action. Absorption of serum with one organism resulted generally in complete inactivation against the homologous organism but little or no loss of activity against antigenically unrelated organisms.

The relationship of these findings to the nonspecific properdin system is of interest. Bactericidal activity of normal serums which were assumed to be devoid of antibody against certain bacteria has been attributed to the properdin system(9). Absorption experiments with *E. coli, S. typhosa*(1), other Gram-negative organisms(8) and also bacteriophage (10) have indicated that substances selectively removed by absorption and which may be regarded as antibodies are invariably present, however, in normal mammalian serums. Because of their low concentration, these antibodies may be unable to effect agglutination reactions, but may be detected by the bactericidal reaction, one of the most sensitive methods for antibody determination(11). In absence of antibody, normal human serum is devoid of bactericidal activity notwithstanding the presence of the components of the properdin system(8). Antibodies of marked specificity appear to play the major part, therefore, in bactericidal action of serum. Possibly, the greater susceptibility of infants to *E. coli* disease may be related to the lower concentration of these antibodies in infant's fluids, but this thesis remains to be tested.

Summary. Resistance to bactericidal action of normal human serum of *E. coli* isolates from normal children and from those with diarrhea was determined. Results indicated that serum resistance did not provide a basis for differentiation of virulent and non-virulent strains. Serum resistance was associated with the O-inagglutinability of the organisms. In addition, bactericidal action of normal human serum against *E. coli* was shown to be dependent upon natural antibodies of marked specificity.

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Desoxyribonucleic Acid (DNA)-Bentonite Flocculation Test for Lupus Erythematosus. (25620)

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It has been found that serum from patients with active systemic lupus erythematosus may flocculate through a wide range of titers with a suspension of a volcanic ash, bentonite, and purified desoxyribonucleic acid. Results of the test agree generally with those of lupus cell preparations. However, significant differences appear to exist especially in some patients with rheumatoid arthritis between the flocculation test and the L. E. test. In such instances, the DNA-bentonite test is negative. The present report describes the procedure for

performing this test and gives some results of its clinical evaluation. More detailed clinical studies will be described later comparing the present test with lupus cell preparation and the bentonite test for rheumatoid arthritis. The test is similar to the bentonite flocculation test for rheumatoid arthritis(1) except that desoxyribonucleic acid, the coating or sensitizing agent, is used rather than gamma globulin (F II fraction). It also resembles the serological test for trichinosis described by Bozicevich *et al.*(2). Holman and Kunkel (3) demonstrated a factor (L. E. Factor) in the serum of lupus erythematosus patients which showed affinity for nucleoprotein and nuclei of cells obtained from a variety of sources. This observation is used as the basis for several tests in diagnosis of systemic lupus erythematosus. Thus, Miescher and Straessle (4) found that the "L. E. Factor" in serum agglutinated tanned red cells sensitized with desoxyribonucleic acid (DNA) or with nucleoprotein. Others applied the fluorescent antibody technic(5,6), complement fixation (7,8,9), latex agglutination(10,11), and isotope tagging procedures(12) to show this relationship.

Materials and Methods. In this test, DNA is substituted for Cohn's Fraction II of the rheumatoid arthritis test as the sensitizing agent in the flocculating system, and the surface acting agent is replaced with a solution of crystallized bovine plasma albumin as a protective colloidal substance. Bentonite, a hydrous aluminum silicate colloidal clay, is the vehicle for the flocculation reactions. Its particles are negatively charged and possess an unusually large surface to mass ratio. Bentonite powder is very stable, and is readily available. *Preparation of stock bentonite suspension.* Suspend 0.5 g of standard Volclay* (Wyoming) bentonite in 100 ml of distilled water and homogenize in a high speed blendor for 2 minutes. Allow the suspension to remain for about 5 minutes and rehomogenize. Transfer the suspension into a glass-stoppered graduate, washing the blendor with distilled water. Add sufficient distilled water to make 500 ml. Shake the graduate for one minute and allow to remain undisturbed for one hour. Place the supernatant in 6 heavy duty Pyrex centrifuge tubes of 100 ml capacity. Discard the sediment. Using an International Centrifuge, Size 2, with a No. 240 head, supernatant is centrifuged for 15 minutes at a speed of 1300 rpm (by tachometer). Relative centrifugal force is approximately 500. Discarding the sediment, supernatant is poured into 6 other 100 ml centrifuge tubes and centrifuged for 15 minutes at 1600 rpm (by tachometer). Relative centrifugal force is approximately 750. Supernatant is poured off and discarded. The accumulated sediment from the 6 tubes is resuspended in 100 ml of distilled water and homogenized for one minute. This is the stock bentonite suspension. Its adsorptive properties remain stable for several months. It is to be noted that the stock bentonite suspension will flocculate spontaneously after a few hours. When ready to use, it must be resuspended by shaking for a few seconds. *Preparation of desoxyribonucleic acid (DNA).* Place 1 mg of highly polymerized DNA† into a 20 ml test tube and add about 0.5 ml of distilled water. Sufficient time should be allowed for the DNA to swell into a translucent gel. Add 4.5 ml of distilled water and shake thoroughly. A clear, colorless, viscous solution is obtained. Prepare 24 hours prior to use and keep in refrigerator. Shake thoroughly before use. *Preparation of DNA-bentonite particles.* Place 1 ml (200 μg) of the DNA solution into a 20 ml test tube and add 10 ml of stock bentonite suspension. Shake vigorously. Add 1 ml of saturated KCl solution. Mix thoroughly and allow to remain at room temperature for one hour with occasional shaking. Add 1 ml of 1% solution in distilled water of crystallized bovine plasma albumin.‡ Invert tube several times. Avoid foaming as much as possible. Albumin solution should be allowed to remain for 10 minutes. It must be pointed out that in the rheumatoid arthritis test a surface-active agent (Tween-80) is employed to prevent the particles from clumping. Since this agent interfered with DNA-benton-

* American Colloid Co., Chicago, Ill.

† Worthington Biochemical Corp., Freehold, N. J.

‡ Armour Labs., Chicago, Ill.

ite particles, a protective colloidal system is used consisting of crystallized bovine plasma albumin. Add 1 ml of 0.1% methylene blue in distilled water. Invert tube several times and centrifuge immediately for 15 minutes at 1300 rpm. Discard supernatant and add 10 ml of distilled water to the flocculated particles. Shake vigorously for one minute, then fill the tube with distilled water. Mix and centrifuge again at 1300 rpm for 15 minutes. Repeat washing once more. After the final centrifuging, discard supernatant and add 5 ml of distilled water to the residue of sensitized dyed bentonite. Shake vigorously for one minute. This is the sensitized bentonite preparation for use in the test. It must be thoroughly shaken each time prior to use. The sensitized bentonite suspension remains stable for 1 week when kept in refrigerator. It may be added that while plasma can be used for L. E. preparations, it cannot be employed for the flocculation test. The plasma often gives false positive reactions. The serum is heated at 56°C for 30 minutes. Ringed microscope slides, such as those employed in Mazzini and Kline flocculation tests (2″ × 3″) with 12 rings are used. Place 0.1 ml of undiluted serum in the first ring and then 0.1 ml of 2-fold serial dilution of serum in saline in the remaining rings. Shake the sensitized bentonite suspension and with a capillary pipette (which should deliver about 40-50 drops per ml) add one drop to each ring on the slide. Place the slide on a Boerner-type rotating machine and rotate at 110-120 times per minute for 20 minutes. Read the slide immediately with the aid of a microscope at low magnification (50×-60×). Drying of serum should be avoided. A known positive and negative serum should first be titrated each day prior to conducting the test on the routine sera, to ascertain sensitivity and specificity of the test. In reading the reaction, a 4-plus flocculation is assigned when all sensitized bentonite particles have clumped. There are 2 types—one in which several large clumps are seen and the other in which many smaller clumps are obtained. Nevertheless, practically all of the small bentonite particles are clumped. A reaction of 3-plus is recorded when approximately three-fourths of the particles are clumped. A 2-plus reaction is one in which half of the sensitized particles are clumped; a 1-plus, when one-fourth of the particles are clumped. In a negative reaction, the sensitized bentonite particles are dispersed in a colloidal suspension. The current test is more difficult to read than the bentonite flocculation test for rheumatoid arthritis, due to the adhesiveness of the DNA with the reactants in the test. Consequently, one must not select one or 2 clumps and regard the test as positive. A thorough scanning of the slide is important and the particles should be evident throughout the preparation. A reaction is regarded as positive when a 1-plus or greater flocculation occurs. With experience, the spurious aggregates can readily be distinguished from true flocculation. It must be emphasized that use of too great a quantity of DNA is to be avoided because hyaline or small fibrinous precipitation is likely to occur in presence of the albumin solution and the methylene blue dye. This makes it difficult to obtain clearcut readings.

Results. Patients with active lupus erythematosus. Sera from 8 patients have been tested. Results of the DNA-bentonite flocculation test and the L. E. preparation findings are listed in Table I. In the 8 cases there was complete agreement between flocculation test and L. E. cell preparation. The Table shows the reciprocal of the highest titer obtained by flocculation test. Serum samples tested at frequent intervals showed variation in titers.§

TABLE I. DNA-Bentonite Flocculation Tests and L.E. Cell Tests in 8 Active Cases of Systemic Lupus Erythematosus.

Age	Sex	L.E. cell	Reciprocal DNA-BFT Titer (flocculation of 1+ or greater)
31	F	Positive	512
34	F	"	256
18	F	"	64
38	F	"	32
36	F	"	32
7	F	"	32
18	F	"	16
37	F	"	8

§ To be reported.

TABLE II. DNA-Bentonite Flocculation Tests and L.E. Cell Tests in 5 Cases of Systemic Lupus Erythematosus in Remission.

Age	Sex	L.E. cell	Reciprocal DNA-BFT Titer (flocculation of 1+ or greater)
16	F	Positive	2
48	M	"	4
22	F	"	Negative
26	F	"	"
47*	F	Negative	"

* Reported as having positive L.E. cells at another hospital.

Patients in remission. Sera from 5 patients were tested (Table II). Two of these gave positive tests by both methods. Two others had positive L. E. cell preparations but gave negative flocculation tests. The remaining case gave negative results by both tests.

Patients without lupus erythematosus. One hundred and thirty-eight sera from patients without evidence of lupus erythematosus were tested. The results are shown in Table III. Patients with related and unrelated diseases were included as well as normal subjects. The DNA-bentonite flocculation test was negative in all 138 cases. The L. E. cell test was conducted on 94 of the above 138 patients, and negative results were obtained in 88 cases. To test the specificity of the flocculation test, 6 individuals, with frank rheumatoid arthritis, were selected who gave positive L. E. cell tests. Their flocculation tests were negative. Thus, it appears that the flocculation test is somewhat more specific than the L. E. cell test. However, based on the results in the remission cases of lupus erythematosus, the L. E. cell test is more sensitive than the flocculation test.

TABLE III. Results of L.E. Cell Tests with Negative DNA-Bentonite Tests on 138 N.I.H. Patients.

Diseases	No. patients	L.E. cell preparation
Rheumatoid arthritis	6	Positive
" "	5	Negative
Juvenile "	1	"
Sarcoidosis	7	"
Fever of unknown origin*	6	"
Systemic fungal disease	4	"
Endocarditis	3	"
Rheumatic heart disease	3	"
" fever, acute	3	"
Cerebral seizures	3	"
Agammaglobulinemia	3	"
Pyelonephritis, chronic	3	"
Infectious hepatitis	2	"
Intestinal parasites	2	"
Cirrhosis of liver	2	"
Empyema	2	"
Thrombocytopenic purpura	2	"
Pericarditis, etiology unknown	2	"
Well individuals	10	"
" "	44	Not tested
Misc.†	25	Negative

* Persistent fever, complete evaluation failed to reveal etiology; fever spontaneously terminated.

† Includes one case of each of following: Dermatomyositis, scleroderma, erythema multiforme, erythema nodosum, chronic glomerulonephritis, polymyositis, Sjoegren's syndrome, allergic vasculitis, chronic osteomyelitis, arteriosclerotic heart disease, asthmatic bronchitis, chronic bronchitis, cerebral palsy, Charcot-Marie-Tooth syndrome, chronic eczema, eosinophilia (persistent, etiology unknown), encephalomeningitis, lethal midline granuloma, malignant melanoma, nephrotic syndrome, osteitis deformans, pulmonary tuberculosis, Salmonella carrier, vaginal trichomoniasis, and pyoderma.

Summary. (1) The test is conducted by adding a suspension of bentonite particles previously coated with desoxyribonucleic acid (DNA) to heat inactivated serum dilutions. (2) Flocculation test showed agreement with L. E. cell test in 8 cases of lupus erythematosus. (3) L. E. cell test appeared to be more sensitive than flocculation test in cases in remission. (4) The flocculation test appeared to be more specific. In 6 selected cases of rheumatoid arthritis, the L. E. cell test was positive; on the contrary, the DNA-bentonite flocculation tests were negative.

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Relation of Mammary Tumors to Mammotropes.* I. Induction of Mammary Tumors in Rats. (25621)

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It has been well established that estrogens and the pituitary gland play a major role in development of mammary gland(1) and in maintenance of growth of mammary tumors (2,3). Our studies point to a cell in the pituitary gland, named mammotrope, which produces a hormone (mammotropin) which is the direct stimulant of the mammary gland(4). Estrogens act indirectly by stimulating mammotropes(4,5). This concept is strengthened by observations described in this and the following papers(6,7), which point to the role of mammotropin in initiating as well as maintaining mammary tumors.†

Methods. Tumor induction. Mammary tumors were induced by 3-MCA in 2 highly inbred strains following Huggins' scheme(3). The rat strains used will be designated F_{Fu} and W_{Fu} following accepted terminology by mouse geneticists. The Fisher strain (F_{Fu}) was obtained from Dr. Wilhelmina Dunning in 1955; the Wistar strain (W_{Fu}) was a partially inbred strain purchased prior to 1945. Both were rigidly inbred and are homozygous from standpoint of transplantation. Breeding and care of rats and features of the W_{Fu} strain have been described(8). *Exp. I.* Four groups of F_{Fu} females (F-I, III, IV and V), one group of castrated F_{Fu} males (F-II), and one group of W_{Fu} females were treated with 3-MCA as indicated in Table I. Two weeks after the last treatment (112 days after start of 3-MCA feeding), a 1 mg pellet of DES was implanted s.c. in 10 rats of the F-II group. *Exp. II.* The influence of MtH on induction of mammary tumors was studied, using functional MtT as a source of this hormone(5). MtT (Strain W6) was grafted s.c. into rats of Groups W-I and W-III, 2 weeks prior to administration of 3-MCA. When a mammary tumor was detected in these rats, a biopsy was made and the tumor resected; parts of it were sectioned and parts transplanted in normal and conditioned rats, as described in the following report(6).

Results. Results of Exp. I are summarized in Tables I and II. Mortality due to toxicity of the carcinogen, indicated by severe fatty degeneration of the liver, was heavy only in Group F-V to which 3-MCA was given 6 times weekly. MT incidence was higher in 50 day old F_{Fu} rats (88 to 100%) than in those 83 days old (50%). All tumors were malignant with one exception, a fibroadenoma in a castrated male that was also given DES. One sarcoma was found in an F_{Fu} female that had multiple carcinomas at death 235 days after treatment. Most animals usually bore more than one tumor; the largest number in one rat was 13. Tumors arose in all mammary glands, perhaps most frequently in those of the thoracic region.

All W_{Fu} 3-MCA treated females developed MT within 5 months, although they received less carcinogen than F_{Fu} rats. Correspondingly, latency periods were shorter in W_{Fu} rats (Table II). All tumors in W_{Fu} rats were carcinomas.

* This investigation was supported by contract from Cancer Chemotherapy Nat. Service Center and grant from Nat. Cancer Inst.

† *Abbreviations*: MT = mammary tumor; Mt = mammotrope; MtH = mammotropic hormone = mammotropin; MtT = mammotropic tumor; 3-MCA = 3-methylcholanthrene; DES = diethylstilbestrol.