*THE STRUCTURE OF A DNA-RNA HYBRID**

By G. Milman, R. Langridge,† and M. J. Chamberlin

HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS; CHILDREN'S CANCER RESEARCH FOUNDATION AND HARVARD MEDICAL SCHOOL, BOSTON, MASSACHUSETTS; AND DEPARTMENT OF MOLECULAR BIOLOGY AND VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY

Communicated by J. D. Watson, April 17, 1967

The model for DNA involving specific base-pairing between antiparallel nucleic acid strands[1] forms the basis for our understanding of polynucleotide structures in general, and of their interactions in many biological processes. However, there are differences in chemical interaction and biological function between DNA and RNA. Presumably some of these differences are due to enzyme specificities which allow the selection of a nucleic acid strand on the basis of its component nucleotides. However physical studies[2] show that other factors must be considered, for differences exist in structural stability even between DNA and RNA helices of identical nucleotide sequence. An understanding of the nature of these differences and their role in nucleic acid behavior requires a detailed knowledge of the three-dimensional structures of the molecules. Fine structural details such as variations of the diameter of the helix and tilt of the bases can at present only be obtained by X-ray diffraction studies of nucleic acid fibers.

In this paper we report the first X-ray diffraction data obtained from a helical structure containing one strand of DNA and a complementary strand of RNA—a DNA-RNA hybrid. This molecule is of structural interest because of the information it may provide about the factors which determine the fine structure of nucleic acid helices, and of biological interest because of the role that base-pairing between DNA and RNA is thought to play in the transcription of DNA nucleotide sequences in RNA synthesis.

Materials and Methods.—Preparation of f1 phage: Bacteriophage f1 is grown and purified by a modification of the method described by Marvin and Schaller[3] for the preparation of bacteriophage fd. The bacterial growth medium contains in 1 liter: 10 gm of Difco Bacto-tryptone, 1 gm of Difco yeast extract, 2.5 gm of NaCl, 0.1 mole of tris (hydroxymethyl)aminomethane (tris), pH 7, and 2 mmoles of $CaCl_2$. *Escherichia coli* strain K12 Hfr is grown with vigorous aeration in glass carboys each containing 10 liters of medium. At a cell density of 2×10^8 to 4×10^8 cells/ml (measured with a Petroff-Hauser counter) a purified stock of f1 phage is added to give approximately 10 phage/cell. Growth is continued for 4 hr with the addition of antifoam (Dow-Corning antifoam C emulsion) to prevent foaming. At the end of this time about 10^{12} plaque-forming units/ml are usually present. To 10 liters of this suspension is added 1 liter of a solution containing ten parts of ethanol to one part of glacial acetic acid. A flocculent precipitate forms, some of which floats to the top (due to entrapment of bubbles) while some settles to the bottom. After 2 hr the intervening clear solution is removed with a glass tube connected to an aspirator pump. The precipitate is sedimented by centrifugation for 30 min at 6000 rpm in a Servall GSA rotor and suspended in 100 ml of 0.5 *M* tris buffer, pH 10.6 by mixing for 1 hr with a magnetic stirring bar. This turbid solution is then centrifuged for 30 min at 20,000 rpm in a Spinco 30 rotor to sediment bacteria and bacterial debris. The supernatant fluid is decanted and the pellet discarded. Further purification is carried out by repeated high and low speed centrifugations. A cesium chloride gradient is prepared (of average density 1.5 gm/cm^3) by adding 4.5 gm of CsCl to 10.0 ml of 1% phage solution and centrifuging for 48 hr in the Spinco SW39 rotor at 37,000 rpm. The phage forms a narrow opalescent band in the middle of the tube, while contaminating materials are usually seen as light brown or white bands on either side of the phage band. The phage band is collected by slicing the tube into sections (using a device kindly provided by M.

Meselson), since the high viscosity of the phage band makes usual collection procedures impractical. A 1% solution of the purified phage is clear and extremely viscous. The phage fraction is dialyzed against distilled water overnight and lyophilized for storage.

Preparation of f1 phage DNA: Lyophilized phage is suspended in a buffer of 0.1 *M* sodium phosphate, 0.1 *M* NaCl, and 0.001 *M* ethylenediaminetetraacetate (EDTA), pH 7.7 (phosphate-NaCl buffer). After an even suspension is obtained, the solution is diluted to an optical density of about 60 at 260 mμ and extracted twice with an equal volume of phenol saturated with phosphate-NaCl buffer. The phenol layers are pooled and extracted once with a small amount of phosphate-NaCl buffer. The aqueous layers are pooled and dialyzed for several days against repeated changes of phosphate-NaCl buffer. Final dialysis is against two changes of 0.01 *M* tris buffer, pH 7.5. The resulting solution is diluted to give 1 to 2 μmoles of DNA nucleotide/ml as determined by optical density measurements (assuming[4] an extinction coefficient of 7.4×10^3).

Boundary sedimentation analysis[5] in 1 *M* NaCl–0.1 *M* NaOH of f1 DNA as extracted from the phage indicates that over 70% of the DNA molecules are circular.[3] As discussed later in this paper, fibers of DNA-RNA hybrid made from this predominantly circular material give poor X-ray diffraction patterns. We therefore prepare linear DNA by treating the f1 DNA with pancreatic DNase for a period sufficient to open all of the circles, yet leave the resulting linear molecules reasonably intact. The DNase reaction mixture contains in 12 ml: 120 μmoles of tris, pH 8, 11 μmoles of $MgCl_2$, 26 μmoles of f1 DNA nucleotide, and 1.2×10^{-3} μg of pancreatic DNase. After 30 min at 30°C the mixture is heated for 2 min at 80°C to inactivate the enzyme. This preparation is used directly for the synthesis of DNA-RNA hybrid.

Preparation of DNA-RNA hybrid: Synthesis of DNA-RNA hybrid is carried out using RNA polymerase fraction IV from *E. coli*.[6, 7] A typical reaction mixture contains the following components in a final volume of 25 ml: 2 mmoles of tris buffer, pH 8.1, 25 μmoles of $MnCl_2$, 20 μmoles each of UTP, GTP, and CTP, 300 μmoles of 2-mercaptoethanol, and 8.5 μmoles of f1 DNA on a nucleotide basis. Since a structure having equimolar proportions of DNA and RNA is desired, a trial reaction is carried out before each large synthesis to determine the length of time needed to produce such a hybrid. A 250-μl aliquot is removed from the large reaction mixture described above, and synthesis initiated by the addition of 200 mμmoles of C^{14}-labeled ATP and 70 μg of RNA polymerase. The mixture is incubated at 37°C, and aliquots are removed at 30-min intervals to determine, by a standard procedure,[8] the amount of RNA synthesized. It is assumed that the RNA product is strictly complementary to the phage DNA added[7] and that the thymine content of f1 is 34%.[4] A reaction period of 2 to 3 hr is needed to allow synthesis of an equivalent amount of RNA. The large synthesis reaction is started by the addition of 20 μmoles of ATP and 7 mg of RNA polymerase and incubated at 37°C for the appropriate length of time. The reaction is terminated by adding 1 ml of 5 *M* NaCl and 2 ml of a 0.1 *M* EDTA solution previously adjusted to pH 7.5. The solution is extracted twice with equal volumes of phenol saturated with phosphate-NaCl buffer, and the phenol layers back-extracted with a minimal volume of phosphate-NaCl buffer. The pooled aqueous layers are dialyzed for 24 hr against a solution of 0.5 *M* NaCl, 0.01 *M* $NaPO_4$, and 0.01 *M* EDTA having a pH of 7.5; for 24 hr against a solution of 0.5 *M* NaCl, 0.01 *M* $NaPO_4$, and 10^{-4} *M* EDTA having a pH of 7.5; and finally for 24 hr against 0.01 *M* $NaPO_4$ and 10^{-4} *M* EDTA having a pH of 7.5. The material is then lyophilized and stored at −20°C.

The f1 DNA-RNA hybrid thus prepared shows a single ultraviolet (UV) absorbing band at a density of 1.510 gm/cc in a cesium sulfate density gradient in the analytical ultracentrifuge.[7] From the density and lack of contaminating DNA or RNA bands and from the precision of the determination of the kinetics of RNA synthesis it is estimated that the ratio of RNA to DNA in the polymer is 1.0 ± 0.1.

Preparation of fibers of the DNA-RNA hybrid: Two glass rods about 1 mm in diameter are mounted in holders which can be moved apart slowly and steadily by means of a fine micrometer screw. The tips of the glass rods are aligned approximately 1 mm apart and a drop of distilled water is placed between them. When a small piece of dry DNA-RNA hybrid is placed in a drop, it rapidly absorbs the water, forming a sticky gel between the tips of the glass rods. The glass rods are then slowly moved apart under continual microscopic observation in polarized light. As the gel slowly dries, it reaches a stage where slow stretching is possible without breakage.

Fibers prepared from lyophilized DNA-RNA hybrid have a granular appearance, and the dif-

fraction pattern shows poor orientation and salt rings indicating that the fibers contain a large amount of salt. Fibers made from alcohol-precipitated DNA-RNA hybrid previously dialyzed against SSC (0.15 *M* NaCl and 0.015 *M* sodium citrate pH 7) also contain excessive amounts of salt. However, good specimens can be obtained by wetting a small amount of lyophilized DNA-RNA hybrid to form a gel and soaking this overnight in a large volume of solution containing 75% ethanol, 22.5% water, and 2.5% SSC. This material is then dried and fibers are prepared as above. Specimens made in this manner show excellent extinction between crossed polaroids and no granular appearance. When observed in a Leitz polarizing microscope using a Leitz-Berek compensator, the best fibers have a negative birefringence of 0.08 which compares well with that given by the best DNA fibers.

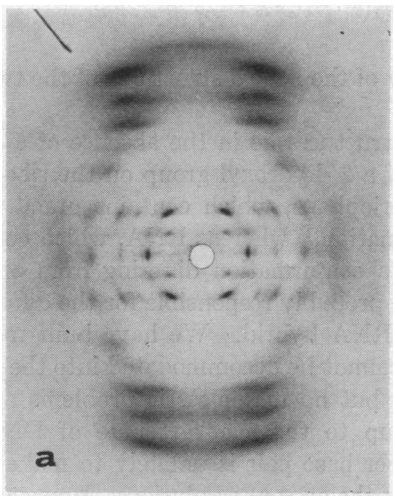
To prestretch a fiber so that it will not bend out of the X-ray beam at high humidities, it is cemented with nail polish to a holder with a phosphor bronze spring acting as a stretcher, and placed in a small sealed box containing a sponge saturated with water. In a few minutes the fiber buckles as water is absorbed. Slack is taken up by adjustment of the spring and the fiber is allowed to dry. After two to three sequences of stretching and drying, the fibers do not change noticeably in length in going from low to high humidity.

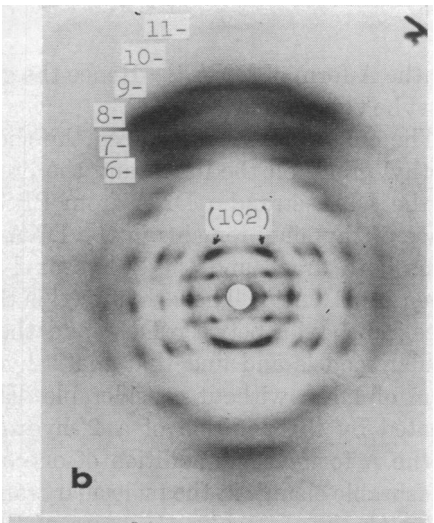
X-ray diffraction apparatus: X rays are generated by Jarrell-Ash line focus units with a spot size of 1.4 × 0.1 mm viewed at a 5 degree take-off angle giving a foreshortened 0.1- × 0.1-mm effective spot size. Copper targets and 0.016-mm nickel filters yield predominantly copper K-alpha radiation of 1.54 A wavelength. The cameras use collimators of lead glass 1 cm in length and 0.06 mm internal diameter. Sheet lead 0.5 mm thick with a hole 0.5 mm in diameter is placed at each end of the collimator; and a platinum electron microscope aperture 0.1-mm hole diameter is placed at the exit. Suitable regions of the fiber specimen are aligned with the collimator using a microscope illuminator. The distance from specimen to film is approximately 27 mm and is calibrated using the diffraction pattern of powdered calcium sulfate. The exact height of the specimen above the platinum guard is measured with the calibrated vertical travel of a microscope. Helium is bubbled through appropriate saturated salt solutions and then through the cameras to control the relative humidity. The saturated salt solutions used are: calcium chloride, 33% relative humidity; sodium nitrite, 66% relative humidity; sodium chlorate, 75% relative humidity; and sodium tartrate, 92% relative humidity. Exposure time is of the order of 30 hr with Ilford Industrial G X-ray film.

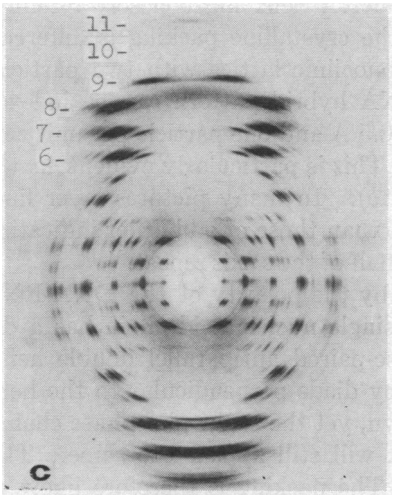
Results.—The DNA-RNA hybrid made from linear DNA, as described above, gives excellent data. Fibers prepared from the predominantly circular material show poor crystallinity and orientation. This may be due to the difficulty of packing and orienting the circles.[9]

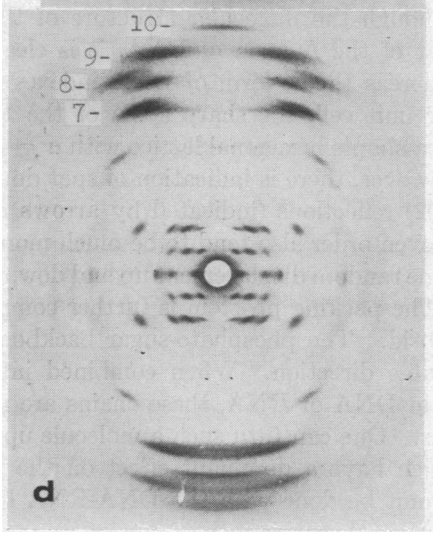
Figure 1(*a*) is a diffraction pattern from a fiber of DNA-RNA hybrid at 75 per cent relative humidity mounted perpendicular to the X-ray beam. At 66 per cent relative humidity the pattern shown in Figure 1*b* is observed. (This fiber is tilted approximately 15° from the perpendicular to the X-ray beam, to bring the meridional spot on the 11th layer line into the reflecting position.) There is little change in the diffraction pattern over the relative humidity range from 33 to 92 per cent, and we conclude that there is no dramatic change in the structure of the DNA-RNA hybrid comparable to the transition from the A form to the B form of DNA.

Figure 1*c* shows the diffraction pattern of the sodium salt of DNA in the A form,[10] and Figure 1*d* that of the sodium salt of double-stranded reovirus RNA.[11] The general intensity distribution of the DNA-RNA hybrid pattern closely resembles that of the A form of DNA (Fig. 1*e*) and differs from RNA. The sixth, seventh, and eighth layer lines are very strong, the tenth is weak and the eleventh appears to be meridional. The maxima on the zeroth through fifth layer lines also match the A pattern almost exactly. The helical repeat distance from the layer line spacing is 28.8 ± 0.5 A which agrees with the latest estimate of 28.15 ± 0.16 A









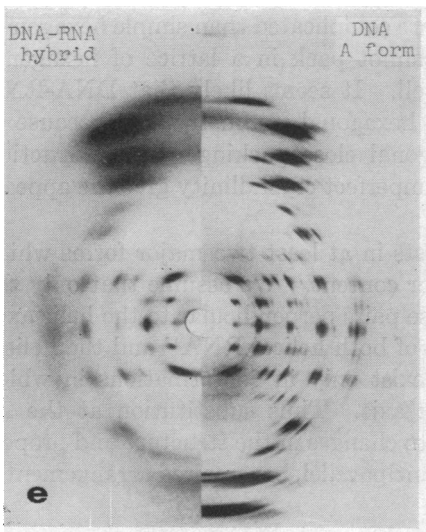


FIG. 1.—(*a*) X-ray diffraction pattern of a fiber of the sodium salt of the DNA-RNA hybrid at 75% relative humidity, mounted perpendicular to the X-ray beam. (*b*) X-ray diffraction pattern of a fiber of the sodium salt of the DNA–RNA hybrid at 66% relative humidity, the fiber is tilted from the perpendicular to the X-ray beam. The higher layer lines are numbered and the (102) reflections indicated. (*c*) X-ray diffraction pattern of a fiber of a sodium salt of calf thymus DNA at 75% relative humidity, the fiber is tilted from the perpendicular to the X-ray beam.[10] The higher layer lines are numbered. (*d*) X-ray diffraction pattern of a fiber of the sodium salt of reovirus RNA at 92% relative humidity, the fiber is tilted from the perpendicular to the X-ray beam.[11] The higher layer lines are numbered. (*e*) A comparison of the diffraction patterns given by fibers of the sodium salt of the DNA–RNA hybrid (*left*) and the sodium salt of DNA in the A form at 75% relative humidity (*right*). Both fibers are tilted from the perpendicular to the X-ray beam.

for the A form of DNA.[12] Hence the geometry of the helical structures of the two must be very similar.[13]

The only differences between the chemical structures lie in the absence of a 5-methyl group on the uracil and the presence of a 2′-hydroxyl group on the ribose of the RNA strand. DNA from PBS2 bacteriophage, which contains uracil in place of thymine, remains in the DNA conformation,[15] whereas RNA, which contains both uracil and the 2′-hydroxyl adopts a conformation differing from any given by DNA.[11] The 2′-hydroxyl is therefore probably responsible for the difference in behavior between DNA and the DNA-RNA hybrid. We have built molecular models and find that the 2′-hydroxyl cannot be accommodated into the B form of DNA without considerable distortion, but no major steric problems are created by the addition of a 2′-hydroxyl group to the sugar residue of DNA in the A form. The addition of one oxygen per base pair is unlikely to make a measurable change in the molecular transform at the present resolution. However, although the molecular structure of the DNA-RNA helix must closely resemble that of the A form of DNA, it is clear that the crystalline packing is different. Whereas the A form of DNA indexes on a monoclinic lattice with two particles per unit cell, the sharp spots of the DNA-RNA hybrid pattern can be indexed on a simple hexagonal lattice with $a = 23.1 \pm 0.5$ A and one particle per unit cell. However, there is indication of spot doubling. This is particularly evident in the (102) reflections (indicated by arrows on Fig. 1*b*). In many pictures layer lines of even order also tend to be much more diffuse than those of odd order indicating some random displacement up and down by one half of the *c* axis repeat.

The packing problem is further complicated by the polarity of the DNA-RNA hybrid. The phosphate-sugar backbone of a single nucleic acid chain has a definable direction. When combined into a base-paired antiparallel double helix, as in DNA or RNA, these chains are related by diads perpendicular to the helix axis. One can turn such a molecule upside-down, yet the sugar-phosphate chains, which have a dominant effect on the packing, will still appear the same. This cannot be done with the DNA-RNA hybrid. The packing is therefore likely to be somewhat irregular.

It is not clear whether a single fiber contains domains having different lattice parameters or whether the actual lattice is more complicated than simple hexagonal. Particles having an elevenfold screw axis cannot pack in a lattice of hexagonal symmetry with only one particle per unit cell. It seems likely that DNA-RNA hybrid helices adopt a lattice of lower than hexagonal symmetry, but because of their cylindrical shape, lie in roughly hexagonal close packing. The diffraction spots would then lie close together and with imperfect crystallinity give the appearance of a simple hexagonal lattice.

Discussion.—The sodium salt of DNA exists in at least two major forms which are interconvertible by variation of the water content. We assume that only the high-humidity form, the B form, with the base pairs perpendicular to the helix axis, exists in solution. In contrast, sodium salts of both helical RNA[11] and the helical DNA-RNA hybrid reported here, seem to exist only in conformations in which the bases are tilted at 70–80° to the helix axis. Thus substitution at the 2′-position of the nucleotide sugar residue leads to changes in the structure and properties of the resulting helix although the basic antiparallel, base-paired arrangement is

TABLE 1

COMPARISON OF DNA, RNA, AND DNA-RNA HYBRID NUCLEIC ACID HELICES

Nucleic acid (sodium salt)	Relative humidity (%)	Helix repeat (A)	Residues per turn	Translation per nucleotide (A)	Base-helix axis angle (degrees)	Ref. no.
DNA A form	75	28.15 ± 0.16	11	2.56	70	10, 12
DNA B form	92	34.6 ± 0.3	10	3.46	90	16
RNA	15–92	30.0 ± 0.15	10 or 11*	3.00 or 2.73*	77 or 73*	11, 14, 17–19
DNA-RNA Hybrid	33–92	28.5 ± 0.5	11	2.60	70	Present work

* See ref. 19 for discussion.

not altered. Marked variations in physical properties between DNA, RNA, and DNA-RNA hybrid helices in solution have also been reported.[2, 7] The parameters of these structures as measured by X-ray diffraction are presented in Table 1.

The thermal stability of a DNA-RNA hybrid in solution is lower than that of the equivalent DNA or RNA helices, and this suggests that the hybrid helix is thermodynamically less stable as well. Since the B form of DNA is more stable in solution than the A form, some of this difference may be due to the energy needed to convert the DNA strand of the hybrid from the B into the A form. Although the ΔF of the B to A transition is not known, one can estimate[20] that a ΔF of about 0.1 kcal/mole of nucleotide for that transition would be sufficient to account for the 4°C difference in melting temperature (T_m) between hybrid and DNA helices. Thus the 2′-hydroxyl on the RNA strand might provide a steric barrier which prevents the DNA strand from assuming the B configuration and could account for the depressed stability of the hybrid helix.

Although fiber analysis by X-ray diffraction is potentially capable of giving a close approximation to the structure of a molecule, the structure in solution may differ from that in the crystalline environment. Hence one can question the validity of any attempts to compare the relative structures of DNA, RNA, and DNA-RNA hybrid helices in solution using data based only on fiber studies. Although ultimately this objection can only be answered by some method of monitoring structure in dilute solution,[22] at 92 per cent relative humidity a nucleic acid molecule in a fiber is already in a highly aqueous environment. In the case of DNA, as many as 30 molecules of water are present per base pair,[10] an amount far in excess of that needed simply to hydrate the molecule. Hence the transition from fiber to solution is not really abrupt, and this should minimize the likelihood of a conformational change occurring during solution. Our conclusion that the DNA-RNA hybrid does not adopt the B conformation in solution is supported by the finding that actinomycin D, which is believed to attach only to the B form, does not bind to DNA-RNA hybrid in solution.[24]

The A form of DNA was previously thought to be no more than an artifact of dehydration, useful in checking the general form of the proposed model. Our work now shows that it may have significance as one of the stable forms of the hydrated nucleic acids.

Summary.—X-ray diffraction patterns of the sodium salt of a DNA-RNA hybrid prepared from single-stranded f1 DNA show that the hybrid possesses a helical conformation almost identical to the A form of sodium DNA, and differing from double-helical RNA. Unlike DNA, no major conformational change occurs

as the relative humidity is varied. We therefore believe that this conformation of the DNA-RNA hybrid is maintained in solution. This restriction is probably imposed by the sugar 2′-hydroxyl groups on one strand.

We thank N. D. Zinder for the original supply of f1 bacteriophage, and A. W. MacEwan for assistance in molecular model building.

* Supported by USPHS NIH predoctoral fellowship 1-F1-GM-20, 817-01A2 (G. M.), USPHS NIH research grant GM-14740 and NSF research grant GB-2330 (R. L.), and USPHS NIH research grant GM-12010 (M. J. C.)

† Present address: Department of Biophysics, University of Chicago, Chicago, Ill. Reprint requests should be directed to R. Langridge at the University of Chicago.

[1] Watson, J. D., and F. H. C. Crick, *Nature*, **171**, 737 (1953).

[2] Chamberlin, M. J., *Federation Proc.*, **24**, 1146 (1965).

[3] Marvin, D. A., and H. Schaller, *J. Mol. Biol.*, **15**, 1 (1966).

[4] Hoffman-Berling, H., D. A. Marvin, and H. Dinwald, *Z. Naturforsch.*, **18b**, 876 (1963).

[5] Studier, F. W., *J. Mol. Biol.*, **11**, 373 (1965).

[6] Chamberlin, M. J., and P. Berg, these PROCEEDINGS, **48**, 81 (1962).

[7] Chamberlin, M. J., and P. Berg, *J. Mol. Biol.*, **7**, 297 (1964).

[8] Josse, J., and A. Kornberg, *J. Biol. Chem.*, **237**, 1968 (1962).

[9] Fiber preparation is an erratic business, and although our results are good, our reasoning may not be. This matter has not been pursued further.

[10] Langridge, R., W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, and L. D. Hamilton, *J. Biophys. Biochem. Cytol.*, **3**, 767 (1957).

[11] Langridge, R., and P. J. Gomatos, *Science*, **141**, 694 (1963).

[12] Fuller, W., M. H. F. Wilkins, H. R. Wilson, and L. D. Hamilton, *J. Mol. Biol.*, **12**, 60 (1965).

[13] This conclusion in no way depends on the model adopted. The inconsistency between the latest model for the A form of DNA constructed by Fuller *et al.*[12] and the infrared dichroism data of Sato *et al.* (ref. 14 p. 189) does not effect our argument.

[14] Sato, T., Y. Kyogoku, S. Higuchi, Y. Mitsui, Y. Iitaka, M. Tsuboi, and K. Miura, *J. Mol. Biol.*, **16**, 180 (1966).

[15] Langridge, R., and J. Marmur, *Science*, **143**, 1450 (1964).

[16] Langridge, R., H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, and L. D. Hamilton, *J. Mol. Biol.*, **2**, 19 (1960).

[17] Langridge, R., M. A. Billeter, P. Borst, R. H. Burdon, and C. Weissmann, these PROCEEDINGS, **52**, 114 (1964).

[18] Tomita, K., and A. Rich, *Nature*, **201**, 1160 (1964).

[19] Arnott, S., F. Hutchinson, M. Spencer, M. H. F. Wilkins, W. Fuller, and R. Langridge, *Nature*, **211**, 227 (1966).

[20] Such an estimate can be obtained[21] by using the Gibbs-Helmholtz relationship and assuming that ΔH is 7 kcal/mole of base pairs. This gives a difference in ΔF of about 0.1 kcal/mole of base for the 4°C difference in T_m which has been reported[7] to exist between a DNA-RNA hybrid and a homologous DNA helix.

[21] Crothers, D., and B. Zimm, *J. Mol. Biol.*, **9**, 1 (1964).

[22] Small angle X-ray scattering on an absolute scale is, in principle, capable of measuring the translation per nucleotide pair of nucleic acids in solution.[23] Due to experimental limitations the results cannot approach the accuracy of most fiber diffraction in this measurement, but the difference we predict between DNA (3.4 A) and the DNA-RNA hybrid (2.6 A) is probably large enough for a significant test by this technique.

[23] Luzzati, V., *Progr. Nuc. Acid Res.*, **1**, 347 (1963).

[24] Haselkorn, R., *Science*, **143**, 682 (1964).