Technical Papers

Fractionation of Human Serum Albumin Using Continuous Filter-Paper Electrophoresis

Daniel L. Larson and Robert Feinberg

Department of Experimental Immunology, Immunology Division, Army Medical Service Graduate School, Washington, D.C.

In the study of proteins, it is often desirable to obtain specimens of a high degree of purity. It has been repeatedly shown that protein preparations that fulfill the requirements for homogeneity by one criterion may not be homogeneous when tested with a different technique. One of the most sensitive tests for the immunological homogeneity of a protein is the gel-diffusion test. In this test, an antiserum is prepared against the material under investigation, and the antigen and antibody are permitted to react in a gel medium under certain standard conditions. In our laboratory, several lots of human serum albumin (*1*), prepared commercially by alcohol fractionation, were examined for homogeneity. The preparations appeared as a single component in the ultracentrifuge and as a single peak with moving boundary electrophoresis. Immunochemically, the preparations gave a smooth quantitative precipitin curve with rabbit antihuman albumin serum. When these lots were tested with the gel-diffusion technique, they were shown to have at least three components, and, in some cases, they had a minimum of six components.

Continuous filter-paper electrophoresis is particularly well suited for the separation of small amounts of mixtures of proteins (*2*). It is a gentle treatment that can be carried out in the cold, and it avoids extreme changes in *p*H and electrolyte concentration. The fractions so obtained can be quantitatively recovered. An important advantage over other techniques of boundary or zone electrophoresis is that, except for the initial contact of the proteins on the paper, the fractions are physically separated throughout the remainder of the run. This reduces to a minimum the interaction between dissimilar proteins. It is the purpose of this communication to report the use of continuous filter-paper electrophoresis for the fractionation of human serum albumin.

Electrophoresis. The large continuous filter-paper electrophoresis cell, developed by Durrum (*2*), was used for the separations. The filter-paper curtain used was Whatman No. 3MM, 18¼- by 22½-in. sheets, cut to provide 28 drip points at the bottom of the curtain. Veronal buffer was used at *p*H 8.6 and ionic strength of 0.05. Power supply was 900 to 1000 v with a current of 20 ma. The entire apparatus was placed in a cold room at 4°C.

Gel-diffusion technique. A modification of the technique described by Oudin (*3, 4*) was used. The final concentration of agar was 0.3 percent. Antiserum concentrations of 50, 25, 12.5, and 6.25 percent by dilution were used. Antigen concentration was 5 percent by weight. Diffusion tubes were 80 mm in length with an internal diameter of approximately 3 mm. Runs were made in a 26°C constant temperature water bath up to 8 days.

Albumin samples. Ten lots of human serum albumin prepared by alcohol fractionation for the American Red Cross were examined. Samples were diluted to 10 percent for electrophoresis and diluted to 5 percent for gel-diffusion tests.

A 10-percent solution of the human serum albumin was allowed to siphon over onto the curtain at the rate of 3 to 6 ml/24 hr and was collected in tubes at the bottom of the curtain. Under these conditions, the albumin was distributed over seven drip points near the center of the curtain at the end of the preliminary run. The samples collected from the drip points were dialyzed at 4°C against distilled water to remove the buffer. The dialyzed fractions were dried in vacuum from the frozen state, and the material was tested for homogeneity using the gel-diffusion test. Material from the leading drip points, containing several bands in the gel-diffusion test, was pooled and run over the curtain a second time under the same conditions. The final products collected from the drip points were dialyzed, dried in vacuum from the frozen state, and retested with the gel-diffusion technique.

The results of a separation of human serum albumin fractions using this procedure are illustrated in Fig. 1.

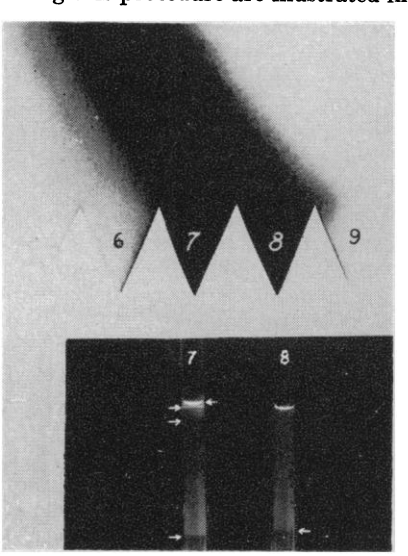

Fig. 1. Continuous filter-paper electrophoresis, second run, human serum albumin. Gel-diffusion tests on material obtained from drip points 7 and 8. Electrophoresis in veronal buffer, *p*H 8.6, ionic strength 0.05, 900 v, 20 ma, 4°C. Gel diffusion 25 percent antibody, 0.3 percent agar, 4 percent antigen, 25°C, 8 days.

Initially, there were four components in this lot of albumin when tested with the gel-diffusion technique. After the initial run, material from the leading three drip points, containing all antigenic components, was pooled, dialyzed, dried in vacuum from the frozen state, and run over the curtain a second time. As can be seen in Fig. 1, material taken from the leading drip point, No. 8, contained a single component; a specimen taken from the next drip point, No. 7, contained four components. The yield of the sample exhibiting a single band in the gel-diffusion test was of the order of 5 to 10 percent by weight of the original material.

It is possible that the final product contained minute amounts of the three other components present in the original material, since there are several recognized limitations to interpretation of gel-diffusion observations (*5*). For example, if the contaminating components were present in sufficiently low concentration, they could be present and still not give rise to a visible band in the gel-diffusion test. Similarly, it is possible that the single visible band represents more than a single antigen. This is unlikely, however, since these antigens would then have to possess the same diffusion rate and be present in similar concentrations, and the antiserum would have to contain homologous antibody at similar concentrations. The final product, representing 5 to 10 percent of the original material, may represent a serum albumin or, possibly, a protein with a faster electrophoretic mobility than serum albumin. It was apparent in the gel-diffusion test that the antibody to the purified material was present in relatively low concentration, but by the criterions we have available for purity, this product appears to be homogeneous.

References and Notes

1. Obtained through the courtesy of J. N. Ashworth of the American Red Cross and E. R. Squibb and Sons.
2. E. L. Durrum, *J. Am. Chem. Soc.* **73**, 4875 (1951); M. D. Paulik, *Can. J. Med. Sci.* **31**, 485 (1953).
3. J. Oudin, *Ann. Inst. Pasteur* **75**, 109 (1948).
4. J. Munoz and E. L. Becker, *J. Immunol.* **65**, 47 (1950).
5. J. Oudin, *Methods in Medical Research* (Year Book Publ., Chicago, 1952), vol. 5, p. 335.

15 April 1954.

Effect of High Fat Feedings on Viscosity of the Blood

Roy L. Swank

Division of Neurology, Department of Medicine, University of Oregon Medical School, Portland

Aggregation and adhesiveness of red blood cells were observed *in vitro* by Swank (*1*) and *in vivo* by Swank and Cullen (*2, 3*) to develop about 4 hr after fat feedings. They reached their maximum intensity 3 to 4 hr later and then subsided slowly to normal. The *in vivo* changes were observed in the cheek pouch of hamsters anesthetized with urethane, using the technique of Fulton, Jackson, and Lutz (*4*), and were recorded in colored motion pictures. The purpose of this report (*5*) is to show that significant increases in the viscosity of blood from the vena cava accompanies the aggregation and adhesiveness of red blood cells observed in the cheek pouch after fat meals.

Blood viscosity was measured by a method based on principles utilized earlier by Fahreus (*6*) and others, and developed for this specific problem (*7*). The time required for 0.1 ml of blood to flow through a standardized orifice at a negative pressure of 100 mm-Hg at 37°C gives a relative measure of viscosity that has proved fairly dependable. This time interval divided by the time required for the same amount of water to pass through the same orifice under the same conditions of temperature and pressure gives the viscosity of blood relative to that of water.

In the present experiments, the standardized orifice was a gage 25 hypodermic needle 1 in. long (vita). The tip of this needle with the bevel down was inserted into the abdominal inferior vena cava of the urethane anesthetized hamster. The negative pressure was applied by opening a stopcock on a 0.1-ml calibrated pipette to which the calibrated hypodermic needle was attached. The flow of blood was timed with a stop watch to 0.1 sec. It is essential in this type of determination that the tip of the needle inside the inferior vena cava be clearly visible; that the flow of blood in the pipette be smooth and steady; that the total time of the flow be less than 30 sec and preferably less than 20 sec; and that duplicate determinations that check to within less than 10 percent deviation be made. The hematocrits were determined on blood drawn from the vena cava into heparin immediately after the viscosity measurements (*1*).

A close correlation was first shown to exist between visible evidences of adhesiveness and aggregation of the red blood cells and an increase in the viscosity of the whole blood in nine hamsters fed a "normal" diet containing mixed grain, vegetable greens, and fox chow, and in 18 experimental animals 5 to 7 hr after being fed 1 to 3 ml of 35-percent cream (3 to 10 g/kg body weight) by stomach tube. Next the duration and extent of the viscosity changes after fat meals were determined.

Hamsters fed a diet containing about 50 percent of their calories as animal fat (*8*) for 5 to 7 days and then given a single fat meal of 2 to 3 ml of 35-percent cream almost always exhibited an increased viscosity of the blood. Animals so fed were anesthetized and their blood viscosities and hematocrits were determined 3, 6, 9, 14, 24, 48, and 72 hr after the single cream meals. The blood viscosity began to increase about 3 hr after the cream feeding and reached its peak in 6 to 9 hr (Fig. 1). During the first 9 hr only minor variations occurred in the hematocrit. The viscosity then returned to normal by the end of 24 hr. This was accompanied by a significant drop in the hematocrit. The hematocrit then returned to normal by the end of 72 hr, accompanied by a rise and then a fall of the viscosity to normal. The low hematocrit at the end of 24 hr is thought to be due to "sticking" of many of the red blood cells in the peripheral circula-