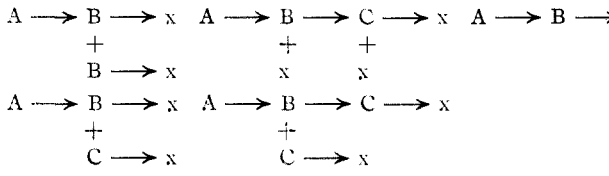
trum are similar to those for the nucleotide formation.

A few pertinent remarks concerning the process of curve fitting will serve to support our proposed mechanism and lend some validity to the velocity constants so obtained. The expressions given in this paper represent the simplest of a large group which were developed for testing. The following are some of the mechanisms for which expressions were developed.



It was found that no one single equation would adequately express the data. The chief difficulty was that the maximum in the rate curve could not be made to coincide with the observed maximum. In the process of combining rate expressions, the type represented by equation 4 was found to be essential. Combination of equation 4 with the equations from the reaction mechanisms just noted were unsatisfactory.

Thus although the set we have chosen is probably not unique, it is likely that the reaction proceeds in an analogous but more complex manner. Perhaps the most significant feature to arise from the analysis of the rate curve is that in all cases examined both a low and a high velocity constant had to be assumed in order to approach a satisfactory fit. If we are to attach any significance to this in terms of phosphate linkages, it would appear that there are at least two types differing a great deal in their lability toward alkali. The ratios of the rates of formation of the isomeric guanylic acids (*a* and *b*) and adenylic acids (*a* and *b*) were constant and similar throughout the entire range of V/u; these were respectively about 1:1 and 1:1.1. There is a possibility for another type of phosphate linkage such as a phosphoester of the uracil or guanine hydroxyl groups which might be sufficiently different from the sugar phosphates. Data which suggest that such a bond exists were presented previously.[3] It should be emphasized that the constancy of the ratio a/b cannot be construed as conclusive evidence that the intact nucleic acid contains the isomers in this ratio. A reversible alkaline-catalyzed isomerization could also account for the results.

Finally it should be noted that all rate curves indicate a definite rate at $V/u = 0$. This may be taken to mean that a definite amount of the nucleotides are formed directly from the nucleic acid and that all nucleotides may therefore serve as end groups.

Acknowledgment.—The author wishes to thank Professor Louis P. Hammett for invaluable advice and for the facilities of his laboratory during the course of this investigation and Dr. George B. Brown for continued interest.

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Studies on the Structure of Nucleic Acids. IV. Investigation of Dye Interactions by Partition Analysis[1]

BY LIEBE F. CAVALIERI, ALICE ANGELOS AND M. EARL BALIS

The interaction of both desoxypentose and pentose nucleic acids with rosaniline and trimethyl-(*p*-(*p*-hydroxybenzeneazo)-phenyl)-ammonium chloride has been investigated by the method of partition analysis. The total number of available sites differs for the two dyes. This effect is interpreted in terms of heterogeneity among the sites. Competitive effects of magnesium ions have been investigated both by the method of equilibrium dialysis and partition analysis. Approximate binding constants for the magnesium ion have been calculated. The competitive interaction of bovine serum albumin with desoxypentose and pentose nucleic acids has been examined in the presence of the above-mentioned dyes. There is no evidence of combination between albumin and either nucleic acid.

In previous communications we have presented studies on the interaction of the cationic dye rosaniline with desoxypentose nucleic acid (DNA)[2] and pentose nucleic acid (PNA)[3] based on the method of equilibrium dialysis. In these studies solutions of relatively high ionic strength were used in order to minimize the Donnan effect. Under such circumstances competitive effects may obscure certain aspects of the binding process. Recently[4] a method of partition analysis has been developed in which solutions of much lower ionic strength are used without the complication of the Donnan effect and it is possible, therefore, to study dye interactions under conditions where the competitive interaction is slight. Briefly, the method consists of determining the distribution of an organic dye between an aqueous phase, which contains the nucleic acid, and an organic phase.[5] The quantity of dye bound and the free equilibrium dye concentration are readily calculated and the results are treated as in the dialysis method.[6,7]

In the present paper we report the results of a study of the interaction of both DNA and PNA

(1) The authors wish to acknowledge the support of the Atomic Energy Commission (contract AT(30-1)-910) and of the National Cancer Institute of the United States Public Health Service.

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

(3) L. F. Cavalieri, S. E. Kerr and A. Angelos, *ibid.*, **73**, 2567 (1951).

(4) F. Karush, *ibid.*, **73**, 1246 (1951).

(5) Irvin, Irvin and Parker, *Science*, **110**, 426 (1949).

(6) F. Karush and M. Sonenberg, THIS JOURNAL, **71**, 1369 (1949).

(7) I. Klotz and Urquhart, *ibid.*, **71**, 847 (1949).

TABLE I

PARTITION ANALYSIS

Temp., °C.	Nucleic acid concn., m./l. × 10^5	Original dye concn. in aqueous phase, m./l. × 10^5	Dye concn. in organic phase, m./l. × 10^5	Free dye concn. in aqueous phase, c m./l. × 10^5	Bound dye, m./l. × 10	r	$r/c \times 10^{-5}$
			*p*H 6.7, DNA, THPA				
4 ± 0.5	2.88	47.6	11.4	11.1	25.0	8.72	0.784
		28.3	6.50	6.08	15.7	5.48	.902
		23.5	5.65	5.24	12.6	4.41	.844
		11.6	2.90	2.24	6.46	2.25	1.02
		4.62	1.26	0.70	2.67	0.93	1.34
		2.36	0.78	0.30	1.27	0.44	1.42
25 ± 0.5	2.60	48.5	10.2	16.8	21.4	8.26	0.492
		37.2	7.80	12.5	16.9	6.51	.520
		25.2	5.24	8.10	11.8	4.56	.564
		20.2	4.28	6.40	9.57	3.68	.575
		10.1	2.24	2.98	4.93	1.89	.636
		5.00	1.16	1.33	2.51	0.965	.725
		2.03	0.554	0.49	0.986	.379	.774
		0.52	0.164	0.11	0.246	.0945	.859
			DNA, Rosaniline				
4 ± 0.5	1.32	29.2	2.35	3.70	23.1	17.5	4.73
		22.9	1.75	2.60	18.5	14.0	5.40
		14.0	1.01	1.18	11.8	8.95	7.57
		11.5	0.825	0.938	9.83	7.45	7.95
		8.79	.594	.630	7.57	5.74	9.10
		5.68	.385	.350	4.94	3.74	10.7
		2.23	.167	.120	1.94	1.47	12.2
25 ± 0.5	1.29	31.8	1.81	5.10	24.8	19.3	3.79
		20.1	1.14	2.90	16.0	12.5	4.31
		21.1	1.18	3.00	16.9	13.2	4.40
		14.2	0.827	1.90	11.5	8.95	4.71
		8.79	.421	0.812	7.56	5.87	7.23
		5.85	.290	.515	5.04	3.91	7.60
		2.40	.130	.19	2.08	1.62	8.52
							$r/c \times 10^{-4}$
			PNA, THPA				
4 ± 0.5	20.6	69.5	18.1	15.8	35.1	1.71	1.07
		48.9	13.9	11.6	23.8	1.14	0.988
		22.1	5.77	4.40	11.9	0.572	1.30
		14.4	4.00	2.8	7.65	.370	1.33
		3.08	1.04	0.410	1.49	.072	1.74
		1.04	0.415	0.135	0.49	.024	1.76
25 ± 0.5	10.25	48.5	14.5	24.3	9.80	.956	0.382
		37.2	11.3	18.8	7.10	.690	.368
		25.2	7.48	12.0	5.70	.556	.464
		20.2	6.06	9.46	4.79	.468	.496
		10.1	3.32	4.70	2.20	.216	.460
		5.0	1.74	2.95	0.31	.0302	1.026

with rosaniline and trimethyl-(*p*-(*p*-hydroxybenzeneazo)-phenyl)-ammonium chloride (THPA). The choice of the latter dye was based on the fact that it possesses a single positive charge which is localized in contrast to rosaniline whose positive charge is directly involved in a resonance structure. To facilitate comparison with the previous results, the interaction of the new dye has also been studied by the method of equilibrium dialysis.

Experimental

Materials.—The nucleic acid samples were those used previously.[3] Trimethyl-(*p*-(*p*-hydroxybenzeneazo)-phenyl)-ammonium chloride (THPA) was generously supplied by Dr. David Pressman and was prepared and purified according to an existing procedure.[8] The *n*-hexanol used in the partition analyses was a redistilled sample of commercial (pure grade) *n*-hexanol. The heptane was of technical quality and was used without purification. The *p*-toluenesulfonic acid hydrate was obtained from the Eastman Kodak Company (EKW) and was used without further crystallization.

Method.—In the partition analyses the aqueous phase was of the same composition for both dyes and was made up as follows: *p*-Toluenesulfonic acid monohydrate (0.01 mole) was dissolved in a liter of water containing sufficient sodium hydroxide to yield a *p*H of 6.8. One hundred ml. of a 0.05 *M* potassium phosphate buffer solution (*p*H 6.7) was diluted to one liter with the solution of sodium *p*-toluenesulfonate. The *p*H of the resulting solution was 6.8. Rosaniline was

(8) D. Pressman, A. L. Grossberg, L. H. Peuce and L. Pauling, *ibid.* **68**, 250 (1946).

dissolved in water and the lower phase consisted of 2 ml. of aqueous dye and 2 ml. of *p*-toluenesulfonate buffer in which was dissolved the nucleic acid (0.2%). THPA was dissolved in the *p*-toluenesulfonate buffer and in this case the nucleic acid was dissolved in water; the aqueous phase consisted of 2 ml. of each of these solutions. The organic phase was different for the two dyes. For rosaniline, batches were made up consisting of 250 ml. of heptane and 252 ml. of *n*-hexanol; for THPA, 80 ml. of heptane and 240 ml. of *n*-hexanol. Distribution coefficients for the dyes were determined under conditions identical to those prevailing for a run. In general the coefficients were determined immediately before a run and were checked frequently. For a new batch of organic phase the distribution coefficients were determined over the entire range.

DISTRIBUTION COEFFICIENTS OBTAINED

T, °C.	Concn., $\times 10^{-6}$ *M*	Distribution coefficient
	Rosaniline (ϵ organic = 79,600 (5400 Å.))	
4 ± 0.5	292.0	0.64
	5.41	1.19
25 ± 0.5	318.0	0.341
	24.1	0.459
	THPA (ϵ organic = 26,700 (3650 Å.))	
	Concn., $\times 10^{-5}$ *M*	
4 ± 0.5	48.6	0.862
	2.07	1.345
25 ± 0.5	48.6	0.592
	2.07	0.933

At the lower temperature equilibration was carried out overnight in a cold room by a rotating device (*ca.* one r.p.s.); at 25° agitation of the solutions was carried out manually. Vigorous shaking for about five minutes was sufficient for complete equilibration. For THPA optical density measurements (Beckman spectrophotometer) of the organic phases were reproducible to within 1%. In the case of rosaniline the error was estimated to be about 3–4%; this may be due to adsorption of this dye on the glassware. In each case the concentration of free dye in the aqueous phase was calculated from the distribution coefficient at the particular dye concentration in the organic phase. Beer's law was found to hold for both dyes throughout the entire concentration range used. When magnesium sulfate was used as a competitive agent new distribution coefficients were determined but the composition of the buffer was otherwise unchanged.

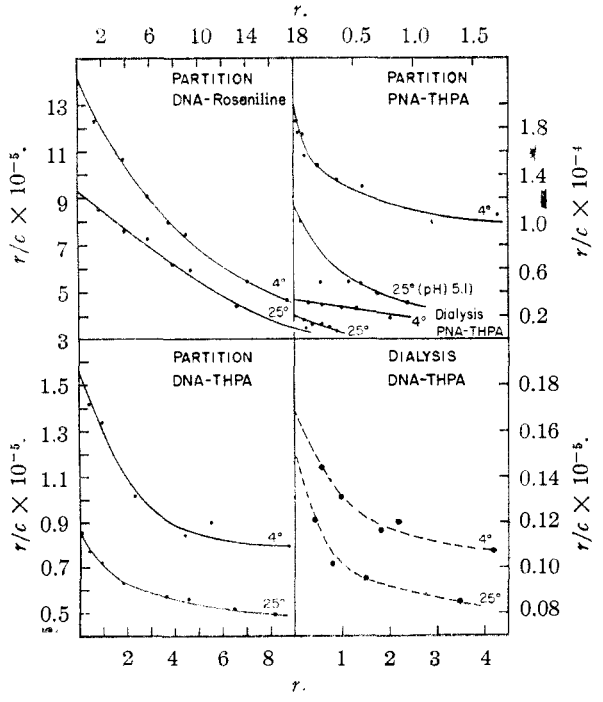

Fig. 1.

The method of equilibrium dialysis was identical to that employed earlier.[2,3] In the case of THPA the quantity of dye bound was small in comparison to the amount present. We have, therefore, a situation in which small differences between large numbers are obtained. Since this greatly magnifies the errors in the r/c values, we did not attempt to construct theoretical curves for these runs. In Fig. 1 the broken line represents the best visual fit rather than a theoretical curve.

Results

The results of the binding process for both dyes with PNA and DNA are plotted in Fig. 1 from the data contained in Tables I and II. r is the number of dye molecules bound per molecule of nucleic acid, n ($= n_1 + n_2$) is the number of available sites per molecule of nucleic acid, k_1 and k_2 are binding constants and c is the free equilibrium dye concentration. In the figure the solid lines represent theoretical curves obtained according to existing procedures.[2,4,9] As before the molecular weight of DNA was chosen for convenience as 35,000; that for PNA as 10,000.

TABLE II

EQUILIBRIUM DIALYSIS

Temp., °C.	Nucleic acid concn. m./l. $\times 10^5$	One-half of original dye concn. m./l. $\times 10^5$	Free dye concn., *c*, m./l. $\times 10^5$ (from optical density of outer soln.)	Free dye concn. plus casing adsorption (*c'*) $\times 10^5$	Bound THPA m./l. $\times 10^5$	*r*	$r/c \times 10^{-4}$
			*p*H 6.7				
			DNA				
4 ± 0.5	4.51	50.9	38.9	41.5	18.9	4.19	0.108
	4.95	25.2	19.4	20.2	11.0	2.22	.120
	4.78	20.6	15.3	16.3	8.60	1.80	.118
	4.51	10.0	7.45	7.84	4.42	0.980	.132
	4.91	5.11	3.74	3.86	2.65	0.540	.144
25 ± 0.5	4.51	5.18	40.6	43.1	15.7	3.48	.0855
	4.51	20.9	20.2	20.0	10.4	2.30	.114
	4.91	20.5	15.89	16.8	7.36	1.50	.0947
	4.51	10.2	7.78	8.25	3.6	0.797	.103
	4.91	5.18	3.84	4.03	2.31	0.471	.123
			PNA				
							$r/c \times 10^{-4}$
3 ± .5	20.0	50.9	40.3	43.0	15.9	0.795	0.197
	19.0	25.7	19.5	20.2	10.0	.526	.270
	19.5	20.6	15.5	16.4	8.16	.418	.270
	20.0	10.0	7.40	7.83	4.44	.222	.300
	20.0	5.11	3.80	3.92	2.38	.119	.314
25 ± .5	23.0	51.8	44.3	47.1	7.70	.335	.757
	20.0	25.9	21.6	22.8	4.9	.245	1.132
	20.0	20.5	17.1	18.2	4.6	.230	1.342
	20.0	10.2	8.38	8.91	2.28	.114	1.36
	18.7	5.18	4.38	4.47	1.43	.0765	1.75

In the case of DNA, partition analysis shows that there are more sites available to rosaniline than to THPA. The ratio n_1/n_2 is larger in the case of rosaniline. In the case of PNA and THPA the method of equilibrium dialysis indicates that a linear relationship exists between r/c and r. The values of n and k correspond to n_2 and k_2, respectively, when compared with those obtained with rosaniline under the same conditions. In the method of partition analysis two types of sites (n_1 and n_2) are evident. The binding capacity

(9) G. Scatchard, I. H. Scheinberg and S. H. Armstrong, *ibid.*, **72**, 535 (1950).

TABLE III

BINDING CONSTANTS: DNA

	Partition analysis				Equilibrium dialysis			
	4°		25°		4°		33°	25°
	Rosaniline	THPA	Rosaniline	THPA	Rosaniline	THPA	Rosaniline	THPA
n_1	6.7	1.4	6.7	0.34	0.16		0.28	
n_2	93.3	53.6	68.3	39.7	23.9	(20–30)	23.7	(15–25)
$k_1 \times 10^{-5}$	1.55	0.58	0.87	0.87	3.92	(0.1–0.2)	3.79	(0.1–0.2)
$k_2 \times 10^{-5}$	0.04	0.014	0.051	0.015	0.065	(0.002–0.004)	0.048	(0.002–0.004)
				PNA[a]				
n_1		0.06			0.05	...	....	...
n_2		4.96			1.95	2.10	(1.95)	0.56
k_1		1.37			2.24	...	....	...
k_2		0.024			0.124	0.016	(0.06)	0.036

[a] This material is sample C used in earlier work.[3]

appears to be slightly higher in the latter case. The ratio k_1/k_2 is quite close to that for DNA.

The results of using magnesium ion as a competing agent in these dye interactions are contained in Tables IV–VI. It can be seen that magnesium ion effectively competes with both rosaniline and THPA for the sites of DNA. The intrinsic binding constants are about one thousandfold smaller than those of the dyes.

Discussion

Since it is highly probable that the interaction of positively charged dyes with nucleic acids occurs at the ionized phosphate groups,[2] it is not unexpected that a decrease in the potassium ion concentration results in an apparent increase in the extent of binding. The observed effect may be ascribed to a competition between the metal ions and the dye for the sites on the nucleic acid molecule. The ionic strength in the partition analyses was about fivefold lower and the increase in the total binding capacity, n, was about fourfold for rosaniline. The value of n for THPA in the dialysis method was not calculated for reasons given in the experimental section but an approximate value appears to be lower than that for the partition method. For rosaniline at 4° the value of n indicates that nearly all of the phosphate groups are available for binding while for THPA about half as many sites are available. This difference in the estimated values of n for the two dyes suggests that there is a heterogeneity among the sites of DNA. If the sites were identical, the value of n should be the same for different dyes. The present data are consistent with the assumption that there exists a number of sites whose binding strength is lower than that represented by the average k_1 and k_2 values obtained for rosaniline. These sites would then presumably interact little or not at all with the weaker THPA.

It is of interest to examine one of the possibilities which could give rise to heterogeneity among the sites. Clusters of the rod-like DNA molecules may be held together by forces interacting along the length of the molecule. If DNA-rosaniline interactions were significantly stronger than DNA–DNA interactions, there would not be effective competition. On the other hand if some of the DNA–DNA bonds were nearly equal in strength to the (weaker) DNA–THPA bonds, effective competition would be expected with the result that the value of n would be lowered.

The binding of rosaniline by DNA as observed with partition analysis shows that n_1 has a value of nearly 7. It will be recalled that the n_1 sites are presumably those involving the divalent phosphate ion.[2] If the divalent ions were present at either end of the DNA molecule n_1 could not be greater than 2. It is suggestive therefore that there are about 4 or 5 nucleotide side-chains per 35,000 molecular weight which possess a divalent phosphate ion.

Comparison of the results obtained with partition analysis with those of equilibrium dialysis in the case of PNA reveals effects similar to those observed with DNA. Interaction of THPA with PNA in the partition method shows about a twofold increase in the total binding capacity over the dialysis method. In the latter technique the high salt concentration totally inhibits interaction of THPA at the divalent (n_1-type) sites.

The ratio of monoester (divalent site) to diester (monovalent site) phosphate obtained from pH titration data (3, 10, 11, 12) is much larger than the ratio n_1/n_2 observed with either rosaniline or THPA. Since the n_1-type sites undoubtedly exist in the PNA molecule the observed lack of interaction with dyes may be due to their unfavorable location. They may be buried within the molecule and are therefore inaccessible or they may jut out far from the general contour of the molecule; in this case their behavior would approach that of the mononucleotides which have been shown not to bind dyes.[3]

Competitive Effects with Mg Ions.—The necessity of salts of divalent metals in desoxyribonuclease action is well recognized. In a preliminary investigation concerned with this matter we have examined the competitive effects of magnesium sulfate on the binding of rosaniline and THPA by DNA both by equilibrium dialysis and partition analysis. Using one initial rosaniline concentration, the free dye concentration has been measured in the presence of varying amounts of magnesium sulfate (Table IV). It can be seen that even at the high buffer concentration (0.05 M) magnesium ions are capable of effective com-

(10) P. A. Levene and H. S. Simms, *J. Biol. Chem.*, **70**, 327 (1926).
(11) E. Jorpes, *Biochem. J.*, **28**, 2102 (1934).
(12) F. W. Allen and J. S. Eiler, *J. Biol. Chem.*, **137**, 757 (1941).

petition with rosaniline. The quantity of magnesium ions bound was obtained by calculation from the increased free dye concentration rather than by direct measurement. In a plot of r/c *vs.* r the typical non-linear curve is obtained which suggests that at least two types of sites might be involved, in the absence of electrostatic effects. A calculation by the procedure used above yields values of approximately 150 and 5 for the two intrinsic binding constants of magnesium ion. These values may be considered only as being of the proper order of magnitude since the question was not investigated in any great detail. It will be noted that the ratio of these constants is approximately equal to that obtained with rosaniline. This is consistent with the fact the magnesium ions compete with rosaniline. Evidence which indicates that the magnesium interacts both at the monovalent (n_2) and divalent (n_1) sites is found in Table V in which it may be seen that the curvature of r/c *vs.* r is essentially the same for both cases.

TABLE IV

EFFECT OF Mg^{++} ON BINDING OF ROSANILINE IN 0.05 *M* POTASSIUM PHOSPHATE, *p*H 6.7, 3°. ORIGINAL CONCN. ROSANILINE = 91.4×10^{-6} *M*; DNA CONCN. = 2.86×10^{-5} *M*; EQUILIBRIUM DIALYSIS

$MgSO_4$, m./l.	Free dye concn.,[a] m./l. $\times 10^6$	Bound dye m./l. $\times 10^6$	r	$r/c \times 10^{-5}$	$\Delta(r/c)$ %[b]	r_1[c]	$r_1/c \times 10^{-2}$
0.000	13.1	58.6	2.05	1.52	0	...	..
.001	13.9	56.6	1.98	1.42	5	0.1	1.0
.003	16.9	49.2	1.72	1.02	24	.5	1.8
.005	18.4	45.4	1.59	0.87	33	.8	1.6
.010	22.1	36.2	1.27	0.59	55	1.6	1.6
.015	23.8	32.0	1.12	0.47	63	2.5	1.6
.020	25.9	26.6	0.03	0.36	71	3.2	1.6

[a] In this range of free dye concentrations there is a 20% correction for the dye adsorbed by the cellophane casing. [b] The quantity $\Delta(r/c)$ represents the difference between the r/c values in column 5 and those obtained in the absence of Mg^{++} at comparable free dye concentrations.[2] [c] The quantity r_1 is the amount of Mg^{++} bound per molecule of DNA. It should be noted that the amount of magnesium bound as calculated by this procedure is not necessarily the total amount bound. It represents that fraction of the bound dye which has been displaced by magnesium ion.

TABLE V

BINDING OF ROSANILINE TO DNA IN 0.05 POTASSIUM PHOSPHATE, 0.006 *M* $MgSO_4$; *p*H 6.7, 3°; DNA CONCN. 2.86×10^{-5} *M*; EQUILIBRIUM DIALYSIS

One-half original dye concn., m./l. $\times 10^6$	Free dye concn., m./l. $c \times 10^6$	Bound dye, m./l. $\times 10^6$	r	$r/c \times 10^{-5}$	In absence of $MgSO_4$ r	In absence of $MgSO_4$ $r/c \times 10^{-5}$
7.76	2.87	8.34	0.29	1.10	0.37	1.99
15.2	5.88	15.7	.55	0.94	0.71	1.77
24.4	9.08	26.0	.91	1.00		
33.3	13.1	33.8	1.18	0.91	1.49	1.57
42.4	17.2	41.8	1.46	.85		
48.6	19.8	47.5	1.66	.84	2.14	1.48

Competitive effects of magnesium ions in the case of THPA were examined by partition analysis since the extent of binding of this dye was low when measured by the method of equilibrium dialysis. It can be seen from Table VI that the system is very sensitive to magnesium ions. Thus 1×10^{-4} *M* magnesium sulfate results in a 60–70% lowering of r/c values, while with 1×10^{-3} *M* the effect is several times greater. Not only is the percentage drop in r/c values greater than in the comparable case of rosaniline, but the absolute number of moles of dye displaced is also greater. This effect is probably associated with the lower intrinsic binding constants of THPA.

TABLE VI

BINDING OF THPA TO DNA BY PARTITION ANALYSES, *p*H 6.7, 3°

Original dye concn. in aqueous phase, m./l. $\times 10^5$	Dye concn. in organic phase, m./l. $\times 10^5$	Free dye concn. in aqueous phase, m./l. $c \times 10^5$	Bound dye, m./l. $\times 10^5$	r	$r/c \times 10^{-5}$
1×10^{-4} *M* $MgSO_4$; DNA concn. = 5.49×10^{-5} *M*					
47.2	8.18	8.99	29.9	5.46	0.609
23.5	4.24	4.10	15.2	2.76	.673
11.7	2.22	1.93	7.61	1.39	.721
4.72	1.00	0.68	3.04	0.55	.82
2.32	.54	0.31	1.47	0.27	.87
1×10^{-3} *M* $MgSO_4$ DNA concn. = 5.42×10^{-5} *M*					
16.3	4.65	5.00	6.70	1.24	0.24
9.90	2.97	2.91	4.02	0.74	.25
4.15	1.41	1.12	1.62	0.29	.26
1×10^{-3} *M* $MgSO_4$ DNA concn. = 2.86×10^{-5} *M*					
16.5	6.58	6.50	3.37	1.23	0.190
8.05	3.58	2.78	1.69	0.616	.221
4.02	1.86	1.10	1.06	.387	.356
1.57	0.78	0.28	0.51	.185	.662

Interaction of DNA with Bovine Serum Albumin.—Since it has been reported[13] that electrophoretic patterns indicate interaction between PNA and ovalbumin we thought it of interest to examine the effect of crystalline bovine serum albumin[14] on the binding of dyes by DNA and PNA. No change in the free rosaniline concentration was observed either with DNA or PNA (*p*H 5.6, 0.05 *M* phosphate buffer) when 1 mg./ml. of bovine serum albumin was present. Interaction of THPA with DNA at *p*H 6.7 was not affected by the presence of the albumin as shown by partition analysis even when the weight ratio of albumin to DNA was 4:1. Unless interaction of albumin with DNA occurs at sites different from those in the dye interactions, it appears that there is no combination between albumin and DNA or PNA. The interaction of albumin itself with these dyes was checked and there was no evidence for binding.

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(13) L. G. Longsworth and D. A. MacInnes, *J. Gen. Physiol.*, **25**, 507 (1941).

(14) Obtained from Armour and Company.