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Partial Purification and Properties of Lupus Erythematosus Cell Promoting Factor. (24448)

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Patients with disseminated lupus erythematosus (DLE) have a substance in their serum which is thought responsible for formation of the characteristic "lupus erythematosus cells." Previous attempts at isolation of this substance have indicated that it is associated with the gamma globulin fraction (1). A useful method for chromatographic fractionation of proteins using cellulose exchangers has recently been developed(2). The technic is carried out under conditions which favor preservation of the structure and biologic activity of the proteins recovered in the fractions. The wide range of applicability of this method is reflected in the reports appearing in the literature(3). A previously reported preliminary study(4) indicated that an anionic cellulose exchanger may effect some separation of the L-E factor. The purpose of this communication is to report on some of the properties of a preparation containing L-E activity by use of a cationic cellulose exchanger.

Methods. Sera obtained from normal individuals and from patients with DLE were stored in frozen state until ready for use. All fractionation procedures were carried out in cold room at 4°C. Isolation of serum gamma globulins was done by electrophoretic convection in 0.15M phosphate buffer at pH 7.4. Nitrogen determinations were done by micro-Kjeldahl technic and phosphorus values were obtained by Schmidt-Thannhauser technic (5). The resulting products contained little or no contaminating beta globulin as determined by moving boundary and paper electrophoresis. However, when the products were examined by agar diffusion(6) using rabbit anti-human gamma globulin as the developing agent, as many as 7 distinct antigenic components could be identified. The gamma globulin isolated from patients with DLE gave strongly positive reactions in the L-E cell preparations(7) and in the DNA-protein coated latex agglutination test(8). Serum samples and gamma globulin fractions from normals and subjects with DLE were prepared for column chromatography by preliminary dialysis against 0.05M phosphate buffer at pH 5.0. A small amount of insoluble material, usually present at end of 24 hours, was removed by centrifugation. When whole serum was to be chromatographed, the dialyzed sample was diluted 1:10 with the starting buffer before placing it on the column. Gamma globulin samples were placed on the column without preliminary dilution. Approximately 15 g of cationic exchange cellulose, CM-cellulose-SF,† were placed in 3 changes of 0.05M phosphate buffer at pH 5 for 48 hours. The slurry was placed in a 20 x 200 mm glass column and packed until a standard height with a flat crown had been achieved. Under these conditions, the flow rate varied from 60 to 80 ml/hour. The pH and salt concentration of the eluting buffers was increased simultaneously. The initial pH was 5 and final pH 7; initial salt concentration was 0.05M and final concentration 0.15M. Salt concentration was increased by step-wise addition of sodium chloride. Six ml samples

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TABLE I.

	L-E γ globulin	Positive column fraction
L-E cells	Yes	Yes
Agglutination	"	"
Total N	100 mg	2.8 mg
N conc.	1.83 mg/ml	.428 mg/ml
Phosphorus conc.	.042 "	.030 "
N/P ratio	44	14.3

were recovered with an automatic fraction collector operating in room at 4°C, at flow rate of 60 to 70 ml/hour. Elution curves were constructed plotting fraction number against optical density at 280 mU. Selected groups of consecutive fractions showing heavy absorption were pooled and concentrated against 0.15M sodium chloride by negative pressure dialysis. The resulting concentrates were characterized as follows: L-E cell promoting activity; DNA-protein coated latex agglutinating activity; nitrogen and phosphorus content; electrophoretic and ultracentrifugal properties; immunologic behavior in gel diffusion studies.

Results. Elution diagrams of normal serum and serum from patients with DLE are qualitatively similar. As indicated, evidence of L-E activity was present in one of the concentrated pooled fractions. Only rosettes were seen in the L-E cell preparation but this material resulted in a strongly positive DNA-protein coated latex agglutination test.

Fig. 1 presents typical elution diagrams of gamma globulin fractions prepared from normal individuals and from patients with DLE.

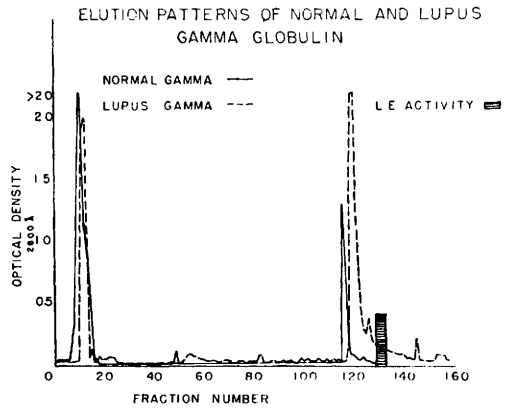

FIG. 1.

The relatively minor differences in the patterns are of questionable significance since they could result from variations in column packing, column size, buffer flow rates, and rates of change in pH and salt concentration. A pooled concentrated fraction could be recovered from L-E gamma globulin which demonstrated L-E activity in both agglutination test and in L-E cell test (Fig. 2). The positive fractions were regularly obtained at a pH between 6.8 and 7.0 and at a salt concentration of 0.15M.

In a typical experiment, 100 mg of L-E gamma globulin nitrogen was placed on the column. L-E activity was recovered in a pooled concentrated fraction with approximately the same activity intensity as the starting material. In Table I, data are shown comparing the properties of the starting material with those of the positive fraction. Activity could be demonstrated in a preparation which had lost 97% of its original total protein nitrogen. Of some interest is the reduction in nitrogen to phosphorus ratio from 44 to 14.3. Since the first major elution peak had a nitrogen to phosphorus ratio of 13, it is presumed that a considerable amount of gamma globulin remained fixed on the column.

The positive pooled fraction was examined in the Tiselius moving boundary electrophoresis apparatus and with conventional paper electrophoresis. A single peak with the mobility of normal gamma globulin was observed. When placed in the ultracentrifuge, the fraction showed a single peak with a sedimentation constant of 7 Svedberg units.

The starting L-E gamma globulin preparation regularly showed as many as 7 distinct antigenic components when examined by the Ochterlony technic; the positive fraction, however, failed to demonstrate more than a single band. In addition, this single band showed a reaction of identity with a component of normal gamma globulin. No band was observed when the positive fraction was set up in the agar diffusion against purified DNA over a pH range of 7.4 to 8.2. The gel diffusion properties of the "leuco-precipitines" described by Seligman are similar to our findings except we were unable to demon-

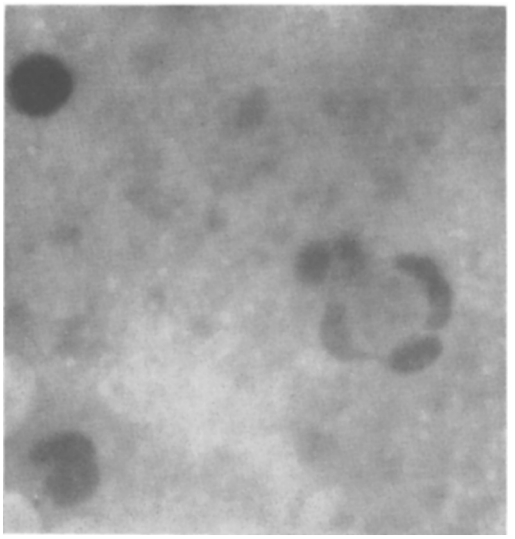

FIG. 2.

strate a precipitin reaction between the L-E factor and DNA(9).

The first major peak, shown in the elution pattern of L-E gamma globulin in Fig. 1, regularly showed from 3 to 5 components when examined by the Ochterlony technic. On 2 occasions, fractions in the first peak demonstrated a positive L-E agglutination test but none of the fractions from this peak produced a positive L-E cell preparation.

Discussion. This study illustrates another use of cellulose exchangers in chromatographic separation of serum proteins. The product showing L-E activity exhibited electrophoretic and sedimentation properties similar to products prepared by other methods(10). Within the limitations of the technics employed, the L-E factor appears to behave as a single component. The data do not support the position that more than one specific factor present in L-E sera is responsible for hematoxylin body formation. The data do suggest that the L-E factor is a substance associated with gamma globulin which shares antigenic groupings with normally occurring gamma globulin. This view receives further support from the observation in our laboratory that rabbit anti-normal human gamma globulin serum is capable of removing the L-E activity from positive preparations (to be published). In addition, fluorochrome studies (11) indicate that, when the L-E factor and nucleoprotein become associated, normal gamma globulin is added to the complex.

The suggestion is often advanced that the L-E factor is an "antibody" to a nuclear component but the evidence available does not permit this conclusion. The amount of protein nitrogen necessary to retain full biologic activity of the L-E phenomenon is small. The unusual sensitivity of the L-E phenomenon to pH, temperature, storage and dilution, and the sequence of events as seen under phase microscopy(12), are not reminiscent of an ordinary antigen-antibody reaction. An alternative suggestion is that L-E activity may be conferred on the normally occurring gamma globulin by its association with a small but highly active abnormal component.

Summary. Partial purification of the L-E cell promoting factor from gamma globulin has been achieved with the aid of a cationic cellulose exchanger. The fraction containing L-E activity is eluted at a pH of approximately 7 and a salt concentration of 0.15M. In the process of purification, total protein nitrogen content was reduced 97% nitrogen to phosphorus ratio was reduced from 44 to 14.3 without notable reduction in biologic activity. This fraction possesses the electrophoretic, sedimentation and immunologic properties of a normal gamma globulin.

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Red Cell and Plasma Transit Through Spleen: the Splenic Hematocrit.* (24449)

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Considerable interest in the physiology of the spleen has been shown since Barcroft and co-workers(1-6) showed its importance as a reservoir for red blood cells. This function has been confirmed by Haam(7) and Kramer (8). Kramer concluded on effect of hypoxia on systemic hemoglobin content and spleen weight, that the spleen consists almost entirely of packed red cells. Reeve(9) postulated from P^{32} blood volume determinations that the spleen concentrates red blood cells. Everell(10) calculated splenic hematocrit in rats, after allowing 15 minutes for red cell mixing with injected labeled red cells. He cound the hematocrit to be 46.9 in female rats and 50.7 in the males. These values would raise a question when compared to Kramer's(8) conclusion that the spleen consists almost entirely of packed red cells. However, Barcroft(1) using CO. had previously demonstrated that 30 to 90 minutes elapses before the general circulation and general mass of hemoglobin in the spleen acquire the same CO content. This indicates that perhaps Everell did not allow sufficient time for red cell mixing to occur. The following experiments were designed to determine the time necessary for equilibrium to be reached between general systemic circulation and splenic venous outflow. This enabled calculation of mean transit time of plasma and red blood cells and provided data from which the splenic hematocrit could be determined.

Methods. Eight healthy mongrel dogs weighing 9 to 12 kg were anesthetized with sodium pentobarbital, 25 mg/kg. A generous midline abdominal incision was made and the spleen exposed. One of the large splenic veins was dissected free, care being taken that adequate collateral venous drainage was left intact. This vein was incised and a polyethylene catheter tied into the proximal segment. The free end of the catheter contained a stopcock. Similar polyethylene catheters were inserted into the femoral artery and femoral vein. In 6 experiments, aortic pressure was monitored with a mercury manometer connected to the femoral arterial catheter. Twelve ml of a saline suspension of the dogs' red cells previously tagged with approximately 500 microcuries of Cr^{51} and washed free of any extracorpuscular Cr^{51} activity was mixed with 125 microcuries of I^{131} tagged human serum albumin. In one experiment, I^{131} gamma globulin was used instead of I^{131} serum albumin. This mixture was then injected over an 8 to 10 second period into the femoral vein. From the start of injection until 5 minutes later, femoral arterial blood and splenic venous blood was collected continuously at 30 second intervals into paraf-

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