SERUM FACTORS IN LUPUS ERYTHEMATOSUS AND OTHER DISEASES REACTING WITH CELL NUCLEI AND NUCLEOPROTEIN EXTRACTS: ELECTROPHORETIC, ULTRACENTRIFUGAL AND CHROMATOGRAPHIC STUDIES

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(Submitted for publication April 12, 1960; accepted June 30, 1960)

In the past decade much interest has been focused on the factors in human serum which in many ways resemble antibodies against components of cell nuclei. The lupus erythematosus (LE) cell, discovered by Hargraves, Richmond and Morton in 1948 (1), is now known to result from the interaction of leukocyte nuclei and a factor(s) most frequently found in the serum of patients with lupus erythematosus. Miescher (2) employed the γ-globulin consumption test to demonstrate a reaction between factors in LE serum and isolated cell nuclei. Since this time, reactions between factors in LE serum and isolated cell nuclei (3, 4), intact nuclei in tissue sections (5–7), extracts containing nucleoprotein (8–10), nucleoprotein precipitates (11), preparations of desoxyribonucleic acid [DNA (4, 12–14)] and histone (15) have been demonstrated. The available information on the anti-DNA and other reactive factors in LE sera has been recently reviewed (16).

As more information concerning the serum-cell nucleus interactions has become available, the nature and the significance of the phenomena have appeared more complex. Anti-cell nucleus factors have been found in the sera of patients with diseases other than lupus erythematosus (7, 11), in sera which do not contain detectable LE cell factor. It appears, therefore, that not all serum-cell nucleus interactions are of the kind that bring about the changes necessary for the production of LE cells. In addition, recent studies (17, 18) indicate that the source of the tissue used in making nucleoprotein extracts is an additional variable affecting the detection of serum anti-nucleus factors. Certain sera were found to react with a *liver* nucleoprotein component which was heat-stable and was destroyed by desoxyribonuclease, whereas other sera reacted with a heat-labile component of nucleoprotein extracts made from *thymus* tissue. The present study was undertaken, therefore, to characterize in physicochemical terms several of the serum anti-nucleus factors, to determine whether the serum factors reacting with what appear to be different nucleoprotein materials might differ also in other properties, and to determine the relationship of the anti-nucleoprotein factors to the LE cell factor and other anti-nucleus factors.

Studies of the γ-globulins in man have emphasized that they are composed of two groups of proteins with ultracentrifugal sedimentation coefficients of 6.6S (molecular weight about 160,000) or of 18S (molecular weight about 1,000,000) (19). These groups contain a range of differing molecules (20). Anion-exchange cellulose chromatography is a useful means of separating the 18S γ-globulins from most of the 6.6S molecules and also provides a means for subdividing the 6.6S γ-globulin group (20). By chromatographic means, antibody activities associated with 18S protein molecules have been separated from smaller 6.6S antibody molecules (21–24).

In a preliminary report (24) on the anti-nucleus activities of LE sera, it was shown that certain anti-nucleus factors, presumably 18S γ-globulins, could be separated by anion-exchange cellulose chromatography from the LE cell factor which appeared in this and other (3, 25, 26) studies to be within the 6.6S γ-globulin group. The present report describes the electrophoretic, ultracentrifugal and chromatographic characterization of the factors in sera from patients with lupus erythematosus and other diseases which react with human leukocytes to form LE cells, with mouse liver cell nuclei as detected by fluorescein-labeled

TABLE I

Results of anti-cell nucleus and anti-nucleoprotein extract tests in sera from patients with systemic lupus erythematosus and other diseases

Diagnosis	No. of patients	No. of patients with positive LE cell test (human leukocyte)	No. of patients with positive anti-nucleoprotein tanned-cell hemagglutination test		No. of patients with positive fluorescein anti-liver nuclei (mouse) test
			Human liver nucleoprotein extract	Calf thymus nucleoprotein extract	
Systemic lupus erythematosus	27	25	15	23	26
Rheumatoid arthritis	30	2	6	3	6
Scleroderma	4	0	0	1	2
Other diseases*	53	0	0	0	6

* Includes idiopathic nephrotic syndrome, 17; dysgammaglobulinemias (principally macroglobulinemia), 13; convalescent mumps, 3.

antibody techniques, and with liver or thymus nucleoprotein extracts in tanned-cell hemagglutination tests.

METHODS

The tannic acid hemagglutination test was performed essentially as described by Boyden (27). Human type O Rh negative red cells or sheep red blood cells were used. The washed cells were "tanned" by mixing with a 1: 25,000 solution of tannic acid in pH 7.2 buffered saline for 30 minutes at room temperature. The tanned red cells, after washing, were coated by mixing with the nucleoprotein extracts for 30 minutes at room temperature. Nucleoprotein extracts were made from human liver obtained at autopsy and from calf thymus; the method for extraction of nucleoproteins with solutions of low ionic strength, described by Chargaff (28), was followed closely. All extracts were clarified by centrifugation in the Spinco model L preparative ultracentrifuge and stored frozen until used. The extracts were diluted from 1: 10 to 1: 400 with 0.15 M NaCl solution buffered at pH 7.2 before coating the tanned red cells; the concentration chosen was the highest that would not produce spontaneous agglutination of the red cells. For determination of anti-thyroglobulin titers, tanned red cells were coated with a 0.02 per cent solution of a thyroglobulin extract. The thyroglobulin was 86 per cent pure.

Quantitative total serum protein measurements, analytic paper electrophoresis and preparative electrophoretic procedures utilizing polyvinyl chloride particle blocks have been described (20). Ultracentrifugal fractionation was carried out in the manner of Robbins, Petermann, and Rall (29) utilizing a Spinco model L preparative centrifuge. Sera were diluted 1: 10 with 0.15 M NaCl solution and serum fractions were dialyzed thoroughly against the same solution prior to ultracentrifugation in the Spinco 40 rotor at 39,000 rpm for 150 minutes. At the end of this time successive samples were collected from the top to the bottom of the ultracentrifuge tube by using a sampling syringe and needle. Determinations were made of the anti-nucleoprotein extract activity, and the results were plotted in terms of the cumulative activity with increasing depth of the tube.

Anion-exchange cellulose chromatography was performed on columns of diethylaminoethyl (DEAE) cellulose (30), employing 2 g of adsorbent and 1 ml of serum. The starting and equilibrating potassium buffer was 0.02 M phosphate, pH 8.0. A 150 ml gradient elution system, providing a progressive rise in phosphate molarity to 0.25 M while maintaining pH 8, was used to elute the adsorbed proteins from the column (20). Effluent from the column was collected in about 45 fractions and the protein content estimated by measuring the optical density at 280 mμ in a Beckman spectrophotometer. The effluent was then combined into 10 or more pools which were tested for anti-nucleoprotein extract activity after dialysis against 0.15 M NaCl and without concentration.

For the fluorescent antibody technique, a $1 \times 1 \times 0.5$ cm piece of mouse liver was placed in a dry ice ethanol bath at $-70°$ C as quickly as possible after removal from the animal. When frozen, the tissue was transferred to a cryostat and sectioned at 5 μ at $-12°$ C. The sections were air-dried and then fixed in acetone for 10 minutes. Various dilutions of the patient's serum to be tested were put on the mouse liver sections and allowed to stand for 20 minutes at room temperature. The serum was then washed off with buffered saline, pH 7.4, with 3 changes of the saline at 5-minute intervals. Fluorescein-labeled anti-human globulin [1] was then placed on the section and allowed to react for 20 minutes at room temperature. The sections were again washed as before with buffered saline, mounted in 25 per cent glycerin in buffered saline and examined microscopically under ultraviolet light at a wave length of 390 to 440 mμ. The greatest dilution of the serum that produced fluorescence of the mouse liver cell nuclei was considered the titer of that serum.

[1] The fluorescein-conjugated horse anti-human globulin was obtained from the Progressive Laboratories, Baltimore, Md. This serum had been previously adsorbed with liver powder to remove nonspecific fluorescence.

RESULTS

Serological studies. Sera from 114 patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma, idiopathic nephrotic syndrome and other diseases were examined. The results of LE cell tests, the titer of reactivity of the sera with nucleoprotein extracts made from human liver and calf thymus, and the titer of reactivity with mouse liver cell nuclei as determined by fluorescein-labeled anti-human globulin tests are listed in Table I. The LE cell test was positive in most cases of SLE and rarely positive in other diseases. Anti-human liver nucleoprotein extract activity was detected in 56 per cent of the patients with SLE and 20 per cent of a selected series of patients with RA. Anti-calf thymus nucleoprotein extract activity was present cent of the patients with SLE and in 20 per cent of RA sera and 25 per cent of the scleroderma sera. The fluorescein-labeled antibody to human γ-globulin revealed anti-cell nucleus activity in 96 per cent of the SLE sera, 20 per cent of the RA sera, 50 per cent of the scleroderma sera and 11 per cent of the other sera tested. The six positive sera in the last group included four patients with idiopathic nephrotic syndrome.

In view of this diversity of results when a variety of serological tests for anti-nuclei and anti-nucleoprotein extract activity was employed, a group of six sera was selected for further study (Table II).

The sera of the second group of three subjects (P.N., S.R. and M.F.) were selected because they reacted very strongly with the nucleoprotein extract made from calf thymus but not at all, or practically not at all, with the human liver nucleoprotein extract. All three sera also reacted with cell nuclei in the fluorescein antibody test, although the titer of serum S.R. seemed lower than the others. Serum M. F., from a patient with scleroderma, did not contain detectable LE cell factor.

The sera of the first group of three subjects jects (A.N., J.B. and H.L.) were chosen because they reacted primarily with the human liver nucleoprotein extract. In addition, the three sera reacted with cell nuclei in the fluorescein antibody test at high titer. Serum H. L. was also found to contain a moderately high titer of anti-thyroglobulin activity, and was the only one of the 15 LE sera tested that contained anti-thyroglobulin activity. A comparison of the results obtained with these six sera shows that it would not be possible to predict, on the basis of the results in one test system, what the behavior of the serum would be in the other four test systems. This suggests that multiple factors were involved in the anti-nucleus and anti-nucleoprotein extract activity detected in these six sera.

Electrophoretic studies. Sera from Subjects A.N., J.B. and P.N. were fractionated by zone (block) electrophoresis. The fractions of the A.N. and J.B. sera were tested for reactivity with

TABLE II

Serological characteristics of six sera with anti-nucleus and anti-nucleoprotein extract activity chosen for further study

Patient	Diagnosis	LE cell test (human leukocyte)	Anti-nucleoprotein extract; tanned cell hemagglutination		Anti-liver nuclei (mouse); fluorescein antibody test	Anti-thyroglobulin extract; tanned cell hemagglutination
			Human liver nucleoprotein extract	Calf thymus nucleoprotein extract		
Group I						
1. P.N.	SLE*	+	80†	60,000†	>128†	<10†
2. S.R.	SLE	+	<10	100,000	16	<10
3. M.F.	Scleroderma	0	<10	3,000,000	>128	<10
Group II						
4. A.N.	SLE	+	1,280	160	>200	10
5. J.B.	SLE	+	1,280	80	>128	<10
6. H.L.	SLE	+	1.280	<10	64	6,250

* Systemic lupus erythematosus.
† Reciprocal of the greatest serial dilution of serum which gave a positive test.

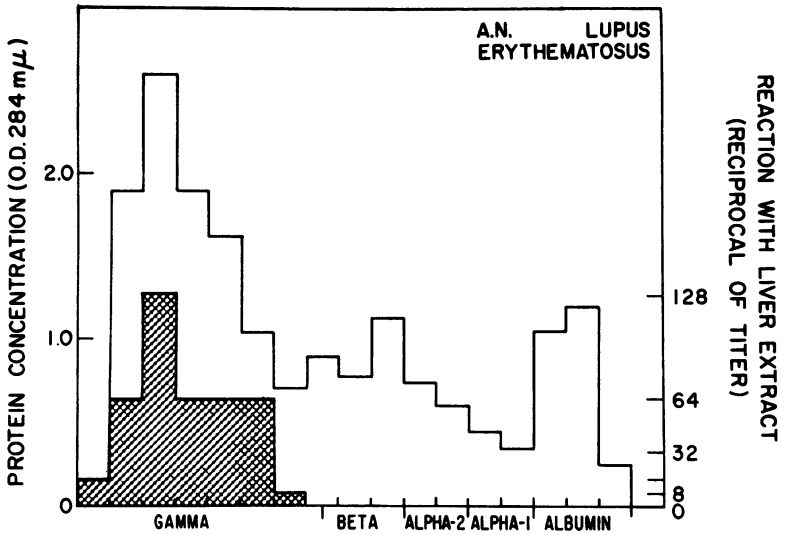

FIG. 1. DISTRIBUTION OF REACTIVITY WITH HUMAN LIVER NUCLEOPROTEIN EXTRACT AFTER ZONE (BLOCK) ELECTROPHORETIC FRACTIONATION OF A.N. SERUM. The shaded area represents the distribution of anti-nucleoprotein extract activity. The distribution of serum proteins was roughly determined by the location of the pigment bands, pink in the β region, blue in the α_2 region, and pale yellow in the albumin region, and was confirmed by analytical electrophoresis of the block sections.

human liver nucleoprotein extract and the fractions of serum P.N. were tested with calf thymus nucleoprotein extract. In each instance, serological activity was associated with the γ-globulin electrophoretic region as illustrated for serum A.N. in Figure 1. This serum contained an increased concentration of γ-globulins, 4.5 g per 100 ml.

Ultracentrifugal studies. Four sera were fractionated in a Spinco preparative ultracentrifuge. Ultracentrifugation was conducted in such a manner that most of the 18S γ-globulins, but little of the 6.6S proteins, would be concentrated at the bottom of the tube. The results are presented graphically in Figure 2.

In this figure, the *cumulative* percentage of serological reactivity is plotted against the distance from the top of the tube. The shaded area represents the behavior of the 6.6S γ-globulin anti-thyroglobulin activity in five sera of patients with chronic thyroiditis which were studied in the same way (23). The sedimentation properties of the anti-calf thymus extract activity in serum P.N. closely resembled those of the 6.6S anti-thyroglobulin activities. Very little of the activity had settled to the bottom of the centrifuge tube.

Anti-human liver nucleoprotein activity, on the other hand, sedimented more rapidly. A large proportion of this activity in sera A.N., J.B. and H.L., the sera of Group II, was found at the bottom of the centrifuge tube, in contrast to the anti-thyroglobulin activity which was also present in H.L. serum, and in contrast to the anti-thymus extract activity of serum P.N. This greater sedimentation of anti-liver nucleoprotein activity in whole serum could be due either to the association of this activity with a larger molecule or the distribution of this activity between both 6.6S and larger molecules. Data to be presented below suggest that the latter explanation is correct.

Chromatographic studies. Whole sera of γ-globulins obtained by preparative (block) electrophoresis from each serum were subjected to anion-exchange cellulose chromatography and the chromatographic fractions were tested for anti-nucleoprotein extract activity, anti-cell nucleus activity, and for capacity to form LE cells. Representative results are illustrated in Figures 3, 4 and 5. Consistent differences were observed between the sera of Group I, reacting primarily with thymus nucleoprotein extracts (Figures 3 and 5), and the sera of Group II, reacting with human liver nu-

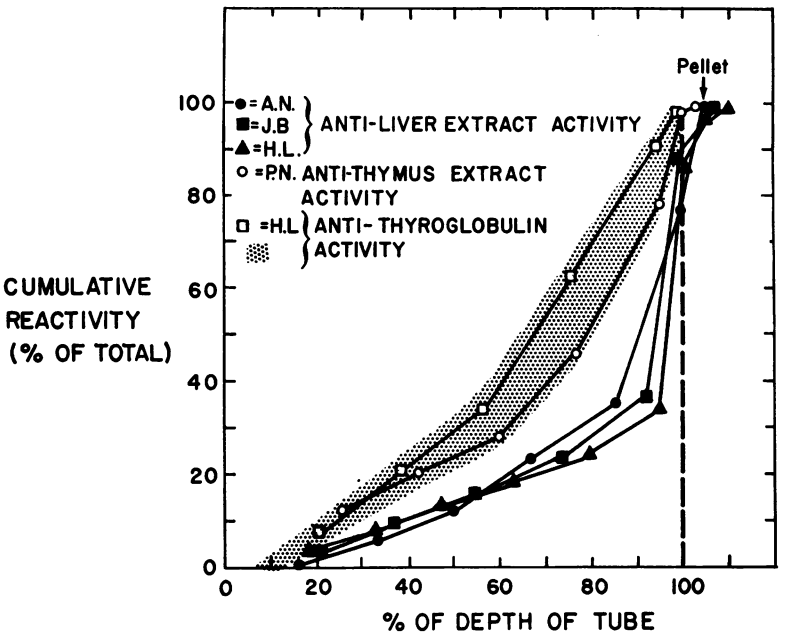

FIG. 2. SEDIMENTATION CHARACTERISTICS OF ANTI-NUCLEOPROTEIN EXTRACT ACTIVITIES IN WHOLE SERUM. Ultracentrifugation was conducted in a preparative centrifuge (39,000 rpm, 150 minutes, 0.15 M NaCl). After centrifugation, successive samples were removed from each tube starting at the top. The distribution of serological reactivity is expressed in terms of *cumulative per cent* of the total in each tube. The shaded area is the distribution of anti-thyroglobulin activities found in a concurrent study of five sera from patients with chronic thyroiditis (23).

cleoprotein extracts (Figures 4 and 5). In the Group I (anti-thymus) sera, almost all reactivity was found between 5 and 15 per cent of the effluent volume and all reactivity was found within 40 per cent of the effluent volume, while in the Group II (anti-liver nucleoprotein) sera the activity (except for LE cell factor) was found in both the 5 to 15 per cent and the 50 to 80 per cent effluent regions.

A comparison of the chromatographic elution patterns for whole serum and for γ-globulins prepared from whole serum (Figures 3 and 4) demonstrates that most of the γ-globulins are eluted in the region of 5 to 15 per cent of the effluent volume. Under the conditions of chromatography employed here, only γ-globulins with sedimentation coefficients of 6.6S are eluted in this region (20). In the Group I sera (P.N., S.R. and M.F.), anti-calf thymus nucleoprotein extract activity was found only in the initial portion of the chromatogram (Figure 3). In these sera, anti-cell nucleus activity was also found only in the 5 to 15 per cent effluent region. These findings, and the sedimentation of the anti-calf thymus extract activity when whole serum was ultracentrifuged, indicate that this activity and the anti-mouse liver cell nucleus activity in these sera are properties of γ-globulins with sedimentation coefficients of 6.6S.

In the Group II sera (A.N., J.B. and H.L.) the factors reacting with liver nucleoprotein extracts were eluted in the region of 5 to 15 per cent and 50 to 80 per cent of the effluent volume. As is seen in Figure 4, the distribution of activity is similar when whole serum or γ-globulin is chromatographed, an observation consistent with the results of electrophoretic fractionation showing the activity to be within the γ-globulin group. The finding of two chromatographic regions with activity indicates that in these sera the anti-nucleoprotein activity was associated with two different groups of γ-globulin molecules.

Ultracentrifugal studies of these two regions from the LE sera chromatograms were undertaken

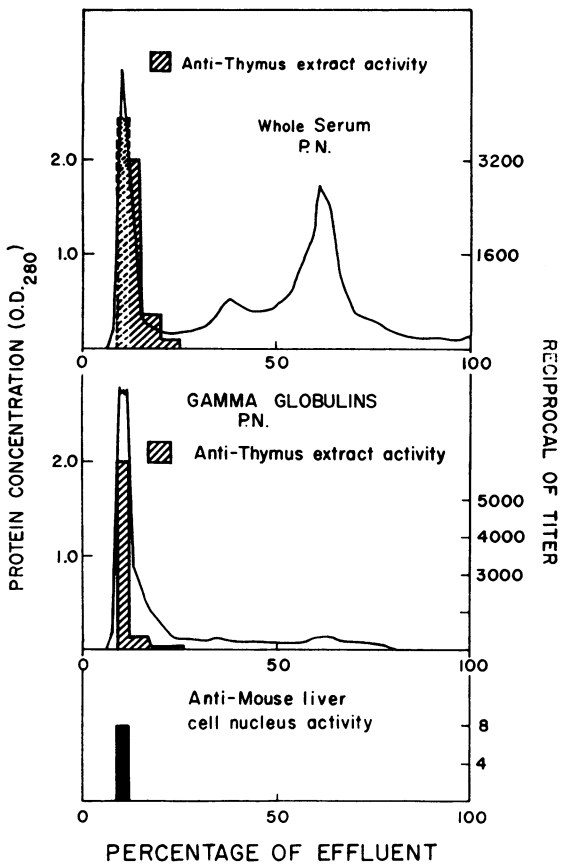

FIG. 3. CHROMATOGRAPHIC DISTRIBUTION OF SEROLOGICALLY DETERMINED ANTI-CALF THYMUS NUCLEOPROTEIN REACTIVITY IS SHOWN (TOP) IN WHOLE SERUM OF PATIENT P.N., AND (MIDDLE) IN THE γ-GLOBULIN OBTAINED BY PREPARATIVE ELECTROPHORESIS OF SERUM P.N. Also shown (bottom) is the anti-cell nucleus activity in the γ-globulin as determined by the fluorescent antibody technique. Chromatography was conducted by standard procedures on diethylaminoethyl-cellulose columns. Proteins were eluted by means of increasing phosphate concentration from 0.02 to 0.25 M, while pH was maintained at pH 8.0.

to determine whether the anti-nucleoprotein activities of these regions were associated with proteins of different sedimentation properties.

Previous studies (20) had shown that the γ-globulin eluted in the 50 to 80 per cent region of the chromatogram was composed primarily of 18S γ-globulins in contrast to the 5 to 15 per cent effluent region which contained only 6.6S γ-globulins. In Figure 6 the ultracentrifugal properties of the proteins contained in the 5 to 15 per cent and the 60 to 80 per cent region of the chromatogram of L.B. γ-globulins is shown. The proteins eluted first are seen to sediment less rapidly ($s^0_{20,w} \cong 18S$) than those eluted in the 60 to 80 per cent effluent region ($s^0_{20,w} \cong 18S$). Confirmation that the anti-nucleoprotein activity was similarly divided between faster and slower sedimenting γ-globulin components was obtained by

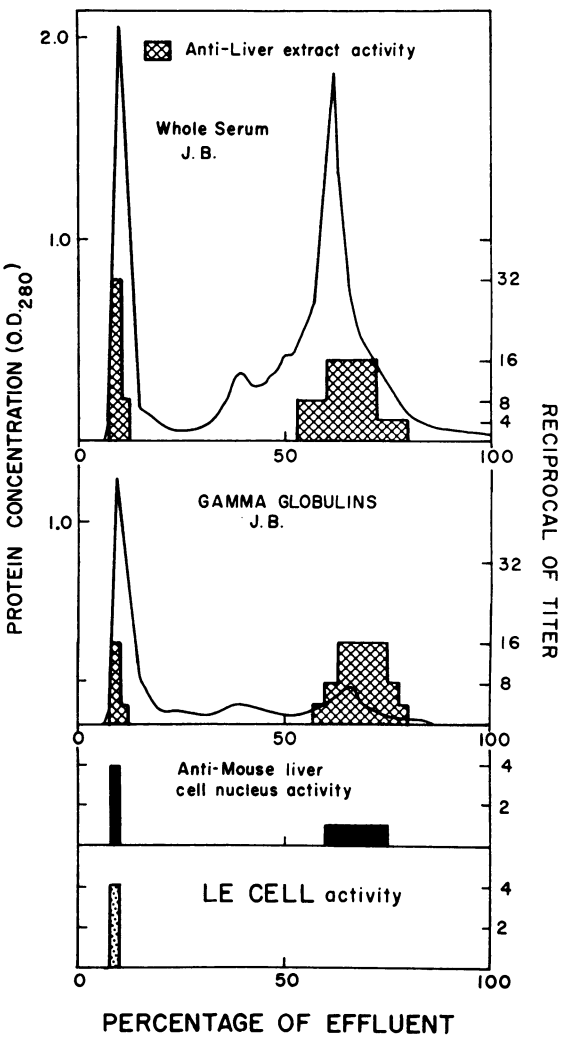

FIG. 4. CHROMATOGRAPHIC DISTRIBUTION OF SEROLOGICAL REACTIVITY WITH HUMAN LIVER NUCLEOPROTEIN EXTRACT IN WHOLE SERUM OF PATIENT J.B. AND IN γ-GLOBULINS PREPARED FROM SERUM J.B. Activity is found in two chromatogram regions. Below, the anti-nucleus activity (by the fluorescent antibody technique) is shown in the two chromatogram regions of the γ-globulins, and (bottom) lupus erythematosus (LE) cell activity is found in only one chromatogram region of the γ-globulins. Chromatography procedure was the same as that described for Figure 3.

preparative ultracentrifugal fractionation. In Figure 7 is illustrated the rapid sedimentation of the anti-human liver nucleoprotein activity in the 60 to 80 per cent effluent fraction which contrasts with the slower sedimentation behavior of the activity in the 5 to 15 per cent chromatogram effluent region. The agreement between the sedimentation behavior of the serological activities and the behavior of the proteins in these two regions supports the conclusions that the anti-liver nucleoprotein activity is associated with two groups of γ-globulin molecules, those with sedimentation

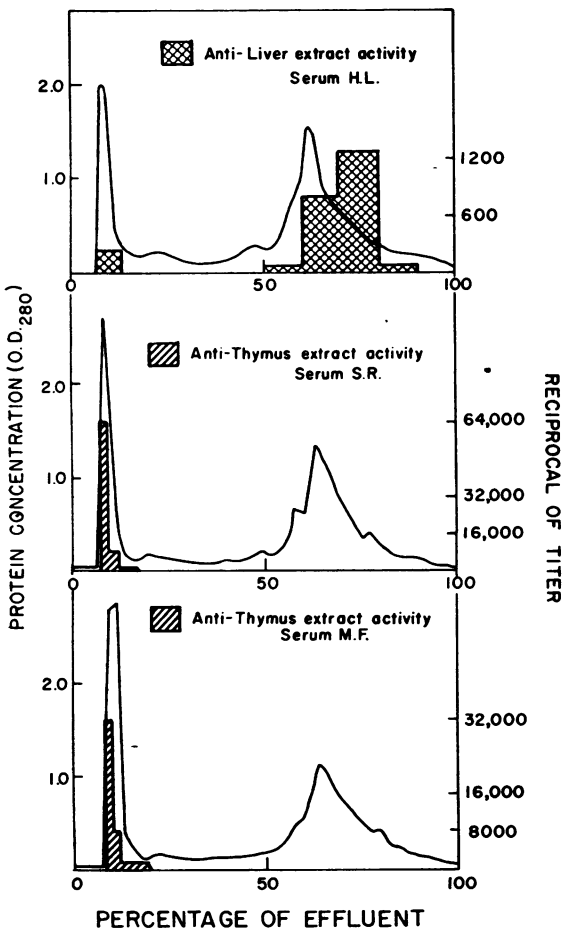

FIG. 5. CHROMATOGRAPHIC DISTRIBUTION OF SEROLOGICAL REACTIVITY WITH HUMAN LIVER EXTRACT IN WHOLE SERUM H.L. WITH ACTIVITY FOUND IN TWO CHROMATOGRAM REGIONS; BELOW IS SHOWN THE CHROMATOGRAPHIC DISTRIBUTION OF REACTIVITY WITH CALF THYMUS EXTRACT IN WHOLE SERA S.R. AND M.F., WITH REACTIVITY IN ONLY ONE REGION. Chromatography procedures were the same as described for Figure 3.

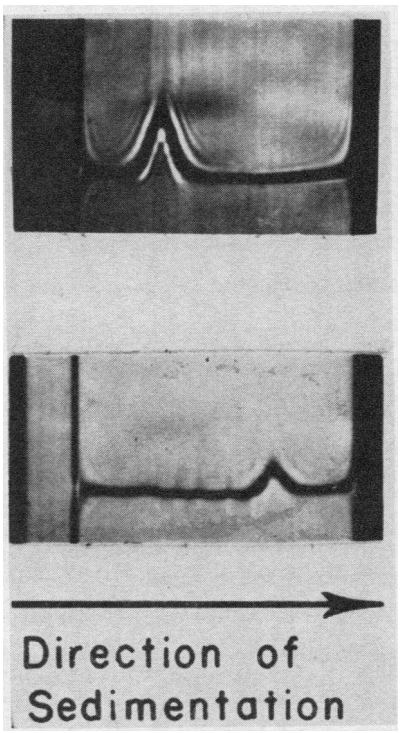

FIG. 6. ULTRACENTRIFUGAL ANALYSIS OF THE 5 TO 15 PER CENT REGION (UPPER) AND IN THE 60 TO 80 PER CENT EFFLUENT REGION (LOWER) FROM AN ANION-EXCHANGE CELLULOSE CHROMATOGRAM OF ELECTROPHORETICALLY PREPARED γ-GLOBULIN FROM L.B. SERUM. The photographs were obtained 26 and 24 minutes, respectively, after reaching rotor speed of 59,780. The direction of sedimentation is to the right. Samples had been dialyzed against 0.15 M NaCl prior to analysis. The sedimentation coefficient was approximately 6.6S for the component in the upper, and 18S in the lower photographs.

coefficients of 6.6S and 18S, and that these two groups have been separated by anion-exchange cellulose chromatography.

If the results of the preparative ultracentrifugal analysis of the two J.B. γ-globulin components illustrated in Figure 7 are compared with the results when J.B. whole serum was similarly analyzed (Figure 2), it is seen that the whole serum results fall between the findings for the individual components. This observation is consistent with the interpretation that the pattern of distribution of anti-nucleoprotein extract activity of this type (anti-human liver) after centrifugation of whole serum (Figure 2) is due to the presence of two components. The existence of two ultracentrifugally different components may be suspected when the whole serum results do not conform to

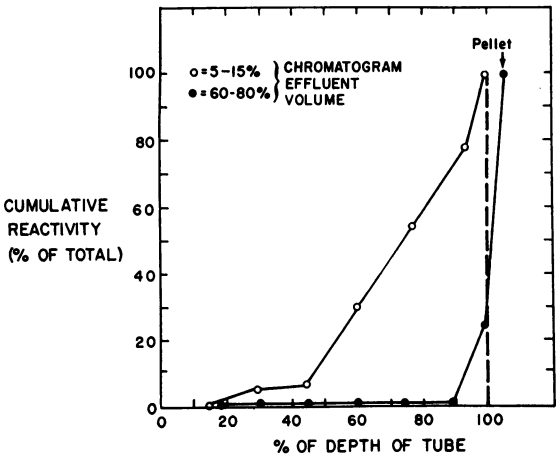

FIG. 7. DIFFERENCES IN THE SEDIMENTATION CHARACTERISTICS OF TWO ANTI-HUMAN LIVER EXTRACT COMPONENTS (5 TO 15 PER CENT AND 60 TO 80 PER CENT) OBTAINED BY CHROMATOGRAPHIC FRACTIONATION OF SERUM J.B. Ultracentrifugation was carried out as in Figure 2.

the expected distribution of activity for 6.6S or 18S components alone. Whole serum preparative ultracentrifugation, however, is a relatively crude technique, and small amounts of one type of γ-globulin activity would be difficult to detect in the presence of large amounts of the other type of γ-globulin activity. Anion-exchange cellulose chromatography provided a better means of separating and detecting the presence of both 6.6S and 18S γ-globulin activities.

Fluorescein-labeled anti-human globulin tests. The γ-globulins of the patient's sera which combined with mouse liver nuclei were detected by their reaction with fluorescein-labeled anti-human globulin. Examples of positive and negative results are illustrated in Figure 8. In the Group I sera (reacting with calf thymus nucleoprotein extract) the anti-cell nucleus factors were found only in the 5 to 15 per cent chromatogram effluent region (Figure 3), whereas in the Group II sera (reacting serologically with liver nucleoprotein extract) the positive fluorescent anti-globulin tests were obtained in both the 5 to 15 per cent and 50 to 80 per cent effluent regions (Figure 4). This shows that at least two physicochemically different γ-globulin components in these latter sera give positive fluorescein antiglobulin reactions and, on the basis of the observations noted above, it seems probable that the two components are 6.6S and 18S γ-globulins.

Some of the activities detected by the fluorescein anti-globulin test may be the same as those detected by the serological anti-nucleoprotein extract tests. Very likely several activities are involved, for inspection of the data in Table II reveals that the activities detected by these two types of tests were not increased equally in LE sera.

LE cell tests. The γ-globulin components responsible for LE cell formation were present only in the 5 to 15 per cent chromatogram effluent region [Figure 4 and Patient H.L. (22)]. As only 6.6S γ-globulins are found in this chromatogram region, the factor(s) responsible for LE cell formation must be 6.6S γ-globulins. The two sera fractionated for detailed study of the factors responsible for LE cell formation were chosen because they had both 6.6S and 18S γ-globulins which reacted with nuclear components by serological and histochemical tests (Group II sera). In view of the absence of LE cell factor in ma-

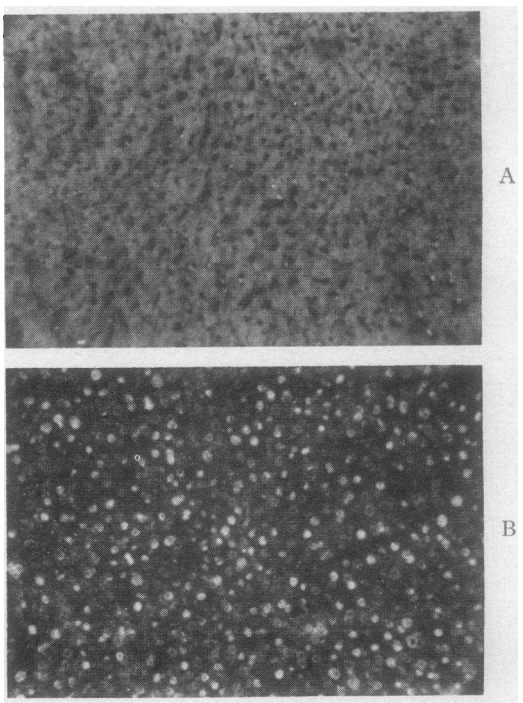

FIG. 8. MOUSE LIVER SECTIONS EXPOSED TO HUMAN SERUM, WASHED, EXPOSED TO FLUORESCEIN-LABELED ANTIBODY TO HUMAN γ-GLOBULIN, AND WASHED AGAIN. A. Lack of fluorescence of the cell nuclei after exposure to normal serum (cell nuclei appear as dark holes) when observed with an ultraviolet light source. B. Bright fluorescence of the nuclei after exposure to lupus erythematosus serum. 150 × magnification.

terial from the 50 to 80 per cent effluent region which contained factors reacting with nucleoprotein extract and cell nuclei, it is clear that the LE cell factor has been separated chromatographically from some of the other anti-nucleus factors in these sera.

DISCUSSION

The discussion of the multiple serum factors reacting with tissue components in diseases such as lupus erythematosus is complicated by the lack of exact knowledge of the tissue substances with which the sera react. The crude nucleoprotein extracts employed could well contain cytoplasmic contaminants, and in fact, Deicher, Holman and Kunkel (31) have recently reported complement fixation reactions with liver cell mitochondrial preparations in the 19S globulins of one of eight SLE sera tested. Although in the present experiments no definitive studies were made of the nature of the active substances extracted from calf thymus because of their lability, the substances in human liver extracts with which the sera reacted were found to be very heat-stable and were destroyed by incubation with desoxyribonuclease (17); they were assumed to be nucleoproteins. In any case, whatever reactions with cytoplasmic constituents may have been present, all six of the sera studied here in detail reacted with components of cell nuclei as shown by the fluorescein-anti-globulin test. Each differed from the others, however, in some aspect of its reactions with cell nucleus components. The sera were considered in two groups. Three of the sera were in Group I, reacting with nucleoprotein extracts of calf thymus and showing activity only within the 6.6S γ-globulins. The three sera of Group II reacted primarily with nucleoprotein extracts from human liver and contained this activity in both 6.6S and 18S γ-globulins. This latter group of sera also contained two types of γ-globulin reacting with the mouse liver cell nucleus.

These observations could be explained either on the basis of a fundamental difference in immune response between the two groups of patients (i.e., those in Group I are limited to the manufacture of 6.6S antibodies, whereas those in Group II can also make 18S antibodies) or on the basis that the two patient groups were subjected to different kinds of immune challenge. Although we cannot yet distinguish between these alternative explanations, there is some evidence that these patients did not have a strict limitation of their capacity to manufacture 6.6S or 18S antibody. Serum M.F., which contained only 6.6S γ-globulins reacting with components of cell nuclei, also contained a high titer of rheumatoid factor which has been shown to be a γ-macroglobulin (32). On the other hand, Patient H.L., who was capable of producing 18S globulins which reacted with human liver nucleoprotein extracts, manufactures only 6.6S γ-globulins reacting with thyroglobulin. Antibodies to thyroglobulin extracts, however, need not be only 6.6S γ-globulins and, indeed, some patients produce largely 18S reactive globulins (23). These observations suggest that the different activities found in LE sera may be related to different tissue substances and that the tissue substances themselves might help to determine whether the antibodies to them are to be 6.6S or 18S γ-globulins.

The finding that the LE cell factor can be separated from some of the other anti-nucleus factors in serum is consistent with the concept of the existence of multiple anti-nucleus factors in the serum of patients with lupus erythematosus (4, 13, 15, 24). It is clear, therefore, that some serum-cell nucleus interactions are of a type which do not lead to the production of LE cells; the reasons for this are not evident. The data showing that other anti-nucleus factors are more widely distributed than is the LE cell factor is consistent with previous reports (7, 11). It will be necessary to test large numbers of sera from each of many disease categories, as well as a large number of normal sera, for the presence of anti-nucleus factors in order to determine their actual distribution.

Although the γ-globulins which react with nuclei in the manner required for the production of LE cells have so far been found only among the 6.6S γ-globulins (3, 25, 26), it is now apparent that 18S γ-globulin components may react with cell nucleus components in lupus erythematosus. Anti-cell nucleus reactivity can be added to the list of antibody-like activities already found in this group of macroglobulins: the isohemagglutinins (22, 33), rheumatoid factor (32) and the anti-thyroglobulin "antibodies" found in the serum of certain patients with thyroiditis (23). The

nomenclature of these macroglobulins is not uniform, and the proteins known as 18S or 19S γ-globulins or γ_1-macroglobulins in the American literature are classified as β_2 M-globulins in the immunoelectrophoretic studies of Burtin and coworkers (34). However, immunochemical studies of the macroglobulins which react with nucleoprotein extracts were not done.

It will be interesting to determine whether any correlations can be made between clinical findings and the serological differences described in the present study. So far as can be determined in a preliminary study (35), no obvious differences in clinical state can be correlated with the different patterns of anti-nucleus factors. The significance of the appearance of these antibody-like proteins in the serum of patients with lupus erythematosus and other diseases is not known. The production in rabbits of what may be somewhat similar auto-antibody-like anti-nucleus factors, after immunization with foreign cell nucleus materials (17, 36), at least suggests the possibility that bacterial or viral infection might result, in certain susceptible individuals, in the production of antibodies to nucleoproteins or other materials which cross react with similar components in human cell nuclei.

The ease with which DEAE cellulose chromatography allows recognition and separation of the 6.6S and 18S reactive factors should facilitate investigation of the mechanism of their production as well as the role, if any, played by these serum factors in the initiation or maintenance of disease processes.

SUMMARY

Multiple factors in sera from patients with lupus erythematosus (LE) and other diseases were found to react with components of cell nuclei. These anti-cell nucleus factors were detected by serological tests employing calf thymus and human liver nucleoprotein extracts and by fluorescent anti-globulin tests with mouse liver tissue and by LE cell tests.

All of the serum factors were in the γ-globulins separated by zone electrophoresis. The anti-nucleus factors were further characterized by anion-exchange chromatography and in some cases by ultracentrifugal studies.

The LE cell factor and the anti-thymus nucleoprotein activity were found only among 6.6S γ-globulins. In contrast, anti-human liver nucleoprotein extract activity was found largely with the 18S γ-globulins, although some activity was also found with 6.6S γ-globulins. The anti-cell nuclei activity detected by the fluorescent anti-globulin test was found with the 6.6S γ-globulins in LE sera reacting with calf thymus nucleoprotein extract and was found with 18S and 6.6S γ-globulins in LE sera reacting primarily with human liver nucleoprotein extract.

The qualitative and quantitative variety of serum factors in lupus erythematosus was notable, although the reasons for their appearance and their role in diseases remains obscure. Anion-exchange cellulose chromatography provided an excellent means for separating and identifying the reactive 6.6S and 18S γ-globulins.

ACKNOWLEDGMENTS

We wish to thank Drs. John Nasou and Kurt Bloch for making available many of the sera utilized in these studies, and Dr. George Brecher and Miss Minna Feld for performing the LE cell tests. We are also indebted to Mr. Robert Bowser for help in anti-nucleoprotein extract assays and ultracentrifugal procedures, Miss Anne P. Horbett for zone electrophoretic procedures, Miss Mary E. Lawrence for assistance with the chromatography, and Mr. Willie Mills for assistance in the fluorescent antibody tests.

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