females regularly cooperate in producing and feeding the young, division of labor among these females exists. Such division of labor involves a variety of modifications of the physiology and behavior of nonsocial ancestors. For queens this is shown by long life, reduction of foraging activities, and continued egg laying, for workers by the relatively short life, frequent failure to mate, and frequent failure of ovarian enlargement, and for both castes, by their cooperative nesting activity. It seems probable that among bees (superfamily Apoidea) there must be a tendency of some sort toward acquisition of these features, hence the noteworthy physiological and behavioral parallelisms which are coordinated to form a functional unit by the individuals of any one colony (*4*).

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21 June 1963

Crystalline Low Molecular Weight γ-Globulin from a Human Urine

Abstract. *A basic protein with a molecular weight near 17,000, related serologically to the normal serum γ_2-globulins, has been isolated in crystalline form from the urine of a patient with multiple myeloma. Proteins of this size along with the Bence-Jones molecules whose molecular weight is about 35,000 can provide subunits for studies of the structure of the serum γ-globulins.*

There are relatively low-molecular weight proteins with serological properties of γ_2-globulins in normal human urine (*1–3*) and in plasma (*3*). Their molecular weights have been reported to be between 10,000 and 40,000 and they have been generally designated as γ_L-globulins (*1*). Recently Berggård and Edelman (*4*) have indicated that these proteins resemble the so-called light (L) chains of normal human 7*S* γ-globulins and that they may be normal counterparts to Bence-Jones proteins.

These γ_L-globulins have been difficult to obtain in sufficient amount and of a degree of purity adequate for physical-chemical characterization. Relatively large amounts have now been obtained from the urine of certain myeloma patients; one of these proteins has been crystallized; the method of preparing this crystalline form and some of its properties are described in this report.

The urine was dialyzed against distilled water and then lyophilized. The proteins recovered were made up into a 5 percent solution in tris-HCl buffer (0.02 ionic strength, *p*H 8.1 ±0.1) and passed over a column of DEAE-cellulose (*5*) previously equilibrated against the same buffer. The γ_L-globulin passed through the column unretarded whereas the more acidic components were retained on the column. When the γ_L-component is present in the urine in minute amounts it may be concentrated first by passing a solution of the urinary proteins in phosphate buffer (*p*H 6 ±0.1, 0.01 ionic strength) over a CM-cellulose (*5*) column equilibrated against this buffer. The column is washed with the same buffer after the addition of the protein until the effluent has an extinction $\left(E^{1\ cm}_{280\ m\mu}\right)$ of less than 0.100. The protein is then sharply eluted by passing a solution of 0.5 to 1.0*M* NaCl over the column. The eluted protein is then dialyzed against tris-HCl buffer (0.02 ionic strength, *p*H 8.1 ±0.1) and then passed over a column of DEAE-cellulose in tris-HCl buffer (0.02 ionic strength, *p*H 8.1 ±0.1). The γ_L-globulin fraction passes unretarded through this column. It is concentrated by lyophilization and a 2- to 5-percent solution was dialyzed against 0.001*M* tris-HCl buffer, *p*H 8. Crystallization usually occurred within a few hours. When crystallization failed to occur the presence of acidic proteins could usually be demonstrated by starch-gel electrophoresis (*6*). These impurities were removed by passage of a 2- to 5-percent solution of the γ_L-globulin in 0.2*M* NaCl at *p*H 5.5 to 7.5 over a column of G-75 Sephadex equilibrated against 0.2*M* NaCl. The γ_L-globulin fraction obtained could then be crystallized at *p*H 8 as described. The crystals have a solubility of between 1 and 2 mg per ml at 1° to 3°C under these conditions. The crystalline γ_L-globulin readily dissolves at *p*H 5 to 6 in 0.1*M* salt and can be recrystallized by dialysis against buffers of low ionic strength at alkaline *p*H. A photomicrograph of the crystals obtained is shown in Fig. 1.

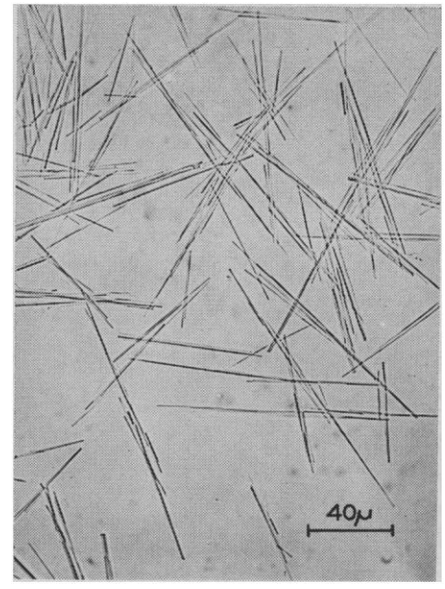

Fig. 1. Twice crystallized γ_L-globulin.

The protein gives a single boundary in the ultracentrifuge and has a sedimentation constant of 1.85*S* in potassium phosphate buffer (*p*H 7.4, 0.2 ionic strength). A velocity sedimentation diagram is shown in Fig. 2. The partial specific volume (0.74) was determined by the method of Drucker (*7*). The molecular weight was determined by the approach to sedimentation equilibrium method (*8*). Average values of 16,900 and 17,700 were obtained at the top and bottom of the cell respectively. These give an average molecular weight of 17,300. The close agreement of the molecular weight determined at the top and bottom of the cell indicate that the γ_L-globulin was essentially homogeneous.

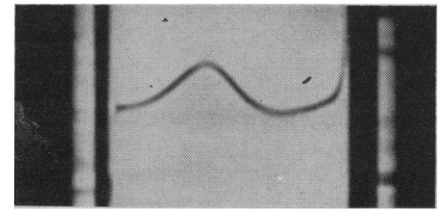

Fig. 2. Sedimentation diagram of crystalline γ_L-globulin after 280 minutes centrifugation at 59,780 rev/min. (The direction of sedimentation is toward the right).

Fig. 3. Descending electrophoretic boundary of crystalline γ_L-globulin after 229 minutes of electrophoresis at a potential gradient of 6.4 v cm^{-1} at 1°C in diethylbarbiturate buffer, 0.1 ionic strength *p*H 8.5. The position of the initial boundary is indicated by the arrow.

The protein migrated as a single component in moving-boundary electrophoresis experiments. A photograph of the descending boundary of an experiment in diethylbarbiturate buffer (0.1 ionic strength, *p*H 8.5) is shown in Fig. 3. The isoelectric point, determined by moving-boundary electrophoresis experiments in buffers (0.1 ionic strength) in which NaCl constituted 80 percent of the salt, was *p*H 8.22. A single component is seen after electrophoresis in starch gel. The protein contained 15.9 percent nitrogen and had an $E^{1\%,\ 1\ cm}_{280\ m\mu}$ value of 10.1.

Precipitin reactions in agar gel by the Ouchterlony (*9*) and microimmunoelectrophoretic (*10*) methods revealed that the γ_L-globulin gave a reaction of partial identity with the normal 7*S* serum γ_2-globulins when reacted with rabbit antisera to γ_2-globulin. It also gave a reaction of partial identity with the classical Bence-Jones protein isolated from the same urine in crystalline form and having a sedimentation constant of 4.5*S*. Both the γ_L-globulin and the Bence-Jones protein react with antibody to the more basic or C-component of papain digests of human 7*S* γ_2-globulin but not with antibody to the acidic or B-component (*11*). These basic and acidic papain-digest components have also been designated as S and F respectively (*12*) to denote their relative electrophoretic mobilities in alkaline buffers.

There may be several proteins of relatively high isoelectric point in the urine of myeloma patients. Multiple components are seen on starch-gel electrophoresis of 7*S* serum proteins from myeloma patients which show single components in moving-boundary electrophoresis experiments at 0.1 ionic strength. A similar number of basic 3.5*S* products are formed by papain digestion of these proteins (*13*). Some relation of these basic 3.5*S* fragments to the γ_L-globulins of urine may exist.

Recent work (*14*) has suggested that the γ_L-globulins may have several serological specificities and the need for the study of these and other of their biological properties on materials that have been more clearly defined in physical-chemical terms is indicated.

Certain myeloma patients may have in their urine and blood serum a series of protein molecules, the 7*S* γ_2-globulins, whose molecular weight is near 160,000, the Bence-Jones proteins near 35,000, and the γ_L components near 17,000. The smaller molecules appear to be subunits of the 7*S* γ-globulins (*15*).

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10 May 1963

Paracrystalline Forms of Fibrinogen

Abstract. *Electron microscope and x-ray diffraction observations on highly ordered tactoids of fibrinogen show that these structures have the same axial period as fibrin. Implications of these results for the analysis of clot formation are discussed.*

The essential step in the vertebrate clotting mechanism is the polymerization of fibrinogen by the proteolytic enzyme thrombin. In order to understand clot formation one must have information about the precise structure of fibrinogen, the conformational changes induced by thrombin, and the architecture of the fibrin clot.

Small-angle x-ray diffraction observations on gels of fibrinogen and fibrin have indicated that these systems have the same axial period (*1*). Therefore, conversion from the soluble monomer to the insoluble clot did not appear to involve large-scale molecular changes in the fibrinogen. Here we describe further studies on paracrystalline structures formed by fibrinogen.

The preparation of needle-shaped "crystals" or tactoids of fibrinogen was reported by Laki (*2*) and Bagdy (*3*). The method we have used is the dialysis of a solution of fibrinogen at neutral *p*H to an ionic strength at which it precipitates. Pentex Fraction 1 Bovine Fibrinogen, purified by ammonium sulfate fractionation or Pentex Purified Fibrinogen ("recrystallized" once) was dissolved in 0.3*M* KCl, 0.05*M* phosphate buffer *p*H 7.0 (protein concentration about 0.4 percent) and dialyzed to a final ionic strength of 0.01 to 0.08. Needle-shaped tactoids result (Fig. 1), which tend to aggregate readily, forming star-shaped clusters or fibrous masses.

Electron-microscope observations of fibrinogen tactoids were made with a Siemens Elmiskop 1 having a double condenser and an acceleration of 80 kv, and a 50-μ objective aperture. The specimens were sprayed with a "Vaponefrin Nebulizer" onto carbon-coated formvar or colloidin films, supported by 150 mesh copper grids. Uranyl acetate or phosphotungstic acid was used as stain.

A highly ordered structure is seen in the electron micrographs. The tactoids, staining well with uranyl acetate, display a regular striation (Fig. 2). Within each period are four fine dark bands and a broader light zone (about 50 Å across). The period is 230 Å ± 5 percent. The broad light zone may be resolved into a doublet. Thin sheet-like structures found in some preparations (see Fig.1) allow high resolution of these features. As yet, staining with phosphotungstic acid has been unsuccessful, and the tactoids appear to break up upon treatment with this agent. Specimens without prior staining show a similar period—probably the result of salt deposition—but a somewhat different intraperiod fine structure (Figs. 3 and 4).

X-ray diffraction photographs were