

tacle, soil erosion. Once scarcely associated with fish production, today its prevention is the hope of thousands of fishermen; in fact, the prevention of soil erosion could be their salvation.

In concluding these remarks, I wish to emphasize the role played today by applied ecology in sport and commercial fisheries. We meet it on every hand. The primary problems in fresh-water fisheries today concern interrelations between species, and population balance. In the ocean, interest centers on the effect environment exerts on population numbers. If, through careful study, the uncertainties can be eliminated, at least in large part, then the road is clear to measure the influence of the fishery on the maintenance of a high economic level of production.

All this investigational subject matter goes by a variety of names, but I can think of none more appropriate than *applied ecology*. The time has come to crystallize our thinking in regard to these particular categories of knowledge, to encourage a uniform terminology, and to utilize an old and well-established

concept—namely, the application of science to human affairs.

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Technical Papers

Ultracentrifugal Studies of γ -Globulins Prepared by Electrophoresis-Convection

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The development in recent years of chemical and physical methods for the fractionation of human and animal blood plasma and serum has made possible the separation of certain components in a state of high purity and others in varying degrees of purity. The physical chemical characterization of the various plasma proteins is important from both a theoretical and a practical point of view. First, it yields information as to the molecular characteristics of these materials, thus giving insight into their reactions and physiological activity and an understanding of those properties that must be employed in the further development of fractionation procedures. It is also indispensable in following the fractionation and assuring uniformity of the products. Electrophoretic analysis has proved well suited as a criterion of purity, and ultracentrifuge studies have been most useful in detecting subtle changes in protein fractions both during preparation and during storage.

Of particular interest to the immunologist and immunochemist is γ -globulin, since this electrophoretic

component of serum contains antibodies against a variety of pathogenic agents and other antigens. A number of ultracentrifugal studies of human γ -globulin, prepared by the low-temperature ethanol fractionation procedure, have been reported. In 1944 Williams and his co-workers (1) reported studies on 35 preparations of Fraction II of plasma. Electrophoretically these materials analyzed about 87%, and in some cases to 95%, γ -globulin, the main impurities being β -globulin and albumin. Ultracentrifugal studies of these preparations revealed about 5% slow-moving component with sedimentation constant of $S=4-5$ Svedberg units, 75% normal component with $S=7$, and 20% fast-moving component with $S=8-18$. The slow-moving component presumably represented albumin, at least in part. The amount of fast-moving component, in large part γ -globulin, was quite uniform in nearly all preparations. Since that time Deutsch and his co-workers (2-4) have studied a number of γ -globulin fractions with different electrophoretic mobilities and purities of 95-99% as judged by electrophoresis. In the ultracentrifuge at least 3 components with average sedimentation constants of $S=7$, $S=8-12$, and $S=18-20$ are discernible. It has been found that as the mobility of these fractions increases from -0.97×10^{-5} cm² sec⁻¹ v⁻¹ to -2.63×10^{-5} at pH 8.6, the percentage of $S=7$ component decreases from about 89 to 57%, with a corresponding increase in the heavier components. Nichol and Deutsch (5) have reported that 93% of one preparation of γ -globulin sedimented with $S=7$.

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TABLE 1
CHARACTERIZATION OF γ -GLOBULIN FRACTIONS

Source	Electrophoretic analysis		Ultracentrifugal analysis
	Percentage γ -globulin	Mean mobility $-10^8 \times \mu$ of the γ -globulin	
(a) Prepared by electrophoresis-convection			
Normal human serum from single donor stored 10 months at 3° C	98	1.32	99% $S = 6$ 1% $S = 18$
Normal human serum from single donor 2 days old	> 98	1.25	99% $S = 6$ 1% $S = 18$
High-titer, human Rh antiserum 1 week old	> 98	0.92	99% $S = 6$ 1% $S = 18$
Pooled human serum from 150 donors 4 days old	96	1.38	95% $S = 6$ 2.5% $S = 8$ 2.5% $S = 18$
Bovine serum from single slaughterhouse steer 4 days after collection	95	1.28	99% $S = 6$ 1% $S = 18$
Bovine serum from single slaughterhouse steer 1 week after collection	82	1.48	99% $S = 6$ 1% $S = 18$
Pooled, high-titer, anti-RBG rabbit serum stored several months in deep freeze	> 98	1.18	> 99% $S = 6$
(b) Prepared by ethanol fractionation			
Armour's Fraction II	> 98	1.47	93% $S = 6$ 7% $S = 8$
Fraction G obtained from Armour's Fraction II	> 98	2.25	75% $S = 6.5$ 25% $S = 8-12$

Recently Smith and D. M. Brown (6) have reported studies of the sedimentation behavior of bovine γ_1 - and γ_2 -globulin, prepared by ethanol fractionation, and two fractions of γ -globulin separated by electrophoresis-convection from Armour's Fraction II of bovine plasma, also prepared by ethanol fractionation. In each instance, the main component, 80-90% of the total sedimenting material, had a sedimentation constant of about $S=7$, and was always associated with a second component of about $S=10$. These workers also found that equine γ - and T-globulins containing high tetanus antitoxic activity likewise contain components that sediment at about 7 S and 10 S . Components with larger sedimentation constants were not detected in these preparations.

On the basis of preliminary studies by Savat (7), which indicate that electrophoretically separated γ -globulin of various species shows little or none of the $S=8-12$ component, Cohn, Deutsch, and Wetter (2) conclude that there is an uncertainty as to whether this component of γ -globulin is a new component formed during the chemical fractionation procedure.

In view of this it appeared desirable to carry out ultracentrifugal studies on γ -globulins separated from the sera of various species by electrophoresis-convection. This method of fractionation of proteins in solution is a mild physical one that achieves separation of the components of a protein mixture by making use of small differences in their mobilities and isoelectric points. The details of construction and operation of the electrophoresis-convection apparatus and its application to the fractionation of the serum proteins have been described previously (8).

γ -Globulin was separated from normal human and bovine sera by fractionation at pH 7.0. Fractions of γ -globulin were obtained from a high-titer, anti-Rh human serum and pooled, high-titer, anti-RBG rabbit serum³ by fractionation at pH 8.1 and 7.5, respectively. Fractionations were carried out in phosphate buffer, ionic strength 0.1, for 48 hr at field strengths of 1.5-2 v/cm. After removal from the electrophoresis-convection apparatus, solutions of the normal human and bovine γ -globulins were dialyzed against Na_2HPO_4 solutions, ionic strength 0.05, for 24 hr at 3° C, and then dried by lyophilization. Samples for electrophoretic analysis were dissolved in distilled water and equilibrated against buffer at 3° C. The solutions of the γ -globulin fractions from the anti-Rh serum and the rabbit antiserum were concentrated by pervaporation. Portions were then equilibrated against either buffer or saline solutions at 3° C.

Electrophoretic experiments were carried out on 0.8-1% protein solutions in barbital buffer, pH 8.6 and ionic strength 0.1, at a field strength of 8 v/cm for 2 hr. The results of the electrophoretic analyses are presented in Table 1. The preparations analyzed from 82% to more than 98% γ -globulin, the principal impurity being β -globulin.

The ultracentrifuge used in these studies is a synchronous-motor, direct-drive instrument designed and built in the Chemistry Department of this institute. A detailed description of it will appear elsewhere. Runs were performed at 53,160 rpm at 21°-22° C on 0.8-1% protein solutions. Sedimentation was followed by the usual Philpot-Schlieren optical method. The sedimentation constants reported in this paper are those determined in the particular solutions studied, and are not values extrapolated to zero concentration. The human and bovine γ -globulins were studied in barbital buffer, pH 8.7 and ionic strength 0.1; and the rabbit γ -globulin fraction in 0.15 M NaCl. Several representative sedimentation patterns are shown in Figs. 1, 2, and 3.

The electrophoretic and sedimentation patterns of a γ -globulin preparation from normal human serum from a single donor are shown in Fig. 1. In the case of the γ -globulin preparations from human sera obtained from single donors, about 99% of the material sedimented as a single component, with a sedimenta-

³ Serum of rabbits immunized against bovine γ -globulin-*p*-azophenylarsonic acid. *R* refers to the azophenylarsonic acid and *BG* to the bovine γ -globulin. This serum and the anti-bovine serum albumin rabbit serum used in this study were kindly supplied by Dan Campbell.

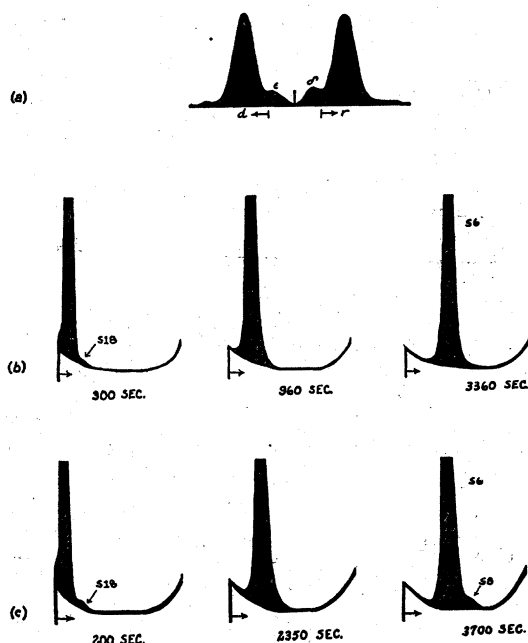


FIG. 1. (a) Electrophoretic pattern of γ -globulin of normal human serum, individual donor; (b) sedimentation patterns of γ -globulin of normal human serum, individual donor; (c) sedimentation patterns of γ -globulin of pooled, normal human serum, about 150 donors.

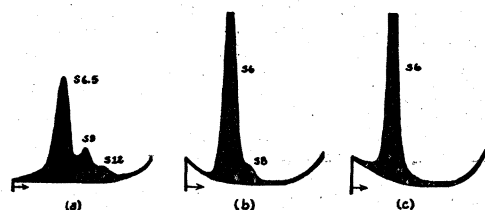


FIG. 2. Sedimentation patterns of (a) Fraction G of Armour's Fraction II, Sample A, 3,100 sec; (b) Armour's Fraction II, Sample B, 3,180 sec; (c) γ -globulin separated from normal bovine serum, individual slaughterhouse steer, by electrophoresis-convection.

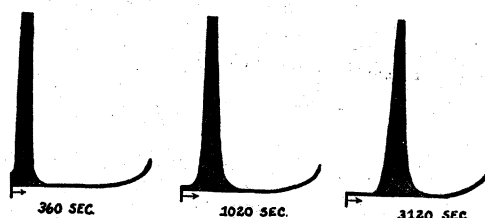


FIG. 3. Sedimentation patterns of a fraction of γ -globulin separated from pooled, high-titer, anti-RBG rabbit serum by electrophoresis-convection.

tion constant of about $S=6$. The trace component had a sedimentation constant of about $S=18$. In the case of the γ -globulin of the pooled normal human serum, 95% sedimented as a single component with $S=6$, 2.5% as a component with $S=8$, and 2.5% as a component of $S=18$.

The bovine γ -globulins prepared by electrophoresis-

convection analyzed greater than 99% of a single component with $S=6$, the trace component having a sedimentation constant of $S=18$. As control experiments, a sample of Armour's Fraction II of bovine plasma, prepared by ethanol fractionation, and Fraction G of bovine γ -globulin were studied. The Armour's Fraction II analyzed greater than 98% γ -globulin, with mean mobility -1.47×10^{-5} at pH 8.7. Fraction G, with mean mobility of -2.25×10^{-5} , had been previously separated by electrophoresis-convection from another sample of Armour's Fraction II (8), and was one of the fractions studied by Smith and Brown (6). Ultracentrifugal studies of Armour's Fraction II and Fraction G were carried out in barbital buffer and 0.1 M NaCl, pH 6.5, respectively. The Armour preparation analyzed 93% of $S=6$ component and 7% of $S=8$ component; Fraction G, 75% of $S=6.5$ and 25% of $S=8-12$. Sedimentation patterns of Armour's Fraction II, Fraction G, and γ -globulin prepared from normal bovine serum by electrophoresis-convection are compared in Fig. 2.

To within the limitations of the resolving power of the optical system, the fraction of γ -globulin from high-titer, anti-RBG rabbit serum sedimented as a single component with sedimentation constant of $S=6$. This result is particularly interesting in that this fraction was 50% precipitable by the R-antigen. Thus, the anti-R bodies sediment at the same rate as normal γ -globulin. Campbell and co-workers (9) have found a light-scattering molecular weight of $158,000 \pm 10,000$ for purified anti-R body prepared by dissociation of specific precipitates. This is the accepted value for normal rabbit γ -globulin. Several samples of γ -globulin prepared by $(\text{NH}_4)_2\text{SO}_4$ fractionation of pooled, high-titer antiserum albumin rabbit serum were also studied. These materials were about 20% precipitable by bovine serum albumin. Ultracentrifugal analyses of these materials, carried out in phosphate buffer, pH 7.6 and ionic strength 0.1, showed about 99% of one component, with a sedimentation constant of about $S=6$ and about 1% of a component with $S=18$. Nichol and Deutsch (5) have studied rabbit γ -globulin prepared by ethanol fractionation. Ninety-five per cent of their preparation sedimented as a component with sedimentation constant of $S=7$.

The results presented here are in contrast to the results of sedimentation studies on γ -globulins prepared by ethanol fractionation. Except for the case of the γ -globulin derived from the pooled human serum, which contained about 5% of material with $S=8-20$, an amount much smaller than is usually found with ethanol fractionated samples, all the γ -globulins prepared by fractionation by electrophoresis-convection were about 99% one component. We have thus shown that this latter method of fractionation does not produce components with $S=8-20$, and have eliminated Seitz filtering, lyophilization, pervaporation, and prolonged storage of the sera either at 3°C or in the deep freeze as causes of their appearance. Although it seems likely that the $S=8-20$ components

of γ -globulins prepared by ethanol fractionation are new components formed either during the fractionation procedure itself or during manipulation of the fractions, the possibility cannot be excluded on the basis of our results that the $S=8-20$ components either appear in connection with the pooling of the individual sera or are present in some, although not all, individual sera.

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Unusual Fracture Traverse in Fluorite

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For the past several years the microscope technique fractography has been studied in the author's laboratory, particularly under sponsorship of the Office of Naval Research, and, in the course of investigating the fractographic technique as a possible tool in mineralogy (1), some interesting and informative patterns were observed. One of these, for fluorite, warrants particular mention.

In Fig. 1 a cleavage traverse appears that is typical of patterns for good crystalline specimens of fluorite. This particular sample was a single crystal provided by N. W. Buerger, of the U. S. Naval Postgraduate School in Annapolis, Md. Cleavage, as is customary, has followed octahedral planes to provide the photographed facet, and an intersecting cleavage profile crosses the field. Instead of fracturing on a single plane, however, the separation has visibly progressed along a succession of parallel lamellae, producing a tearline structure of rather regular features. The angularity of the markings suggests a relationship of the tearline phenomenon to crystal structure, and close observation of the fractograph will disclose a fine structure both as superficial striae and as a minute stepwise progression of the tearlines. These suggest a subtle imperfection structure of regular type, such as that postulated by the micellar theory (2), but the pattern is otherwise conventional.

In Fig. 2, however, an elaborate structure is displayed, one that does not submit readily to conventional theories for crystal structure and fractological behavior of the solid state. The specimen is polycrystalline fluorite from Ferry County in the state of Washington, submitted by Grant M. Valentine, of the Department of Conservation and Development. The



FIG. 1. Fractograph of octahedral cleavage in fluorite, revealing crystallographic features and suggesting a lamellated structure of the crystal. ($\times 175$.)

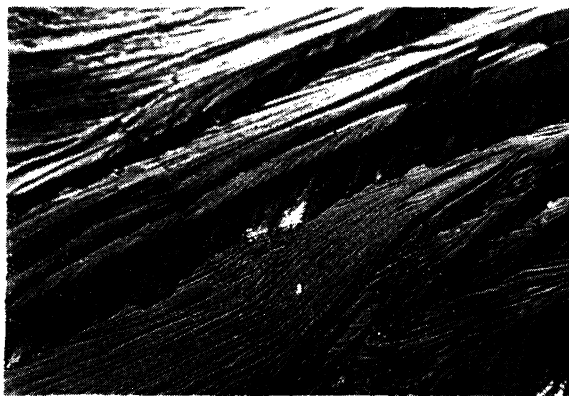


FIG. 2. Unusual fracture traverse in a fluorite crystal, showing an elaborate pattern of unsolved origin. ($\times 237$.)

field of the fractograph lies within the boundaries of a single grain. Accordingly, because the entire field has a common molecular orientation, the pattern seems clearly to indicate either (a) growth imperfection of the lineage or dendrite type, or (b) complex stress-strain relationships whose cause is unknown.

If it is a matter of growth imperfection, the over-all molecular structure can be understood to have achieved an approximately uniform orientation at the time of the original growth of the crystal; but throughout that growth there became distributed the observed elaborate pattern of imperfection. The micellar theory (3) accounts for such patterns on the basis of a deposition of molecular clusters, or micelles, from the liquid state. The molecular structures of the individual micelles maintain the approximate orientation common to the field, thereby constituting the defined monocrystal, but a displacement is suffered among the micelles themselves. Fracture, influenced by paths of minimum cohesion, tends strongly to proceed along the intermicellar and interlineage boundaries, thereby depicting the imperfection architecture, of which the fractograph in Fig. 2 may be an excellent example.

If the pattern is not directly referent to historical features of crystal formation, it must then express