A HYBRID HELIX CONTAINING BOTH DEOXYRIBOSE AND RIBOSE POLYNUCLEOTIDES AND ITS RELATION TO THE TRANSFER OF INFORMATION BETWEEN THE NUCLEIC ACIDS

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One of the central problems in molecular biology today is the mechanism whereby genetic information which is stored in the deoxyribosenucleic acid (DNA) is transferred to other molecular species. It is quite clear that genetic information is contained in the DNA molecules. This was initially demonstrated by work on the bacterial transforming factor and more recently by the analysis of the mechanism of bacteriophage infection. It is equally clear at the present time that the major mode of expressing a genetic potentiality is by governing protein synthesis. Protein synthesis is carried out in the microsomal particles. However, the information bearing elements in protein synthesis are believed to be found in the ribosenucleic acid (RNA), which constitutes over one half of the microsomal particle. The other component of these particles is protein, and it is unlikely that this has an important role in ordering the sequence of amino acids because the microsomal protein component seems to be common to all particles even though they are synthesizing widely different proteins.[1]

This has led to the hypothesis that DNA "makes" RNA. The implication in this statement is that there exists a mechanism whereby the DNA molecule can act as a template for determining the order of ribonucleotides, so that the DNA nucleotide sequence (hence information) is related to that found in the RNA molecule. Up to the present there has been no experimental evidence regarding the mechanism of this transfer. The purpose of this paper is to show that it is possible to form a specific and complementary helical complex involving a synthetic DNA strand and a synthetic RNA strand. In this we demonstrate that this is a *possible* method for the transfer of the information from DNA to RNA.

Synthetic polynucleotides were used in carrying out this study. For several years, the synthetic polyribonucleotides have been available as a result of the work of Ochoa and his collaborators on the enzyme polynucleotide phosphorylase.[2] In the presence of a proper substrate, this enzyme has the ability to polymerize a variety of nucleotides which have the same ribosephosphate backbone as that found in naturally occurring RNA. These materials have been extremely useful in carrying out a variety of experiments on polynucleotide interaction. In particular, it has been possible to demonstrate with them the formation of a variety of two and three stranded helical complexes, such as polyriboadenylic acid plus one or two strands of polyribouridylic acid.[3, 4]

More recently synthetic polynucleotides containing the deoxyribose backbone have been polymerized by Khorana and his associates.[5] Using a new polymerization mechanism, they have been able to make a series of deoxyribose polymers with chains containing up to 20 residues. Although these are considerably shorter than many of the synthetic polyribonucleotides, nonetheless it was decided to attempt to form helical complexes with these materials.

Methods and Materials.—The author is indebted to Professor Khorana who generously made available a reaction mixture from one of his polymerizations of polydeoxyribothymidylic acid.* This reaction mixture was chromatographed on a diethyl-amino-ethyl cellulose column using a lithium chloride gradient at neutral pH. This method is essentially a modified form of that used earlier by Khorana and his collaborators.[5] From the eluate it is possible to obtain reasonably pure fractions which have various degrees of polymerization. Optical density measurements at room temperature were made in a Cary Recording Spectrophotometer. A Beckman spectrophotometer equipped with thermal spacers was used for making optical density measurements at other temperatures. Sedimentation constants were determined in a Spinco analytical ultracentrifuge. Extinction coefficients were measured using a modified orcinol reaction for the ribose polymers and acidic hydrolysis for the deoxyribose polymer.[6]

Results.—Initially, three different experimental methods were used to demonstrate the formation of two and three stranded helical complexes among the synthetic polyribonucleotides.[3, 4] When two polynucleotide species combine to form a helical complex there is usually a drop in the optical density of the absorption band. The hypochromicity in the ultraviolet arises in conjunction with the packing of the purine and pyrimidine residues. This drop can be used as a quantitative measure of the extent of complex formation and as a means of measuring the stoichiometry by using the method of continuous variation. Another experimental method is the demonstration of aggregation shown by an alteration of the hydrodynamic properties of the solution reflecting the change in molecular weight. This is most easily accomplished by using the analytical ultracentrifuge. Finally, X-ray diffraction studies of fibers provide a direct demonstration of the helical nature of the complex and are used to deduce the geometry and structure of the complex.

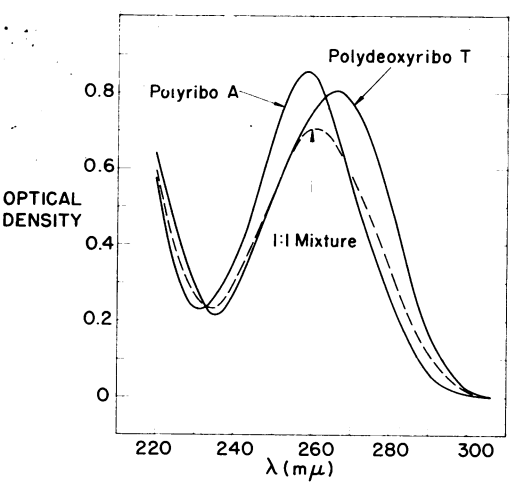

FIG. 1.—The spectra of polyriboadenylic acid, polydeoxyribothymidylic acid and a 1:1 mixture. All solutions are in 0.6 *M* NaCl, 0.01 *M* sodium cacodylate at pH 6.9. T = 24°C.

In the present communication, we report spectrophotometric and ultracentrifugal experiments which demonstrate the existence of the complex. X-ray diffraction studies have been initiated but are still in a preliminary stage and the results of those experiments will be reported elsewhere.

The polydeoxyribothymidylic acid used for the spectrophotometric studies consisted of a mixture of polynucleotides with 11 to 13 residues in the polymer chain. The polyriboadenylic acid was a much longer material containing around 2,000 nucleotides. The ultraviolet absorption spectrum of these materials is illustrated in Figure 1. Polyriboadenylic acid has an absorption maximum at

2,590 Å, while polydeoxyribothymidylic acid has a maximum at 2,660 Å. The 1:1 mixture of these materials at neutral pH in 0.6 *M* NaCl shows a lowering of optical density which can be seen very clearly where the two individual absorption spectra cross at 2,625 Å. There is a 12 per cent lowering of the optical density at that wavelength. This is somewhat lower than the hypochromicity which has been reported for other polynucleotide complexes,[4, 7] and is related to the fact that one of the polynucleotides is very short. The hypochromicity is in fact related to the degree of polymerization and this will be discussed more thoroughly in another paper.

An indication of the type of complex found can be shown by measuring the absorption coefficient at 2,625 Å for a variety of mixtures of the two polynucleotide

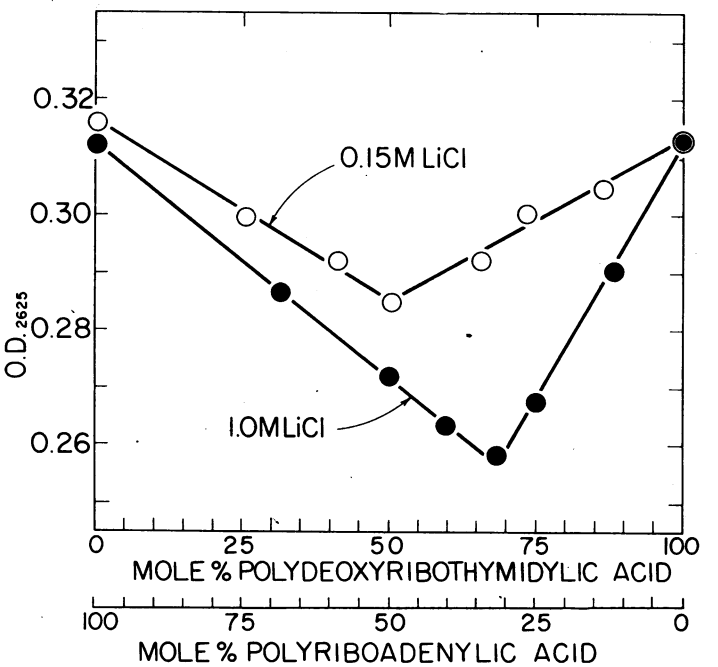

FIG. 2.—The optical density at 2625 Å of solutions containing polyriboadenylic acid and polydeoxyribothymidylic acid. The total nucleotide concentration is the same for all points. The solutions are at pH = 7.0. T = 24°C.

species. The results are illustrated in Figure 2. The concentration of polymeric materials is the same at all points but the mole ratio is altered in a continuous fashion in order to illustrate the formation of the hybrid complex. It can be seen that at neutral pH and room temperature a 1:1 complex forms in 0.15 *M* LiCl while a 2:1 complex forms when the salt concentration is increased to 1 *M* LiCl. The results are analogous with those found earlier for the two and three stranded helical complexes formed between polyriboadenylic acid and polyribouridylic acid, or polyriboadenylic acid and polyriboinosinic acid.[4, 7] A higher ionic strength promotes complex formation and more electrolyte is required in the solution for the addition of the third strand than is needed for the second strand. These experiments were carried out using LiCl solutions but similar results are found with NaCl.

Divalent cations are more effective in promoting the formation of this hybrid complex; a result which has been noted previously with the ribonucleotide complexes.[4]

Another property of the complex can be demonstrated by measuring the hypochromicity as a function of temperature. By this means the thermal denaturation or "melting out" of the complex can be followed by observing the rise in optical density as the temperature is raised. The results of this experiment are illustrated in Figure 3. In Figure 3*a* the optical density is plotted for 0.6 *M* LiCl at pH 7. These show that the spectrum of polyriboadenylic acid itself rises gradually as the temperature is increased. However, there is no change in the extinction coefficient of the polydeoxyribothymidylic acid with temperature. The dotted

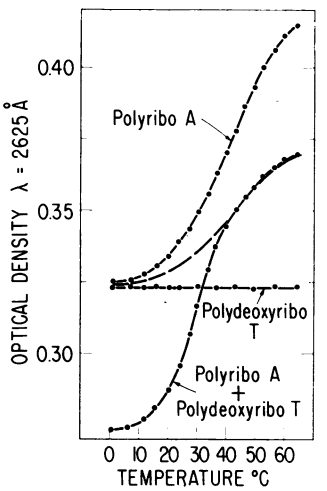

FIG. 3*a*.—The optical density of polyriboadenylic acid, polydeoxyribothymidylic acid, and a 1:1 mixture as a function of temperature. The solutions are in 0.6 *M* LiCl, pH = 7.0.

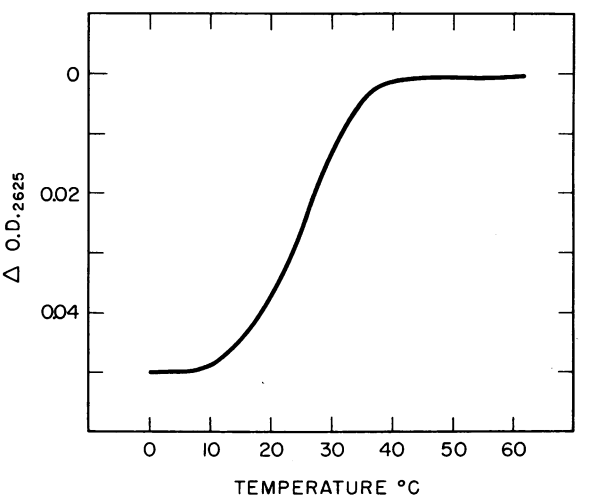

FIG. 3*b*.—The difference between the dashed curve in 3(*a*) and the observed curve for polyriboadenylic acid plus polydeoxyribothymidylic acid (Δ O.D.) is plotted as a function of temperature. The difference is zero about 40°C showing complete dissociation.

line in Figure 3*a* is the mean of these two curves and accordingly represents the temperature dependence of a nonreacting mixture of the two polynucleotides. Plotted in the same figure is the observed change in the optical density of the 1:1 complex as a function of temperature. It can be seen that at low temperatures, the complex has a lower optical density. However, it rises sharply around 27° until finally near 40° the curve is coincident with the dotted line showing that there is no longer any complex present. The "melting out" or thermal destruction of the helical complex can be shown by plotting the difference between the nonreacting, dashed curve and the experimentally observed curve. The difference, Δ O.D., is plotted as a function of temperature in Figure 3*b*, and it has the value of zero beyond 40° which shows that the complex is fully broken.

Ultracentrifugal studies provide clear evidence for complex formation. These studies were carried out in 1 *M* LiCl; the high ionic strength was used to insure that the small polydeoxyribonucleotides would be attached to the longer ribosepoly-

nucleotide. Under these conditions the sedimentation constant of the polyriboadenylic acid was $S_{20} = 11.7$. The polydeoxyribothymidylic acid had a sedimentation constant of less than 0.1 since the material never left the meniscus even though the centrifuge rotor was operating at its highest speed for a prolonged period. On forming the 1:1 complex a sedimentation constant of 13.7 was observed. Under similar conditions, the 2:1 complex had a sedimentation constant of 16.8. Thus the experiments are clearly consistent with the spectrophotometric results in demonstrating the existence of a complex.

Several tests were carried out to check on the specificity of the interaction to ascertain whether or not the hydrogen bonding potential of the thymine residue is used. These experiments were carried out in two ways. A variety of polynucleotide mixtures were made and spectrophotometric tracings were obtained at room temperatures using neutral solutions of 0.6 LiCl. In addition, the effect of divalent cations was tested by using solutions containing 0.01 *M* $MgCl_2$. Another type of spectrophotometric experiment was carried out at a low temperature (0.5°C) with a high ionic strength, (1 *M* NaCl, 0.01 *M* $MgCl_2$). These conditions favored the formation of complexes and a lowering of the optical density was used as an indication that a reaction had occurred. The results of these tests are shown in Table 1.

Before discussing Table 1 it is worth recalling the results of experiments with a variety of synthetic polyribonucleotides. Polyriboadenylic acid has the ability to take on an additional strand of polyribouridylic acid to make a two stranded helix. It can also take on a second strand of polyribouridylic acid to make a three stranded helix. It is believed that the essential features necessary for this interaction are the presence of a keto oxygen on C_6 of the uracil residue and a proton on N_1. When this tautomeric form is present, there are two sites at which the uracil can form a pair of hydrogen bonds with the adenine ring:

(1) Uracil keto oxygen on C_6 bonding to the amino group of adenine and the N_1-H bonding to the N_1 of adenine. These are the bonds which are used in the Watson-Crick structure of DNA.

(2) However, adenine can form a second set of hydrogen bonds with the uracil residue.[4] These involve a hydrogen bond between the adenine amino group and the keto oxygen on the C_6 of uracil, and a hydrogen bond formed between the N_1-H of uracil and the imidazole N_7 of adenine. This type of arrangement was first postulated for the combination of polyriboadenylic acid plus two-polyribouridylic acids. However, this hydrogen bonding has recently been found in a crystal containing adenine and thymine derivatives.[8] In addition to a three-stranded helix involving uracil residues, polyriboadenylic acid can form analogous structures with two strands of polyriboinosinic acid or two strands of polyribothymidylic acid.[7, 9] In these latter examples, a keto oxygen is found on C_6 and a proton is attached to N_1 of thymine or hypoxanthine, and it is likely that they form an analogous set of hydrogen bonds.

In this light we can now consider the results of the survey for the specificity of polydeoxyribothymidylic acid. Table 1 has three columns. Column 1 shows reactions which might result in the formation of a two stranded helix. Here, deoxy T reacts only with ribo A (using the abbreviations in the table). Column 2 lists reactions which might result in the formation of a three stranded helix.

TABLE 1

THE REACTIVITY OF POLYDEOXYRIBOTHYMIDYLIC ACID

Two-stranded	Reaction	Three-stranded	Reaction	Four-stranded	Reaction
ribo A + deoxy T	+	(ribo A + ribo U) + deoxy T	+	$(\text{ribo I})_3$ + deoxy T	−
ribo U + deoxy T	−	(ribo A + ribo I) + deoxy T	+	(ribo A + 2 ribo U) + deoxy T	−
ribo I + deoxy T	−	(ribo A + ribo T) + deoxy T	+	(ribo A + 2 ribo I) + deoxy T	−
ribo C + deoxy T	−	(ribo A + deoxy T) + ribo U	+	(ribo A + 2 ribo T) + deoxy T	−
		(ribo A + deoxy T) + ribo I	+		
		(ribo A + deoxy T) + ribo T	+		
		(ribo A + deoxy T) + ribo C	−		
		(ribo I + ribo C) + deoxy T			

A plus sign indicates a reaction occurred as measured by a lowering of optical density. Parentheses implies that the complex was formed before the addition of the testing substance. The experiments with $(\text{ribo I})_3$ were carried out under conditions in which the three stranded helix is stable.[11]

Abbreviations: deoxy T = polydeoxyribothymidylic acid, ribo A = polyadenylic acid, ribo I = polyriboinosinic acid, ribo C = polyribocytidylic acid, ribo T = polyribothymidylic acid.[10]

Thus deoxy T will add as a third strand to several two stranded helices: ribo A + ribo U, ribo A + ribo I, ribo A + ribo T as well as ribo A + deoxy T. In addition, the two stranded helix of ribo A + deoxy T can take on as a third strand ribo U, ribo I, or ribo T. In the third column are listed experiments designed to test for the formation of four stranded helices. The results of these tests were all negative.

We may summarize these tests for specificity by stating that polydeoxyribothymidylic acid acts in a manner which is analogous to polyribouridylic acid or polyribothymidylic acid. In short, the absence of the addition oxygen atom on carbon-2′ of the sugar does not change the specificity which is inherent in the structure of the pyrimidine residue in these polynucleotides. This does not, of course, mean that it does not alter the readiness with which these reactions occur, either measured in terms of rate or free energy changes. However, at the present time we are only concerned with the question of the specificity of the reaction.

Discussion.—X-ray diffraction work which has been carried out on the synthetic polyribonucleotides amply demonstrates that they form molecular complexes which are helical and which are built along lines roughly similar to those found in DNA, namely a helix in which the purine and pyrimidine bases are located in the center and the ribose-phosphate chain on the outside. However, despite the similarities between the complex of polyriboadenylic acid + polyribouridylic acid and DNA it is worth emphasizing that there are significant differences. The major difference results from the presence of the ribose group in the backbone of the synthetic polynucleotides which significantly alters the helical configuration. Thus, for example, the polyriboadenylic acid + polyribouridylic acid complex has a diameter 6 Å greater than that found in DNA.[12] This in turn means that the helical axis of the molecule is not in the same position relative to the purine-pyrimidine base pair as it is in DNA. These differences are, of course, due to the presence of the additional oxygen on carbon-2′ of the ribose residue. DNA in its accepted configuration could not have an additional oxygen on this site because there is not enough room for it. The oxygen atom with its Van der Waals radius of 1.4 Å significantly

alters the configuration of the ribose sugar relative to that of deoxyribose. Despite these differences a prediction was made that it would be possible to produce hybrid helices with both ribose and deoxyribose backbone chains because the similarities between the two backbones were believed to be greater than the differences.[13] The present experimental demonstration of this implies that the two different backbones are somehow able to accommodate each other and it is quite likely that X-ray diffraction studies will show that a compromise is reached regarding the diameter of the helix as well as in the position of the helix axis relative to the purine-pyrimidine base pair. In this regard it is quite likely that the ribose-phosphate chain will be at a greater distance from the helix axis than the more closely packed deoxyribose-phosphate chain.

It is likely that the hydrogen bonding in the hybrid complexes is similar to that described above for polyriboadenylic acid plus two polyribouridylic acid. Further information on this hydrogen bonding arrangement should come from X-ray diffraction work on these complexes.

With the increasing availability of synthetic techniques or possibly with the use of the DNA polymerase enzyme it may be possible to extend this type of study by utilizing other polydeoxyribonucleotides. In this way it should be possible to explore more fully the effect of the additional hydroxyl group on the ribonucleotide part of the hybrid helix. For example, it would be of great interest to know whether it is possible to make a hydrogen bond involving that hydroxyl which bonds to one of the oxygen atoms on an adjacent nucleotide, such as oxygen-1′ of the next ribose ring.

Relation to the transfer of information between nucleic acids: We have no direct experimental information at the present time which demands that the DNA nucleotide sequence influences the ordering of the nucleotides on RNA. Nonetheless this is widely believed because of a large number of indirect experiments which point to this as the major route for expressing genetic potentialities. However, the manner in which this is done is quite unknown.

When three-stranded polynucleotides were first discovered using synthetic ribonucleotides, it was suggested that this might be the analogue of a mechanism whereby a two-stranded DNA molecule might serve as a template for the manufacture of a single-stranded RNA molecule.[4] Indeed, the fact that there is a deep groove in DNA just large enough to accommodate a single polynucleotide strand was extremely suggestive. However, despite work by many individuals for several years no adequate structural solution has been found. That is, using our presently accepted stereochemical concepts, no satisfactory molecular arrangement has been devised whereby the hydrogen bonding potentiality of an incoming ribonucleotide strand would be specified by one complementary pair of a DNA molecule.[13]

In addition to this somewhat negative evidence, some new results have come to light recently which have underlined the importance of a single polynucleotide strand in relation to its biological activity. These have been first of all the recognition by Kornberg and his associates that the best primer for the replication of DNA was a single strand of DNA rather than the double-stranded complementary molecule.[14] The single strand polymer, however, results in the production of a double-stranded DNA molecule. Thus a single-stranded DNA is the template for its own replication. The second important development was the discovery

by Sinsheimer that the DNA within the small virus ΦX-174 is itself single stranded.[15] This single strand of DNA contains all the biological information necessary to replicate the virus. Both of these events have reinforced the concept that the biological production of RNA may proceed through an analogous mechanism whereby a single strand of DNA serves as a template for the production of a complementary RNA strand.[13] After polymerization, the RNA strand is separated from the original template molecule and it then carries genetic information. This, of course, presupposes an enzymatic system capable of utilizing ribonucleotide di- or triphosphates and which is dependent upon the presence of a single stranded DNA primer for its activity. Although there are some recent indications which suggest that there may be more than one type of enzyme for polyribonucleotide synthesis, the situation is unclear at the present time.

Some interesting indirect experimental evidence is available which is pertinent to the question of how DNA influences the RNA of the cell. First we may mention the experiments of Belozersky and Spirin[16] who have analyzed the base ratios of (guanine + cytosin)/(adenine + thymine) in the DNA of a variety of microorganisms and compared them to the comparable base ratios in RNA [(guanine + cytosine)/(adenine + uracil)]. The results do not show a 1:1 correspondence but nonetheless they do show a *weak* dependence of the RNA base composition on the DNA base composition of the same cell. The suggestion can be made that this reflects the fact that there are several different types of RNA inside the cell, only some of which may be important in conveying the genetic information present in DNA, while the other types of RNA may have a variety of other functions. We are, of course, aware of the fact that there are several types of RNA in the cell, such as microsomal, soluble, nucleolar, and other nuclear types. Which of these may reflect the base composition of DNA is as yet unknown.

More direct evidence is available from the experiments of Volkin and Astrachan which were carried out on bacteriophage infected cells of *E. coli*.[17] Shortly after the invasion of the bacterial cell by the DNA of the T2 bacteriophage, they could demonstrate the production of a new type of RNA in the cell. This RNA has a gross base composition which is identical to that of the invading phage DNA and quite different from that of the host RNA. The newly synthesized RNA was identified by means of isotopic phosphorous or carbon and they showed that the amount of newly synthesized adenine was equal to the uracil while cytosine was equal to the guanine in the same ratios as were present in the invading DNA if we equate uracil to thymine. These experiments were again repeated using another bacteriophage (T7) which contains a different base ratio and again the newly synthesized RNA had the same base composition as the invading DNA. Thus it is quite likely that these results reflect something which is fundamental in the metabolism of the phage infected bacterial cell.

It is reasonable to believe that the incoming bacteriophage DNA has a direct role in promoting the synthesis of the new type of RNA which Volkin and Astrachan have identified. The fact that the RNA composition is identical to that of the invading DNA has some interesting consequences. This is, of course, compatible with a mechanism in which the invading DNA is split into single strands, each strand of which serves to produce a complementary type of RNA. This would directly explain the composition of the new RNA. However, there is another

possibility. If one strand of the invading DNA were inhibited from acting as a template for synthesizing RNA, then only a single strand of RNA could be polymerized. However, if this occurred it is quite unlikely that the resulting RNA would have a composition identical to that of the intact DNA. It is improbable (but not impossible) that a single strand of a DNA duplex has by itself a complementary base ratio. In the case of the single strand of DNA in ΦX-174 the base ratios are not complementary.[15]

However another alternative is possible if only a single strand of DNA operates to make a single strand of RNA. It is conceivable that this single strand of RNA produces more RNA using the same type of mechanism, i.e., one in which it acts as a template for making two stranded complementary RNA. This would, of course, produce an RNA with base ratios such as Volkin and Astrachan have observed. At the present state of our knowledge we do not have enough information to chose between these various alternatives. However, we are left with an interesting question regarding the physiological role of the two RNA strands which appear to be present on the basis of the bacteriophage experiments. For instance, are both of these complementary RNA strands used in protein synthesis and by what mechanism? We can only speculate at the present time.

The demonstration that a hybrid helix is possible involving a synthetic DNA and a synthetic RNA strand should further stimulate attempts to find a mixed species of this type within the cell. Such a complex may, of course, have only a very transient existence and the molecule may be very unstable. Nonetheless this would represent a fruitful type of research to pursue at the present time.

Conclusions.—In this paper we have presented evidence which shows that it is possible to have a hybrid helix in which one strand with a DNA backbone can be made to wrap around another strand with an RNA backbone in such a manner that the strands are held together by complementary hydrogen bonds formed between the purine and pyrimidine residues. This finding may have relevance in the process whereby the genetic information or nucleotide sequence in DNA is transferred to an RNA molecule and suggests experiments which should be carried out to search for the existence of such transfer mechanisms in intact cellular systems.

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* To avoid confusion, a convention is adopted whereby references to a nucleotide polymer contains explicitly the name of the sugar involved in the backbone. Thus the terms polyriboadenylic acid, polydeoxyribothymidylic acid, or polyribothymidylic acid are used.

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ADENOSINE 5'-PHOSPHOSULFATE AS AN INTERMEDIATE IN THE OXIDATION OF THIOSULFATE BY THIOBACILLUS THIOPARUS

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Orthophosphate is required for the complete oxidation of thiosulfate to sulfate by whole cells of *Thiobacillus thioparus*. Arsenate can replace phosphate in this process.[1-3] Santer[4] observed that, during the oxidation of thiosulfate in the presence of O^{18}-labeled orthophosphate, O^{18} is transferred to the sulfate produced in the oxidation. This transfer is also insensitive to 2,4-dinitrophenol. These results suggest that one or more sulfur-containing nucleotides are intermediates in the conversion of thiosulfate to sulfate. Adenosine 5'-phosphosulfate (APS) and 3'-phosphoadenosine 5'-phosphosulfate (PAPS) have been shown to be intermediates in the metabolism of sulfate by yeast and mammalian tissue. PAPS is the "active sulfate" of mammalian tissue and can transfer sulfate to phenols, carbohydrates, and steroids.[5] In addition to sulfurylation reactions, extracts of yeast can reduce PAPS to sulfite and 3',5'-diphosphoadenosine (PAP) in the presence of TPNH (eq. (1)).[6, 7]

$$\text{PAPS} + 2e \rightarrow \text{PAP} + SO_3^{--} \qquad (1)$$

Although APS has not been shown to participate in sulfate transfer reactions, APS reductase can reduce the sulfate of APS directly to sulfite in extracts of *Desulfovibrio desulfuricans* (eq. (2)).[8]

$$\text{APS} + 2e \rightleftharpoons \text{AMP} + SO_3^{--} \qquad (2)$$

This reaction seems to be reversible since APS can be formed from AMP and sulfite in the presence of partially purified preparations of APS reductase from this organism.[9] The observations that the sulfate of PAPS and APS can be reduced to sulfite (eqs. (1) and (2)) and the reduction of APS is reversible suggest a mechanism for the participation of sulfur-containing nucleotides in the oxidation of thiosulfate. If it is assumed that sulfite can be produced in the oxidation of thiosulfate, the oxidation of sulfite by the reversal of either APS or PAPS reduction leads to the formation of one or more sulfur-containing nucleotides.

Santer's O^{18} data, although indicating that a sulfur-containing nucleotide is an