

*Summary.*—Streptomycin decreased the polyuridylic acid-dependent incorporation of C<sup>14</sup>-labeled phenylalanine into acid-insoluble products in a cell-free system containing ribosomes of streptomycin sensitive *E. coli* and supernatant of either sensitive or resistant cells. There was no inhibition when the system contained ribosomes of streptomycin resistant *E. coli* and supernatant of either resistant or sensitive cells. The experiments show that streptomycin interferes with ribosomal function in sensitive bacteria and point to the ribosomes as the site of streptomycin sensitivity. They also show that the genetic locus determining streptomycin sensitivity is part of a ribosome-specific region of the bacterial chromosome.

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† International Postdoctoral Fellow of the National Institutes of Health, U. S. Public Health Service. Permanent address: Instituto de Química Fisiológica y Patológica, Universidad de Chile, Santiago, Chile.

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*INTERACTION OF NUCLEOSIDES AND RELATED COMPOUNDS  
WITH NUCLEIC ACIDS AS INDICATED BY THE CHANGE OF  
HELIX-COIL TRANSITION TEMPERATURE*

BY PAUL O. P. TS'O, G. K. HELMKAMP, AND C. SANDER

CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, AND UNIVERSITY OF CALIFORNIA AT RIVERSIDE

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Recent advances in the study of *in vitro* replication of nucleic acid have indicated that the base composition and perhaps the base sequence of the newly synthesized polymeric nucleic acids are under the specific direction of the template-DNA.<sup>1-8</sup> Thus, monomeric nucleoside-triphosphate must interact specifically with the template DNA in order to attain proper alignment of the contiguous monomers during the polymerization process. The high degree of specificity in this interaction, which is perhaps common and basic to all biological systems, raises an interesting question as to its chemical mechanism. Since the sugar and phosphate moieties are common to all the nucleoside-triphosphates, the specificity must reside in the variation of the pyrimidine and purine bases. Therefore, it appears to be reasonable to first study the interaction of these bases and their nucleosides with the nucleic acids. Complications due to charge effects of the phosphate groups of the nucleic acids and the triphosphate moieties of the nucleotides are avoided when the bases and the nucleosides are employed. The nucleosides in most cases are preferable to the bases for this type of study because of solubility problems. We are aware also of the fact that the interaction takes place in the presence of enzymes.

The specificity of interaction appears, however, to be dependent only on the template nucleic acid and not on the enzyme. Thus, studies of the interaction of nucleic acids with nucleosides constitute a meaningful approach.

Most of the nucleic acids and synthetic polynucleotides exhibit a helix-coil transition within a defined narrow temperature zone ( $T_m$ ) at a given ionic strength and pH.<sup>9-16</sup> There are reasons to suspect that interaction of bases or nucleosides with a nucleic acid will affect  $T_m$ . Thus, nucleosides could be bound preferentially to the coil form of the polymer, and in so doing hinder the formation of the helix. In this situation the  $T_m$  will be lowered as analyzed by Peller<sup>17, 18</sup> who used polypeptides as the model. The lowering of  $T_m$  will vary directly with concentration of added interacting substance.<sup>18</sup> On the other hand, the presence of the nucleoside in solution may cause an interaction, the detailed nature of which is not yet understood, but which lowers the stability of the helix. The denaturing effect of certain organic solvents may be of this category.

Polyadenylic acid (poly A) and thymus DNA have been chosen as the experimental material. Poly A, a homopolymer, has the advantage of simplicity for the structural interpretation of the interaction. The helix-coil transition and the helical structure of poly A are the best known among all synthetic polynucleotides.<sup>11-14, 16, 19, 20</sup> The stability of the poly A helix is sensitive to ion concentration and pH, and these factors must be controlled accurately in order that they do not mask the effect of added nucleoside. DNA, on the other hand, is a natural heteropolymer and the stability of its helical form is relatively less sensitive to pH and ionic concentration.

The helix-coil transition of these polymers can be conveniently followed by changes of optical properties such as the increase of absorption near 260  $m\mu$  region (hyperchromicity) or the loss of optical rotatory power.<sup>9-16</sup> Optical rotatory power measured at 436  $m\mu$  has the special feature that many of the added, highly u.v. absorbing substances which are optically inactive, such as purine and pyrimidine, do not interfere with the measurement.

*Materials.*—Compounds of the highest degree of purity available commercially were used without further purification. The following A grade (unless specific otherwise) compounds were obtained from California Corporation for Biochemical Research, Los Angeles: purine, cytidine, thymidine, uridine, adenosine, adonitol, inosine, 2,6-diaminopurine hemisulfate (B) and caffeine (C). Pyrimidine, uridine, and riboflavin were from Nutritional Biochemicals Corporation. 2,6-dichloro-7-methyl purine was from Cyclo Chemical Corporation, Los Angeles. Phenol (chromatographic, 88%) was from Mallinckrodt Co., and cyclohexanol (reagent grade) was from Fisher Scientific Co. All other chemicals were reagent grade.

Methyl D-riboside was synthesized from D-ribose with methanolic HCl according to the method of Jackson and Hudson.<sup>21</sup> The preparation was recrystallized.

Calf thymus DNA (highly polymerized) was purchased from Nutritional Biochemical Corporation. Some of its properties have been previously described.<sup>15, 16</sup> Poly A<sup>16</sup> was purchased from Miles Chemical Co., Clifton, N. J. The value of  $S_{w,20,50\%}$  of the poly A was 6.5 S in 0.1 *M* NaCl, 0.05 *M* Tris (tris(hydroxymethyl)aminomethane hydrochloride buffer).

*Instruments and Measurements.*—Optical rotation measurements were made with a Rudolph Model 200S polarimeter equipped with an oscillating polarizer and xenon and mercury arc lamps.<sup>15, 16</sup> The 10 cm polarimeter tubes were of unitized glass construction with water jacket and quartz window. Temperature of the polarimeter tube was read directly with a thermometer and held at any desired temperature to  $\pm 0.1^\circ\text{C}$  by the flow of water through both the compartment and the tube. Concentrations of poly A and DNA of 1-1.4  $\mu\text{g/ml}$  gave 1-1.4 degree rotation for poly A and 0.4-0.6 degree for DNA at 436  $m\mu$ .

Optical density measurements were made with a Beckman DK-2 u.v. recording spectrophotometer fitted with a modified temperature control device. Quartz cells were fitted with 20 mm immersion standard taper thermometers for direct reading of solution temperatures to  $\pm 0.1^\circ\text{C}$ .

pH measurements were made with Radiometer pH meter 22, Copenhagen, Denmark, and to an accuracy of  $\pm 0.01$  pH units.

Resistance of the solutions was measured to an accuracy of  $\pm 1\%$  at  $22.2 \pm 0.05^\circ\text{C}$  with a portable a.c. electrolytic conductivity bridge manufactured by Leeds and Northrop Co., New York.

Viscosities were determined with a four-bulb Ubbelohde-dilution-type viscometer designed and constructed by the Cannon Instrument Co., State College, Pennsylvania. The viscometer had a water flow time of 350 seconds for each bulb, and its maximum shear gradient for water varied from  $40 \text{ sec}^{-1}$  to  $210 \text{ sec}^{-1}$ . Measurements were performed at  $22.6 \pm 0.05^\circ\text{C}$ .

*Definition and measurement of  $T_m$  and  $\Delta T_m$ .*  $T_m$  is defined as the temperature at the midpoint of the helix-coil transition, measured optically.<sup>9</sup>  $\Delta T_m$  is defined as the lowering of  $T_m$  at constant pH and ionic strength brought about by the presence of an interacting substance. In the poly A system, where the temperature-invariant regions at low temperature (helical form) and high temperature (coil form) can be easily obtained,  $T_m$  can be measured experimentally with an accuracy of  $\pm 0.5^\circ\text{C}$ . In the DNA system, the temperature-invariant region at high temperature (near  $95^\circ\text{C}$ ) is not conveniently reached in routine optical rotation measurements. Consequently, the determination of  $T_m$  is made by approximation of the region at which the rate of change of an optical property is largest. The accuracy of  $T_m$  determination for DNA is  $\pm 1^\circ\text{C}$ . However, as shown later, the interacting substances lower  $T_m$  without appreciably changing the breadth of the transition. The melting curves of the polymer with and without the interacting substances parallel each other and the displacement of one curve with respect to the original can be measured precisely even though the midpoints cannot be determined with great accuracy. Consequently,  $\Delta T_m$  for DNA should be reliable to  $\pm 1^\circ\text{C}$ . For the poly A system, the  $\Delta T_m$  should also be within  $\pm 1^\circ\text{C}$ . The higher accuracy of determination is counteracted by the sensitivity of the system to small fluctuations in pH and ionic strength.

In the presence of an u.v. absorbing substance such as purine,  $T_m$  cannot be measured by the absorbance method (hyperchromicity), but can be conveniently determined by measuring rotations at  $436 \text{ m}\mu$  (Figs. 1 and 2). Nucleosides<sup>22</sup> such as uridine, do possess an optical rotation. The

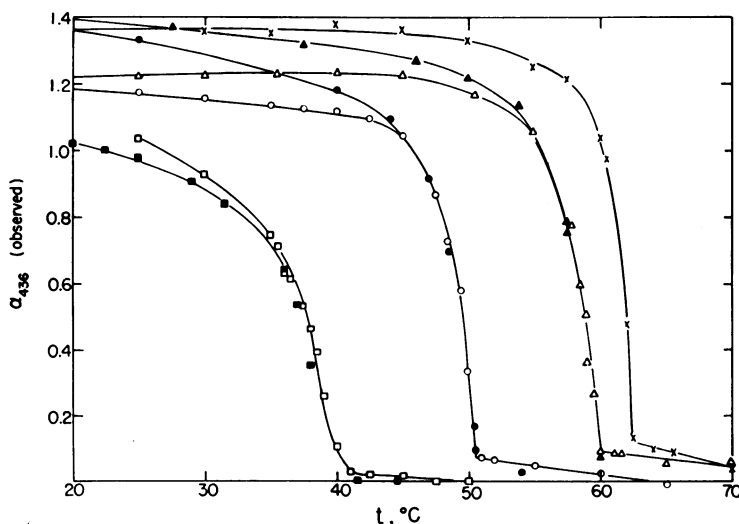


FIG. 1.—Helix-coil transition of poly A measured by optical rotation at  $436 \text{ m}\mu$  in various concentrations of purine. Buffer:  $0.2 \text{ M}$  Na-acetate, pH 4.85; poly A ( $-\times-\times-$ ); with  $0.05 \text{ M}$  purine ( $-\Delta-\Delta-$ ); with  $0.3 \text{ M}$  purine ( $-\circ-\circ-$ ); with  $0.6 \text{ M}$  purine ( $-\square-\square-$ ); empty symbols designate the heating curves and solid symbols designate cooling curves.

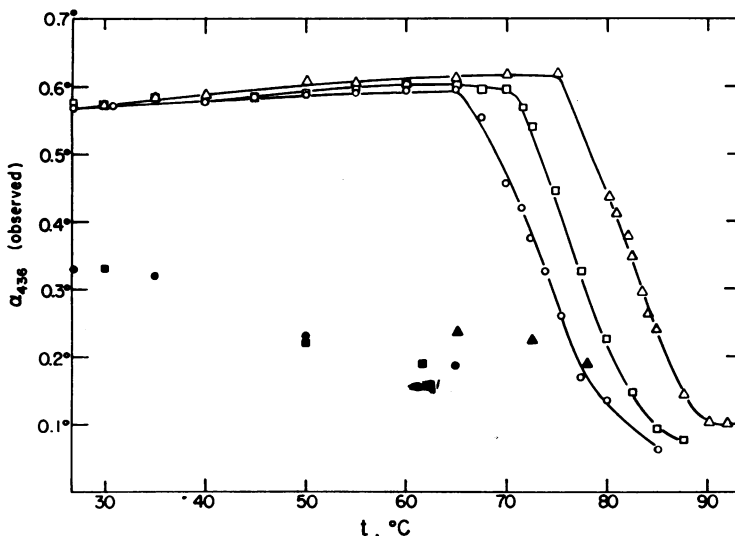


FIG. 2.—Helix-coil transition of DNA measured optical rotation at 436  $\mu$ . Buffer: 0.1  $\mu$  Na-phosphate, pH 6.5. DNA ( $\Delta$ ); with 0.3  $M$  pyrimidine ( $\square$ ) and 0.3  $M$  purine ( $\circ$ ). Empty symbols designate the heating curves and solid symbols designate the cooling curves.

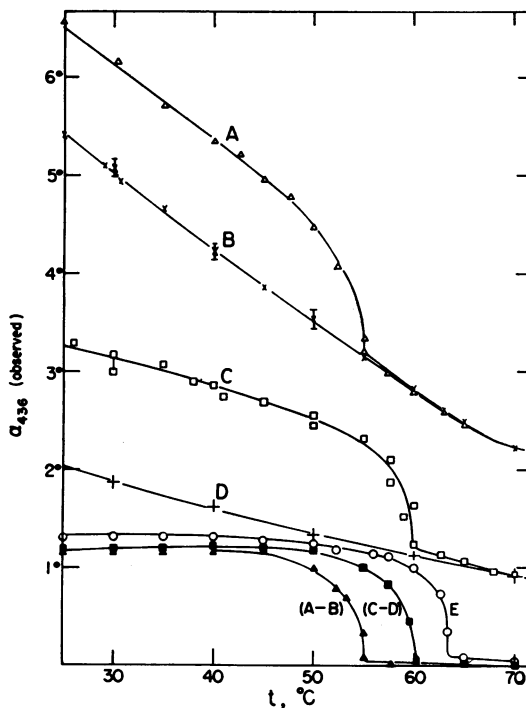


FIG. 3.—Helix-coil transition of poly A measured by optical rotation at 436  $\mu$ . Buffer: 0.2  $M$  Na-acetate, pH 4.85. Poly A and 0.6  $M$  uridine, A ( $\Delta$ ); 0.6  $M$  uridine alone, B ( $\times$ ); poly A and 0.15  $M$  uridine, C ( $\square$ ); 0.15  $M$  uridine alone, D ( $+$ ); poly A alone, E ( $\circ$ ); helix-coil transition of poly A in the presence of 0.6  $M$  uridine, (A-B) ( $\blacktriangle$ ); helix-coil transition of poly A in the presence of 0.15  $M$  uridine, (C-D) ( $\blacksquare$ ).

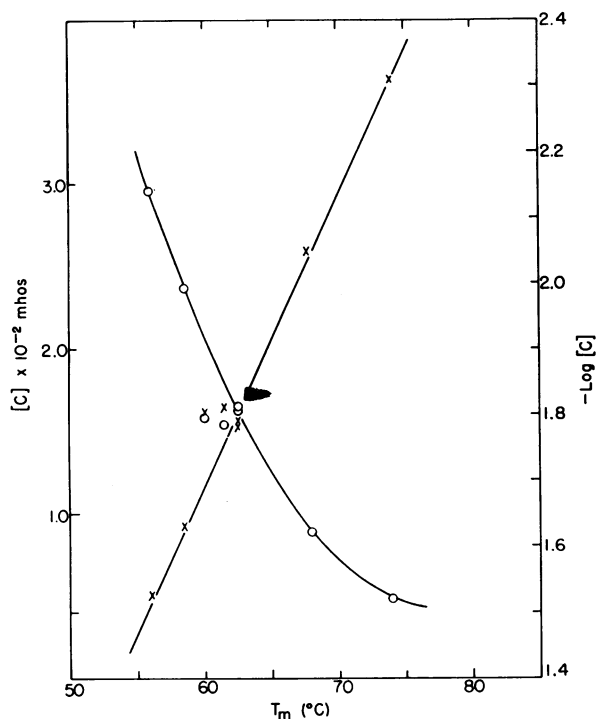


FIG. 4.—The  $T_m$  of poly A versus specific conductance. Specific conductance expressed in linear scale ( $\circ$ — $\circ$ — $\circ$ ) and expressed in logarithmic scale ( $\times$ — $\times$ — $\times$ ). Change of the conductance of the system was done by dilution of 0.4 M Na-acetate solution. pH in all solutions was pH 4.85.

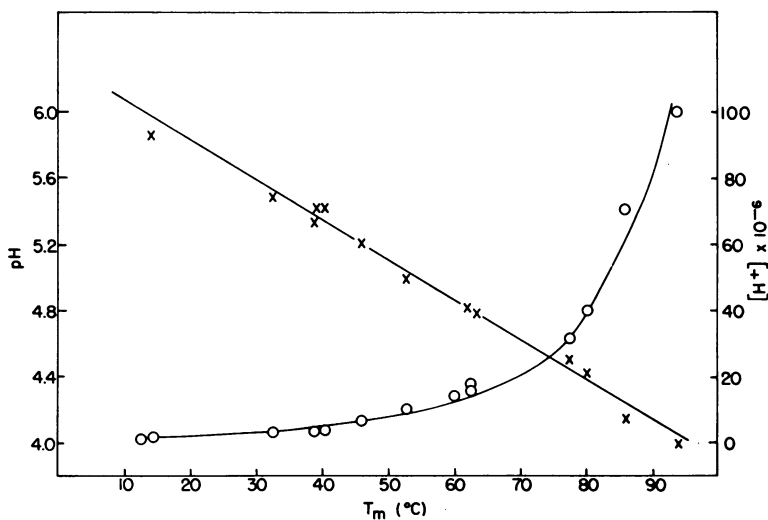


FIG. 5.—The effect of pH on the  $T_m$  of poly A. Hydrogen ions concentration in ( $\circ$ — $\circ$ — $\circ$ ); pH in ( $\times$ — $\times$ — $\times$ ). Change of the pH of the system was done with the addition of acetic acid to constant concentration of Na ions (0.2 M).

specific rotations are, however, not large and they change gradually with temperature (curves B, D of Fig. 3). The rotation of the nucleic acid, and therefrom the helix-coil transition temperature, can be separated by correcting the total rotation for the contribution of the added nucleosides (curves A-B and C-D in Fig. 3).

No differences in the  $T_m$  or  $\Delta T_m$  have been detected so far as between measurements with optical absorbance and optical rotation.

*Results and Discussion.*—*Effect of ionic concentration and pH on the  $T_m$  of poly A and DNA:* The effects of ionic concentration and of pH on the  $T_m$  of poly A and of DNA were studied so that these effects might be isolated from those of added interacting substances. Ionic concentration of the solutions is expressed by their specific conductance.

Figures 4 and 5 illustrate the dependence of  $T_m$  of poly A on conductance and pH. Such behavior has been observed before though perhaps less systematically.<sup>11, 12, 14–16, 23, 24</sup> The increased stability of poly A at low pH may be attributed to the presence of positively charged adenine groups which contribute to the stability of the helical form by attracting the phosphate group of the opposite chain.<sup>20, 24</sup> The reduced stability of helical poly A in high ionic concentration may be due to a lowering of the pK and thus of the charge of the adenine groups.<sup>24</sup>

The change of  $T_m$  of thymus DNA over a narrow range of pH and ionic strength is shown in Table 1. As might be expected, within the range of 6.5–7.5, the  $T_m$

TABLE 1  
THE MELTING TEMPERATURE,  $T_m$ , OF THYMUS DNA AT VARIOUS IONIC CONCENTRATIONS\* AND pH

Ionic strength $\mu$	Specific conductance mhos $\times 10^{-3}$	pH	$T_m$ (°C)
0.05	3.76	6.5	76.5
0.1	7.51	"	81.5
0.15	9.80	"	84.5
0.1	6.88	7.4	80.5

\* Na-phosphate buffer.

of DNA is not affected by change of pH. As for the effect of ionic concentration, our limited data on thymus DNA (Table 1) and more extended data on T-7 DNA<sup>25</sup> indicate that the  $T_m$  of DNA is proportional to the logarithm of ionic concentration. Similarly, the logarithmic dependence of  $T_m$  of poly A on ionic concentration is clearly shown in Figure 4. The effect on the  $T_m$  of poly A is the reverse of that on the DNA system. An effect of ionic concentration on the  $T_m$  of TMV-RNA similar to that on DNA has been reported.<sup>26</sup> This logarithmic relationship may be qualitatively anticipated from the theory of polyelectrolytes through the viewpoint of the distribution of counter ions,<sup>27, 28</sup> and specifically by changing the energetic term in the expression for the ratio of the partition functions of random and helical elements.<sup>29</sup>

Conductance measurements confirm that addition of nonionic substances does not change the ionic concentration of the systems enough to affect the  $T_m$  of either DNA or poly A noticeably. The pH of the poly A system, on the other hand, exerts a critical effect on  $T_m$ . The pH of the poly A system after addition of neutral interacting substances such as urea, uridine, purine, etc., was therefore always controlled to within 0.02 pH of that of the original system by the addition of small amounts of acetic acid. By careful measurement and control of pH and ionic

strength, we believe that these side effects have been reduced to less than one degree.

*Interaction of nucleosides and related compounds with nucleic acids as measured by  $\Delta T_m$ :* Data on the lowering of  $T_m$  (i.e.,  $\Delta T_m$ ) of poly A and of DNA in the presence of nucleosides and related compounds are summarized in Tables 2 and 3

TABLE 2  
INTERACTION OF NUCLEOSIDES AND RELATED COMPOUNDS WITH POLYADENYLIC ACID AS MEASURED BY  $\Delta T_m$ \*

Compounds	Concentrations used $M \times 10^2$	Experimental $\Delta T_m$ obtained at the maximum concentration used	Extrapolated† $\Delta T_m$ caused by interactant at molar concentration
Pyrimidine derivatives			
Pyrimidine	15, 30	6.5	17-20
Uridine	10, 15, 30, 60	9	15-30
Cytidine	15, 30	9	28-30
Thymidine	15, 30	10	33-40
Purine derivatives			
Purine	5, 15, 30, 60	23	40-80
Adenosine	6	3.5	55
Deoxyguanosine	5	5	100
Inosine	6, 15	6.5	50-100
Caffeine	10, 20‡	26	90-130
2,6-Dichloro-7-methylpurine	1§	3	300
Other compounds			
Phenol	25	4	16
Cyclohexanol	15, 30	2.5	8
Coumarin	2	3.5	180
Urea	300, 500	2	1
Adonitol	60	0	0
1-Methyl-ribose	60	0	0**

\* See text for the definition of  $\Delta T_m$ .  $\Delta T_m$  is experimentally accurate within one degree.

† Range given in this column is obtained from the range of experimental  $\Delta T_m$  obtained at both the highest and lowest concentration of interactant employed. Higher concentrations of interactant tend to give lower values for the extrapolated  $\Delta T_m$  per mole of interactant.

‡ A 0.20 molar caffeine solution can be obtained at 35°C. The  $\Delta T_m$  at 0.2 M is obtained from the cooling curve.

§ A 0.01 molar 2,6-dichloro-7-methylpurine solution can be obtained at 40°C.

|| Measured by both absorption and rotation method.

\*\* Measured by only absorption method.

TABLE 3  
INTERACTION OF NUCLEOSIDES AND RELATED COMPOUNDS WITH THYMUS DNA AS MEASURED BY  $\Delta T_m$ \*

Compounds	Concentration used $M \times 10^2$	Experimental $\Delta T_m$ C°	Extrapolated $\Delta T_m$ caused by interactant at one molar concentration
Pyrimidine	30	5	17
Thymidine	30	5.5	18
Uridine	30	5	17
Purine	30	9	30
Inosine	15	4.5	30
Caffeine	10	6	60
Urea	300, 400, 500	15 (at 5 M)	3

\* See text for the definition of  $\Delta T_m$ .  $\Delta T_m$  is experimentally accurate to within one degree.

respectively, and the effects of concentration of the various compounds on  $\Delta T_m$  are shown in Figure 6. The data indicate that  $\Delta T_m$  increases with concentration of the interacting substances employed and with a deviation from linearity which grows greater at higher concentrations. It should be noted, however, that the relative experimental error in  $\Delta T_m$  at low concentration is much higher than that at high concentration. The effectiveness of added compounds in lowering the  $T_m$  of poly A can be arranged as follows: adonitol, 1-methyl riboside,<sup>30</sup> urea (all with

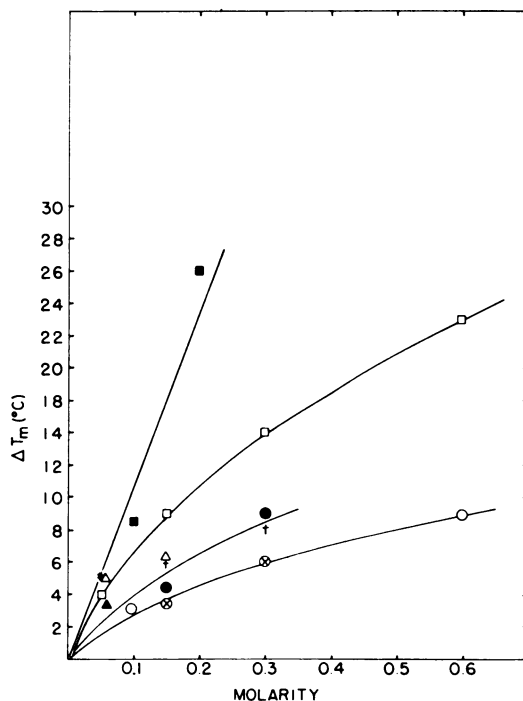


FIG. 6.—The  $T_m$  of poly A determined in various concentrations of interacting substances. Caffeine (■); deoxyguanosine (\*); purine (□); ionsine (Δ); deoxyadenosine (▲); cytidine (●); thymidine (+); uridine (O); and pyrimidine (×).

negligible effects) < cyclohexanol < phenol, pyrimidine, uridine < cytidine, thymidine < purine, adenosine, inosine, deoxyguanosine < caffeine, coumarin, 2,6-dichloro-7-methylpurine. It should be noted that since the effect of the last two substances can be studied only up to 0.01 *M* concentration,  $\Delta T_m$  again is subject to a large per cent of experimental error. There is no doubt that they lower the  $T_m$  of poly A, but it is not certain whether they are more effective than caffeine.

The same series holds also for the effectiveness of compounds tested in the DNA system. The extrapolated  $\Delta T_m$ /per mole of interactant tends to be 30–40% smaller for DNA with compounds such as thymidine, purine, and caffeine. Mandell and Hersey have also observed that phenol lowers the denaturation temperature of DNA.<sup>31</sup> 2,6-Diaminopurine at 0.01 *M* (limited by the solubility) was also found to lower the  $T_m$  of poly A by 5–6°C, a large value. Since this molecule has one pK at 5.1,<sup>32</sup> at the experimental pH 4.85, a considerable portion of the molecules in solution are in the cationic state. Therefore, the interaction of 2,6-diaminopurine with poly A may be assisted by the charge interaction and thus differ from the interactions of the uncharged molecules.

Interestingly enough, as shown in Tables 2 and 3, 5 *M* urea has an appreciable effect on  $T_m$  of DNA (15°C lowering), while 5 *M* urea has a negligible effect on  $T_m$  of poly A (no more than 2°C lowering). The result with thymus DNA is similar to that previously reported.<sup>33</sup> Steiner and Beers<sup>14</sup> had also noticed that the titration curve of poly A is not changed appreciably in the presence of 7 *M* urea.



Thus, it appears to be important to study more extensively the ineffectiveness of urea on  $T_m$  of poly A. If the pH of the solutions varies with temperature and if the heat of ionization is affected by urea, it might be that the urea effect is cancelled out by the pH effect. However, it has been found that acetate buffer (0.2  $M$ ) adjusted to pH  $4.85 \pm 0.01$  at  $26^\circ$  in the presence and absence of 5  $M$  urea shows no detectable change in pH at  $40^\circ$ . The effect of temperature on the pH of the system is therefore small, and the presence of urea does change it noticeably. A specificity in the interaction of urea with poly A and DNA was still not rigorously demonstrated, however, because the temperature needed for DNA melting is about  $85^\circ$  in the presence of urea. It is possible however to raise  $T_m$  for poly A to about the same point ( $86^\circ$  in 0.2  $M$  acetate buffer at pH 4.10), and under these conditions to make a more meaningful comparison between the urea effects on the two polymers in case the effect of urea is temperature dependent. After the heating of such a solution in a helix-coil transition experiment the urea was found to be partially hydrolyzed and the pH to have changed from 4.10 to 4.30. With 5  $M$  urea, the observed  $\Delta T_m$  for poly A was about  $4^\circ$ , but after correction for the pH change the effect was not more than  $2^\circ$ . Indeed all experimental evidence indicates little effect of urea on the stability of the poly A helix.

Certain conclusions in regard to the relationship of the structure of various interactants to their respective effectiveness in  $T_m$  lowering can be drawn from the data in Tables 2 and 3 and from the series presented before. These interpretations are especially definitive for poly A, a homopolymer system. (1) The interaction between nucleic acids and bases or nucleosides which results in lowering of  $T_m$  does not require the complementary structure of Watson-Crick (A to U). (2) Though other pairing schemes between bases have been proposed,<sup>34, 35</sup> the present interaction may not even involve hydrogen bonding with the ring substituents (the amino and keto groups). This conclusion is suggested by the fact that pyrimidine is not less effective than uridine and purine is not less effective than the nucleosides. (3) Though the present data do not exclude the possibility of hydrogen-bonding with the ring itself, results on pyrimidine and coumarin (which have only hydrogen-bonding acceptor sites) as well as 2,6-dichloro-7-methylpurine and caffeine (the hydrogen-bonding ability of the rings of these compounds is much less than that of purine) do not suggest that this is an important contribution. (4) Planar and aromatic rings are more effective than nonplanar and saturated rings. (5) Two-ring compounds are more effective than one-ring compounds. Methylation and chlorination also appear to increase the effectiveness. These data suggest that the system obeys Traube's rule in a broad sense.

*The helix-coil transition profile of nucleic acids in the presence of purines and pyrimidines:* Figures 2 and 6 show the helix-coil transition profile of DNA and poly A in the presence of purine and pyrimidine as studied by optical rotation at 436  $m\mu$ . It can be seen from these two figures that the lowering of  $T_m$  of DNA and poly A is large and significant. On the other hand, the effect of purine and of pyrimidine on the breadth of the transition is not very noticeable under the present experimental conditions, except in the case of poly A with 0.6  $M$  purine where the  $T_m$  has been lowered from  $62$  to  $38^\circ\text{C}$ . It has been observed that in the lowering the  $T_m$  of poly A as caused by increase in ionic strength and pH, the transition also has a tendency to be broadened. The present data are perhaps not precise enough

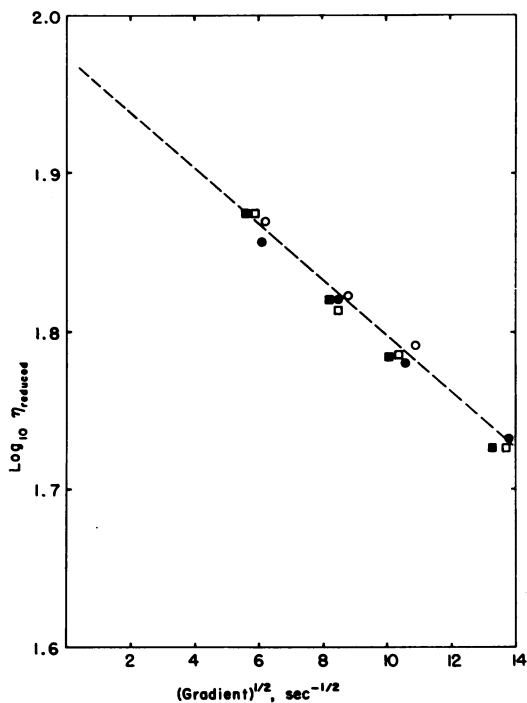


FIG. 7.—The reduced specific viscosity of calf thymus DNA in the presence of 0.3 *M* purine, 0.1  $\mu$  phosphate, pH 6.5. DNA, 31.7 micrograms/ml, (○); 43.7 micrograms/ml (□). The solid symbols indicate the presence of 0.3 *M* of purine in the same DNA solution.

for a critical analysis of the influence of these compounds on the breadth of the transition, but this aspect has considerable theoretical interest.<sup>29, 36</sup>

*Viscosity of DNA and poly A in 0.3 M purine solution:* Recent work of Lerman<sup>37</sup> has indicated that the viscosity of DNA increases significantly (up to more than 100%) in the presence of various cyclic cationic dyes such as acridine orange. Based on this and other observations, he suggests that these compounds are intercalated between adjacent nucleotide-pair layers by extension and unwinding of the deoxyribose-phosphate backbone.

Our investigation on the viscosity of thymus DNA in 0.3 *M* purine, a purine concentration high enough for a  $\Delta T_m$  of 9°C shows that the viscosity of DNA is unchanged in the presence of purine (Fig. 7). A similar result was also obtained for the poly A solution (0.2 *M* Na-acetate, pH 4.85) in the presence of 0.3 *M* purine, a concentration which elicits a  $\Delta T_m$  of about 15°C. The reduced specific viscosity of poly A (0.8 mg/ml, at about 400 sec<sup>-1</sup> gradient) was 0.85 dl/g, and in the presence of purine was 0.88 dl/g, a difference within experimental error.

*Comments.*—These investigations were initially based on the premise that the denaturation of nucleic acids by nucleosides may reflect the same degree of specificity as that of nucleotide incorporation in the enzymatic system. This is not observed. It is true that the condition in which the nucleoside triphosphates interact with the template DNA may be quite different from those in which nucleoside and the nucleic acid interact at elevated temperature. On the other hand,

the results do suggest that the recognition process in nucleic acid replication may involve more complicated effects than simple monomer-polymer interaction.

At present, the denaturation effect of the nucleosides and bases does not appear to be involved with hydrogen-bonding but rather with hydrophobic interaction. The term "hydrophobic interaction" adopted here includes van der Waals forces, the interaction of the  $\pi$ -electron systems, and the clustering tendency of nonpolar groups owing to the strong solvent-solvent interaction of water. Recently, the importance of the contribution of hydrophobic interaction to the stability of the helix of nucleic acids has been recognized. Various lines of evidence which support this contention are outlined below. (1) The denaturation of DNA and poly A in methanol and ethanol has been reported<sup>38, 39</sup> and explained in terms of hydrophobic interaction.<sup>38</sup> (2) The solubilization and formation of molecular complexes of various cyclic compounds by and with purine as well as the solubilization of these cyclic compounds by DNA has been reported.<sup>40, 41</sup> (3) Solubilization of adenine and thymine by purine, pyrimidine, uridine, and cytidine has been recently observed in our laboratory.<sup>42</sup> (4) The solubility of adenine and thymine has been found to be 3-6-fold higher in pyridine than in water (solubility expressed on the basis of moles of solute per mole of solvent).<sup>43</sup> This evidence together with the data presented here suggest to us that purines and pyrimidines interact with nucleic acids through hydrophobic bonds and thus weaken the hydrophobic interaction between adjacent bases. Before any definite understanding of this interaction can be achieved, the binding of interacting substances to nucleic acid must be quantitatively studied. Currently, this is being investigated.

It is interesting to note the ineffectiveness of urea as compared to the effectiveness of the purines and pyrimidines in lowering  $T_m$  of DNA and especially of poly A. Recently, questions have been raised concerning the mechanism of urea denaturation.<sup>44, 45</sup> It is not certain that the action of urea in denaturing these natural polymers is due to the breaking of hydrogen-bonding as heretofore stressed. Thus, it would be premature to suggest that hydrogen-bonding is not important in holding the poly A helix together. However, the difference between DNA and poly A in their responses to urea is noteworthy.

It is perhaps also tempting to suggest that a connection may exist between the type of interaction reported here and the mechanism of carcinogenesis and mutation of many cyclic compounds.<sup>46-48</sup> The results in this paper certainly warrant further investigation in this direction.

*Summary.*—A series of compounds has been tested for effectiveness in lowering the melting temperature of poly A and of thymus DNA. The order of increasing activity was found to be: adonitol, methyl riboside (both negligible) < cyclohexanol < phenol, pyrimidine, uridine < cytidine, thymidine < purine, adenosine, inosine, deoxyguanosine < caffeine, coumarin, 2,6-dichloro-7-methylpurine. Urea was ineffective with poly A and only slightly effective with DNA. At a concentration of 0.3 *M*, purine lowered the  $T_m$  of DNA about 9°.

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## THE ISOLATION OF YEAST RIBOSOMES ASSOCIATED WITH TRIOSE PHOSPHATE DEHYDROGENASE\*

BY WILLIAM A. WARREN AND DAVID A. GOLDTHWAIT†

DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE, WESTERN RESERVE UNIVERSITY,  
SCHOOL OF MEDICINE, CLEVELAND

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The role of the ribosome in the synthesis of proteins has been clarified with the development of *in vitro* systems which incorporate isotopically labeled amino acids into peptide linkages.<sup>1</sup> Recently, the older concept that the ribosome contained template RNA for the synthesis of specific proteins has been expanded by the demonstration of a metabolically active RNA designated as "messenger" RNA, which exists in bacterial cells both in the soluble fraction and associated with ribosomes.<sup>2, 3</sup> The ability of reticulocytes to synthesize protein in the absence of DNA<sup>4</sup> suggests that there may be cases in which the "messenger" RNA is stable. If it were possible to isolate a family of ribosomes which was involved in the synthesis of a specific enzyme, it might be possible to demonstrate that the RNA of these ribosomes differed in structural and functional properties from that of the total population. The data reported in this paper concern an immunological method of isolation. Antiserum to crystalline yeast triose phosphate dehydrogenase was used to precipitate a small percentage of the total ribosomal population. By this method, enrichment of the triose phosphate dehydrogenase activity per unit of ribosomal RNA was obtained.<sup>5</sup>

*Materials and Methods.*—Triose phosphate dehydrogenase (TPD) was prepared from baker's yeast (Red Star Yeast and Products Co., Cleveland, Ohio) by the method of Krebs.<sup>6</sup> Different preparations of the enzyme were recrystallized either two or three times. Crystalline aldolase, prepared from rabbit muscle by the method of Taylor *et al.*,<sup>7</sup> was provided by H. Z. Sable. The assay for triose phosphate dehydrogenase activity was as follows. To a cuvette with a 1 cm light path were added 0.6 ml of 0.06 *M* sodium pyrophosphate pH 8.5, 0.04 ml of 0.17 *M* sodium arsenate, 0.01 ml of 0.05 *M* DPN, 0.144 ml of 0.05 *M* cysteine, 0.27 mg of aldolase, an aliquot of the enzyme, and water to a final volume of 1.1 ml. After preincubation for 3 min, 0.1 ml of 0.05 *M* fructose diphosphate was added and the rate of reduction of DPN at 340  $m\mu$  between 30 and 90 sec was measured. Preparations with an activity of approximately 25  $\mu\text{m}/\text{min}/\text{mg}$  of protein, when assayed by this procedure, were obtained.

Alcohol dehydrogenase was prepared from baker's yeast (Red Star), recrystallized three times, and assayed by the methods of Racker.<sup>8</sup> For protein determinations, the Folin-phenol method<sup>9</sup> was employed as well as that of McDuffie and Kabat,<sup>10</sup> employing an extinction coefficient at 277  $m\mu$  of 0.01 per  $\mu\text{g}$  of protein nitrogen per ml.

Antisera to triose phosphate dehydrogenase and alcohol dehydrogenase were prepared by immunization of rabbits. This was done either by intravenous injections (12 injections of 5 mg each over a 4-week period) or by subcutaneous administration of enzyme in adjuvant. Thirty-two mg of protein in 4 ml of Bacto-Adjuvant Complete, Difco Laboratories, Detroit, Michigan, were injected into 4 loci on the back. One month after injection, 2 mg of enzyme were injected intravenously and 8 days later the rabbits were bled. The amounts of antibody obtained by the two methods were comparable. Sera were heated at 56°C for 30 min. Preparations of antisera