could act more directly on these other receptors so that only a low degree of protection would result.

The results with vagotomized monkeys reported in this communication after i.v. challenge are in general agreement with the findings in vagotomized cats given enterotoxin by the same route(3).

Summary. Rhesus monkeys subjected to transthoracic vagotomy were challenged with staphylococcal enterotoxin and observed for vomiting. A significantly higher tolerance was shown against intragastric administration than against intravenous injection of enterotoxin. Complete protection was not obtained even against oral challenge. Responsiveness to enterotoxin-induced emesis in vagotomized monkeys and cats is similar if the challenge is by the intravenous route.

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Antibodies in Systemic Lupus Erythematosus and Myasthenia Gravis Which React with Thermally Denatured DNA-Coated Bentonite. (28882)

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Antibodies to native deoxyribonucleic acid (DNA) have been demonstrated in the sera of some patients with systemic lupus erythematosus (SLE) by several techniques including precipitation, complement fixation, and aggregation of tanned red blood cells or of bentonite particles coated with native DNA(1,2,3,4). The diagnostic usefulness of these tests has been limited by the low incidence of anti-DNA antibodies detected.

Levine *et al*(5) first reported enhanced immunologic reactivity of DNA after thermal denaturation. Stollar *et al*(6) have shown by complement fixation that sera from patients with SLE react with thermally denatured DNA more frequently than they react with native DNA. Because of its technical simplicity, we have employed a modification of the bentonite particle technique described by Bozicevich *et al*(4) to compare the reactivity of native and thermally denatured DNA with sera from patients with SLE and certain other diseases.

The finding by White and Marshall(7) of an antinuclear antibody in some patients with myasthenia gravis led us to look for antibodies to thermally denatured DNA and certain other antibodies in the sera of patients with this disease.

Materials and methods. Serum. Most sera were kept frozen for various periods up to several months prior to testing. Some of the sera from the patients with myasthenia gravis had been kept at 4°C for several months.

Native DNA bentonite test. The method of Bozicevich *et al*(4) was used.

Thermally denatured DNA bentonite test.

Highly polymerized calf thymus DNA* was dissolved in distilled water (200 μg/ml), heated in a boiling water bath for 45 minutes and quickly cooled in ice water. Spectrophotometric analysis of the DNA solution at 2600 Å showed a 40% increase in absorbance after heating. This method of thermal denaturation of DNA and spectrophotometric determination of the resulting molecular changes has been described by others(8,9). The suspension of bentonite particles was prepared according to the procedure decribed by Bozicevich *et al*(4). Two hundred micrograms of thermally denatured DNA in 1.0 ml of distilled water was added to 10 ml of bentonite particle suspension and allowed to stand for 1 hour at room temperature with occasional shaking. One ml of 1% crystalline bovine plasma albumin† was added and mixed carefully to avoid foaming. After 10 minutes, 1.0 ml of 0.1% methylene blue was added to color the particles. The suspension was centrifuged at 500 × *g*, resuspended in distilled water, washed twice more in this manner and finally resuspended in 2.5 ml of distilled water. The reagent is stable at 4°C for at least one week. One-tenth ml of serially diluted sera, previously heated to 56° for 30 minutes, and approximately 0.025 ml of denatured DNA-coated bentonite reagent were placed in ringed glass slides. The slides were rotated 120 times per minute for 20 minutes on a Boerner type rotator and observed for flocculation under 100 × magnification.

Determination of rheumatoid factor. The method used was the bentonite flocculation test of Bozicevich *et al*(10).

Determination of thyroglobulin antibody. A modification of the hemagglutination test of Boyden(11) was used in which tannic acid-treated human type O, Rh negative erythrocytes coated with human thyroglobulin served as the antigen.

Test for antinuclear antibody. A modification of the technique of Friou *et al*(12) was used. Four micron thick frozen sections of mouse liver, mounted on glass slides and air dried, served as a source of nuclei. The sections were flooded with serial 4-fold dilutions of the test sera in phosphate buffered saline, reacted for 15 minutes in a moist chamber, and then washed in 3 changes of buffered saline for five minutes each. The sections were then exposed to a fluorescein isothiocyanate conjugated rabbit anti-human 7S gamma globulin‡ for 15 minutes following which the slides were washed as before, mounted under buffered glycerine and a cover slip and examined for nuclear fluorescence. An Osram HBO 200 light source and a UG-1 exciter filter was used with a Leitz Ortholux microscope, a dark field condenser and Wratten 2A barrier filter.

Results. The incidence of reactivity with thermally denatured DNA and native DNA and of antinuclear antibody in sera of patients with SLE, rheumatoid arthritis, scleroderma, Sjögren's syndrome and of normal and disease controls is shown in Table I. Sera from 35 patients with typical SLE were studied. All the patients had positive LE cell preparations sometime in the course of their disease. Sera from 23 (66%) of these patients flocculated bentonite particles with thermally denatured DNA while only 10 (29%) flocculated the particles coated with native DNA. Antinuclear antibody was found in all 34 sera tested.

Reactivity with thermally denatured DNA was found in sera from 7 (25%) of 27 patients with rheumatoid arthritis. However, LE cell preparations were positive in 6 of these patients, and of these, 4 (67%) reacted with thermally denatured DNA. By contrast sera of only 3 (14%) of the 21 patients with rheumatoid arthritis, without demonstrable LE cells, flocculated denatured DNA-bentonite. Antinuclear antibody was found in the sera of 4 (25%) of 17 patients with rheumatoid arthritis without demonstrable LE cells and in 5 (83%) of 6 sera from patients with rheumatoid arthritis and positive LE cell preparations.§

* Worthington Biochemical Corp., Freehold, N. J.
† Armour Co., Chicago, Ill.
‡ Kindly supplied by Dr. James Coldberg, Laboratory of Immunology, NIAID, NIH, Bethesda, Md.
§ These patients had characteristic deforming arthritis and were not thought to have SLE.

TABLE I. Results of Tests for Serum Reactivity with Denatured DNA, Native DNA and Whole Nuclei.

	Denatured DNA bentonite		Native DNA bentonite		Antinuclear antibody	
	Patients tseted	% positive	Patients tested	% positive	Patients tested	% positive
Systemic lupus erythematosus (SLE)	35	66	35	29	34	100
Probable SLE*	4	100	4	50	4	"
Discoid lupus	"	0	N.T.§	N.T.	N.T.	N.T.
Rheumatoid arthritis with LE cells	6	67	6	17	6	83
" " without LE cells	21	14	21	5	17	25
Sjögren's syndrome	8	38	7	12.5	5	60
Scleroderma	7	0	N.T.	N.T.	7	57
Neuromuscular diseases†	3	100	3	0	3	0
Disease controls‡	124	3	38	0	100	1
Normal "	26	0	N.T.	N.T.	N.T.	N.T.

* Patients with signs and symptoms of SLE but without positive LE cell preparations.
† No specific neurological diagnosis could be made in these 3 patients. One had a positive LE preparation on one occasion and another had arthralgia.
‡ Tuberculosis(33), nephrosis(7), fever of unknown origin(5), granuloma(5), psoriasis(5), others(65).
§ Not tested.

Reactivity with thermally denatured DNA was found in sera from 3 (38%) of 8 patients with Sjögren's syndrome but in none of 7 patients with scleroderma. By contrast, the incidence of antinuclear antibody in these sera (Sjögren's syndrome: 60%, scleroderma: 57%) was strikingly similar. Anti-DNA antibody was not found in sera of 2 patients with dermatomyositis.

Sera of 4 patients with arthritis, fever and skin rash and other signs and symptoms characteristic of SLE, but without positive LE cell preparations, flocculated bentonite coated with thermally denatured DNA. All 4 sera also contained antinuclear antibody. Sera from 3 patients with neuromuscular disorders of unknown etiology flocculated bentonite coated with thermally denatured DNA. None of these 3 sera contained demonstrable antinuclear antibody. Sera from 2 of 4 siblings of patients with SLE reacted with denatured DNA-bentonite.

Sera from 26 normal people and 124 patients with a variety of diseases were studied. None of the normal sera flocculated denatured DNA-bentonite particles. Only 4 (3%) of the disease control sera reacted positively. One of these was from a patient with periodic fever due to hypothalmic disease, 2 were from patients with pulmonary tuberculosis, one of whom also had rheumatoid spondylitis, and the other was from a patient with hypergammaglobulinemia and a multi-form skin eruption.

The titers of antibody determined with denatured DNA and native DNA, of rheumatoid factor and of antinuclear antibody in sera from patients with SLE are compared in Table II. Reactions with thermally denatured DNA were more frequent and in higher titers than the reactions with native DNA in almost every case. The highest titer observed in the reactions with native DNA was 1:64, whereas titers of 1:1024 were reported for the reaction with thermally denatured DNA. In no instance did a serum react with native DNA and not with denatured DNA. The antinuclear antibody titers were frequently, but not always, higher than the anti-denatured DNA titers and they were occasionally lower. Rheumatoid factor in a titer of 1:32 to 1:256 was found in 12% of the patients with SLE.

The results of studies of sera from 44 patients with myasthenia gravis are shown in Table III. Nine (20%) of the sera reacted with denatured DNA bentonite while none reacted with native DNA. Antinuclear antibody was present in sera of 16 (37%) of the patients with myasthenia gravis and rheumatoid factor and thyroglobulin antibodies were occasionally present in low titer.

Serum from one patient with SLE was fractionated on diethylaminoethyl (DEAE)-

TABLE II. Antibody Titers in Sera of Patients with SLE.

Patient	Denatured DNA bentonite	Native DNA bentonite	Antinuclear antibody	Rheumatoid factor
G.W.	1:1024	1:16	1:64	neg
E.H.	"	neg	1:256	"
T.W.	"	1:4	"	"
C.W.	"	1:16	"	1:32
N.B.	1:256	1:64	1:64	neg
G.J.	"	1:16	1:256	"
J.B.	"	"	"	"
H.V.	1:64	neg	1:64	1:256
H.L.	"	1:16	1:1024	"
A.H.	"	1:4	1:16	N.T.*
S.R.	"	"	1:4096	neg
A.N.	"	neg	1:256	N.T.
F.G.	1:16	"	1:64	neg
J.G.	"	"	1:256	"
D.B.	"	"	"	"
E.W.	"	undil	1:1024	"
B.B.	"	neg	1:64	"
A.M.	"	"	1:256	"
S.P.	"	"	1:64	"
H.F.	1:4	"	"	"
D.E.	"	"	1:256	"
L.H.	"	"	1:1024	"
M.P.	"	"	N.T.	"
R.S.	neg	"	1:64	"
M.W.	"	"	1:256	1:32
C.L.	"	"	1:400	neg
M.L.	"	"	1:256	"
S.M.	"	"	1:200	"
R.E.	"	"	1:256	"
H.N.	"	"	1:16	"
A.N.	"	"	1:1024	"
J.S.	"	"	"	"
T.N.	"	"	1:16	"
H.N.	"	"	"	"
C.B.	"	"	1:4096	"
No. positive/ (% pos) No. tested	23/35 (66%)	10/35 (29%)	34/34 (100%)	4/33 (12%)

* Not tested.

cellulose(13) and then tested for reactivity with denatured DNA. The first fraction, which contained the purified 7S gamma globulin, was the only fraction which flocculated the bentonite particles coated with denatured DNA. This fraction from pooled normal serum did not flocculate the particles. The flocculation of bentonite particles coated with denatured DNA was inhibited by absorbing several reactive sera, including those from myasthenia gravis as well as SLE, with either calf thymus DNA or salmon sperm DNA, but histone did not inhibit flocculation. Absorption of a reactive serum with the nucleotides adenosine diphosphate (ADP) or guanosine diphosphate (DGP) also inhibited flocculation.

Discussion. The increased reactivity of thermally denatured DNA is apparently due to the exposure of formerly masked groups which occurs when the double helix of native DNA is separated by rupture of hydrogen bonds to form a "single strand"(5,14). Coating bentonite particles with this denatured DNA provided a more sensitive serologic reagent for detection of antibodies to DNA than is provided by bentonite coated with native DNA. Only 29% of sera from patients with SLE reacted with native DNA-bentonite while 66% flocculated bentonite coated with thermally denatured DNA. The flocculation test described is simpler than complement fixation and may also be more sensitive since Stollar *et al*(6), using complement fixation tests, found antibodies to thermally denatured DNA in only about 30%

TABLE III. Antibody Titers in Sera of Patients with Myasthenia Gravis.

Patients	Denatured DNA bentonite	Rheumatoid factor	Thyroglobulin antibody	Antinuclear antibody
1. M.K.	1:64	neg	neg	neg
2. J.G.	1:16	N.T.*	N.T.	1:64
3. H.R.	"	neg	neg	1:4
4. H.G.	"	"	"	1:64
5. R.S.	"	N.T.	N.T.	N.T.
6. S.C.	"	neg	neg	1:64
7. D.P.	1:4	"	"	"
8. E.V.	undil	N.T.	N.T.	undil
9. H.K.	"	neg	neg	1:4
10. D.S.	neg	1:128	"	1:4
11. R.P.	"	1:32	1:8	neg
12. A.K.	"	1:16	neg	1:16
13. A.J.	"	1:8	neg	1:256
14. D.B.	"	neg	1:32	neg
15. E.C.	"	"	"	1:64
16. R.L.	"	"	neg	1:4
17. J.S.	"	"	"	"
18. B.S.	"	"	"	1:16
19. N.K.	"	"	"	1:4
20. R.L.	"	"	"	"
21. S.G.	"	"	"	"
22.-44. (incl)	"	"	"	neg
No. positive/ (% pos) No. tested	9/44 (20%)	4/41 (10%)	3/41 (7%)	16/43 (37%)

* Not tested.

of patients with SLE.

The increase in sensitivity has led to the demonstration of anti-DNA antibody in the serum of patients with diseases other than SLE. Kayhoe *et al*(15), using native DNA bentonite, found positive reactions only in SLE. In the present study thermally denatured DNA bentonite was used, and positive reactions were found in sera from patients with rheumatoid arthritis, especially in those with positive LE cell preparations, Sjögren's syndrome and myasthenia gravis. This is not unexpected since these diseases have known clinical similarities and share a variety of serological abnormalities,(16,17, 20).

Antinuclear antibody was found in all the patients with SLE and in virtually every instance where anti-DNA antibody was demonstrable. Since antibody to DNA should react with DNA of cell nuclei, this was expected. On the other hand, some sera contained antinuclear antibody and no detectable anti-DNA antibody. Perhaps the indirect fluorescent antinuclear procedure is more sensitive than the bentonite flocculation test. However, it has been shown that sera from patients with SLE contain multiple factors which react with different nuclear components (16,17). Since all these factors are not present in the serum of every patient with SLE, it is not paradoxical to find an occasional serum with a very high antinuclear antibody titer and no detectable anti-DNA antibody (Table II). The antibodies in such sera are apparently reactive only with nuclear components other than DNA. The conspicuous lack of detectable anti-DNA antibody in the sera of 7 patients with scleroderma, 4 of which contained antinuclear antibody, suggests that it is not merely a difference in sensitivity between the techniques and that perhaps the circulating antibodies in scleroderma are directed against nuclear components other than DNA. The finding of anti-DNA antibodies in the absence of antinuclear antibody in the serum of 3 patients with obscure neuromuscular disease and one with myasthenia gravis was unexpected. A possible explanation for this seemingly paradoxical finding is that a low concentration of anti-DNA antibody is insufficient to produce detectable fluorescence when other antinuclear factors are not present in the serum.

Our data support the finding of White and Marshall(7) of antinuclear antibody in the serum of some patients with myasthenia gravis. In addition, the impressive reactivity of many of the sera with thermally denatured DNA, the finding of rheumatoid factor and anti-thyroglobulin antibodies in the same series, the demonstration by Strauss *et al*(17) of a muscle binding, complement fixing globulin in the serum of patients with myasthenia gravis (recently confirmed by Beutner *et al* (18)), and the anti-thymus antibodies described by Van der Geld *et al*(19) constitute a remarkable array of serological abnormalities in this disease. The relation of these serological abnormalities to the pathogenesis of myasthenia gravis remains to be determined.

The DEAE cellulose fractionation data show that the anti-DNA serum factor is 7S gamma globulin. The inhibition studies with DNA, histone, ADP and GDP indicate a certain specificity in the reaction. Together these data support the concept that the serum factor detected by denatured DNA-bentonite is antibody directed against DNA.

This modification of the DNA-bentonite test provides a simple technique for the study of patients with SLE and related disorders as well as a useful test for detection of experimentally produced anti-DNA antibodies and for study of the antigenic determinants of DNA.

Summary. Sera from 66% of 35 patients with SLE flocculated bentonite particles coated with thermally denatured DNA while only 29% of these same sera flocculated bentonite particles coated with native DNA. All of these sera contained antinuclear antibody. Twenty per cent of sera from 44 patients with myasthenia gravis flocculated the bentonite particles coated with denatured DNA while none reacted with the native DNA bentonite. Thirty-seven per cent of the sera from patients with myasthenia gravis contained antinuclear antibody. Rheumatoid factor and antibodies to thyroglobulin were occasionally seen in low titer in the serum from patients with myasthenia gravis.

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