STUDIES ON THE MECHANISM OF THE LUPUS ERYTHEMATOSUS (L.E.) PHENOMENON *

By ALAN C. AISENBERG †

(From the Department of Medicine, Harvard Medical School, and the Medical Services of the Massachusetts General Hospital, Boston, Mass.)

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Since the original demonstration by Hargraves of the Feulgen positive nature of the lupus erythematosus (L.E.) cell (1), it has become increasingly evident that the specific nature of the L.E. phenomenon resides in the reaction of the cell nucleus with a gamma globulin-like protein from the L.E. patient's serum (2–8). Friou, Finch and Detre in particular (3, 4), and others (5, 6) have shown by means of the fluorescent antibody technique of Coons and Kaplan (9) that there is nuclear localization of serum gamma globulin of patients with L.E.

The present communication on the L.E. phenomenon is divided into two parts. The first part, utilizing L. E. tests done with isolated cell nuclei and purified L.E. gamma globulin, presents evidence for the participation of an accessory serum factor necessary for phagocytosis in the L.E. phenomenon. The second part contains quantitative studies with fluorescent-labeled L.E. gamma globulin which suggest that the L.E. factor is not identical in all patients, and that the L.E. factor may react with a protein of the cell nucleus.

METHODS AND MATERIALS

Preparation of gamma globulin and nuclei. Sera were obtained from normal controls and from clinically typical cases of L.E.D. (lupus erythematosus disseminata) with strongly positive L.E. tests diagnosed on the wards of the Massachusetts General Hospital. Gamma globulin was prepared from these sera by ethanol fractionation, employing a modification of Methods 6 and 10 of Cohn (10).

Nuclei from the livers of 200 to 300 Gm. male rats (Tables III through VI) or from human buffy coat (Tables I and II) were freshly prepared each day except where noted, by fractionation in 0.25 sucrose (11) following homogenization in a glass Potter-Elvejhem homogenizer. Following the customary wash in isotonic sucrose, the liver nuclei were washed two additional times in buffered $CaCl_2$ sucrose (42.8 Gm. sucrose, 0.37 Gm. $CaCl_2$, 4.0 Gm. NaCl, 0.69 Gm. $NaH_2PO_4 \cdot H_2O$ per L., adjusted to pH 7.0 with NaOH).

Studies utilizing the L.E. test. L.E. tests were performed by a modification of the procedure of Zinkham and Conley (12) which employs glass beads to traumatize the leukocytes. Buffy coat (0.5 ml.) was obtained from heparinized normal blood by the addition of bovine fibrinogen (13) and the pellet obtained by centrifugation was resuspended in 0.2 ml. of an isotonic NaCl solution of the gamma globulin to be tested, 0.05 ml. of 0.1 M pH 7.4 phosphate buffer, and 0.15 ml. of isotonic NaCl. After addition of two glass beads, the tubes were shaken at a rate of 240 oscillations per minute with an Eberbach shaking machine for 50 minutes at room temperature. At the end of this time the tubes were centrifuged, and the pellets smeared on cover slips, stained with Wright's stain, and the number of L.E. cells per 1,000 leukocytes was determined.

Experiments designed to demonstrate the necessity of an accessory serum factor for the L.E. phenomenon (Table I) were done in the following manner. Leukocytes from 0.5 ml. of normal buffy coat were washed five times with buffered saline (8 Gm. of NaCl and 1.38 Gm. of $NaH_2PO_4 \cdot H_2O$ per L. adjusted to pH 7.0 with NaOH) and incubated with 0.3 ml. of isotonic saline (first column of Table I), 0.2 ml. of L.E. gamma globulin heated to 56° C. for 30 minutes (second column), and with 0.2 ml. of heated L.E. gamma globulin with 0.1 ml. of fresh normal serum added (third column). The phagocytic index was determined with particulate starch (14) on separate tubes, by counting the number of granules per 100 white blood cells with the aid of a phase contrast microscope.

The experiments designed to demonstrate the two-stage nature of the L.E. phenomenon (Table II) were done in a somewhat different manner. Nuclei either from 1.0 ml. of fresh buffy coat, or 1.2 mg. of lyophilized leukemic nuclei, were incubated with 0.2 ml. of L.E. gamma globulin at room temperature for 30 minutes with occasional agitation. The nuclei were then washed twice with buffered saline to remove unbound L.E. gamma

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† United States Public Health Service Research Fellow of the National Institute of Allergy and Infectious Diseases (1956–1957).

TABLE I

Studies on the effect of lupus gamma globulin, normal serum factor, desoxyribonuclease and $MgCl_2$ on L. E. cell formation

Expt.	Addition	Control		L. E. gamma globulin		L. E. gamma globulin + normal serum	
		L. E. cells	Phagocy.	L. E. cells	Phagocy.	L. E. cells	Phagocy.
1	None	0		0		327	
	0.005 M $MgCl_2$	0		78		1,020	
2	None	0		33		2,460	
3	None		0		8		1,420
4	0.005 M $MgCl_2$	0		440		4,140	
	0.005 M $MgCl_2$ + 0.1 mg. DNAse	0		0		0	

globulin, and then reincubated with leukocytes from 0.5 ml. of buffy coat suspended in 0.2 ml. of normal serum. At the end of the second 30 minute incubation the tubes were centrifuged, the sediment smeared and stained, and the number of L.E. cells counted. As controls for unbroken cells in the preparation of fresh nuclei, duplicate preparations were run and smeared and counted after the initial incubation.

Studies with fluorescent lupus gamma globulin. Gamma globulin was conjugated with fluorescein isocyanate by the method of Coons and Kaplan (9). Nonspecific fluorescence was removed by dialysis for 24 hours in the cold against buffered saline, and by one or two passages through Dowex-1 chloride (15). Liver powder was not used because it removed specific nuclear fluorescence. Following passage through Dowex, the conjugate was concentrated about threefold in a dialysis bag by evaporation in the cold, and then redialyzed against buffered saline. A normal gamma globulin preparation was conjugated with each L.E. globulin. Conjugates could be kept for several weeks in the cold room at 0–2° C., or for several months in the frozen state at − 20° C. without deteriorating.

Incubations with fluorescent gamma globulin were 30 minutes in duration and were performed in test tubes agitated occasionally at room temperature. Preliminary studies showed nuclear uptake of gamma globulin to be essentially complete under these conditions. It was also found in preliminary studies that nuclear uptake of fluorescent gamma globulin [lupus serum L.E. (BO)] was proportional to nuclei over a wide range of nuclear additions (additions of nuclei equivalent to 90 to 900 mg. of liver), and was proportional to gamma globulin over a wide range of gamma globulin additions (0.1 to 4.0 mg.). For convenience the standard reaction mixture contained 0.1 ml. of rat liver (nuclei equivalent to 300 mg. of wet rat liver in buffered $CaCl_2$ sucrose), 0.1 ml. of buffered saline, and 0.05 ml. of conjugate. The protein content of the aliquot varied between 0.7 and 2.4 mg. from experiment to experiment, but in any particular experiment the protein of the control and the L.E. gamma globulin were quite similar (Table III). Following the incubation the excess conjugate was removed by washing the nuclei five times with 3.0 ml. of buffered $CaCl_2$ sucrose at room temperature. Prior to each centrifugation the suspended nuclei were permitted to stand five minutes at room temperature.

The uptake of fluorescent protein by the nuclei was determined by measuring the fluorescence of the washed nuclei in the Aminco-Bowman spectrophotofluorometer, using an exciting wave length of 365 mμ and measuring the fluorescence at 520 mμ. Because of the fluorescence of the turbid nuclear suspension itself, it was found desirable to scan each sample's emitted fluorescence spectrum from a wave length of 450 to 550 mμ, and to measure the height of the 520 mμ peak over the baseline fluorescence

TABLE II

L. E. cell formation after incubation of isolated nuclei with normal and L. E. gamma globulin, and after reincubation of the treated and washed nuclei with normal white blood cells

Expt.		Incubation		Reincubation	
		Control	L. E.	Control	L. E.
1	Nuclei from fresh buffy coat	0	0	0	470
2	Lyophilized leukemic nuclei			0	280
	Lyophilized leukemic mitochondria				0
3	Nuclei from fresh buffy coat	0	21	0	378

TABLE III

Uptake by nuclei of fluorescent gamma globulin from patients with disseminated lupus erythematosus and from normal controls

1	2	3	4	5	6	7	8	9
Expt.	Conjugate	Protein in aliquot	Fluoresc. in aliquot	Degree of fluoresc.	Corrected exptl. reading	Uptake exptl.	Gamma globulin of serum	Uptake calc.
no.	*no. name*	*mg.*	*units*	*units/gamma protein*	*units*	*gamma/ mg. protein*	*mg. protein/ ml.*	*gamma/ ml. serum*
1	1. Control (AA)	0.77	1,630	2.1	9	5.6	5.2	29
	2. L. E. (BO)	0.67	2,020	3.0	119	59.2	26.3	1,560
2	3. Control (JD)	2.2	2,870	1.3	17	5.9	4.7	28
	4. L. E. (SU)	2.2	2,530	1.1	22	9 1	24.0	218
	5. L. E. (VI)	2.1	2,980	1.4	54	18.4	16.7	307
	6. L. E. (KR)	1.7	1,960	1.1	23	12.3	9.8	121
	7. L. E. (MA)	2.4	3,260	1.4	145	43.2	16.3	705
3	8. Control (WB)	2.0	3,880	1.9	23	6.1	4.9	30
	9. L. E. (LE)	2.0	4,560	2.3	92	20.0	12.4	248
4	10. Control (AA)	1.1	3,210	2.9	10	3.1	5.1	16
	11. L. E. (TU)	1.9	7,410	3.9	221	29.8	14.5	432

(Figure 1). All measurements were performed in a 1.2 ml. volume using slit set No. 3, photomultiplier tube IP-28 at maximal or next to maximal sensitivity, and were recorded on a Bristol wide-strip recorder. On the Aminco-Bowman spectrophotofluorometer the potentiometer reading is a linear function of the fluorescence. An arbitrary fluorescence unit was defined as the amount of fluorescence in 1.2 ml. of a turbidity-free medium which would cause a 1 per cent potentiometer fluctuation at 520 mμ when excited at 365 mμ with the photomultiplier functioning at maximal sensitivity. The turbidity of the nuclear suspensions absorbed about two-thirds of the fluorescence, but to determine this absorbtion accurately, a correction factor was obtained by comparing the observed fluorescence of a standard aliquot of conjugate in 1.2 ml. of wash solution and in 0.1 ml. of nuclei diluted to 1.2 ml. with wash solution. An individual correction factor was obtained for each preparation of nuclei and for preparations of nuclei altered in various ways; this factor was used to correct the observed fluorescence. All the values recorded in the tables have been corrected in this manner. Since the fluorescence remains stable after dissolving the nu-

TABLE IV

Specific inhibition of nuclear uptake of fluorescent-labeled lupus gamma globulin

Expt.	Pretreatment source of unlabeled globulin	Source of labeled globulin	Experimental uptake	
			units	*gamma*
1	NaCl	Control (AA)	15	7.1
	NaCl	L. E. (BO)	122	40.7
	Control (AA)	L. E. (BO)	125	41.7
	L. E. (BO)	L. E. (BO)	28	9.3
	L. E. (MA)	L. E. (BO)	90	30.0
	L. E. (KR)	L. E. (BO)	120	40.0
2	NaCl	L. E. (BO)	76	25.3
	L. E. (BO)	L. E. (BO)	20	6.7
	L. E. (VI)	L. E. (BO)	105	35.0
	NaCl	L. E. (LE)	92	38.6
	L. E. (LE)	L. E. (LE)	33	13.8
	L. E. (BO)	L. E. (LE)	73	30.6
	NaCl	L. E. (VI)	72	51.1
	L. E. (VI)	L. E. (VI)	30	21.3
	L. E. (BO)	L. E. (VI)	66	46.8
	NaCl	Control (AA)	5	2.4

TABLE V

Uptake of fluorescent-labeled lupus gamma globulin by nuclei after pretreatment with desoxyribonuclease and trypsin

Expt.	Pre-treatment	Uptake (gamma) Control (AA)	Uptake (gamma) L. E. (BO)	DNA Klett units ($E_{600}-E_{650}$)
1	Control	3.2	38.0	73
	DNAse	9.4	47.0	5
2	Control	6.7	57.9	
	DNAse		47.0	
	Trypsin		7.0	

clei in 1 N NaOH, the absorbtion measurements could be checked by adding NaOH. (The fluorescence is lost in acid solution, but recovered on neutralization.)

Tabulation of the uptake of fluorescent normal and L.E. gamma globulin was done in the following manner (Table III): The protein content (mg.) and the total fluorescence (units) of the 0.05 ml. aliquots of the various conjugates are reported in Columns 3 and 4 of this table, and the degree of fluorescence of the conjugates (units per gamma protein) are reported in Column 5. Column 6 presents the experimentally observed corrected fluorescence of nuclei (units) incubated with conjugate in the standard manner. In Column 7 this uptake of gamma globulin has been converted to gamma protein taken up per mg. protein in the aliquot by dividing the value in Column 6 by the degree of fluorescence (Column 5) and by the protein content of the aliquot (Column 3). The uptake per ml. serum (Column 9) is obtained by multiplication of the per mg. uptake (Column 7) by the concentration of gamma globulin of the original serum (Column 8).

Quantitative studies on the specificity of the nuclear staining reaction (Table IV) were performed by first incubating nuclei with 0.2 ml. of various unlabeled gamma globulin preparations, washing them twice at 0° C. and then reincubating them with labeled conjugate in the standard manner. The uptake in gamma is obtained by dividing the uptake in units by the degree of fluorescence of the appropriate conjugate.

Two types of quantitative studies on the nature of the nuclear component reacting with the lupus gamma globulin were done. In the first type (Table V), nuclei equivalent to 300 mg. of tissue were suspended in 0.3 ml. of buffered saline and pretreated for one hour at 30° C. with 0.5 mg. of desoxyribonuclease (DNAase) or trypsin in the presence of 0.005 M $MgCl_2$. The nuclei were then washed twice to free them of enzyme and incubated under standard conditions with fluorescent gamma globulin. Controls were pretreated with saline in the absence of enzyme.

The second type of experiment on the nature of the reacting component of the nucleus was done in a somewhat different manner (Table VI). Multiple duplicate aliquots of nuclei were incubated under standard conditions with the same fluorescent lupus gamma globulin and the initial uptake measured in the usual way. The labeled nuclei were then treated in one of the following ways: treated for 2 hours at 30° C. after suspending in 0.3 ml. of buffered saline with 0.5 mg. of DNAase or 0.2 mg. of ribonuclease (RNAase) in the presence of 0.005 M $MgCl_2$; extracted twice at 0° C. for 30 minutes with 1.2 ml. of 0.5 per cent citric acid in 1 M NaCl; or extracted three times at 0° C. over a 24 hour period with 1.2 ml. of 1 M NaCl. In each case the extracted fluorescence and the fluorescence of the residue was measured, and a control tube was run in which the nuclei were treated under identical conditions with buffered saline.

Nuclear fluorescence was frequently checked with a fluorescence microscope employing a Reichert 200 watt mercury vapor lamp and housing, a dark field condensor, and a filter system which passed light of 365 mμ wave length. Transient drying of nuclei was found to result in a marked loss of fluorescence.

Miscellaneous. The desoxyribonucleic acid (DNA) content of nuclei was determined with diphenylamine in the Klett colorimeter, using dichromatic readings (E_{600} to E_{650}) to reduce nonspecific color (16). A micromodification of the Kjeldahl procedure was used for the determination of protein concentration (17). DNAase, RNAase and trypsin were recrystallized materials obtained from the Worthington Corporation. Fluorescein amine was obtained from the Sylvania Chemical Company, Orange, N. J., and was converted to the isocyanate by the method of Coons and Kaplan (9). Other chemicals were commercial products of reagent grade. All experiments were performed with gamma globulin preparations from at least two different lupus patients.

RESULTS

Accessory serum factor

Table I presents data indicating that in addition to the L.E. factor, a second serum factor present in normal serum is necessary for the L.E. phenomenon. It can be seen in Experiments 1 and 2,

TABLE VI

Release of fluorescent lupus gamma globulin bound to nuclei by various treatments of the nuclei

Expt.	Treatment	Initial	Extract	Residue
		gamma	*gamma*	*gamma*
1	Control	30.0	2.3	20.3
	DNAse	30.0	1.0	21.0
2	Control	35.0	1.0	31.7
	0.5% citric acid in 1 M NaCl	35.0	0.7	27.7
3	Control	41.3	5.7	42.3
	1 M NaCl	41.3	29.0	12.7
4	Control	38.7		27.0
	RNAse	38.7		23.3

(in the absence of added $MgCl_2$) that washed white cells incubated with heated gamma globulin give a negative or weakly positive L.E. test. In contrast to this result, strongly positive L.E. tests are obtained when 0.1 ml. of normal serum is added. It is necessary to heat the gamma globulin to 56° C. to clearly demonstrate the requirement for normal serum factor. An accessory serum factor was previously described by Kurnick (18) who ascribed to it a role in the DNAase, DNAase-inhibitor system.

Under the conditions of the present experiments, heating the normal serum factor to 56° C. for 30 minutes reduced the activity to 29 per cent of that of the unheated control, and exposure to 0.03 N NH_4OH for 90 minutes at 37° C. reduced the activity to 27 per cent. However, activity of the combined heat-inactivated and NH_3-inactivated sera (19) was only 30 per cent. The failure to obtain complete activity from the combined inactivated sera suggests that the accessory serum factor is not identical with serum complement, but comprehensive studies in this regard have not been done. However, it is felt that this accessory serum factor acts as a phagocytosis-promoting factor similar to that described by Tullis (14). The evidence for this is given in Experiment 3 of Table I where studies on the phagocytosis of neutral starch granules in the presence and absence of normal serum factor are presented. It can be seen that washed leukocytes do not phagocytose starch granules in the presence of heated L.E. gamma globulin, while phagocytosis is marked when normal serum is added.

Table I also contains data on the effect of the addition of $MgCl_2$ and of DNAase on L.E. cell formation. $MgCl_2$ addition causes an increase in the number of L.E. cells in both the system with and the system without added normal serum factor, while the addition of DNAase results in a complete inhibition of L.E. cell formation.

Evidence of an initial reaction of nuclei with the L.E. gamma globulin prior to the phagocytosis of the nuclear material is presented in Table II. In these experiments, nuclei, after treatment with normal or L.E. gamma globulin, were washed to remove excess globulin, and then reincubated with normal serum and leukocytes. Nuclei treated with L.E. gamma globulin and then exposed to white cells and normal serum in a medium free of

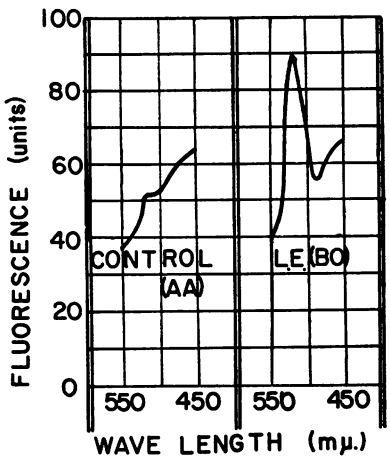

FIG. 1. REPRODUCTION OF AMINCO-BOWMAN SPECTROPHOTOFLUOROMETER TRACING OF EMISSION SPECTRUM FROM 450 TO 550 Mμ OF LIVER NUCLEI AFTER TREATMENT WITH FLUORESCENT GAMMA GLOBULIN FROM NORMAL CONTROL (AA) AND LUPUS PATIENT L. E. (BO)

L.E. factor went on to L.E. cell formation, while numerous controls, including untreated nuclei, nuclei treated with normal gamma globulin, and mitochondria treated with L.E. globulin, did not show L.E. cell formation. The small number of L.E. cells found after the initial incubation in Experiment 3 is caused by the few undisrupted cells (5 to 10 per cent) which are present in the preparations of fresh nuclei from normal buffy coat.

This then suggests that the accessory serum factor is necessary for the phagocytosis of the altered nuclear material (nuclear material which has reacted with L.E. gamma globulin) by normal leukocytes. That this extracellular nuclear material is actually altered by its reaction with L.E. factor is suggested by its morphologic appearance. Wright's stain of the control incubation without L.E. gamma globulin demonstrates a considerable amount of extracellular nuclear material (Feulgen positive) which is fine, lacelike and irregular in appearance (Figure 2). In the presence of heated L.E. gamma globulin, but without added normal serum, the nuclear material is dense, globular, purplish and homogeneous (Figure 3). This latter appearance is characteristic of the extracellular nuclear material and intracellular inclusions produced by L.E. sera as described by others (12, 20), but in the present situation the evolution of the L.E. cell is prevented by the absence of the phagocytosis-promoting factor.

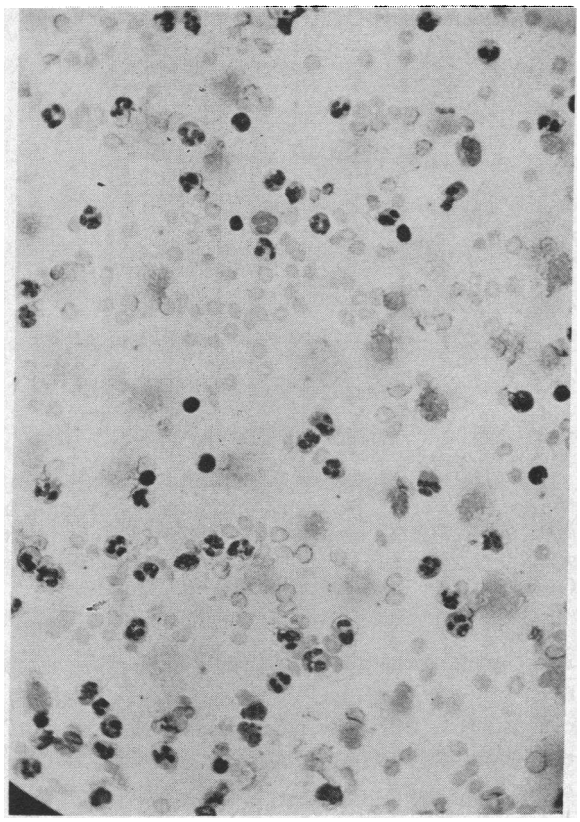

FIG. 2. EXTRACELLULAR NUCLEAR MATERIAL OBTAINED BY SHAKING WASHED WHITE BLOOD CELLS WITH GLASS BEADS IN THE PRESENCE OF ISOTONIC SALINE

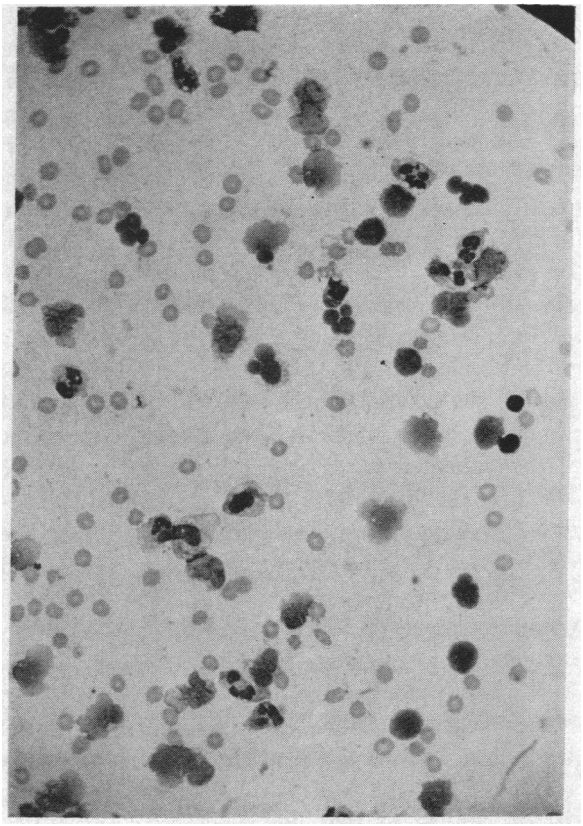

FIG. 3. EXTRACELLULAR NUCLEAR MATERIAL OBTAINED BY SHAKING WASHED LEUKOCYTES WITH GLASS BEADS IN THE PRESENCE OF HEATED LUPUS GAMMA GLOBULIN

Quantitative studies with fluorescent lupus globulin

Quantitative studies on the uptake by rat liver nuclei of fluorescent gamma globulin from four normal controls and seven patients with L.E.D. are presented in Table III. The maximum uptake of the controls is 6.1 gamma per mg. protein, while the lupus conjugates vary from 8.9 to 59 gamma per mg. The highest uptake of the lupus sera represents an uptake of 5.9 per cent of the protein of the aliquot. The gamma globulin uptake by nuclei when expressed per ml. of serum (Column 9) shows a more striking difference between L.E. patients and controls. This uptake does not exceed 30 gamma per ml. for the controls, while L.E. patients vary from 0.12 to 1.5 mg. per ml. serum.

Table IV presents quantitative studies on the specificity of the nuclear staining reaction performed by preincubating the nuclei with unlabeled gamma globulin. In Experiment 1 of Table IV it can be seen that specific inhibition of the uptake of labeled L.E. (BO) globulin is obtained by pretreatment with labeled globulin from this patient but not by pretreatment of the nuclei with normal gamma globulin or gamma globulin from two other lupus patients. To be certain that the difference in the inhibition of labeled L.E. (BO) uptake by the unlabeled gamma globulin preparations of the various L.E. patients used in Experiment 1 is not merely a reflection of a greater quantity of L.E. factor in the blood of L.E. (BO), Experiment 2 was done in which labeled and unlabeled gamma globulin from several patients with L.E. were reciprocally tested. It can be seen that the uptake of each patient's labeled gamma globulin is inhibited by prior treatment with unlabeled material from that patient, but not by unlabeled gamma globulin from other lupus patients.

Tables V and VI are concerned with studies on the nature of the nuclear component reacting with the lupus gamma globulin. In both Experiments

1 and 2 of Table V it can be seen that pretreatment of nuclei with DNAase, sufficient to remove more than 93 per cent of the DNA of the nuclei, does not reduce the subsequent uptake of fluorescent lupus gamma globulin by nuclei. Trypsin treatment does reduce the uptake of gamma globulin, but since trypsin completely destroys nuclear morphology this observation must be interpreted with caution (DNAase does not alter nuclear morphology).

In the experiments presented in Table VI, the nature of the reacting nuclear component was approached by studying the release of nuclear-bound lupus gamma globulin by various treatments of the nuclei. Experiment 1 confirms the above observation with DNAase, since there is no release of fluorescent material by the action of this enzyme on fluorescent gamma globulin-labeled nuclei. (The removal of DNA was checked as in Table V by direct determination after DNAase treatment.) In Experiment 2 of Table VI treatment with 0.5 per cent citric acid in 1 M NaCl fails to remove appreciable fluorescence, while Experiment 3 shows that repeated extractions with 1 M NaCl in the cold over a 24 hour period removes about two-thirds of the fluorescence bound to nuclei. The extracted fluorescent gamma globulin is still bound to the nuclear component in the extract, since dialyzing the 1 M NaCl extract against isotonic NaCl results in the formation of a fluorescent precipitate and a supernatant solution which is free of fluorescence. (In Experiment 3 of Table VI 40 per cent of the extracted fluorescence, all contained in the precipitate, could be recovered after dialysis.)

DISCUSSION

The present work supports the idea that a two-stage process is involved in L.E. cell formation. The first stage, specific for serum of patients with L.E., involves the reaction of a component of the cell nucleus with the L.E. factor and the morphologic alternation of the nucleus associated with this reaction. This first step is facilitated by rotation with glass beads, or in the present experiments, by presenting to the system isolated nuclei obtained by cell fractionation. The second stage in the L.E. phenomenon is the phagocytic step in which the altered nuclear material is phagocytosed by normal white cells to give L.E. cells. This step involves a heat-labile factor present in normal serum, believed to be a phagocytosis-promoting cofactor, but does not require L.E. gamma globulin. This two-stage formulation is consistent with the work of others (20–22), and certain of the present studies on this aspect of the L.E. phenomenon are similar to the work of Holman and Kunkel (8).

The present studies with fluorescent-labeled L.E. gamma globulin differ in two significant respects from the studies of other investigators (3–6). First, while in the past a fluorescent anti-gamma globulin rabbit serum was used, in the present work lupus gamma globulin was labeled directly. Secondly, because of the nature of the studies that were contemplated, a quantitative technique was developed. Aside from the disadvantage of requiring separate conjugation of each serum studied, the technique employed in the present work has one other important limitation.

Because absorption of the conjugate with liver powder removes some specific nuclear fluorescence, this step had to be omitted in the present studies. This has resulted in nuclei showing some uptake of control gamma globulin which repeated efforts have failed to eliminate. Furthermore, alterations in nuclei tend, in general, to enhance this non-specific uptake (Table V). The result of this limitation is that for the more refined measurements, such as the nature of the reacting nuclear component, only the most strongly reacting lupus conjugates can be studied with conclusive results.

Nonetheless interesting information on the L.E. phenomenon has been obtained by the present quantitative technique. First, it has been possible to demonstrate that nuclei show a greater uptake of L.E. than of normal gamma globulin, and to obtain a quantitative estimate of the amount of L.E. globulin reacting (Table III). Secondly, the specific inhibition studies bring out the interesting conclusion that the L.E. factor in various patients is not identical, since inhibition of labeled gamma globulin uptake by nuclei is only obtained by prior treatment of the nuclei with unlabeled gamma globulin from the same patient. Presumably, this difference of the L.E. factor in various patients is minor, since the experience of other investigators indicates that the L.E. factor reacts with anti-serum to normal gamma globulin (3–6) and has physical properties similar to normal

gamma globulin (2). Löw and Zetterström, employing an agar diffusion technique, also found minor differences in the L.E. factor in various patients (23). It is not unlikely that the major part of the L.E. factor molecule is identical with normal gamma globulin, while a minor part differs from normal gamma globulin and varies from patient to patient. Further, it is possible that the varied clinical picture of L.E.D. is a reflection of the difference in the L.E. factor in various patients.

The present studies suggest that the L.E. factor reacts with the nonhistone protein rather than the DNA of the nucleus. DNAase treatment fails to alter the reactability of nuclei with L.E. gamma globulin, and L.E. gamma globulin is not removed when fluorescent globulin-labeled nuclei are treated with DNAase. Extraction of histone with 0.5 per cent citric acid in 1 M NaCl (8) from the fluorescent globulin-labeled nuclei fails to extract fluorescence, while 1 M NaCl alone does extract the lupus globulin-nuclear component complex. This 1 M NaCl extract is composed largely of DNA, histone and nonhistone protein (24). It is of interest that the lupus globulin is evidently tightly bound to the nuclear component, since the complex survives 1 M salt extraction and subsequent dialysis.

The above evidence that the L.E. factor reacts with the nonhistone protein rather than with the DNA is in disagreement with the work of a number of other investigators (3, 4, 8, 25, 26). With regard to this point, the possibility exists in the present studies that artifacts may be caused by the development of nonspecific absorption by nuclei following enzyme treatment (Table V). Furthermore, because of considerations presented earlier, only the few most strongly reacting lupus sera could be studied with regard to the reacting nuclear component, and the results obtained may therefore not be generally applicable to lupus sera. It is of interest in this regard that Robbins, Holman, Deicher and Kunkel (25) have reported that certain lupus sera fix complement in the presence of whole nuclei, but not in the presence of DNA.

SUMMARY AND CONCLUSIONS

1. Studies on the lupus erythematosus (L.E.) phenomenon have shown that in addition to L.E. factor, nuclei, and normal leukocytes, an accessory serum factor present in normal serum is necessary for the L.E. phenomenon. This accessory factor is believed to be necessary for the phagocytosis of nuclear material which has been specifically altered by reaction with L.E. factor.

2. The reaction of fluorescent labeled lupus gamma globulin with nuclei has been quantitatively studied. Evidence for the heterogeneity of the L.E. factor in various patients has been obtained.

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