

ANTIBODIES TO POLYNUCLEOTIDES IN HUMAN SERA:  
ANTIGENIC SPECIFICITY AND RELATION TO DISEASE\*

By D. KOFFLER, M.D., R. CARR, M.D., V. AGNELLO, M.D., R. THOBURN, M.D.,  
AND H. G. KUNKEL, M.D.

(From The Rockefeller University, New York 10021, and the Department of Pathology,  
Mount Sinai School of Medicine of the City University of New York,  
New York 10029)

(Received for publication 12 April 1970)

A variety of anti-polynucleotide antibodies directed against native DNA [NDNA]<sup>1</sup> (1-3), single-stranded DNA [SDNA] (4-6), double-stranded RNA [DSRNA] (7-9), polyribonucleotides (10), ribonucleoprotein derived from thymic nuclear extracts (11), and ribosomes (12) have been detected in human sera. Each of these antibodies is characteristically found at high levels in the sera of patients with systemic lupus erythematosus (SLE), and except for anti-NDNA antibodies, they also have been observed in the sera of patients with a variety of diseases associated with active tissue destruction (8). Experimental studies indicate that an appropriate immunogen may induce antibodies to SDNA (13, 14), DSRNA (15), polyribonucleotides (16, 17), and ribosomes (18), but not to NDNA. Anti-NDNA antibodies are found only in the spontaneous NZB/W disease of mice (19, 20). These observations indicate that most polynucleotides are potentially immunogenic.

Although it is known that antibodies to NDNA and SDNA are heterogeneous, the cross-reacting specificities and the interrelationship of these antibodies in human sera have not been defined. Less information is available concerning the specificities of antibodies directed against DSRNA and ribonucleoprotein and their cross-reactions with other polynucleotides. The major objective of the present study was to elicit information concerning the types of immunogens most likely responsible for inducing the formation of the various anti-polynucleotide antibodies found in human sera.

*Materials and Methods*

*Polynucleotides Employed for Immunological Tests.*—Native calf thymus DNA (NDNA), *Escherichia coli* DNA, *Clostridium perfringens* DNA and salmon sperm DNA (Worthington

\* Supported by U.S. Public Health Service Grants AM 04761 and AM 13721.

<sup>1</sup> *Abbreviations used in this paper:* anti-A, rabbit anti-adenosine-bovine albumin; anti-T, rabbit anti-thymidine-bovine albumin; DSRNA, double-stranded RNA; FTRBC, formalinized O Rh positive tanned red blood cells; ME, mercaptoethanol; NDNA, native DNA; PBS, phosphate-buffered saline; RBC, red blood cells; SDNA, single-stranded DNA; SLE, systemic lupus erythematosus; UVDNA, ultraviolet irradiated DNA.

Biochemical Corp., Freehold, N. J.), and *Micrococcus lysodeikticus* DNA (Miles Laboratories, Inc., Elkhart, Ind.). Poly A, Poly U, Poly G, Poly C, Poly A·Poly U, and Poly I·Poly C (Miles Laboratories, Inc.). Poly dA, Poly dT, Poly dG, and Poly dC (Biopolymers, Inc., Chagrin Falls, Ohio).

Native SV 40 DNA was generously supplied by Dr. R. Klett. Adenovirus 2 DNA and T<sub>4</sub> phage DNA were generously supplied by Dr. W. Doerfler and Dr. W. Schweiger, respectively. Ultraviolet irradiated DNA (UVDNA) was prepared following the method of Tan et al. (21). Denatured DNA was prepared by heating native DNA at 100°C for 10 min and transferring directly to an ice bath.

Ribosomes were prepared from cytoplasmic supernatant of Krebs ascites tumor cells (22) by centrifugation at 100,000g for 1 hr. The cytoplasmic supernatant was kindly supplied by Dr. A. O. Pogo.

*Serum Specimens.*—Human sera were obtained from patients with the following diseases: SLE [from 60 patients], rheumatoid arthritis (32), chronic active hepatitis (43), procainamide administration<sup>2</sup> (19), primary biliary cirrhosis (20), infectious mononucleosis (20), chronic glomerulonephritis (40), and carcinoma of the cervix (13). In addition, 280 normal sera and 65 sera were obtained randomly from hospitalized patients with a variety of diseases.

Rabbit anti-sera were prepared to adenosine-bovine albumin (anti-A) and thymidine-bovine albumin (anti-T) conjugates (13). The following polynucleotides were complexed to methylated bovine serum albumin (14) in the proportion of 1 mg of polynucleotide to 1 mg of albumin and emulsified in complete Freund's adjuvant before immunization of rabbits: NDNA, SDNA, Poly A·Poly U, Poly I·Poly C, and UVDNA. Two rabbits were immunized with each antigen. Serial serum specimens were obtained from 25 patients with SLE studied for periods of 6 months to 4 yr.

*Procedures.*—Actinomycin-labeled DNA was prepared and utilized in a gamma globulin-binding assay as previously described (23). The amount of labeled DNA bound to red blood cells (RBC) was determined by solubilizing RBC from 1 cc of a 4% suspension in NCS reagent (Amersham-Searle Corp., Des Plaines, Ill.) and decolorizing with a saturated solution of benzoyl peroxide. Agar gel diffusion was performed in 0.6% agarose at pH 7.4 using well sizes as previously described (24).

Hemagglutination tests employed formalinized O Rh positive red blood cells (25) which were tanned (26) [FTRBC] and incubated with polynucleotide antigens. Equal volumes of antigen in McIlvaine's buffer and a 3% suspension of FTRBC were used. Optimal time for incubation, pH of the buffer system, and concentration of the antigen were determined for each system. The amount of tritiated actinomycin-labeled NDNA and SDNA bound to FTRBC with different buffer systems and incubation times is shown in Table I.

Antigen concentrations of 50 µg/ml were used for NDNA and UVDNA. SDNA coats autoagglutinate at these concentrations, and therefore, lower concentration (10 µg/ml) of the actinomycin-complexed form of SDNA was used (23). Poly A·Poly U, Poly I·Poly C, and Poly A were incubated with cells at 20 µg/ml. All coats were incubated for 60 min except for SDNA which was incubated for 15 min.

Human and rabbit sera were inactivated for 30 min at 56°C. A 1:4 dilution of 0.2 cc of serum was diluted serially with 1% normal rabbit serum in pH 7.2, 0.02 M phosphate-buffered saline (PBS) and 0.1 cc PBS was added to each tube for a total of 0.3 cc. 1 drop (0.05 cc) of a 3% suspension of coated cells was added to each tube. The settling pattern was read after 2 hr.

The specificity of the hemagglutination reaction was determined by inhibition of the hemagglutination reaction with appropriate antigen. Before serial dilution of the test serum, 0.1 cc of antigen was substituted for the PBS added to the first tube. The specificity of the NDNA coat was determined by the absence of reactivity of NDNA-coated cells with rabbit

<sup>2</sup> Patients with evidence of anti-nuclear antibodies.

antisera (anti-T, anti-A, and anti-SDNA) and with two SLE sera containing antibodies reactive with SDNA. Antibodies to SDNA were assayed by prior adsorption of sera with NDNA so that all reactivity with NDNA-coated cells was eliminated.

Quantitative inhibition studies were performed with an end point dilution of antiserum added to a serial dilution of antigen. The tube at which no significant inhibition of hemagglutination occurred was considered maximal inhibition by antigen in terms of micrograms per milliliter or nanograms per milliliter. The test was used to quantitate and compare the inhibitory activity of different polynucleotides with both human and rabbit antisera.

Soluble saline extract of calf thymus nuclei was prepared as previously described (27) and coated on fresh human type O Rh positive tanned red blood cells for assay of anti-ribonucleo-protein antibodies.

TABLE I  
*Binding of Native DNA and Single-Stranded DNA to Formalinized, Tanned RBC*

Antigen incubation	Buffer system	Time of incubation	Amount bound to 1 cc of 4% RBC
	(pH)	(min)	( $\mu$ g)
SDNA (10 $\mu$ g)	4.7	5	1.5
		15	4.9
		30	6.0
		60	5.9
DNA (50 $\mu$ g)	4.9	5	4.5
		15	6.0
		30	7.5
		60	7.0
	7.2	60	1.7
	4.9*	60	3.4
DNA (10 $\mu$ g)	4.9	5	1.9
		15	2.1
		30	2.7
		60	3.6

\* Formalinized only.

Enzymatic treatments: Ribonuclease incubation of nuclear extract containing ribonucleo-protein was performed as previously described (24). Protease conjugated to carboxymethyl-cellulose (Enzite-Pro, Miles Laboratories, Inc.) was hydrated at 37°C for 1 hr and incubated with nuclear extract for 1 hr at 37°C with agitation, in ratio of 1 mg of Enzite-Pro to 2 mg of protein.

Mercaptoethanol treatment was performed on selected sera by treating serum with 0.2 M mercaptoethanol for 1 hr at 37°C. DEAE-column chromatography was performed for the isolation of fractions containing Sm antigen (28). A linear 10-40% sucrose gradient in pH 7.2 phosphate-buffered saline was performed for separation of 7S and 19S gamma globulins.

## RESULTS

### *Specificities of Anti-Polynucleotide Antibodies*

*Sensitivity of Test Systems.*—Sera with precipitin reactions with NDNA uniformly gave positive hemagglutination and DNA-binding tests. The hemag-

glutination assay was less sensitive than the DNA-binding test for detecting anti-NDNA antibodies in sera without precipitins. 17 of 24 of these sera showed reaction with labeled DNA whereas 9 of 24 showed positive hemagglutination reactions. Such sera, with negative hemagglutination tests, had low levels of DNA-binding, between 10 and 20%. Several sera showed greater hemagglutinating activity than DNA-binding. A comparison of the results of the hemagglutination test and DNA-binding assay for individual sera is shown in Fig. 1.

*Anti-NDNA and Anti-SDNA Antibodies.*—The reactivity of anti-NDNA antibodies with NDNA, denatured DNA, and various synthetic polydeoxyribonucleotides was studied primarily with the hemagglutination-inhibition

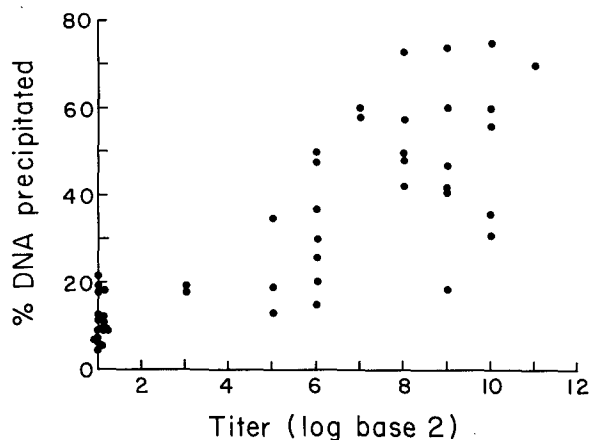


FIG. 1. Comparison of % actinomycin-labeled DNA precipitated by  $(\text{NH}_4)_2\text{SO}_4$  and hemagglutination titers of 47 SLE sera.

system using NDNA as the red cell coat. Single-stranded DNA inhibited this system equally as well or better than NDNA in 16 of 17 sera tested (Table II). One serum (UH) showed stronger reaction with NDNA. Less reactivity with polydeoxyribonucleotides was demonstrated. Poly dA was the most reactive nucleotide with anti-NDNA antibody-coated cells, whereas dT, dG, and dC were less reactive. In contrast, polyribonucleotides, both single- and double-stranded (Poly A, Poly G, Poly C, Poly U, Poly A·Poly U, and Poly I·Poly C) were unreactive with anti-NDNA antibodies in this system.

No clear differences in reactivity among bacterial, viral, salmon sperm, and mammalian DNA were demonstrated (Table III). It was found that native circular SV 40 DNA did not react as well as native calf thymus DNA. After denaturation, however, SV 40 DNA reacted comparably with denatured mammalian and bacterial DNA. Glucosylated T<sub>4</sub> phage DNA was also less reactive only before denaturation. No correlation between the guanosine-

cytosine content of the various DNA preparations (29) and reactivity with anti-NDNA antibody was evident.

The specificity of antibodies reactive with SDNA was studied primarily by agar gel diffusion in order to differentiate antibodies reactive with individual bases and with common determinants between NDNA and SDNA. The sera were divided into two groups, those giving precipitin reactions with SDNA and those giving precipitin reactions with both SDNA and NDNA. Both groups of sera gave clear precipitin reactions with different synthetic polydeoxyribo-

TABLE II  
*Hemagglutination-Inhibition Reactions of NDNA, SDNA, and Synthetic Polydeoxyribonucleotides Using 17 Different SLE Sera*

	NDNA	SDNA	dA	dC	dG	dT
GS	312*	20	—‡	—	—	—
LM	625	312	2500	2500	—	1250
MC	625	39	156	—	—	5000
UH	1	625	2500	—	—	—
IS	625	156	—	—	—	—
SV	39	39	2500	—	—	—
JB	5000	312	—	—	—	—
BA	2500	39	625	39	1250	156
BR	39	39	—	—	—	—
JB	312	5	2	—	—	—
EG	39	1	20	312	2500	1250
PW	20	39	78	—	—	—
DS	625	625	156	—	—	—
AE	78	10	—	—	—	—
AH	2500	39	—	—	—	—
EU	156	78	—	—	—	—
DB	312	156	—	—	—	—

\* Minimal concentration of antigen (ng/ml) inhibiting positive hemagglutination reaction between test serum and native DNA-coated cells.

‡ Represents no inhibition at 20,000 ng/ml.

nucleotides (Table IV). Only four of the 26 sera showed no reactivity with any of the polydeoxyribonucleotides as was the case for the control sera. Several sera showed slight spurring of SDNA over NDNA or NDNA over SDNA, but no serum was reactive only with NDNA. The precipitin reactions with synthetic polydeoxyribonucleotides were useful in defining the multiple specificities of antibodies reactive with SDNA. Antibodies with single-base specificities were observed in addition to antibodies directed against determinants common to one or more polydeoxyribonucleotides and SDNA.

Two sera illustrating several specificities are shown in Fig. 2. Serum Ro (Fig. 2 A) contained antibodies specific for dC and dT. The weakening of the dC precipitin reaction after absorption with dT indicates that antibodies re-

TABLE III  
*Hemagglutination-Inhibition Reactions of Mammalian, Bacterial, Viral, and Salmon Sperm DNA Using Six Different Sera*

	UH	JB	PW	AE	BA	EG
CT (native)	312*	312	39	2,500	2,500	39
CT (denatured)	5,000	20	78	312	39	10
SV 40 (native)	1	312	625	—‡	10,000	1,250
SV 40 (denatured)	1,250	625	39	625	10	625
Ad. 2 (native)	5,000	10,000	5	2,150	625	156
Ad. 2 (denatured)	—	625	<1	625	10	78
T <sub>4</sub> phage (native)	2,500	—	156	—	10,000	5,000
T <sub>4</sub> phage (denatured)	—	10,000	2	10,000	39	1,250
T <sub>4</sub> phage (native) without glucose	156	10,000	<1	5,000	78	156
<i>E. coli</i> (native)	625	625	156	2,500	312	156
<i>E. coli</i> (denatured)	10,000	625	5	5,000	10	78
<i>Cl. perf.</i> (native)	1,250	312	312	1,250	39	625
<i>Cl. perf.</i> (denatured)	5,000	78	2	1,250	20	156
<i>M. lys.</i> (native)	5,000	5,000	156	5,000	312	156
<i>M. lys.</i> (denatured)	5,000	1,250	20	5,000	39	20
Salmon testes (native)	312	78	1,250	2,500	625	78
Sal. testes (denatured)	625	39	156	5,000	10	39

\* Minimal concentration of antigen (ng/ml) inhibiting positive hemagglutination reaction between test serum and native DNA-coated cells.

‡ Represents no inhibition at 20,000 ng/ml.

TABLE IV  
*The Reaction of SLE Sera Containing Precipitins to DNA with Polydeoxyribo- and Polyribonucleotides*

	No. of Patients	Polydeoxyribonucleotides				Polyribonucleotides				
		dC	dT	dA	dG	rC	rU	rA	rG	rI
Sera with precipitins to NDNA and SDNA	1	+	+	—	—	—	—	—	—	+
	3	+	+	—	—	—	—	—	—	—
	3	+	—	—	—	—	—	—	—	—
	4	—	+	—	—	—	—	—	—	—
	4	—	—	—	—	—	—	—	—	—
Sera with precipitins to SDNA only	2	+	+	+	—	—	—	+	—	+
	1	+	+	—	—	—	—	—	—	+
	4	+	+	—	—	—	—	—	—	—
	1	+	—	—	—	—	—	—	—	—
	3	—	+	—	—	—	—	—	—	—
Totals	26	15	18	2	0	0	0	2	0	4
		58%	65%	8%	0	0	0	8%	0	15%

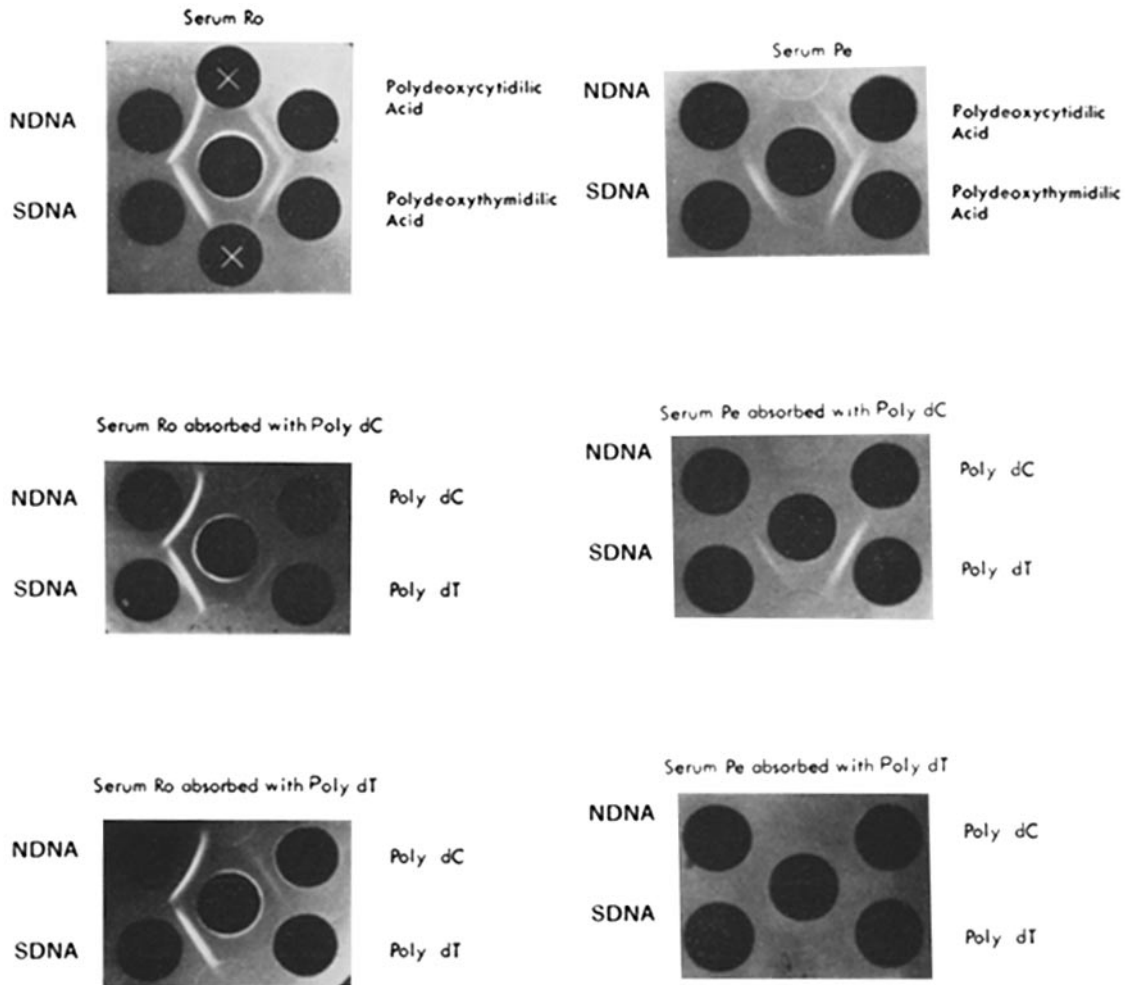


FIG. 2 A. Studies on serum Ro. The lines formed with Poly dC and Poly dT show faint spurs through each other. Absorption with either polynucleotide removes reactivity with that polynucleotide and weakens but does not eliminate reactivity with the other.

FIG. 2 B. Studies on serum Pe. The line formed with Poly dT spurs over the line with Poly dC, and absorption with Poly dC removes only the line with Poly dC. Absorption with Poly dT removes all reactivity.

active with common determinants on polydeoxyribonucleotides are also present. Absorption with both dC and dT did not eliminate the reaction with NDNA or SDNA, indicating the presence of a third population of antibodies. A second serum, Pe (Fig. 2 B) showed one population of antibodies specific for dT; a

second reactive with dT, dC, and SDNA; and a third reactive with dC and SDNA. Absorption with dC eliminates the reactions with dC and weakens the precipitin reaction with SDNA. Absorption with dT removes reactions with SDNA, dC, and dT. Another pattern was observed in a third serum (Go) which contained antibodies reactive with dC and dT. Absorption with either eliminates reaction with both and with SDNA, but not with NDNA. This serum contains antibodies to common determinants of single-stranded polydeoxyribonucleotides and to SDNA.

*Anti-DSRNA.*—Antibodies reacting with DSRNA have been demonstrated by agar gel diffusion and hemagglutination tests, raising the possibility that these antibodies were specific for the double-stranded conformations of RNA. Antibodies reactive with Poly A·Poly U, however, were found to cross-react not only with Poly I·Poly C, but with Poly A and denatured DNA (Table V). Poly I·Poly C showed greatest cross-reactivity; Poly A and denatured DNA

TABLE V  
*Inhibition of Hemagglutination Reaction of Anti-Poly A·Poly U Antibodies by Polynucleotides*

Inhibition	Antigen			
	Poly A·Poly U	Poly I·Poly C	SDNA	Poly A
Complete	20*	11	5	8
Partial	2	5	3	4
None	0	6	14	10

\* Number of SLE sera.

showed less cross-reactivity. Most sera inhibited by denatured DNA were also inhibited by Poly A, whereas lack of inhibition by denatured DNA was usually accompanied by failure of Poly A to inhibit. Rabbit anti-sera, however, showed greater specificity for the homologous double-stranded polyribonucleotide. The quantitation of the inhibitory activity of Poly A·Poly U, Poly I·Poly C, Poly A, and denatured DNA in selected human sera and in four rabbit sera is shown in Table VI. None of the human sera showed complete specificity for Poly A·Poly U or Poly I·Poly C, whereas three of the four rabbit sera exhibited considerably greater specificity for the homologous immunogen.

*Anti-Ribonucleoprotein.*—Several nuclear antigens have been identified in a soluble fraction obtained by extraction of nuclei with buffered saline solutions. The present study measured antibodies to an antigen which has been previously demonstrated to be active in a hemagglutination assay (11, 27). This antigen is sensitive to ribonuclease and protease and is distinct from another nuclear antigen, the Sm antigen, which is not sensitive to ribonuclease. Fractions obtained from DEAE-column chromatography which contain the Sm antigen do



not contain the ribonucleoprotein. Column isolated fractions containing the Sm antigen were not active in a direct hemagglutination assay system.

Anti-ribonucleoprotein antibodies showed precipitin reactions with nuclear extract utilizing agar gel diffusion. Sm antigen and ribonucleoprotein showed independent precipitin lines with sera containing antibodies to both antigens. Several sera which had high hemagglutination titers ( $>1:500,000$ ) of anti-ribonucleoprotein antibodies exhibited precipitin reactions only when diluted to 1:50 or 1:100, indicating that precipitins were not observed when a serum contained significant antibody excess.

Hemagglutinating activity of anti-ribonucleoprotein antibody was inhibited

TABLE VI  
*Quantitation of Hemagglutination-Inhibition Reaction of Anti-Poly A·Poly U Antibodies*

	Inhibiting antigens			
	Poly A ·Poly U	Poly I ·Poly C	SDNA	Poly A
SLE sera				
BA	0.3*	0.15	—‡	—
BR	0.7	0.7	—	12
PW	6	6	—	—
CA	3	—	—	0.3
PA	0.07	0.7	25	3
Rabbit anti-sera				
Anti-Poly A·Poly U (331)	0.3	—	—	—
Anti-Poly A·Poly U (332)	0.07	—	—	—
Anti-Poly I·Poly C (333)	6	0.7	—	—
Anti-Poly I·Poly C (334)	—	3	—	—

\* Minimal concentration of antigen ( $\mu\text{g}/\text{ml}$ ) inhibiting positive hemagglutination reaction between test serum and Poly A·Poly U-coated cells.

‡ Represents no inhibition at 100  $\mu\text{g}/\text{ml}$ .

by a ribonuclease-protease-sensitive antigen obtained from nuclear extract. In addition, ribosomes isolated from cytoplasmic supernatant were reactive with these antibodies. NDNA, SDNA, Poly A·Poly U, Poly I·Poly C, Poly A, and nuclear fractions containing Sm antigen were not reactive with anti-ribonucleoprotein antibodies.

*Interrelationships of Anti-Polynucleotide Antibodies in Serial Studies of SLE Patients*

Antibodies to NDNA, SDNA, and DSRNA were found to appear some time during the course of SLE in most patients (Table VII). In contrast, anti-ribonucleoprotein antibodies were absent from eight of 25 serial studies. Peaks of NDNA antibody were usually correlated with episodes of disease activity and serum complement depression. High titers of anti-SDNA and anti-Poly

A·Poly U antibodies frequently occurred during periods of clinical activity, but rises in the titers of these antibodies also occurred during clinically quiescent periods. Approximately one-half of the patients with anti-ribonucleoprotein antibodies showed no significant fluctuation of antibody titers, which persisted

TABLE VII  
*Serial Studies of 25 Patients with SLE*

	Antibody			
	DNA	SDNA	Poly A ·Poly U	RNA Pr
All sera positive	3	3	2	7
All sera negative	3	2	6	8
Peaks of antibody activity	19	20	17	10

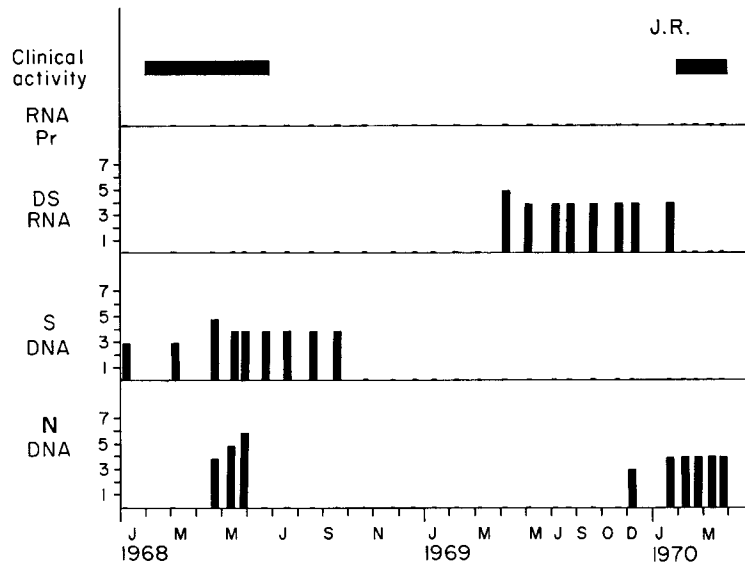


FIG. 3. Two episodes of clinical activity associated with rises in anti-NDNA antibody titers. Steroid treatment was associated with a rapid decline in clinical symptoms with a concomitant decrease in anti-NDNA antibody. Anti-SDNA antibodies persisted after clinical symptoms abated. Anti-DSRNA antibodies appeared during a clinically quiescent period. Note the absence of anti-ribonucleoprotein antibodies in all sera tested.

in all sera tested. These antibodies did not disappear during periods of inactive disease or after steroid therapy. Two serial studies showing a typical relationship between the antipolynucleotide antibodies and clinical activity are shown in Figs. 3 and 4. These patients illustrate the sporadic persistence of anti-SDNA and anti-DSRNA antibodies during clinically quiescent periods and also demonstrate the concomitant rise in titer which may occur when the titer

of anti-NDNA antibodies increases. Anti-ribonucleoprotein antibodies were present in high titer throughout the course of one patient and absent from another.

In three patients with classical SLE, anti-NDNA antibodies were not observed. In one patient with severe renal disease, anti-NDNA, SDNA, and DSRNA antibodies were absent and anti-ribonucleoprotein antibodies persisted in high titer. A second patient with renal disease did not have anti-NDNA antibodies but did demonstrate antibodies to other polynucleotides.

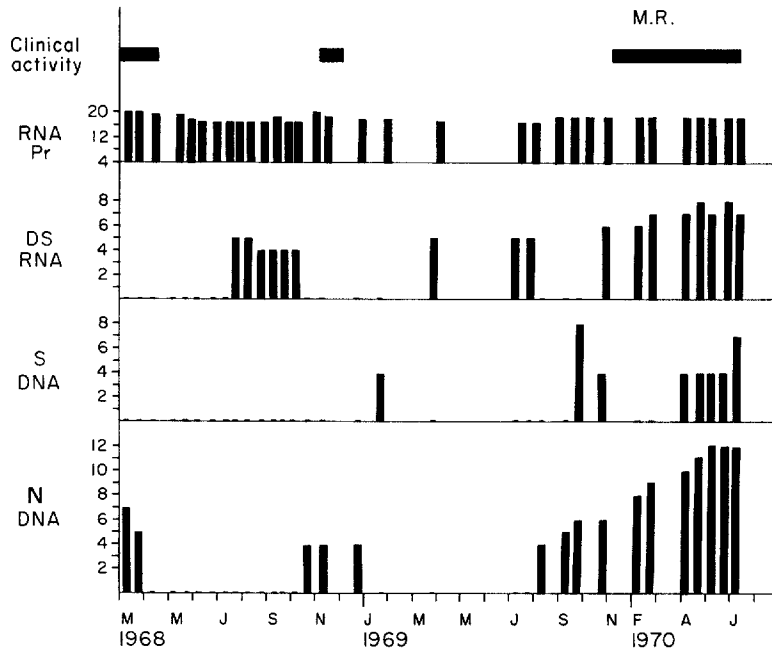


FIG. 4. Three episodes of clinical activity associated with rises in anti-NDNA antibody. The third clinical episode of activity shows parallel increases in SDNA, DSRNA, and NDNA antibody titers. Anti-DSRNA antibodies persist during quiescent periods. Note anti-ribonucleoprotein antibodies persist in high titer throughout the course of the disease.

#### *Distribution of Antibodies to Polynucleotides in Human Sera in Different Conditions*

Antibodies to NDNA are mainly restricted to patients with SLE, whereas antibodies to SDNA, Poly A·Poly U, and Poly I·Poly C are found in sera obtained from patients with other diseases (Table VIII). Antibodies to SDNA are present in most patients with SLE and are also present in high incidence in individuals treated with procainamide, rheumatoid arthritis, infectious mononucleosis, and chronic active hepatitis. Antibodies reactive with DSRNA preparations are present in lower incidence in diseases other than SLE. Anti-

bodies to ribonucleoprotein were limited mainly to patients with SLE, rheumatoid arthritis, and mixed connective tissue disease.

Antibodies reactive with Poly A were found in 14 of 60 SLE sera with a titer of 4.6 (log base 2). Inhibition of the reaction with Poly A was present in all cases. Poly A·Poly U inhibited equally as well as Poly A in 12 of the 14 sera tested.

SLE sera were also studied for the presence of antibodies specific for UVDNA. 11 sera obtained from patients with photosensitivity reactions and 46 serial serum specimens from six patients with active SLE showed no evidence of antibody to UVDNA. Two rabbit anti-sera prepared to UVDNA used as positive controls had titers of 1:160 and 1:320. The reaction of these sera was

TABLE VIII  
*Incidence of Antibodies to Polynucleotides*

Sera		Antibodies positive %				
Source	No. tested	NDNA	SDNA	Poly A·Poly U	Poly I·Poly C	RNA Pr
SLE	60	60 (7.3)*	87.0 (5.1)	55.0 (5.3)	21.6 (5.0)	66.6 (12.0)
Normal	110	0.3 (5.0)‡	3.7 (4.0)	0	1.1 (4.0)	0
Hospital§	65	0	16.8 (4.3)	7.7 (4.1)	9.2 (4.3)	3.1 (9.0)
Procainamide	19	0	52.6 (4.5)	10.5 (6.0)	5.3 (7.0)	0
Chronic active hepatitis	43	2.3 (3.0)	58.2 (5.1)	27.2 (6.0)	—	0
Infectious mononucleosis	20	0	40.0 (5.2)	10.0 (4.0)	5.0 (4.0)	0
Rheumatoid arthritis¶	32	3.1 (3.0)	39.5 (5.5)	3.0 (5.0)	0	15.5 (10.0)
Chronic glomerulo-nephritis	40	2.5 (4.0)	7.5 (4.6)	0	—	2.5 (9.0)
Primary biliary cirrhosis	20	0	15.0 (3.6)	—	—	—
Carcinoma of cervix	13	0	0	0	0	0

\* Mean titer of group of serums expressed as log base 2, shown in parenthesis.

‡ 280 normal sera were tested.

§ Random hospital serums obtained from patients with a variety of diseases.

|| 11 sera were tested.

¶ Two patients had clinical evidence of mixed connective tissue disease in addition to rheumatoid arthritis.

completely inhibited by UVDNA, minimally inhibited by SDNA, and showed no inhibition by NDNA.

#### *Characteristics of Anti-Polynucleotide Antibodies*

Anti-DNA antibodies were mainly mercaptoethanol (ME) insensitive. Of 18 sera tested, six sera showed no change in titer, 10 showed a moderate decrease, and the reactivity of two sera was eliminated by ME treatment. Sucrose gradient ultracentrifugation of one serum (MR) also demonstrated activity of anti-NDNA antibodies in both 19S and 7S fractions. In contrast, the titers of anti-ribonucleoprotein antibodies were unaffected by ME treatment. Anti-SDNA and anti-Poly A·Poly U antibodies were predominantly ME sensitive. These sera showed a marked decrease in titer or loss of reactivity following ME treatment. Several high-titered sera, with anti-SDNA and anti-Poly A·Poly U antibodies, however, showed minimal change after ME treatment,

indicating that certain sera contained antibody populations unaffected by incubation with ME.

#### DISCUSSION

Previous studies have demonstrated that anti-NDNA and SDNA antibodies are heterogeneous (30-33) and that antibodies with specificity for both the native conformations as well as exposed nucleotides of the denatured molecule are present in the sera of patients with SLE. The present studies with the hemagglutination-inhibition system have permitted a broader survey and indicate that most antibodies reactive with NDNA react equally as well or more avidly with SDNA. Only one of 17 sera tested showed predominant specificity for the double-stranded conformation. Similar antibodies reactive with NDNA have been described in the NZB/W mice with a disease similar to SLE (19, 20). They have not as yet been produced in rabbits after various types of immunization which result in antibodies specific for SDNA. The possibility is raised by the present experiments that in susceptible hosts, i.e. patients with SLE and NZB/W mice, some type of SDNA is the immunogen involved but that here antibodies reactive with NDNA are also produced.

The reaction of anti-NDNA antibodies with intact circular SV 40 DNA indicates that interaction of antibodies with NDNA does not require strand breaks or significant denaturation of the native molecule. Although NDNA preparations may contain small amounts of SDNA (34), several experiments indicate that in the present experiments the SDNA was not responsible for the NDNA reactions. Rabbit anti-sera to SDNA determinants and SLE sera containing antibodies directed only against SDNA did not cause agglutination of NDNA-coated cells. The weaker reactions with intact circular DNA were most probably related to a unique conformation, since the denatured strands reacted in a fashion similar to other types of DNA in the NDNA system. The reaction of mammalian, bacterial, viral, and salmon sperm DNA with DNA antibodies showed a variable pattern. Sera reacting more strongly with SDNA than NDNA usually exhibited similar reactions with several types of DNA. Although the guanosine-cytosine content ranged widely for the various types of DNA, the antibodies were not able to distinguish differences among the DNA substrates. These studies indicate that neither host tissue, bacterial, nor viral nucleotides have preferential reactivity with anti-NDNA antibodies found in human sera.

Precipitin tests with synthetic polydeoxyribonucleotides revealed antibodies with base specificity in addition to antibodies reactive with determinants common to SDNA and polydeoxyribonucleotides, and to SDNA and NDNA. Inhibition with polydeoxyribonucleotides of precipitin and hemagglutination reactions of one serum (Ro) failed to eliminate reactivity with NDNA, whereas absorption with SDNA inhibited both the precipitin and hemagglutination

reaction with NDNA. The reaction of another serum (Go) with NDNA was not inhibited by Poly dT and dC, but did show elimination of the reaction with NDNA by absorption with SDNA. Hemagglutination inhibition also showed that anti-NDNA antibodies had more avid reaction with SDNA than with polydeoxyribonucleotides. Therefore, both precipitin and hemagglutination inhibition tests indicate that SDNA contains determinants for most antibodies reactive with NDNA and that synthetic polydeoxyribonucleotides, although showing many properties of SDNA, are not "complete" antigens either because a unique conformation or nucleotide sequence is needed for the reaction of anti-DNA antibodies.

Antibodies to DSRNA also exhibit marked heterogeneity in human sera in contrast to rabbit anti-sera which have predominant specificity for homologous double-stranded polyribonucleotides. Antibodies from human sera reactive with Poly A·Poly U cross-react strongly with Poly I·Poly C and Poly A and less strongly with SDNA. A quantitative comparison of the inhibitory activity of double-stranded polyribonucleotides, Poly A, and denatured DNA for five high-titered sera containing antibodies reactive with Poly A·Poly U revealed only one serum which reacted preferentially with Poly A·Poly U. The strong reactivity with Poly A suggests that certain antibodies reactive with double-stranded polyribonucleotides may be induced by single-stranded polyribonucleotides and lack specificity for the double-stranded conformation. The antibodies reactive with DSRNA have analogies with anti-NDNA antibodies with respect to the reaction with single-stranded molecules. Further evidence for this hypothesis is derived from the observation that most anti-Poly A antibodies are also reactive with Poly A·Poly U. In this study it was not possible to assess the contribution of the Poly U moiety of the double-stranded molecule because of the ease of degradation of this molecule by ribonuclease. It has been suggested that double-stranded RNA virus is responsible for stimulating the formation of antibodies reactive with synthetic DSRNA (7). The lack of reactivity with transfer RNA and ribosomal RNA has been presented as evidence indicating that single-stranded RNA is an unlikely immunogen for anti-DSRNA antibodies (35). Present studies indicate that single-stranded polyribonucleotides, such as Poly A, which are more resistant to ribonuclease digestion, are reactive and that several types of antigens other than DSRNA are potential immunogens.

Antibodies reactive with the special ribonucleoprotein obtained from a phosphate-buffer extract of nuclei showed no cross-reactions with NDNA, SDNA, DSRNA, or polyribonucleotides. The antigen here is sensitive to ribonuclease. The antibodies involved are also reactive with cytoplasmic ribosomes, and these relationships are currently under study.

In order to obtain further evidence concerning the role of these various polynucleotide antibodies, serial assays were carried out on serum specimens

obtained from 25 patients with SLE followed for periods of 6 months to 4 yr. Three general conclusions could be derived from the data obtained from those experiments. Firstly, anti-NDNA antibodies were closely related to episodes of active disease in most cases. However, three of the 25 patients with classical SLE did not have anti-NDNA antibodies, although multiple episodes of clinical activity were observed. This would appear to indicate that this system is not necessarily involved in the mediation of tissue lesions in all instances. One of these patients without anti-NDNA, SDNA, or DSRNA antibodies had several episodes of severe nephritis which were associated with the appearance of a rheumatoid factor containing cryoglobulin and a C1q precipitin reaction in serum, implicating an independent antigen-antibody system in this patient (36, 37). Secondly, titers of antibodies reactive with SDNA and polyribonucleotides were frequently elevated during periods of clinical exacerbation in association with anti-NDNA antibodies. They were also found to rise during periods when the patients were clinically inactive in contrast to anti-NDNA antibodies. Thirdly, titers of antibodies to ribonucleoprotein did not appear to fluctuate markedly and were not associated with disease activity. The majority of patients either exhibited elevated titers of this antibody in all sera or showed a complete absence throughout their course, although occasional patients showed peaks of activity indicating that this antigen-antibody system is less frequently involved in complex formation.

The variability in the types of antibodies associated with episodes of disease activity suggests that several types of immune complexes may be involved in the renal lesions. The results of fluorescent antibody studies of glomeruli showing deposition of native DNA (24), DNA with single-stranded determinants (38), and rheumatoid factor (36) also suggest the heterogeneous nature of the immune complex deposits. Furthermore, elution studies indicate that although antibodies to NDNA predominate, other types of anti-nuclear antibodies are concentrated in glomeruli, and in several cases anti-NDNA antibodies were not found in glomerular eluates.

Previous studies on the incidence of antibodies to NDNA and SDNA in various diseases indicated that antibodies to NDNA were mainly limited to patients with SLE (8). These studies were extended to include antibodies reactive with DSRNA and nuclear ribonucleoprotein. Antibodies to DSRNA were found in SLE and in low incidence in several other diseases, following a distribution similar to that of anti-SDNA antibodies. In contrast, antibodies to ribonucleoprotein were mainly limited to patients with SLE and other connective tissue disorders. Therefore, antibodies to NDNA and ribonucleoprotein have a restricted distribution, whereas, antibodies to polyribonucleotides and single stranded deoxyribonucleotides have a more widespread distribution in a variety of diseases where evidence of tissue injury exists. The results of these studies, the characterization of antibody specificities, and the serial studies of

SLE sera, suggest that SDNA is probably a major immunogen in man, which may be responsible for a variety of anti-polynucleotide antibodies in SLE and other diseases.

#### SUMMARY AND CONCLUSIONS

The specificities of anti-polynucleotide antibodies found in human sera were studied using several immunological procedures. Anti-native DNA (NDNA) antibodies and certain anti-double-stranded RNA (DSRNA) antibodies were found to react with single-stranded DNA (SDNA), and anti-NDNA antibodies were observed to react more avidly with SDNA than with NDNA in most sera tested. Antibodies to NDNA showed no preferential reactivity with NDNA or SDNA derived from mammalian tissue, bacterial, or viral sources. Precipitating antibodies reactive with individual bases, with common determinants of bases, and with common determinants of SDNA and NDNA were detected utilizing synthetic polydeoxyribonucleotides. Antibodies to DSRNA were also heterogeneous and reactive with both Poly A·Poly U and Poly I·Poly C in addition to reactivity with Poly A and SDNA. In contrast, antibodies to a ribonucleoprotein determined by hemagglutination and by precipitation showed no reaction with NDNA, SDNA, or DSRNA.

Serial studies of serum specimens from patients with systemic lupus erythematosus (SLE) indicated that anti-NDNA antibodies were closely associated with disease activity. Titers of antibodies to SDNA or DSRNA were also frequently increased during these periods but in addition showed peaks during quiescent periods. Anti-NDNA antibodies were detected in most patients' sera at sometime during the course of the disease. Three patients were observed with active SLE, who did not develop anti-NDNA antibodies, even in the presence of severe renal disease. Evidence that other antigen-antibody systems may also play a role in the pathogenesis of the renal disease was particularly apparent in these patients. Anti-ribonucleoprotein antibodies were not well correlated with the peaks of antibody activity of other polynucleotide antibodies, suggesting that an independent immunogen was responsible for induction of these antibodies.

The close association of certain populations of anti-polynucleotide antibodies during the course of active SLE, the presence of cross-reacting antigenic determinants of SDNA, NDNA, and DSRNA, the preferential avidity of anti-NDNA antibodies for SDNA, and the frequent increase of anti-SDNA antibodies in SLE and other diseases associated with active tissue destruction suggest that SDNA is a ubiquitous antigen that may stimulate the formation of antibodies reactive with a variety of polynucleotides.

The authors are gratefully indebted to Miss Lydia Legrand for her excellent technical assistance.



## REFERENCES

1. Robbins, W. C., H. R. Holman, H. R. Deicher, and H. G. Kunkel. 1957. Complement fixation with cell nuclei and DNA in lupus erythematosus. *Proc. Soc. Exp. Biol. Med.* **96**:575.
2. Seligmann, M. 1957. Mise en évidence dans le sérum de malades atteints de lupus érythémateux disséminé d'une substance déterminant une réaction de précipitation avec l'acide désoxyribonucléique. *C.R.H. Acad. Sci.* **245**:243.
3. Deicher, H. R., H. R. Holman, and H. G. Kunkel. 1959. The precipitin reaction between DNA and a serum factor in systemic lupus erythematosus. *J. Exp. Med.* **109**:97.
4. Barbu, E., M. Seligmann, and M. Joly. 1960. Réactions entre des acides désoxyribonucléiques diversement dénatures ou dégradés et les anticorps antiacide désoxyribonucléique du sérum de malades atteints de lupus érythémateux disséminé. *Ann. Inst. Pasteur (Paris)*. **99**:695.
5. Stollar, B. D., L. Levine, and J. Marmur. 1962. Antibodies to denatured DNA in lupus erythematosus serum. II. Characterization of antibodies in several sera. *Biochim. Biophys. Acta.* **61**:7.
6. Stollar, B. D., L. Levine, H. I. Lehrer, and H. Van Vunakis. 1962. The antigenic determinants of denatured DNA reactive with lupus erythematosus serum. *Proc. Nat. Acad. Sci. U.S.A.* **48**:874.
7. Schur, P. H., and N. Monroe. 1969. Antibodies to ribonucleic acid in systemic lupus erythematosus. *Proc. Nat. Acad. Sci. U.S.A.* **63**:1108.
8. Koffler, D., R. I. Carr, V. Agnello, T. Feizi, and H. G. Kunkel. 1969. Antibodies to polynucleotides: distribution in human serums. *Science (Washington)*. **166**:1648.
9. Steinberg, A. D., S. Baron, and N. Talal. 1969. The pathogenesis of autoimmunity in New Zealand mice. I. Induction of antinucleic acid antibodies by polyinosinic polycytidylic acid. *Proc. Nat. Acad. Sci. U.S.A.* **63**:1102.
10. Carr, R. I. 1969. DNA antibodies and DNA in serum. Doctorate Thesis. The Rockefeller University, New York.
11. Sharp, G. C., W. S. Irvin, R. L. LaRoque, C. Velez, V. Daly, A. D. Kaiser, and H. R. Holman. Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. *J. Clin. Invest.* In press.
12. Schur, P. H., L. Moroz, and H. G. Kunkel. 1967. Precipitating antibodies to a ribosomal antigen in the serum of patients with systemic lupus erythematosus. *Immunochemistry*. **4**:447.
13. Erlanger, B. F., and S. M. Beiser. 1964. Antibodies specific for ribonucleosides and ribonucleotides and their reaction with DNA. *Proc. Nat. Acad. Sci. U.S.A.* **52**:68.
14. Plescia, O. J., W. Braun, and N. C. Palczuk. 1964. Production of antibodies to denatured deoxyribonucleic acid (DNA). *Proc. Nat. Acad. Sci. U.S.A.* **52**:279.
15. Lacour, F., A. M. Michelson, and E. Nahon. 1968. Specific antibodies to polynucleotide complexes and their reaction with nucleic acids: importance of the

- secondary structure of the antigen. *In Nucleic Acids in Immunology*. O. J. Plescia and W. Braun, editors. Springer-Verlag, New York. 98.
16. Seaman, E., H. Van Vunakis, and L. Levine. 1965. Antigenicity of polyribonucleotides. *Biochemistry*. **4**:1312.
  17. Karol, M. H., and S. W. Tanenbaum. 1967. Antibodies to hapten-conjugated proteins which cross-react with RNA. *Proc. Nat. Acad. Sci. U.S.A.* **57**:713.
  18. Barbu, E., and J. Panijel. 1960. Présence d'anticorps anti-acide ribonucléique dans les immunsérums anti-ribosomes. *C. R. H., Acad. Sci.* **250**:1382.
  19. Lambert, P. H., and F. J. Dixon. 1968. Pathogenesis of the glomerulonephritis of NZB/W mice. *J. Exp. Med.* **127**:507.
  20. Steinberg, A. D., T. Pincus, and N. Talal. 1969. DNA-binding assay for detection of anti-DNA antibodies in NZB/NZW F<sub>1</sub> mice. *J. Immunol.* **102**:788.
  21. Tan, E. M. 1968. Antibodies to deoxyribonucleic acid irradiated with ultraviolet light: detection by precipitins and immunofluorescence. *Science (Washington)*. **161**:1353.
  22. Pogo, A. O., V. G. Allfrey, and A. E. Mirsky. 1966. Evidence for increased DNA template activity in regenerating liver nuclei. *Proc. Nat. Acad. Sci. U.S.A.* **56**:550.
  23. Carr, R. I., D. Koffler, V. Agnello, and H. G. Kunkel. 1969. Studies on DNA antibodies using DNA labelled with actinomycin-D (3H) or dimethyl (3H) sulphate. *Clin. Exp. Immunol.* **4**:527.
  24. Koffler, D., P. Schur, and H. G. Kunkel. 1967. Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. Exp. Med.* **126**:607.
  25. Jokinen, G. J., and H. Julkunen. 1965. DNA haemagglutination test in the diagnosis of systemic lupus erythematosus. *Ann. Rheum. Dis.* **24**:477.
  26. Campbell, D. H., J. S. Garvey, N. E., Cremer, and D. H. Sussdorf. 1964. *Methods in Immunology*. W. A. Benjamin, Inc., New York. 162.
  27. Holman, H. R. 1965. Partial purification and characterization of an extractible nuclear antigen which reacts with SLE sera. *Ann. N. Y. Acad. Sci.* **124**:800.
  28. Tan, E. M., and H. G. Kunkel. 1966. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J. Immunol.* **96**:464.
  29. Sober, H. A., editor. 1968. *Handbook of Biochemistry. Selected Data for Molecular Biology*. The Chemical Rubber Co., Cleveland, Ohio. H30.
  30. Tan, E. M., P. H. Schur, R. I. Carr, and H. G. Kunkel. 1966. Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J. Clin. Invest.* **45**:1732.
  31. Kunkel, H. G., and E. M. Tan. 1964. Autoantibodies and disease. *Advan. Immunol.* **4**:351.
  32. Seligmann, M., and R. Arana. 1968. The various types of DNA antibodies in lupus sera. *In Nucleic Acids in Immunology*. O. J. Plescia and W. Braun, editors. Springer-Verlag, New York. 98.
  33. Arana, R., and M. Seligmann. 1967. Antibodies to native and denatured deoxyribonucleic acid in systemic lupus erythematosus. *J. Clin. Invest.* **46**:1867.

34. Tan, E. M., and P. G. Natali. 1970. Comparative study of antibodies to native and denatured DNA. *J. Immunol.* **104**:902.
35. Talal, N., and A. D. Steinberg. 1971. Inhibition of anti-RNA antibodies in human and mouse lupus by viral and synthetic ribonucleic acids. 16th Interim Scientific Session of the American Rheumatism Association. Washington, D.C. 14. (Abstr.)
36. Koffler, D., V. Agnello, R. Thoburn, and H. G. Kunkel. 1970. Antibodies to polynucleotides, Clq precipitins and rheumatoid factor in systemic lupus erythematosus. *Immunopathol. Int. Symp.* 6th. In press.
37. Agnello, V., R. J. Winchester, and H. G. Kunkel. 1970. Precipitin reactions of the Clq component of complement with aggregated gamma globulin and immune complexes in gel diffusion. *Immunology.* **19**:909.
38. Seegal, B. C., L. Accinni, G. A. Andres, S. M. Beiser, C. Bruni, C. L. Christian, B. F. Erlanger, and K. C. Hsu. 1968. Immunofluorescent studies of lupus nephritis in man. *Fed. Proc.* **27**:544.