Histamine has been reported to exert an inhibitory effect on the growth of transplanted tumors(5). The present results are in accord with this observation.

Since tumor growth was more favorable in histamine depleted animals, it is tempting to assume that tissue reactivity or inflammatory potential regulates tumorigenesis. In order to determine to what extent this hypothesis could further be verified, experiments are now underway to investigate whether compound 48/80 might influence the rate of tumor induction in rats.

Summary. The growth of Walker Tumor 256 is increased in rats receiving compound 48/80 in progressive dosage. This is significant only in animals pretreated with the histamine releaser.

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A DNA-Reacting Factor in Serum of a Patient with Lupus Erythematosus Diffusus.* (23544)

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The question whether DNA can elicit specific antibodies in experimental animals is still controversial, although some positive evidence has been presented(1). Our own experiments have been inconclusive(2). Because of the possibility that DNA might behave as an hapten, it was nevertheless decided to test whether DNA preparations would react against human pathological sera. Our attention was mainly directed towards Lupus Erythematosus for: 1) the nucleolysis and subsequent nucleophagocytosis typical of this condition, liberate and possibly make accessible to immunological processes an abnormally large amount of nuclear material; 2) these patients are exceptionally apt to produce auto and iso-antibodies; 3) the immunological nature of the L.E. phenomenon is supported by some well established observations.

The following sera have been tested against DNA: a) 9 from Lupus Erythematosus diffusus, b) 60 from normals, c) 15 from luetic patients, d) 30 from miscellaneous diseases showing high level of gamma globulins, e) 7 from collagenous diseases (1 Liebman-Sachs endocarditis, 3 cutaneous Lupus Erythematosus, 1 Schoenlein-Henoch, 1 macroglobulinaemia). A definite positive reaction on complement fixation and on precipitin test was observed only with the serum of a patient with acute, untreated, Lupus Erythematosus diffusus (serum E-2); all other sera did not react. A short account of the observations herein presented has been previously published(3).

Materials and methods. DNA was obtained from i) normal human leucocytes, ii) leucocytes from human lymphatic leukemia, iii) leucocytes from human myeloid leukemia, iv) calf thymus, v) rabbit spleen. DNA was extracted according to the Kay, Simmons, Dounce technic as modified by Chargaff(4), using 10^{-3} M NaCl as the solvent wherever water was prescribed (Cavalieri *et al.*, 1956). Moreover the crude sodium nucleate was only washed with 95% ethanol. The fibrous DNA was stored at -20°C. The chemical analysis *i.e.* % H_2O, N, P(5), the u.v. absorp-

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tion data at various pH, the average molecular weight as determined by light scattering (6) and the sedimentation experiments before and after chymotrypsin showed the high degree of purity of these undenaturated DNAs. As a control the following antigens were tested against E-2 serum: RNA from yeast and from human leucocytes; purified Vi and O polysaccharides from *S. typhi;* purified H blood group substance from human meconium; polyvinylpyrrolidone; dextran; cardiolipine; an aqueous extract from *Treponema pallidum,* Reiter's non-pathogenic strain. The sodium salt of the DNA used for the immunological test was dissolved in 0.14 M NaCl by standing overnight at 4°C (concentration 500 g/ml). Further dilutions in the range of 100-0.5 μg/ml were prepared immediately before use; none of our DNA preparations up to a concentration of 100 μg/ml showed any anticomplementary activity. The anticomplementary activity of the E-2 serum was compensated for by titrating the complement in presence of 1 volume of diluted (1:4) inactivated serum. Equal amounts (0.10 ml) of: 1) guinea pig complement containing two 50% units, 2) diluted (1:4) E-2 serum, 3) various dilutions of antigen, were mixed; 0.20 ml of veronal buffered saline were added and the mixture left overnight at 4°C. The following morning 1 ml of sensitized red cells was added and the hemolysis read after 45′ incubation at 37°C. A full range of serum and antigen controls was set up in all tests. A positive reaction was scored when hemolysis was reduced from 90% to 30% or less. The precipitin tests were carried out in capillary tubes (bore 2 mm) stratifying the antigen at various concentrations over the serum usually diluted 1:4 and clarified by removal of lipids. A reaction was judged positive when a sharp opaque disk appeared at the interface within 2 hours; in general the reaction was visible after 15′ and reached its maximum in 45′.

Results. A) The E-2 serum gave a positive reaction both as complement fixation and as ring precipitation with all DNA preparations tested. Within the sensitivity of the methods no differences in reactivity among DNAs

TABLE I. Schematic Representation of Serological Reactions (Precipitin Ring Test and Complement Fixation) Given by Different Sera against Pertinent Materials. Note disappearance of reactivity after treatment of DNAs and Treponemic extract with DNAase.

Sera	DNAs (1-100 μg/ml)			Treponemic extract (1:5-1:20)			Cardiolipine	Other macromolecular antigens RNA antigens (1-100 μg/ml)
	Untreated	+ DNAase	+ chymotrypsin	Untreated	+ DNAase	+ chymotrypsin		
Lupus Erythematosus (Patient E-2)	++	0	++	+	0	++	0	0
Lues	0	0	0	++	++	±	++	0
Normals, 9 other L.E. sera and miscellaneous diseases	0	0	0	0	0	0	0	0

++ = strongly positive; + = positive; ± = weakly positive; 0 = negative.

from different sources were detected, all preparations reacting positively up to a concentration of about 1 μg/ml. Among the other above listed materials only the treponemic extract behaved like DNA.

B) The finding that E-2 serum reacted with the treponemic extract, but did not with cardiolipin and gave a negative treponemic immobilization test led us to investigate if nucleic acids were present in the spirochetal material; using the Ceriotti method it was possible to show the presence of DNA (10-20 μg/ml).

C) Absorption of E-2 serum for one week at 4°C with an excess of any one of the 5 DNA preparations and of the treponemic extract, neutralized all precipitating reactivity, while absorptions with unrelated materials, including RNA, were ineffective.

D) One DNA preparation and the treponemic extract were again tested after treatment under appropriate conditions with crystallized DNAase and Chymotrypsin; the results are particularly significant (Table I) and strongly suggest that only DNA was involved in the reactivity of the E-2 serum.

E) The reacting factor was still active after 1 hour heating at 56°C. The precipitate from the E-2 serum at ½ saturation of $(NH_4)_2SO_4$ reacted strongly on ring test with DNA, but could not be used for the complement fixation because of a very high anticomplementary activity. Preliminary experiments of recovery of the factor from the different fractions of the serum, isolated by means of zone electrophoresis, have been up to now unsuccessful.

Conclusions. While further investigations are in progress, the following conclusions may be provisionally drawn: 1) in serum of a patient with acute Lupus Erythematosus diffusus a factor has been found specifically reacting with purified DNA; 2) this factor does not discriminate between DNAs from different sources and species; 3) under usual criteria it seems to behave like an antibody; 4) the positive reaction of this peculiar serum against a treponemic extract appears to be due to the presence of desoxypentose nucleic acids in the extract. 5) It has not been possible to test whether the serum of this patient, which gave a strongly positive L. E. phenomenon, would retain this ability after absorption with DNA; hence it is impossible now to decide how closely this factor is related to the Hargrave Haserick's globulin. Because no reactivity against DNA was demonstrated in 8 other patients with a positive L.E. phenomenon, the DNA reacting factor of this peculiar serum might be regarded as an occasional expression of the disease, related to the wellknown auto-immune hyperreactivity of the L.E. patients. If the factor is demonstrated not to be an antibody the alternative explanation could be of a material of nuclear origin still able to form a complex with DNA.

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