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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-

pearance of petechiae after application of a tourniquet on the arm. Thrombocyte count 70,000; bleeding time 8 minutes; coagulation time 5½ minutes; prothrombin value normal; fibrinogen 0.57%; serum vit. C normal. Anemia remained largely unchanged in spite of repeated blood transfusions and treatment with liver and folic acid. Erythrocyte sedimentation rate and hyperglobulinemia remained likewise unchanged during about 2 years. No additional symptoms indicating myeloma or leukemia were observed during this period. The patient died in his home in July 1949. Autopsy not performed.

Experimental. Serum samples were obtained on 2 occasions and stored in the refrigerator at +4°C. The euglobulin fraction was separated by dilution with 19 volumes of distilled water. A voluminous glassy, gelatinous precipitate was formed, adherent to the walls of the vessel. After centrifugation the supernate was discarded and the precipitate redissolved in the original volume of 0.9% sodium chloride solution. The procedure was repeated three times and the final precipitate redissolved in 1.25 times the original volume of saline. The solution was centrifuged for 30 minutes at 10000 r.p.m. in a Servall angle centrifuge. The inconsiderable amount of sedimentable material was discarded. The slightly opalescent solution was stored in the refrigerator until used. Immediately before use it was recentrifuged as above.

In a series of dilutions with different salt concentrations the rate of formation of a precipitate was studied. As the most suitable for the present purposes a concentration of 0.074% sodium chloride was chosen. At this level of electrolyte content a slight increase in opalescence was noted after a couple of minutes.

Three small test tubes received each 0.1 ml of a 4% osmium tetroxide solution. The macroglobulin solution was rapidly diluted with distilled water to the suitable salt concentration and 0.1 ml samples removed after 0, 2, and 15 minutes and immediately mixed with the osmic acid solutions. After fixation for 15 minutes, drops were mounted on collodion screens. Excess fluid was removed after 5 minutes and the screens were dried under an electric bulb. After drying they were washed repeatedly with distilled water and again dried, shadow-cast with palladium-platinum and examined in an RCA EMU electron microscope.

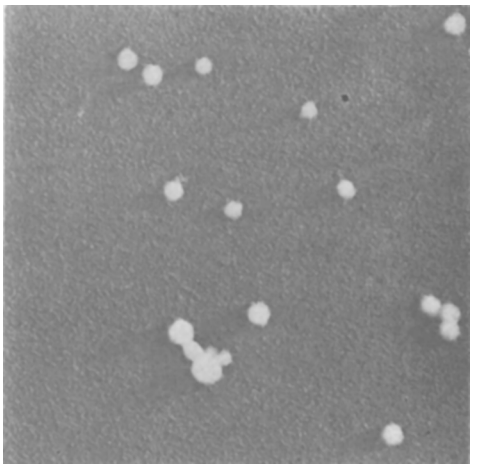

FIG. 1. Preparation of euglobulin obtained by fixation with osmium tetroxide immediately after incitation of precipitation. Shadowcast with palladium-platinum under an angle of 5°. Electron-optic magnification 6000 ×. Final magnification 17000 ×.

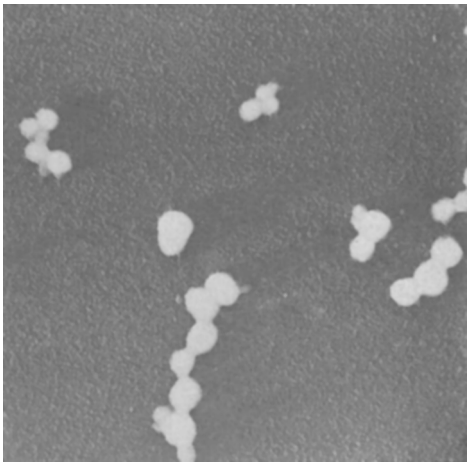

FIG. 2. The same preparation 2 min after incitation of precipitation.

Fig. 1-3 are representative micrographs of the specimens removed after 0, 2, and 15 minutes, respectively. It is of interest to note that the size of the aggregates is rather uniform in each individual specimen but shows a pronounced increase with the time after incitation of precipitation. This fact seems to indicate that fixation with osmic acid inter-

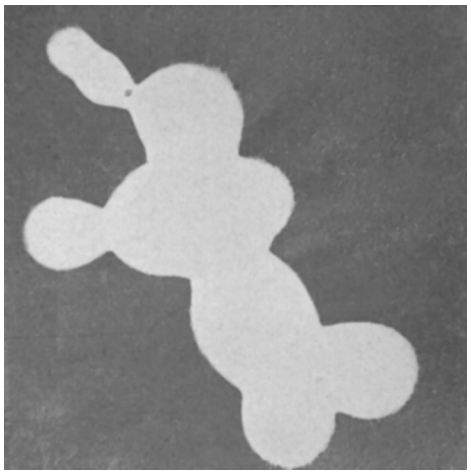

FIG. 3. The same preparation 15 min after incitation of precipitation.

rupts the formation of aggregates or at least slows down the process sufficiently to make a registration of the different stages possible.

The surface relief of the aggregates does not show but their contours suggest a granulated structure. although it is hardly possible to draw any conclusions regarding the size or shape of the individual molecules in the aggregates. However, a scrutiny of the micrographs reveals the presence of other structures besides the aggregates, *viz.*, small practically spherical bodies measuring ca. 120-180 Å. In the 0-minute specimen they are very numerous and not easily distinguishable from a mere coarseness of the carrier film. With the growth of the aggregates they decreased considerably in number and appear, in the 15-minute specimen, scarce and evenly distributed over the membrane. It would seem, therefore, that these structures represent particles present in the specimen and not only a rough surface of the collodion film. The obvious inverse correlation between the size of the aggregates and the number of small particles indicates that the larger structures are formed by aggregation of the latter. The size of the small particles is not incompatible with the assumption that they represent molecules of the macroglobulin.

Summary. A case of anemia combined with hyperglobulinemia is described. In the serum the presence of a macroglobulin with an estimated molecular weight of about one million was demonstrated. Electron micrographs of the partially purified macroglobulin under certain experimental conditions show the presence of spherical particles measuring approximately 120-180 Å.

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The Neuromuscular Mechanism of Alkalotic and Hypocalcemic Tetany.* (19367)

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The motor phenomena which characterize tetany consist of muscle fasciculations and tonic and clonic muscle spasms. Tetany is usually caused by a reduction in the concentration of ionized blood calcium or by an elevation in blood pH. Either of these alterations in the milieu interne apparently diminishes the stability of the nerve cell membrane, which diminution is reflected in an increased electrical excitability, altered afterpotentials and a reduced capacity of accommodation(1,2). At a critical level, iterative spontaneous discharges occur which are propagated along the nerve fiber(1,3). While

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