be so characteristic that identification was possible beyond doubt. The results showed clearly that elimination of the lines belonging to one component by pretreatment of the medium with either antigen or antibody had no influence whatever upon the formation of the precipitate characteristic of the other. It would seem, therefore, that the procedure can be safely applied in the analysis of complex antigenic systems.

Preliminary experiments along these lines with antigens prepared from rabbit bone-marrow gave promising results. In a subsequent paper the application of the procedure in the analysis of the antigenic structure of different organs will be described(9).

Summary. A modification of Ouchterlony's gel diffusion method for antigen analysis is described. It is based on the following observations: 1. By pretreatment of the diffusion medium with sufficient amounts of one component of a complex immunologic system the subsequent appearance in the medium of precipitates corresponding to this particular component could be completely inhibited. 2. The diffusion of the other components of the same system and the formation of other immunoprecipitates was not influenced. 3. By incorporation in the diffusion medium of individual components of a complex immunologic system it is thus possible, by elimination of some of the lines, to simplify the precipitation spectrum and facilitate the antigen analysis.

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Serological Analysis of Components in Hemopoietic Tissue.* (19365)

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In a previous paper(1) by Björklund and Hellström the biological effects of a horse hyperimmune serum against rabbit bone-marrow were described. Rabbits, after receiving intravenous injections of this serum, displayed symptoms referable on one hand to a histopathologically demonstrable aplasia of the hemopoietic system and degeneration of the circulating blood cells, on the other hand to a general toxic effect. Administration of large serum doses invariably caused the death of the animals. It seemed probable that antibodies were primarily responsible for the effects observed.

It might be assumed that the preparations of bone-marrow used for immunization of the horses contained a number of substances with distinct immunological properties. Among these, some are presumably specific for different elements of the hemopoietic system, while others originate in capillary endothelium, connective tissue, serum and similar sources not intrinsically connected with the hemopoietic apparatus proper. By means of serological *in vitro* studies it should be possible to differentiate between the antigenic components and examine their distribution in different organs and tissues. The same methods might also be applied as a guide in attempts at isolation and purification of the biologically interesting antigens. The present paper reports

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some preliminary experiments along these lines.

Materials and methods. Antigens. Red bone-marrow, liver, spleen, lung and adrenals were obtained from rabbits used for pregnancy tests according to Friedmann-Schneider. The organs were removed within 2 hours after the animals had been sacrificed and immediately frozen at −20°C. Batches of tissue of each kind were ground and extracted 3 times with ether at +5°C. After removal of the ether, the residue was lyophilized, ground in a ball mill, and again extracted with ether. One gram of the dry powder, obtained after removal of the ether, was finally extracted with 16 ml of physiologic saline at pH 7.2 and centrifuged. The clear supernatant was used as antigen. Rabbit red cells were washed 3 times in saline and then subjected to the treatment described above. Rabbit plasma obtained from healthy animals was stored at −20°C and diluted in 3 parts of saline before use. *Hyperimmune serum.* Horses were immunized by subcutaneous inoculations of bone-marrow antigen as described previously(1). Two sera were available: The horse Nobert treated with a total amount of 21.3 g (dry powder) during a period of 8 months and Plym immunized with 32.75 g in the course of 4 months. *Diffusion medium.* The same medium was used as previously described (9). *Antigen analysis.* Ouchterlony's gel diffusion method(2-8) for serological analysis of precipitating antigen-antibody systems was used in combination with the inhibition method as described previously(9). This latter method was employed because of the antigenic complexity of the systems under study. By addition of one particular antigenic component to the medium the appearance of a precipitate corresponding to this antigen could be inhibited. It was thus possible, by adding extract of a certain organ to the medium, to suppress the formation of all precipitation lines belonging to this particular antigenic system. Inhibiting concentrations in the medium were established in either of two ways: a) by adding the antigen to the agar before pouring the plates or b) by diffusion from the basins. The former method was applied only when normal rabbit plasma was used as inhibiting factor. Plasma was added to a concentration of 25%, replacing equal amounts of saline. In the latter method the basins were filled on 5 consecutive days with the inhibitory preparation and the liquid allowed to diffuse completely into the gel(9).

TABLE I. Precipitation Spectrum of Antigens Tested in Diffusion Plates Inhibited with Rabbit Plasma.

Antigens	No. and character of precipitates
Bone-marrow	++++
Liver	++(+)
Spleen	+++
Lung	++(+)
Adrenal	++
Red cells	—
Plasma	—

+ = a strong line; (+) = a weak line; — = no line.

Experimental. Preliminary studies with the Nobert serum indicated that the bone-marrow of rabbit contains antigenic components, which are not demonstrable in plasma from the same species. To confirm this and to study the distribution of the non-humoral antigens in other tissues the following experiments were set up using serum from Plym. Bone-marrow, liver, spleen, lung, adrenal, red cells and plasma from rabbit were tested against anti-bone-marrow serum in diffusion plates inhibited with rabbit plasma. Efficacy of inhibition was shown by the fact that rabbit plasma gave rise to no lines of precipitate. Nor did the red cells. With the other preparations clearcut lines were obtained in varying numbers (Table I).

To check whether the extracts contained other antigens with precipitation optima outside the range of concentrations established, all extracts were tested also in dilutions of 1/5, 1/25 and 1/125. No further lines of precipitate were seen. Attempts were now made to identify the components in the different organs.

In 30 diffusion plates, inhibited as above, each extract was tested against each one of the others and the patterns of precipitation were compared. All extracts were also tested against rabbit plasma as a control. In most cases it was possible to differentiate between

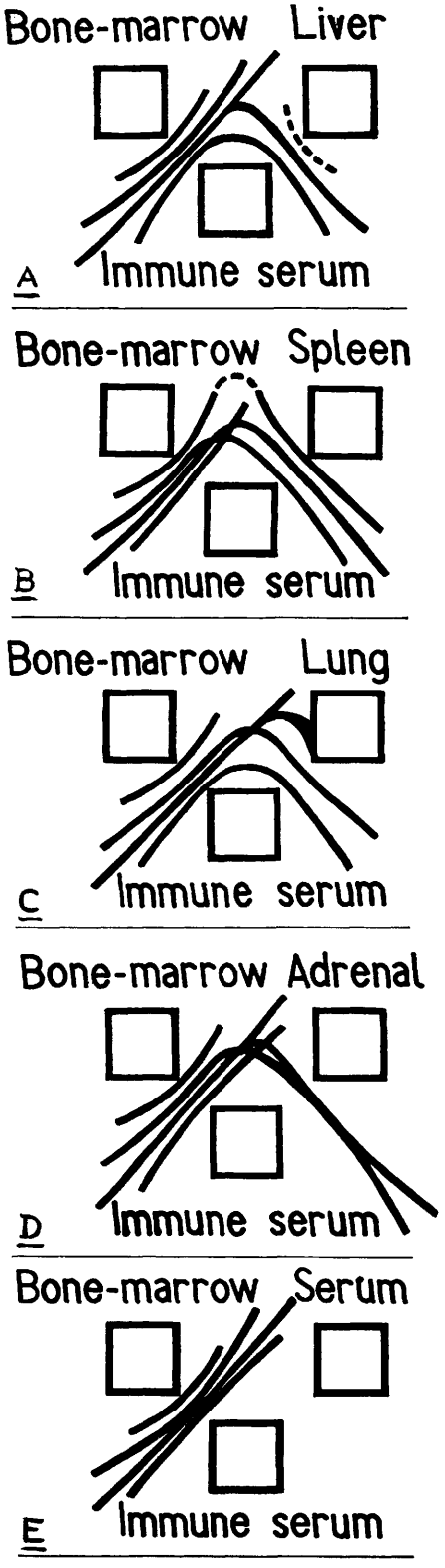

FIG. 1. A to E. Tracings of precipitation patterns in diffusion plates inhibited with normal rabbit plasma. The squares indicate the basins serving as diffusion centers of the reactants: a horse-rabbit bone-marrow hyperimmune serum and the diverse antigens as indicated in the figures.
N.B. Upper right reagent of Fig. 1E should read plasma instead of serum.

the various components and by means of the reactions of identity(5,8) to study the composition of different extracts. In some cases, however, fusion of the individual lines made such an analysis difficult or impossible. It was found that a better resolution of the precipitation patterns was obtained on dilution of the extracts. By means of this procedure clearcut results were obtainable. In all, 4 different precipitates could be distinguished, designated in the following as A, B, C and D. In Fig. 1 tracings of the different patterns are reproduced. From these it is obvious that the bone-marrow extract contained all 4 components. In the spleen A, B and C were present but not D. Liver and adrenal contained a substance designated B_1 giving a reaction of partial identity with B. Traces of B_1 were also found in the lung together with C and D. Besides B_1 the liver contained D and traces of C and in the adrenal C was found in large amounts. It was also interesting to note that the bone-marrow contained more B than the spleen but the spleen on the other hand more C than the bone-marrow.

If the statements made were correct one should expect the different extracts to inhibit precipitation to a degree corresponding to their content of antigens as shown above. For instance the bone-marrow should inhibit all lines, while spleen extract, containing only A, B and C, should leave the D-precipitate, present in bone-marrow, liver and lung, intact. The latter extracts should, therefore, give a single line in a medium inhibited with spleen extract.

Using bone-marrow, liver, spleen, lung, adrenal and rabbit plasma 6 groups of diffusion plates, totalling 162, were inhibited by the method (b). (See Methods) All plates in each group were inhibited with one of the extracts. Thus the plates of the first group were inhibited with bone-marrow, those of the second group with liver and so on. In each

TABLE II. Results of Cross-Inhibition Tests. The composition of antigens, as suggested by the analysis illustrated in Fig. 1, is indicated by the letters in the top row and the left column under the respective headings. The precipitation patterns obtained in cross-inhibition and cross-precipitation tests are indicated by the corresponding letters.

Antigens used as inhibitors	Antigens tested against anti-bone-marrow serum: Bone-marrow A,B,C,D	Liver B_1,(C),D	Spleen A,B,C	Lung (B_1),C,D	Adrenal B_1,C	Plasma —
Plasma —	A,B,C,D	B_1,(C),D	A,B,C	(B_1),C,D	B_1,C	—
Liver B_1,(C),D	A,B,C	—	A,B,C	C	C	(±)
Bone-marrow A,B,C,D	—	—	—	—	—	(±)
Spleen A,B,C	D	D	—	D	—	(±)
Lung (B_1),C,D	A,B	—	A,B	—	(B_1)	(±)
Adrenal B_1,C	A,B,(D)	D	A,B	D	—	(±)

group pairs of extracts were tested in all combinations. As expected no precipitates appeared in plates inhibited with bone-marrow extract. As a whole the results confirmed entirely the previous observations (Table II.).

In a special control series normal horse serum was compared to the hyperimmune serum against all antigenic preparations. On no occasion did the normal horse serum give rise to any visible reaction.

Discussion. The appearance of multiple lines has been explained as phenomena of the type of Liesegang rings. However, the selective removal of the lines, one by one by specific inhibition, makes the assumption of the Liesegang phenomena as explanation of the multiplicity of the lines invalid. Therefore every single line has to be considered as a reaction between one particular antigen and its corresponding antibody. Thus, by means of Ouchterlony's gel diffusion method combined with a special inhibition technic it was possible to demonstrate the presence of 4 antigenic bone-marrow components and to examine their distribution in liver, spleen, lung and adrenal. None of the components could be demonstrated in normal rabbit blood indicating that they are probably cellular constituents not appearing in the circulating blood in appreciable concentrations. Different organs seem to share certain common non-humoral antigens. The actual pattern, however, differs from one organ to another.

None of these tissue antigens were bone-marrow specific in the strict sense of the word. However, the complete set of antigens was found only in the bone-marrow. The spleen containing three antigens comes next; an interesting fact with regard to the role played by this organ in hemopoiesis.

It might be assumed that there is a connection between the tissue antigens demonstrated and the serious anti-hemopoietic biological effect of anti-bone-marrow serum described in a previous paper(1). However, in attempts to explain the biological effect of anti-bone-marrow serum it must be borne in mind that there might also exist humoral antigenic substances, intrinsically connected with the hemopoiesis. Such substances, if any, could not be demonstrated by means of the method used, as the serological reactions of humoral antigens were either inhibited or so complex that a differentiation was impossible.

Summary and conclusions. 1. In a previous paper it was described how serious damage to the hemopoiesis could be produced in rabbits by injections of horse antiserum to rabbit bone-marrow. 2. As a basis for the present study it was presumed that the hemopoietic tissue contains several antigenic components, some of which may be connected with function or structure of the bloodforming system. 3. Using a special serological method, four different non-humoral antigenic components were demonstrated in the bone-marrow. None of these components was strictly specific to this organ. They were found also in liver, spleen, lung and adrenal although none of these organs contained the complete set of these antigens. Of the organs examined the spleen came closest to the bone-marrow in respect of antigenic structure. 4. It is suggested that the biological effect of the anti-bone-marrow hyperimmune serum is connected with a reaction between serum anti-

bodies and the antigens described.

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Electron Microscopy of Euglobulin from a Case of Hyperglobulinemia.* (19366)

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The appearance of proteins, not present in normal serum in demonstrable concentrations, has been described for a number of pathologic conditions. Bence Jones protein in myeloma is a well known example. Waldenström(1,2) described a special type of hyperglobulinemia regarded by him as an independent syndrome and designated as "essential" hyperglobulinemia. Pedersen and Waldenström(3) studied the proteins in hyperglobulinemic sera and found as the most remarkable feature a fast sedimenting globulin component the molecular weight of which was estimated at about one million. Electrophoretically this macroglobulin migrated with the $\beta 2$ globulin. Serum showed a very high viscosity and in one case spontaneous congelation at temperatures below +7°C was observed. Waldenström, discussing this phenomenon, tentatively assumes an elongated shape of the globulin molecules and advances also a hypothesis on the viral nature of the macroglobulin.

Since one of us (H.M.) had an opportunity to examine a similar case it seemed of interest to attempt the application of electron microscopy in the study of the pathologic protein in order to obtain some additional information about its properties.

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Case history. A 78-year male admitted to Örebro county hospital September 1947, diagnosed anemia. He had suffered from repeated profuse bleedings from the nose. Examination disclosed a pronounced anemia and a very high E.S.R. (167 mm/1 hr). The former gel reaction was strongly positive on account of which the serum proteins were analyzed. The total protein content was 12%. By electrophoresis a considerable increase in the amount of γ globulin was found. The viscosity was very high, $\frac{\eta_i}{\eta_{io}} = 5.7$ at 37°C, 13.3 at 13°C (J. Waldenström). On storage at +4°C the serum congealed spontaneously. Euglobulin reaction strongly positive. Sedimentation analysis revealed the presence of a macroglobulin component with a sedimentation constant of $s_{20} = 19$ S (Pedersen). X-ray examination did not disclose any skeletal abnormalities. The urine was free of protein. On sternal puncture large numbers of small lymphocytes but only 0.4% plasma cells were found. Differential count of blood white cells showed 50% lymphocytes and no abnormal cells. Axillary and inguinal lymph nodes were moderately enlarged. Biopsy showed diffuse proliferation of lymphatic parenchyma with the follicular structure maintained; the sinus endothelium swollen and proliferating; catarrhal lymphadenitis without signs of a specific process (Behring). The patient showed a hemorrhagic diathesis with epistaxis and ap-