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IMMUNOLOGICAL DETECTION OF NUCLEIC ACIDS AND ANTIBODIES TO NUCLEIC ACIDS AND NUCLEAR ANTIGENS BY COUNTERIMMUNOELECTROPHORESIS

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SUMMARY

A counterimmunoelectrophoresis (CIEP) technique has been developed for the rapid, simple, specific detection of nucleic acids as antigens, or for the detection of precipitating antibodies to nucleic acids or nuclear antigens. The majority of precipitins could be detected within 1 hr. As little as 0·0015 μg of antigen per ml (e.g. poly A : poly U) could be detected. Specificity of rabbit antisera to nucleic acids was demonstrated by selective reactions using a panel of polynucleotides. 1091 patient sera were examined for precipitins to DNA, single-stranded DNA, nucleoprotein and calf thymus nucleoprotein. Precipitins to DNA were found in 42% of systemic lupus erythematosus sera, 9% of rheumatoid arthritis sera and 4% of juvenile rheumatoid arthritis sera. Results with the CIEP method showed equal sensitivity as results obtained by complement fixation or binding assays, but were more sensitive than double diffusion in agar (Ouchterlony).

INTRODUCTION

Numerous immunological techniques are currently available for the detection in serum of nucleic acids, antibodies to nucleic acids and nuclear antigens. The methods vary as to their sensitivity, complexity, length of time involved, the cost of the equipment and the reagents needed. The techniques of complement fixation, haemagglutination, haemagglutination inhibition, immunofluorescence, binding assays, and double diffusion in agar (Ouchterlony) (Friou, Finch & Detre, 1958; Koffler *et al.*, 1969; Pincus *et al.*, 1969; Schur & Sandson, 1968; Stollar, 1970; Tan *et al.*, 1966) all vary in sensitivity and ease of performance and have numerous advantages as well as disadvantages. The purpose of this study was, therefore, to develop a technique for the immunological detection of (1) nucleic acids and

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(2) antibodies to nucleic acids and nuclear antigens, which technique would be simple, sensitive, specific, rapid and inexpensive. Counterimmunoelectrophoresis appears to offer these advantages and was found to be useful for screening large numbers of sera for either antibodies or antigens. This technique, as first applied to the detection of hepatitis-associated antigen (Gocke & Howe, 1970) was modified to explore its sensitivity for the detection of antibodies to different nucleic acids and nuclear proteins, as well as its sensitivity for detecting nucleic acids as antigens.

MATERIALS AND METHODS

Counterimmunoelectrophoresis (CIEP) method

The following modifications of the counterimmunoelectrophoresis technique (Gocke & Howe, 1970) were assessed for their capacity to increase sensitivity: well diameters ranged from 3–8 mm; the distance between wells, edge to edge, varied from 3–12 mm; electrophoresis was performed at both 4°C and room temperature with a constant current ranging from 15–45 mA; the time of electrophoresis varied from 30 min to 2 hr. The following buffers were used and compared: 0·025 M veronal with 0·04 M NaCl, pH 7·4–8·6; 0·025 M veronal with 0·15 M NaCl, pH 7·4–8·6; 0·05 M veronal, pH 8·0–8·8; 0·025 M veronal, pH 8·0; 0·01 M phosphate-buffered saline, pH 6·0–8·6; 0·1 M Tris with 0·06 M HCl, pH 7·0; 0·45 M Tris with 0·025 M boric acid with 0·001 M EDTA, pH 8·7; and 0·001 M Tris with 0·1 M NaCl with 0·001 M EDTA, pH 9·6. Different forms of agar were used, including: agarose (L'Industrie Biologique Française, Lot number 8631, number 8632, number 8633), agar–agar (Baltimore Biological Laboratories, Lot number 902635), and Noble agar (Difco, Lot number 542175), in concentrations ranging from 0·7 to 2·0% in the above buffers. After electrophoresis representative slides were incubated at 4°C for 18 hr and/or washed in veronal, veronal with saline or 0·01 M phosphate-buffered saline, pH 7·2 (PBS) buffers, for 1–36 hr. After the buffer wash, the slides were washed in distilled water from 1 to 16 hr. Buffer and distilled water washes were carried out both at 4°C and at room temperature. One per cent Amido Black and 1% tannic acid were compared as stains.

Optimal method

The system offering the greatest sensitivity was found to be the following: 8·3 × 10·3 cm glass lantern slides (Eastman–Kodak) were precoated around the edge with 0·3% agarose, dissolved in water and allowed to dry. Then, 10 ml of 0·85% agarose (L'Industrie Biologique Française, Lot number 8633) in 0·1 M Tris with 0·06 M HCl buffer, pH 7·0, were melted over a boiling water bath, poured evenly onto the precoated slides and allowed to gel at room temperature. The slides were stored up to 2 days at 4°C in a moist chamber prior to use. Two vertical columns of paired wells were cut; the wells were 5 mm in diameter and 3 mm apart from edge to edge. The agarose plugs were removed by suction. Antiserum, heat-inactivated at 56°C for 30 min, was placed in the anodic well of each paired column and antigen in the cathodic well. The wells were filled to capacity (0·025 ml, to the level of the agarose) with a Pasteur pipette. The electrophoresis chambers were filled with the Tris–HCl buffer. Paper wicks (Whatman chromatography paper 3 MM) were then affixed to the slide and a constant current of 30 mA was applied across the slide for 60 min. Voltage varied from 70–80 V. Electrophoresis was carried out at room temperature. The slides were read,

incubated overnight at 4°C in a moist chamber, read again, washed for 7 hr in PBS and 1 hr in distilled water at 4°C, covered with filter paper (Whatman Filter Paper number 1) and then dried overnight at 37°C. The following day the slides were stained with 1% Amido Black, decolourized and the results recorded. The intensity of the precipitin lines was graded from 1–4+.

Sensitivity

The above variables were compared for antigen sensitivity (i.e. the ability of antisera to detect low concentrations of antigen): decreasing concentrations of poly A:poly U, poly I:poly C, polydTrA (obtained from Miles Laboratories, Elkhart, Indiana) and single-stranded deoxyribonucleic acid (SS-DNA) were electrophoresed against antisera from rabbits immunized with these antigens and against sera from patients with systemic lupus erythematosus (SLE) who had high titre antibodies to these same antigens. The antigen sensitivity of double-stranded DNA was assessed by testing decreasing concentrations of DNA against sera from systemic lupus erythematosus (SLE) patients with high titres of anti-DNA antibodies.

For the assessment of antibody sensitivity, two-fold serial dilutions of rabbit antisera or SLE sera were tested against ten-fold dilutions of the above antigens.

Other methods

Antinuclear antibodies (ANA) were detected by the immunofluorescent technique using cryostat sections of snap-frozen mouse liver (8 μm) (Rothfield, Frangione & Franklin, 1965). Serial dilutions of sera were made whenever a positive reaction was detected in sera diluted 1:10.

Polynucleotides were diluted in PBS and their concentrations estimated by examining their optical density at 260 nm. Polynucleotides were aliquoted at concentrations of 0·1–1 mg/ml and stored at 4°C or −20°C for variable lengths of time.

Nucleoprotein (NP) was obtained from Worthington Labs (Freehold, New Jersey), dissolved in PBS and usually sonicated.

Deoxyribonucleic acid (calf thymus DNA) was obtained from Worthington Labs and dissolved in PBS.

SS-DNA was prepared from DNA by heat denaturation at 100°C for 15 min followed by immersion in an ice bath.

Calf thymus nucleoprotein (CTN) was prepared from calf thymus nuclei as described by Tan & Kunkel (1966).

Ouchterlony assays were performed in 0·6% agarose in PBS as described previously (Tan *et al.*, 1966).

Rabbits were immunized with complexes of methylated bovine serum albumin and polynucleotides emulsified with Freund's complete adjuvant (Plescia, Braun & Palczuk, 1964). Booster injections were given at intervals until test bleedings from the rabbits gave optimal precipitin lines with the antigen as assessed by CIEP.

Patients were studied at the Robert B. Brigham Hospital, Boston, Massachusetts. Diagnoses were based on clinical impressions and conform to the guidelines proposed by the ARA (Brewer *et al.*, 1972–3; Cohen *et al.*, 1971; Ropes *et al.*, 1958).

RESULTS

Antigen sensitivity (Table 1)

Using whole rabbit antisera, as little as 0·03 μg/ml of SS-DNA could be detected by CIEP. Other rabbit antisera could detect as little as 0·015 μg/ml of poly I:poly C, 0·0015 μg/ml of poly A:poly U, or 0·07 μg/ml of polydTrA. Using SLE sera, as little as 0·015 μg/ml of DNA could be detected. Similar concentrations could be detected when these nucleic acids were added to normal serum instead of PBS.

TABLE 1. Relative antigen and antibody sensitivity of Ouchterlony and CIEP techniques

	Antigen sensitivity				Antibody sensitivity			
	CIEP		Ouchterlony		CIEP		Ouchterlony	
	Rabbit	SLE	Rabbit	SLE	Rabbit	SLE	Rabbit	SLE
Nucleic acids								
DNA	X	0·15 μg/ml	X	10 μg/ml	X	1:32	X	Undiluted
SS-DNA	0·03 μg/ml	0·07 μg/ml	10 μg/ml	10 μg/ml	1:8	1:8	1:4	Undiluted
Poly I : poly C	0·015 μg/ml	0·1 μg/ml	7·5 μg/ml	10 μg/ml	1:16	1:16	1:8	Undiluted
Poly A : poly U	0·0015 μg/ml	0·0125 μg/ml	12·5 μg/ml	10 μg/ml	1:32	1:2	1:16	Undiluted
PolydT : polyrA	0·07 μg/ml	0·025 μg/ml	3 μg/ml	1·5 μg/ml	1:4	1:16	1:4	1:4
Nuclear antigens								
NP	X	0·07 μg/ml	X	3 μg/ml	X	1:16	X	Undiluted
CTN	X	1:4096	X	1:64	X	1:256	X	1:64

TABLE 2. Antibody specificity of rabbit antisera

	DNA	SS-DNA	Poly I: poly C	Poly A: poly U	PolydT: polyrA	Poly I	Poly U	Poly A
Anti-SS DNA	0	+	0	0	0	0	0	0
Anti-poly I : poly C	0	0	+	0	0	+*	0	0
Anti-poly A : poly U	0	0	+	+	0	0	0	+†
Anti-polydTrA	0	0	0	0	+	0	0	0

* Positive *vs* poly I at 100 μg/ml, negative at 10 μg/ml.
† Positive *vs* poly A at 1 μg/ml, negative at 10 and 0·1 μg/ml.

Antibody sensitivity and specificity (Tables 1 and 2)

Three hundred and ninety-seven patient sera which contained antinuclear antibodies with titres of 1/20 or greater, were examined by CIEP to four nuclear antigens to determine whether overnight incubation, washing and staining significantly increased the incidence of precipitins. Of those 272 positive reactions (i.e. after staining), 62% were seen after electrophoresis for 1 hr and 79% after overnight incubation at 4°C. Some precipitin bands seen after washing were not visualized after staining.

Serial dilutions of rabbit antiserum and SLE sera were tested against a panel of the nucleic acids and the nuclear antigens. Sera from animals immunized with SS-DNA reacted only with SS-DNA and not with the other nucleic acids tested (see Table 2). Antisera could be diluted 1/8 and still precipitate with antigen (1–10 μg/ml). Sera from animals immunized with poly I:poly C reacted with poly I:poly C and weakly with poly I at 100 μg/ml but not with other nucleic acids. Antisera could be diluted 1/16 and still precipitate with antigen (1–10 μg/ml). Sera from animals immunized with poly A: poly U reacted with poly A: poly U, poly I:poly C, and weakly with poly A, but not with other nucleic acids. Antisera could be diluted 1/32 and still precipitate with antigen (1–10 μg/ml). Sera from animals immunized with poly dTrA reacted only with poly dTrA (see Table 2).

A strong SLE serum (not shown on Table 1) could be diluted 1/16 and still react with NP at a concentration of 10 μg/ml and 1 μg/ml. The serum could be diluted 1/64 and still react with SS-DNA at concentrations of 0·6, 1, and 10 μgI ml. The serum could be diluted 1/128 and still react with 10 μg/ml of DNA.

When SLE sera were absorbed with DNA, they no longer reacted with DNA. The preparations of DNA did not contain any SS-DNA as assessed by CIEP using rabbit anti-SS-DNA. Digestion of DNA with desoxyribonuclease eliminated the reaction between SLE sera and DNA while trypsin had no effect. When CIEP plates were extensively washed with PBS and then overlaid with fluoroscein-labelled anti-human gamma-globulin, the precipitin lines formed between SLE (and rheumatoid arthritis (RA)) sera and DNA fluoresced. Precipitin lines formed between human sera and anti-complement antisera did not fluoresce.

Comparison of CIEP with other methods for detecting antigen or antibody

Ouchterlony (Table 1). The CIEP and Ouchterlony methods were compared for antibody and antigen sensitivity to DNA, SS-DNA, poly I: poly C, poly A:poly U, poly dTrA, CTN and NP using representative rabbit and SLE sera. Examples of results with different sera are given in Table 1. The CIEP method was considerably more sensitive in detecting small concentrations of antigen than was a sensitive Ouchterlony method. For instance, a rabbit antisera could detect as little as 0·0015 μg/ml of nucleic acid (e.g. poly A:poly U) by the CIEP method but only 12·5 μg/ml by the Ouchterlony method. Other rabbit antisera could also detect much lower concentrations of nucleic acids by the CIEP method than by the Ouchterlony method as outlined in Table 1. SLE sera could also detect much lower concentrations of nucleic acid by the CIEP method than by the Ouchterlony method, *viz.* 0·15 μg/ml DNA by CIEP and 10 μg/ml by Ouchterlony.

The CIEP method was only somewhat more sensitive in detecting antibodies to nucleic acids and nuclear antigens than was the Ouchterlony method as shown in Table 1. Rabbit antisera could usually be diluted (two-fold serial dilutions) one more dilution and still precipitate with antigen by the CIEP method as opposed to the Ouchterlony method. For instance, rabbit antisera to SS-DNA could be diluted 1/8 and still react with SS-DNA in the CIEP method but only 1/4 in the Ouchterlony method. The CIEP method was much more sensitive in detecting antibodies in the SLE sera than was the Ouchterlony method. A representative SLE serum (see Table 1) which would precipitate with DNA by the Ouchterlony method only if not diluted, could be diluted 1/32 and still react with DNA by the CIEP method. The results of other representative SLE sera are given in Table 1, where in most instances studied sera could be diluted further in the CIEP method than in the Ouchterlony method and still precipitate with nucleic acids or nuclear antigens. A strong SLE serum (not

shown in Table 1) which could be diluted 1/128 and react in the Ouchterlony method with DNA, could not be diluted any further and still react in the CIEP method with DNA. There were many additional sera studied which reacted with antigens in the CIEP method but reactions were not detected by the Ouchterlony method. Of eighty-eight sera with precipitins to DNA demonstrated by CIEP, only one precipitated when tested by the Ouchterlony method. Of 139 sera with precipitins to SS-DNA by CIEP, only four were positive by Ouchterlony. Of seventy-one sera with precipitins by CIEP to CTN, twenty-eight of these same sera had positive precipitin lines demonstrated by the Ouchterlony method. Of sixty-six sera demonstrating antibodies to NP by the CIEP method, only four were positive when tested by Ouchterlony.

Complement fixation. Ninety-four SLE sera were examined by both complement fixation with DNA (Schur & Sandson, 1968) and by CIEP with DNA. The geometric mean complement-fixing titre of those sera that precipitated with DNA by CIEP was 5·5, while the mean complement-fixing titre for those that failed to precipitate was 3·4. Four sera that did not fix complement precipitated and seventeen sera that did not fix complement did not precipitate.

Binding assay. Fifty SLE sera were assayed for anti-DNA by both CIEP and a radioactive binding assay (kindly performed by Dr Norman Talal, University of California, San Francisco). Twenty-two sera giving negative results by CIEP were also negative by the binding assay and all of the twenty-eight sera giving positive results by CIEP were positive by the binding assay.

Clinical studies

Sera from 7794 patients with suspected rheumatic diseases were sent to this laboratory from seven hospitals and were examined in a 12-month period. 53% did not have detectable ANA, while 20% were positive with a titre of 1:20 or greater. Precipitins to DNA were seen in 21%, to SS-DNA in 35%, to CTN in 12% and to NP in 18% of those sera with an ANA titre of 1:20 or greater. Sera from patients without ANA, or whose ANA had a titre of 1:10 or less, did not have precipitins to DNA, NP or CTN; an occasional such sera did have precipitins to SS-DNA. Of six hundred and twenty-eight sera (with an ANA titre of 1:20 or greater) with precipitins to either DNA, SS-DNA, or NP, seventy-three precipitated with DNA alone, two hundred & eighty-three with SS-DNA alone, thirty with NP alone, sixty with both DNA and SS-DNA, fifty-three with DNA and NP, twenty-three with SS-DNA and NP and a hundred and six with all DNA, SS-DNA and NP.

Of 448 sera from patients with RA, 30% had a negative ANA, 33% were positive ANA with a titre of 1:10 and 37% had a titre of 1:20 or greater. Nine per cent of sera from patients with RA had precipitins by CIEP to DNA and will be the subject of a separate report. Thirteen per cent of RA sera had precipitins to SS DNA, 2% of sera had precipitins to CTN and 4% to NP.

Of 162 sera from patients with JRA, 52% had a negative ANA, 31% had a positive ANA with a titre of $\leqslant$1:10 and 17% had a titre of 1:20 or greater. Four per cent of the JRA sera had precipitins by CIEP to DNA, 6% to SS-DNA, 1% to CTN and 1% to NP.

Of 228 sera from patients with SLE, none had a negative ANA, 15% had a positive ANA with a titre of 1:10 and 85% had a titre of 1:20 or greater. Thirty-seven per cent of sera had a titre of 1:640 or greater. Of 277 sera, 42% had precipitins by CIEP to DNA, 56% to SS-DNA, 23% to CTN and 35% to NP.

Of twenty-eight sera from patients with scleroderma, twenty-three had a positive ANA with a titre of 1:10 or greater. None of these sera had positive precipitins by CIEP to DNA or NP, two were positive against SS-DNA and two against CTN.

Of forty-two sera from patients with Sjögren's syndrome, twenty-seven had a positive ANA. Two of these sera demonstrated positive precipitins by CIEP to DNA, six to SS-DNA, one to NP and three to CTN.

DISCUSSION

The present study describes a counterimmunoelectrophoresis (CIEP) technique for the immunological detection of nucleic acids, antibodies to nucleic acids, and nuclear antigens. As little as 0·0015 μg of polynucleotides per ml could be detected. Previous studies employing another CIEP method could detect 0·2 μg/ml of DNA (Davis & Davis, 1973) or 0·12 μg/ml of SS-DNA (Davis, 1971). Only 1 μg/ml could be detected by chemical means (Williams & Chase, 1968), 0·01 μg/ml by complement fixation (Stollar, 1970) and 0·4 μg/ml by haemagglutination inhibition (Koffler *et al.*, 1969). The advantages of the CIEP technique are that it is extremely rapid and one can detect antigens at the end of 1 hr, that is, at the end of electrophoresis, although maximum sensitivity was accomplished by an overnight incubation of the slide followed by washing and staining. In addition, the technique is extremely inexpensive, requiring only immunological reagents, glass, agar and a power pack. The technique of counterimmunoelectrophoresis is now widely employed for the detection of hepatitis-associated antigens (Gocke & Howe, 1970) and very likely will be employed because of the previously mentioned advantages for the detection of other antigens. The primary limitations of CIEP are the availability of specific antiserum, lack of quantitation and occasionally negative results in large antigen excess.

There are, in addition, numerous ways for detecting antibodies to nucleic acids and nuclear antigens. The technique of double diffusion in agar (Ouchterlony) (Tan *et al.*, 1966) is simple, can demonstrate specificity, but is insensitive and may take up to 72 hr to demonstrate results. Complement fixation is very specific, moderately rapid, relatively inexpensive but is somewhat tedious to perform (Schur & Sandson, 1968). However, some sera are anticomplementary and not all sera will fix complement equally. Haemagglutination and haemagglutination inhibition are very sensitive, specific, rapid and inexpensive (Koffler *et al.*, 1969) but again, somewhat tedious to perform. Sera will also vary in their ability to promote agglutination. Immunofluorescence is relatively simple, specific, rapid and inexpensive but not very sensitive (Gonzalez & Rothfield, 1966). The new binding assays are very sensitive and specific but are not, on the whole, very rapid (Pincus *et al.*, 1969). In addition, the assays are somewhat complex and as radioactive material is employed, requires quite expensive equipment. The present technique of CIEP has the advantages of ease of performance, speed and economy when compared to these techniques and compared favorably in sensitivity in detecting antinuclear antibodies.

The sensitivity of the CIEP technique for detecting anti-DNA antibodies was compared to an anti-DNA binding assay. Previous studies have utilized a different CIEP method for detecting anti-DNA and anti-SS-DNA antibodies (Arquembourg *et al.*, 1972; Davis, 1971; Dorsch & Barnett, 1972). Anti-DNA precipitins were noted in 75% of SLE sera by one group (Williams & Chase, 1968), while another group (Dorsch & Barnett, 1972) stated that their CIEP method was less sensitive than a Farr binding assay for detecting anti-DNA

antibodies. In the present study, twenty-two out of twenty-two sera that gave negative results by CIEP were also negative by the binding assay and all twenty-eight of those sera giving positive results by CIEP were also positive by the binding assay.

Utilizing the CIEP method on 1091 sera, precipitins to DNA were found in 227 sera. Precipitins to DNA were found in 9% of sera from patients with RA, 4% of patients with juvenile rheumatoid arthritis (JRA) and 42% of sera from patients with SLE. Previously, precipitins (by Ouchterlony) to DNA have been noted in 25% of sera from patients with SLE (Schur & Sandson, 1968) and not in sera from patients with other diseases (Anderson *et al.*, 1962). Antibodies to DNA have been found in 61% of SLE patients by complement fixation (Schur & Sandson, 1968), 60% by passive haemagglutination (Koffler *et al.*, 1969), 75% by binding assay (Pincus *et al.*, 1969), 64% by bentonite flocculation (Kayhoe, Nason & Bozicevich, 1960), and in 40% indirectly by immunofluorescence (peripheral pattern) (Gonzalez & Rothfield, 1966). Using the bentonite flocculation technique, antibodies to DNA were found in 4% of sera from patients with 'other diseases' (Sturgill *et al.*, 1964). Anti-DNA antibodies in patients with RA have been noted only sporadically (Bickel, Barnett & Pearson, 1968; Franco & Schur, 1971; Koffler *et al.*, 1969; Robitaille & Tan, 1973; Tan, 1967). The patients with RA and JRA who were found to have anti-DNA antibodies will be the subject of a separate report. Anti-DNA antibodies in patients with scleroderma have been noted by some (Hansom, Drexler & Kornreich, 1970) but not by others (Sharp *et al.*, 1971). Anti-DNA has also been seen occasionally in patients with Sjögren's syndrome (Pincus *et al.*, 1969).

Precipitins to SS-DNA were noted in 385 of 1091 sera examined (i.e. those with an ANA titre of 1:20 or greater). They were noted in 13% of all RA sera, 6% of all JRA sera and 56% of all SLE sera examined. Previous studies have noted complement fixing antibodies in 75% of patients with SLE (Schur & Sandson, 1968), 41% by double diffusion in agar (Schur & Sandson, 1968) and 92% of haemagglutination (Koffler *et al.*, 1969). Antibodies to SS-DNA have been noted in 60% of patients with RA as assessed by haemagglutination (Koffler *et al.*, 1969) but not in patients with scleroderma (Sharp *et al.*, 1971) and occasionally in patients with Sjögren's (Talal & Schur, 1968).

Precipitins to CTN (Schur & Sandson, 1968), which is similar to a mixture of the Sm (Tan & Kunkel, 1966), RNP (Reichlin & Mattioli, 1973), and ENA (Sharp *et al.*, 1971) antigens, were noted in 128 of 1091 sera examined (i.e. sera with an ANA titre of 1:20 or greater). They were found in 2% of all RA sera, 1% of all JRA sera, and 23% of all SLE sera examined. Previously, precipitins to CTN were noted in 13% of SLE sera (Schur & Sandson, 1968) to Sm in 57–75% of SLE sera and not in sera from patients with RA (Tan, 1967; Tan & Kunkel, 1966). Antibodies to ENA have been noted in 50–55% of patients with SLE but not in RA (Sharp *et al.*, 1971) or scleroderma (Sharp *et al.*, 1971). Antibodies to RNP have been noted in 25% of SLE sera but not in sera from patients with scleroderma, Sjögren's syndrome or RA (Reichlin & Mattioli, 1973).

Precipitins to NP were noted in 194 of 1091 sera examined (i.e. sera with an ANA titre of 1:20 or greater). They were noted in 4% of all RA sera, 1% of all JRA sera and 35% of all SLE sera examined. Previously, precipitins to NP were noted in 51% of SLE sera and 12% of RA sera (Tan, 1967).

The frequency of finding antinuclear antibodies in the diseases examined in this study is similar to those noted by others (Bianco *et al.*, 1971; Gonzalez & Rothfield, 1966; Rothfield & Rodnan, 1968; Talal & Schur, 1968).

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