Structural Changes in Solution[1]

BY LIEBE F. CAVALIERI AND AUDREY STONE

RECEIVED AUGUST 8, 1955

The interaction of the basic dye rosaniline with calf thymus deoxyribonucleic acid (DNA) isolated according to the procedure of Signer and Schwander has been examined and the results compared to those obtained with Hammarsten DNA. Two fundamental differences exist: (a) in the former, the bound dye causes structural alterations of the DNA; (b) the binding capacity of the former is significantly lower. The results are discussed in terms of structure. It is suggested that DNA in solution exists as a rather tight aggregate whose smaller units are joined by lateral forces, perhaps hydrogen bonds. It is further suggested that cleavage of this aggregate leads to degraded DNA. This type of aggregate is distinguished from one which is dependent upon concentration, *i.e.*, reversible aggregation.

In two[2,3] earlier investigations the results of the interaction of the basic dye rosaniline with deoxyribonucleic acid (DNA) were presented. These studies have now been extended using calf thymus DNA isolated according to the procedure of Signer and Schwander[4] (denoted as the SS sample), and we have shown that substantial differences exist between the present preparation and the Hammarsten sample[5] (denoted as the H sample) used previously.[2,3] The most striking difference is that the binding capacity of the SS sample varies with dye concentration in a manner indicating that structural changes occur as a result of the binding process. In addition, its binding capacity is lower.

On the basis of the earlier work[2,3] where it was shown that DNA degraded by acid or alkali had a higher binding capacity, the present results indicate that the SS sample is less degraded. It remains to be shown whether such effects have any biological significance. In this connection, it is unjustified from a biological point of view to consider any particular sample of DNA as being "better" than another because less degradation has apparently occurred during its preparation; this is obvious since the relation between physical structure and biological activity is quite nebulous at present. While this view is appreciated for the most part, there nevertheless appears to be some confusion in the literature.

Experimental

Materials.—The calf thymus DNA (sodium salt) was prepared according to the method of Signer and Schwander[4] and was the same sample used in another investigation.[6] The rosaniline was the same sample employed previously.[2,3]

Method.—Binding studies were carried out at 25 ± 0.1° and *p*H 7.2, using the method of partition analysis.[7] The details of the procedure have been given previously.[3]

Results

Figures 1 and 2 show plots of r/c *vs.* r for typical experiments where r is the average number of molecules of dye bound per phosphate group at a free dye concentration c. The upper curve in Fig. 1 is taken from previous data[3] and replotted for comparison; it was obtained using the H sample. The lower curve represents the SS sample. The broken curve in this figure approximates the binding process if both samples had behaved similarly. In Fig. 3, r *vs.* c is plotted for different DNA concentrations, showing that for any c, r increases with decreasing DNA concentration.

Discussion

In general a simple binding process is represented by eq. 1

$$r/c = k(n - r) \qquad (1)$$

where r is the average number of bound dye molecules at a free dye concentration c and k is the association constant assumed to be identical for all n available sites per DNA molecule. In the present paper r is the amount of dye bound per mole of phosphorus, rather than per mole of DNA. This has been done to avoid the use of a particular assigned molecular weight. Consequently, the maximum theoretical value of n is one, assuming that only one dye molecule is capable of being bound at each phosphate group, although it is apparent from previous and present data that the calculated value of n is less than one. That is, not all phosphates are available for binding, a situation which is discussed below.

Deviations from a straight line (eq. 1) in the r/c *vs.* r plots indicate that (a) electrostatic interactions occur among bound dye ions,[8] (b) the association constants of the sites are different[9] or (c) structural

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. C-471) and from the Atomic Energy Commission (Contract No. AT(30-1)-(910).

(2) L. F. Cavalieri and A. Angelos, THIS JOURNAL, **72**, 4686 (1950).

(3) L. F. Cavalieri, A. Angelos and M. E. Balis, *ibid.*, **73**, 4902 (1951).

(4) R. Signer and H. Schwander, *Helv. Chim. Acta*, **33**, 1521 (1950).

(5) E. Hammarsten, *Biochem. Z.*, **144**, 383 (1924).

(6) L. F. Cavalieri and B. Hatch, THIS JOURNAL, **75**, 1110 (1953).

(7) F. Karush, *ibid.*, **75**, 1246 (1953).

(8) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).

(9) F. Karush, THIS JOURNAL, **72**, 2705 (1950).

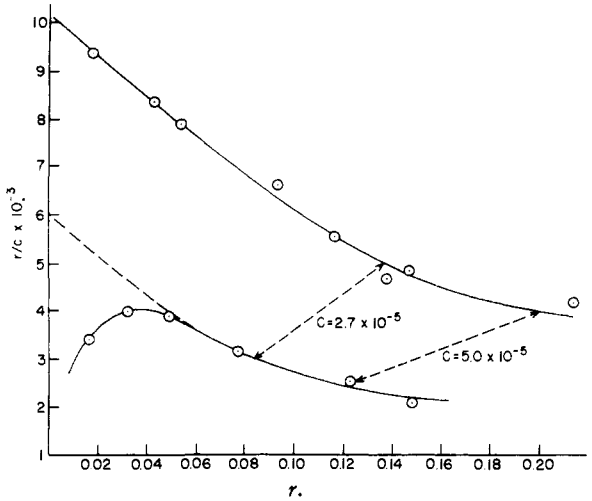

Fig. 1.—Upper curve, H sample 0.45 mg. DNA/ml.; lower curve, SS sample 0.50 mg. DNA/ml. In the lower curve the broken portion plus the unbroken descending portion is the theoretical curve discussed in the text. Two sets of r/c values are indicated at comparable c's.

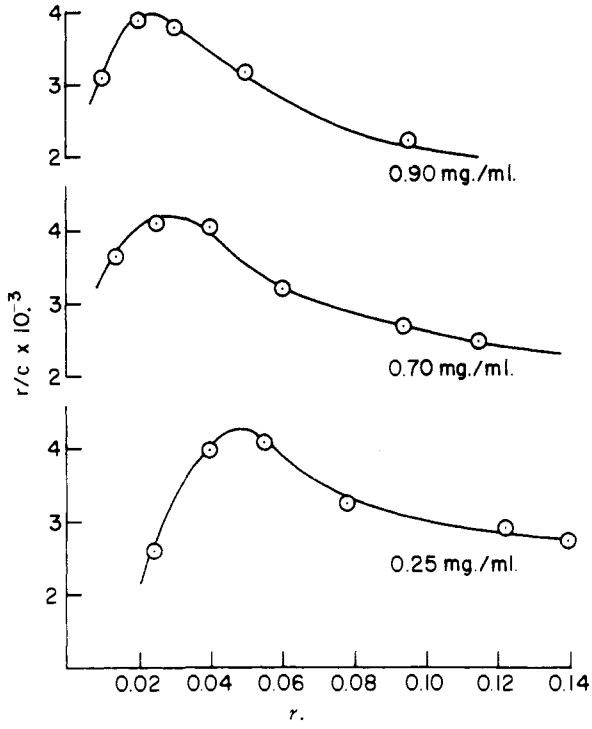

Fig. 2.—Binding curves at several DNA concentrations (SS sample).

alterations take place during the binding process. Possibilities (a) and (b) would yield curves which are concave upward. Since our curves show a maximum,[10] the explanation is to be sought in the third possibility.

It is clear from eq. 1 that for a maximum to occur in an r/c *vs.* r plot there must be an increase in either k or n or both. Stated in other terms, the situation requires that interaction of a dye ion at

(10) A similar effect was first observed and discussed by Karush (*J. Phys. Chem.*, **56**, 70 (1952); *cf.* THIS JOURNAL, **76**, 5536 (1954)).

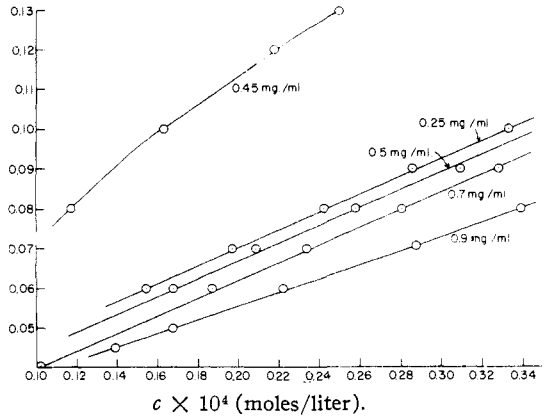

Fig. 3.—Upper curve, H sample; lower four curves, SS sample. The binding capacity can be ascertained by comparing r values at the same c.

one phosphate site either increases the strength of binding or liberates other phosphate sites. It is important to decide whether the increase in binding capacity results from an inter- or intramolecular change in the DNA. There are two facts which indicate that the former takes place. First, the position of the maximum is a function of DNA concentration. It will be seen from Fig. 2 that the maximum shifts toward lower r values with increasing DNA concentration. Second, the increase in r/c occurs when only a few per cent. of all phosphates are bound. This tends to rule out the contraction of a single molecule. In addition, alteration of a phosphate *per se* because of dye interaction at an adjacent position appears unlikely because of the large distance between phosphates.[11]

If the inference is correct that intermolecular changes occur as a result of the binding process we would expect that changes in the DNA concentration should affect the binding capacity. Results bearing on this are contained in Fig. 3, where it is shown that the binding capacity is greater at lower DNA concentrations. This is not an ionic strength effect since the contribution of the DNA to the ionic strength is low.[12] The increase in r with decreasing DNA concentration and the initial increase in r/c, *i.e.*, the maximum, may result from similar processes. Conceptually, we may have the following: in the case of the maximum, the dye may interact at a site on one DNA molecule displacing (*i.e.*, dissociating) another DNA molecule which in turn is free to bind dye. In the case of the general increase in binding in dilute solution, Fig. 3, the effect may be one of separation of DNA molecules, thus liberating sites. The identity of the sites liberated in each instance cannot be ascertained from our data. There is further evidence which indicates that DNA molecules interact. It was shown previously[3] that the value of n for a more weakly bound dye is significantly less than that for rosaniline. This was taken to mean that interaction among DNA mole-

(11) M. H. F. Wilkins, A. R. Stokes and H. P. Wilson, *Nature*, **171**, 739 (1953).

(12) In these experiments the ionic strength of the buffer system was 0.03; that of the DNA varied from 8×10^{-4} to 3.2×10^{-3}. In earlier experiments[2] where a similar concentration dependence was shown, the ionic strength of the buffer was 0.2.

cules resulted in effective competition with respect to the weaker dye.

It is pertinent to consider in more detail the nature of the association among DNA entities. We wish to consider this question within the framework of the double helical structure.[13,14] The greater binding capacity of the H sample suggests that more phosphate groups are available in this material than in the SS sample (Fig. 3). It is highly improbable that these samples are fundamentally different; the difference lies principally in their relative sizes.[15] On this basis, the greater binding capacity of the H DNA appears to result from cleavage of a larger molecule along its length. We infer this because the phosphates lie outside the helix[11] along its entire length and these would have to be liberated in order to give a higher binding capacity. To restate this, the availability of sites would not change much if a long molecule were simply cleaved in halves, quarters, etc., by a plane perpendicular to the long axis. We envisage cleavage which results in the separation of one double helix from another (*i.e.*, not cleavage of the double helix itself). This leads to the conclusion that DNA is actually a rather tight aggregate of perhaps two double helices joined together by lateral forces; this is to be distinguished from a head-to-tail type. We suggest that this entity is the one which we identify as the DNA molecule and that it is quite distinct from a micelle; in other words, the aggregate may be thought of as an integral structure. A similar aggregate was suggested earlier[16] on the basis of the apparent dissociation constants of DNA.

In order to consider more closely the origin of the maxima in Fig. 2, it is necessary to discuss briefly the nature of the binding process itself. It was shown[2] that the data obtained with the H sample could be accounted for in terms of two widely differing association constants. The interpretation presented was that the higher constant represented binding at doubly charged phosphate groups while the lower was for the singly charged phosphates. Regardless of the validity of this view, certain conclusions regarding the difference between the H and SS samples appear warranted. The sites with the higher association constants are those involved in producing the maximum. We have calculated the theoretical curve for the SS sample, neglecting the maximum. The broken line in Fig. 1 indicates the theoretical binding process had the maximum not existed. The association constants turn out to be the same as for the H sample. For the SS sample, n_1 (higher k) is 0.04; n_2 (lower k) is 0.43. For the H sample n_1 is 0.06; n_2 is 0.66. Although the calculations are approximate, they suggest that fewer sites are available in the SS sample.

(13) J. D. Watson and F. H. Crick, *Nature*, **171**, 739 (1953).

(14) M. Feughelman, R. Langridge, W. E. Seeds, A. R. Stokes, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, R. K. Barclay and L. D. Hamilton, *ibid.*, **175**, 834 (1955).

(15) The molecular weight of the H DNA is 2.3 million; the SS sample has a molecular weight of 5.8 million; both determined by light scattering. These results will be given more fully in another publication.

(16) L. F. Cavalieri and A. Stone, THIS JOURNAL **77**, 6499 (1955).

Summary

We have suggested that the DNA molecule is a tight aggregate composed of units smaller than those observed, say, by light scattering. We have further implied that this aggregate acts as a unit. The dependence of binding capacity on DNA concentration indicates that interaction occurs among these aggregates. The lateral aggregate of the type suggested would also provide electrostatic heterogeneity among the phosphate sites, a condition which must be fulfilled to account for our data. The fact that the binding capacity increases after DNA has been exposed to acid or alkali suggests that the lateral forces involve, in part at least, hydrogen bonds in which the phosphate groups participate. In this connection if a sample such as the H DNA appears to be degraded as a result of the isolation procedure it may be that the aggregate has been disrupted but that no covalent bonds have actually been cleaved. If our interpretations are correct, an interesting biological possibility arises: namely, that the bacterial transformations which are caused by DNA may be due to the smaller units of the aggregate.

Acknowledgment.—The authors wish to thank Dr. George B. Brown for continued interest and helpful discussions.

NEW YORK 21, N. Y.