IN THE LABORATORY

Dialysis of Protein Solutions for Electrophoresis

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Blood serum and other biological systems containing proteins as well as low-molecular, diffusible ions and organic compounds must be equilibrated against a suitable buffer mixture prior to study in the electrophoresis apparatus. By establishing Donnan equilibrium between the colloid and the supernatant solution, boundary anomalies due to pH and conductivity differences are eliminated to a certain extent (cf. *4*). At present it is standard procedure to dialyze the protein solution, after diluting it with buffer, against a large volume of the same buffer through Cellophane tubing at low temperature and for a considerable period of time. Thus, Longsworth, *et al.* (*3*), in their electrophoresis experiments on normal and pathological human blood serum and plasma, employed static dialysis at 0°–2° C for 3 days, changing the outside buffer solution every day. As an alternative procedure, these workers rotated the buffer solution for 1 hr at room temperature, replaced it by fresh buffer, and allowed dialysis to proceed for 24 hrs or more at 0°–2° C.

In clinical work, the time required for the equilibration process represents a factor of major importance since it delays appreciably the examination of the serum or plasma in the electrophoresis apparatus and hence the availability of the results to the medical staff. At the suggestion of Kurt G. Stern and with his help we have studied the time course of dialysis with a view of reducing it to a minimum compatible with the requirements of precise electrophoresis experiments. Various forms of mechanical dialyzers were built by the American Instrument Company, Silver Spring, Maryland, through the active cooperation of Gerald H. Lovins and Alfred Henley, of the Company's Research Department, and tested for performance under routine conditions in our laboratory. The construction of the dialyzer adopted for use as a result of this experimentation is shown in Fig. 1. For details of the design and components, reference is made to the figure. The Cellophane tubing employed in the experiments was seamless Visking sausage casing (Batch No. 295) placed at our disposal through the courtesy of the Visking Corporation, Chicago.

For the experiments reported here we used pooled human serum samples and sodium barbiturate buffer of 0.1 ionic strength and pH 8.5, as recommended by Longsworth (*2*) for electrophoretic experiments on human serum. For dialysis, one part of serum was diluted with two parts of barbiturate buffer. It was then placed in the Cellophane tubing and equilibrated with mechanical stirring against 25 volumina of the same buffer under the conditions to be described. In order to follow the process quantitatively, it was interrupted at frequent intervals, and aliquots of the protein solution were withdrawn from the dialysis bag into a Shedlovsky conductivity cell (of

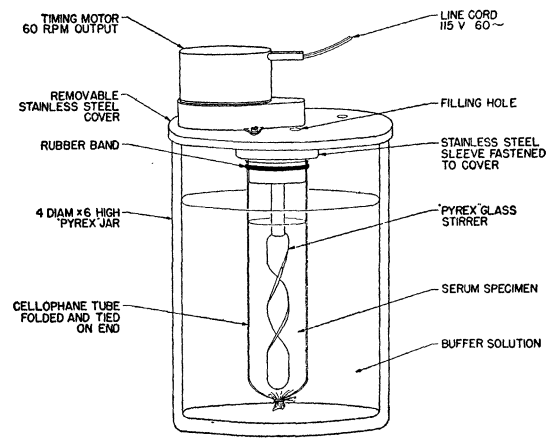

FIG. 1. Mechanical dialyzer (longitudinal section), showing Cramer synchronous motor, 115 v, 60 cycles AC, 60 r.p.m.; gear box of motor; stainless-steel cover with two holes; metal sleeve for dialysis bag, welded to cover; glass stirrer, made of 5/16" rod, 5" long; coupling of stirrer to motor axle, made of plastic tubing; protein solution, 30-ml volume; dialysis bag, made of Cellophane tubing, approximately 5" long and 1 1/8" in diameter, tied at bottom, and attached to sleeve by rubber band; outside buffer solution, approximately 770-ml volume; and Pyrex glass jar, 4" wide and 6" high (Corning No. 850).

the type shown in *3*, p. 403), which was immersed in an ice-water mixture contained in a Dewar vessel. As soon as temperature equilibrium of the sample had been reached, the conductance was measured with the aid of an electronic conductivity bridge of the design of Frederick Kavanagh, kindly placed at our disposal by the Klett Manufacturing Company. This instrument consists of an R–C oscillator, giving signals of 1,000 as well as 2,200 cycles/sec frequency, a three-stage resistance-capacitance coupled amplifier, and the bridge proper incorporating a series of General Radio decade resistors of high precision. As indicators of balance between the unknown electrolytic resistance and the decade resistors, a 6E5 "magic eye" tube as well as a headphone is employed. The latter will detect an imbalance as small as about 0.002%, while the sensitivity of the "magic eye" indicator amounts to only 0.1% of imbalance. Before measurements, the bridge was checked against a cali-

brated General Radio precision resistor of 10,000 ohms. The conductivity cell had a cell constant of C = 6.69, as determined by calibration with 0.1 N KCl solution. Each time when dialysis was interrupted by removal of the Cellophane bag from the dialyzer, a 5-ml aliquot was removed from the outside buffer solution for subsequent conductance measurements. Upon completion of the measurement of the protein sample, it was returned to the solution in the bag and dialysis was promptly resumed. In this manner, appreciable volume changes of the serum samples during the course of the experiment were avoided. While conductance measurements were mainly relied upon for determining the rate of equilibration of the serum against the buffer, pH-measure-

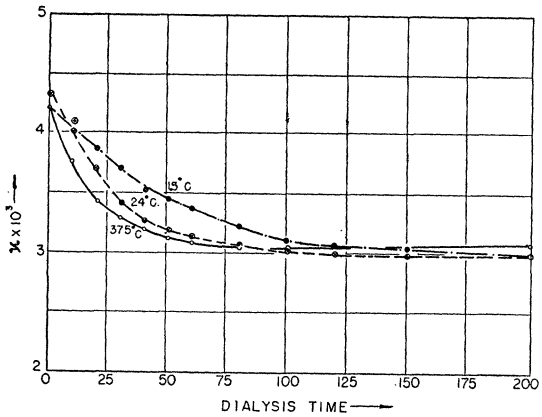

FIG. 2. Equilibration of human blood serum against barbiturate buffer through Cellophane at three different temperatures, with mechanical stirring.

ments were also performed on the inside and outside solutions before and after the experiment as additional checks.

In order to study the temperature dependence of the equilibration process, dialysis experiments were conducted at 1.5, 24, and 37.5° C by immersing the entire dialyzer into water baths of the appropriate temperature and allowing the outside buffer solution to attain the temperature of the bath before starting the experiment. While the conductance of the buffer solution changed little throughout the experiment, that of the diluted serum decreased and approached a value slightly below that of the buffer solution at a rate which was a function of temperature. Changes in conductance of the serum samples throughout the course of dialysis for the three temperatures mentioned above is shown graphically in Fig. 2. It is evident that the initial slope of the conductance curves increases with increasing temperature. While equilibration at 37.5° C is practically complete after 60 min of dialysis, 100 min are required at 24° C and 150 min at 1.5° C. For practical purposes, room temperature appears to be preferable to either 1.5° or 37.5°, since the rate of equilibration at 20°–24° C is sufficiently rapid to allow for the dialysis of 6 serum samples in three dialyzers per 8-hr working day without involving the risk of secondary changes which might conceivably occur during dialysis at 37° C.

The pH and specific-conductance values of the diluted serum and buffer solutions before and after equilibration are shown in Table 1. The small but consistent differences in the final conductance values are due to the Donnan effect caused by the presence of nondiffusible colloid ions, viz. the serum proteins (cf. *1*).

TABLE 1

EQUILIBRATION OF HUMAN SERUM AGAINST BARBITURATE BUFFER BY DIALYSIS THROUGH CELLOPHANE WITH MECHANICAL STIRRING

	Human serum (3 times diluted)		Barbiturate buffer ($\mu = 0.1$)	
Dialysis	Initial	Final	Initial	Final*
Temperature	pH $\kappa \times 10^3$	pH $\kappa \times 10^3$	pH $\kappa \times 10^3$	pH $\kappa \times 10^3$
1.5° C	... 4.20	8.54 2.98	8.55 3.08	8.55 3.14
24° C	8.62 4.31	8.60 3.00	8.58 3.09	8.59 3.15
37.5° C	8.49 4.23	8.51 3.09	8.53 3.08	8.52 3.21

* All final values were determined after 200 min of dialysis, all conductivities were measured at 0° C, and pH values were determined with a Cambridge pH-Meter at room temperature.

Next, the effect of mechanical stirring on the rate of equilibration was determined by performing two experiments at 24° C, in one of which the stirrer was kept stationary. The results (see Fig. 3) showed clearly the

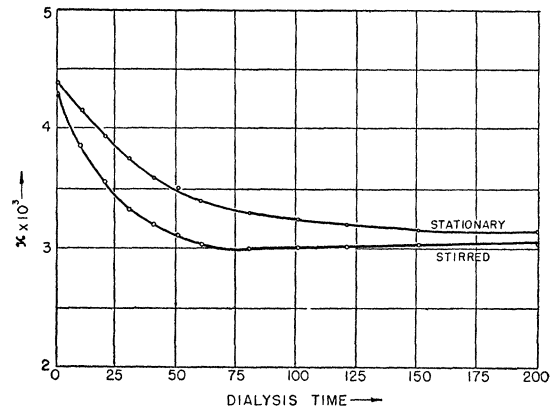

FIG. 3. Effect of mechanical stirring on rate of equilibration of serum against barbiturate buffer at 24° C.

increase in equilibration rate with mechanical stirring: while in the latter case equilibrium was reached after approximately 60 min of dialysis, equilibrium was incomplete even after 200 min without stirring.

For the sake of economy, changes of the outside buffer solution were omitted; this is permissible in the instance of blood serum, the chief inorganic components of which are sodium chloride and sodium bicarbonate. When ammonium sulfate or sodium sulfate containing protein fractions are dialyzed prior to electrophoretic examination, the outside buffer solution should be renewed before electrophoresis because of the considerable increase in conductance caused by the neutral salt.

The experiments described show that it is possible to equilibrate protein solutions against a buffer within 2 hrs, using Cellophane tubing and a simple mechanical dialyzer. This reduces considerably the time required for electrophoretic examination of clinical material, e.g. blood serum.

References

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A Simple Aid for the Cannulation of Small Blood Vessels

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Cannulation of the larger blood vessels (1 mm or more in diameter) offers no special difficulty. The most common method is to pick up a small portion of the wall of a vessel with tissue forceps, snip part way through the wall (about one-third of the circumference), lift up the triangular flap with the tissue forceps, and insert the cannula through the opening. If the head of the cannula is not too large for the size of the opening in the vessel, no difficulty is encountered. In the case of smaller blood

FIG. 1. Forceps to aid cannulation of small blood vessels.

vessels, however, such as the artery of the rabbit's ear, the caudal or carotid artery or jugular vein of the rat or mouse, or the aorta of a small frog or toad, cannulation may prove difficult and time consuming. Making the usual cut in the wall of a very small blood vessel is difficult, even with small sharp-pointed scissors, and when this is accomplished, the cut vessel is frequently torn across during manipulation for the insertion of the cannula. For this reason, either a sharp metal needle or sharp-pointed small glass cannula of appropriate size has usually been inserted into the lumen by direct puncture of the wall of the vessel. This has the obvious disadvantage of leaving in the vessel a sharp point which frequently punctures the wall at some other site, if the animal moves or if the cannula is manipulated. For these reasons it is considered of interest to report on the development of a simple contrivance (Fig. 1) which enables the rapid insertion of a blunt glass, metal, or plastic cannula into a small blood vessel.

The lower portions of the two blades of a small tissue forceps are bent at a right angle and sharpened into two thin, short, (about 3 mm) sharp-pointed prongs with flat inner surfaces which can be brought into perfect contact by pressure on the handles. The distance which the blades of the forceps are permitted to separate is first adjusted with the set screw, as shown in Fig. 2. A slight pressure exerted on the handles approximates the prongs of the blades to form a thin, sharp needle with which the wall of the vessel is pierced. Upon release of the pressure on the handles the prongs separate to the predetermined distance, creating an opening through which the head of the cannula can be introduced. The

FIG. 2. Position of forceps during insertion of cannula into blood vessel (×5).

prongs also act as guides for the insertion of the head of the cannula. With the blood vessel held in position, a slight upward pull of the forceps results in an enlarged opening which further facilitates insertion of the cannula. The blades of the forceps are then withdrawn from the vessel, leaving the head and neck of the cannula in place, to be tied in in the usual fashion. Precaution to keep the vessel moist is observed throughout the course of these manipulations. If the head of the cannula is not too large for the opening in the wall and for the lumen of the vessel, the insertion is accomplished easily and expeditiously.

In experiments in which it was frequently necessary to cannulate small arteries and veins as small as 0.3 mm in diameter, this instrument proved of great help. The device can also be used for the cannulation of large vessels, because it facilitates the insertion of a cannula with a head of even larger diameter than that of the natural, undistended lumen of the vessel, and because this is accomplished with a minimum of injury to the wall of the vessel.