kinase, on the other hand, the interactions between the enzyme and the adenine ring or the ribose hydroxyls are less exacting than with AMP aminohydrolase and a given alteration in the ribose–adenine torsion angle should therefore be better tolerated; such an alteration would also tend to be better tolerated by AMP kinase because the concomitant rotation of the 4′,5′ bond which can occur would tend to protect the phosphate reaction center from unfavorable effects on catalysis. It is concluded that the substrate properties of 8,2′- and 8,3′-*O*-cyclo-AMP with AMP aminohydrolase and AMP kinase are consistent with the previous postulate that in enzyme-bound AMP H-8 is located in the area above C-4′ and, further, these properties provide evidence that rotation of the ribose–adenine bond so as to position H-8 first over H-3′, then over H-2′, progressively reduces substrate activity.

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Specific Binding of Ribonucleic Acid by Antiadenosine Antibodies†

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ABSTRACT: Antibodies to the four major RNA bases were elicited by immunization with nucleoside–protein conjugates. Reactivity of RNA with antinucleoside antibody was investigated by the double-antibody technique which measures the primary interaction between antibody and antigen. In order to demonstrate a reaction between antinucleoside antibody and RNA, serum ribonuclease activity had to be eliminated, and at the levels of RNase found in the antisera this could be done with Na_2SO_4. In the presence of 0.2 M Na_2SO_4, antiadenosine (anti-A) reacted with all RNA preparations tested, except tRNA. No reaction with RNA could be detected with antibody to guanosine, cytidine, or uridine under identical conditions although these were at least as reactive with DNA as was anti-A. The specificity of RNA–anti-A interaction was characterized further by inhibition experiments. Only adenosine and adenine-containing nucleosides, nucleotides, or polynucleotides inhibited the binding of tritiated *Escherichia coli* RNA. The inhibitory activity of poly(A) was lost when it was complexed with poly(U) in a hydrogen-bonded duplex.

Immunochemical techniques have been of great value in studies on the structure and function of proteins and polysaccharides. Recently, nucleic acid reactive antibodies have been obtained, both experimentally and from sera of patients with systemic lupus erythematosus, thereby making possible similar immunochemical studies of nucleic acids.

One method for obtaining antibodies that react with nucleic acids involves the use of hapten–protein conjugates (Butler *et al.*, 1962; Erlanger and Beiser, 1964; Sela *et al.*, 1964; Halloran and Parker, 1966; Karol and Tanenbaum, 1967). A general procedure, based on periodate oxidation of the free vicinal hydroxyl groups, was developed by Erlanger and Beiser (1964) for coupling any ribonucleoside or ribonucleotide to a protein carrier. Specific antibodies were obtained for the five common bases present in RNA and DNA. The specificity of these antisera, determined by precipitation, complement fixation, and hapten inhibition, was directed primarily against the homologous purine or pyrimidine base. The antisera cross-reacted with denatured DNA from all species tested, but did not react with native DNA. All attempts to demonstrate direct reaction of the antisera with

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native RNA or denatured RNA were unsuccessful. However, evidence for the binding of RNA to these antisera was inferred from the finding that RNA inhibited DNA–antibody and homologous antigen–antibody reactions.

Antibodies that react with RNA have been obtained by immunization with other hapten conjugates. Conjugates of 5′-carboxylic acid derivatives of nucleosides with a polypeptide were used to elicit hapten-specific antibodies (Sela *et al.*, 1964; Ungar-Waron *et al.*, 1967). The antisera precipitated with denatured DNA, and after ribonuclease activity was inhibited, antiadenosine and antiuridine precipitated with denatured RNA, but did not precipitate with native RNA. Anticytidine and antiguanosine were not tested. In a similar study, Karol and Tanenbaum (1967) conjugated uridine, dihydrouridine, and pseudouridine carboxylic acid derivatives to bovine serum albumin. Sulfate-fractionated globulins reacted with denatured DNA and with native and denatured RNA.

The purpose of this investigation was to study further the reactivity of antinucleoside antibody with RNA. Since a reaction of RNA with antibody could not be demonstrated by precipitation or complement fixation, which reflect secondary phenomena, methods that measure the primary interaction were investigated. Moreover, Minden *et al.* (1969) have shown that secondary tests frequently fail to detect significant amounts of antibody demonstrable by primary binding tests. In this study, the double antibody method was used to measure the reaction of RNA with antinucleoside antibodies. The Farr technique (Farr, 1958) itself was not feasible because of the insolubility of ribosomal RNA in 50% saturated ammonia sulfate.

Methods and Materials

Chemicals. Purine and pyrimidine bases, nucleosides, and nucleotides were purchased from either Sigma Chemical Co., St. Louis, Mo., or Calbiochem, Los Angeles, Calif. Iodine-125 (carrier free) was purchased from New England Nuclear Corporation, Boston, Mass., and [^{3}H]uridine (8 Ci/mmol) was purchased from Schwarz/Mann, Orangeburg, N. Y. Soluene was purchased from Packard Instrument Co., New York, N. Y., and Omnifluor from New England Nuclear, Boston, Mass.

Enzymes. Pancreatic ribonuclease (5× recrystallized) and electrophoretically purified deoxyribonuclease I (ribonuclease free) were purchased from Worthington Biochemical Corp., Freehold, N. J.

Nucleic Acids. In crude material, concentrations of DNA and RNA were determined by the diphenylamine reaction (Burton, 1956) and a modified orcinol procedure (I-San Lin and Schjeide, 1969), respectively. Concentrations of polynucleotides and purified nucleic acids were determined spectrophotometrically. The following molar extinction coefficients (E_p), in TBS,[1] were used: native DNA, 6.6×10^3; native RNA, 6.8×10^3; poly(A), 10.5×10^3; poly(C), 6.6×10^3; poly(U), 9.2×10^3. Calf thymus DNA, *Micrococcus luteus* DNA, and *Clostridium perfringens* DNA were purchased from Sigma Chemical Co. Solutions (1 mg/ml) of DNA in 0.15 M NaCl–0.01 M citrate, pH 7.0, were treated with pancreatic ribonuclease (10 μg/ml) for 1 hr at 37°, dialyzed against 1 M NaCl–0.01 M citrate, pH 7.0, and deproteinized with chloroform–isoamyl alcohol. The procedure for the preparation of IDNA was published previously (Rosenberg *et al.*, 1972).

Poly(A), poly(C), and poly(U) were purchased from Biopolymers, Inc., Dover, N. J. Low molecular weight contaminants were removed by dialysis against TBS. They were stored in TBS at −20°.

RNA *Escherichia coli* Ra 19 (RNase$^-$ Met$^-$) was grown in a minimal salt medium supplemented with methionine (50 μg/ml). The mean generation time for the strain in this media is 55 min. Cells (2 l.) were harvested during late exponential growth, washed with 0.01 M Tris–0.01 M $MgCl_2$, pH 7.4, and disrupted in a French pressure cell at 10,000 psi. The lysate was then used for preparing total RNA by the procedure of Bolton (1966). RNA so prepared contained 6–12% DNA as determined by the diphenylamine reaction. Electrophoretically purified DNase (10 μg/ml) was added to total RNA (4.1 mg/ml) in 0.01 M $MgCl_2$–0.01 M Tris, pH 7.4. After 1 hr incubation at 37° the solution was dialyzed first against 1.0 M NaCl and then against TBS and extracted with phenol followed by precipitation of total RNA with ethanol. This total RNA preparation contained less than 1% DNA. RNA at a concentration of 4.1 mg/ml in TBS was stored in small amounts at −20°. [^{3}H]Uridine-labeled *E. coli* RNA was prepared as above except that [^{3}H]uridine (250 μCi/l.) was added to the culture medium 30 min before harvesting the cells. ^{32}P-Labeled Qβ RNA, tritiated f2 RNA and ^{14}C-labeled embryonic chick RNA were gifts from Drs. D. Mills, T. August, and H. Rosenkranz, respectively.

Denaturation of Nucleic Acids. Solutions of DNA or RNA in TBS were heated for 10 min at 100° and then rapidly chilled in an ice bath. When indicated in the text, formaldehyde at a concentration of 1% was included during denaturation.

Ribonuclease Assay. [^{3}H]RNA (50 μl; *ca.* 3500 cpm) was mixed with 0.05–0.5 ml of enzyme solution in TBS and incubated at 37° for 30 min. Cold 5% Cl_3CCOOH (0.5 ml) was then added and the tubes were kept at 4° for 30 min. The concentration of ribonuclease present in test material was determined from a standard curve which had previously been obtained using pancreatic ribonuclease.

Preparation of Antisera. The preparation of the nucleoside–bovine serum albumin conjugates and the protocol for immunization have been described previously (Erlanger and Beiser, 1964). Bovine serum albumin conjugates of the four major nucleosides of RNA—adenosine, cytidine, uridine, and guanosine—were coupled to bovine serum albumin and injected into groups of four–six rabbits. Antisera of each specificity were pooled and γ-globulin fractions were prepared using sodium sulfate (Kekwick, 1940). Their specificity was determined by agar–gel diffusion analysis (Lacour *et al.*, 1962). The globulins were adjusted to a concentration of 20 mg of protein/ml. These preparations of antiadenosine, anticytidine, antiguanosine, and antiuridine bovine serum abumin will be referred to as anti-A, anti-C, anti-G, and anti-U, respectively. Anti-RGG was obtained from an adult sheep that was immunized with RGG in complete Freund's adjuvant. The serum from several bleedings was pooled and a globulin fraction was prepared. One milliliter of anti-RGG maximally precipitated 205 μg of RGG.

Quantitative microprecipitin reactions were performed as described by Mage and Dray (1965), except that the total volume was 0.15 ml.

The procedure for precipitation of antibody–nucleic acid

[1] Abbreviations used in this work are: TBS, 0.15 M NaCl–0.01 M Tris, pH 7.2; IDNA, iodinated calf thymus DNA; RGG, rabbit γ-globulin.

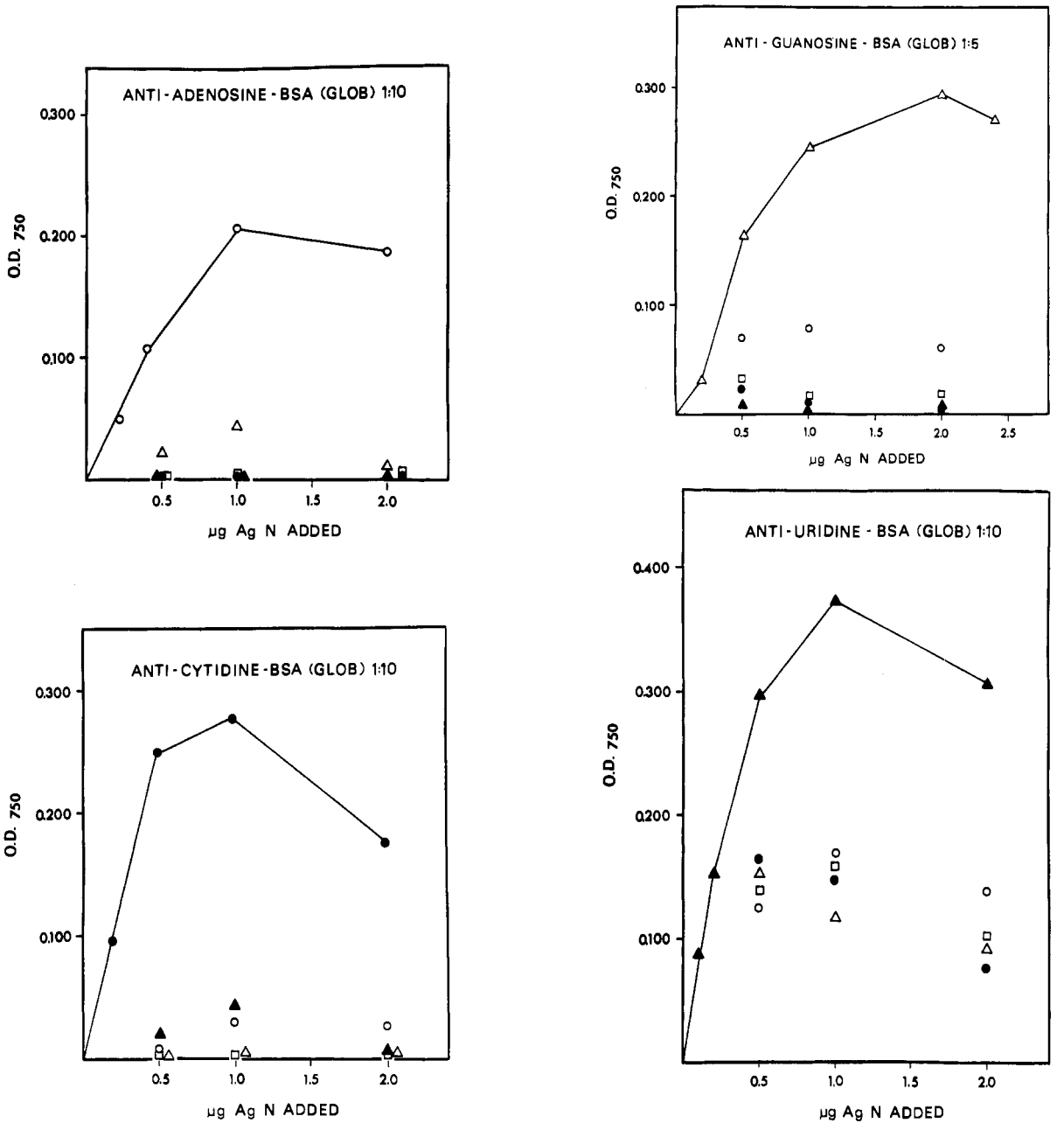

FIGURE 1: Microprecipitin reactions between nucleoside–bovine serum albumin (BSA) conjugates and 50 μl of antibody globulin diluted with TBS; total volume 0.15 mμ: (○) A–bovine serum albumin (BSA); (●) C–bovine serum albumin (BSA); (△) G-bovine serum albumin (BSA); (▲) U-bovine serum albumin (BSA); (□) bovine serum albumin (BSA).

complexes with anti-RGG is described elsewhere (Rosenberg *et al.*, 1972).

Radioactivity Determination. ^{3}H, ^{14}C, and ^{32}P were counted in a Beckman liquid scintillation spectrometer. Aqueous samples from 50 μl to 1 ml were counted in Bray's scintillation mixture (Bray, 1960). Immune precipitates, Cl_3CCOOH precipitates, and aqueous samples up to 50 μl were solubilized with 0.5–1.0 ml of Soluene and counted in a standard toluene mixture (4 g of Omnifluor/l. of toluene). Efficiencies were determined either by the use of internal standards or channel-ratio external standardization. In Bray's solution, efficiencies for ^{3}H ranged from 9 to 18%, and for ^{14}C from 48 to 75%. Solubilized samples in toluene had efficiencies for tritium of 28–40% and for carbon from 80 to 90%. The efficiencies of ^{32}P-labeled samples were identical in both scintillation mixtures and were not affected by the presence of Cl_3CCOOH. One hundred per cent efficiency was assumed.

Results

The globulins were characterized by precipitin reactions using the nucleoside conjugates of the four RNA bases and also the carrier protein (Figure 1). In all cases, the homologous antigens reacted best. Anti-A and anti-G cross-reacted with the heterologous purine conjugate. Anti-U cross-reacted equally with all the heterologous test antigens including bovine serum albumin. Antibody concentrations as determined by precipitation with the homologous antigens at equivalence are listed in Table I. Maximal amounts of antibody precipitated by HCHO-denatured calf thymus DNA are also listed in

TABLE I: Antibody Nitrogen Precipitated at Equivalence by Homologous Antigens and Formaldehyde-Denatured Calf Thymus DNA.

Antiserum	μg of Antibody N/ml for Homologous Antigen	μg of Antibody N/ml for HCHO-Denatured DNA
Anti-A	636	400
Anti-C	952	330
Anti-G	433	210
Anti-U	1260	165

Table I. No precipitate was obtained with *E. coli* RNA or with yeast tRNA either native, heat denatured, or formaldehyde denatured. Globulin fractions prepared from preimmunization bleedings did not precipitate with either DNA or RNA.

The primary interaction of labeled nucleic acids with antibody was examined using the double-antibody procedure. In initial experiments, RNA did not react significantly with antinucleoside antibody. The slight binding of native, heat-denatured, or formaldehyde-denatured *E. coli* [^{3}H]RNA to immune globulins did not differ significantly from that found with controls. The maximum quantities of added native RNA bound to anti-A, anti-C, anti-G, anti-U, pooled normal RGG, and anti-bovine serum albumin were 10, 6, 8, 5, 7, and 4%, respectively.

The possibility that antibody–RNA interaction was not detected because of RNA degradation by RNase was investigated. RNase was detected in all antibody globulin preparations at concentrations ranging from 0.0005 to 0.005 μg/ml. Several procedures for removing the enzyme were tried. Bentonite removed ribonuclease, but also removed large amounts of γ-globulin. The enzyme was not completely removed by passage through Sephadex G-100 or through DEAE-Sephadex. An attempt was also made to remove RNase by repeated sodium sulfate precipitation of the antibody preparations. During the course of these precipitations, it was observed that sodium sulfate inhibited ribonuclease activity (Rosenberg, 1970). This finding, we later learned, had been reported previously (Saroff and Carroll, 1962). An enzyme concentration of 0.01 μg/ml, considerably above that found in any of the globulin fractions, was inhibited completely by 0.2 M Na_2SO_4.

The interaction of anti-A with native tritiated total RNA in the presence of sodium sulfate is summarized in Table II.

TABLE II: Effect of Sodium Sulfate on Primary Reaction of Anti-A with Total RNA.[a]

0.2 M Na_2SO_4	Immune Ppt (%)	Cl_3CCOOH Ppt (%)
+	75	100
−	10	33

[a] 50 μl of anti-A (1:4) and 1.7 μg of tritiated total *E. coli* RNA were incubated in the presence and absence of Na_2SO_4 at a final concentration of 0.2 M.

TABLE III: Reaction of Antinucleoside Antibody with Labeled RNA (Per Cent Bound).

RNA	μg Added	Antisera A (1:10)	C (1:4)	G (1:4)	U (1:2)	BSA (1:4)
E. coli						
Native	1.7	35	7	5	6	4
Heat denatured	1.7	38	6	8	5	2
HCHO denatured	1.7	10	9	8	12	8
Embryonic chick	5.0	14	2	6	7	
Qβ phage	0.03	82	5	7	9	6
f2 phage						
Native	1.0	54	5	10	8	4
HCHO denatured	1.0	21	8	9	12	8

[a] Bovine serum albumin.

In the absence of sulfate, most of the RNA was degraded and no longer precipitable by Cl_3CCOOH. In its presence, all RNA was acid insoluble and 75% of added RNA was found in the immune precipitate. Heat denatured *E. coli* RNA reacted similarly.

In contrast to the ability of anti-A to react with *E. coli* total RNA in the presence of sulfate, anti-C, anti-G, and anti-U failed to react. In all cases, RNA was completely Cl_3CCOOH precipitable. Several additional antibody preparations of each specificity were tested with native and heat denatured *E. coli* [^{3}H]RNA. Antisera to adenosine always reacted with RNA whereas no reaction was obtained with antisera to the other nucleosides. Absorption of anti-A with adenosine–bovine serum albumin or with denatured DNA completely eliminated its ability to bind with RNA.

To eliminate the possibility that the reaction of anti-A with RNA was merely a quantitative effect, the titers of all sera were determined with IDNA (Figure 2). The titers of anti-C, anti-G, and anti-U used to study the reaction with RNA were at least as high as that of anti-A, as determined by reactivity with labeled DNA.

Anti-A reacted with native and heat-denatured RNA from all species tested (Table III). Anti-C, anti-G, and anti-U did not bind detectably. It is important to note that ^{32}P-labeled Qβ RNA had a high specific activity, and therefore was used at 1/50th the amount required for the other RNA preparations. In spite of this increase in sensitivity, reaction could be detected only with anti-A. Formaldehyde denaturation eliminated the reactivity of *E. coli* RNA and drastically reduced the reactivity of bacteriophage f2 RNA. There was a slight but probably insignificant reactivity of HCHO-denatured RNA with anti-U. However, it was not possible by hapten inhibition to demonstrate that the reaction was specific for uridine.

^{3}H-Labeled total RNA was separated into its components by sucrose gradient ultracentrifugation, and fractions containing RNA were assayed with anti-A (Figure 3). Anti-A reacted equally well with all samples taken from the faster sedimenting, 16 S and 23 S, regions of the gradient. In contrast, samples taken from the 4S region of the gradient varied in reactivity with antibody. Samples taken from within the peak reacted very little with anti-A. The only sample that reacted appreciably with anti-A was taken from a fraction which sedi-

TABLE IV: Inhibition of the Binding of RNA to Anti-A.[a]

Inhibitor	nmol for ~50% Inhibition
Adenine	0.5
Adenosine	0.2
5′-AMP	0.2
2′,3′-AMP	0.5
Deoxyadenosine	0.5
UpA	1.0
ApU	0.5
Purine riboside	500.0 (18)
Inosine	500.0 (43)
Ribose	500.0 (9)
Deoxyribose	500.0 (21)
Cytidine	500.0 (35)
Guanosine	500.0 (10)
Uridine	500.0 (43)
	μg for ~50% Inhibition
Poly(A)	0.2
Poly(C)	25.0 (20)
Poly(U)	100.0 (18)
Calf thymus DNA	
Native	50.0 (38)
Heat denatured	2.5
M. luteus DNA	
Native	50.0 (45)
Heat denatured	10.0
Cl. perfringens	
Native	25.0
Heat denatured	2.5
E. coli rRNA	2.0
E. coli tRNA	500.0 (7)

[a] The concentrations required for approximately 50% inhibition are reported. Where this was not possible the highest concentration is reported and parenthetically the corresponding percentage of inhibition. Fifty microliters of anti-A (1:10) was mixed with inhibitor and kept 1 hr at 4°, and then 1.7 μg of tritiated total RNA was added. Thirty-four per cent of the added RNA was precipitated by anti-A in the inhibition control tubes.

mented slightly faster than the 4S peak. This material is probably 5S ribosomal RNA.

The specificity of RNA–anti-A interaction was characterized further by inhibition experiments. Only adenine- and adenosine-containing nucleosides, nucleotides, dinucleotides, and polynucleotides caused inhibition (Table IV). The three DNA species tested have widely different contents of adenosine. *M. luteus*, which contains the least amount of adenosine, was the least inhibitory of the three denatured DNAs. Native DNA also inhibited, but at much higher concentrations. The finding that tRNA did not inhibit RNA–anti-A binding is in accord with its inability to bind directly with anti-A.

When poly(A) and poly(U) are mixed, they spontaneously form a hydrogen-bonded duplex (Rich and Davies, 1956). Such duplexes were used, therefore, as a model system to demonstrate that hydrogen-bonded poly(A) was unreactive with antibody. A concentration of poly(A) was chosen which

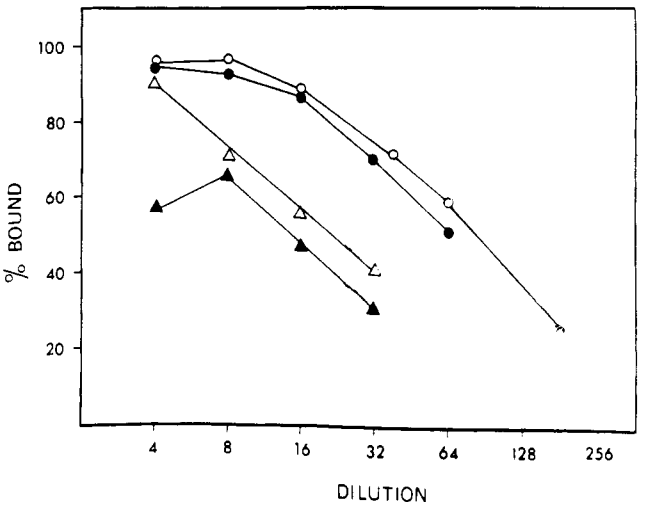

FIGURE 2: The binding of ^{125}I-labeled calf thymus DNA to antinucleoside antibody. Per cent of 0.13 μg of heat-denatured IDNA (2950 cpm) bound by different dilutions of antinucleoside antibody. Final volume of 0.15 ml in TBS contained 0.2 M Na_2SO_4: (○) anti-A; (●) anti-C; (△) anti-G; (▲) anti-U. (From Rosenberg *et al.*, 1972.)

inhibited the binding of anti-A to RNA by about 80%. Various concentrations of poly(U), incubated with poly(A) for 1 hr at 4°, were tested for their effect on the inhibitory activity of poly(A). As can be seen from Figure 4, with the formation of increasing amounts of duplex (increasing mole per cent poly(U)) there was a decrease in the ability of poly(A) to inhibit the binding of RNA to anti-A. At approximately equimolar amounts, the inhibition by poly A was completely eliminated.

Based on the observations with poly(A)·poly(U) duplexes, RNA was incubated with poly(U) prior to reaction with antibody. If extended sequences of adenosine are involved in RNA–antibody interaction, poly(U) should inhibit the reaction. Weight ratios of poly(U) to RNA of 1:1, 20:1, and 50:1 were incubated for 1 hr at 4°. These samples containing 1.0 μg of RNA were then allowed to react with anti-A in the usual

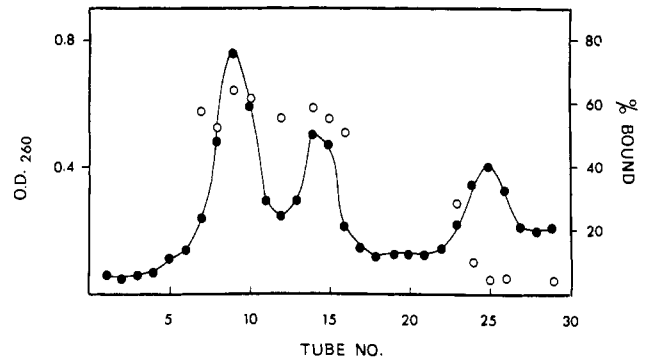

FIGURE 3: The binding of anti-A with sucrose gradient fractionated *E. coli* RNA. [^{3}H]RNA (100 μg) was loaded onto a 5–20% sucrose gradient buffered with TBS. The material was centrifuged in a SW 27 rotor for 18 hr at 23,000 rpm and 4°. The absorbance of 0.5-ml fractions, collected from the bottom, was determined (●, OD_{260}). Approximately 2 μg of RNA from each fraction was then allowed to react with 50 μl of anti-A (1:4) in TBS containing 0.2 M Na_2SO_4; ○, % of added RNA precipitated using anti-RGG.

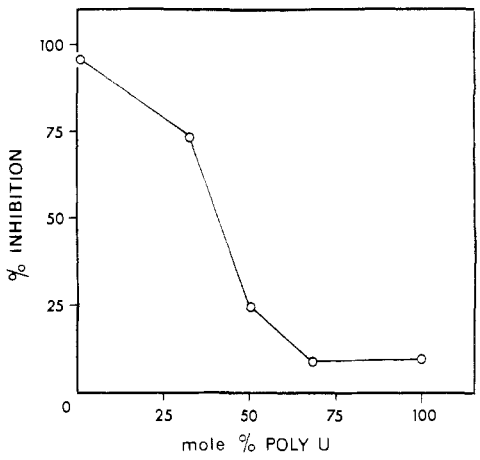

FIGURE 4: The effect of poly(A)·poly(U) mixtures on the binding of total RNA by antibody. Poly(A) (2.5 μg) was mixed with 1.2, 2.4, and 4.8 μg of poly(U), and incubated at 4° for 1 hr. Anti-A (1:10) (50 μl) was added and incubated for an additional 30 min before the addition of 1.7 μg of tritiated total RNA. Control points were 2.5 μg of poly(A) (0 mol % poly(U)) and 4.8 μg of poly(U) (100 mol % poly(U)).

manner. Poly(U) had essentially no effect upon the reaction. The maximum inhibition was 20%. The order of addition of reagents is in fact immaterial, since in Table IV it was shown that prior incubation of poly(U) with anti-A gave rise to an 18% inhibition of RNA binding.

Discussion

The reactivity of RNA with antibodies to adenosine, but not with antibodies directed against cytidine, guanosine, or uridine, was surprising. It was expected, if a reaction between RNA and antinucleoside antibody could be demonstrated, that all four bases, as was found with DNA, would be reactive. The hydrogen-bonded bases of native DNA had previously been shown to be incapable of reacting with antinucleoside antibody (Erlanger and Beiser, 1964; Garro *et al.*, 1968), but they become available to react with antibody upon heat denaturation of the DNA. A majority of the bases of ribosomal and bacteriophage RNA are hydrogen bonded by DNA type base pairing (A with U, G with C); the pairing results from folding of the polynucleotide chain to bring bases into positions in which they could hydrogen bond. Interspaced among these folded regions are non-base-paired regions. In DNA, it has been shown that antibodies react with unpaired cytosines in an otherwise double-stranded molecule (Garro *et al.*, 1968). It was therefore expected that antinucleoside antibody would detect residues in RNA that were not hydrogen bonded.

The inability of the antisera, other than anti-A, to react with RNA did not result from differences in antibody content of the antisera. The antisera, at the dilutions tested with RNA, contained comparable concentrations of DNA-reactive antibody. This was determined both from precipitin curves between the binding curves of the antisera with IDNA (Figure 2).

It is possible that antisera to adenosine are of considerably higher affinity than antisera to the other nucleosides. The affinity constants of the antisera in their reaction with homologous haptens have not been determined. However, relative affinity constants of DNA–antinucleoside antibody binding were estimated from saturation curves (Rosenberg *et al.*[2]) by comparing the concentrations of free DNA at which 50% of the antibody combining sites were filled. Anti-A, anti-C, and anti-G had similar affinities, whereas anti-U was less by a factor of ten. Therefore, it seems unlikely that affinity plays an important role in explaining why only anti-A binds to RNA.

The structure of various RNA species has been investigated intensively over the past several years (Zachau, 1969; Attardi and Amaldi, 1970). Most RNA species consist of one linear single-stranded polynucleotide chain. These include tRNA, ribosomal RNA, and most RNAs of viral origin. RNA structure is classified on several levels: primary, the linear sequency of bases; secondary, hydrogen bonding and stacking of bases; and tertiary, the overall folding of the molecule into its three-dimensional form found in solution. All these levels of structure must be considered in attempting to explain the binding of RNA by antisera to adenosine. This reactivity cannot be explained solely by the base composition of the RNAs tested because they do not contain adenosine as the preponderant base (Attardi and Amaldi, 1970).

Poly(U) interacts with *E. coli* RNA, and internal adenylate sequences in the RNA have been suggested as the site of such binding (Hayes *et al.*, 1966; Millar and MacKenzie, 1967). The interaction of poly(U) with rRNA did not prevent the binding of the RNA by anti-A. However, poly(U) can prevent binding of poly(A) by anti-A as shown by the fact that poly(A)·poly(U) did not interfere with the ability of anti-A to react with RNA. The primary sequence of *E. coli* rRNA has been partially determined (Fellner *et al.*, 1970), and so far does not support the notion of long runs of adenosine. The interaction between rRNA and poly(U) may, therefore, be confined to short runs of adenosine with a concomitant low association constant as compared with poly(A)·poly(U). If this association constant is much lower than that of antibody with RNA, poly(U) would not inhibit the observed binding of antibody with RNA.

The secondary structure of tRNA has been better characterized than that of any other RNA (Zachau, 1969). The cloverleaf model has been established as the best representation of the secondary structure of tRNA. The majority of the bases are hydrogen bonded, and others, at specific areas which loop out, are non-base-paired. A base sequence of biological importance, the anticodon, is always in such a loop. The anticodon loop, the aminoacylation site, and possibly the undefined sites for synthetase recognition and ribosome binding could have been available for binding to antibody. There is evidence that some adenosine residues are exposed (Cramer *et al.*, 1968). Perphthalic acid which reacts specifically with adenosine reacts with some adenosine residues in native tRNA. However, adenosines available for reaction with perphthalic acid may not be equally available for reaction with a macromolecule such as an antibody. Since the tRNA molecule is compactly folded, its tertiary structure may prevent antibody from binding with the bases. tRNA undergoes a conformational change upon acylation (Chatterjee and Kaji, 1970); it would be of interest to determine whether antinucleoside antibody can bind to aminoacylated tRNA.

Antiuridine antibodies have been reported to react with RNA by other investigators. Karol and Tanenbaum (1967)

[2] Rosenberg, B. J., Erlanger, B. F., and Beiser, S. M. unpublished data.

and Sela *et al.* (1964) immunized animals with uridinecarboxylic acid derivatives and found that the antisera reacted with denatured RNA. Karol and Tanenbaum also noted reactions with native RNA and with tRNA digested with RNase. The fact that the uridine conjugates used by both groups were different from the uridine–bovine serum albumin conjugate studied here might account for the disparity in observed antibody reactivity. Although all antisera are specific for uridine, conjugation at the 5′ position may expose a different antigenic portion of the ribonucleoside.

The decreased reactivity of formaldehyde-denatured RNA with anti-A could be attributed to modification of adenosine residues. Formaldehyde reacts primarily with the amino groups (Fraenkel-Conrat, 1954) that are present in adenosine, guanosine, and cytidine, but not in uridine. The amino group of adenosine, so altered, might not react with anti-A. The decrease in reactivity of formaldehyde-denatured RNA is in accord with the results of Garro *et al.* (1971), who demonstrated that HCHO-denatured DNA did not react as well with anti-cytidine as did heat-denatured DNA, but the reactions were reversed with anti-thymidine; HCHO-denatured DNA reacted better than did heat-denatured DNA. Furthermore, formaldehyde-treated cytidine–bovine serum albumin did not fix complement with anti-cytidine, whereas thymidine–bovine serum albumin, so treated, fixed complement with anti-thymidine as effectively as did untreated thymidine–bovine serum albumin. A consequence of the interaction of formaldehyde with the bases is the complete disruption of RNA secondary structure (Boedtker, 1967; Feldman, 1967). It would be expected that uridine residues in formaldehyde-treated RNA should be available to antibody. There is a slight but probably insignificant reactivity of HCHO-denatured RNA with anti-U. Uridine did not inhibit the reaction, thus indicating further its lack of specificity. It is also possible that the RNA was degraded by the formaldehyde denaturation conditions used (Boedtker, 1968; Fenwick, 1968). The RNA was Cl_3CCOOH precipitable but limited digestion could have occurred.

The specificity of the reaction of antinucleoside antisera with RNA has not yet been explained. The importance of the conformation of polynucleotide chains in their reaction with various antinucleic acid antibodies has been well documented (Seaman *et al.*, 1965; Schwartz and Stollar, 1969; Lacour *et al.*, 1968; Panijel *et al.*, 1966). For example, Panijel and coworkers observed that antiribosome antisera, which precipitates all ribopolymers, is best precipitated by polymers that contain the least secondary structure. A study of homopolynucleotides of known conformation and of heteropolynucleotides of known base composition may provide insights into the types of conformation and base sequence with which antibody can react.

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