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*).

H. F. DEUTSCH
Department of Physiological Chemistry, University of Wisconsin, Madison

References and Notes

1. T. Webb, B. Rose, A. H. Sehon, *Can. J. Biochem. Physiol.* **36**, 1167 (1958); E. C. Franklin, *J. Clin. Invest.* **38**, 2159 (1959); J. S. Remington, E. Merler, A. M. Lerner, D. Gitlin, M. Finland, *Nature* **194**, 407 (1962).
2. O. T. Stevenson, *J. Clin. Invest.* **39**, 1192 (1960); I. Berggård, *Clin. Chim. Acta* **6**, 413 (1961).
3. ———, *ibid.*, p. 545.
4. ——— and G. D. Edelman, *Proc. Natl. Acad. Sci. U.S.* **49**, 330 (1963).
5. E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.* **78**, 751 (1956); DEAE, diethylaminoethyl-; CM, carboxymethyl-.
6. O. Smithies, *Biochem. J.* **7**, 585 (1959).
7. C. Drucker, *Trans. Faraday Soc.* **34**, 1214 (1938).
8. W. J. Archibald, *J. Phys. Chem.* **51**, 1204 (1947).
9. J. Oudin, in *Methods in Medical Research*, A. C. Corcoran, Ed. (Year Book Publishers, Chicago, 1952), p. 360.
10. J. J. Scheidegger, *Intern. Arch. Allergy Appl. Immunol.* **7**, 103 (1955).
11. H. F. Deutsch, E. R. Stiehm, J. I. Morton, *J. Biol. Chem.* **236**, 2216 (1961).
12. G. M. Edelman, J. Heremans, M. Heremans, H. Kunkel, *J. Exptl. Med.* **112**, 203 (1960).
13. N. O. Thorpe, M. R. MacKenzie, H. F. Deutsch, *Acta Chem. Scand.*, in press.
14. K. Takatsuki and E. F. Osserman, *Federation Proc.* **22**, 327 (1963).
15. Supported in part by grant C-1786 from the U.S. Public Health Service.

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

taken of wet tactoids packed into thin-walled quartz capillaries. A Philips fine focus sealed tube, nickel-filtered CuKα radiation, and a small-angle camera with slit collimation were used. The small-angle x-ray pattern shows four orders of a 226 ± 5 Å repeat. The first order is weak and may have non-meridional components. This is seen even though the x-ray patterns were taken with slit collimation. The third order is strongest and very sharp. The axial period is thus identical to that of fibrin and to gels of fibrinogen produced by centrifugation (*1*), although the distribution of intensity on the diagram is different.

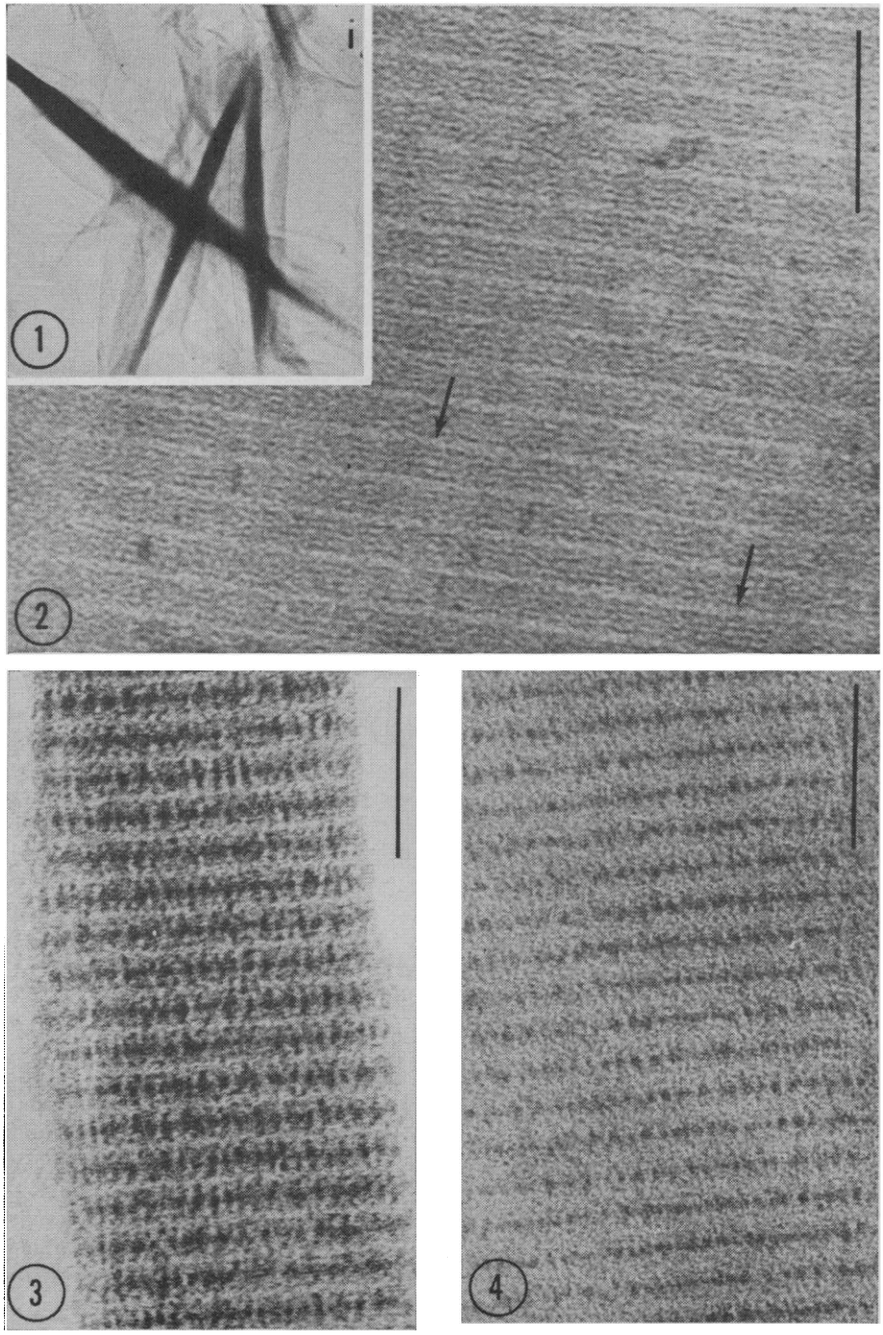

Fig. 1. A low-power electron micrograph of several fibrinogen tactoids. Note the sheet-like structures associated with the paracrystals. Fig. 2. A very thin area stained with uranyl acetate. The arrows point to regions where the intraperiod fine structure can best be seen. A similar preparation is shown unstained in Fig. 4. Fig. 3. An unstained relatively thick tactoid. The period is the same as that observed in the stained preparations but the density distribution is somewhat different. The densities observed here are probably due to the deposition of salts. Note the suggestion of a longitudinal striation, which probably indicates the orientation of the fibrinogen molecules in the paracrystals. Fig. 4. An unstained area of about the same thickness as that shown in Fig. 2. The high electron-density structures seen in Fig. 3 can be recognized in Fig. 4. All magnification bars represent 0.1 μ.

There need be no absolute correspondence between the appearance of paracrystals in the electron microscope, and the electron-density distribution deduced from the x-ray diagrams. The electron microscope yields, in effect, a two-dimensional projected image, whereas the x-ray diffraction method reveals also the transverse location of scattering density. For example, the x-ray pattern of the projected structure in Figs. 2 and 3 would be expected to have a meridional first order. Furthermore, relatively small fluctuations in electron density may be detected by x-ray diffraction, but for adequate contrast in the electron-microscope image, fairly large amounts of heavy metal must be deposited on a thin specimen. Heavy-metal staining therefore may cause significant changes in the electron-density distribution of the structure which would affect intensities on the x-ray diagrams.

The relationship between the fibrinogen gels (*1*) and the tactoids reported here is of considerable interest. We have found that birefringent gels produced by centrifugation of fibrinogen at high ionic strength also contain oriented tactoids. The x-ray diagrams of the gels showed a strong meridional or near-meridional first-order reflection (*1*); however, diagrams from the packed low ionic-strength tactoids have a broad, relatively weak first order. The differences in the x-ray diagrams may be due to structural changes caused by manipulation of the low ionic-strength tactoids, or to actual differences between the tactoids produced by these two methods.

The results thus far indicate that there is an identity in the axial period of fibrin and paracrystalline structures built from fibrinogen. This fact is an argument for the rigidity of the monomeric packing unit in the fibrinogen-fibrin system. There may, however, be more than one stable aggregate which the fibrinogen packing unit can build, as shown by differences in the intensity distribution on the x-ray diagrams. The aggregates would have the same axial repeat but differ in lateral contacts. The favored structure would be determined

by specific environmental conditions. Thus, in the conversion of fibrinogen to fibrin, it seems likely that the loss of highly charged fibrinopeptides (*4*) alters the interaction properties of the building units without causing profound changes in internal structure.

Preliminary observations indicate that fibrin stained with uranyl acetate (*5*) shows a density distribution in the electron microscope similar to that reported here for stained fibrinogen tactoids (*6*).

CAROLYN COHEN
Children's Cancer Research Foundation and Harvard Medical School
JEAN-PAUL REVEL
Harvard Medical School
JOSEPH KUCERA
Children's Cancer Research Foundation Boston, Massachusetts

References and Notes

1. L. Stryer, C. Cohen, R. Langridge, *Nature* **197**, 793 (1963).
2. K. Laki, *Studies Inst. Med. Chem., Univ. Szeged* (1942).
3. D. Bagdy, *Hung. Acta Physiol.* (1948 p. 18.
4. F. R. Bethelheim and K. Bailey, *Biochim. Biophys. Acta* **9**, 578 (1962); K. Laki, J. A. Gladner, J. E. Falk, *Nature* **187**, 758 (1960); B. Blomback and J. Sjoquist, *Acta Chem. Scand.* **14**, 493 (1960).
5. After discussion with Drs. C. Hall and H. Slayter.
6. Supported in part by U.S. Public Health Service grants A-2633 (C.C.) and SG-13,979 (J-P.R.) from the National Institutes of Health. We thank Professor R. S. Bear, and Drs. D. L. D. Caspar and R. Langridge for useful discussions.

22 April 1963

Elastic Membrane: Effect of Increasing Tension on the Absorptive Capacity

Arterial walls contain a large proportion of elastin, a tissue polymer with long-range elasticity similar to that of rubber. In arterial hypertension the arterial walls are under constantly elevated tension. In atherosclerosis, a disease considered to be enhanced by arterial hypertension (*1*), adsorption of blood lipids to and into the arterial wall is thought to be an important pathogenic factor (*2*). Moreover, it has been shown, in electron-microscopic studies, that one of the earliest steps in the pathogenesis of experimental atherosclerosis is the inhibition of lipid by the internal elastic lamina (*3*). Therefore it seems reasonable that enhancing of adsorptive capacity of the internal elastic lamina may be one of the means by which hypertension predisposes to atherosclerosis.

Before exploring this idea, it would be desirable to know whether increasing the tension of elastic substances in general would increase their ability to adsorb molecules from solution. Experiments were conducted with models of heavy latex rubber dam (*4*) to simulate the elastic adsorbent membrane; Evans blue was used for the substance to be adsorbed.

A 0.3 percent solution of Evans blue in McIlvaine's buffer with *p*H of 4.8 was prepared. Pyrex test tubes with lips having an outside diameter of 2.8 cm and with their closed ends cut out were used as receptacles for the dye solution. Circles with diameters of 2.2, 2.4, 2.6, and 2.8 cm were traced in black on sheets of heavy latex-rubber dam with a ball-point pen. Each sheet, with the drawn circle on the outer surface, was stretched over the lipped end of the test tube so that the inked line of the circle coincided with the outer border of the lip, and was fixed in position by rubber bands wound tightly around the dam and the tube just below the lip. After the dam had been stretched and fixed to the tube, the traced circle was always 2.8 cm in diameter. Therefore the ratios of the stretched to the unstretched diameters of the various sized circles were: 2.8/2.8, 2.8/2.6, 2.8/2.4, and 2.8/2.2. Two milliliters of dye solution was added to each tube covered by dam and left there for 6 hours. The inside of the tube and the inner latex surface were rinsed thoroughly with distilled water and 3 ml of distilled water was left in each tube for 15 minutes. After the water was completely drained from the tube, the dye adherent to the dam was eluted by leaving 3 ml of 1 percent aqueous solution of sodium carbonate in the tube for exactly 1 hour. The eluate was then poured into a cuvette and its optical density read at 580 mμ. The optical density was converted to micrograms of dye by a regression equation obtained by plotting the optical densities of several dye solutions of known and varying concentration. The data were expressed as micrograms of dye per square centimeter of stretched membrane for each "area-stretch" ratio (the area of the stretched circle on the membrane divided by the area of circle before stretching).

There was significant increase in adsorptive capacity ($p < .01$) by the stretched membrane with increasing tension up to an area-stretch ratio of 1.36 (Table 1). Also, up to this point the increase was found by semilogarithmic plot to be exponential ($y = 3.67e^{.577}$). Beyond a stretch ratio of 1.36 increase in adsorptive capacity of the stretched membrane ceased. Although the shape of the experimental curve plotted from the data in the table seems to suggest an equilibrium, it is not clear whether this phenomenon involves equilibria or rates of adsorption and diffusion in the membrane.

Table 1. Effect of increasing tension on the capacity of latex rubber dam to adsorb Evans blue.

Area stretch ratio	Trials (No.)	Mean quantity of dye adsorbed by stretched dam (μg/cm^2)	Standard error
1.00	14	5.22	.10
1.16	17	6.42	.08
1.36	18	8.32	.05
1.62	16	8.58	.14

The reason for the increase of adsorptive capacity of the rubber membrane with increasing tension may possibly be found in the explanation of the phenomenon of elasticity of rubber-like substances (*5*).

JOHN H. LUNSETH
Veterans Administration Hospital, Fargo, North Dakota

References and Notes

1. I. H. Page, *Circulation* **10**, 1 (1954).
2. G. L. Duff and G. C. McMillan, *Am. J. Med.* **11**, 9 (1951); S. L. Wilens, *Science* **114**, 389 (1951).
3. J. Parker, *Am. J. Pathol.* **36**, 19 (1960).
4. Obtained from Hygienic Dental Manufacturing Company, Akron 10, Ohio.
5. P. J. Flory, *Principles of Polymer Chemistry* (Cornell University Press, Ithaca, 1953); M. L. Huggins, *Physical Chemistry of High Polymers* (Wiley, New York, 1958).

29 April 1963

Renal Tubular Localization of Chlormerodrin Labeled with Mercury-203 by Autoradiography

Abstract. *An autoradiographic technique has been developed for determining the intrarenal distribution of therapeutic doses of chlormerodrin labeled with mercury-203. Highest concentrations of mercury were detected in the straight portion of the proximal tubule in the rat and in the convoluted portion of the proximal tubule in the dog.*

It is generally accepted that mercurial diuretics act by inhibiting renal reabsorption of sodium and water, although the mechanisms of action and tubular site at which this effect occurs remain controversial. Conventional studies of renal function and histochemical methods have failed to resolve these questions (*1, 2*). If it is assumed