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Meeting of the RNA Tie Club, Cambridge, U.K., fall 1955. Left to right: Francis Crick, Alex Rich, Leslie Orgel, Jim Watson.

On looking back at a Biochimica et Biophysica Acta paper published 32 years ago: a commentary by

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on 'Studies on the formation of two- and three-stranded polyribonucleotides'
by G. Felsenfeld and A. Rich
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In the early 1950s I was a postdoctoral fellow working for Linus Pauling at Caltech. In 1952 I started to take X-ray diffraction patterns of fibers of DNA. The fiber diffraction cameras available at that time were quite primitive and the pictures I got revealed a helical diffraction pattern but without enough resolution to be useful. In early 1953 the Watson and Crick paper came out, so I turned to the structure of RNA. However, the role of RNA at that time was obscure. Later in 1953 Jim Watson returned from Cambridge to Caltech and we decided to work together on this problem. We collected samples of RNA from many different investigators and tried to draw fibers from them to look at their diffraction patterns. We obtained some fiber diffraction patterns that indicated that RNA was helical and published papers describing these patterns together with some general observations about the possible relationship between DNA and RNA [1,2]. In retrospect, those early diffraction patterns clearly showed what we now call an A-type diffraction pattern, which is the form of the RNA double helix.

Research in the field of RNA structure was painfully slow and it was very difficult to get samples that were useful. This changed dramatically in 1955, when Severo Ochoa and Marianne Grunberg-Manago isolated polynucleotide phosphorylase [3]. That enzyme would take ribonucleoside diphosphates and convert them into RNA polymers. I had moved to the National Institutes of Health in 1954, and when Severo Ochoa came there to give a seminar in the spring of 1955, he agreed to give me some of his RNA polymers for physical studies. Suddenly the pace of work in this field began to change dramatically. Fibers drawn from polyadenylic acid (poly(A)) itself showed a curious pattern that was obviously helical, with a 3.8 Å meridional reflection. The pattern showed some interesting features. But it was clear that I was limited by the low intensity of X-rays available in the sealed X-ray tube with which I was working. Two years earlier, Francis Crick had visited Caltech and we had come to know each other. At that time he had invited me to come to Cambridge, U.K., if I wanted to use the rotating anode high-intensity X-ray tube that had been developed there. Once I had these oriented poly(A) fibers I wrote to him and he agreed to act as my host while exploring those diffraction patterns. At Cambridge we worked on these patterns in the fall of 1955. Jim Watson had then returned to Cambridge, U.K., and the three of us puzzled at length about this pattern. The structure was ultimately elucidated as a double-stranded parallel helix in which the poly(A) chains were protonated. David Davies joined in the final solution of this structure that was eventually published in great detail [4].

On returning to NIH in early 1956, I concentrated on the interaction of poly(A) and poly(uridylic acid) (poly(U)). Robert Warner, working in Severo Ochoa's department, had shown that when these two polymers are mixed together, the optical absorbance drops [5]. The meaning of hypochromism in the ultraviolet was not understood in those days, but it was clear that something was happening. David Davies and I, working together at NIH, found that if we mixed these two molecules together in equimolar amounts we could draw out a fiber that produced a helical diffraction pattern somewhat similar to the rather fuzzy patterns that Jim Watson and I had described a few years earlier. These were indeed A-type RNA patterns, which RNA double helices are known to adopt normally in a hydrated environment (DNA will also adopt an A-type conformation, but only when the DNA fiber is dried somewhat). We concluded from these experiments that poly(A) plus poly(U) made a double helix [6]!

At that time I remember walking down one of the long corridors at NIH and I bumped into Herman Kalckar, who was working in another part of the building.

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"Herman," I told him, "We've just discovered that mixing poly(A) and poly(U) forms a double helix rather like DNA."

Herman replied, somewhat incredulously, "What? You mean without an enzyme?"

This was the first time I realized that the mind-set of the biochemist was somewhat different from mine. It seemed apparent to me that these two molecules were forming a more stable structure at a lower energy and so the reaction should proceed spontaneously. Herman was quite surprised that this reaction could occur without an enzyme, since it was clear that an enzyme was needed to make DNA, as Arthur Kornberg and his colleagues were demonstrating during that time period.

David Davies and I continued working on the reaction of poly(A) plus poly(U). About that time, Gary Felsenfeld, who had been working at Oxford, joined our laboratory at NIH. In fact, David Davies, Gary Felsenfeld and I were all together at Caltech, since David had been another postdoctoral fellow of Linus Pauling's while Gary was his Ph.D. student.

Gary set about analyzing the molecular weights of the complex of poly(A) plus poly(U) compared to the molecular weights of the individual polymers. He did this using the then rather new Spinco Model E ultracentrifuge, measuring their sedimentation constants. What Gary found was that occasionally when he mixed these together with a somewhat greater excess of poly(U) than poly(A), a more rapidly sedimenting complex formed. This seemed somewhat puzzling. One of the things we then explored was the role of changing the ionic strength. When we went from a solution in which there was only 0.1 M NaCl to a solution to which we added 0.01 M $MgCl_2$, a remarkable transformation occurred. This transformation was visible by using what we called the "method of continuous variation," that is, making a series of mixtures of poly(A) and poly(U) in which the total number of phosphate residues remains constant but the ratio of poly(A) to poly(U) changes continuously. With this system, we saw that in the 0.1 M NaCl solution, the optical absorbance in the ultraviolet came to a sharp minimum at a 1:1 mixture of poly(A) and poly(U). This was the 1:1 complex. However, upon the addition of the divalent cation, the mixture changed and came to a new lower minimum with a mole ratio of 2 uridylic acid residues to 1 adenylic acid residue. Thus it was making a complex involving 1 poly(A) and 2 poly(U) molecules. Together with David Davies, we made 2:1 mixtures and drew fibers from them and discovered that the diameter of the molecule did not change appreciably, even though there was an alteration in the intensity distribution. Thus it was likely that the second poly(U) strand filled the major groove of the double helix. Inspection of the structures and hydrogen bonding potentialities of both adenine and uracil suggested to us that the second strand of uridylic acid was being added to the adenine-uracil base-pair through the formation of two hydrogen bonds, one from the uracil O-4 to the adenine amino group N-6, and the other from the uracil N-3 to the adenine imidazole N-7. This type of hydrogen bonding would neatly account for the fact that the diameter of the molecule did not change but the sedimentation constant increased 50%. We wrote a short note describing this first triple-stranded molecule together with this unusual type of base-pairing [7]. This type of hydrogen bonding was later found in a single crystal X-ray diffraction analysis carried out by Karst Hoogsteen at Caltech with 9 ethyladenine and 1 methylthymine [8]. It is now widely known as Hoogsteen pairing.

Gary Felsenfeld and I then carried out a series of solution studies on the formation of two- and three-stranded polynucleotide molecules in the system of poly(A) plus poly(U). The results of that investigation were published in BBA in volume 26, 1957 [9]. The BBA paper considers in some detail the nature of the spectroscopic changes when poly(A) and poly(U) are mixed together as a function of adding various salts. We found that the addition of one strand of poly(U) to one strand of poly(A) formed a 1:1 complex that was quite stable. It required only monovalent cations. From the sharpness of the drop in optical absorbance in a stoichiometric mixture, it could be shown that there was an effective equilibrium constant of 10^6 or 10^7. Kinetic studies showed that the reaction occurs very rapidly, so that special methods would be required to study it, since it is complete in less than 1–2 s in 0.1 M NaCl at neutral pH. The role of the cation is quite important, since lowering the cation concentration slows up the reaction.

Addition of very small amounts of divalent cations to the system produced a striking effect with a new deeper minimum at 2U:1A. The triple-stranded complex requires a divalent cation, largely to overcome the electrostatic repulsion which the third polynucleotide chain experiences as it starts to fold around the double-stranded helix. We showed that a number of divalent cations are effective, including Mg^{2+}, Mn^{2+}, Zn^{2+} and Ca^{2+}. If Na^+ alone is used to try to add the third strand, very high concentrations are required. This effect was interpreted as indicating the role that the cations had in overcoming the electrostatic repulsion. The electrostatic analysis considered both nonspecific ionic effects such as the Debye–Hückel screening, or specific complexing with particular cations. It was concluded that the effectiveness of the divalent cations was greater than could be accounted for by simple Debye–Hückel screening, and specific complexing was likely to be found. In recent years, using high-resolution single crystal X-ray diffraction analysis, it has been possible to identify specific sites on double-stranded RNA and DNA at which magnesium and other divalent cations complex. In a physiological salt solution, the

first two strands of poly(A) plus poly(U) are tightly bound, while the third strand is somewhat loosely bound.

This paper represented the first solution study of polynucleotide interactions leading to helix formation. With the availability of other polynucleotides, it was possible to show, using both X-ray and solution techniques, that a number of other complexes formed. Thus, within a short time I found that poly(adenylic acid) plus poly(inosinic acid) would form two-stranded and three-stranded structures [10]. Likewise, poly(inosinic acid) itself would form a multiple-stranded parallel helix that the data suggested could either be three-stranded or four-stranded [11]. Although my early preference was for a triple-stranded helix, subsequent work indicates that it is a four-stranded helix. Likewise, poly(inosinic acid) and poly(cytidylic acid) were found to form a helical complex analogous to the guanine-cytosine base-pair [12]. The same approach was used to show that a DNA-RNA hybrid double helix could be made containing poly(riboadenylic acid) and poly(deoxythymidylic acid) [13]. This was cited as a DNA-RNA hybrid, representing an example of how one strand of DNA could be used to code for a complementary polyribonucleotide to make a double helix. These polynucleotide studies were carried out well in advance of the discovery of RNA polymerase and the hybridization reactions that later became commonplace in studying relations between DNA and RNA.

It is interesting to write a paper about three-stranded molecules that were discovered 32 years ago. Three-stranded molecules have again come into vogue. There are certain homopurine-homopyrimidine sequences that are found in DNA. These sequences, under negative torsional strain, undergo a conformational change in which one segment undergoes strand separation and the liberated polypyrimidine strand is believed to fold back upon a double-helical homopurine-homopyrimidine to form what is believed to be a triple-stranded molecule. These homopurine-homopyrimidine sequences are found in regulatory regions and so it is surmised at present that their formation may have some relevance to the regulation of transcription. This may be the case, but details of that transformation and the biological role that it plays are yet to be elucidated.

What young research workers today find hard to understand is the extent of the ignorance that prevailed in the mid and late 1950s about major issues of molecular biology. For example, many scientists felt that DNA makes RNA makes proteins, but did not know how this came about. It took a considerable time period before the relevant enzymes were isolated. Even then, there was some question about how, for example, a enzyme that makes RNA would obtain its information. Would it obtain its information simply by using a single strand of DNA and assembling complementary nucleotides on it? Or would it make the RNA strand by using the double-stranded DNA as a template? Even though the latter seemed less probable, it could not be ruled out.

TABLE I

Possible Types of Nucleotide Polymerase Enzymes

Enzyme	Primer	Substrate	Product
A	Single-chain DNA	Desoxy ATP Desoxy GTP Desoxy CTP Desoxy TTP	DNA polynucleotide chain
B	Single-chain DNA	ATP (ADP) GTP (GDP) CTP (CDP) UTP (UDP)	RNA polynucleotide chain
C	Singel-chain RNA	ATP (ADP) GTP (GDP) CTP (CDP) UTP (UDP)	RNA polynucleotide chain
D	Single-chain RNA	Desoxy ATP Desoxy GTP Desoxy CTP Desoxy TTP	DNA polynucleotide chain

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In a later piece written in 1959 dealing with An Analysis of the Relations Between DNA and RNA [14], I speculated at length about possible reading mechanisms involving either double-stranded templates or single-stranded templates. In that same article I outlined the various kinds of polymerase that could occur using the probable single-stranded templates. Here I reproduce a table from that article outlining the four different types of polymerase enzyme that could be found (Table I). At that time, we had evidence only for enzyme A, that is, a DNA polymerase that had been isolated by Arthur Kornberg and his collaborators that used deoxynucleoside triphosphates and made a double-stranded helix from a single-stranded template. The other three enzymes were unknown. Of course, today we recognize enzyme B as RNA polymerase and enzyme C is used for certain types of viral RNA replication, while enzyme D is the well-known reverse transcriptase that was not discovered for another decade or so. At that stage in the late 1950s it was easy to think about many possibilities, but it was very hard to assign a probability to them. What we know today is that nature is very flexible. If something can happen, nature is likely to have used it in one way or another to make the system work. Thus, all of the enzymes in Table I are known to be active in biological systems.

In that same 1959 paper [14], I speculated about the evolution of the nucleic acids. Today there is great interest concerning the role of RNA in early evolution. This short excerpt speaks for itself:

Evolution of the Nucleic Acids

One of the really remarkable things about the nucleic acids is that there are two of them. These molecules are very similar, differing only by a systematic hydroxyl group and an occasional methyl group. Nevertheless, we see that they appear to have quite different functions in the cell. The DNA molecule is used in nature as the major carrier of genetic information; the RNA molecule seems to be used in the conversion of genetic information into actual protein molecules that carry out, as it were, the functions implicit in the genetic material.

Nothing is known about the survival of nucleic acids in the evolutionary process. However, because we see these two closely related molecules with different functions, we are, of course, tempted to ask whether they may have originated historically from a common molecule that then specialized in the course of evolution into the two different classes of molecules we see today. To pursue this argument further, we note the fact that the RNA molecule is also able to carry genetic information, as mentioned above in the case of the tobacco mosaic virus infection. Hence it may be reasonable to speculate that the first polynucleotide molecule that nature used was an RNA-like molecule that is able both to convey genetic information and to organize the amino acid molecules to produce specific types of proteins. DNA might then be regarded as a specialized derivative molecule that evolved in a form that carried out only the molecular replicating cycle that is an inherent part of the transmission of genetic information. DNA is less reactive metabolically, perhaps because of the absence of the hydroxy group, and this may have a selective advantage in an evolving biochemical system.

A major interest during the late 1950s that occupied many of us was the problem of information transfer. What coded for the sequence of amino acids? If it was RNA, what was the relationship between the number of nucleotides in the RNA strand and the number of amino acids? This point was addressed most graphically initially by the noted astrophysicist George Gamow, who understood problems of information very well, even though he was unfamiliar with the fine details of molecular biology. He suggested a coding for amino acids directly on the DNA double helix [15]. When George Gamow came to visit Max Delbruck at Caltech in 1954, he was immediately intrigued by the work that Jim Watson and I were doing on RNA structure, and we spent some time collectively wondering about ways in which the coding problem could be solved. Gamow had a great flair for unusual activities. He concluded that this problem needed concentrated effort to obtain a solution, an effort that required the formation of a dedicated group or 'club.' Gamow decided to found the RNA Tie Club. He found a haberdasher in Los Angeles who could make an elegant black woolen tie with a woven wiggly bright green strand that Gamow designed as the backbone of RNA and attached to it were yellow purine and pyrimidine rings for the bases. The members of this club were largely friends of George Gamow, but it did include a number of well-known biochemists and molecular biologists. Gamow decided that there should be 20 members, one for each amino acid, and four additional members, one for each base in RNA. There were various meetings of this club.

The accompanying photograph shows a meeting of the RNA Tie Club in Cambridge, U.K. in the fall of 1955. The photograph was taken in Francis Crick's sitting room in Portugal Place in Cambridge. The members are wearing their RNA Tie Club tie (except for Leslie Orgel, who left his tie at home). Members used to prepare papers dealing with the coding problem, and indeed Gamow and I, together with Martynas Ycas, wrote a rather extensive review in 1956 that brought together all the information available then dealing with the coding problem [16]. Other papers were just circulated to members without actually being published. One notable paper was written by Francis Crick and circulated to members, describing the adaptor hypothesis, that is, a small RNA molecule that might serve as an intermediary between the coding RNA (messenger RNA) and the growing polypeptide chain. This was in essence a description of what we now know to be the transfer RNA molecule.

Looking back over 30 years, I can see that the spirit of that time was quite exhilarating. There were large areas that we knew nothing about, and we could speculate about the nature of biological systems. These speculations covered basic phenomena that had to be elucidated before the molecular nature of these systems could be described. What is remarkable is that most of these problems were solved within a decade or so, so that we rapidly developed a very coherent picture of how biological systems function.

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References

1 Rich, A. and Watson, J.D. (1954) Nature 173. 995–996.
2 Rich, A. and Watson, J.D. (1954) Proc. Natl. Acad. Sci. 40, 759–764.
3 Grunberg-Manago, M., Ortiz, P.J. and Ochoa, S. (1955) Science 122. 907–910.
4 Rich, A., Davies, D.R., Crick, F.H.C. and Watson, J.D. (1961) J. Mol. Biol. 3. 71–86.
5 Warner, R.C. (1956) Fed. Proc. 15. 379.
6 Rich, A. and Davies, D.R. (1956) J. Am. Chem. Soc. 78. 3548.
7 Felsenfeld, G., Davies, D.R. and Rich, A. (1957) J. Am. Chem. Soc. 79. 2023–2024.
8 Hoogsteen, K. (1963) Acta Cryst. 16. 907–916.
9 Felsenfeld, G. and Rich, A. (1957) Biochim. Biophys. Acta 26. 457–468.
10 Rich, A. (1958) Nature 181. 521–525.
11 Rich, A. (1958) Biochim. Biophys. Acta 29. 502–509.
12 Davies, D.R. and Rich, A. (1958) J. Am. Chem. Soc. 80. 1003.
13 Rich, A. (1960) Proc. Natl. Acad. Sci. USA 46. 1044–1053.
14 Rich, A. (1959) Ann. N.Y. Acad. Sci. 81, 709–722.
15 Gamow, G. (1954) Nature 173. 318–320.
16 Gamow, G., Rich, A. and Ycas, M. (1956) in Advances in Biological and Medical Physics. IV, pp. 23–68, Academic Press, New York.

STUDIES ON THE FORMATION OF TWO- AND THREE-STRANDED POLYRIBONUCLEOTIDES

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INTRODUCTION

The synthetic polyribonucleotides are a series of nucleotide polymers that can be formed enzymically. They have been shown to be linked by the same 3'–5' ribose phosphate backbone which is found in naturally occurring nucleic acids[1]. Thus, a study of their chemical reactivity may be instructive in helping us to understand how the nucleic acids carry out their chemical activities *in vivo*.

One of the first reactions which was observed with the synthetic polyribonucleotides was the combination of polyadenylic acid (poly A)* with polyuridylic acid (poly U)* in aqueous solutions[2]. These two molecules interact to form a new species, which is identifiable through an alteration in the sedimentation coefficient, electrophoretic mobility, and ultraviolet spectrum. Fibers have been drawn from mixtures of these two polynucleotides and they have been shown to produce an X-ray diffraction pattern differing from that of either component[3–5]. This diffraction pattern has been interpreted in terms of a two-stranded helical structure in which the adenine is hydrogen bonded to uracil, the base pairs being helically stacked at right angles to the fiber axis. The geometry of this complex is very similar to that seen in the desoxyribosenucleic acid (DNA) molecule.

More recently, a new reaction has been discovered involving the complex poly (A + U), and poly U[6]. It has been shown that these two species will interact under appropriate conditions to form a three-stranded molecule. This reaction is also specific, in that (A + U) will react with poly U, but not with polyadenylic acid or polycytidylic acid. In view of the similarities in the structures of (A + U) and DNA, it has been suggested that the three-stranded structure may be a prototype of a biologically significant molecule in which a single stranded RNA is wrapped about a two-stranded DNA.

THE HYPOCHROMIC EFFECT

The spectrophotometric method used in this study depends upon the fact that the complexes formed between poly A and poly U have optical densities at 259 mμ which are lower than the optical densities of their individual components. This general phenomenon is called the hypochromic effect, and it is commonly observed in the naturally occurring nucleic acids. It is usually measured by comparing the optical density of the nucleic acid (*e.g.* $O.D._{260}$) to the optical density of the hydrolyzed material. Thus, it is a lowering of optical density relative to the constituent nucleotides.

* In some cases, A or U is used instead of poly A or poly U to stand for the polynucleotides.

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In the synthetic polynucleotides, hypochromic effects have already been reported[2]. Thus, poly A is described as having an optical density 61% as large as that of its nucleotides in the absence of salt at pH 7. Poly U has a small hypochromic effect (95%).

In these experiments poly A is mixed with poly U, and upon combination they exhibit further lowering of optical density. That this is really a hypochromic effect, rather than a shift in the absorption maximum, is seen in Figs. 1a and 1b.

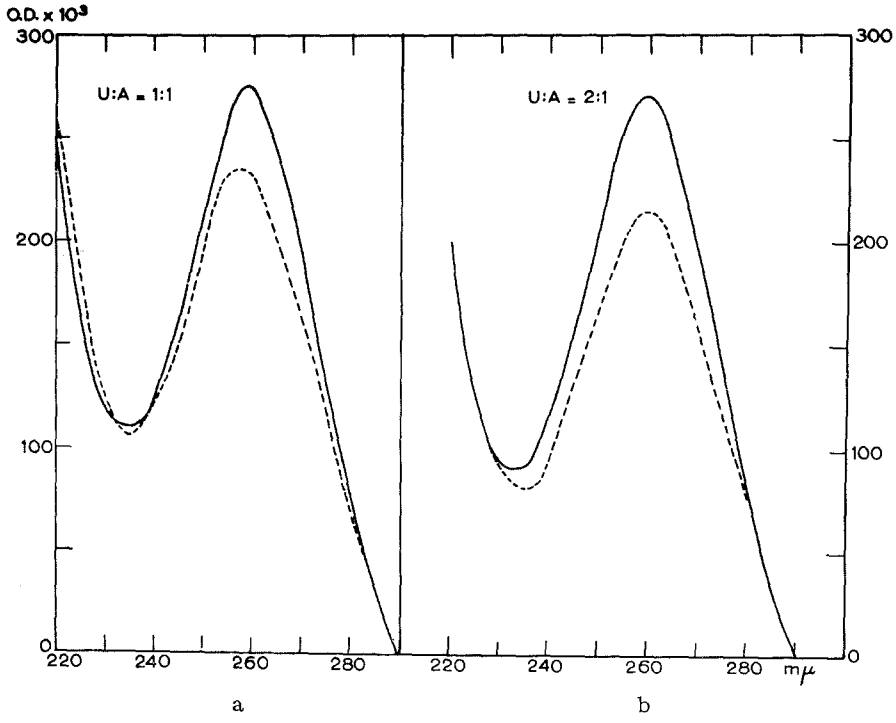

Fig. 1. The U.V.-absorption spectrum of mixtures of polyadenylic acid (A) and polyuridylic acid (U) at pH 7.8. (a) A 1:1 mixture of A and U. ——— No ions added (distilled water); – – – – 0.1 *M* NaCl. (b) A 1:2 mixture of A and U. ——— No ions added (distilled water); – – – – 0.1 *M* NaCl, 0.001 *M* $MgCl_2$.

X-ray diffraction studies of poly A and of poly U have shown that while the former produces a sharply defined diffraction pattern, the latter is amorphous[4,5]. It is possible that this is correlated with the hypochromic effects described above, in that poly U does not have a well defined structure, and has approximately the same optical density as its constituent nucleotides. However, the combination with poly A produces a well-ordered complex as shown by the sharply defined X-ray diffraction pattern. This mutual ordering may be related to the lowering of the optical density which we observe when the complexes form.

There is no agreement regarding the physical basis of the hypochromic effect. Suggestions have been made relating it to hydrogen bonding, altered tautomeric forms in the bases, or a crowding of the flat purine and pyrimidine rings so that their π-electron systems interact. For the purposes of this study, we need only assume that this phenomenon occurs to an extent which is proportional to the number of nucleotide units involved in the interaction. For long polymeric molecules, this is

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quite a reasonable assumption. Thus, by measuring the optical density at 259 mμ, it is possible to measure the extent of a reaction involving a change in the hypochromic effect.

EXPERIMENTAL

The optical density of solutions of poly A and poly U was measured in most cases with a Beckman Model DU spectrophotometer. In those experiments in which it was necessary to observe an optical density that rapidly changed with time, a Cary recording spectrophotometer (Model 14) was used. In such cases mixing of the solutions of poly A and poly U was accomplished by injecting one solution into the other with a hypodermic needle and syringe. Since the mixing occurred directly in the optical cell, which was in place in the spectrophotometer (with the hypodermic needle projecting through a light-tight plasticine seal into the cell along one of its edges), it was possible to follow the course of reactions from within three seconds of the beginning of mixing.

The poly A and poly U used in these experiments was synthesized in our laboratory with the use of the polynucleotide phosphorylase prepared from *E. coli* by the method of LITTAUER AND KORNBERG[7]. In addition, other samples of poly A were kindly provided by Prof. S. OCHOA[8].

The polymers were harvested by ethanol precipitation and centrifugation. They were redissolved in water and, except where otherwise stated, were given a final dialysis against $5 \cdot 10^{-3} M$ NaCl before freeze-drying. In this way, the dried material contained of the order of one mole of NaCl for each mole of polymer phosphate.

Analyses of phosphate concentration were made by the method of LOWRY *et al.*[9], and in the case of poly A ribose analyses were made by the method of MEJBAUM[10]. These gave values for the molar optical densities in 0.1 M NaCl, pH 6.4 to 7.4, of 9,180 for poly A and 9,430 for poly U.

Sedimentation studies were carried out in the Spinco Model E Analytical Ultracentrifuge equipped with ultraviolet optics. Sedimentation constants were determined on solutions with optical densities at 259 mμ of 0.3–0.6. These were the same solutions which were used in the spectrophotometric studies. The sedimentation coefficient appeared to be independent of concentration in this range. The values of the sedimentation coefficient are mean values, as the materials are all polydisperse.

Solutions stored at 5° C remained unchanged in combining power and sedimentation properties over a period of a week or more. All experiments were performed at 22–25° C.

The formation of complexes was followed by the continuous variation method[11]. Separate solutions of poly A and poly U were made up to equal concentration (on a molar phosphate basis), and the reaction was studied by making a series of mixtures in which the total volume (and, therefore, total concentration of phosphate) was kept constant while the ratio of the volumes of each solution added was varied.

In the following section, we will derive an expression showing how the optical density will vary as a function of composition in such a series of mixtures. Such a plot of optical density is referred to as a mixing curve.

THEORY

Let the sum of the concentrations of A and U polymer phosphate in solution be kept constant at a, and let the optical density of poly A (per mole of phosphate) be denoted by Q_1, that of poly U by Q_2, and that of some complex poly (A + nU) by Q_3. Then in the limit of large excess of U, where the reaction is driven to completion, the addition of one unit of poly A will involve the removal of one unit of poly U to keep a constant and the chemical reaction of n units of poly U to form one unit of poly (A + nU). Therefore, as a function of the amount of poly U added, the optical density D will be given by the straight line

$$D = [-Q_2 (1 + n) + Q_3] [a - \mathrm{U}] + Q_2 a \qquad (1)$$

where U is the total amount of poly U in the solution (in all forms). Similarly, in the limit of a large excess of A the addition of one unit of poly U will involve the removal $(1 + 1/n)$ units of poly A, so that

$$D' = \left[-Q_1 \left(1 + \frac{1}{n}\right) + \frac{Q_3}{n}\right] \mathrm{U} + Q_1 a \qquad (2)$$

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If we plot D and D' against the mole fraction of poly U in all forms in solution, U/a, it is easy to show that these two lines intersect at $U/a = n/(n+1)$ so that the extrapolation of the straight-line portions of the continuous varation plot will lead to the value of n. If the equilibrium constant for the reaction is very large, as is true for much of the data to be discussed below, the two lines will be straight for all values of U and no extrapolation will be necessary. If the constant is so small that there is observable dissociation when $U/a = n/(n+1)$, then the two lines will form a continuous curve here rather than a sharp point. If $Q_1 = Q_2$, a minimum or maximum will still occur at $U/a = n/(n+1)$, though for other cases this is not true. In any event, the extrapolations of the straight-line portions of the curve give the correct value $n/(n+1)$.

The preceding discussion applies to the formation of a single complex, $(A+nU)$. If more than one complex is formed simultaneously, the problem becomes more complicated. Let us assume that a second complex, $(A+mU)$, also forms (where $m>n$), but that the $(A+nU)$ molecule is almost completely undissociated and much more stable than the $(A+mU)$ molecule, so that no $(A+mU)$ is formed unless the ratio of U to A is greater than n.

If we compare the plot of optical density *vs.* U/a for this model system with that for the system in which $(A+nU)$ alone is formed, it is clear that the curves must coincide in the region in which U/a is less than $n/(n+1)$ (*i.e.* the region in which $U/A < n$). The behavior for $U/a > n/(n+1)$ will depend upon the molar optical density of the complex $(A+mU)$, which we call Q_4. Using the same arguments employed to derive eqns. (1) and (2), we find that the limiting straight line determined by the mixing curve for the region in which U/a is slightly greater than $n/(n+1)$ is

$$D'' = \left[-Q_3\left(1+\frac{n+1}{m-n}\right)+\frac{n+1}{m-n}Q_4\right]\left[U-\frac{an}{n+1}\right]+\frac{Q_3 a}{n+1} \qquad (3)$$

and the straight line for the region in which U/a is close to unity is

$$D''' = [-Q_2(1+m)+Q_4][a\text{-}U]+Q_2 a \qquad (4)$$

These two lines intersect at $U/a = m/(m+1)$, making it possible to determine m.

We will show in the next section not only that poly A and poly U react in the manner described in the preceding paragraphs (with $n=1$ and $m=2$), but that the change in optical density is twice as great when $(A+2U)$ is formed from poly A and poly U as when $(A+U)$ is formed, so that the straight lines defined by eqns. (2) and (3) coincide.

RESULTS

The two-stranded complex. The first set of continuous variation mixing experiments was performed in 0.1 *M* NaCl buffered at pH 7.4 with glycyl glycine. The result is shown in Fig. 2. The straight lines are the best least square lines through the points, and have correlation coefficients of 0.992 (for U < 50%) and 0.990 (for U > 50%). There is no observable deviation from linearity in either curve near the center, indicating that the association of the two polymers is very strong. The position of the minimum clearly demonstrates the formation of a 1:1 complex, in confirmation of the X-ray diffraction studies.

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We can estimate a lower limit for an "effective" equilibrium constant for the reaction (A) + (U) = (A + U) by the sharpness of the optical density curve at 50% mole ratio. The data is sufficiently accurate to detect a rounding off of the minimum by an amount equal to 5–10% of the total optical density drop. Hence, the reaction must be at least 90–95% completed, yielding an "effective" equilibrium constant of 10^6 or 10^7. However, as will be shown below, the combination of these two polymers requires additional cations, so that the equation as written above is incomplete.

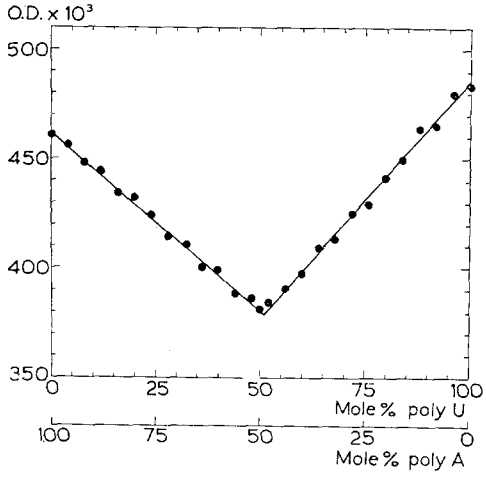

Fig. 2. The O.D. at 259 mμ of mixtures of polyadenylic acid and polyuridylic acid. The optical densities were measured within 15 min of mixing. All solutions are in 0.1 *M* NaCl, 0.01 *M* glycyl-glycine, pH 7.4, T = 25° C. The least-square lines are shown.

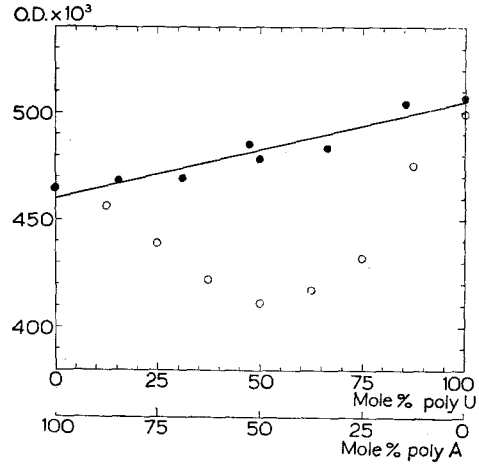

Fig. 3. Formation of the (A + U) complex in 0.01 *M* sodium cacodylate buffer at pH 6.5. ● Zero time O.D.; ○ final O.D. (24 h).

The result shown in Fig. 2 is also obtained in 0.1 *M* NaCl buffered with 0.01 *M* sodium cacodylate adjusted to pH 6.5. However, the poly A-poly U interaction disappears entirely in 0.1 *M* NaCl if the pH has been lowered to 5.2 by the addition of 0.01 *M* sodium acetate–acetic acid buffer. The presence of a set of titratable groups[12] in poly A with a p*K* at about 5.7 suggests that a group important to the specific interaction of the poly A with the poly U is being blocked by hydrogen ion in this pH region.

The optical density change in 0.1 *M* NaCl at pH 6.4–7.4 occurs with a rapidity which is not measurable by the simple hypodermic injection technique described in the section on METHODS. That is, the reaction is complete in less than 1–2 seconds. However, if the reaction is studied in solutions of 0.01 *M* cacodylate at pH 6.5 to which no NaCl has been added, the rate of reaction is slowed to a point where it is possible to use the injection technique.

Fig. 3 shows the optical densities extrapolated to zero time with the use of this technique. It also shows the optical densities after the solutions have reached equilibrium. Most of the drop in optical density occurs within the first hour. The rounded character of the mixing curve suggests an incomplete association of the complex.

The dependence of the optical density on the presence of cations is demonstrated further by experiments in solutions free of excess ions. Samples of poly A and poly U

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prepared as described in the section on METHODS were redissolved in triple distilled water and dialyzed against a solution of ethylenediaminetetraacetic acid (EDTA) disodium salt which had been adjusted to pH 6.8 with NaOH. The polymer solutions were then dialyzed against triple distilled water to remove the EDTA, and lyophilized. Working solutions were made from these dried samples in triple distilled water, and the pH of each was adjusted to about 6.6 with NaOH solution that had been prepared with precautions to avoid the absorption of CO_2. This addition does not raise the ionic strength appreciably ($\sim 10^{-5}$ M). These solutions show no hypochromic effect upon mixing*.

The addition of very small amounts of a divalent cation to this system produces a striking effect. Fig. 4 (solid circles) shows the optical density drop upon the addition of manganous ion to a 1:1 mole mixture of the two polymers. In each case 0.1 ml of a concentrated solution of $MnCl_2$ was added to 3.0 ml of the ion-free mixture. There is already an observable hypochromic effect when the molar ratio of Mn^{++} to phosphate is 2:1, and the reaction is completed at a molar ratio near 3:1. The (A + U) complex shows a very high affinity for manganous ion, and preliminary evidence shows a similar affinity for Mg^{++}. Experiments carried out at pH 7.8 yield the same results as those above at pH 6.6.

The effect of sodium ion on the "ion-free" mixture of poly A and poly U is similar to that of maganous ion, but the concentration of sodium ion necessary to produce interaction is about a hundred times greater. Fig. 4 shows the effect of the addition of NaCl solutions in an experiment analogous to that described above for $MnCl_2$.

It is interesting to note that the preparation of "ion-free" poly A and poly U by dialyzing against EDTA and distilled water does not affect their combining properties irreversibly. The normal hypochromic effect is observed in 0.1 M NaCl, 0.01 M cacodylate.

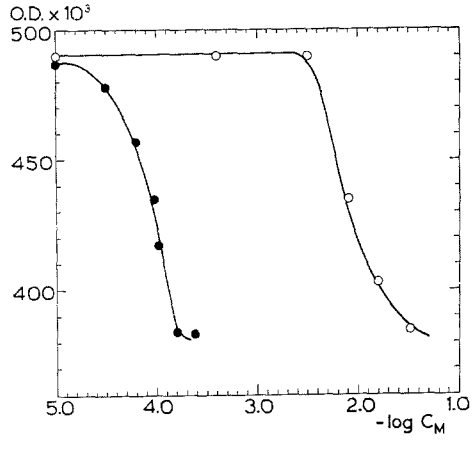

Fig. 4. Addition of metal ions to a 1:1 A:U mixture in distilled water. O.D. is plotted against — log (total metal ion conc.). ● $MnCl_2$; ○ NaCl.

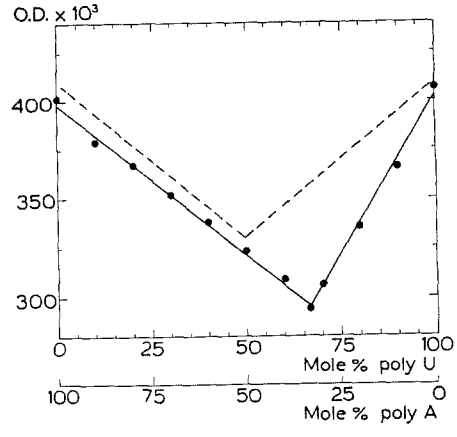

Fig. 5. The O.D. of various mixtures of poly A and poly U. The solid lines are for solns. that have 0.1 M NaCl, 0.01 M glycyl-glycine (pH 7.4) and $1.2 \cdot 10^{-2} M$ $MgCl_2$. The dashed line shows the O.D. before the addition of the $MgCl_2$. Measurements were made 2 h after mixing.

* The absence of a hypochromic effect in ion-free A + U mixtures has also been reported by WARNER[13].

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The reaction with Mn^{++} can be reversed by the addition of EDTA. For example, when the solution in Fig. 4 containing $2.4 \cdot 10^{-4}$ *M* Mn^{++} is made $3 \cdot 10^{-3}$ *M* in EDTA, the optical density rises to near the original "ion-free" value.

It is possible to demonstrate the combination of poly A with poly U in the ultracentrifuge. Thus, if we combine in 0.1 *M* NaCl a poly A sample with an $S_{20} = 8.0$ with a poly U sample of $S_{20} = 3.2$, we produce a complex with an S_{20} of 12.6. It is also possible to demonstrate the reversal of the combination in the ultracentrifuge. In a 1:1 mixture of ion-free A and U in $2 \cdot 10^{-4}$ *M* Mn^{++} (OD = 0.373), the sedimentation coefficient is 13.9 for the (A + U) complex. If this solution is made 10^{-3} *M* in EDTA, the mean sedimentation coefficient changes from 13.9 to $S_{20} = 4.4$, which is near the mean of the individual sedimentation velocities of the poly A and poly U separately.

We believe that it is reasonable to conclude on the basis of these data that the metal ions are playing a role in the formation of the (A + U) complex, and that without excess cations the complex is probably completely dissociated*.

The three-stranded complex. In the presence of 10^{-2} *M* Mg^{++} or other divalent cations, the mixing curve assumes a new shape[6] in which a sharp minimum occurs at 67% U, 33% A (Fig. 5), indicating complete formation of a new complex involving two strands of U for each strand of A. It is interesting to note that the slow partial formation of this new complex is seen in 0.1 *M* NaCl with no divalent cations added, since after 48 hours the points on the right side of Fig. 2 fall below the straight line.

Ultracentrifuge studies of this system[6] show an increase of nearly 50% in the sedimentation coefficient of the new complex compared with that of the (A + U) complex. This increase in S_{20} of approximately 50% would be expected if the two stranded (A + U) complex were to take on a third strand to become (A + 2U). The third strand could fill the deep helical groove in A + U (similar to the deep groove seen in DNA). Since it would displace water molecules from that site, and not appreciably alter the frictional forces or shape factor of the molecule, the net density increment of the molecule over the solvent would result in approximately a 50% increase in sedimenting velocity.

In short, the evidence for the formation of a three stranded polynucleotide complex rests upon the demonstration of a new sharp minimum in the optical denisty curves with an accompanying change in sedimentation properties.

The conversion of the 1:1 complex into the 2:1 complex may be followed by observing the change in the optical density *vs.* composition-curve. In Fig. 6, this curve is shown for two different concentrations of NaCl at time of mixing (zero time) and at equilibrium. The "zero time" curves were obtained by using the rapid mixing technique described above. Both zero time curves show the formation of a 1:1 complex with a minimum at 50% mole fraction. (It should be noted that the open circles and triangles at the left half of Fig. 6 fall directly upon the solid circles and triangles, and they are not shown separately in the figure.) The fact that the mixing curve at zero time is that of the two-stranded complex demonstrates clearly that the reaction which forms (A + U) from A and U is much faster than that which forms (A + 2U) from (A + U) and U under the same conditions.

At equilibrium (48 h), the optical density reading in 0.35 *M* NaCl shows a marked

* It is possible that the two chains are loosely associated in the absence of excess cations in such a way that there is no change in optical density and sedimenting properties. However, we consider this unlikely.

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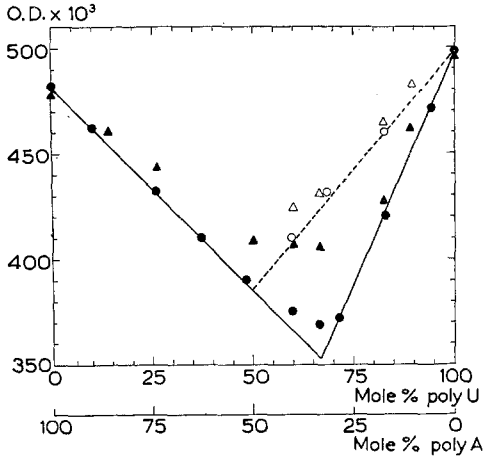

Fig. 6. O.D. curves showing formation of (A + U) and (A + 2U) complexes in 0.01 *M* sodium cacodylate, pH 6.5. △ 0.35 *M* NaCl, zero time; ○ 0.70 *M* NaCl, zero time; ▲ 0.35 *M* NaCl, 48 h; ● 0.70 *M* NaCl, 48 h. (Solid and open symbols coincide for U < 50%.)

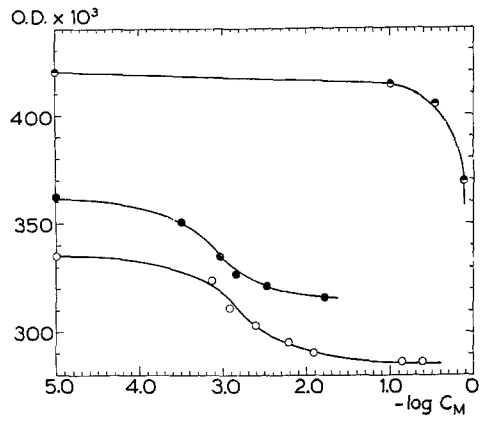

Fig. 7. O.D. changes in solns. with mole ratios A:U = 1:2. Before the addition of further salt, the solns. were in 0.1 *M* NaCl, 0.01 *M* cacodylate, pH 6.5. Salt was added to reach the concentrations shown in the abscissa. ● $MnCl_2$; ○ $MgCl_2$; ◓ NaCl (for NaCl, the concentration C_M refers to total sodium ion). In the case of NaCl, the solutions of poly A and poly U were made up directly with the concentrations of NaCl shown. The total base concentration varies slightly from one point to another, but all points have been adjusted to the same scale for a given curve.

bowing of the curve on the right side with a shift of the minimum. In 0.70 *M* NaCl the change is more pronounced, and the formation of the 2:1 complex is almost complete.

Further studies of the effect of cations on the formation of (A + 2U) have been made in a manner analogous to that described for (A + U) as shown in Fig. 4. Solutions with a 1:2 ratio of A to U were prepared in 0.1 *M* NaCl, 0.01 *M* cacodylate at pH 6.5. As shown in Figs. 2 and 5, such solutions contain an equimolar mixture of (A + U) and U molecules. To this, a small volume of concentrated salt solution is added. In Fig. 7, the equilibrium optical density is plotted as a function of the negative logarithm of the final ion concentration. Results are plotted for $MgCl_2$, $MnCl_2$, and NaCl. Different initial concentrations of polymer were chosen, so that the curves do not coincide near $C_M = 0$. As in the case of the formation of the (A + U) complex, the results show that divalent cations are more effective than monovalent cations in bringing about the formation of the three stranded complex. We have also carried out experiments with Zn^{++} and Ca^{++}, both of which have an activity similar to that shown for Mn^{++} and Mg^{++}. It should be pointed out in comparing Figs. 3–5 that the concentrations necessary to bring the reactions to completion are about 100-fold greater for the three-stranded complex than for the two-stranded complex.

In the case of magnesium and manganese ions, (M), it is possible to fit the data for the formation of (A + 2U) with a single equilibrium constant if we assume a reaction of the form

$$(A + U) + U + iM = (A + 2U)\cdot M_i, \qquad (5)$$

and choose $i = 2$. If i is chosen equal to 1, 3, or 4, the "constant" varies over factors

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of ten. Table I shows the values of the equilibrium constant for this reaction as a function of divalent cation concentration for several values of i. No correction is made for the binding of metal ions by the buffer, which is at most 10% as estimated from the pH shift on addition of metal ion to the cacodylate. This correction was calculated for glycyl glycine from available data[12] and was also less than 10%. The affinity of Mn^{++} in most complexes is larger than that of Mg^{++}, so that it is not surprising that here also, the equilibrium constant for manganese ion is greater than that for magnesium ion.

TABLE I

APPARENT EQUILIBRIUM CONSTANT FOR THE REACTION

$$(A + U) + (U) + iM = (A + 2U \cdot M_i)$$

$$K_i = \frac{[A + 2U \cdot M_i]}{[A + U]\,[U]\,[M]^i}$$

Total metal ion concn.	K_i *Magnesium*			
	$i = 1$	$i = 2$	$i = 3$	$i = 4$
$7.3 \cdot 10^{-4}$	$3.6 \cdot 10^{7}$	$5.2 \cdot 10^{10}$	$7.7 \cdot 10^{13}$	$1.2 \cdot 10^{17}$
$1.2 \cdot 10^{-3}$	$1.1 \cdot 10^{8}$	$9.6 \cdot 10^{10}$	$8.1 \cdot 10^{13}$	$7.0 \cdot 10^{16}$
$2.4 \cdot 10^{-3}$	$1.6 \cdot 10^{8}$	$6.7 \cdot 10^{10}$	$2.8 \cdot 10^{13}$	$1.2 \cdot 10^{16}$
$6.1 \cdot 10^{-3}$	$2.8 \cdot 10^{8}$	$4.7 \cdot 10^{10}$	$7.6 \cdot 10^{12}$	$1.3 \cdot 10^{15}$
$1.2 \cdot 10^{-2}$	$8.2 \cdot 10^{8}$	$6.8 \cdot 10^{10}$	$5.7 \cdot 10^{12}$	$4.7 \cdot 10^{14}$
Total metal ion concn.	K_i *Manganese*			
	$i = 1$	$i = 2$	$i = 3$	$i = 4$
$3.23 \cdot 10^{-4}$	$8.2 \cdot 10^{7}$	$2.5 \cdot 10^{11}$	$8.6 \cdot 10^{14}$	$2.9 \cdot 10^{18}$
$9.04 \cdot 10^{-4}$	$2.5 \cdot 10^{8}$	$2.7 \cdot 10^{11}$	$3.3 \cdot 10^{14}$	$3.9 \cdot 10^{17}$
$1.77 \cdot 10^{-3}$	$4.8 \cdot 10^{8}$	$2.7 \cdot 10^{11}$	$1.6 \cdot 10^{14}$	$9.7 \cdot 10^{16}$
$3.23 \cdot 10^{-3}$	$1.5 \cdot 10^{9}$	$4.7 \cdot 10^{11}$	$1.5 \cdot 10^{14}$	$4.5 \cdot 10^{16}$

K_2 av. (Magnesium) $= 6.6 \cdot 10^{10}$; K_2 av. (Manganese) $= 3.2 \cdot 10^{11}$.

We have not yet determined the order of the sodium ion in the (A+2U) reaction or the order of manganese ion and sodium ion in the reaction to form (A+U). In these cases, the effective range of concentration of cation from that producing barely detectible association to that producing complete association is rather narrow, and consequently the determination of the equilibrium constant is made more difficult than in the systems shown in Table I. In addition, these more difficult cases involve large variations in ionic strength as metal ions are added, so that interpretation of results in terms of a single constant becomes even more complicated.

It should be pointed out that the fact that the equilibrium expression is a constant for $i = 2$ implies nothing about the number of metal ions bound per nucleotide, since we can also interpret the data in terms of a reaction

$$(A + U)_m + U_m + 2M = (A + 2U)_m \cdot M_2 \qquad (6)$$

where $(A+U)_m$ refers to some effective site on the molecule with a size of m units. The data merely show that the extent of reaction is dependent upon the square of the divalent cation concentration. It is not possible to determine the value of m from

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this kind of experiment alone so long as the total concentration of M^{++} is much greater than that of $(A+2U)_m \cdot M_2$.

Reaction velocity. As mentioned above, the formation of the (A+U) complex is complete in less than 1 sec when the two polynucleotides are mixed in 0.1 *M* NaCl. In the formation of (A+2U), the rate at which the reaction proceeds as well as the extent of the reaction can be controlled by the amount of added cation. In Table II, we have listed the time required for the reaction to go halfway to equilibrium, as a function of various cations. All of the cations listed in Table IIa accelerate the formation of A+2U at roughly comparable rates. As expected, the time required to get halfway to equilibrium grows longer as the cation concentration decreases.

TABLE II

TIME REQUIRED FOR THE REACTION

$$(A + U) + (U) + 2M = (A + 2U \cdot M_2)$$

TO GO HALF WAY TO EQUILIBRIUM

(a) Final concn. of added salt	$t_{1/2}$ *(min)*	*(a) Final concn. of added salt*	$t_{1/2}$ *(min)*
$7.3 \cdot 10^{-4} M$ $MgCl_2$	8	$3.23 \cdot 10^{-4} M$ $MnCl_2$	4
$1.2 \cdot 10^{-3} M$ $MgCl_2$	6	$9.04 \cdot 10^{-4} M$ $MnCl_2$	3
$2.4 \cdot 10^{-3} M$ $MgCl_2$	4	$3.23 \cdot 10^{-3} M$ $MnCl_2$	1.5
$6.1 \cdot 10^{-3} M$ $MgCl_2$	1.3	$1.62 \cdot 10^{-2} M$ $MnCl_2$	< 0.5
$1.2 \cdot 10^{-2} M$ $MgCl_2$	0.5		
0.12 M $MgCl_2$	0.2	$8 \cdot 10^{-3} M$ $CaCl_2$	0.9
0.24 M $MgCl_2$	< 0.1	$8 \cdot 10^{-3} M$ $ZnCl_2$	1.0

The initial concentrations of (A + U) and (U) were both equal and approximately $(5 \cdot 10^{-5} M)$. All solutions were in 0.1 *M* NaCl and at pH 6.4–7.4.

(b) Final total concn. of NaCl	$t_{1/2}$ *(min)*
0.35 *M*	6
0.60 *M*	1.2
1.1 *M*	0.4

The initial conditions were as above, but more NaCl was added.

All of the preceding experiments were carried out with samples of poly A and poly U which had sedimentation coefficients of 8.0 and 3.2 respectively. With this material, the reaction proceeds quite slowly in 0.1 *M* NaCl as noted above ($t_{1/2}$ several hours). In Table IIb, we have listed $t_{1/2}$ as a function of total NaCl concentration showing a behavior similar to that seen with divalent cations, but requiring concentrations roughly one hundred times as great. In preliminary experiments using the same poly U but a smaller poly A ($S_{20} = 3.5$), we found that the reaction occurs much more rapidly in 0.1 *M* NaCl, even though the equilibrium position is unchanged.

DISCUSSION

In the theoretical section, we have pointed out that the optical density mixing curve for the formation of a second complex need not have the same slope between 50% and 67% mole fraction of U as it has at U < 50%. However, the fact that the curve

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for U > 50% is collinear with the curve for U < 50% implies that the hypochromic effect attendant upon the addition of the second U chain is the same as that occurring for the first chain. This suggests that the second strand of poly U may assume a configuration not unlike that of the first strand, *i.e.* helically wound with the plane of the uracil residues normal to the helix axis.

The experimental results establish the existence of a 1:1 complex and a 1:2 complex of poly A and poly U and in addition permit us to draw some conclusions about their relative strengths of association. The formation of both complexes is dependent upon the presence of small cations. In both cases, divalent cations are effective in much smaller concentrations than are monovalent cations, but the range of concentrations in which the cations exert their effect differs markedly in the two complexes. The reaction forming the (A + U) complex is driven to completion by concentrations of Mn^{++} or Mg^{++} which are of the same order of magnitude as the concentration of bases; the reaction in which the third strand is added does not occur until the concentration of divalent cation is between 50 and 100 times as great as the base concentration. In the case of sodium ion, the concentration required to form the two-stranded complex is between a tenth and a hundredth of that required to add the third strand.

It appears that in solutions of any given ionic composition, the addition of the first strand of poly U to poly A occurs much more readily than the addition of the second, with a faster rate of formation and a larger affinity.

In solutions which approximate the natural physiological range of ionic strengths we would expect the third strand to be bound relatively weakly to a two-stranded "core" of (A + U), but in such solutions the two strands of the (A + U) structure would be bound to each other so tightly that they would behave as an entity in all reactions. This is of interest in view of the possibility that the formation of this three-stranded complex may be similar to a process whereby a two stranded DNA (similar to (A + U)) has wrapped around it a single stranded RNA.

The effect of cations can be understood in terms of models which have been proposed for the two-stranded and three-stranded structures*[3,4,6]. It seems likely that the second poly U ribose-phosphate backbone lies considerably closer to the poly A backbone than does that of the first poly U. The high concentration of negative charge on the chains will tend to oppose the formation of any structure in which two strands lie close together. It is not surprising, therefore, that the three-stranded structure should form less readily than the two-stranded structure in which the chains are relatively far apart.

The role of cations is to neutralize the negative charges, permitting the chains to approach each other. There are two ways in which these cations can bring this about: a Debye-Huckel screening, which is measured by the ionic strength, or a more specific interaction with negatively charged groups in the polymer. The disparity between the ionic strengths at which divalent, in contrast to monovalent cations, are effective makes us believe that the former explanation is inadequate to account for all of the observed phenomena. The fact that the reaction forming (A + 2U) is second order in magnesium or manganese ions suggests that these ions, at least, are being bound specifically to one or more negative sites on the polymer molecules.

The ability of cations to act as a bridge between negatively charged sites has

* We are indebted to Dr. DAVID R. DAVIES for the discussion contained in this paragraph.

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been demonstrated in the binding of small anionic molecules to proteins[15]. It is possible that cations have a similar role in the polynucleotides.

Studies of solutions of DNA in distilled water have shown that their optical density is lowered by the addition of ions[16]. Since DNA denatures in distilled water[17] it is difficult to interpret these reactions. It has been suggested that the initial stages of DNA denaturation involve a partial separation of the two chains[18]. If this is so, the role of the added cations in this system may be related to bringing these chains closer together. In this regard, it should be noted that the effect of monovalent and divalent cations upon optical density is quite similar to that shown in Fig. 4 for (A + U).

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We wish to thank Mrs. JEAN JOHNSON for technical assistance, and Miss ANN ROLLER for phosphate analyses. We are indebted to Prof. NORMAN DAVIDSON and Dr. DAVID R. DAVIES for helpful discussions.

SUMMARY

The interaction of the synthetic polyribonucleotides, polyadenylic acid and polyuridylic acid has been studied spectrophotometrically by means of the continuous variation technique. The effect of the ionic composition of the solution upon the tendency to form two-stranded and three-stranded complexes is discussed, and it is shown that an excess of small ions is necessary to both reactions. It is found that manganese and magnesium ions have a greater effect than sodium ion in promoting these reactions. It is shown that the two-stranded complex is more stable than the three-stranded complex.

REFERENCES

1 S. OCHOA AND L. HEPPEL, *The Chemical Basis of Heredity*, Johns Hopkins Press, Baltimore, 1957, p. 615.
2 R. C. WARNER, *Federation Proc.*, 15 (1956) 379.
3 A. RICH AND D. R. DAVIES, *J. Am. Chem. Soc.*, 78 (1956) 3548.
4 A. RICH, *The Chemical Basis of Heredity*, Johns Hopkins Press, Baltimore, 1957, p. 557.
5 A. RICH, *Ann. N.Y. Acad. Sci.*, (in the press).
6 G. FELSENFELD, D. R. DAVIES AND A. RICH, *J. Am. Chem. Soc.*, 79 (1957) 2023.
7 U. Z. LITTAUER AND A. KORNBERG, *J. Biol. Chem.*, 226 (1957) 1077.
8 M. GRUNBERG-MANAGO, P. J. ORTIZ AND S. OCHOA, *Science*, 122 (1955) 907.
9 O. H. LOWRY, N. R. ROBERTS, K. Y. LEINER, M. L. WU AND A. L. FARR, *J. Biol. Chem.*, 207 (1954) 1.
10 W. MEJBAUM, *Z. Physiol. Chem.*, 258 (1939) 117.
11 P. JOB, *Ann. Chim.*, 9 (1928) 113.
12 R. F. BEERS, JR. AND R. F. STEINER, *Nature*, 179 (1957) 1076.
13 R. C. WARNER, personal communication.
14 C. B. MONK, *Trans. Faraday Soc.*, 47 (1951) 297.
15 I. M. KLOTZ AND W. C. LOH MING, *J. Am. Chem. Soc.*, 76 (1954) 805.
16 J. SHACK, R. J. JENKINS AND J. M. THOMPSETT, *J. Biol. Chem.*, 203 (1953) 373.
17 R. THOMAS, *Biochim. Biophys. Acta*, 14 (1954) 231.
18 P. DOTY AND S. A. RICE, *Biochim. Biophys. Acta*, 16 (1955) 446.
19 J. R. FRESCO AND P. DOTY, *J. Am. Chem. Soc.*, 79 (1957) 3928.

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Note added in proof. It has been pointed out recently[19] that poly A exists as a random coil in salt-free solution at pH > 6.5 and in 0.15*M* NaCl at pH > 5.7. All of our experiments (except that at pH 5.2, for which no reaction was observed) were performed in the region where poly A is a random coil.