occurs is not known. Consideration should be given to the fact that increased sensitivity of such parameters as heart rate and blood pressure to injected amines after reserpinization has been shown by several investigators (13,14,15,16). One may also speculate that the cardiovascular system becomes more sensitive to amines released by adrenal stimulation after reserpinization. However, a more detailed study needs to be made on sensitivity changes before conclusions can be drawn on this point.

Summary. Increments in plasma levels of epinephrine resulting from nicotine stimulation of the adrenal medulla are markedly reduced by reserpine. However, increments in heart contractile force and arterial blood pressure produced by adrenal stimulation, normally proportional to catechol amine levels, are not significantly altered following reserpine.

1. Woods, E. F., Richardson, J. A., Richardson, A. K., Bozeman, R. F., Jr., *J. Pharmacol. Exp. Therap.*, 1956, v116, 351.

2. Woods, E. F., Richardson, J. A., *ibid.*, 1955, v114, 445.

3. Richardson, J. A., Richardson, A. K., Brodie, O. J., *J. Lab. Clin. Med.*, 1956, v47, 832.

4. Weil-Malherbe, H., Bone, A. D., *J. Biochem.*, 1952, v52, 311.

5. Boniface, K. J., Brodie, O. J., Walton, R. P., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 263.

6. Cotten, M.deV., Maling, H. M., *Am. J. Physiol.*, 1957, v189, 580.

7. Kroneberg, G., Schumann, H. J., *Arch. Exp. Path. Pharmacol.*, 1958, v234, 133.

8. Burn, J. H., Rand, M. J., *Brit. Med. J.*, 1958(1), 903.

9. Callingham, B. A., Mann, M., *Nature*, 1958, v181, 423.

10. Coupland, R. E., *J. Endocrinology*, 1958, v17, 191.

11. Muscholl, E., Vogt, M., *J. Physiol.*, 1958, v141, 132.

12. Maling, H. M., Cohn, V. H., Jr., Highman, B., *J. Pharmacol. Exp. Therap.*, 1959, v127, 229.

13. Bejrablaya, D., Burn, J. H., Walker, J. M., *Brit. J. Pharmacol.*, 1958, v13, 461.

14. Bein, H. J., *Experientia*, 1953, v9, 107.

15. Bein, H. J., Gross, F., Tripod, J., Meier, R., *Schweiz. Med. Wochenschr.*, 1953, v83(2), 1007.

16. Trapold, J. H., Plummer, A. J., Yonkman, F. F., *J. Pharmacol. Exp. Therap.*, 1954, v110, 205.

Received October 29, 1959. P.S.E.B.M., 1960, v103.

Inhibition of Hexamminecobaltic Chloride, DNA Interaction by Sera of Patients with Disseminated Lupus Erythematosus.* (25561)

GERALD WEISSMANN† (Introduced by Lewis Thomas)

Dept. of Medicine and Study Group for Rheumatic Diseases, N. Y. Univ. College of Med., and Third (N.Y.U.) Medical Division, Bellevue Hospital, N. Y. City.

Evidence obtained by immunologic technics suggested that serum of patients with disseminated lupus erythematosus (DLE) contains several factors reacting with various nuclear materials. Holman and Kunkel(1) and Friou(2) indicated that gamma globulin of such sera is bound to both nuclei and nucleoprotein, while Miescher's studies(3) showed that nuclei of several sources can absorb from DLE sera the factor responsible for L. E. phenomenon, and also remove from such sera measurable amounts of gamma globulin. Classical precipitin curves have been obtained between protein-free desoxyribonucleic acid (DNA) and DLE serum(4) while latex particles(5) and red cells(6) coated with such material have been agglutinated by DLE serum. The reaction between highly polymerized DNA and the polyvalent cobalt salt hexamminecobaltic chloride ($Co(NH_3)_6Cl_3$) results in production of a clot of insoluble DNA. While studying this reaction, it was observed that DNA which had been treated with serum from patients with DLE, failed to clot, while normal serum had no such effect. The present paper deals with a simple method for demon-

* Aided by grant from N.I.H.

† Fellow of Arthritis and Rheumatism Fn., 1958-59.

stration of clot inhibition, and studies with a variety of sera are reported.

Materials and methods. Hexamminecobaltic chloride was prepared by the method of Bjerrum and McReynolds(7).‡ Purity was established by nitrogen analyses and constancy of absorption spectrum upon reprecipitation, as well as by powder pattern X-ray diffraction. A 0.5% w/v aqueous solution was routinely employed, having absorbance of 1.004 at 470 μ in Beckman DU spectrophotometer. Such solutions were prepared weekly, and showed little loss of DNA clotting activity if stored at 4°C. Aqueous solutions of calf thymus DNA, stated to be highly polymerised and protein free§ (500 μg/ml) were prepared and stored at 4°C. Immediately prior to use, aliquots were diluted 1:1 with distilled water. Such preparations were assayed for relative viscosity at 37°C. Relative viscosity was 4.45 ± 0.10 in Ostwald viscosimeters with water-flow time of 92 seconds. Constant viscosity after addition of $MgSO_4$ to 0.005 M was presumed to indicate absence of desoxyribonuclease (DNase). Histone of calf thymus origin and protamine sulfate§ were prepared as 0.02% w/v serum solutions. Dried cartilage powder was prepared by homogenizing 2 g of beef nasal septal cartilage in a Vir-Tis 45 mixer at top speed in 250 cc of absolute ethanol. The residue was washed 3 times each with absolute ethanol and ether, and then dried *in vacuo* over $CaCl_2$. Whole dried calf thymus and guinea pig nuclei were obtained through the courtesy of Dr. Peter Miescher. Pancreatic DNase‖, 100,000 units, was dissolved in 9 ml of 0.05 M tris (hydroxymethyl) aminomethane buffer pH 7.01. For activation of the enzyme, 1 ml of 0.1 M $MgSO_4$ was added, and resultant solution stored at 4°C prior to use. For routine testing, 0.4 ml of DNA (100 μg) were placed in small serology tubes, 0.5 ml of test serum was added with gentle agitation, and resultant mixture incubated at 37°C for 30 minutes. Thereafter, the tubes were brought to room temperature, 0.2 ml of hexamminecobaltic chloride solution added, and the tubes vigorously tapped with the forefinger, setting the solution spinning. In all "negative" sera, an easily visible, strand-like clot appeared immediately. This was graded $^{+}$ to $^{++++}$ depending on quantity of the clot formed. In positive sera, mainly from patients with DLE, no such clot formed. Instead, a slightly turbid solution resulted upon addition of the cobalt salt. All determinations were made in duplicate. Known inhibitory and normal sera were tested with each group of unknown sera. In some experiments clot inhibition was tested with various concentrations of DNA. To test whether inactivation of serum DNase would affect clot inhibition, 6 inhibitory sera were heated at 60°C for 30 minutes, prior to use. Patients diagnosed as DLE had signs and symptoms of that disease as well as positive L.E. cell preparations by the method of Hargraves(8). Patients diagnosed as probable DLE had similar signs and symptoms but did not have positive L.E. cell preparations. Sera were also obtained from 44 patients with rheumatoid arthritis, 2 of whom had positive L.E. cell preparations. The inhibitory effect of protamine sulfate or histone was tested by adding 0.1 ml of 0.02% solutions of these materials to 0.5 ml samples of normal serum mixed with 0.4 ml of standard DNA solution; the mixtures were incubated at 37°C, then 0.1 ml of a 1% w/v solution of cobalt salt was added. Absorption of sera was tested with nuclei or whole cartilage powder by incubating 50 mg (dry weight) of these materials for 4 hours at 37°C with 1.0 ml of serum; the mixture was centrifuged, and 0.5 ml of the supernatant was used for clot inhibition studies. For enzyme studies, 0.05 ml of DNase stock previously diluted 1:100 with 0.9% saline solution was incubated with 0.4 ml of 250 μg/ml DNA solution and 0.5 ml serum samples for 30 minutes at 37°C. Similar solutions were prepared with addition of sodium versenate (EDTA) 0.75 mg/ml to inactivate DNase as controls.

Results. Experiments summarized in Table I showed that in the presence of 0.5 ml of normal serum, at least 100 μg of DNA in aqueous solution were necessary before a clot appeared after addition of cobalt salt. Smaller amounts did not produce a visible

‡ Generously supplied by Dr. Maxwell Schubert.

§ Nutritional Biochemicals Corp., Cleveland, O.

‖ Lederle Laboratories, Pearl River, N. Y.

TABLE I. Hexamminecobaltic Chloride-DNA Clots in 0.5 ml of Serum (See Text).

μg DNA in 0.4 ml H_2O	DLE serum		Normal serum		
	Loc.	Ben.	Nun.	Rod.	McC.
25	0	0	0	0	0
50	0	0	0	0	0
100	0	0	0	2+	3+
150	0	0	0	3+	3+
200	1+	0	3+	4+	4+
250	2+	3+	3+	4+	4+

clot. When similar experiments were performed in the presence of 3 DLE sera, no clot was visible with less than 200 μg. Using the technic described above, 20 of 31 DLE sera were positive, 5 of 12 probable DLE sera were positive, and 9 of 139 miscellaneous sera were positive (Table II). These included 4 sera from patients with convalescent hepatitis, 2 from patients given protamine (see below) and one each from patients with diagnoses of uncomplicated cirrhosis, penicillin allergy, and hepatic decompensation. Sera from 6 of 44 patients with rheumatoid arthritis were positive. Sera from patients with hypergammaglobulinemia due to sarcoidosis, cirrhosis, hepatoma, myeloma, macroglobulinemia or tuberculosis were negative.

When 6 positive sera were heated at 60°C for one half hour to inactivate serum DNase, no change in their inhibitory property was noted. Such heating did not cause negative sera to become inhibitory. Frequent thawing and freezing of some normal sera caused them to inhibit clot formation, while turbid sera interfered with a clear end-point. Sera showing an absorbance over .150 at 600 μμ in a Coleman Jr. spectrophotometer were not employed. Attempts to quantitate the reacting factor in DLE sera by dilution of whole sera were relatively unsuccessful. No inhibitory activity was observed at greater than 1:8 dilutions of several positive DLE sera.

TABLE II. Sera Inhibiting Hexamminecobaltic Chloride-DNA Clot Formation.

Diagnosis	DNA clot inhibition		L.E. cell preparation	
	Positive	Total tested	Positive	Total tested
Disseminated lupus erythematosus	20	31	31	31
Probable DLE	5	12	0	12
Rheumatoid arthritis	6	44	2	44
Misc.	9	139	0	9

Studies of inhibition of clot formation (Table III) indicated that DNA which had been depolymerised by pancreatic DNase no longer formed a clot on reacting with hexamminecobaltic chloride. This was true even in absence of serum, with saline used as diluent. When the action of DNase was inhibited by addition of EDTA, a clot readily formed. EDTA by itself had no effect upon clot formation or inhibition. When protamine sulfate or calf thymus histone were added to normal serum, clot inhibition similar to that observed with DLE serum was noted. If the specified concentrations were exceeded, these basic proteins formed DNA clots resembling those of cobalt salt in serum or saline. Incubation of positive DLE serum with whole calf thymus or guinea pig nuclei resulted in loss of inhibitory activity. To exclude a non-specific effect of polyanion, cartilage powder as a source of relatively insoluble chondromucoprotein was used in similar incubation experiments.

TABLE III. Hexamminecobaltic Chloride-DNA Clot Inhibition by Test Substances.*

Calf thymus DNA preparation in 0.4 ml H_2O		Whole serum	
		DLE, Loc.	Normal, Rod.
Polymerised	100 μg	0	3+
Depolymerised with DNase†	"	0	0
Polymerised (DNase + EDTA‡)	"	0	3+
		Absorbed with calf thymus or guinea pig nuclei	
Polymerised	"	2+	3+
		Absorbed with cartilage powder	
"	"	0	3+
		Protamine sulfate§ added to both sera	
"	"	0	0
		Calf thymus histone§ added to both sera	
"	"	0	0

* Clot formation graded + to 4+. Inhibition indicated: 0.

† Pancreatic DNase 10 units/ml + .005 M $MgSO_4$ for activation.

‡ EDTA 0.75 mg/ml to inactivate DNase by binding Mg^{++} ions.

§ 0.1 ml of 0.02% w/v solution.

No loss of inhibition resulted. In some DLE sera not giving a clot with DNA following addition of the cobalt salt, there was precipitate after 12 hours of standing at 4°C. This precipitate was no greater in those sera to which DNA *and* the cobalt salt had been added, than in those to which DNA alone had been. These precipitates, soluble in 1M NaCl, reacted strongly in capillary precipitin tubes with anti-human gamma globulin. Presumably these resembled the DNA-DLE gamma globulin precipitates described by Deicher *et al.*(4).

Discussion. There are 2 possible explanations for DNA clot inhibition following the addition of hexamminecobaltic chloride. Either the DNA could be depolymerised (as in the case of prior incubation with DNase) or the anionic groups of the polynucleotide could be linked by salt-like bonds to a factor in positive sera making these groups unavailable to the polyvalent cobalt cation. The latter possibility is supported by inhibition of DNA clot formation following addition of basic proteins like histone and protamine to normal serum. Failure of EDTA alone to block inhibition, and studies suggesting removal of the reactant from positive sera by incubation with calf thymus or guinea pig nuclei argue against the enzyme theory. Soluble DNA-protein complexes derived from calf thymus nuclei do not yield clots upon addition of hexamminecobaltic chloride, but do exhibit turbidities directly related to the amount of such complex present in solution. Since evidence from agglutination and precipitin studies indicates that globulin bound to nuclear extracts may well be a true antibody to such materials, it seems likely that formation of a soluble DNA-DLE serum complex prevents the polynucleotide from being precipitated in visible strands by cation. These observations resemble those of Lee(9), who used the agglutination of leukocytes by quinacrine as the phenomenon inhibited by DLE serum. The method described above deals with much more highly purified materials, has a more definite end-point, and seems of promise in assaying discrete serum fractions.

Summary. Normal sera do not inhibit the grossly visible clot formed by addition of hexamminecobaltic chloride to aqueous solutions of calf thymus DNA. The sera of approximately 66% of patients with DLE inhibit such clot formation. Prior treatment of DNA by active DNase also prevents clot formation, as does addition of histone or protamine to normal sera. The serum factor responsible for such inhibition is removed by prior incubation with whole calf thymus or guinea pig nuclei.

1. Holman, H. R., Kunkel, H. G., *Science,* 1957, v126, 162.
2. Friou, G., *J. Clin. Invest.,* 1957, v36, 890.
3. Miescher, P., Fauconnet, M., *Experientia,* 1954, v10, 252.
4. Deicher, H. R. G., Holman, H. R., Kunkel, H. G., *J. Exp. Med.,* 1959, v109, 97.
5. Christian C. L., Mendez-Bryan, R., Larson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 820.
6. Miescher, P., Strassle, R., *Vox Sanquinis,* 1957, v2, 283.
7. Bjerrum, J., McReynolds, J. P., *Inorg. Synth.,* 1946, v2, 216.
8. Hargraves, M. M., Opfell, R. W., *Progr. Hemat.,* 1956, v1, 249.
9. Lee, S. L., *Blood,* 1958, v13, 778.

Received October 30, 1959. P.S.E.B.M., 1960, v103.

Influence of Unbalanced Diets on Nitrogen Content and Enzymatic Activities of Hepatic Mitochondria of Rats. (25562)

NICOLA DIOGUARDI AND GIAN CARLO SECCHI (Introduced by G. Pozza)
Istituto di Clinica Medica Generale e di Terapia Medica, University of Milano

The aim of this work is to study whether an unbalanced dietary protein intake may cause changes in nitrogen content of the hepatic mitochondria. Previous observations(1) demonstrated that in normal rats fed protein deficient diet or fasted, some enzymatic activi-