from an action of the "antiviral" protein (*13*) on the ribosomes of toxoplasma which interferes with translation of toxoplasma messenger RNA by toxoplasma ribosomes. Among alternative explanations is the possibility that host cell ribosomes are used in toxoplasma replication, and in the interferon-treated cells they fail to translate toxoplasma messenger RNA. Either of these mechanisms may account for the observed action of interferon on cells infected with psittacosis (*1*) and TRIC agents (*2, 3*). Certainly the current definition of interferon in terms of its antiviral properties alone must be broadened to include its action against phylogenetically higher organisms.

Variations in virulence of certain viruses appears to be correlated with the ability to induce interferon production (*14*). The wide variation in virulence among different strains of toxoplasma (*15*) may, in a similar manner, be related to their ability to induce interferon production. As toxoplasma have been shown to stimulate interferon production, the role of interferon as a determinant in recovery from protozoal intracellular infection (such as toxoplasma, malaria, leishmania) in vivo must be considered. The general nature of interferon action against intracellular protozoa and its possible role in vivo are suggested by the independent findings of Jahiel *et al.* (*16*) on the protective effect of interferon-inducing agents in the mouse against *Plasmodium berghei* infection.

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7. Each 100 ml contained 36 ml of media 199, 36 ml of Earle's balanced salt solution, 14 ml of fetal calf serum, 14 ml of chick embryo extract and antibiotics as in the L cell medium.
8. Each 100 ml contained 55 ml of medium 199, 20 ml of medium 199 (double strength), 2 ml of tryptose phosphate broth, 1 ml of fetal calf serum, 1 ml of chick serum, 1 ml of a 2.8-percent solution of sodium bicarbonate and 20 ml of a 4.75-percent solution of Noble special agar (Difco).
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Deoxyribonucleic Acid Antibody: A Method to Detect Its Primary Interaction with Deoxyribonucleic Acid

Abstract. *Antibody to DNA in human serums can be detected by the ammonium sulfate method. This sensitive and specific technique, which measures the primary interaction between DNA and antibody to DNA, is based on the observation that free DNA is soluble in 50-percent saturated ammonium sulfate whereas antibody-bound DNA is insoluble.*

Antibodies specific for DNA in the serums of patients with systemic lupus erythematosus have been demonstrated by several immunologic techniques including quantitative precipitation, immunodiffusion in agar, complement fixation, and hemagglutination (*1*). These techniques measure a secondary manifestation which may or may not occur after a primary reaction between antigen and antibody has taken place. Since some serums, especially in the human, have low precipitating, complement-fixing, or hemagglutinating efficiencies (*2*), a technique which measures the primary interaction between DNA and antibody should provide information indicative of total antibody. We now report on a primary binding technique to measure DNA antibody by the ammonium sulfate method which was originally described for bovine serum albumin (*3*). Antibody-bound DNA can be separated from free DNA by this technique because DNA is soluble in 50-percent saturated ammonium sulfate solutions, whereas DNA-antibody complexes are insoluble.

Human serums were obtained from patients with systemic lupus erythematosus and from normal subjects. All patients' serums had antibodies to nuclear material as demonstrated by the indirect immunofluorescent technique; some serums had precipitins to DNA as detected by immunodiffusion in 0.4 percent agarose against calf thymus DNA (*4*). None of the normal serums had demonstrable DNA precipitins or antibodies to nuclear material.

Tritiated DNA (H^3-DNA) was prepared by growing *Bacillus subtilis* in medium (*5*) containing tritiated adenine or thymidine (*6*) and was isolated and purified as reported (*7*). The ammonium sulfate test has been described (*3*). DNA from calf thymus (20 μg in 1 ml of borate buffer at *p*H 8.4; 0.1 ionic strength) or H^3-DNA from *B. subtilis* (2 μg in 0.5 ml borate buffer) were added to equal volumes of serum diluted 1 :10 in borate buffer. Subsequent dilutions of serums were made

Table 1. DNA binding by normal human serums and systemic lupus erythematosus serums. Results are expressed as the percentage of calf thymus DNA or *Bacillus subtilis* tritiated DNA (H^3-DNA) detected by diphenylamine or isotopic assay in the 50-percent saturated ammonium sulfate precipitates (ppt) of the serums diluted 1:10. DNA precipitins were detected in patients' serums Nos. 1 to 4 but not in the remaining patients' or normal human serums.

Serums (No.)	20 μg DNA (% in ppt)	2 μg H^3-DNA (% in ppt)
Normal human		
1	7.5	3.7
2	2.5	2.3
3	7.5	2.0
4	5.0	1.4
5	2.5	2.1
6	7.5	4.5
7	5.0	8.8
8	5.0	1.7
9	7.5	2.8
Pool	2.5	2.6
Systemic lupus erythematosus		
1	97.5	71.7
2	77.5	57.2
3	72.5	51.6
4	15.0	25.3
5	10.0	10.7
6	10.0	14.1
7	7.5	5.0
8	5.0	5.0

with normal human serum diluted 1 : 10 in borate buffer. After incubation at 4°C for 18 hours, an equal volume of saturated ammonium sulfate was added. The precipitates were washed with 50-percent saturated ammonium sulfate in borate buffer, redissolved and analyzed for calf thymus DNA by a diphenylamine method (*8*) and for H^3-DNA in a Packard Tri-Carb liquid-scintillation spectrometer (*9*). The mean of duplicate determinations is expressed as the percentage of DNA added to serum which was detected in the ammonium sulfate precipitate.

When either 20 μg of calf thymus DNA or 2 μg of H^3-DNA were added to normal serums diluted 1 : 10, less than 10 percent of the DNA added was detected in the ammonium sulfate precipitates (Table 1). However, patients' serums (Nos. 1 to 4) containing DNA precipitins bound 15 to 97 percent of the added DNA. The percentage of DNA bound by the patients' serums lacking detectable DNA precipitins was either normal or slightly greater than normal.

To investigate the specificity of DNA antibodies as measured by the ammonium sulfate method, we determined the effect of unlabeled nucleic acids and immunologically unrelated polyanions (hyaluronic acid and heparin) on the H^3-DNA binding by patients' serums. Those serums which bound more than 50 percent of the H^3-DNA at a 1 : 10 dilution were further diluted so that approximately 50 percent of the H^3-DNA was bound. Calf thymus DNA, yeast RNA, hyaluronic acid, or heparin (*10*) was added to the serums and incubated at 4°C for 24 hours before the addition of H^3-DNA. The final concentration of these inhibitors was 50 μg/ml or 25 times the concentration of the H^3-DNA. Under these conditions, antibody combining sites are occupied by specific unlabeled antigen and therefore are unavailable to bind labeled antigen when subsequently added (*11*). Patients' serums which bound more H^3-DNA than normal serums did were significantly inhibited by calf thymus DNA (Table 2). By contrast, H^3-DNA binding was slightly decreased in only two serums in the presence of heparin and RNA. Thus the inhibition technique has a high degree of specificity and can be applied to detect free DNA in serum or other biological fluids.

The sensitivity of the ammonium sulfate method for measuring DNA antibody is limited primarily by the specific activity of radioactive DNA. When this was increased so that less H^3-DNA (0.1 μg) could be added to serums, it was possible to dilute a serum approximately 1 : 5000 before the percentage of H^3-DNA bound approached normal (Fig. 1). More importantly, the results in Fig. 1 show that both precipitating and nonprecipitating antibody to DNA can be detected by this technique. Although we have observed 97 percent binding of mammalian DNA (Table 1), maximum binding of tritiated bacterial DNA has not exceeded 72 percent.

Our results indicate that it is feasible to measure antibody to DNA by precipitation of DNA-antibody complexes with 50-percent saturated ammonium sulfate. This technique detects the primary interaction between DNA and antibody, whereas secondary tests measure only those antibodies capable of precipitation, complement fixation, or hemagglutination. With a sensitive, specific, quantitative primary binding technique, it may be found that the incidence and heterogeneity of DNA antibodies in human serums may be greater than is believed.

Table 2. Effect of unlabeled nucleic acids, hyaluronic acid, and heparin on H^3-labeled DNA binding by systemic lupus erythematosus serums. Results are expressed as the percentage of 2 μg of H^3-DNA in the 50-percent saturated ammonium sulfate precipitates of the serums when no inhibitor was present or when calf thymus DNA, yeast RNA, hyaluronic acid, or heparin was added at a final concentration of 50 μg/ml. Serums were diluted as follows: No. 1, 1:75; No. 2, 1:18; No. 3, 1:12; Nos. 4 to 8, 1:10.

Serum (No.)	Control	Calf thymus DNA	Yeast RNA	Hyaluronic acid	Heparin
1	44.4	3.9	45.9	47.5	49.3
2	50.3	5.5	50.2	51.1	49.7
3	48.2	6.3	46.5	48.1	46.8
4	21.5	2.6	17.4	17.9	13.9
5	9.5	1.4	8.5	9.4	7.7
6	19.4	2.3	10.7	17.2	17.8
7	3.3	0.7	2.2	5.6	5.1
8	5.5	1.4	3.0	4.9	5.8

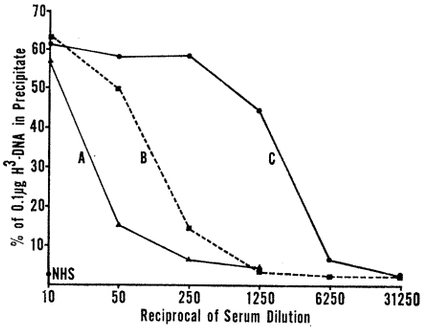

Fig. 1. Percentage of 0.1 μg of H^3-DNA in the 50-percent saturated ammonium sulfate precipitates of dilutions of serums (fivefold, serially) from patients with systemic lupus erythematosus. *A*, serum with precipitins only to heat-denatured calf thymus DNA; *B*, serum with no detectable precipitins to DNA; and *C*, serum with precipitins to both native and heat-denatured calf thymus DNA. Less than 3 percent (3 ng) H^3-DNA was detected in the precipitate of normal human serum (*NHS*).

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10. Calf thymus DNA and yeast RNA were obtained from Worthington Biochemical Corp., Freehold, N.J.; human umbilical hyaluronic acid from Mann Research Laboratories, Inc., New York, N.Y.; and heparin sodium from Upjohn Co., Kalamazoo, Mich.
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