THE SPECIFICITY AND APPLICATIONS OF ANTIBODIES TO HELICAL NUCLEIC ACIDS

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I. INTRODUCTION

The definition of binding site determinants of antigens has provided a basis for our understanding of the range and specificity of immune recognition. It has also established the use of antibodies as specific reagents for the assay and structural study of a large number of molecules of interest in biology and medicine. The investigation of nucleic acid antigens includes these aspects and other special interests as well. One relates to the more general question of how proteins recognize specific sites in nucleic acids. Such recognition is important for the function of repressors, amino acyl-tRNA synthetases, restriction and modification enzymes, polymerases, enzymes involved in the processing of messenger RNA, and receptors for interferon induction. These proteins, and perhaps immunochemical probes as well, may predict the presence of fine structural features that are not readily detected by physical measurements alone. A second special interest concerns the mechanisms underlying the disease systemic lupus erythematosus (SLE), in which the production of antibodies to nucleic acids is a characteristic feature and part of a picture of unusual immunological reactivity.

Other features of nucleic acid immunochemistry also require special comment. With few exceptions, nucleic acids are not effective immunizing agents by themselves; therefore, a variety of complexes of protein carriers with either macromolecular nucleic acids or with small fragments of the DNA or RNA have been used to induce antibody formation. The antibodies can then recognize and combine with the nucleic acid component alone; that is, the nucleic acids usually act as haptens. The requirements for immunogenicity have been reviewed previously.[1-6] Previous reviews have also described the criteria necessary to establish that one is measuring a true antigen-antibody reaction that involves nucleic acid determinants.[3,5]

The present article will focus on questions of how precisely the antigenic determinants of helical nucleic acids have been defined, to what extent antibodies can differentiate various helical conformations, and how these antibodies may be used as specific reagents. Some of these aspects form the subject matter of other reviews as well.[5,6] While this discussion will consider primarily helical nucleic acids, antigenic properties of nonhelical nucleic acids have also been extensively studied;

some basic differences to be expected in antigenic properties of helical and nonhelical structures (with illustrative comparisons drawn from protein immunochemistry) will therefore be described before specific antibody-antigen systems are presented.

A. Sequential and Conformational Determinants in Nucleic Acids

In attempts to define antigenic specificities of the major classes of macromolecules, it is possible to identify different kinds of determinant structures having a lesser or greater degree of dependence on the overall configuration of the polymer. Those that involve a small fragment with minimal dependence on the overall polymer conformation have been termed "sequential determinants."[7] They may be short oligosaccharide, oligopeptide, or oligonucleotide sequences, up to the size of about 5 to 7 residues.[8] The corresponding antibodies combine readily with the oligomers either when the latter are isolated as such or when they are accessible in a larger polymer, but not when they are masked within complex polymer structures. For example, antibodies to some specific polypeptide sequences of ribonuclease may react with denatured ribonuclease but not with the native protein containing the same sequence.[9] Similarly for nucleic acids, the pyrimidine or purine portions of single nucleotides or oligonucleotide base sequences may serve as sequential determinants for some of the antibodies in SLE sera and for many types of experimentally induced antibodies.[5] The corresponding antibodies react only with single-stranded portions of nucleic acids,[5] in which the bases are exposed, and not with regions in which they are inaccessible within a base-paired helix.

If the pentose-phosphate backbone of a sequence of nucleotides in a single chain formed the antigenic site, however, it could be exposed in both random coil and helical structures, so that both forms would be reactive with the corresponding antibodies. Secondary structure would have only a modifying effect on quantitative aspects of the reaction, and this could still be considered a sequential determinant.

Other determinants depend not only on accessibility but also on the overall shape of a region of the polymer for their integrity. These "conformational determinants"[7] have been considered to be especially prominent in globular protein antigens since many antiprotein antibodies will react with native but not with denatured protein,[10] even though the constituent primary structures are more available in the denatured form. As further examples, antibodies can distinguish between the reduced and oxidized conformations of hemoglobin[11] and the closed loop or open chain conformations of amino acid residues 64 to 80 in lysozyme.[12] An elegant model system for defining sequential and conformational determinants is one built on the tripeptide Tyr-Ala-Glu.[13] If it exists as tripeptide side chains on a carrier protein, it serves as a sequential determinant. If it exists in the polymerized form $(Tyr\text{-}Ala\text{-}Glu)_n$, it forms a helix when n is greater than 13, and the same primary structure is then involved in a conformational determinant. Antibodies to either form are specific and will not react with the other form. This serves in part as a model for polynucleotide antigens as well, in terms of the possibility of new antigenic sites being generated by the helical configuration while some sequential sites are simultaneously masked.

An important feature of conformational determinants is that a given antigenic site may depend in part on relationships between parts of a molecule that are not contiguous in the primary sequence but are close to each other in the native configuration. Such relationships may be important in the polypeptide or polynucleotide helices described above and are suspected to be involved in native protein determinants. In the case of nucleic acids, nearby regions of adjacent chains in a double- or triple-helix could participate together in forming one antigenic structure.

It is much more difficult to precisely delineate a conformational determinant than a sequential one. For the latter, it is possible to isolate a small monovalent fragment that maintains the structural features essential for its reactivity. Native conformation, on the other hand, is nearly always destroyed during an attempt to isolate a small reactive fragment. Even though the lysozyme loop can be isolated and shown to be monovalent, the extent of the antigenic determinant is probably smaller than the total loop, but smaller fragments cannot be obtained without breaking the loop. Similarly with nucleic acids, the smallest fragments that will maintain native helical shape may well be greater than the size of a single antigenic specificity site.

For these reasons, a second approach is usually

TABLE 1

Systems of Antibodies That React with Helical Nucleic Acids

Source of serum or immunogen	Major reactivity
1. Systemic lupus erythematosus sera (immunogen unknown)	i. Native DNA, synthetic ds polydeoxyribonucleotides, RNA-DNA hybrids ii. ds viral RNA, synthetic polyribonucleotides, RNA-DNA hybrids
2. Ribosomes	i. Single-, double-, or multistranded RNA, synthetic polyribonucleotides
3. Methylated BSA complexes of polynucleotides	
a. ds polyribonucleotides	i. poly(A) · poly(U); poly(I) · poly(C); ds viral RNA (some cellular RNA) ii. poly(G) · poly(C); (some cellular RNA)
b. ds hybrid	i. poly(A) · poly(dT); poly(I) · poly(dC); RNA-DNA hybrid
c. ds polydeoxyribonucleotide*	i. poly(dG) · poly(dC)
d. triple-stranded polynucleotide	i. poly(U) · poly(A) · poly(U); poly(U) · poly(A) · poly(dT) ii. poly(U) · poly(dA) · poly(U); poly(U) · poly(dA) · poly(dT)

*MBSA complexes of native DNA, poly(dAT) · poly(dAT), and poly(dA) · poly(dT) have been tested but were not effective immunogens.

taken for the study of conformational determinants. This involves measuring degrees of cross-reaction between slightly differing structures. If they are found to be antigenically distinguishable and the differences in their three-dimensional structures are known, as from X-ray diffraction studies, one can hope for some insight into the shape of the antigenic site itself.[13a] Still, the description will usually be less complete than for sequential sites, especially in terms of the extent of the determinant.

Antibodies that react with helical nucleic acids do show varying degrees of dependence on the overall conformation of the helix for reactivity. Unlike antibodies specific for denatured DNA, they appear to react with the backbone on the outside of the helix. Some require a specific combination of two or more kinds of polynucleotide chains, while others react with the backbone of a single strand and can recognize it either as a single chain or in varying double-stranded or triple-stranded combinations. The systems that have been found to react with helical polynucleotides are listed in Table 1.

II. SLE ANTIBODIES TO DNA

A. Evidence for Reactivity with Native DNA

The anti-DNA immunoglobulins present in the sera of SLE patients were among the first of any antinucleic acid antibodies to be described.[14-17] These sera may, in fact, contain several populations of such antibodies. Some resemble experimentally induced types that are specific for bases or base sequences and react only with denatured DNA.[5] Unlike the latter sera, however, SLE sera may also contain additional antibodies which react with native DNA.

Since it has been very difficult to induce antibodies to native DNA in experimental animals, considerable effort has been expended in showing that the SLE sera are not simply reacting with regions of denatured DNA in the native samples. In fact, antibodies that react specifically with denatured DNA can detect such regions (Figure 1A), even when as little as 0.1% of a presumably native DNA sample is single stranded. The latter situation can be revealed by quantitative precipitin or complement fixation studies, as a large total amount of the native DNA is required for reaction. Some SLE sera, however, react with low concentrations of native DNA;[18,19] if denatured regions were involved, one would have to conclude that nearly all the DNA was single stranded. This was ruled out for the antigen samples that were used. Indeed, some SLE sera react with lower concentrations of native than of denatured DNA (Figure 1B), and others precipitate or fix complement

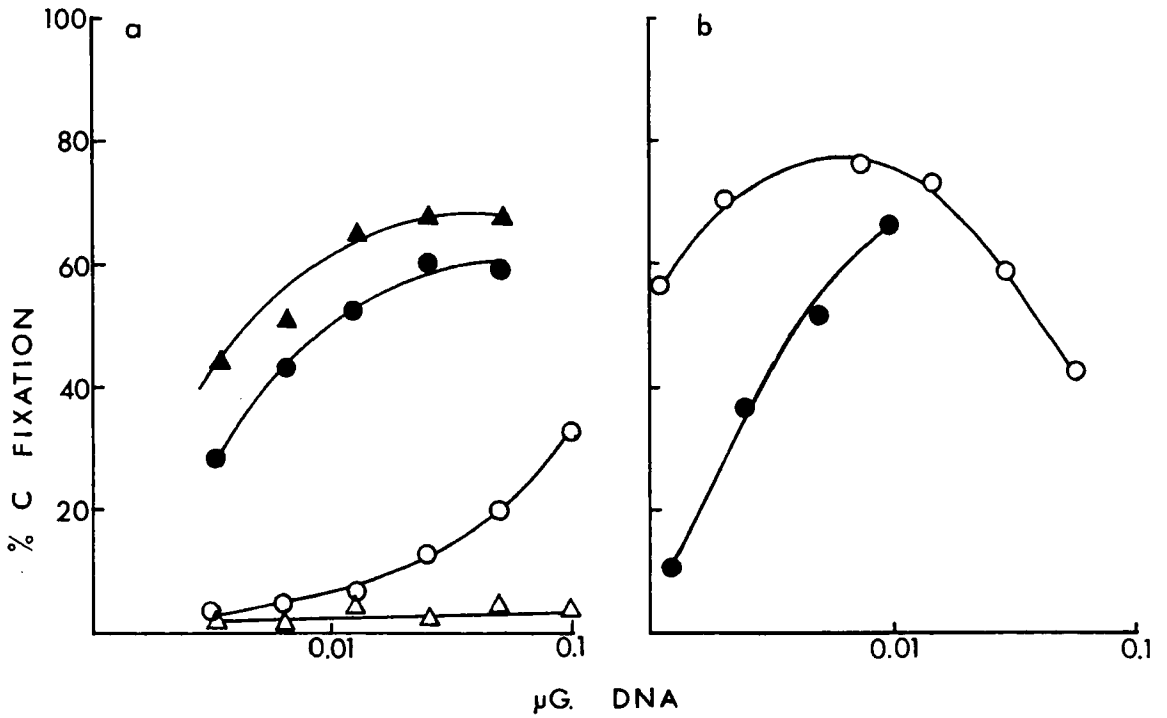

FIGURE 1. Quantitative microcomplement fixation reactions of (A) antidenatured DNA antiserum and (B) SLE anti-DNA serum, with native *E. coli* DNA (○), denatured *E. coli* DNA (●), native calf thymus DNA (△), and denatured calf thymus DNA (▲). The reaction of antidenatured DNA serum reflects the presence of small amounts of denatured DNA in the native DNA samples, as judged from the requirement of high concentrations of total native DNA for reactivity. This reactivity was eliminated by treatment of the sample with a nuclease specific for denatured DNA. The same treatment did not destroy the activity of low concentrations of native DNA with SLE serum.

with native DNA but not at all with denatured DNA.[19]

In further characterizations, the antibodies in some SLE sera were shown to precipitate and fix complement with DNA from which denatured material had been removed by a methylated BSA-kieselguhr column.[20,21] They bound radioactive closed circular helical DNA that could not be bound by antibodies to denatured DNA,[22] and they specifically agglutinated erythrocytes coated with SV40 DNA, a closed circular form.[23]

Separate antidenatured and antinative antibody populations have also been identified in some sera by two-dimensional immunodiffusion analysis (Figure 2).[19] When such a serum was absorbed by precipitation with denatured DNA, the residual supernatant could precipitate native but not denatured DNA. Further, the appearance and disappearance of reactivity on denaturation and renaturation were reciprocal as measured with SLE antidenatured and antinative DNA populations (Figure 3).[19] Thus, there is extensive evidence indicating that some SLE antibodies do react with native DNA. A particular immunofluorescence system allows the assay of antibody to native DNA without the problem of possible formation of single-stranded regions during preparative procedures.[24] This assay uses the hemoflagellate *Crithidia luciliae* or related trypanosomes as substrate. These organisms contain DNA concentrated, free of histone, in a large kinetoplast. With an indirect immunofluorescent technique, SLE sera that contain antibody to native DNA lead to specific staining of the kinetoplast.[24-26]

B. The Antigenic Sites of Native DNA

Identification of the determinants for antinative DNA sera has been attempted in two ways. As expected, the reactions were not inhibited by mononucleotides;[27] oligonucleotides containing all bases, as a partially digested DNA preparation, were poorly inhibitory.[28] Thus, bases or base sequences are not involved, and the approach of studying cross-reactions with naturally occurring or synthetic polynucleotides has been used for further exploration.

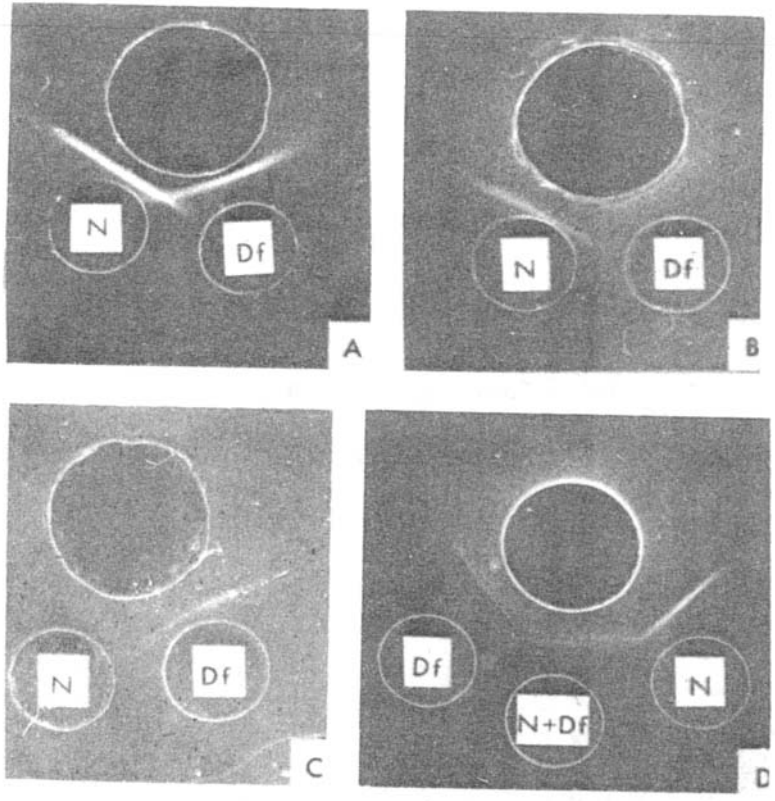

FIGURE 2. Immunodiffusion reactions of an SLE serum containing separate antinative and antidenatured DNA antibody populations. Large unlabeled wells contained: A and D, whole serum; B, serum absorbed with denatured DNA; C, serum absorbed with native DNA. The antigen wells contained: N, native DNA; Df, DNA denatured in the presence of formaldehyde; N + Df, a mixture of native DNA and DNA denatured in the presence of formaldehyde. (From Arana, R. and Seligmann, M., *J. Clin. Invest.*, 46, 1876, 1967. With permission.)

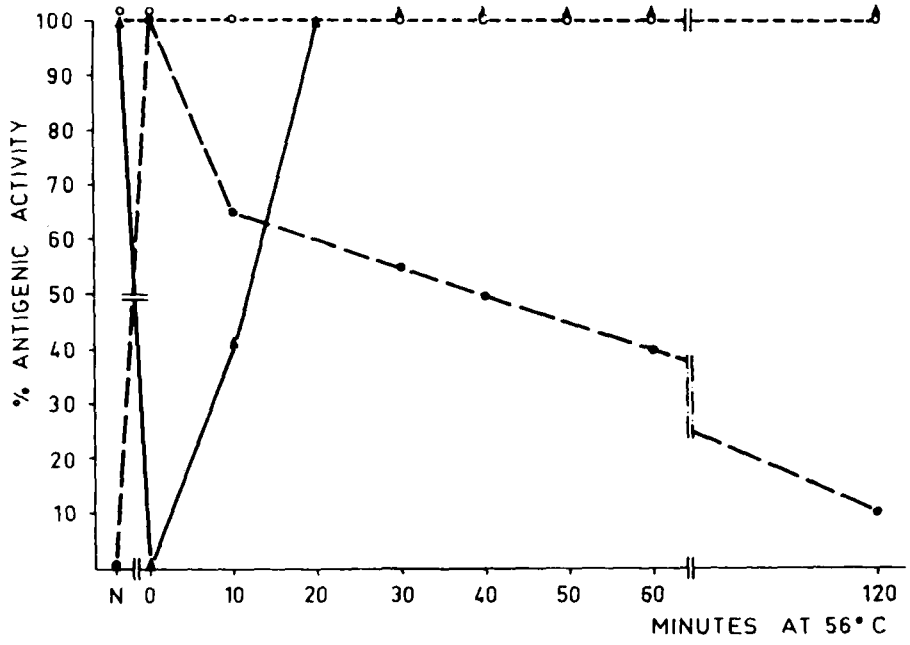

FIGURE 3. T7 phage DNA renaturation, as measured by complement fixation reactions with three different SLE sera containing antibodies to native DNA (▲), denatured DNA (●), or determinants common to native and denatured DNA (○). 100% antigenic activity was determined with the sample before denaturation (N) or at 0 time of incubation at 56° after denaturation, as appropriate for each serum. An increase or decrease in antigen reactivity during renaturation was measured reciprocally with antinative and antidenatured DNA sera, respectively, while no change was measured with a serum that fixed complement on both forms of DNA. (From Arana, R. and Seligmann, M., *J. Clin. Invest.*, 46, 1876, 1967. With permission.)

In precipitation or complement fixation experiments, such sera react with native DNA of any plant, animal, bacterial, or viral source tested.[18] Synthetic double-stranded polydeoxyribonucleotides, such as poly(dAT)·poly(dAT) and poly(dA)·poly(dT), are strongly reactive, and the rather different structure poly(dG)·poly(dC) is only slightly less effective.[29] A weaker cross-reaction occurs with the RNA-DNA hybrid poly(A)·poly(dT),[29,30] and no reaction at all is seen with double-stranded RNA forms such as poly(A)·poly(U), poly(I)·poly(C), or reovirus RNA.[27-29] (Other SLE antibody populations do react with the double-stranded RNA forms and not with DNA, as described below.) From these studies and the lack of direct involvement of the bases, it appears that the common and distinguishing feature of the reactive forms is the deoxyribose-phosphate backbone and that this is a major feature of the determinant.

There remains the question of whether the backbones of both strands are directly required for one specific site. Some insight comes from cross-reactions measured by inhibition experiments. Arana and Seligmann reported that even if some sera would precipitate only native DNA, the precipitation could be inhibited by denatured DNA.[19] This finding has been reported in other assay systems, notably inhibition of the passive agglutination of erythrocytes coated with native DNA[23] and inhibition of the binding of radioactive native DNA.[27,31] For most SLE sera, therefore, the backbone of a single chain seems sufficient, unless base-paired regions within the denatured DNA coil are actually responsible for the inhibition. This latter possibility could be tested further since such regions melt progressively at relatively low temperatures, and inhibition by denatured DNA should then show a more marked temperature dependence than that caused by native DNA. In occasional cases the native double-helical form was truly required for effective inhibition;[23] for these antibodies, the precise orientation of the two DNA strands would form one determinant.

It is still necessary to consider the apparent contradiction in the facts that some sera precipitate only native DNA, that this activity cannot be absorbed by precipitation with denatured DNA, and yet that denatured DNA can inhibit the precipitation of the native form. This could reflect the fact that the inhibition reactions require only primary binding of antigen by antibody, while precipitation and complement fixation require aggregation as well. Perhaps the spacing of determinants is such that some antibodies are able to cross-link and thus aggregate the extended rodlike structure of native DNA more effectively than the collapsed random coil of denatured DNA, even if the single pentose-phosphate chain is accessible and reactive in both forms. That is, both binding sites of these antibodies may attach to determinants on a single molecule of denatured DNA. The theory of the balance between binding events that do or do not lead to cross-linking of antigen by antibody has been discussed by Crothers and Metzger.[32]

The inhibition results cannot be further interpreted in terms of whether native or denatured DNA is in fact the truly homologous antigen. While these data do show that some of the determinants are present on both native and denatured DNA, they do not provide direct measures of the relative affinities for these two antigen forms at individual binding sites. Relative affinities can be deduced when univalent haptens are used as inhibitors,[33] but in most of the above studies the nucleic acid inhibitors were multivalent macromolecules. The inhibitory effectiveness can be greatly magnified by the advantages of multivalence,[32,34,34a] and it cannot be assumed that the same amplification factor can be applied to the two very different structures of native and denatured DNA.

That multivalence is playing a role and enhancing the apparent avidity can, in fact, be surmised from the amounts of inhibitor required. Several sera were inhibited by DNA concentrations of 1 to 10 ng/ml. This is a molar concentration of about 10^{-12} *M* for a DNA preparation with a molecular weight of 10^6 to 10^7. However, with monovalent inhibitors of antibodies to nucleosides or base sequences, inhibition of precipitation or complement fixation requires concentrations in the range of 10^{-8} to 10^{-4} *M*,[35-38] and we have found that a 10^{-4} to 10^{-5} *M* concentration of mononucleoside was required to inhibit the binding between homologous antinucleoside antibody and radioactive DNA. It is probably the multivalence of the DNA that leads to effective inhibition at molar concentrations several orders of magnitude lower than those required with monovalent haptens.

The precise nature and origin of the immuno-

gens in SLE are unclear. The formation of the anti-DNA antibodies may be a response of an abnormal immune system to normally occurring cellular breakdown products or to products of viral infection. Alternatively, viral or chemical derepression of normally suppressed clones of antibody forming cells may occur. Some or all of these factors may be dependent on the appropriate genetic background of the subject. These possible bases for the immunopathogenesis of SLE have been discussed elsewhere.[5,39-41]

An interesting recent finding is related to this question and to the possibility that one backbone may form the determinant for most sera. Anticardiolipin antibodies, induced by micelles with cardiolipin determinants on the surface, cross-reacted with micelles that incorporated native DNA.[42] The structure of the cardiolipin (diphosphatidyl glycerol) could bear some resemblance to that of a DNA backbone, with phosphodiester groups separated by an equal spacing of carbon atoms.[42] It has long been known that SLE sera give "false positive" serological tests for syphilis, in a test that depends on a reaction with cardiolipin.

Also of interest in this regard is another recent finding that administration of bacterial lipopolysaccharide to mice led to a release of DNA (mainly denatured) into the circulation within hours and then, after several days, to the formation of antibodies that reacted with both native and denatured DNA.[43] Perhaps the released DNA became associated with residual lipopolysaccharide to form a micellar immunogen.

Whatever the immunogen, the nature of the immune system itself probably does play a significant role. In several systems, the intensity of responses of animals to nucleic acid antigens has been shown to be under genetic control,[44-47] and the patterns of occurrence of the disease in animals[48,49] and humans[50,51] suggest the importance of genetic background of susceptible individuals.

C. Applications of SLE Antinative DNA Antibodies

Antibodies that react with native DNA have served as reagents for studies of the organization of DNA in isolated chromatin or nuclei.[52-56] The sera reacted in complement fixation with very small amounts of free native DNA, reaching equivalence with 2 to 10 ng of the nucleic acid. With chromatin, about 50-fold higher DNA concentrations were required for equivalent reactivity, indicating that only about 2% of the DNA in chromatin was available to react with antibody.[52] After dissociation of the nucleoprotein in solutions of high salt concentration and reassociation by dialysis against buffered normal saline, about 10 to 20% of the DNA became available for reaction. A sharp transition for this unmasking occurred between 0.4 and 0.6 *M* NaCl, a concentration at which mainly the F1 histone would be dissociated. The masking of DNA reactive sites by the addition of isolated histone could also be measured.[52] In a more specific case, an SLE serum was used to measure a difference in the association of phosphorylated and unphosphorylated F1 histone with DNA.[53]

SLE sera that precipitated native but not denatured DNA (or purified antinative DNA antibody) gave a typical pattern of nuclear staining when assayed by immunofluorescence.[54,55] The staining was located at the peripheral rim of the nucleus, suggesting that the small amount of native DNA that is accessible to antibody is exposed mainly near sites of association with the nuclear membrane.

Tan and Lerner studied the fate of the material during the cell cycle in synchronized cells.[56] In the G_1 phase the exposed native DNA was located at the nuclear rim. In the S phase, staining was stronger at the periphery, but some occurred in the center of the nucleus as well. In the M phase, several clumps of stained material were seen in the cytoplasm adjacent to the plasma membrane. In contrast to these results with antinative DNA, antidenatured DNA sera detected the single-stranded material only during the S phase.

With greater resolution, including the use of electron microscopy,[57] the SLE sera can thus be useful reagents. The more widespread application is limited in part by the limited availability of suitable sera. Since most sera contain more than one antibody population, only occasional samples have the desired specificity directly. With others, suitable removal of unwanted antibodies by absorption may be successful, or the antinative DNA antibodies may be purified. With most sera, however, relatively small amounts of the antibody are present, and usually small samples of active serum are available since the antibodies occur mainly during acute episodes of illness and not during periods of remission.

Hoping to control both specificity and availability, workers in several laboratories have tried to induce antinative DNA antibodies in normal laboratory animals. With or without protein or particulate carriers, however, native DNA has not been an effective hapten for immunization.[5] The major occurrence of antinative DNA antibody other than in human SLE is in the NZB/NZW hybrid strain of mice.[58-61] These animals develop a disease very much like human SLE, with similar immunopathology. Their sera also have mixtures of antibody populations and have not, as yet, served as large supplies of monospecific reagents. Perhaps larger animals will respond to lipopolysaccharide or micellar immunogens as discussed above and provide the desired supplies of reagent.

III. ANTIBODIES TO RNA INDUCED BY RIBOSOMES

The antinative DNA antibodies of SLE sera were specific for the deoxyribose-phosphate backbone and did not react with RNA. Conversely, antibodies specific for the ribose-phosphate backbone or RNA have been obtained by immunization with either bacterial or mammalian ribosomes.[62,63]

When horses or rabbits were immunized with ribosomes, they produced antibodies to both protein and RNA components.[62,63] The antibodies to RNA included several populations that could be distinguished in cross-reactions with various synthetic polynucleotides.[64] One population was purified from specific precipitates formed with poly(A);[64] the washed precipitates were dissociated in 0.5 *M* $MgCl_2$ and 7S immunoglobulin was recovered by gel filtration. This purified antibody precipitated with all forms of RNA, including ribosomal, transfer, or viral RNA, and with all synthetic polyribonucleotides.[64,65] It precipitated with known single-stranded, double-stranded, and triple-stranded forms, such as poly(A), poly(A) · poly(U), and poly(A) • 2 poly(U).[65,66] Since base composition was not critical and since determinants were still available in helical forms, it was concluded that the ribose-phosphate backbone formed the antigenic site. This interpretation was supported by the finding that neither native nor denatured DNA was reactive. While the conclusion holds for some of the anti-RNA antibody induced by ribosomes, such as this purified fraction, the reactions of other populations in these sera were only partly absorbed by synthetic polyribonucleotides, so that other specificities also occur.[67]

The cross-reaction patterns of the purified antibody suggested that the backbone of only one chain was sufficient to form a determinant. This conclusion was supported by the finding that extended single-stranded forms of poly(A), poly(U), poly(I), or poly(G) in distilled water would precipitate with this antibody.[65] (A fraction of antibody that itself remained soluble on dialysis against distilled water was used.)

From the quantitative analysis of the precipitation it would appear that antibody molecules could be densely packed along the extended backbone. In antibody excess, the weight ratio of antibody to antigen in the precipitates formed with poly(C), poly(A), or poly(I) was about 40:1,[65] corresponding to one antibody molecule for every ten nucleotides. In the reaction with poly(U) the extreme ratio was 120:1, or one antibody molecule for every three or four nucleotides. Thus, unless some antibody was coprecipitated without directly binding to an antigenic site, an adequate determinant would have to be a very short segment of the backbone. The antigen-antibody precipitation was partly inhibited by tetraadenylate but not by mono-di- or trinucleotides.[68] With double-stranded helical polymers, the maximal antibody: antigen weight ratios in the precipitates were 10 to 15,[66] corresponding to one antibody molecule for each 12 to 20 base pairs.

Further quantitative analysis showed that secondary structure did have a modifying effect on the serological reactivity of the polynucleotides;[66,69] measurement of both antibody and antigen in the precipitate was used to study polynucleotide organization.[66,69] At very low ionic strength at neutral pH, other homopolyribonucleotides were similar to poly(U) in terms of the amount of antibody that was precipitated at equivalence and the maximal amount of antigen precipitated (either at equivalence or in antigen excess). As polynucleotides formed more ordered structures, under different conditions of ionic strength or pH or in polymer mixtures, there was a decrease in the total amount of antibody precipitated and, in some cases, an increase in the maximal amount of antigen precipitated.[66,69] Formation of a single-stranded helix (poly(A) at low pH) caused just a decrease in antibody precipitation.[66]

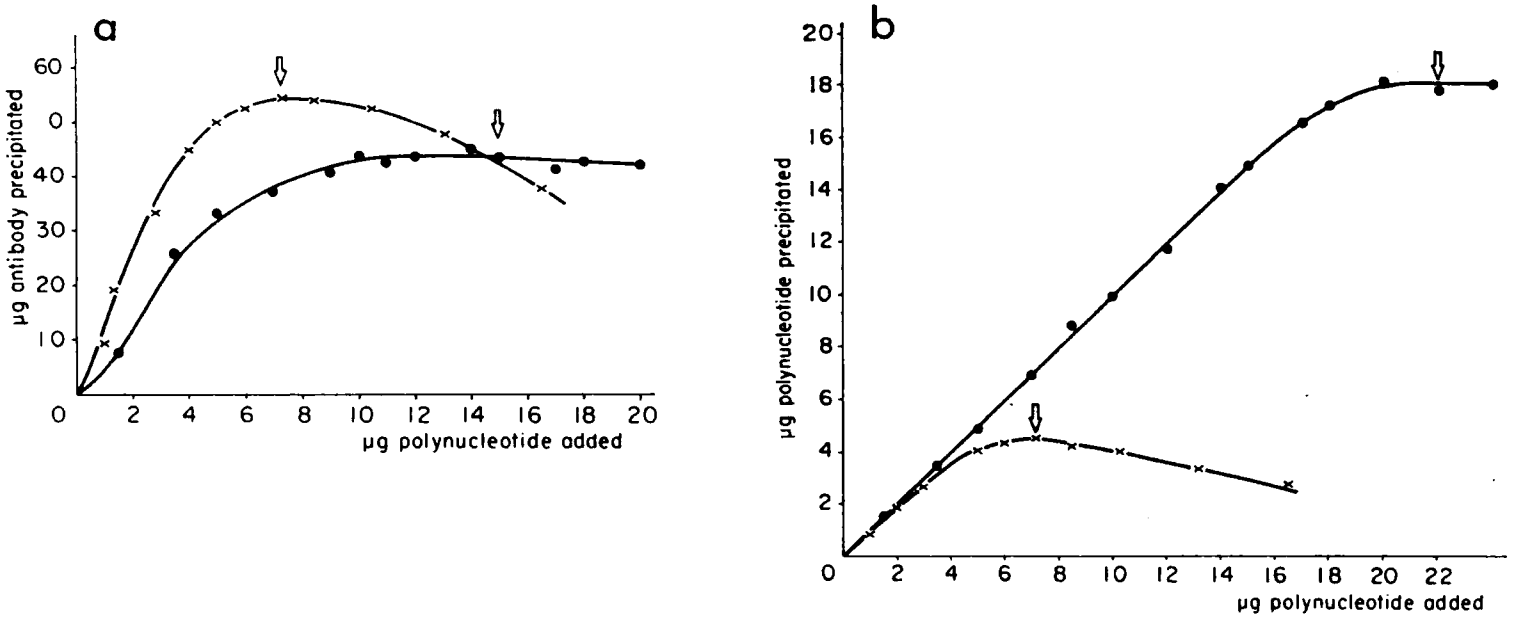

FIGURE 4. Reactions of purified anti-RNA antibodies from horse antiribosome sera. Measurements of antibody (a) and antigen (b) precipitated on mixing constant amounts of antibody with varying amounts of poly(U) (X) or poly(I) (•) in 5×10^{-4} *M* Mg^{++}-Tris (5 m*M*), pH 7.1. The arrows indicate the values chosen as representing the equivalence zone and used in the construction of Figure 5. The poly(I) precipitated less antibody than poly(U), but the equivalence complexes contained four times as much poly(I) as poly(U). (Reprinted with permission from Souleil, C. and Panijel, J., *Biochemistry*, 7, 7, 1968. Copyright by the American Chemical Society.)

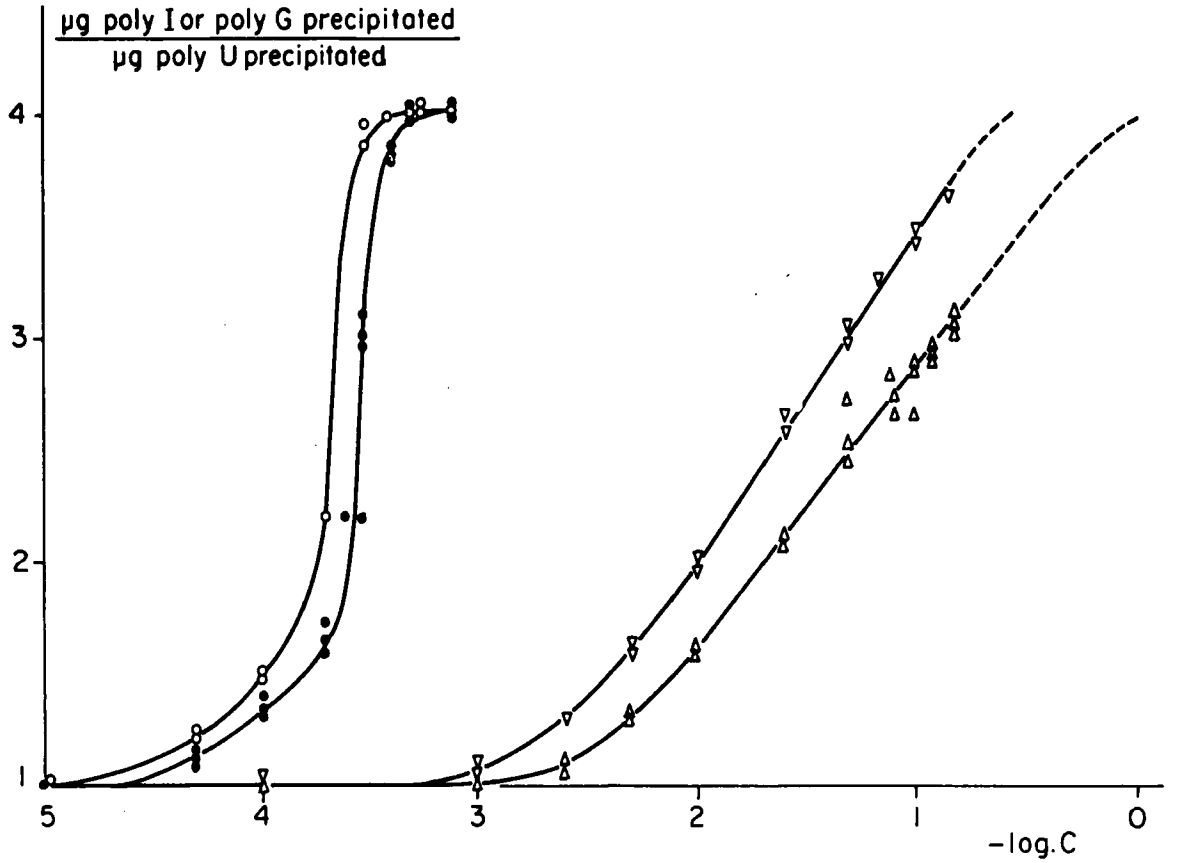

FIGURE 5. Ratios of the maximal amount of poly(I) or poly(G) precipitated to the maximal amount of poly(U) precipitated under the same conditions by purified anti-RNA antibodies from horse anti-ribosome sera. The experiments were carried out at 20° in Tris (5 m*M*, pH 7.1) in increasing concentrations of either $MgCl_2$ or NaCl + 0.5 m*M* EDTA. Poly(I) in $MgCl_2$ (•), poly(G) in $MgCl_2$ (○), poly(I) in NaCl (△), and poly(G) in NaCl (▽). (Reprinted with permission from Souleil, C. and Panijel, J., *Biochemistry*, 7, 7, 1967. Copyright by the American Chemical Society.)

Formation of double-stranded structure (poly(A) · poly(U)) caused a similar decrease in antibody precipitation, but in addition twice as much polynucleotide was precipitated by a given amount of antibody as compared with poly(U). For the triple-helical poly(A) · 2poly(U), three times as much antigen was precipitated. Thus, there was a correspondence between the number of strands in the polymer and the weight of polynucleotide precipitated by a given amount of antibody. This relationship was then applied to the study of poly(I) and poly(G) (Figures 4 and 5).[69] With increasing ionic strength or magnesium ion concentration, the polynucleotide precipitation increased to a value four times that obtained with poly(U) under the same conditions. It was concluded that poly(I) exists in a four-stranded structure in such solutions, a possibility that had been suggested by physical measurements.

These antibodies were also used to study the organization of RNA in ribosomes.[70] The RNA of *E. coli* ribosomes was accessible to the antibodies, while that of mammalian ribosomes was not. From antibody:antigen ratios in precipitates formed with *E. coli* ribosomal subunits, it was concluded that most of the RNA is accessible in the 30S subunit but only part of it is so in the 50S subunit. In this situation, helical or nonhelical structures were not distinguished, but the results suggest that the ribose-phosphate backbone is the portion that is accessible in the ribosome, while the bases may be masked within secondary structure.

IV. ANTIBODIES TO DOUBLE-HELICAL POLYRIBONUCLEOTIDES

The role of helical conformation in several

nucleic acid immune systems was a negative one; that is, it masked the antigenic sites of base-dependent sequential determinants.[5] In other cases, described above, it had a modifying effect on reactions of sequential backbone determinants. Its positive role in the formation of reactive sites has become apparent through immunization of animals with synthetic double- and triple-stranded helical copolymers or with viral double-stranded RNA. While the copolymer poly(I) · poly(C) has been found to be one of the few forms of nucleic acid that can be immunogenic without a protein carrier,[71] nearly all working sera have been obtained by immunization with methylated BSA complexes of the copolymers,[72] as the amount of antibody produced is much greater than with the polynucleotide alone.[71]

A. Antibodies to Poly(A) · Poly(U) and Poly(I) · Poly(C)

Nahon et al. were the first to use methylated BSA complexes of double-stranded and triple-stranded polynucleotides as immunogens.[73] They obtained antibodies that were distinct from those induced by complexes of single-stranded homopolymers. Several other laboratories have followed this lead and have described antibodies with varying patterns of specificity. In all cases, there has been extensive reciprocal cross-reactivity between poly(A) · poly(U) and poly(I) · poly(C) and double-stranded viral RNA, while antibodies to either of the above synthetic polynucleotides showed little cross-reactivity with poly(G) · poly(C).[73,75,78] Anti-poly(I) · poly(C) antisera may also contain antibodies that precipitate poly(I).[73,79]

Differences in results arose in assays of cross-reaction with other forms of naturally occurring RNA. Measuring direct precipitation and absorption, Nahon-Merlin et al. found that anti-poly(I) · poly(C)[79] and anti-poly(A) · poly(U)[73] sera reacted with mammalian, bacterial, and yeast ribosomal or transfer RNA and with single-chain or double-stranded viral RNA. The total RNA of mammalian cells gave almost as much precipitation of anti-poly(I) · poly(C) antibody as did the homologous antigen. The ability of the sera to react with whole cell RNA was eliminated by prior absorption with poly(I) · poly(C) or reovirus RNA, so that double-helical regions in the RNA single chain were considered to be determinant structures.[79]

Animals immunized in other laboratories have yielded sera with an apparently narrower cross-reactivity, at least in several assay systems. In these cases, anti-poly(A) · poly(U) or anti-poly(I) · poly(C) antibodies still react with either of these synthetic helical forms, with double-stranded RNAs of reovirus, rice dwarf virus, mycophage, other plant viruses, and arbovirus replicative forms. They do not, however, react with ribosomal or transfer RNA of animals, plants, or bacteria or with single-stranded viral RNA, even though these RNAs have secondary structure that includes some base-paired regions.[79a,b] This suggests that the shapes of such regions in these RNAs are different from the shape of a more extended helix such as a viral replicative form. Alternatively, tertiary structure may mask the base-paired regions; in transfer RNA, the loops may fold into a more compact structure and overlie the base-paired stems.[79a] In fact, a large excess of ribosomal or transfer RNA did not inhibit the reaction of purely double-stranded forms.[80] The requirement for the double-helical structure was also evident from the fact that the active antigens lost reactivity upon thermal denaturation;[73,75] the serologically measured denaturation profiles gave Tm values that were appropriate for the given helices tested (Figure 6).[29,75] The sera may show some cross-reactivity with an RNA-DNA hybrid such as poly(A) · poly(dT),[29] a reaction that has been more prominent with anti-poly(I) · poly(C) sera than with anti-poly(A) · poly(U) sera, but variable from serum to serum.

An additional feature may complicate the interpretation of some data on cross-reactivity. Mixtures of poly(A) and poly(U) may contain single-stranded, double-stranded, and triple-stranded polymers.[81] Corresponding sera can be similarly complex, and it may be necessary to absorb or purify separate populations of antibodies to clarify whether true cross-reactions occur. The sera may include, for example, an anti-poly(A) population that cross-reacts with denatured DNA; absorption with poly(A) removes reactivity with both the poly(A) and the denatured DNA, and antibody purified from precipitates with poly(A) will react with the denatured DNA, but not with double-stranded poly(A) · poly(U).[5] A separate population of antibodies specific for double-stranded structure could also be purified.[82] For this, a precipitate was formed with anti-poly(A) · poly(U) serum and poly(I) ·

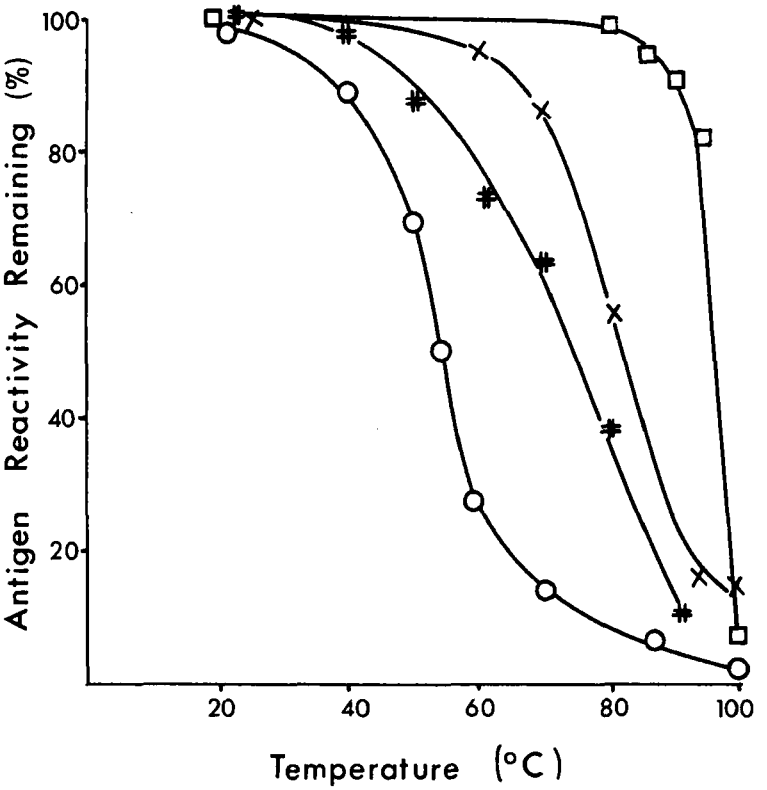

FIGURE 6. Immunochemical measurement of the thermal denaturation of helical copolymers with corresponding antisera. Poly(A) · poly(U) (○) and reovirus RNA (□) were measured with anti-poly(A) · poly(U) antisera; poly(A) · poly(dT) (#) and poly(dG) · poly(dC) (X) with corresponding antisera. The poly(A) · poly(U) and poly(A) · poly(dT) were heated in a buffer of 0.14 *M* NaCl, 10 m*M* Tris, pH 7.4, with 3% formaldehyde; the reovirus RNA and poly(dG) · poly(dC) were heated in 0.014 *M* NaCl, 1 m*M* Tris, pH 7.4. (From Stollar, B. D., *Science*, 169, 609–611, 7 August 1970. Copyright 1970 by the American Association for the Advancement of Science. With permission.)

poly(C) as antigen; the latter contains only double-stranded material that would react with the antiserum (no triple-helix is formed,[81] and the homopolymer components would not react with antibodies to poly(A)).[83] The precipitate was washed and then suspended in distilled water at about 50°C. Under these conditions the polynucleotide strands dissociated and were no longer bound by antibody. The free polynucleotides were digested with pancreatic ribonuclease and the released antibody was dissolved in saline and separated from the ribonuclease and oligonucleotides by gel filtration. The purified antibody reacted with poly(A) · poly(U), poly(I) · poly(C), or double-stranded RNA[82] and still showed a cross-reaction with RNA-DNA hybrid poly(A) · poly(dT). It also detected some double-helical regions in a 1:2 mixture of poly(A) and poly(U) but did not react with single-stranded poly(A) or with native or denatured DNA preparations.

Clearly the antibodies to these helical forms did not react with a specific base or base sequence, and the determinant probably involves the backbone structure on the outside of the helix. It is likely that both strands are involved in forming a determinant since single-stranded forms did not inhibit the reaction, and addition of a third strand usually masked the determinant.[5,82] Further, the two strands have to be in proper orientation with respect to each other since poly(G) · poly(C) also contains two polyribonucleotide backbones but was sharply distinguished from poly(A) · poly(U) and poly(I) · poly(C).[73,75] This conclusion is supported by recent findings that chemical modifications of the ribose of one chain (such as

2′-O-methylation) drastically reduced reactivity with anti-poly(A) · poly(U) antibody.[84]

While poly(I) · poly(C) and poly(A) · poly(U) showed extensive mutual cross-reactivity, it was not complete; there was usually greater reactivity with the homologous form[23,29,79] so that fine structural differences between the two were detectable. Similarly, antibodies induced by poly(A) · poly(U) or poly(I) · poly(C) were generally less reactive with double-stranded viral RNA than with homologous antigen,[29] and antibodies induced by viral double-stranded RNA were more reactive with the viral RNA than with the synthetic copolymers.[77] This lack of total reciprocal cross-reactivity may indicate the presence of a variety of regional structural features in double-stranded RNA and the potential for development of a range of specific antibody reagents to explore these structures. Still, in the applications of these antibodies to biological problems, the emphasis so far has been to take advantage of their ability to react with a wide range of double-stranded RNA forms and to distinguish these, as a class, from other types of nucleic acids. Little attention has been paid to the exploration of subpopulation specificities other than that for poly(G) · poly(C) (Section IV.B).

Antibodies have been used to study replicative forms of viral RNA.[80,82,87] They detected nanogram quantities of the double-stranded replicative forms of Sindbis and dengue viral RNA even in the presence of a several thousand-fold excess of host ribosomal and transfer RNA, which did not interfere.[80] Very high concentrations of RNA from normal uninfected host cells did show some reactivity; it could be accounted for if 0.02 to 0.1% of the cellular RNA were double stranded. Such quantities have been detected by other means, and at least a portion of this has been identified as heterogeneous nuclear RNA.[88] The infected cells contained up to 50-fold more double-stranded RNA than the uninfected cells. Immunochemical assays of sucrose density gradient ultracentrifugation fractions detected the normal cell double-stranded RNA in the 8S to 11S region and the viral replicative forms in the 12S and 20S regions (Figure 7). The immunochemical assay provided one of the first measures of a 12S form for this virus RNA replicative form and aided in the further characterization of its parent material as a component of defective virus particles.[89]

The sensitivity of the immunochemical assay for double-stranded replicative forms of RNA was of particular importance in a study of dengue virus mutants. It allowed determination of the phenotypes of two classes of temperature sensitive mutants: One type could form double-stranded RNA and the other could not.[90] The overall yields of mutant viruses were low, and techniques depending on the incorporation of labeled uridine in the presence of actinomycin D had not been able to clearly define these phenotypes (Lubiniecki, A. S., personal communication).

With immunofluorescent techniques, the antisera or purified antibodies have also detected the intracellular location of double-stranded RNA in virus-infected cells, without significant staining of host cell RNA. Silverstein and Schur identified the mature double-stranded form of reovirus in infected cells.[91] In another instance, the question of whether the double-stranded replicative form of Sindbis virus RNA exists in vivo was approached.[82] Virus-infected cells were fixed in acetone at −20°C and dried and stained with purified anti-double-stranded RNA antibody and fluorescent anti-rabbit globulin (Figure 8). In cells sampled several hours after infection, antigen was detectable in granules or clumps in the cytoplasm, often near cell membranes. As cold acetone fixation and drying should not cause deproteinization, this suggests that the double-stranded form does exist in the cell as well as in extracted RNA. It remains possible that the plus and minus strands are in very close proximity but not paired, perhaps associated with a protein, and that the acetone denatured the protein and allowed the strands to anneal.

A further biological question that has been studied with these antibodies concerns the mechanism of induction of interferon production by double-stranded polyribonucleotides. Incubation of poly(I) · poly(C) with antibody to double-stranded RNA inhibited the ability of the copolymer to induce interferon formation.[92,93] Further, even after the poly(I)· poly(C) had been absorbed to the cell surface in the cold, and excess polymer washed off, the antibody could inhibit the interferon production if it was added before the cells were warmed.[93] It was then possible to determine how long the poly(I) · poly(C) had to remain associated with the membrane at 37° in order that the steps that initiate interferon production could be completed beyond the point where the process could be inhibited by the antibodies. Of related interest is the finding that when animals

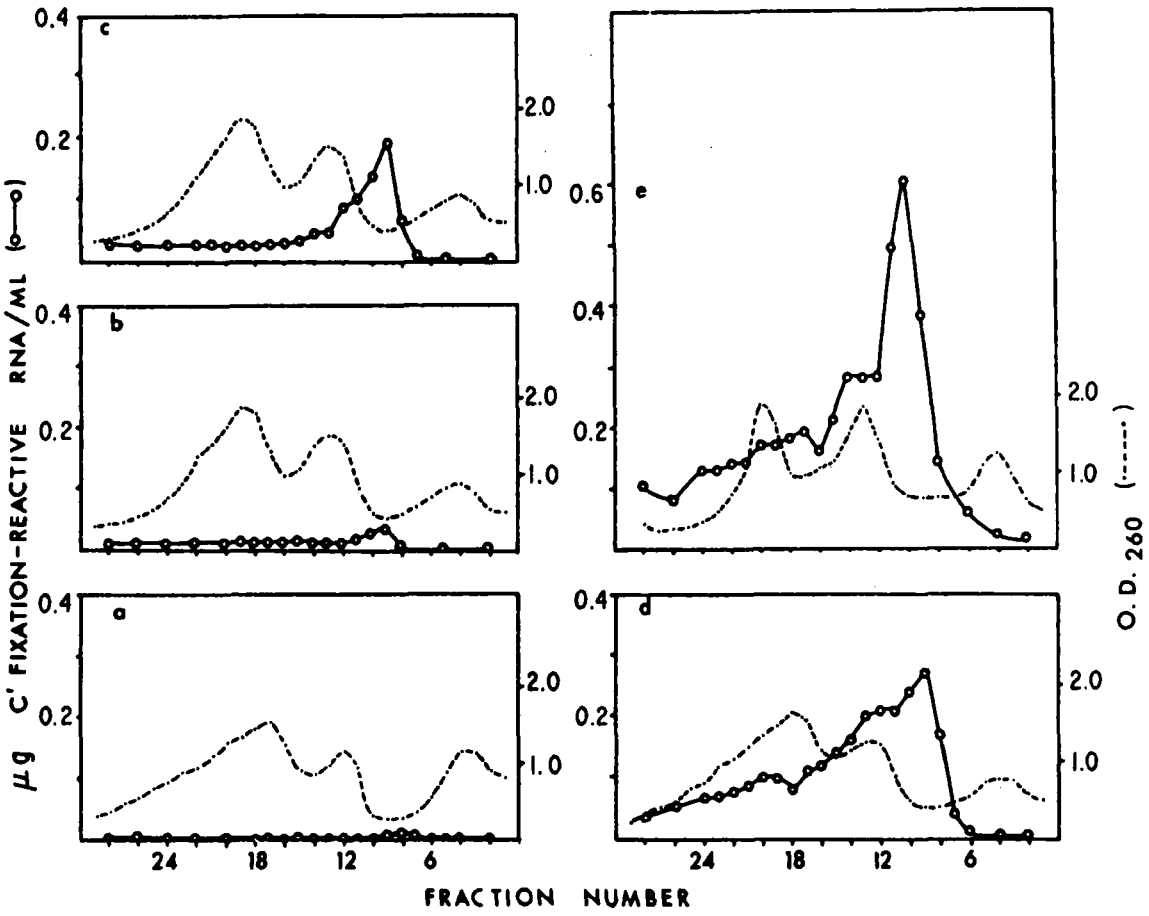

FIGURE 7. Immunochemical sucrose gradient analysis of RNA from uninfected BHK-21 cells and from Sindbis virus-infected cells harvested at different times after infection.[80] RNA was extracted from uninfected cells (a) and from cells infected for 4 hr (b), 8 hr (c), 13 hr (d), and 24 hr (e), applied to linear sucrose gradient (5 to 20% w/w) and centrifuged at 22,500 r/min for 16 hr. Fractions of 1 ml were assayed for A_{260} and for double-stranded RNA reactive with antibody to poly(A) poly(U) (o). The data were normalized so as to represent the application of 1 mg of RNA to each gradient.

had been preimmunized with poly(I) · poly(C), they did not form interferon in response to a further intravenous injection of the copolymer, while unimmunized animals did so.[92]

Another type of application of the antibodies has been to search for the presence of double-stranded RNA in biological samples. Ikegami and Francki used immunodiffusion with two types of serum to demonstrate the double-stranded RNA in preparations of rice dwarf virus (RDV) and related viruses. Sera induced by RDV contained antibodies that precipitated with poly(I) · poly(C) and gave identical reactions with poly(I) · poly(C), the sap of Fiji disease virus (FDV)-infected leaves, and free FDV nucleic acid (Figure 9).[94] Thus, the preparation used for immunization must have contained double-stranded RNA that was free or became available after injection. More direct was the use of antibodies induced by poly(I) · poly(C) itself to detect double-stranded RNA forms, including the replicative form of tobacco mosaic virus and a viral RNA form in the nucleic acid extracted from FDV-infected sugar cane leaves.[77] The same antibodies were used elsewhere to screen extracts of cultures of several species of *Penicillium* for the presence of mycophage double-stranded RNA.[95] While two-dimensional immunodiffusion was sensitive enough for detection of the double-stranded RNA in unconcentrated cell sap in some cases, lower concentrations of the antigen were missed in other cases unless the extracts were concentrated. In quantitative assays, it was possible to rule out the presence, or at least place a limit on the maximum amount, of double-stranded

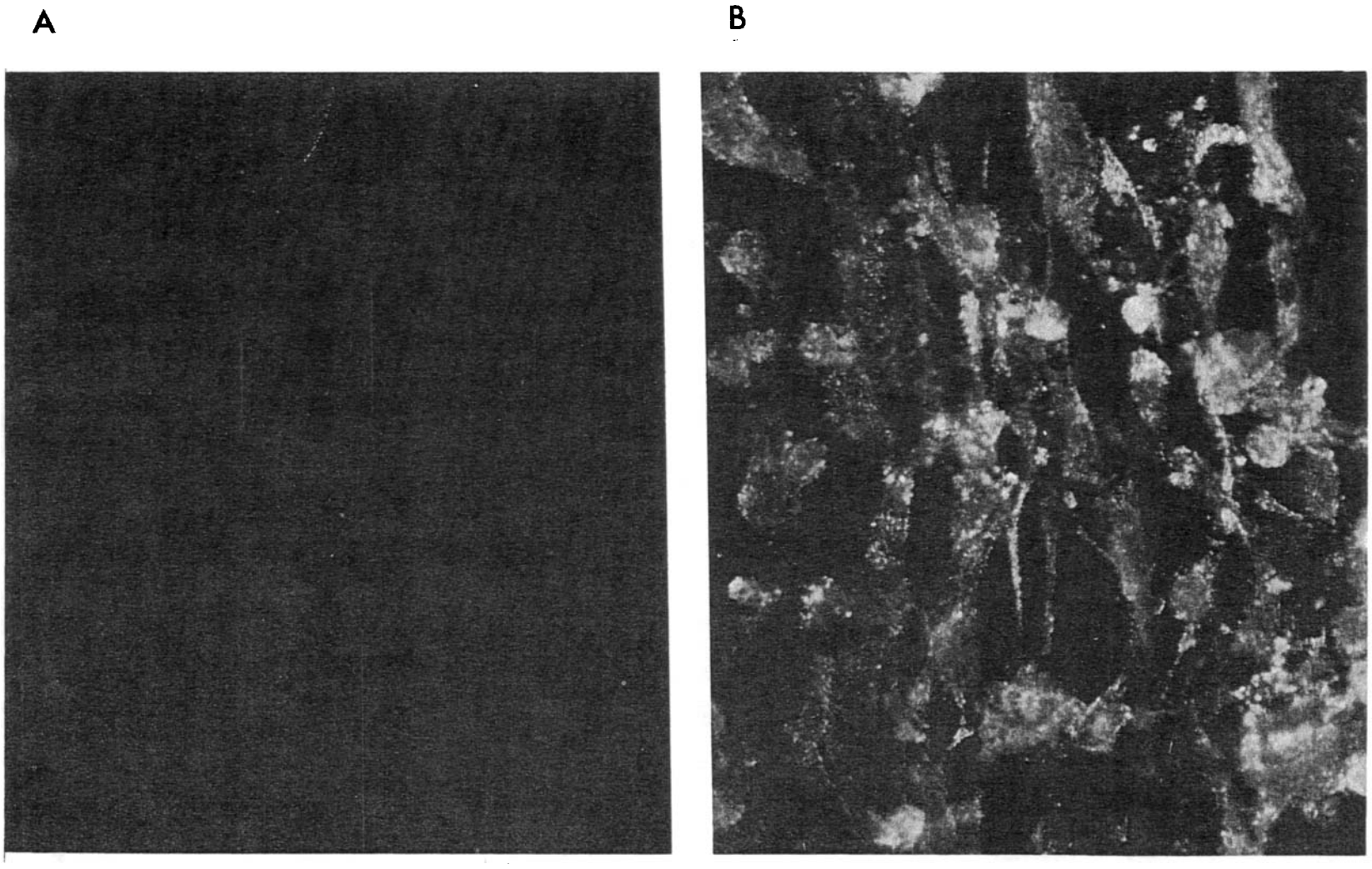

FIGURE 8. Fluorescent antibody staining of Sindbis virus-infected BHK21 cells, with purified antibody to double-stranded RNA followed by fluorescein-labeled goat anti-rabbit globulin. (A) 1.5 hr after infection; (B) 24 hr after infection. (From Stollar, B. D. and Stollar, V., *Virology*, 42, 276, 1970. With permission.)

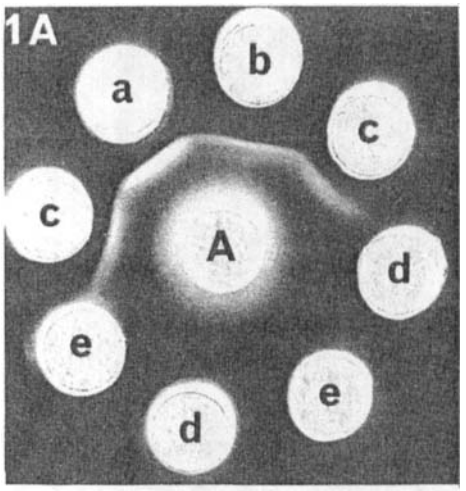

FIGURE 9. Two-dimensional immunodiffusion of antiserum to rice dwarf virus. Antivirus antiserum was in the center well, and antigens in the peripheral wells were (a) Fiji disease virus-infected cell sap, (b) Fiji disease virus nucleic acid, (c) poly(I) poly(C), (d) uninfected cell sap, and (e) uninfected host cell nucleic acid. (From Ikegami, M. and Francki, R. I. B., *Virology*, 56, 404, 1973. With permission.)

RNA in a preparation of infectious potato spindle tuber viroid.[96]

Antibodies to poly(I) · poly(C) were also used as a convenient way to follow the purification of a virus that contained double-stranded RNA, when little material and no antiserum specific for viral protein was available.[86] Samples were extracted with phenol and tested in immunodiffusion with anti-poly(I) · poly(C) serum, and the amount of virus was considered to be parallel to the amount of double-stranded RNA present. Once the virus was purified, it was used as an immunogen and induced antibodies to both the protein and to the double-stranded RNA components. Antiviral nucleic acid serum gave stronger precipitation with the viral nucleic acid than with poly(I) · poly (C), as discussed above, and absorption of the serum with poly(I) · poly(C) still left some specific anti-FDV RNA antibodies free in the serum.

It should be noted that this discussion has emphasized the portion of the antibody population that reacts selectively with one conformational type of double-helical polyribonucleotide. The total antibody response may include a richer variety of specificities within one serum,[6] and the specificity pattern may vary from animal to animal[97] or species to species.[98]

Antibodies that react with double-stranded RNA also occur in sera of patients with SLE[23,78,99] and some other diseases[23,100] and in the NZB/NZW and other mice.[61,101] The immunogenic stimulus is not known; while it may be a viral replicative form, it could also be endogenous double-stranded RNA. Studies of cross-reactions again showed extensive similarities among poly(A) · poly(U), poly(I) · poly(C), and viral double-stranded RNA.[23,78,85] The latter tended to be more effective than the synthetic copolymers;[85,101] this may reflect the presence of additional conformations that could result from the presence of all four bases in any natural double-stranded RNA rather than unique specificity for a viral form as such. The SLE anti-double-stranded RNA antibodies are distinct from those directed against DNA, as measured by their independent occurrence[78] and by differing patterns of inhibition.[30,101] With several sera, additional reactions also occurred with poly(A), poly(I), poly(G), or poly(C),[23,78,85,101] but it was not clear whether these were cross-reactions or were due to distinct antibody populations.

B. Antibodies to Poly(G) · Poly(C)

Poly(G) · poly(C) presents a different helical shape and reacts poorly with anti-poly(A) · poly(U) or anti-poly(I) · poly(C) sera.[75,75] The reactions may be too weak to appear in immunodiffusion assays but may be found in quantitative precipitation assays.[6] We have recently surveyed nine anti-poly(A) · poly(U) antisera and found that seven did give a weak precipitation in gels with poly(G) · poly(C). The immunodiffusion patterns suggested that these reactions involved a group of antibodies distinct from those that reacted with poly(I) · poly(C). This was supported by the finding that antibodies purified from the poly(I) · poly(C) precipitation gave no reaction with the poly(G) · poly(C).

Lacour and co-workers have studied antibodies induced by methylated BSA complexes of poly(G) and poly(G) · poly(C).[102,103] While the former reacted only with poly(G) and not with the duplex form, the anti-poly(G) · poly(C) sera reacted with a variety of double-helical polyribonucleotides, with poly(dG) · poly(dC), and, in some cases, with poly(G) or poly(C) alone.[102] They also reacted with animal cell ribosomal RNA and with reovirus and Qβ RNA but not with any bacterial RNA, mammalian tRNA, or three different single-stranded viral RNAs.[103] The reactions of animal

cell RNA and the Qβ were found to depend on secondary structure, as reactivity was destroyed by heating the RNA in 1% formaldehyde. The spectrum of reactivity was different from that of anti-poly(I) · poly(C) or anti-poly(A) · poly(U) antisera but was still rather broad.

V. ANTIBODIES TO RNA-DNA HYBRIDS

One of the motivations for studying antibodies to synthetic helical polymers was the hope of obtaining antibodies to native DNA, to replace the dependence on SLE sera. Antibodies to double-stranded RNA did not provide the desired anti-native DNA reactivity. Since they did, however, show some recognition of RNA-DNA hybrids, it was felt that perhaps antibodies induced by the hybrids could carry the recognition the rest of the way to double-stranded DNA. Instead, poly(A) · poly(dT) induced antibodies that were completely specific for hybrid structure and recognized neither double-stranded RNA nor double-stranded DNA.[29] They did react with hybrids of varying base composition, including poly(I) · poly(dC), or hybrids of natural RNA and DNA.[104] The specificity of these sera provided a clear example of the involvement of both strands of a double-helix in an antigenic determinant; since neither poly(dA) · poly(dT) nor poly(A) · poly(U) reacted with anti-poly(A) · poly(dT) antisera, it appears that the presence of just one of the component chains in a helix could not form a determinant.

The specificities of the antihybrid and anti-double-stranded RNA antibodies were distinct enough to allow their use for the quantitation of each form in a mixture containing both.[104] When denatured DNA was used as a template for *E. coli* RNA polymerase, a major product was an RNA-DNA hybrid. Since the product of the reaction could induce interferon production by mammalian cells, it was questioned whether there may also be some double-stranded RNA in the mixture. Corresponding antisera measured both helical forms in the mixture and were also used to assay fractions from an equilibrium density gradient ultracentrifugation experiment in which the two forms were separated.[104] Quantitation of the separated helical structures was similar to that obtained with the mixture; thus, there had not been any major interference in one antigen-antibody reaction by the presence of the second antigen.

Perhaps because there are several sensitive assays for RNA-DNA hybridization, the antihybrid antibodies have not found wide application. It is likely that they could prove uniquely useful in some experiments, including assays of materials that do not bear radioactive labels, or for *in situ* hybridization studies in tissue sections.

VI. ANTIBODIES TO TRIPLE-HELICAL POLYNUCLEOTIDES

The formation of triple-helical structures presents new antigenic conformations, as first noted by Lacour et al. in comparisons of antibodies to poly(A) · poly(U) and to triple-helical poly(A) · 2poly(U).[105] In these and later experiments,[5,105] antibodies to poly(A) · 2poly(U) reacted poorly with poly(A) · poly(U) and not at all with poly(I) · poly(C) or double-stranded reovirus RNA. In fact, the residual reactivity that did occur with a poly(A) · poly(U) preparation could have been due to the presence of a small amount (5 to 10%) of triple-helix in the 1:1 mixture of the two homopolymers, as suggested from assays of mixtures of the homopolymers in continuously varying proportions.[5,106]

In reciprocal experiments, purified antibodies from different antisera to the double-stranded forms (purified from precipitates made with poly(I) · poly(C)) showed one of two patterns of reactions with the triple-helix. In most cases, much larger amounts of poly(A) · 2poly(U) than of double-stranded copolymer were required for a given level of complement fixation; for this pattern the reaction may have been due to a small amount of double-stranded structure in the 1:2 mixture of homopolymers, with addition of the third strand masking 90% or more of the determinants for this anti-double-helix antibody.[5,82] A different purified antibody, however, reacted with low concentrations of both the double- and the triple-stranded forms;[82] this antibody may have been directed against a region of double-helix that does not become distorted or masked as the third strand is added.

Comparisons of other triple-stranded structures have revealed a number of antigenic distinctions.[106] Antibodies to poly(U) · poly(A) · poly(U) were fully reactive with poly(U) · poly(A) · poly(dT), but completely unreactive with poly(U) · poly(dA) · poly(U) or poly(U) · poly(dA) · poly(dT). In turn, antibodies to either of these triple-helical forms containing poly(dA)

were completely unreactive with the forms containing poly(A) but recognized the two structures built on poly(dA) as being serologically identical. Thus, the nature of the pentose associated with the purine determined the specificity, regardless of which two polypyrimidine polynucleotides were present. A triple-helix containing only purines, poly(I) · poly(A) · poly(I), was not recognized by the anti-poly(U) · poly(A) · poly(U) serum.

These patterns again indicate that the overall conformation is critical and that all three strands may contribute to the formation of a determinant. This was supported by other relationships as well, as may be seen with the anti-poly(U) · poly(dA) · poly(dT) serum. It did not react at all with double-stranded poly(dA) · poly(dT), consisting of two of the homologous three strands, nor with poly(U) · poly(A) · poly(dT), in which another combination of two of the homologous three strands was also present. Thus, only the appropriate combination of all three strands was recognized, and the determinant must involve a specific steric interrelationship of structural features along the three backbones.

Antibodies specific for a triple-helix can be used, with the method of continuously varying proportions, to determine whether the triple-stranded form is the sole product of a mixture of two homopolymers.[106] Antibodies to poly(U) · poly(A) · poly(U) readily demonstrated that little triple-helix appeared until after double-helical structure was formed in a mixture of the homopolymers poly(A) and poly(U) (Figure 10). In contrast, mixtures of poly(dA) and poly(U) in any proportion contained an amount of triple-helix that would be expected if it were the only product and no double-stranded structure were formed (Figure 10). This finding agreed with results of physical chemical measurements on mixtures of these two polymers as well.[107]

Only brief attempts have been made to use the anti-triple-helix antibodies to probe for the presence of such structures in naturally occurring nucleic acids, as in replicative intermediates, for example. To date the findings have been negative.

VII. STRUCTURAL BASIS FOR SPECIFICITY AND RECOGNITION

Known differences among the polynucleotide structures provide some suggestions as to the nature of the critical determinant regions for specificity. The most direct and complete descriptions of these structures have come from X-ray diffraction studies. Several features differentiate the predominant B-DNA from the A-RNA and A′-RNA helix forms.[108] In the RNA helix, the 2′-hydroxyl group is at the outside of the helix and its presence or absence could provide part of a recognition site directly. Another important aspect of the furanose is its nonplanar shape. In B-DNA it is in a C3-exo configuration,[109] while in double-stranded RNA and RNA-DNA hybrids it is in a C3-endo form (Figure 11).[108,110] In addition, the furanoses lie in different planes in the DNA and RNA helices and form different angles with the planes of the base pairs. These aspects could provide a large enough steric difference to be recognized by antibodies if the pentose is a major part of the determinant. These may be important factors especially in the mutual lack of cross-reactivity of the general anti-RNA antibodies induced by ribosomes and the SLE anti-DNA antibodies. In each case, a single polynucleotide chain appears to provide the determinant.

On the other hand, the differentiation between poly(I) · poly(C) and poly(G) · poly(C) and the sharp differentiation between double-stranded RNA, triple-stranded RNA, and RNA-DNA hybrids mean that determinants can involve more than the hydroxyl groups or the pentose configurations on one pentose-phosphate chain. For the anti-poly(A) · poly(U) or antihybrid sera, and for a few of the SLE anti-DNA sera, the double-stranded structure was required even for inhibition of reactivity, and with the anti-triple-helical poly(U) · poly(dA) · poly(dT) even the double-stranded poly(dA) · poly(dT) portion could not inhibit the reaction of the homologous form.

The helical shapes differ in respects other than the nature of the pentose itself.[108] In DNA, the axis of the base pairs passes through the center of the fiber axis, while in double-stranded RNA it lies toward the periphery. The base pairs are perpendicular to the DNA fiber axis but lie at an acute angle to the RNA fiber axis. The DNA helix has 10 base pairs per full helical turn, while the RNA helix may be 11-fold or 12-fold.[110] These features influence the orientation of the pentose-phosphate backbones of two adjacent chains relative to each other and the size and shape of the major and minor grooves. Thus, if an antigenic determinant involved portions of both strands of double-helical RNA (or all three strands of a

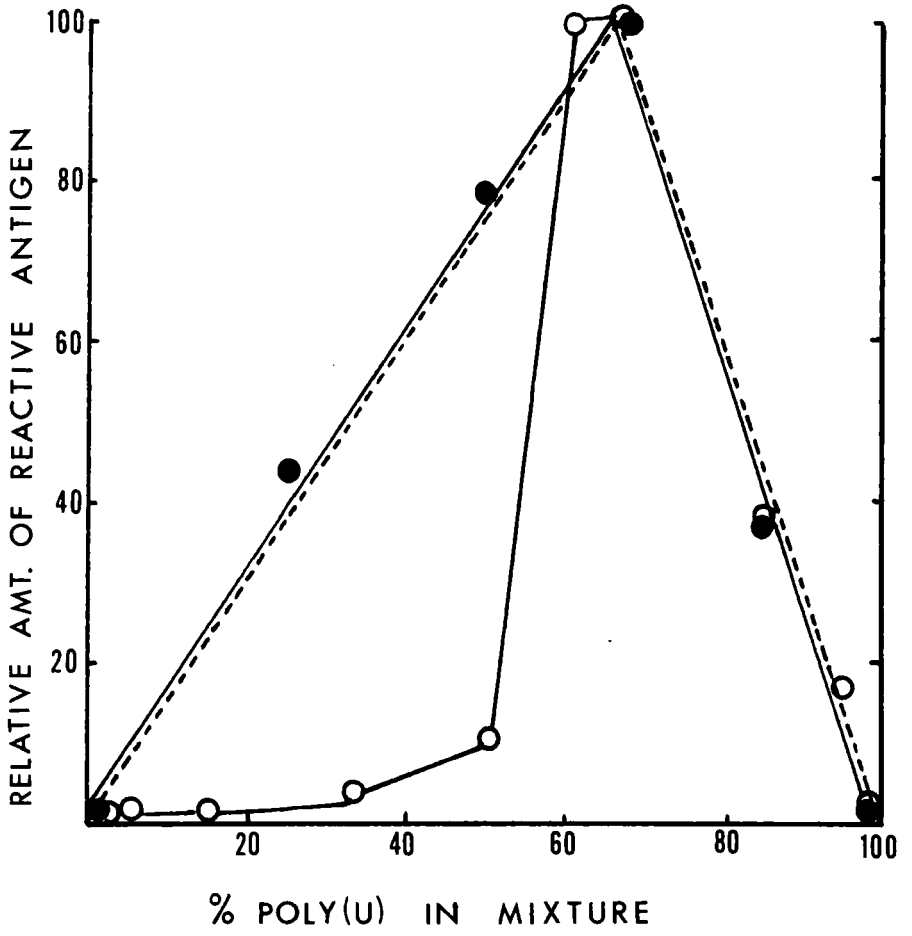

FIGURE 10. Measurement of the formation of triple-stranded polymers in mixtures of poly(U) and poly(A), assayed with anti-poly(A) · 2poly(U) antiserum (○), and in mixtures of poly(dA) and poly(U) with anti-poly(dA) · 2poly(U) antiserum (●). Homopolymers were mixed in varying proportions at concentrations of $1 \times 10^{-5} M$ nucleotide in 0.14 *M* NaCl, 1 m*M* Tris, pH 7.4, with 5 m*M* $MgSO_4$ and 1.5 m*M* $CaCl_2$. Each mixture was diluted and assayed in quantitative complement fixation reactions. Relative amounts of reactive antigen were calculated from the total amount of polynucleotide required to reach a given point on the complement fixation curve. The broken line represents the maximum theoretical value expected if only triple-helical copolymer were formed in all mixtures. (From Stollar, B. D. and Raso, V., *Nature*, 250, 231, 1974. With permission.)

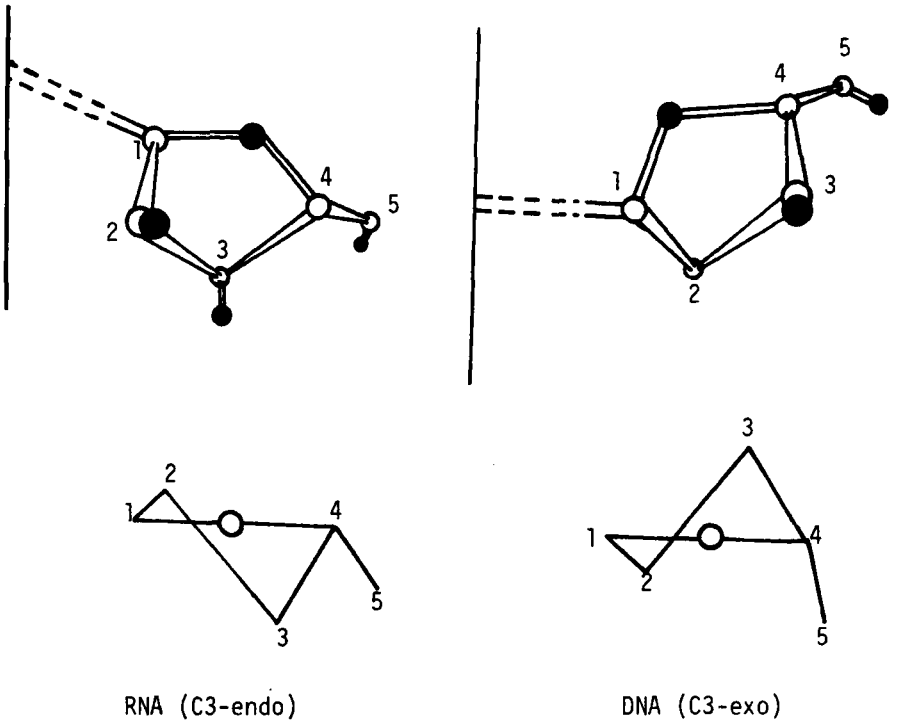

FIGURE 11. Aspects of the furanose conformations and the relation of the plane of the furanose to the plane of the bases in double-stranded A-RNA and double-stranded B-DNA. (Drawn after Arnott, S.[108] and Arnott, S. and Hukins, D. W. L.[109] With permission.)

triple-helix), changes in the orientations of the strands would clearly be able to affect serological reactivity.

In picturing the ways in which antibodies may recognize these features, it is important to consider the potential dimensions of one combining site. Several sequential antigenic determinants were estimated to be about 25 X 17 X 6.5 Å, with a maximum of 34 X 12 X 7 Å.[8] These dimensions are similar to or slightly larger than those of the binding sites of myeloma proteins that bind menadione[111] or phosphorylcholine.[112] The Fab portion of a myeloma protein had a binding surface of about 25 X 20Å,[111] which could also encompass two or three polynucleotide chains (Figure 12) if the antinucleic acid antibody combining sites formed grooves on such a surface.

The particular shape of the furanose or the influence of its shape on the overall helical configuration plays a major role in the immune recognition of the nucleic acids as well as in serological specificity. This involves a remarkably sharp recognition of helical shape since methylated BSA complexes of a number of helices were potent immunogens, while complexes of native DNA, poly(dA) · poly(dT), or poly(dAT) · poly(dAT) induced no antinucleic acid antibody at all. These nonimmunogenic forms all have the C3-exo furanose configuration, while the immunogenic forms, including the triple-stranded polymer built on poly(dA), have the C3-endo configuration.[113] A further striking example is that of poly(dG) · poly(dC). This copolymer retains a large amount of A-DNA helical form, with the C3-endo furanose structure,[114] and is immunogenic in MBSA complexes.[29] Like poly(dAT) · poly(dAT), it is an eightfold helix, but the poly(dAT) · poly(dAT) has the furanose configuration of native B-DNA and is not immunogenic. When a helical polymer *is* immunogenic, only the features that differ from native DNA are recognized, as none of the resulting sera will cross-react with native DNA.

VIII. CONCLUSION

Varying antibodies with several types of

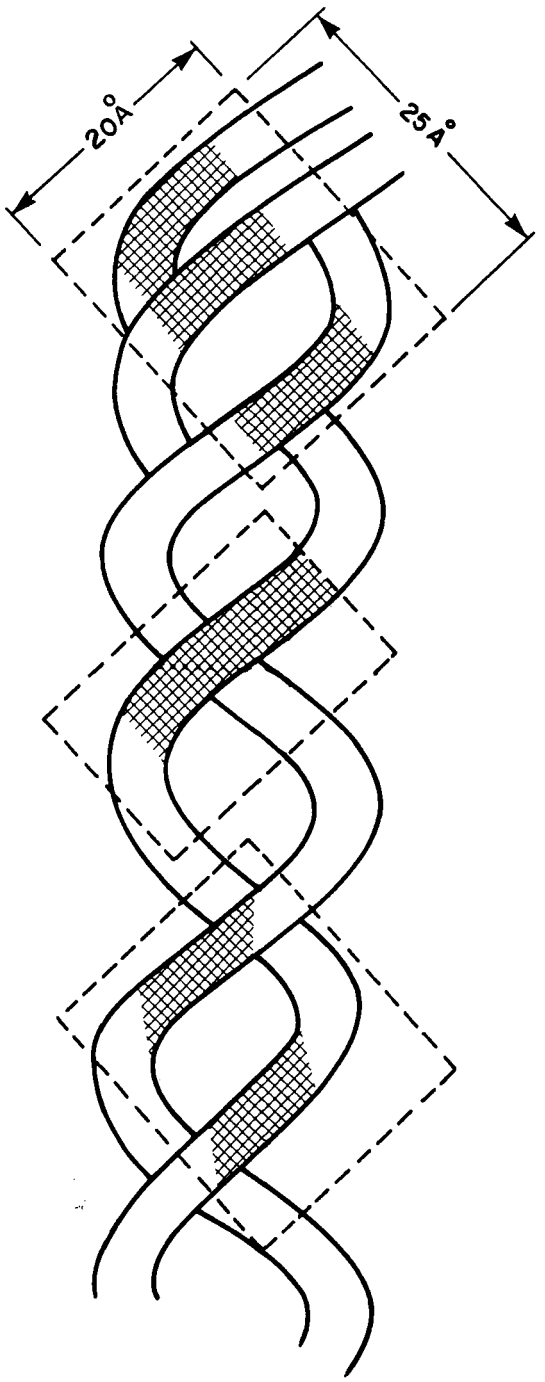

FIGURE 12. Possible specificity determinants encompassed in an area of 25 × 20 Å, encompassing either a single polynucleotide chain or the backbones of two or three adjacent chains.

specificity patterns have been described. Some antibodies can react with both helical and random coil nucleic acids, and others react with only the helical forms. The latter antibodies can differentiate between double- or triple-stranded polynucleotides with minor variations in helical shape. Their analysis has not reached a stage at which numerous antiserum fractions are available as specific reagents for unique localized structures in a given nucleic acid. Still, distinctions between helical classes can be remarkably sharp, both in the immune recognition process and in serological reactions, and these distinctions do allow important applications of the antisera as class reagents.

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REFERENCES

1. Plescia, O. J. and Braun, W., Nucleic acids as antigens, *Adv. Immunol.*, 6, 231, 1967.
2. Levine, L. and Van Vunakis, H., Serological activities of nucleic acids, in *Antibodies to Biologically Active Molecules*, Cinader, B., Ed., Pergamon Press, Oxford, 1967, 25.
3. Levine, L. and Stollar, B. D., Nucleic acid immune systems, *Progr. Allergy*, 12, 161, 1968.
4. Goldfarb, D. M., *Immunology of Nucleic Acids*, Nauka, Moscow, 1968.
5. Stollar, B. D., Nucleic acid antigens, in *The Antigens*, Vol. I, Sela, M., Ed., Academic Press, New York, 1973, 1.
6. Lacour, F., Nahon-Merlin, E., and Michelson, M., Immunological recognition of polynucleotide structure, *Current Topics in Microbiology and Immunology*, 62, 1, 1973.
7. Sela, M., Antigenicity: some molecular aspects, *Science*, 166, 1365, 1969.
8. Kabat, E. A., The nature of an antigenic determinant, *J. Immunol.*, 97, 1, 1966.
9. Brown, R. K., Studies on the antigenic structure of ribonuclease. III. Inhibition by peptides of antibody to performic acid-oxidized ribonuclease, *J. Biol. Chem.*, 237, 1162, 1962.
10. Landsteiner, K., *The Specificity of Serological Reactions*, Harvard University Press, Cambridge, Mass., 1945.
11. Reichlin, M., Bucci, E., Antonini, E., Wyman, J., and Rossi-Fanelli, A., The immunochemical difference between horse oxy- and deoxyhaemoglobin, *J. Mol. Biol.*, 9, 785, 1964.
12. Arnon, R. and Sela, M., Antibodies to a unique region in lysozyme provoked by a synthetic antigen conjugate, *Proc. Natl. Acad. Sci. U.S.A.*, 62, 163, 1969.
13. Schechter, B., Schechter, I., Ramachandran, J., Conway-Jacobs, A., Sela, M., Benjamini, E., and Shimizu, M., Synthetic antigens with sequential and conformation-dependent determinants containing the same L-tyrosyl-L-alanyl-L-glutamyl sequence, *Eur. J. Biochem.*, 20, 309, 1971.

13a. Reichlin, M., Amino acid substitution and the antigenicity of globular proteins, *Adv. Immunol.*, 20, 71, 1975.

14. Seligmann, M., Mise en évidence dans le serum de malades atteints de lupus erythémateux disséminé d'une substance déterminant une réaction de précipitation avec l'acide désoxyribonucléique, *C.R. Acad. Sci. Paris*, 245, 243, 1957.
15. Cepellini, R., Polli, C., and Celada, F., A DNA-reacting factor in serum of a patient with lupus erythematosus diffusus, *Proc. Soc. Exp. Biol. Med.*, 96, 572, 1957.
16. Miescher, P. and Strassle, R., New serological methods for the detection of the lupus erythematosus factor, *Vox Sang.*, 2, 283, 1957.
17. Robbins, W. C., Holman, H. R., Deicher, H. R., and Kunkel, H. G., Complement fixation with cell nuclei and DNA in lupus erythematosus, *Proc. Soc. Exp. Biol. Med.*, 96, 575, 1957.
18. Stollar, D., Levine, L., and Marmur, J., Antibodies to denatured deoxyribonucleic acid in lupus erythematosus serum. II. Characterization of antibodies in several sera, *Biochim. Biophys. Acta*, 61, 7, 1962.
19. Arana, R. and Seligmann, M., Antibodies to native and denatured deoxyribonucleic acid in systemic lupus erythematosus, *J. Clin. Invest.*, 46, 1867, 1967.
20. Tan, E. M. and Natali, P. G., Comparative study of antibodies to native and denatured DNA, *J. Immunol.*, 104, 902, 1970.
21. Winfield, J. B. and Davis, J. S., IV, Anti-DNA antibody in procainamide-induced lupus erythematosus. Determinations using DNA fractionated by methylated albumin-kieselguhr chromatography, *Arthritis Rheum.*, 17, 97, 1974.
22. Aarden, L. A. and Feltkamp, T. E. W., personal communication.
23. Koffler, D., Carr, R. I., Agnello, V., Thoburn, R., and Kunkel, H. G., Antibodies to polynucleotides in human sera, antigenic specificity and relation to disease, *J. Exp. Med.*, 134, 294, 1971.
24. Aarden, L. A., De Groot, E. R., and Feltkamp, T. E. W., Immunology of DNA III: *Crithidia luciliae*, a simple substrate for the determination of anti-ds-DNA with the immunofluorescent technique, *Ann. N.Y. Acad. Sci.*, in press.
25. Beck, J. S. and Walker, P. J., Antigenicity of trypanosome nuclei: evidence that DNA is not coupled to histone in these protozoa, *Nature*, 204, 194, 1964.
26. Thivolet, J., Monier, J. C., Lalain, F., and Richard, M. M., Récherche quantitative simultanée des anticorps antinucleaires totaux et anti-acide desoxyribonucleique sur frottis de sang de souris parasitees par *Trypanosoma gambiense*, *Ann. Inst. Pasteur*, 109, 817, 1965.
27. Ginsberg, B. and Keiser, H., A millipore filter assay for antibodies to native DNA in sera of patients with systemic lupus erythematosus, *Arthritis Rheum.*, 16, 199, 1973.
28. Stollar, B. D., unpublished observations.
29. Stollar, B. D., Double-helical polynucleotides: immunochemical recognition of differing conformations, *Science*, 169, 609, 1970.
30. Talal, N. and Gallo, R. C., Antibodies to a DNA-RNA hybrid in systemic lupus erythematosus measured by a cellulose ester filter radioimmunoassay, *Nat. New Biol.*, 240, 240, 1972.
31. Cohen, S. A., Hughes, G. R. V., Noel, G. L., and Christian, C. L., Character of anti-DNA antibodies in systemic lupus erythematosus, *Clin. Exp. Immunol.*, 8, 551, 1971.
32. Crothers, D. M. and Metzger, H., The influence of polyvalency on the binding properties of antibodies, *Immunochemistry*, 9, 341, 1972.

33. Pauling, L., Pressman, D., and Grossberg, A. L., The serological properties of simple substances. VII. A quantitative theory of the inhibition by haptens of the precipitation of heterogeneous antisera with antigens and comparison with experimental results for polyhaptenic simple substances and for azoproteins, *J. Am. Chem. Soc.*, 66, 784, 1944.
34. Hornick, C. and Karush, F., Antibody affinity. III. The role of multivalence, *Immunochemistry*, 9, 325, 1972.
34a. Gopalakrishnan, P. V. and Karush, F., Antibody affinity. VII. Multivalent interaction of anti-lactoside antibody, *J. Immunol.*, 113, 769, 1974.
35. Erlanger, B. F. and Beiser, S. M., Antibodies specific for ribonucleosides and ribonucleotides and their reaction with DNA, *Proc. Natl. Acad. Sci. U.S.A.*, 52, 68, 1964.
36. Jaton, J. C., Ungar-Waron, H., and Sela, M., Comparison of IgG and IgM antibodies specific for uridine, *Eur. J. Biochem.*, 2, 106, 1967.
37. Halloran, M. J. and Parker, C. W., The production of antibodies to mononucleotides, oligonucleotides and DNA, *J. Immunol.*, 96, 379, 1966.
38. Stollar, D., Levine, L., Lehrer, H. I., and Van Vunakis, H., The antigenic determinants of denatured DNA reactive with lupus erythematosus serum, *Proc. Natl. Acad. Sci. U.S.A.*, 48, 874, 1962.
39. Talal, N., Immunologic and viral factors in the pathogenesis of systemic lupus erythematosus, *Arthritis Rheum.*, 13, 887, 1970.
40. Christian, C. L. and Phillips, P. E., Viruses and autoimmunity, *Am. J. Med.*, 54, 611, 1973.
41. Ziff, M., Viruses and the connective tissue diseases, *Ann. Intern. Med.*, 75, 951, 1971.
42. Guarnieri, M. and Eisner, D., A DNA antigen that reacts with antisera to cardiolipin, *Biochem. Biophys. Res. Commun.*, 58, 347, 1974.
43. Fournie, G. J., Lambert, P. H., and Miescher, P. A., Release of DNA in circulating blood and induction of anti-DNA antibodies after injection of bacterial lipopolysaccharides, *J. Exp. Med.*, 140, 1189, 1974.
44. Lambert, P. H. and Dixon, F. J., Genesis of antinuclear antibody in NZB/NZW mice. Role of genetic factors and viral infections, *Clin. Exp. Immunol.*, 6, 829, 1970.
45. Stollar, B. D., Fuchs, S., and Mozes, E., Immune response of mice to nucleic acids; strain-dependent differences in magnitude and class of antibody production, *J. Immunol.*, 111, 121, 1973.
46. Scher, I., Frantz, M. M., and Steinberg, A. D., The genetics of the immune response to a synthetic double-stranded RNA in a mutant CBA mouse strain, *J. Immunol.*, 110, 1396, 1973.
47. Mozes, E. and Fuchs, S., Linkage between immune response potential to DNA and X chromosome, *Nature*, 249, 167, 1974.
48. Ghaffar, A. and Playfair, J. H. L., The genetic basis of autoimmunity in NZB mice studied by progeny-testing, *Clin. Exp. Immunol.*, 8, 479, 1971.
49. Lewis, R. M. and Schwartz, R. S., Canine systemic lupus erythematosus. Genetic analysis of an established breeding colony, *J. Exp. Med.*, 134, 417, 1971.
50. Grumet, F. C., Coukell, A., Bodmer, J. G., Bodmer, W. F., and McDevitt, H. O., Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus, *N. Engl. J. Med.*, 285, 193, 1971.
51. Larsen, R., Familial studies in systemic lupus erythematosus. III. Presence of LE factor in relatives and spouses, *Acta Med. Scand. Suppl.*, 543, 30, 1972.
52. Stollar, B. D., Immunochemical measurement of DNA in nucleoprotein with the use of anti-DNA antibodies from patients with systemic lupus erythematosus, *Biochim. Biophys. Acta*, 209, 541, 1970.
53. Adler, A. J., Schaffhausen, B., Langan, T. A., and Fasman, G. D., Altered conformational effects of phosphorylated lysine-rich histone (f1) in f1-deoxyribonucleic acid complexes. Circular dichroism and immunological studies, *Biochemistry*, 10, 909, 1971.
54. Casals, S. P., Friou, G. J., and Teague, P. O., Specific nuclear reaction pattern of antibody to DNA in lupus erythematosus sera, *J. Lab. Clin. Med.*, 62, 625, 1963.
55. Tan, E. M., Relationship of nuclear staining patterns with precipitating antibodies in systemic lupus erythematosus, *J. Lab. Clin. Med.*, 70, 800, 1967.
56. Tan, E. M. and Lerner, R. A., An immunological study of the fates of nuclear and nucleolar macromolecules during the cell cycle, *J. Mol. Biol.*, 68, 107, 1972.
57. Beck. J. W., Scott, G-B., Munro, H. N., Waddington, S., and MacSeveney, D., A new immunochemical technique for electron microscopic study of nuclear structure using human antinuclear antibodies, *Exp. Cell Res.*, 28, 406, 1962.
58. Lambert, P. H. and Dixon, F. J., Pathogenesis of the glomerulonephritis of NZB/NZW mice, *J. Exp. Med.*, 127, 507, 1968.
59. Zeleznick, L. D., Holm, M. S., and Barnett, E. V., *Proc. Soc. Exp. Biol. Med.*, 131, 716, 1969.
60. Steinberg, A. D., Pincus, T., and Talal, N., DNA-binding assay for detection of anti-DNA antibodies in NZB/NZW F1 mice, *J. Immunol.*, 102, 788, 1969.
61. Thoburn, R., Koffler, D., and Kunkel, H. G., Distribution of antibodies to native DNA, single-stranded DNA and double-stranded RNA in mouse serums, *Proc. Soc. Exp. Biol. Med.*, 136, 711, 1971.
62. Barbu, E. and Panijel, J., Presence d'anticorps anti-acide ribonucléique dans les immunsérums antiribosomes, *C. R. Acad. Sci. Paris*, 250, 1382, 1960.
63. Lacour, F., Harel, J., Harel, L., and Nahon, E., Etude immunologique de l'acide ribonucléique (RNA) par la microméthode de double diffusion dans l'agar, *C. R. Acad. Sci. Paris*, 255, 2322, 1962.

64. **Panijel, J.,** Précipitation immunologique des polynucléotides synthetiques et multiplicité des anticorps anti-RNA, *C. R. Acad. Sci. Paris,* 256, 4540, 1963.
65. **Panijel, J., Souleil, C., and Cayeux, P.,** Immunochimie des polyribonucléotides. I. Homopolymères à une ou plusieurs chaines, *Biochim. Biophys. Acta,* 123, 221, 1966.
66. **Souleil, C., Cayeux, P., and Panijel, J.,** Immunochimie des polyribonucléotides. II. Complexes d' homopolymères différents, *Biochim. Biophys. Acta,* 123, 235, 1966.
67. **Nahon, E., Lacour, F., and Harel, J.,** Identification immunologique de diverses fractions de RNA cellulaire et de polynucléotides de synthèse, *C. R. Acad. Sci. Paris,* 260, 357, 1965.
68. **Panijel, J.,** Précipitation des polyribonucléotides homopolymères par les divers anticorps du serum anti-DNA, *C. R. Acad. Sci. Paris,* 257, 3064, 1963.
69. **Souleil, C. and Panijel, J.,** Immunochemistry of polyribonucleotides. Study of polyriboinosinic acid and polyriboguanylic acid, *Biochemistry,* 7, 7, 1968.
70. **Panijel, J. and Delaunay, M-C.,** An immunochemical approach to the structure of the ribosomal particles, *Biochem. Biophys. Res. Commun.,* 29, 158, 1967.
71. **Parker, L. M. and Steinberg, A. D.,** The antibody response to polyinosinic polycytidylic acid, *J. Immunol.,* 110, 742, 1973.
72. **Plescia, O. J., Braun, W., and Palczuk, N. C.,** Production of antibodies to denatured deoxyribonucleic acid (DNA), *Proc. Natl. Acad. Sci. U.S.A.,* 52, 279, 1964.
73. **Nahon, E., Michelson, A. M., and Lacour, F.,** Antigénicité des complexes de polynucléotides, *Biochim. Biophys. Acta,* 149, 127, 1967.
74. **Plescia, O. J., Strampp, A., and Kwiatkowski, Z.,** Hybridization between poly-uridylate and oligoadenylate: an immunochemical analysis, *Fed. Proc.,* 28, 695, 1969.
75. **Schwartz, E. F. and Stollar, B. D.,** Antibodies to polyadenylate-polyuridylate copolymers as reagents for double-strand RNA and DNA-RNA hybrid complexes, *Biochem. Biophys. Res. Commun.,* 35, 115, 1969.
76. **Poverenny, A. M., Podgorodnichenko, V. K., and Saprygin, D. B.,** The secondary structure of nucleic acids which induce the immunological response, *Immunochemistry,* 10, 577, 1973.
77. **Francki, R. I. B. and Jackson, A. O.,** Immunochemical detection of double-stranded ribonucleic acid in leaves of sugar cane infected with Fiji disease virus, *Virology,* 48, 275, 1972.
78. **Schur, P. H. and Monroe, M.,** Antibodies to ribonucleic acid in systemic lupus erythematosus, *Proc. Natl. Acad. Sci. U.S.A.,* 63, 1108, 1969.
79. **Nahon-Merlin, E., Moulart, L., and Lacour, F.,** Immunochemical studies of anti-poly(I) · poly (C) antibodies and of their reactions with RNA, *Immunochemistry,* 10, 571, 1973.
79a. **Cramer, F.,** *Progr. Nucleic Acid Res. Mol. Biol.,* 11, 391, 1971.
79b. **Hindley, J.,** *Progr. Biophys. Mol. Biol.,* 26, 271, 1973.
80. **Stollar, V. and Stollar, B. D.,** Immunochemical measurement of double-stranded RNA of uninfected and arbovirus-infected mammalian cells, *Proc. Natl. Acad. Sci. U.S.A.,* 65, 993, 1970.
81. **Michelson, A. M., Massoulie, J., and Guschlbauer, W.,** Synthetic polynucleotides, *Progr. Nucleic Acid Res. Mol. Biol.,* 6, 83, 1967.
82. **Stollar, B. D. and Stollar, V.,** Immunofluorescent demonstration of double-stranded RNA in the cytoplasm of Sindbis virus-infected cells, *Virology,* 42, 276, 1970.
83. **Seaman, E., Van Vunakis, H., and Levine, L.,** Antigenicity of polyribonucleotides, *Biochemistry,* 4, 1312, 1965.
84. **Johnston, M. I., Torrence, P., Stollar, B. D., and Witkop, B.,** in preparation.
85. **Talal, N., Steinberg, A. D., and Daley, G. G.,** Inhibition of antibodies binding polyinosinic polycytidylic acid in human and mouse lupus sera by viral and synthetic ribonucleic acids, *J. Clin. Invest.,* 50, 1248, 1971.
86. **Ikegami, M. and Francki, R. E. B.,** Purification and serology of virus-like particles from Fiji disease virus-infected sugar cane leaves, *Virology,* 61, 327, 1974.
87. **Stollar, V., Shenk, T. E., and Stollar, B. D.,** Double-stranded RNA in hamster, chick, and mosquito cells infected with Sindbis virus, *Virology,* 47, 122, 1972.
88. **Jelinek, W. and Darnell, J. E.,** Double-stranded regions in heterogeneous nuclear RNA from Hela cells, *Proc. Natl. Acad. Sci. U.S.A.,* 69, 2537, 1972.
89. **Shenk, T. E. and Stollar, V.,** Defective-interfering particles of Sindbis Virus 1. Isolation and some chemical and biological properties, *Virology,* 53, 162, 1973.
90. **Lubiniecki, A. S. and Tarr, G. C.,** Characterization of dengue virus type 2 temperature-sensitive mutants, *Am. Soc. Microbiol. Abstr.,* 1975, in press.
91. **Silverstein, S. C. and Schur, P. H.,** Immunofluorescent localization of double-stranded RNA in reovirus-infected cells, *Virology,* 53, 162, 1973.
92. **Dianzani, F., Forni, G., Negro-Ponzi, A., Pugliese, A., and Cavallo, G.,** Decreased interferon response to polyinosinic polycytidylic acid in rabbits immunized against the inducer, *Proc. Soc. Exp. Biol. Med.,* 139, 93, 1972.
93. **Vengris, V. E., Stollar, B. D., and Pitha, P. M.,** Interferon externalization by producing cell before induction of antiviral state, *Virology,* in press.
94. **Ikegami, M. and Francki, R. I. B.,** Presence of antibodies to double-stranded RNA in sera of rabbits immunized with rice dwarf and maize rough dwarf viruses, *Virology,* 56, 404, 1973.

95. Moffitt, E. M. and Lister, R. M., Detection of mycoviruses using antiserum specific for ds RNA, *Virology*, 52, 301, 1973.
96. Stollar, B. D. and Diener, T. O., Potato spindle tuber viroid. V. Failure of immunological tests to disclose double-stranded RNA or RNA-DNA hybrids, *Virology*, 46, 168, 1971.
97. Lacour, F., Fourcade, A., and Camus, G., Etude de la spécificité des anticorps anti-poly(I) · poly(C) chez différentes souches de souris, *C.R. Acad. Sci. Paris*, 278, 1793, 1974.
98. Lacour, F., Deleon, I., and Nahon-Merlin, E., Pouvoir immunogène du poly(I) · poly(C)-sérumalbumine bovine methylée chez le lapin, le hamster, et la souris, *Ann. Inst. Pasteur*, 121, 633, 1971.
99. Schur, P. H., Stollar, B. D, Steinberg, A. D., and Talal, N., Incidence of antibodies to double-stranded RNA in systemic lupus erythematosus and related diseases, *Arthritis Rheum.*, 14, 342, 1971.
100. Epstein, W. V., Tan, M., and Easterbrook, M., Serum antibody to double-stranded RNA and DNA in patients with idopathic and secondary uveitis, *N. Engl. J. Med.*, 285, 1502, 1971.
101. Steinberg, A. D., Baron, S., and Talal, N., The pathogenesis of autoimmunity in New Zealand mice. I. Induction of antinucleic acid antibodies by polyinosinic polycytidylic acid, *Proc. Natl. Acad. Sci. U.S.A.*, 63, 1102, 1969.
102. Michelson, A. M., Lacour, F., and Nahon-Merlin, E., Propriétés immunochimiques du polyG · polyC et du polyG, *C.R. Acad. Sci. Paris Ser. D.*, 272, 669, 1971.
103. Nahon-Merlin, E., Michelson, A. M., Verger, C., and Lacour, F., Specificity of anti-polyG · polyC antibodies, *J. Immunol.*, 107, 222, 1971.
104. Colby, C., Stollar, B. D., and Simon, M. I., Interferon induction: DNA-RNA hybrid or double-stranded RNA? *Nat. New Biol.*, 229, 172, 1971.
105. Lacour, F., Michelson, A. M., and Nahon, E., Specific antibodies to polynucleotide complexes and their reaction with nucleic acids: importance of the secondary structure of the antigen, in *Nucleic Acids in Immunology*, Plescia, O. J. and Braun, W., Eds., Springer-Verlag, Berlin and New York, 1968, 32.
106. Stollar, B. D. and Raso, V., Antibodies recognize specific structures of triple-helical polynucleotides built on poly(A) or poly(dA), *Nature*, 250, 231, 1974.
107. Riley, M., Maling, B., and Chamberlin, M., Physical and chemical characterization of two- and three-stranded adenine-thymine and adenine-uracil homopolymer complexes, *J. Mol. Biol.*, 20, 359, 1966.
108. Arnott, S., The geometry of nucleic acids, *Progr. Biophys. Mol. Biol.*, 21, 267, 1970.
109. Arnott, S. and Hukins, D. W. L., Refinement of the structure of B-DNA and implications for the analysis of x-ray diffraction data from fibers of biopolymers, *J. Mol. Biol.*, 81, 93, 1973.
110. Arnott, S., Hukins, D. W. L., Dover, S. D., Fuller, W., and Hodgson, A. R., Structures of synthetic polynucleotides in the A-RNA and A'-RNA conformations: x-ray diffraction analyses of the molecular conformations of polyadenylic acid · polyuridylic acid and polyinosinic acid · polycytidylic acid, *J. Mol. Biol.*, 81, 107, 1973.
111. Amzel, L. M., Poljak, R. J., Saul, F., Varga, J. M., and Richards, F. F., The three-dimensional structure of a combining region-ligand complex of immunoglobulin NEW at 3.5 A resolution, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 1427, 1974.
112. Segal, D. M., Padlan, E. A., Cohen, G. H., Rudikoff, S., Potter, M., and Davies, D. R., The three-dimensional structure of a phosphorylcholine-binding mouse immunoglobulin Fab and the nature of the antigen binding site, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 4298, 1974.
113. Arnott, S. and Selsing, E., Structures for the polynucleotide complexes poly(dA) · poly(dT) and poly(dT) poly(dA) · poly(dT), *J. Mol. Biol.*, 88, 509, 1974.
114. Arnott, S. and Selsing, E., The structure of polydeoxyguanylic · polydeoxycytidylic acid, *J. Mol. Biol.*, 88, 551, 1974.
115. Arnott, S., Chandrasekaran, R., Hukins, D. W. L., Smith, P. J. C., and Watts, L., Structural details of a double-helix observed for DNA's containing alternating purine and pyrimidine sequences, *J. Mol. Biol.*, 88, 523, 1974.