# STUDIES ON ISOLATED CELL COMPONENTS

## XI. RAT BLOOD PROTEINS

N. G. ANDERSON, R. E. CANNING, M. L. ANDERSON[1] and R. H. SHELLHAMER[2]

*Biology Division, Oak Ridge National Laboratory,[3] Oak Ridge, Tenn., and Department of Anatomy, University of Indiana, U.S.A.*

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During the course of a series of studies on tissue proteins of various rat organs, it became necessary to identify individual plasma proteins that may be present in organ extracts either as contaminants or as cellular products. Such identification requires both biophysical and serological data on as many serum proteins as possible and the use of specific antisera. This in turn necessitates the preparation of purified protein fractions. Since insufficient information is available for such a fractionation, and since large differences exist in the reported composition of rat serum and in the nomenclature of the electrophoretically observed components or peaks, we found it necessary to begin with a study of whole serum in the Tiselius electrophoresis apparatus and in the ultracentrifuge. In this paper, the results of these orienting studies are presented, together with data on purified rat serum albumin and $\gamma$-globulin. In subsequent papers, additional studies on the purification and properties of other serum proteins will be presented.

Electrophoretic studies have been made on rat serum and plasma proteins by investigators using the Tiselius apparatus [2, 3, 5–8, 11–13, 15, 19, 22, 23, 25, 26, 31–33, 36, 37, 41, 42], filter paper electrophoresis [4, 14, 16, 18, 20, 38, 45], or starch zone electrophoresis [9, 10]. Considerable variation in the ratios of the various protein fractions has been reported, and the nomenclature of the components or peaks has not been consistent. Furthermore, the so-called albumin peak of the early literature has been shown to be double under certain conditions, whereas precipitation studies have suggested that it may contain three components [33]. On paper, a large $\alpha$ peak is often observed that contains the highest concentration of protein-associated sulfate in serum [38]. Few ultracentrifuge studies have been reported [33, 41].

[1] Consultant, under Subcontract No. 780.
[2] Consultant, under Subcontract No. 1183.
[3] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

Keltz and Mehl [24] purified rat serum albumin from Cohn's fraction V. Electrophoretic studies indicated homogeneity at pH 8.5 but not at lower pH's. No ultracentrifuge studies were reported. Goldstein and Anderson [17] prepared $\gamma$-globulin using an adaptation of Cohn's method *X* and found this to be relatively pure by paper electrophoresis at one pH [27].

## METHODS

*Preparation of serum.*—Male Sprague-Dawley rats (200–400 g) were stunned and exsanguinated by drawing the neck across a sharp blade. Blood from each rat was collected in a beaker containing 0.5 ml of 0.1 *M* sodium ethylenediamine tetraacetate at pH 7.5. The beakers were chilled and the blood from 4–40 animals was pooled. After centrifugation in the cold to remove cellular elements, the plasma was collected, calcium chloride added, and the fibrin removed by stirring with a glass spiral. The serum was then dialyzed against the appropriate buffers or stored by freezing at −20°C.

*Electrophoretic analysis.*—Most electrophoretic studies were made in the Spinco Model H Tiselius apparatus at 0.90°C in 11-ml cells with quartz center sections. Since the effect of temperature on pH varies with different buffers, all pH measurements as well as conductivity measurements were made at bath temperature with a Beckman Model G pH meter and the procedure outlined in Beckman Bulletin 95-B. Unless otherwise specified, the 0.1 ionic strength buffers of Miller and Golder [28], which contain 0.08 ionic strength NaCl, were used. Purification of fractions was occasionally followed with a Model 38 Perkin-Elmer Tiselius apparatus with 2-ml open cells. Paper electrophoresis analyses were routinely done with the Spinco Model R Durrum type apparatus with pH 8.6, 0.075 ionic strength buffers, except as indicated. The prescribed staining and drying procedures outlined in Spinco Manual OIR-1 were followed and the strips scanned with the Analytrol Model RA recorder-integrator.

*Analytical ultracentrifugation.*—The Spinco Model E analytical ultracentrifuge used was equipped with a phaseplate schlieren diaphragm [40]. A rotor temperature indicator and control unit was used, which permits measurement (± 0.02°C) and continuous automatic control of rotor temperature during operation to within 0.1°C and all values given are corrected to 20° in water. Kodak Metallographic plates were used for photographing patterns. Standard 12-mm 4° cells were employed. The sedimentation rates at zero concentration were computed from the regressions, calculated by the least squares method [30].

*Designation of proteins.*—The present designation of serum proteins as albumin, $\alpha$- (with subscripts), $\beta$-, and $\gamma$-globulins is unsatisfactory for general application to sera other than human. As is evident from these and further studies, several proteins in rat serum cannot be conveniently designated under the old $\alpha,\beta$ system. For example, in many sera, additional components not

apparent in biophysical studies have been obtained serologically [34]. A new method for designating proteins in mixtures will therefore be suggested.

Since the electrophoretic mobility of a protein varies with pH, ionic strength, temperature, and ionic composition, it is not easy to use mobility data to specify a protein. For the present series of studies and a parallel series of investigations on tissue proteins, however, a standard unit, $T$, is proposed that is equal to the mobility in $cm \times 10^5$/volt/cm/sec. under conditions specified as follows:

$$\begin{matrix}\text{buffer} \\ \text{temperature}\end{matrix} \; T \; \begin{matrix}\text{ionic strength} \\ \text{pH}\end{matrix}$$

A protein such as rat serum albumin with a mobility of $-5.2$ $cm^{-5}$/volt/cm/sec. in a phosphate buffer, 0.1 ionic strength, at 0.9°C, at pH 7.5, would be indicated as:

$$\begin{matrix}\text{phosphate} \\ 0.9\end{matrix} \; T \; \begin{matrix}0.1 \\ 7.5\end{matrix} \; -5.2$$

For most proteins, the value of $T$ would lie between $+10$ and $-10$. In the present series, the following simplifications are introduced. If the temperature is not indicated, it is assumed to be 0.9°C; if the buffer is not indicated, it is assumed to be a Miller-Golder buffer containing NaCl, and if the ionic strength is not indicated, it is assumed to be 0.1. The sign after the mobility symbol, $T$, is ordinarily a minus indicating that the protein moves toward the anode. It is replaced by a plus when the protein moves toward the cathode. In this simplified version, the mobility of the protein given above is simply $T_{7.5} - 5.2$. Quite obviously a protein may be designated by a variety of $T$ values depending on pH. It appears useful to employ the pH values of 5.0, 7.5, and 8.6 whenever practical to facilitate comparisons. Sedimentation constants are indicated in the usual manner [39]. The isoelectric point in Miller-Golder buffers, 0.1 ionic strength, is indicated by the symbol $I$. If other buffers are used, subscripts similar to those used in mobility designations are used. Rat serum albumin may be designated according to this system as follows:

$$T_{7.5} - 5.2, \; S_{20,w} \; 4.7, \; I \; 4.6.$$

This will appear unduly complicated until it is realized that what appears to be the albumin peak in rat serum, observed electrophoretically at pH 8, is actually at least five different proteins with different sedimentation constants and isoelectric points. Only one of these appears to have all the properties listed for serum albumin.

The advantage of this system of designating proteins or protein fractions is that considerable information is included in the designation, without implying that the preparation is one single pure substance. When blood or tissue proteins described in this manner have been found to have specific functions or enzymic activities, the designation will be replaced by a specific name. Or, if a protein is purified until it becomes homogeneous by all criteria available, then even though its function may not be known, it may be assigned a name, number, or letter.

If, instead, letters or numbers are attached to complex fractions, considerable confusion arises. Almost without exception, simple lettering or numbering sequences applied to biological systems, whether in genetics, hematology, or biochemistry, have had an initial appealing simplicity and have ended in great complexity. Certainly the simple $\alpha, \beta, \gamma$-system of designating blood proteins breaks down completely when applied to lower vertebrates. The system proposed here, although complex initially, is capable, in principle, of including as many protein species as can be detected by the biophysical methods here used. The practice of using sedimentation rates for designating serum macroglobulins ($S_{20}$ 20 components) and lipoproteins ($S_f$ components of Gofman) is already widespread.

## RESULTS

*Electrophoresis of whole serum.*—For electrophoretic studies, 6 ml of rat serum was diluted to 21 ml with the appropriate buffer and dialyzed in the cold for 48 hours. The results obtained over the range from pH 3.6–9.1 are shown in Fig. 1. The magnification of the electrophoretic patterns has been adjusted so that the mobilities of all components may be read off the same abscissa. Magnification of the patterns on the left (cathode) limb were calculated by using the conductivity of the protein solution, whereas mobilities in the right limb (anode) were calculated by using the buffer conductivity.

It is strikingly evident that the "albumin" peak seen at pH 8.0 is composed of at least three components that separate when the pH is lowered to approximately 5.0. These components are designated as follows: (1) $T_{5.1} - 2.9$, $I$ 4.2; (2) $T_{5.0} - 2.3$, $I$ 4.4; and (3) $T_{5.0} - 1.4$, $S^{\circ}_{20,w} - 4.4$, $I$ 4.6.

The first two are not homogeneous but contain several proteins easily distinguished ultracentrifugally.

The area under the electrophoretic patterns was divided into Gaussian curves as far as possible, by the method of Hoxter *et al.* [21] (Table I). Material that appeared to be in the "albumin" peak at pH 8–9, or migrated

faster than the albumin at lower pH's, is totaled with the albumin as the "albumin group". It varies in amount from 56 to 72 per cent depending on the pH. When comparison is made with the values previously reported for rat serum albumin (Table II), it appears that in many instances, all the com-

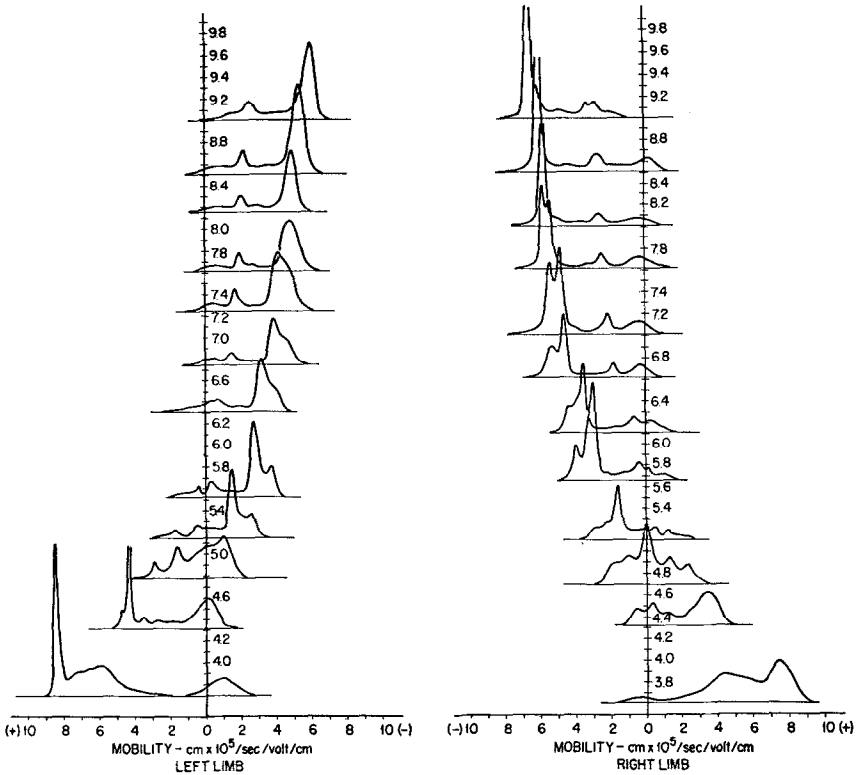


Fig. 1.—Electrophoretic patterns of rat serum at various pH values in 0.1 ionic strength buffers [28]. Magnifications of the various patterns have been adjusted so that the mobilities indicated along the abscissa apply to all diagrams. Mobilities in the left limb were calculated by using the conductivity of the protein solution, whereas mobilities in the right limb were based on the buffer conductivity. Conductivity and pH measurements were made at 0.9°C.

ponents in the albumin group have been treated as being serum albumin. It is of interest that in previous work (Table II), the percentage of serum albumin reported has varied from 44 to 76 per cent when free electrophoresis was used, and from 31 to 89 per cent when paper electrophoresis was used. Although strain differences may well exist (note for instance the markedly different pattern seen by Moore [31] in the cotton rat), the results are more likely caused by variations in the amount of albumin group included in the so-called $\alpha$ fractions.

The best estimate of the amount of serum albumin from the present studies is 36 per cent, or approximately half the average value previously obtained (Table II). The material migrating ahead of the albumin at pH 7.6 and below accounts for approximately 27 per cent of the serum proteins. The faster

TABLE I. *Summary of electrophoretic studies on rat serum.*

| pH | *I* – 4.2 | *I* – 4.4 | Albumin | Albumin group | Post-alb. peak | α Zone | β Zone | γ Zone | No. of expts. |
|---|---|---|---|---|---|---|---|---|---|
| 9.1 | — | — | — | 56.4 | 11.3 | 13.1 | 9.5 8.0 | 7.2 | 2 |
| 8.6 | — | — | — | 64.4 | — | 12.5 | 10.6 | 12.1 | 1 |
| 8.1 | | | — | 64.1 | — | 11.3 | 10.8 | 13.5 | 2 |
| 7.6 | 32.9 | | 33.9 | 71.0 | — | 9.7 | 7.5 | 14.6 | 2 |
| 7.1 | 33.4 | | 38.0 | 71.9 | — | 8.7 | 7.7 | 11.6 | 2 |
| 6.6 | 28.6 | | 38.1 | 67.7 | — | 9.1 | 9.1 | 14.2 | 3 |
| 6.1 | 18.9 | 8.9 | 33.9 | 61.7 | — | 12.4 | 9.4 | 13.6 | 2 |
| 5.6 | 16.6 | 8.6 | 37.5 | 63.4 | — | 13.3 | 10.5 7.3 | 6.1 | 2 |
| 5.1 | 13.9 | 14.6 | 37.2 | 64.1 | — | 16.5 | 8.3 3.9 | 5.5 | 3 |
| 4.7 | 15.6[a] | 17.8[a] | 31.6 | 65.9 | — | 9.5 | 16.6 9.8 | — | 2 |

[a] Data from right limb only.

peak, designated *I* 4.2, contains about 15 per cent, whereas the second peak, *I* 4.4, contains approximately 12 per cent of the total. Since *I* 4.2 contains at least three components ultracentrifugally, and *I* 4.4 is also multiple, it would not be expected that all components found in one peak at one pH would be in the same peak at another pH. Rather, material may shift from one area to another. Evidence for this is seen in the increase in the area of the *I* 4.4 peak as the pH is lowered from 6.1 to 4.7.

The area between the albumin peak and the β peak was treated in two ways. When a large easily definable peak appeared (*X* or $\alpha_1$), it was simply listed as the postalbumin peak. This was done because it may well be identical with either *I* 4.4 or *I* 4.2. Further work will be required to establish its identity. The remaining area, which cannot be unambiguously divided up into peaks, is listed as the α zone that varies from 8.7 to 16.5 per cent (average 12 per cent). This area has been characterized as "electrophoretically heterodisperse" [33].

The β-globulin peak is double at pH 9.1, single from pH 8.6 to 6.1, and double again from pH 5.6 to 4.7. This presents an interesting puzzle since the amount of material found in the two peaks does not equal the amount

present when only one peak is seen. Possibly the $\gamma$-globulins and the salt boundary contribute to the $\beta$ peaks betwen pH 5 and 6. A double $\beta$ peak at pH 8.6 has been previously reported by Fisher *et al.* [13]. The best estimate for the percentage of $\beta$ peak material present is about 9 per cent.

The percentage of $\gamma$-globulin present varies considerably at different pH's owing to the presence of the salt boundary and to incomplete separation from the $\beta$ peak. The best estimate is taken from the experiments performed at pH 9.1 and is 7 per cent. Previously recorded values ranged from 3 to 11

TABLE II. *Summary of electrophoretic*

Superscripts on buffers indicate composition as follows: LV = Longsworth veronal;

| | Authors | Strain | pH | Ionic strength |
|---|---|---|---|---|
| P* | Deutsch & Goodle [8] | Sprague-Dawley | $8.6^{LV}$ | — |
| S* | Moore [31] | Long-Evans | $8.6^{LV}$ | 0.1 |
| S | Moore [31] | Long-Evans | 8.6 | 0.1 |
| S | Moore [31] | Long-Evans | $7.4^{P}$ | 0.2 |
| S | Moore [31] | Long-Evans | $7.4^{P}$ | 0.2 |
| S | Moore [31] | Long-Evans | $7.4^{P}$ | 0.2 |
| S | Moore [31] | Long-Evans | $7.4^{P}$ | 0.2 |
| S | Moore *et al.* [33] | Long-Evans | $7.4^{P}$ | 0.2 |
| S | Cohen & Thompson [5] | — | — | — |
| S | Cook *et al.* [6] | Holtzman Sp.-Daw. | 8.3 | 0.1 |
| P | Gjessing & Chanutin [15] | Wistar M | $8.6^{V}$ | 0.1 |
| P | de Lamirande & Cantero ]7] | Wistar | $8.3^{V}$ | 0.1 |
| S | Westphal *et al.* [42] | Sprague-Dawley M | $8.6^{V}$ | 0.1 |
| P | Westphal *et al.* [42] | Sprague-Dawley M | $8.6^{VAc}$ | 0.1 |
| S | Mackay [27] | — | 8.0 | 0.2 |
| S | Fischer *et al.* [13] | Wistar M | $8.6^{V}$ | 0.1 |
| S | Schultz *et al.* [37] | Wistar F | $8.6^{V}$ | 0.1 |
| S | Roberts & White [36] | Sprague-Dawley M | $8.6^{V}$ | — |
| S | Gleason & Friedberg [16] | — | $8.6^{V}$ | — |
| S | Eymer (1954) | — | — | — |
| S | Arbouys *et al.* [2] | — | $8.6^{V}$ | — |
| | Berg (1955) | — | — | — |
| | Berg (1955) | — | — | — |
| S | Winkler & Pasehke [45] | — | $8.6^{VAc}$ | 0.1 |

per cent for free electrophoresis and from 3 to 20 per cent when paper was used. Determinations of rat serum $\gamma$-globulin on paper are not accurate since, as will be discussed in a subsequent paper, the heavy component ($S_{20}$ 20) moves with serum albumin in free electrophoresis (pH 8.6) but adheres to paper and does not move at the same pH.

The peaks that migrate cathodically, especially at the lower pH's, cannot be identified until larger amounts of purified serum proteins are available. The finding that bovine serum albumin breaks up into several peaks in acid

*studies on rat plasma and serum.*

P = phosphate; VAc = veronal acetate; P = phosphate. *P = plasma; S = serum.

| FREE ELECTROPHORESIS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Mobility ($cm^2 \times 10^5$ $sec^{-1}$ $v^{-1}$) | | | | | Percentage composition | | | | |
| ɔumin | $\alpha$ | $\beta$ | $\Phi$ | $\gamma$ | Albumin | $\alpha$ | $\beta$ | $\Phi$ | $\gamma$ |
| 6.1 | — | 2.7 | — | 1.6 | 59.1 | 15.4 | 19.4 | — | 4.8 |
| 5.9 | 5.1 4.7 | 2.9 | — | 1.8 | 58 | 20 2 | 15 | — | 5 |
| 5.9 | 5.5 4.0 | 2.7 | — | 1.5 | 72 | 6 8 | 11 | — | 3 |
| 4.7 | — | 2.2 | — | 0.9 | 72 | — | 17 | — | 11 |
| 4.8 | 3.2 | 2.4 | — | 1.3 | 70 | 9 | 12 | — | 9 |
| 4.6 | 3.0 | 2.0 | — | 0.7 | 76 | 9 | 12 | — | 3 |
| 4.4 | 2.8 | 2.0 | — | 0.5 | 76 | 6 | 11 | — | 7 |
| — | — | — | — | — | 71.4 | — | 16 | — | 11 |
| — | — | — | — | — | 50.6 | 18.6 10.6 | 15.4 | — | 4.8 |
| 5.6 | 3.5 | 2.6 | — | 1.5 | 66 | 14 | 13 | — | 6 |
| 6.4 | 5.6 4.7 | 3.4 | 2.9 | 2.0 | 44 | 26 6 | ←19→ | | 6 |
| 6.8 | 4.7 | 3.8 | 2.5 | 1.1 | 53 | 8 | 8 | 22 | 9 |
| — | — | — | — | — | 58.9 | 12.3 | 17.9 | — | 10.9 |
| 5.4 | — | — | — | — | 62.0 | 12.4 | 14.3 | — | 11.3 |
| — | — | — | — | — | 63 | 13 | 18 | — | 6 |
| 5.64 | 5.12 3.77 | 2.73 | — | 1.93 | 54.9 | 9.5 10.4 | 14.8 | — | 10.6 |
| 5.92 | 5.25 4.06 | 2.90 | — | 2.05 | 48.6 | 13.7 10.0 | 14.4 | — | 13.2 |
| 6.6 | 5.1 3.6 | 2.1 | — | 1.8 | 50.4 | 19.2 7.2 | 14.6 | — | 8.6 |
| PAPER ELECTROPHORESIS | | | | | | | | | |
| — | — | — | — | — | 88.6 | 1.2 .36 | 6.9 | — | 2.9 |
| — | — | — | — | — | 30.6 | 13.1 12.2 | 27.1 | — | 19.6 |
| — | — | — | — | — | 55.5 | 7.4 | 22.9 | — | 14.2 |
| — | — | — | — | — | 46.4 | 12.1 9.5 | 20.4 | — | 11.8 |
| — | — | — | — | — | 46.5 | 12.5 8.8 | 21.1 | — | 11.5 |
| — | — | — | — | — | 41.6 | 10.5 11.0 | 21.6 | — | 15.5 |

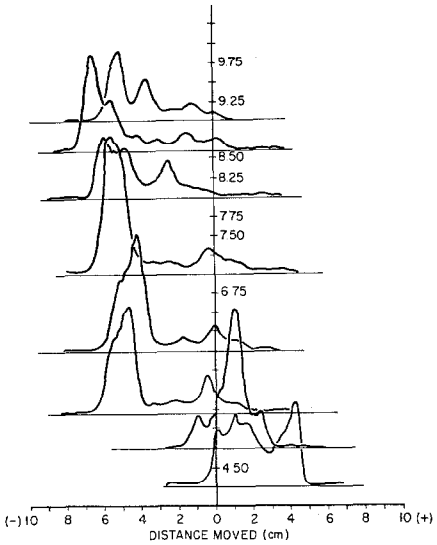


Fig. 2.—Tracings of paper electrophoresis strips run in 0.02 ionic strength buffers at the pH's and times shown. Point of application of the protein is shown by arrow.

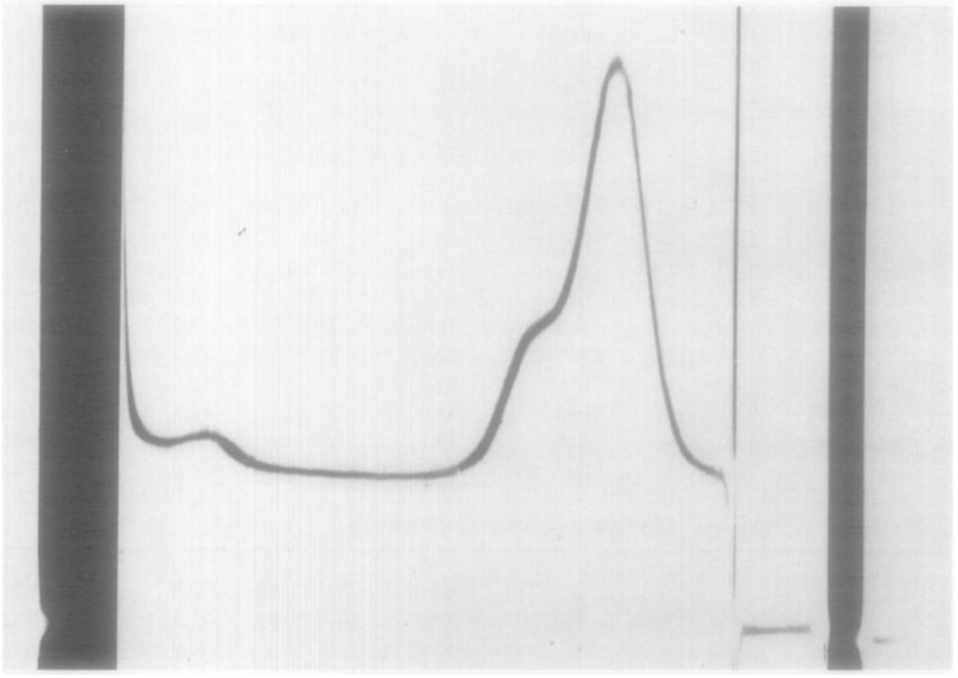

Fig. 3.—Sedimentation diagrams of whole serum after 40 minutes at 56,100 rpm. Phase plate angle was 55°. Pooled rat serum was mixed with three parts of buffer (0.2 ionic strength, pH 7.5) and dialyzed against the same buffer 48 hours.

solutions [1] suggests additional difficulties in the interpretation of serum protein patterns at low pH.

It should be emphasized that mobility measurements and isoelectric points derived from studies on whole serum should be considered as guides and not as definitive values. Accurate measurements of these parameters should be made on the purified protein itself.

A similar set of experiments was attempted with paper electrophoresis, by using 0.02 ionic strength buffers over the pH range 4.2 to 9.0 (Fig. 2). The shift of material migrating behind the albumin peak at pH 9.0 to a position in front of the albumin at pH 5.2 is evident. The differences between these results and those obtained in free solution are probably caused by adsorption of the proteins on filter paper.

*Ultracentrifugation of whole serum.*—Rat serum was prepared for ultracentrifugation by either dialysis against 0.1 or 0.2 ionic strength buffer at pH 7.5 or, for comparative purposes, by mixing the serum with three volumes of 0.1 ionic strength buffer. A typical pattern is shown in Fig. 3. The results of a systematic series of sedimentation studies conducted with various dilutions of the same pooled serum sample in 0.2 ionic strength buffer are shown in Fig. 4. Measurement of the areas under the three main peaks in two separate experiments gave the following concentrations expressed as percentages of the total: $S_{20,w}$ 20 – 3.5 and 3.7 per cent, $S_{20,w}$ 9 – 19.2 and 19.7 per cent, and $S_{20,w}$ 4.8 – 77.2 and 77.6 per cent. In one experiment at pH 5.0 in 0.1 ionic strength buffer, 5.3 per cent was found in the heavy component, 33.2 per cent in the $S_{20,w}$ 9 peak, and 61.5 per cent in the $S_{20,w}$ 4.8 peak, suggesting that pH may affect the distribution of material between the two large peaks. The sedimentation rates of the three peaks at zero concentration were 4.78, 9.08, and 19.95 at 20°C. These results are subject to the errors that occur with multicomponent systems [35]. Results of studies on the isolated heavy component will be presented in a subsequent paper.

*Preparation of γ-globulin.*—The continuous curtain electrophoresis cell (Spinco Model CP) was used in fractionation of 200 ml of normal rat serum in approximately 50-ml batches. A potential of 900 volts was maintained across the curtain. The serum was dialyzed against the 0.02 *M* sodium phosphate buffer (pH 7.5) used and then mixed with equal volumes of the buffer. When a feed rate of approximately 2.0 ml per hour was used separations such as the one shown in Fig. 5 were obtained. The protein analysis was done on the basis of ultraviolet absorption at 280 mμ of samples from the collecting tubes diluted 1:26 with buffer. The protein in the first five or six tubes was pooled from all runs and precipitated in the cold with 70 per

cent saturated ammonium sulfate. The precipitate was redissolved in buffer, dialyzed, and run again at the same pH and buffer composition in the CP cell. The separation achieved is shown in Fig. 6. The protein in tubes 8 to 17 was precipitated with ammonium sulfate and dialyzed against 0.1 ionic strength Miller-Golder buffer [28] at pH 7.5. The purity is indicated in the sedimentation diagrams and electrophoretic patterns shown in Fig. 7. Con-

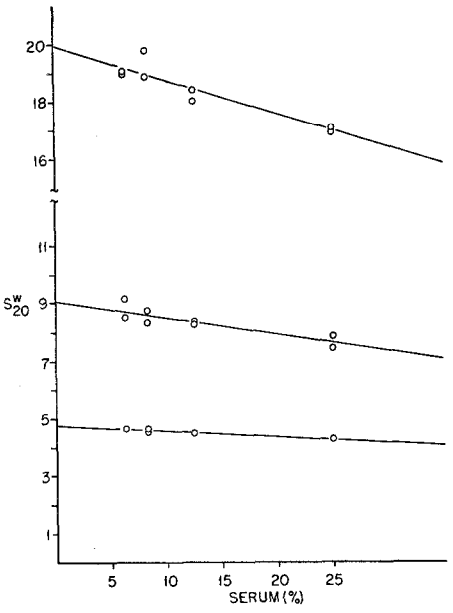


Fig. 4.

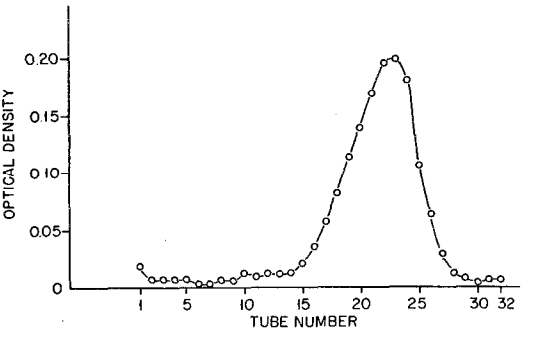


Fig. 5.

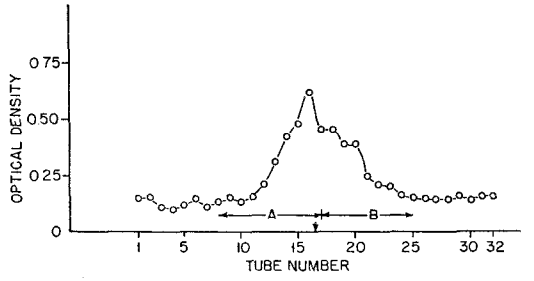


Fig. 6.

Fig. 4.—Sedimentation rates of peaks in whole rat serum at various dilutions. Undiluted serum contained 5.14 mg protein/ml. Measurements made at 20.0°C and corrected to conditions obtaining in water. $S_{20,w}$ values at zero concentration were calculated to be 4.78, 9.08, and 19.9 for the three peaks.

Fig. 5.—Separation of rat serum components achieved at pH 7.5 in 0.02 ionic strength phosphate buffer with continuous paper curtain electrophoresis cell. Serum dialyzed against buffer and diluted with equal parts of buffer. Cell data: 900 volts, ~48 ma, feed rate = 2 ml/hr (setting 5.5 with 30 mm feed tube), overflow, left, and right capillaries all set at 7.0.

Fig. 6.—Separation of $\gamma$-globulins on second run through continuous paper curtain electrophoresis cell. pH 7.5; ionic strength, 0.1 phosphate buffer; 900 volts; ~40 ma; flow rate, 2 ml/hr (setting 5.5 with 30 mm feed tube); overflow, left, and right capillaries at 6.5.

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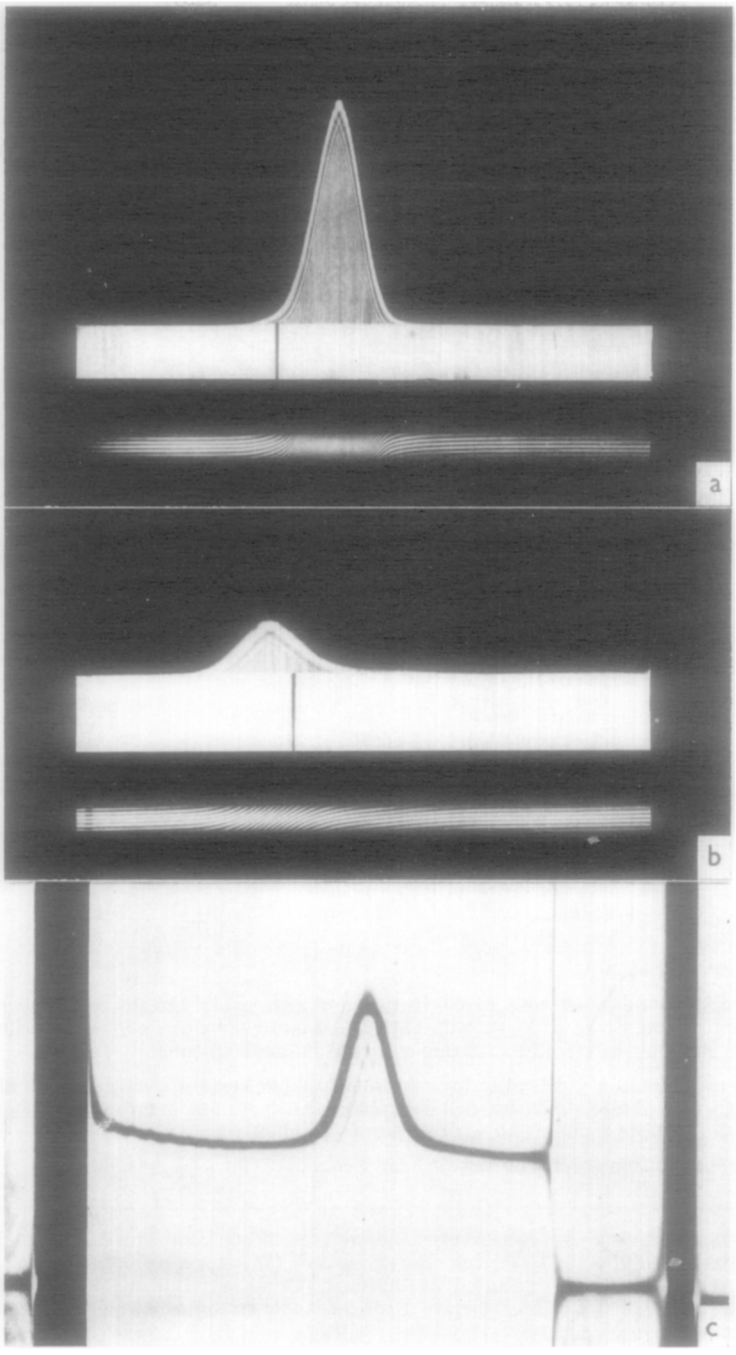
Fig. 7.—Purified rat $\gamma$-globulin (*a*) after electrophoresis for 1200 seconds in pH 7.58; ionic strength, 0.1, phosphate-NaCl buffer in 11 ml cell, 55° bar angle, (*b*) after electrophoresis for 15,300 seconds at pH 5.39, ionic strength, 0.1 in 2 ml cell. 55° angle, (*c*) after ultracentrifugation at 56,100 rpm in pH 7.5; ionic strength, 0.1 buffer at 20° for 42 minutes. Phase plate angle = 55°.


a
b
c

tamination by $\beta$-globulin or faster-moving protein at pH 7.5 is less than 0.01 per cent from Rayleigh fringe data.

The second $\gamma$-globulin fraction from the CP cell separation shown in Fig. 6 was purified by an additional run through the same cell, concentrated, and studied ultracentrifugally and electrophoretically. Since no significant differ-

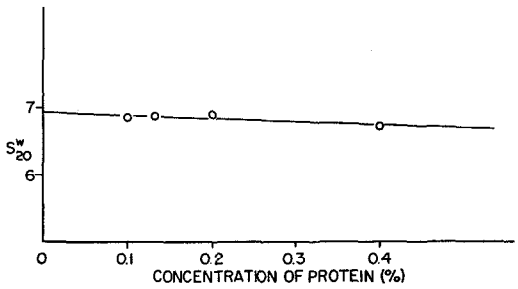



Fig. 8.

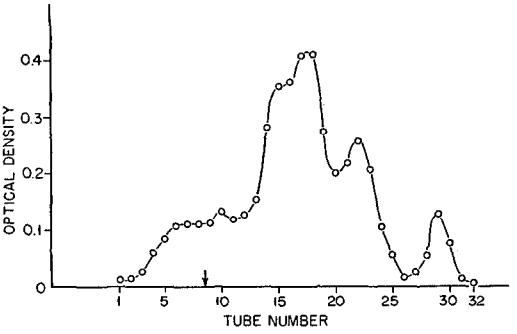



Fig. 9.

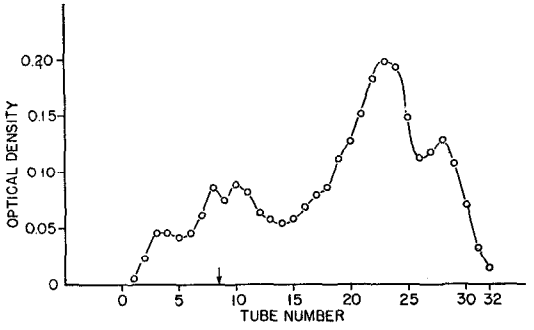



Fig. 10.

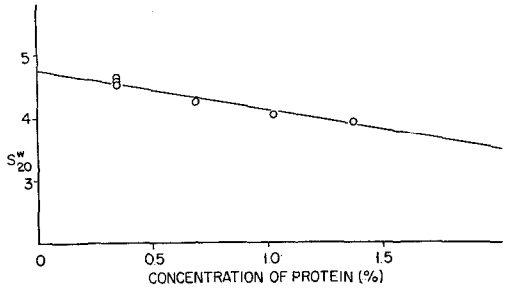



Fig. 12.

Fig. 8.—Sedimentation studies on purified rat serum $\gamma$-globulin. Sedimentation rate at zero concentration was 6.94 at 20°C.

Fig. 9.—Separation obtained with pooled rat serum with paper curtain electrophoresis at pH 5.0 in sodium acetate, ionic strength, 0.02 buffer. Cell data: 980 volts, ~ 70 ma, overflow and capillaries set at 8.0, flow rate 3 ml/hr (setting 8.0 with 30 mm feed tube).

Fig. 10.—Serum albumin purification by continuous paper curtain electrophoresis at pH 7.5 in ionic strength, 0.02 phosphate buffer. Cell data: 900 volts, $\simeq$ 40 ma, overflow, left, and right capillaries at 7.0, flow rate 2 ml/hr (5.5 with 30 mm feed tube).

Fig. 12.—Sedimentation studies on purified rat serum albumin. $S_{20,w} = 4.75$.

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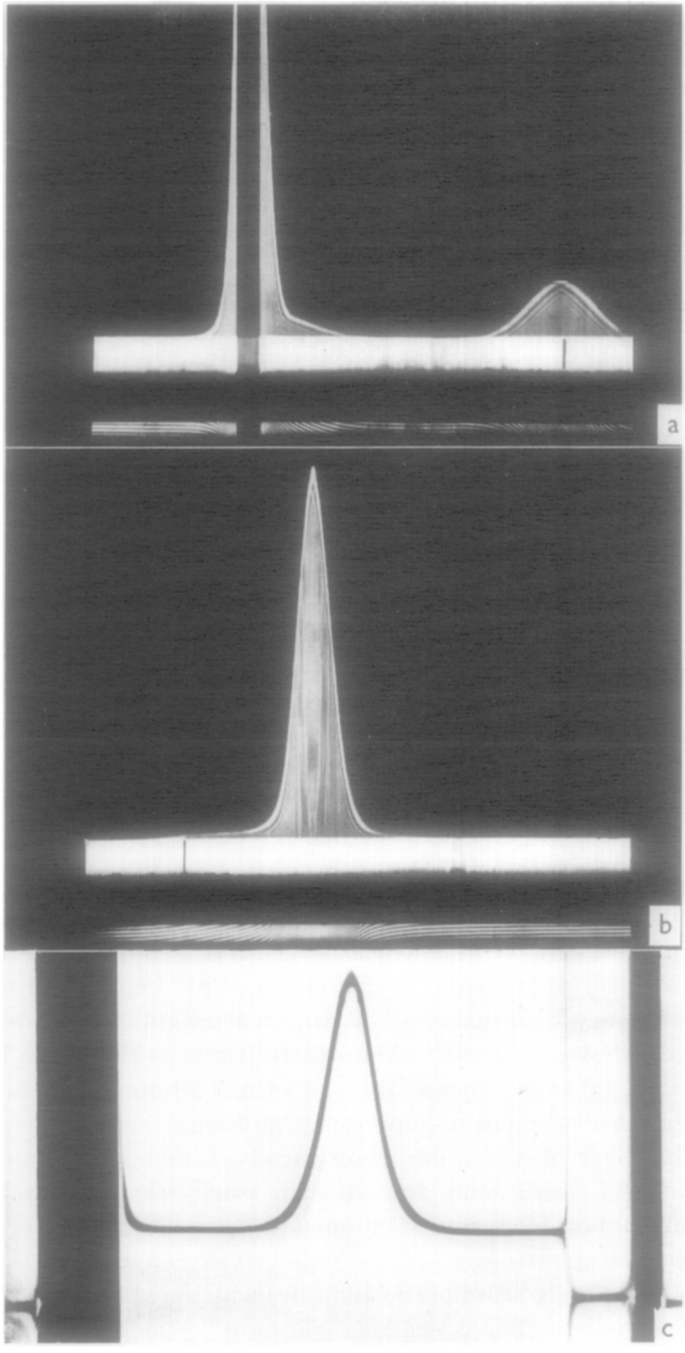
Fig. 11.—Rat serum albumin (*a*) after electrophoresis for 12,600 sec. in pH 7.53; ionic strength, 0.1 phosphate-NaCl buffer in 11 ml cell. Bar angle 55°. (*b*) After electrophoresis for 12,000 sec. in pH 5.16, ionic strength 0.1, acetate-NaCl buffer in 11 ml cell. Bar angle 55°. (*c*) After ultracentrifugation for 6720 sec. at 56,100 rpm in pH 7.5, ionic strength, 0.1 phosphate-NaCl buffer. Phase plate angle = 70°.


a
b
c

ence was noted in the sedimentation of these two preparations, the results of a series of sedimentation studies are shown for the second preparation alone in Fig. 8. The $S_{20,w}$ was found to be 6.94. The average mobility was $T_{7.5} - 0.75$.

*Preparation of rat serum albumin.*—Approximately 250 ml of pooled frozen rat serum was dialyzed against 0.02 *M* sodium acetate buffer at pH 5.0 and run through the CP cell by using a potential of 980 volts across the curtain. The separation obtained is shown in Fig. 9. The solution in tubes 15 to 20 under the albumin peak was collected, concentrated with 70 per cent ammonium sulfate, taken up in the same pH 5.0 acetate buffer, and run through the curtain, lyophilized, and stored at −20°C. Final purification was done by reconstituting with distilled water, and running through the CP cell at pH 7.5 in 0.02 ionic strength sodium phosphate buffer. The material from the largest peak was concentrated and run through the CP cell again at pH 7.5. The results are shown in Fig. 10. The purity of the preparation is indicated by the ultracentrifuge diagram and electrophoretic patterns shown in Fig. 11. The Rayleigh fringe data show that the contamination with $\gamma$- and $\beta$-globulins at pH 7.5 was less than 4 per cent. The contamination with proteins moving faster than serum albumin at pH 5 was less than 0.1 per cent. Sedimentation studies at pH 7.5 in 0.2 ionic strength buffer are shown in Fig. 12. The $S_{20,w}$ for rat serum albumin is 4.74. Additional studies on the effect of pH on the $S_{20,w}$ of rat serum albumin are planned.

## DISCUSSION

Data on the properties of serum proteins in normal experimental animals are a prerequisite to studies on the function of individual protein species and on alterations occurring in pathological states. Rat serum has therefore been characterized electrophoretically over a range of pH values and ultracentrifugally over a range of dilutions. The apparent albumin peak observed at pH 8 has been shown to break up into three peaks as the pH is lowered to pH 5, suggesting that the concentration of serum albumin is actually much lower than has been generally supposed. Additional heterogeneity in these three peaks has been observed ultracentrifugally. Data on the relative mobilities of the various constituents and on their isoelectric points provides the necessary information for serum fractionation using either electroconvection, zone, or free electrophoresis.

Comparison of the electrophoretic and ultracentrifugal findings reveals several interesting points. The so-called "albumin" peak in the serum ultracentrifuge diagram contains approximately 77 per cent of the serum protein

and has a sedimentation coefficient of 4.78 Svedberg units. This compares favorably with the $S_{20,w}$ 4.74 obtained for purified serum albumin but not with the lower value of 36 per cent serum albumin found electrophoretically. This implies that a considerable protein fraction exists with the sedimentation coefficient of serum albumin but with a different mobility. Whether there exists in rat serum a number of "albumins" remains an open question.

The globulin ultracentrifuge peak contains approximately 19 per cent of the serum protein; however, its sedimentation rate ($S_{20,w}$ 9) is considerably higher than the value of 6.94 obtained for purified $\gamma$-globulin. A component or components sedimenting faster than $\gamma$-globulin must therefore be present in addition to the macroglobulin observed. These conclusions are not invalidated by the small shift in the ratios between the "albumin" and "globulin" peaks observed with changes in serum concentration.

With almost any given peak, the possibility exists that one or more minor constituent may be included under it. This is evident when areas under homologous peaks are compared at different pH's. The percentage $\beta$-globulin, for example, varies considerably betweeen pH 9 and 5. Attempts to use electrophoretic or ultracentrifugal data from mixtures analytically must therefore be treated with reservation although such data are valuable for comparative work. It should be emphasized that attempts to demonstrate homogeneity must include at least electrophoresis at several different pH values and ultracentrifugal analysis.

Further studies on the biophysical properties of serum proteins, together with serological investigations will be presented in subsequent papers.

## SUMMARY

Serum obtained from adult male Sprague-Dawley rats was examined by using moving boundary electrophoresis over the pH range 3.6–9.0. The "albumin" peak at pH 8.0 is found to break up into three peaks as the pH is lowered to 5. These constitute 15, 12, and 36 per cent of the serum proteins and have isoelectric points at pH 4.2, 4.4, and 4.6, respectively. The slowest moving peak (isoelectric point at pH 4.6) is identified as serum albumin. The $\beta$-globulin region is double peaked at pH 9, is single from pH 8.6 to 6.1, and double again from pH 5.6 to 4.9. The $\beta$-globulins account for approximately 9 per cent and the $\gamma$-globulins 7 per cent of the serum proteins.

In the ultracentrifuge, three peaks are observed when whole serum is used. These have sedimentation coefficients of 4.78, 9.08, and 19.95 at 20°C in water extrapolated to zero concentration. The first value compares favorably with the value of 4.74 obtained with purified serum albumin, but the

second is considerably higher than the value of 6.94 obtained for purified rat $\gamma$-globulin.

The preparation of rat serum albumin and $\gamma$-globulin by continuous paper curtain electrophoresis is described.

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