duced at all. In this regard it is interesting to point out that while ZCe F_1 hybrids became tolerant in all instances to either CBA or C57 Bl tissue, hybrids of the same kind failed to become tolerant when they are treated with either BALB/C or A tissue. Although the number of animals in these latter groups is small, the observation that the same strain of mice (ZCe F_1) may become tolerant of cells from one donor strain but not from another is of interest.

The observations of Billingham and Brent would best be interpreted as indicating differences in susceptibility to immunological tolerance on the part of the recipient strain. Our observations must be interpreted as evidence of differences in capacity of donor cells to induce tolerance upon injection in a given strain. Although apparently contradictory, these observations are not really in conflict and might indeed be based on similar underlying mechanisms.

It is clear that these striking differences in both strain susceptibility to the development of tolerance and capacity to induce tolerance were responsible for difficulties originally encountered in our laboratory when we attempted to reproduce the original observation of the British investigators.

Summary. Mice of the Ce, ZBC, and ZCe F_1 stocks have been made tolerant to homologous skin transplantation of Z(C3H), Ce. C57 Bl and CBA strains by intravenous injection at birth of homologous spleen cells. However, the same procedure failed to induce tolerance in ZCe F_1 mice when treated with homologous cells of either BALB/C or A strains.

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Improved Method for Measuring Lupus Globulin Nucleoprotein Interaction.*† (23864)

GEORGE J. FRIOU (Introduced by P. B. Beeson)

Medical Service of Veterans Admin. Hospital, West Haven, and Department of Medicine, Yale University School of Medicine, New Haven, Conn.

In our recent studies a globulin factor with marked affinity for nuclei was demonstrated in lupus erythematosis serum by use of fluorescent antibody technic(1,2). Further investigation revealed that the nuclear factor in this reaction was nucleoprotein. A method for titration of the factor in lupus serum was devised. Using this test it was found that the factor was present in all serums of a large group of patients with disseminated lupus. It was also present in low titer in serum of a small proportion of patients with other syndromes generally thought to be closely allied to lupus, including especially rheumatoid arthritis, and individuals with chronic biologic false positive serologic tests for syphilis, but rarely in other human serums. In patients with positive tests there was general correlation between titer of the factor and manifestations usually associated with active disseminated lupus(3,4). These findings led to a more comprehensive clinical and laboratory study of the reaction. The possibility that some other method might be better suited to

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continued study of the reaction was investigated. In this report a method is described in which iodine^{131}-labelled antibody to human gamma globulin provided an effective means of measuring the reaction of the lupus factor with nucleoprotein.

Methods. In unpublished studies it has been shown that serums reacting with natural nucleoprotein also react with artificial complexes of DNA and histone. Natural nucleoprotein has not been sufficiently stable for use over long periods of time. Commercially available DNA and histone‡ are quite stable and the artificial complexes were therefore selected for use. DNA and histone were dissolved separately in water at concentration of 2 mg/ml. Sufficient NaCl was added to DNA solution to provide a 2 molar concentration. When this had dissolved, an equal amount of histone was added slowly with constant mixing. Salt concentration was then reduced to 0.5 molar by slowly mixing in appropriate amount of water. The final material was opalescent but contained no visible precipitated complex. Preliminary tests indicated that the most satisfactory results were obtained with glass surfaces coated with DNA-histone complex. The largest practical area was obtained by coating outside of closed end of 12x100 mm test tubes. These were cleaned thoroughly, dried and dipped in the solution of complex to depth of 6 cm. They were then suspended in horizontal position and rotated until dry. Immediately before use coated surfaces were washed by dipping quickly several times in 0.1 M NaCl to remove excess salt without dissolving the DNA-histone complex. For most studies one lot of rabbit antiserum to human gamma globulin (Fraction II) was used. This reacted at dilution of 1:256 in a ring test with 1:1000 dilution of antigen. The antiserum was fractionated with ammonium sulfate, and aliquots sufficient for several weeks work iodinated as needed by procedure described by Masouredis(5). Use of the same iodinated solution in more than one test on several occasions indicated that, under conditions used, antibody content was not a limiting factor. In the test procedure 2 ml sample of human serum was placed in tube, 16 mm outside diameter, in ice bath. The coated 12 mm tube was drained briefly, after washing as described above, and placed in serum for 10 minutes. During this period the serum was agitated several times by lifting the coated tube momentarily. At end of 10 minutes the tubes were removed from serum, rinsed gently with pH 7.0 isotonic phosphate-saline buffer and placed for 5 minutes in a larger tube containing 20-25 ml of buffer at room temperature. They were moved occasionally to facilitate washing. This step was repeated 2 more times with fresh buffer. The tubes were then placed in 2 ml of iodinated antibody solution in 16 mm tubes for 30 minutes at room temperature, then washed as before, placed in 15 x 125 mm tubes and counted in a well type scintillation counter. All tube dimensions described were based on a counter receiving a 15 mm tube. Counting of upper portion of coated area was incomplete due to instrument design, and error due to variation in coated area was therefore minimal. In one series of experiments, serums of different activities were simulated by preparing a graded series of dilutions, and tested to determine relation of recorded counts/minute to concentration of lupus factor. For most other purposes 20% concentration of serum was satisfactory. Counts/minute recorded were corrected for background radioactivity, and traces of iodine^{131} bound in absence of

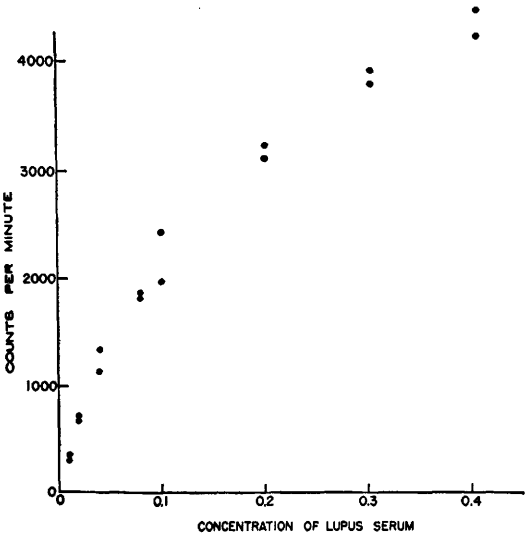

FIG. 1. Relation of concentration of lupus factor to counts/min. recorded.

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

TABLE I. Results of Tests with Iodinated Antibody as Measure of Lupus Globulin Nucleohistone Interaction.

Serum*	Conc., %	Comments	Counts/min.	
1. DLE	20		1998	2230‡
	10		1354	1546
2. "	20		584	
3. "	20	a. Control, no DNA added	4323	4215
		b. DNA added, 400 γ (μg)/ml	4084	3858
4. "	15	a. Control, no DNA or histone added	2102	2382
		b. DNA and histone added, 200 γ/ml each†	770	532
		c. DNA added, 200 γ/ml	2010	1900
5. RA	20	Low titer of affinity for	50	
6. "	20	nucleohistone with flu-	63	
7. BFP	20	orescent antibody test	49	
8. N	20	Negative against nucleo-	5	
9. "	40	histone with fluores-	3	0
10. "	40	cent antibody test	9	6
	10		0	5

* DLE, disseminated lupus erythematosis; RA, rheumatoid arthritis; BFP, chronic biologic false positive serologic test for syphilis; N, normal.

† Insoluble DNA—histone complex removed by centrifugation.

‡ Duplicate determinations. Antibody solution iodinated 17 days previously.

serum, by subtraction of results obtained in simultaneous blank determinations.

Results. In Fig. 1 results are shown which indicate relation of counts minute to relative concentrations of the serum factor, obtained by testing a series of dilutions of a single serum specimen. Similar curves were obtained with other serums, the slope varying with activity of the particular serum. In Table I representative results of tests on selected serums are shown. In investigations of conditions which influence interaction of lupus globulin with nucleoprotein, or DNA-histone complex, controls may be included which eliminate the need for further standardization. For clinical studies the need for day-to-day standardization is apparent. Preliminary results indicate that variables can be adequately controlled for this purpose.

Our purpose has been to devise a system with which to study factors influencing the reaction of lupus globulin with nucleoprotein. Some conditions of test as now conducted have been chosen rather arbitrarily and are likely to be modified as more is learned about the reaction itself. Even in its present state of development this method appears superior to others now available.

Summary. An improved procedure has been described for measurement of interaction of globulin from lupus erythematosis serum with nucleoprotein, or artificial complexes of desoxyribonucleic acid and histone. Relatively large glass surface areas coated with these materials bound maximum amounts of the serum factor, and allowed most efficient removal of unbound serum protein by washing. Antibody for human gamma globulin labelled with iodine^{131} provided a sensitive means of measuring amount of bound globulin when used under proper conditions. Measurement of relative concentrations of serum factor was achieved in test system employing a single tube of serum at 20% concentration.

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