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An Electrophoretic Analysis of Normal And Pathological Sera

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Abstract. The technique of closed-strip filter paper electrophoresis was used to analyze and compare normal and pathological human sera, and to study the effects of age, species, and stress on animal sera. Problems encountered were overcome by varying pressure, buffer, dye, electrodes, sources of voltage of electric current, and the denaturing and dyeing process.

The type of zone electrophoresis with which this project is concerned is a relatively simple technique called the closed strip or "sandwich" method. The apparatus used consisted of plate glass (4 in. by 9 in.); Whatmann 3 mm. filter paper; clamps for pressure; 2 beakers, 2 ring stands and clamps to support the 2 carbon electrodes; vaseline to prevent evaporation from the plates; and a power source of five, 45 volt "B" batteries to supply the DC—225 volts at low amperage.

Chemicals used included a sodium borate electrolyte with a pH of 8.6; an alcohol solution of 1 percent bromophenol blue saturated with mercuric chloride; and alcohol, distilled water, acetic acid for rinsing away excess dye.

The glass plates were set on the beakers so that the filter paper (3 in. by 12 in.) extended beyond the ends of the plates about 1½ in. to dip into the electrolyte.

Before the strip was placed on the lower plate it was marked with a pencil about 2½ in. from the end; the strip was dipped into the electrolyte and blotted uniformly. After the strip was laid on the lower plate, the sample of serum (about 5 lambda) was applied with a capillary tube at the pencil mark. Since the serum and electrolyte were alkaline, the point of application was placed near the cathode to allow the serum to travel toward the anode (Figure 1*A*). According to the kind and number of surface charges on each protein, depending on its molecular structures, the proteins will be separated into distinct spots. In normal human serum five spots can be distinguished. Albumin moves most rapidly and is most abundant; alpha$_1$

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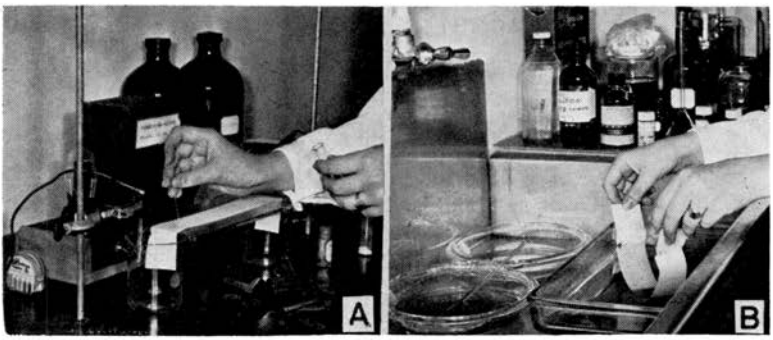

Figure 1. A. Serum is applied by means of a capillary tube. The point of application is near the cathode. B. Dyeing and rinsing is done after the proteins in the strip have been denatured.

globulin follows albumin closely and sometimes remains with it; alpha$_2$ globulin and beta globulin follow next; and gamma globulin remains at the point of application.

The second plate was laid on top of the strip; vaseline sealed the edges; and pressure was applied with clamps. After the battery had supplied current for an 8-hour run, the strips were removed and placed in the dye. After 5 minutes they were removed from the dye and rinsed. Ammonia vapor was used to bring out the stain more effectively (Figure 1*B*).

Problems Encountered

Since the first results were not entirely satisfactory, methods of improvement were initiated. Instead of using just one sample on the 3-inch wide strip, three samples, about one-half inch apart, had been used. Strips of polyethylene had been used between three such filter papers. After the dyeing process, the strip closest to the top plate was the best; more important, however, was the fact that the three samples of serum on a single filter strip had not traveled the same distance, and this pattern of irregularity was consistent for the three strips. The first correction, therefore, was to apply and maintain uniform pressure on the plates. The 3-inch width strip was reduced to 1½ inches.

To eliminate "washing" and spread of liquid between plates, the use of vaseline was abandoned and a fine coat of silicone grease substituted. The coat covered the entire surface of both plates.

After experimentation with a more sensitive buffer—a barbiturate—there was a great change in the pH of the small volume of the buffer in the beaker at the anode. To correct this, larger beakers and platinum electrodes were substituted.

With continued use of the batteries there was not the assurance

of a constant amperage, thus introducing another variable in the experiment. The batteries were replaced by a transformer-rectifier set-up salvaged from a radio. This proved convenient and efficient, having a range from 110-260 volts at 12½ volt intervals. It delivered 35-40 milliamps.

The drying procedure was also improved. The proteins in the strips were denatured before dyeing by drying the strips in an oven for 30 minutes at 110° F.

Testing Pathological Sera

The patterns for pathological sera differ from the normal because certain physiological conditions of the body result in a change of serum constituents. Albumin, usually the most abundant protein, is produced by the liver. If the liver fails, the other tissues make up for the lower albumin level by producing more globulins. An increase in gamma globulin could indicate a liver disorder; it could also indicate an infection, for most of the antibodies appear as gamma globulin-like proteins. An increase in alpha globulin results when there is a breakdown of tissue proteins as in a fever-producing disease.

Before testing any pathological sera, a study was made of a number of patterns of serum from people with specific diseases. This necessitated translating these from a diagram of a scanning pattern (a curve obtained when a light beam is passed through the strip) to the approximate pattern if the spot technique were used. These patterns served as a reference and an aid to interpreting results.

The first pathologic serum used for experimentation came from a patient suffering from a kidney disease. A large increase in $alpha_1$ globulin showed up.

Serum from a very old woman suffering from recticulum cell sarcoma was obtained. Electrophoretic analysis indicated a noticeable increase in $alpha_2$ globulin. There also appeared to be two fractions present in the gamma globulin area.

Testing Animal Sera

Animal sera differ from human sera in the kind and abundance of proteins. Whereas there were five visible separations in human serum, bovine serum showed only four separations and dog serum showed six. Further study revealed that in addition to species effects, factors of age, sex, or special stress also produced a difference in serum within the same species.

Research further uncovered a new method of denaturing the

proteins on the strips prior to dyeing. This required a solution consisting of 9 parts methanol and 1 part acetic acid in which the strip was soaked. Both bromocresol green and amidoschwartz 10B were used to dye strips denatured in this way. Amidoschwartz worked very well when prepared by dissolving 0.5 grams of dye in 300 ml. of ethanol, 50 ml. of acetic acid, and 100 ml. of water. The strip was left in the bath for 25 minutes, then removed and placed in the same solution *without* dye for about one hour.

Guinea pigs were used to study the effect of centrifugal force, rapid succession of heat and cold, immobile suspension, and various diets. Difficulty in interpreting the results, which varied only slightly from the normal, led to investigation of the more specific action of immunoelectrophoresis. In addition to the electrophoresis of the antigen (guinea pig serum), there was the highly specific immune reaction between the antigen and an antibody (antiserum produced in rabbits). This analysis differed from the technique of electrophoresis in that it was carried out in an agar gel and the arcs of precipitate made dyeing unnecessary.

Filter paper electrophoresis is valuable because of the simplicity of operation once the technique is perfected. Experimentation in this field uncovers facts about the complexity of proteins, and thus allows more concentrated study on individual proteins.

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