THE PRECIPITIN REACTION BETWEEN DNA AND A SERUM FACTOR IN SYSTEMIC LUPUS ERYTHEMATOSUS

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The participation of nucleic acids, particularly of DNA[1] in serological reactions has been studied for a number of years. Earlier reports have dealt with interactions between bacterial nucleic acids and antisera directed against these bacteria (1–3). More recently, a number of laboratories have presented evidence for complement-fixing (4, 5) and precipitating (6, 7) antisera obtained by the immunization of experimental animals with various preparations of mammalian or bacterial DNA. The problem of the importance of protein contaminants or small units of amino acids for the antigenicity of these DNA preparations, however, has not been entirely resolved; although the DNA appears to be necessary for the specificity of these reactions, the antigenicity of DNA itself is still a matter of debate.

Studies of serological reactions encountered in the human disease systemic lupus erythematosus[1] have recently yielded increasing evidence for an immune reaction involving nuclear materials and DNA. SLE is a disease characterized by the appearance of numerous types of antibodies to various cellular constituents (8), and particularly by a γ-globulin which induces specific morphologic changes in cell nuclei (9, 10). The latter substance, called the "LE factor," has been shown to react directly with isolated cell nuclei (11, 12) and nuclear nucleoprotein (12–14), and appears to be one of a number of related circulating serum factors which react against different constituents of the nucleus (15).

A specific interaction between highly purified DNA preparations from a variety of sources and SLE serum utilizing precipitation (16–18), complement fixation (19, 20), and passive hemagglutination (21) techniques has been suggested by work in different laboratories during the last year. The abolition of the reaction by treatment of the DNA with DNAase has pointed to the integral

[1] The following abbreviations will be used:—
DNA, desoxyribonucleic acid.
DNAase, desoxyribonuclease.
RNA, ribonucleic acid.
RNAase, ribonuclease.
SLE, systemic lupus erythematosus.
LE cell, lupus erythematosus cell (9).

participation of the DNA itself. Again, however, the antigen-antibody nature of these reactions has not been conclusively established, and the properties of the participating serum factor remain somewhat obscure.

The present study was undertaken to define more clearly the nature of the reaction between DNA and SLE serum, particularly with the objective of distinguishing between specific and non-specific interactions of DNA and serum proteins and of obtaining more evidence as to the immune nature of the reaction.

Materials and Methods

1. Sera from patients with acute SLE were collected under sterile conditions and stored at 4°C. until used; the sera were obtained from patients in the acute phase of the illness.

2. DNA preparations.—calf thymus DNA and salmon sperm DNA were obtained commercially from Mann Research Laboratories, Inc., New York, and showed the following analyses: Calf thymus DNA: C: 37.18 per cent N: 16.54 per cent, phosphorus: 9.91 per cent; salmon sperm DNA: phosphorus: 8.46 per cent, DNA from normal human liver was prepared from a specimen obtained within 5 hours after death using the method of Kirby (22); 2×10^{-3} molar NaCl was used instead of distilled water in all instances. DNA from myelocytic leukemia leucocytes and from lymphocytic leukemia lymphocytes was obtained from Dr. E. Polli and Dr. R. Ceppellini, Universitá di Milano, Italy. A purified phage DNA preparation from type T6 phage was kindly furnished by Dr. Marjoris Jesaitis, The Rockefeller Institute. The protein content of the different DNA preparations never exceeded 1 per cent and ranged from 0.46 to 0.60 per cent as measured by a modified Folin method employing an albumin standard. The RNA was yeast nucleic acid obtained from the California Foundation for Biochemical Research, Los Angeles. All samples, except for the human liver DNA, were lyophilized. Stock solutions of 0.50 mg./ml. were made and stored in the ice box until used. DNA solutions of the desired concentration were obtained from these stock solutions for immediate use.

3. Enzymes and enzyme inhibitors.—The pancreatic DNAase was a once crystallized preparation from Worthington Chemical Corporation. For the use in agar tube precipitin tests, 0.5 ml. of a DNA solution containing 0.50 mg./ml. was mixed with 0.25 ml. of a DNAase solution of 0.40 mg./ml. in a pH 6.8 phosphate buffer containing 5 μM $MgCl_2$/ml. The reaction mixture was incubated for 60 minutes at 37°C. Sufficient sodium citrate was then added to bind the Mg^{++} ions present and to inactivate the DNAase. The undialyzed solution was then used as antigen after diluting it so that the concentration resembled the original concentration of the positive control.

The RNAase was a crystallized preparation from Worthington Biochemical Corporation. The procedure used for treating the DNA with RNAase was the same as described for DNAase.

Trypsin was obtained from Worthington Biochemical Corporation as a once crystallized preparation. To 0.5 ml. of a DNA solution containing 0.5 mg./ml., 0.25 ml. of pH 7.6 phosphate buffer containing 0.025 mg. of trypsin was added. The mixture was then incubated at 37°C. for 60 minutes. 0.07 mg. soybean trypsin inhibitor was then added to inactivate the enzyme. DNA solutions treated in these ways were then used as "antigen" in the tube precipitin test.

For the dissociation of precipitates, 0.1 ml. of a solution containing 0.5 mg. DNAase/ml. was added to the washed precipitate which had been suspended in the 0.3 ml. of 0.14 molar NaCl. The mixture was then made 0.02 molar with respect to magnesium chloride by the addition of 0.1 ml. of a 1.0 molar $MgCl_2$/0.14 molar NaCl solution. It was then incubated at 37°C. for 60 to 90 minutes and at 4°C. for periods up to 18 hours while dialyzing against large

amounts of 0.14 molar NaCl–0.02 molar $MgCl_2$ solution. The effect of the digestion and dialysis was tested by analyzing the contents of the dialysis bag for DNA.

4. Agar double diffusion tests.—The double diffusion precipitin technique was modified from the original method of Oakley and Fulthorpe (23) as described by Preer (24) and Doniach and Roitt (25). 100 x 10 mm. tubes were used. 0.15 ml. of serum was carefully put into the bottom of the tube by means of a capillary pipette and 0.3 ml. of 0.5 per cent agar in 0.14 molar NaCl (containing 1:10,000 merthiolate) was then layered over the serum after heating it to 80° and subsequent cooling to 55°. After the agar had cooled down to room temperature, 0.15 ml. of the desired antigen dilution was layered over the agar. The tubes were then closed with a plastic cover and incubated at 37°C. for periods up to 3 weeks. They were read daily until the 7th day and left for occasional observation for 2 more weeks. Precipitin bands appeared usually after 30 to 72 hours and generally showed little migration. For the determination of the highest serum dilution still giving a positive precipitin reaction, the gradient diffusion technique of Augustin (26) was also employed.

5. Precipitin reactions in agar plates.—A modified Ouchterlony technique (27) was used employing 1 per cent agar in 0.14 molar NaCl.

6. For the quantitative precipitin curves (28), sera were inactivated at 56°C. for 30 minutes. All curves were set up with duplicates, each tube containing 0.25 ml. of the serum, 0.25 ml. of the desired buffer, and 0.5 ml. of the "antigen" solution made up in the buffer employed in the particular experiment. Thus, a final serum dilution of 1 to 4 was used regularly. Adequate serum and antigen controls were included. A barbital buffer was used at pH 8.6 while at pH 5.1 and 7.45, phosphate buffers were employed. The buffer solutions were all brought to an ionic strength of 0.15 by the addition of NaCl if not otherwise indicated. After addition of antigen and buffer to the serum, the tubes were incubated at 37°C. for 60 minutes and subsequently at 4°C. for 96 hours. The tubes were then spun, decanted, and the precipitate was washed twice with cold saline. Protein determinations on the precipitates were done using a modified Folin-Ciocalteu (29) method. Since the DNA preparations did not show a positive Folin reaction up to 0.5 mg./ml., the amount of protein in the precipitate could be used as a direct measure of the precipitated serum protein and was converted into μg. of γ-globulin nitrogen using a standard curve obtained with micro Kjeldal analyses of a preparation of normal fraction II human γ-globulin (Lederle No. C-439). DNA determinations on the precipitates were done by suspending the washed precipitate in 0.5 ml. of 10 per cent perchloric acid; the mixture was then heated to 70°C. for 20 minutes, cooled to room temperature, and the extinction read at $\lambda = 260$ mμ in the Beckman spectrophotometer using the micro cells described by Lowry and Bessey (30). A standard curve with known amounts of DNA was obtained for the particular DNA preparation by the same method.

7. Immunoelectrophoresis.—A modification of the original method of Grabar and Williams (31) was employed using a 1 per cent agar in a barbital buffer of pH 8.2 and an ionic strength of 0.05. Serum samples (up to 0.25 ml.) were mixed 1:1 with the agar and put into a round well which had been cut into the agar layer at the desired point of origin. Electrophoresis was then performed in the cold room at 300 to 350 volts and 40 to 60 ma. for 1 to 6 hours. After completion of the separation, a DNA solution of 125 μg./ml. was filled into a long well cut out parallel to the separated serum. Usually horse antiserum to whole human serum or rabbit antiserum to human γ-globulin was put into a similar well on the other side of the separated serum, thus indicating the location of the different serum protein fractions. A normal serum sample separated simultaneously on the same plate served as control.

8. Assay for lupus erythematosus cells.—For the determination of LE cell forming capacity in sera and dissolved precipitates, the technique of Snapper and Nathan (32) was used.

9. Complement fixation tests.—Complement fixation tests were performed according to the methods of Casals and Palacios (33) using two 100 per cent units per tube and including appropriate serum and antigen controls at all times.

RESULTS

1. Double Diffusion Agar Precipitation.—When solutions of DNA between 10 and 500 μg. per ml. were employed as antigen in the double diffusion agar

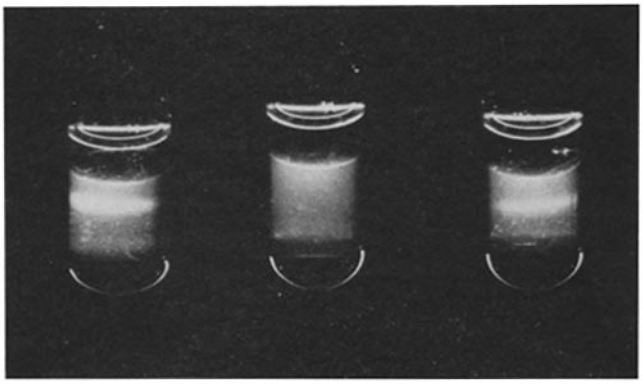

FIG. 1. The precipitin reaction in agar double diffusion tubes, DNA (calf thymus) 100 μg./ml. in 0.15 M NaCl layered over the agar with serum below the agar layer. Tubes photographed on the 6th day. Proceeding from left to right: SLE serum Me, normal control serum SLE serum Te.

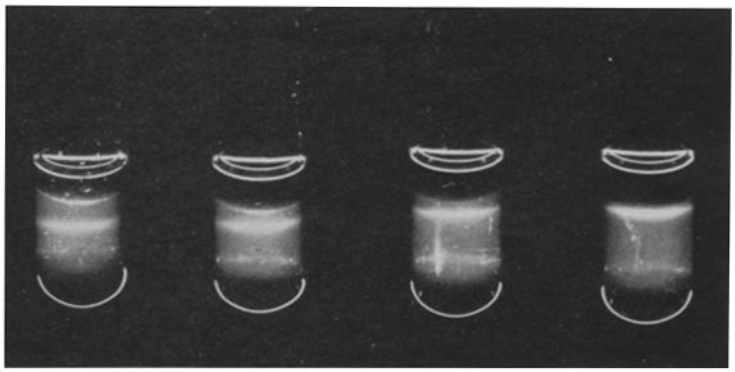

FIG. 2. The influence of the DNA concentration on the location of the precipitin band. DNA (calf thymus) concentrations from left to right: 500, 125, 50, and 12.5 μg./ml. in saline. Serum Te. Tubes photographed on the 6th day.

precipitation tubes, a definite precipitin band was observed in the agar layer after incubation for 2 to 6 days. The formation of the precipitate occurred with the DNA suspended in various buffers between pH 6.9 and 8.6 as well as in distilled water. A typical example of such a precipitin reaction is shown in fig. 1. The precipitin band migrated slowly through the agar until equilibrium was

established, and could then be observed unchanged for 2 to 3 weeks thereafter. No precipitate could be observed in any of the control tubes or in tubes in which DNA was replaced by the same concentration of RNA. The reaction was encountered with the serum of eight patients but was absent in sera from six other patients with SLE giving positive LE cell tests. The reaction appeared to be specific for the SLE sera and could not be shown with any other sera tested; these included sera from six normal persons, from patients with rheumatic fever with high sedimentation rates, rheumatoid arthritis with high serum euglobulin levels and high titer sheep cell agglutination, cirrhosis or chronic hepatitis with hypergammaglobulinemia, advanced scleroderma, and

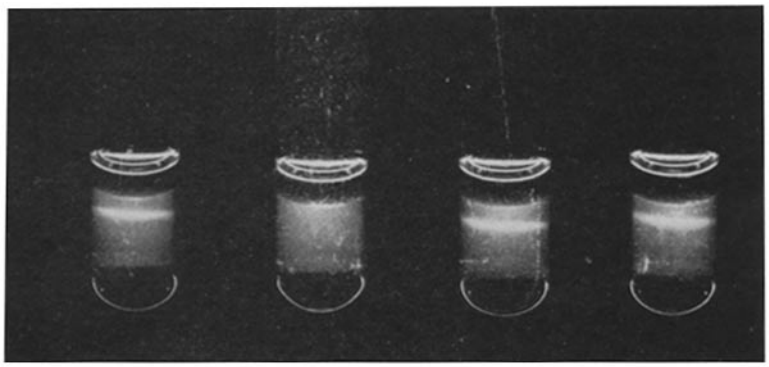

FIG. 3. The effect of different enzymes on the reactivity of the DNA (for details see text). Proceeding from left to right, the control reaction with 100 μg. DNA/ml. (1), the effect of pretreatment of the DNA solution with DNAase resulting in abolition of the precipitin line (2), the negative effect of RNAase (3), and trypsin (4) treatment. Serum: Te. Tubes photographed on the 6th day.

solutions of different concentrations of human fraction II γ-globulin (Lederle No. C-531). Some of these control sera had previously shown a precipitate in a simple ring test with DNA (19); with the double diffusion tube technique, however, these same sera showed a precipitate at the interphase between agar and serum regardless of the presence or absence of DNA thus indicating the presence of a readily precipitable protein; but no bands which formed in or moved through the agar were observed.

The specificity of the precipitin reaction with DNA could be further demonstrated in several ways. The position of the precipitin band could be shown to depend on the DNA concentration used (Fig. 2). At a concentration of 10 to 12 μg. DNA per ml., the band would usually form at the interface of the DNA and agar layers thus determining the lowest possible antigen concentration for the particular serum. Identical reactions could be encountered using

the gradient diffusion technique of Augustin (26); in this technique, a concentration gradient of the serum proteins is created in the agar layer by incubating the tubes without antigen for some hours and adding the antigen after the serum proteins have diffused into the agar. The strongest reacting sera still gave visible precipitin bands at dilutions of 1:4. Treatment of the DNA lution with DNAase prior to use resulted in the abolition of its precipitating power while treatment with RNAase or trypsin had no effect (Fig. 3).

No differences could be observed using DNA from different sources including calf thymus, salmon sperm, and type T6 phage. DNA isolated from human liver and human leucocytes also showed the same reaction, and no specificity of the human DNAs could be demonstrated. This is in accordance with the non-specific reactivity of different DNAs observed earlier with the complement fixation reaction (19).

The capacity of these sera to form precipitin bands with DNA could be abolished by absorbing the sera prior to testing with DNA as well as with calf thymus nuclei and isolated calf thymus nucleoprotein. This finding is consistent with recent evidence (34) that nucleoprotein will absorb the factors responsible for both the complement fixation reactions with DNA and with nuclei from many SLE sera, and that there seems to exist a variety of related but slightly different factors reacting with different sites of the nucleoprotein antigen in different sera; one of these would be the factor reacting with isolated DNA.

As already mentioned, the formation of the precipitin band was found independent of the pH of the buffer solutions used for the DNA dilution within the range of pH 6.9 to 8.6. However, the precipitin band tended to be denser at the lower pH, and precipitation was also enhanced by dissolving the DNA in distilled water instead of buffer. An ionic strength up to 0.25 did not interfere with precipitation. Further investigations on the influence of pH and ionic strength were undertaken with the precipitin curve system.

2. Studies with Precipitin Curves.—While the double diffusion technique furnished evidence for a specific interaction of DNA and a serum constituent in SLE, further evidence for the quantitative character of this reaction and the nature of the reactive serum protein was secured from the studies with precipitin curves obtained with three of the positive sera. These sera had been shown to contain larger amounts of the precipitating protein as determined by the gradient diffusion technique. Attempts to obtain precipitin curves from the other positive sera were less successful because of the very small amount of precipitate obtained.

Two typical precipitin curves, each characteristic for the particular serum, are shown in Fig. 4. At no other concentration of DNA up to 500 μg. per ml. could a precipitate be obtained from the serum. The curves show the characteristics of a usual precipitin curve; zones of antibody excess, equivalence, and

antigen excess can clearly be separated. As shown in Table I, an assay of the precipitates obtained at different points of the curve showed that the DNA added could be quantitatively removed from the precipitate in the antibody excess region and up to the equivalence point, while with increasing antigen

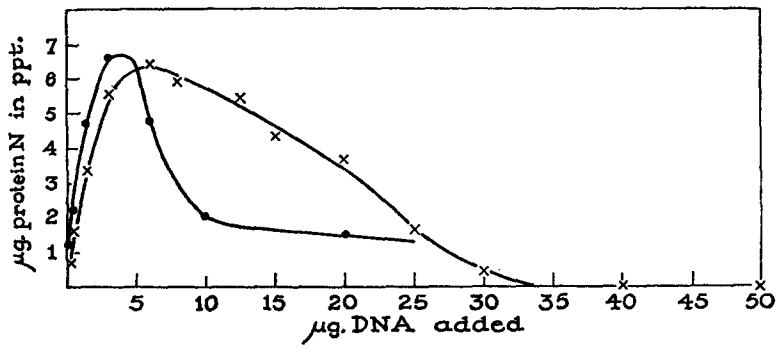

FIG. 4. Two precipitin curves obtained with DNA and two different sera. Serum dilution: 1:4.

×——×, serum: Te.

●——●, serum: Me.

TABLE I

Relation between the Composition of the Precipitate and the Amount of Added DNA

DNA added	Protein in ppt.	DNA found in ppt.
μg.	μg.	μg.
0.25	7.0	0.25
0.5	13.5	0.50
1.5	29.0	1.625
3.0*	41.3	2.90
5.0*	41.3	4.025
10.0	16.0	4.25
20.0	12.5	1.625
50.0	7.5	1.501

* Equivalence zone.

excess, progressively smaller fractions of the added DNA appeared in the decreasing amounts of precipitate. As in the agar double diffusion tubes, no precipitates were obtained using RNA at equal concentrations, and none of the control sera employed (including those with unusually high γ-globulin levels) gave a precipitin curve. Occasionally, small amounts of protein could be found in precipitates obtained from the hypergammaglobulinemic serum of one patient with cirrhosis in the control series; again however, 80 to 90 per cent of this

precipitate occurred regardless of the presence of DNA, and since this serum contained large amounts of euglobulin the greater part of this non-specific precipitation could be accounted for by euglobulin precipitation on dilution and standing.

TABLE II

Complement Fixation Tests on Serum Supernatants at Different Points of the Precipitin Curve

Precipitin curve data		Complement fixation titers on serum supernatants after removal of precipitate					
Total amount of DNA added	Protein in ppt.	Antigen used	Titer*				
			4	8	16	32	64
μg.	μg.						
None‡	—‡	DNA, 20 μg./ml.	4	4	4	4	c§
		Nucleoprotein‖	4	4	4	4	c
0.125	1.0	DNA	4	4	4	3	c
		Nucleoprotein	4	4	4	1	c
0.50	13.2	DNA	4	4	3	c	c
		Nucleoprotein	4	4	4	c	c
1.25	25.0	DNA	c	c	c	c	c
		Nucleoprotein	4	4	2	c	c
3.0¶	35.5	DNA	c	c	c	c	c
		Nucleoprotein	4	4	2	c	c
6.0	33.0	DNA	c	c	c	c	c
		Nucleoprotein	4	4	1	c	c

* Numbers represent reciprocal serum dilutions.

‡ Serum control tube.

§ Fixation tubes are graded from 4 = complete fixation to c = no fixation (complete hemolysis).

‖ Calf thymus nucleoprotein, precipitated from 1 M NaCl solution and homogenized for 5 minutes in a Potter homogenizer.

¶ Equivalence point.

Changes of the total volume in the individual precipitin tube between 0.75 and 2.0 ml. total volume did not alter the amount of precipitate or the shape of the curve appreciably.

A close relationship between the DNA-precipitating and complement-fixing factors in the serum was suggested both by complement fixation reactions on serum supernatants after removal of the specific precipitates as well as by complement fixation and precipitin tests performed on the protein recovered from equivalence point precipitates. The result of a typical experiment of the

former kind is shown in Table II. As the precipitin curve approaches the equivalence zone, the complement fixation titer against DNA decreases and disappears near the equivalence point while the complement fixation titer against nucleoprotein is only partially removed, indicating the presence of more than one factor reacting with nucleoprotein. Also, no further precipitation in the

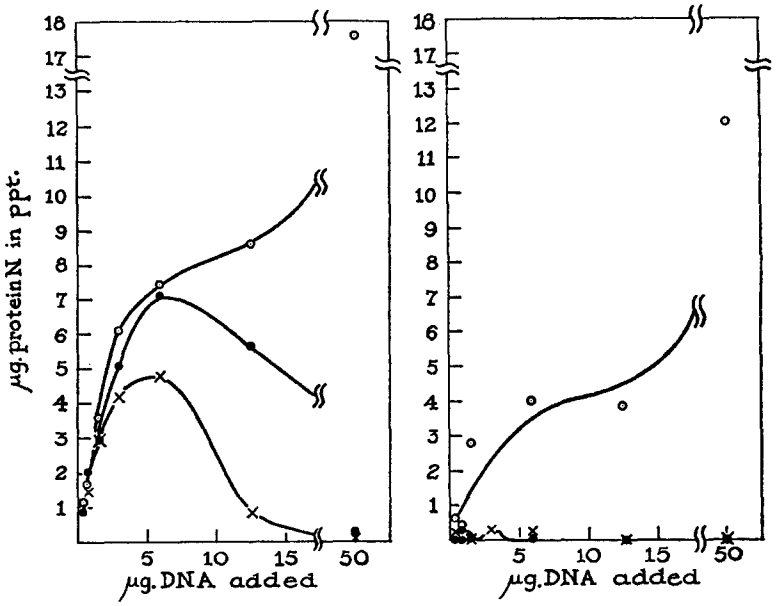

FIG. 5. Comparison of the precipitin curves obtained with a positive SLE serum (Te) (left graph) and a normal serum (right graph).
○——○, saline-phosphate buffer: pH 5.1.
●——●, saline-phosphate buffer: pH 7.45.
×——×, saline-barbital buffer: pH 8.6.
Ionic strength: 0.15.
The normal serum only shows non-specific precipitation at pH 5.1, but no precipitates at the other pH levels. The SLE serum shows precipitates at all three pH levels; at pH 5.1, the precipitate is obviously composed of both specific and non-specific precipitates.

agar double diffusion tube could be obtained after removing the precipitating factor from the serum with amounts of DNA equal to those precipitated at equivalence. Thus the conclusion can be drawn that the factor responsible for the precipitin reaction in these sera is intimately related to and perhaps identical with the factor causing the fixation of complement with DNA. Because of the higher sensitivity of the complement fixation test, it is not surprising that a positive reaction had been encountered more frequently with this test (18, 19); all sera with positive precipitin reactions have been shown to give

positive complement fixation titers with DNA (as well as in most instances with other nuclear constituents).

3. Influence of pH and Ionic Strength on the Precipitin Curve.—It has already been stated that the precipitin band formation in the agar double diffusion system could be facilitated by decreasing the pH or ionic strength of the solvent of the DNA, but that within a certain pH the reaction would not be altered qualitatively by such changes. Further information about these influences was obtained from precipitin curve studies. Fig. 5 shows the influence of changing the pH of the reaction mixture between pH 5.1 and 8.6. There appears a non-

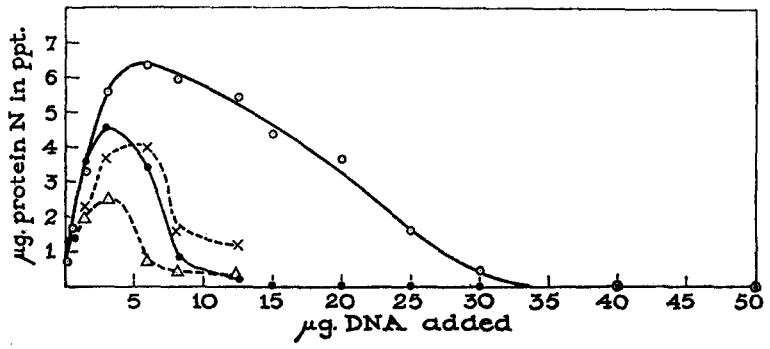

FIG. 6. The influence of pH and NaCl concentration on the amount of precipitate. Serum: Te.

O——O, pH 7.45, ionic strength: 0.15
●——●, pH 7.45, ionic strength: 0.25
×-----×, pH 8.6, ionic strength: 0.15
△-----△, pH 8.6, ionic strength: 0.25

specific precipitate of serum protein and DNA at pH 5.1, a pH clearly below the isoelectric point of all gamma and some of the beta globulins. Such non-specific precipitation is known to occur between many basic proteins and DNA *e.g.*, references 35, 36). At pH 7.45 or 8.6 (*i.e.* above the isoelectric point of most γ-globulins) no precipitate appears with the normal serum, while a precipitin curve is obtained with the SLE serum. The non-specific precipitation appears to occur only under conditions of opposite net charge, but at the higher pH levels at which both serum proteins and DNA have the same over-all charge, precipitation occurs only with the SLE sera. On the other hand, the important role of the participation of charged groups, in the system under investigation is illustrated by Fig. 6. By increasing the ionic strength from 0.15 to 0.25 at the different pH levels, the amount of precipitate obtained could be shown to decrease appreciably over the entire curve. Further investigations on this point are in progress.

4. The Nature of the Serum Factor.—While attempts to dissociate the pre-

cipitate with highly concentrated NaCl or with acid buffers were largely unsuccessful, some dissociation could be achieved using DNAase and employing the procedure described above. The protein removed from the precipitate by this method reacted strongly with rabbit antiserum to human γ-globulin in a capillary tube; it had a complement fixation titer against DNA very similar to the original serum, and also showed fixation of complement when whole nuclei were employed as antigen. When a large precipitate from 5 ml. of one serum was treated in this manner, and the protein concentration of the resulting

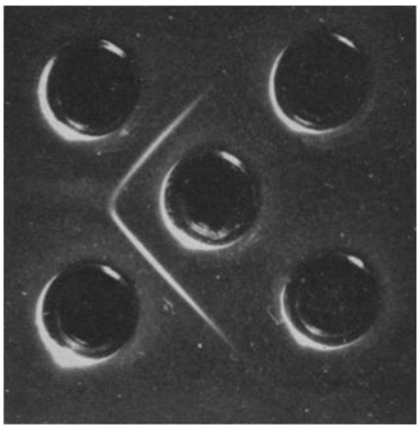

FIG. 7. Immunological similarity of the protein recovered from the specific precipitate by the action of DNAase, with normal human γ-globulin. Center well: supernatant of the dissolved specific precipitate (equivalence point) from 0.50 ml. of SLE serum, after treatment with DNAase and dialysis. Left upper well: horse antiserum to whole human serum. Left lower well: rabbit antiserum to fraction II human γ-globulin. Right upper well: rabbit antiserum to human albumin. Right lower well: saline.

supernatant so adjusted that its γ-globulin content equalled the amount of γ-globulin precipitated at equivalence point, 50 per cent of this γ-globulin could be precipitated by the addition of an appropriate amount of DNA. The remaining solution contained γ-globulin as determined by qualitative capillary precipitin tests, but could not be precipitated by the addition of further DNA.

The protein recovered by the DNAase method represented between 30 and 50 per cent of the total amount precipitated and gave a single precipitin line with rabbit antiserum to human γ-globulin in an Ouchterlony plate; this line showed a reaction of identity with a single precipitin line obtained with horse antiserum to whole human serum (Fig. 7) and also with human fraction II γ-globulin, by complete fusion of the lines. Precipitates were also dissolved in 0.14 molar NaCl containing 6 molar urea, followed by the addition of DNAase after the precipitate had dissolved, and immediate dialysis for 18 hours against

0.14 M NaCl — 0.02 M $MgCl_2$. The results were not different from those obtained with DNAase treatment alone. Analysis of the remaining precipitate after DNAase treatment showed that approximately 90 per cent of the DNA had disappeared after DNAase treatment and dialysis, while about 50 per cent of the total amount of protein present in the original precipitate remained insoluble. A further small fraction of this "remaining precipitate" could then be recovered by heating to 56° for 30 minutes in 0.14 M saline. This fraction, which could also be shown to give a reaction of identity with fraction II γ-globulin, did not show any complement-fixing activity, but occasionally exhibited a weak capacity to form "LE cells." However, even after this procedure about 25 to 30 per cent of the protein would remain precipitated. It is probable that this fraction represents denatured protein, which becomes denatured by the successive isolation procedures; it could be shown to contain γ-globulin since it would take up protein from rabbit antiserum to human γ-globulin. However, further investigation on this problem appears indicated because there remains some possibility of the participation of a second protein with low solubility properties.

The electrophoretic mobility of the serum factor was determined by preparative zone electrophoresis on Geon resin 426 (37) performed on whole SLE serum giving positive reactions with DNA. Precipitin reactions in agar double diffusion tubes on concentrated eluates from the electrophoresis block showed that the DNA precipitating factor migrated with the γ-globulin fraction; likewise, in complement fixation tests on the recovered fractions against DNA as antigen, only the γ-globulin fraction gave positive results. In the latter experiments, the difficulty of the anticomplementary behavior of isolated γ-globulin was overcome by the addition of an equal volume of normal serum to the concentrated eluates. On immunoelectrophoresis, however, the factor consistently showed a considerably faster mobility; within 48 to 72 hours, one clear line of precipitin developed well ahead of the γ-globulin peak, but was still within the limits of the long γ-globulin line. Although this difference in mobility is as yet unexplained, the migration of this particular protein may have been influenced by the acidic agar medium.

5. The Relationship of the DNA-Precipitating γ-Globulin to the Formation of LE Cells.—Investigations on the question of the relation of the DNA-precipitating factor and the capacity of these sera to form lupus erythematosus cells proved difficult because of the non-quantitative nature of the LE test. All sera continued to show definite LE cell formation after removing DNA precipitates at the equivalence point, or by adding antigen excess concentrations of DNA to the serum. In some cases, however, the total number of lupus erythematosus cells formed after removal of the equivalence point precipitate appeared to be decreased. The eluates obtained from equivalence point precipitates by DNAase digestion never showed any LE cell–forming capacity, regardless of the pres-

ence or absence of normal serum. However, since it was found that the presence of DNAase in these eluates, by its action on cell nuclei, would itself interfere with the formation of LE cells, the eluates were heated to 65°C. for 20 minutes; this treatment destroyed the DNAase activity but did not severely interfere with the LE cell-forming capacity in controls. Also after this treatment, the eluates did not induce the formation of LE cells. On the contrary, some of the eluates obtained from 56°C. heating of the "remaining" precipitate (*i.e.* after removal of the fraction liberated by DNAase and subsequent washing), exhibited a very weak capacity to induce LE cell formation. Thus, while the LE factor appears to require the presence of the nuclear DNA for the interaction with such nuclei (10), it shows little or no reaction with DNA alone.

DISCUSSION

Non-specific interactions of DNA with a number of different proteins has been the subject of several reports (35, 36); it has been shown that such interactions are predominantly determined by the acidic properties of the DNA and the nature of the reaction medium providing for the necessary difference in net charge (36). Thus, these DNA-protein linkages appear to be largely of a salt-like nature, a fact which also applies to native nucleohistone complexes. Even with such systems, however, precipitin curves have been observed under the proper conditions by Björnesjö and Teorell (38) in their studies on the interaction of histone and albumin with DNA. Normal fraction II γ-globulin will also interact with DNA under conditions of opposite net charge of protein and nucleic acid; at pH 5.1 and an ionic strength of 0.15, a precipitin curve can be observed with this system, while no such interaction occurs at any pH above 7.0. The reaction between DNA and SLE serum factor described here, then differs from the formation of salt-like complexes in that the formation of precipitates occurs at pH levels at which the over-all charge of the DNA and the serum proteins including the γ-globulins is the same. The observation that at an ionic strength of 0.25 the amount of protein precipitated by DNA from the SLE serum is reduced by about one-half need not argue against an immunologically specific interaction, although the influence of salt concentration appears to be more pronounced than is usual with other systems. Heidelberger and associates (39) have shown in their studies of rabbit antisera to pneumococcal polysaccharides that the presence of salt concentrations above 0.15 ionic strength resulted in decreasing the amount of precipitate, and similar observations have been made by Oudin and Grabar (40) for egg albumin and homologous rabbit antibody. The results of the present study suggest a considerable participation of charged groups in the DNA-SLE serum factor interaction, and this is further emphasized by the fact that a change of the pH of the reaction mixture from 7.45 to 8.6 also decreases the amount of precipitate. Since one can well expect the participation of the prominent acidic phosphate groups of

the DNA molecule in any interaction, they also may be responsible in part for the specificity.

Immunological analyses of the protein recovered from the DNA precipitate by DNAase treatment indicate that the serum factor behaves antigenically like a γ-globulin. This is in accordance with observations recently reported by Seligman (41). Zone electrophoresis on a polyvinyl supporting medium has furnished further evidence that the factor is a γ-globulin. On the other hand, a more rapid mobility has been obtained by immunoelectrophoresis in 1 per cent agar used to permit diffusion of the DNA. The different migration in this medium remains unexplained; the possibility exists that the migration of this particular protein is influenced by the acidic agar medium. The electrophoretic results provide good evidence against a hypothetical, unusually basic protein of histone character which would be expected to migrate more slowly than the γ-globulin.

The relation between the DNA-precipitating factor and the LE cell factor is of considerable interest. It has been shown that treatment of isolated nuclei with DNAase prevents adsorption of LE cell factor on such nuclei (12). Friou (42), Holborow (43), and Bardawil (44) and their coworkers utilizing the fluorescent antibody technique, have demonstrated a reaction between a serum γ-globulin in SLE and cell nuclei in tissue slices. It appears that in most instances the reacting serum γ-globulin was the LE cell factor, and these workers have found that DNAase treatment of those tissue preparations abolished the reaction with SLE serum. These findings have suggested that DNA is essential for the reaction of LE cell factor and nuclei.

The accumulated evidence now suggests that the DNA-precipitating and the LE cell-inducing factors are two separate γ-globulins: certain sera containing the LE cell factor do not contain the DNA-precipitating factor, serum supernatants after removal of the DNA-precipitating factor are still capable of inducing the formation of LE cells to much the same degree as before, and the LE cell factor requires both the protein and the DNA of nucleoprotein for its reaction (34). Thus, while both the DNA-precipitating factor and the LE cell factor react with DNA, in the latter instance protein, probably histone, must also be present for the reaction to occur.

Polli and coworkers (45) have recently presented evidence for the absorption of LE cell factor from one serum by DNA alone, and Seligman (41) has reported recovery of protein capable of inducing LE cell formation from precipitates obtained with DNA, although much of the LE cell-forming activity remained in the supernatant serum. A clear explanation of these findings is not at hand, but they may be due to a weak "cross-reaction" between LE cell factor and DNA alone, or to coprecipitation of some LE cell factor with the DNA precipitate, or variations in the specificity of the LE cell factor in some sera. In order to settle this question further knowledge will have to be acquired concerning the antigenic sites of nuclear constituents.

The evidence presented above as well as that collected in other laboratories regarding the reactions between DNA and SLE serum strongly suggests that DNA possesses antigenic properties. Final proof however for this concept has still to await further animal experimentation. Work of this nature so far published has suggested such antigenicity, but none of these studies has reported the broad reactivity of DNA preparations from widely divergent sources noted in the present study. This lack of species specificity is strong evidence against any important role of small protein contaminants in the DNA preparations. Difficulties encountered by many in achieving experimental antibodies against DNA (with certain exceptions (4, 46)) indicate that it is a relatively poor antigen under ordinary experimental conditions. In the case of SLE, which may well be described as a basic disorder of the entire immune system with the resulting production of many unusual antibodies, it is conceivable that substances which are poorly antigenic normally, assume such qualities and therefore give rise to rare antibodies. Thus, the presence of antibodies directed against DNA, as well as related ones against different nuclear antigens (34), would appear to fit the concept that the disease SLE is characterized by the appearance of a wide assortment of antibodies against tissue components.

SUMMARY

The sera of certain patients with systemic lupus erythematosus contain an antibody-like substance capable of reacting with highly purified DNA preparations from widely divergent sources. Precipitin reactions have been demonstrated by double diffusion in agar and quantitative precipitin curves have been obtained. Complement was observed to be fixed in the reaction.

Evidence was obtained that the serum factor possessed antigenic properties similar to those of γ-globulins and migrated with this fraction on zone electrophoresis. The interaction of this factor with DNA exhibited certain specific characteristics which differ considerably from non-specific reactions between DNA and proteins in general.

The DNA-precipitating factor appeared to be one of a number of related factors reacting with nuclear constituents of many different cells. It differed in certain respects from the "LE factor" which is responsible for the formation of "LE cells."

The accumulated evidence, although not yet conclusive, favors the concept that the precipitating factor represents an antibody to DNA, and that it is one of a number of autoantibodies elicited in this disease.

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