IMMUNOSEDIMENTATION PROTEIN ASSAY

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Sedimentation analysis of proteins has undergone long development not only along the line of utilization of the analytical ultracentrifuge, with its optical recording system, but also with the use of preparative ultracentrifuges, followed by assay of fractions withdrawn from the centrifuge tube (CT) by means of peristaltic pumps [4, 6, 7]. Protein fractions thus obtained are analyzed mainly by immunodiffusion methods. It must be noted that immunodiffusion analysis [3, 5, 9, 13] has so far been carried out in separate stepwise-collected fractions, and no method yet exists analogous to that in [8] which would give a continuous, smooth analysis, for the technique of continuous transfer of all layers of the column of liquid with sucrose gradient from CT has not yet been developed. The advantage of continuous assay is widely known [3, 8]. During stepwise collection of fractions two proteins, which have begun to separate during ultracentrifugation, may be collected in the same fraction and be mixed again. During continuous analysis all minor degrees of separation which have appeared in the first stage are well preserved and provide a wealth of information after immunodevelopment [8].

The writers suggest a method based on the properties of a cuvette with agar walls. In a vertical cuvette whose walls consist of agar gel, any substance can pass from the walls into the lumen of the cuvette (into solution) smoothly, without mixing of the layers of the solution and, conversely, may pass back from the solution into the walls. This is facilitated by a concentration gradient of sucrose in both the solution and the agar walls (vertical gradient). One such diffusion method of transport of mulecules from the agar wall of a vertical cuvette into solution (and in the opposite direction) without disturbance of the laminarity of the liquid in the cuvette was described previously [1]. On the basis of this method a number of design solutions are possible. One of them is examined in this paper.

After sedimentation of proteins the tube in which ultracentrifugation was carried out was connected end to end with the lower opening of a "sluice" (a channel in an agar block), and the column of liquid from CT was drawn up into the "sluice" by hydraulic displacement, by injecting 60% sucrose through a needle, inserted into CT as far as the bottom, by a peristaltic pump or syringe [12]. The liquid was raised into the "sluice" together with the protein zones. Short-term electrophoresis was carried out in the horizontal direction to incor-

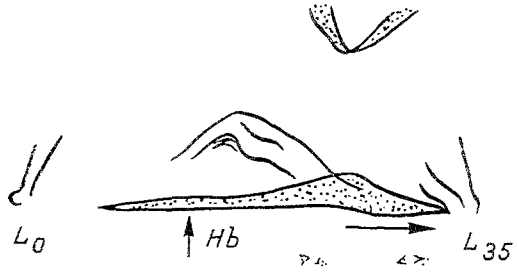

Fig. 1. Scheme of pattern obtained by immunoelectrophoresis. 0) Meniscus, L_{35}) segment from meniscus to point at a depth of 35 mm; Hb) postiion of hemoglobin zone. Horizontal arrow indicates direction toward bottom of CT.

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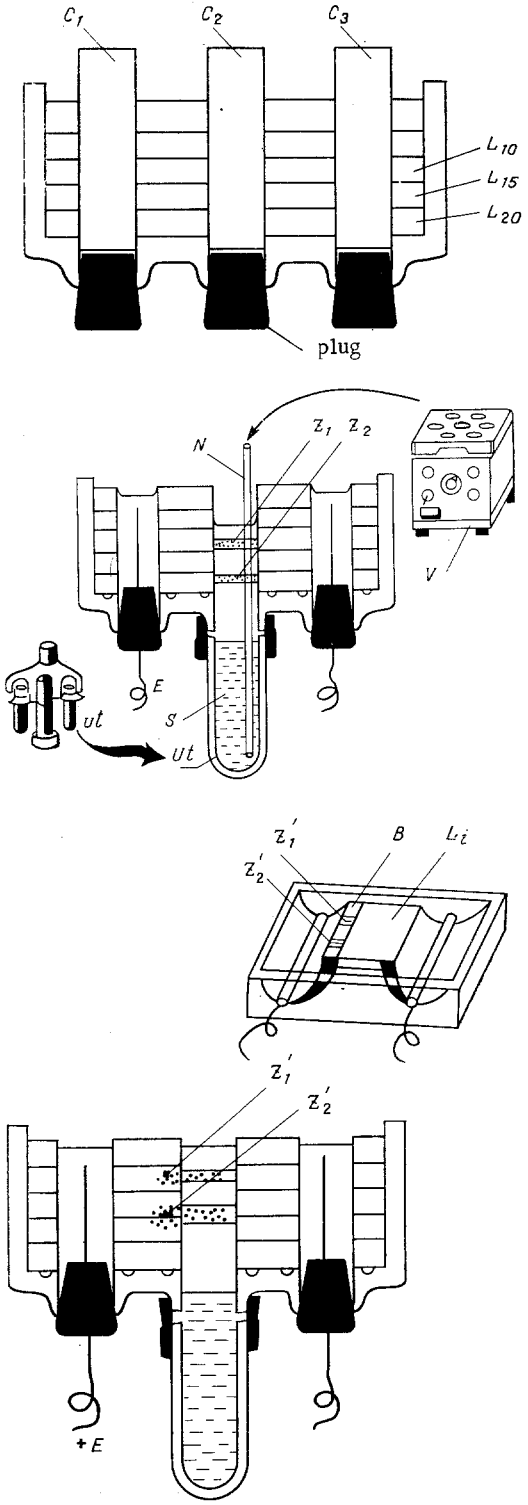

Fig. 2. Successive stages of immunosedimentation. A) Pouring the agar block; T) tubes; C_1-C_3) cylinders around which channels are formed in agar (C_1 and C_3 for electrodes, C_2 for "sluice"; L_{20}) layer containing 20% sucrose; L_{15}) 15% sucrose, and so on B) electrodes (E) are inserted. CT (Ut) connected to "sluice." Sucrose (S) introduced on bottom of tube through needle (N) for peristaltic pump (V). Protein zones (Z_1, Z_2) rise into "sluice"; C) zones have risen into "sluice". Penetration electrophoresis carried out. Zones migrate into agar and have formed zones in agar (Z_1', Z_2'). Bar of agar (B) transferred into cuvette for horizontal electrophoresis, in which electrophoresis by Laurell's method is carried out (Li).

porate the protein zones into the agar wall of the "sluice." Two vertical fragments were cut from the anodal wall of the "sluice" and from the cathodal wall. The fragments were placed on a slide which was covered with agar solution containing antiserum.

Crossed immunoelectrophoresis was carried out by Laurell's method with the formation of continuous (uninterrupted) precipitation lines for each protein.

The pattern of precipitation obtained in the experiments is illustrated systematically in Fig. 1.

The experimental conditions were as follows. A sample of 0.1 ml human blood serum was obtained and applied to a sucrose gradient. A tube measuring 12.5 × 50 mm was used. A gradient of 20 to 5% sucrose was prepared in a mixer in buffer of 0.012 M Tris and 0.095 M glycine, adjusted to pH 8.3. Sedimentation was carried out for 3.5 h at 40,000 rpm on a Beckman (USA) L-65 centrifuge in SW-50 rotor.

The tube in which ultracentrifugation was carried out was joined end to end by means of a rubber sleeve with the lower opening of a "sluice" channel 11 mm in diameter in a 1% agar block 45 mm high, with horizontal section of 20 × 90 mm; the agar was made up in the same buffer. Preliminary experiments showed that after displacement of the liquid from CT into the "sluice" the zones began to fall slowly. The hemoglobin zone, which was visible, fell in this case by 20 mm/h. This evidently took place through dilution of the sucrose in the "sluice" near the agar walls and by upward movement of the solution in those places, and for that reason this part of the zone which was closer to the axis of the channel fell. Under these conditions an analytical experiment was impossible. To stabilize the zone, an agar block was poured in the form of five layers containing sucrose in concentrations of 20, 15, 10, 7, and 5%. Protein zones were stabilized in such an agar block with a sucrose gradient. It was found that after 45-60 min the stepwise gradient had become smooth. Protein zones raised in to the channel with this kind of sucrose gradient remained *in situ*. Mixing of the layers of sucrose gradient from the tube with those in the agar called for no special measures, and all that was needed was exact coincidence of the meniscus with the upper surface of the agar block.

To form the channel of the "sluice" and the channels for electrodes, three plastic cylinders (11 × 65 mm) were inserted into the hole at the bottom of the cuvette (Fig. 2) during pouring.

The cuvette was filled with liquefied agar. After the agar had solidified, the cylindrical molds were removed, with the formation of three channels in the agar block. The middle channel was used as the "sluice," the other two as electrode reservoirs. They were closed with rubber stoppers, each carrying an electrode.

After CT had been joined to the "sluice" 60% sucrose solution was introduced on the bottom with a needle 12 cm long by means of a syringe or (better) a Vario Perpex peristaltic pump (from LKB, Sweden).

After the liquid containing the zones had been raised from CT into the vertical cuvette ("sluice"), electrophoresis was carried out.

Protein zones migrated in the horizontal direction and entered the agar walls of the vertical cuvette ("sluice"). Electrophoresis was complete after 60 min at 20 V and with a distance of 68 mm between the axes of the electrode reservoirs (channels).

A vertical agar fragment along the whole wall of the sluice was excised. The piece was placed from the anode side on a glass plate. The surface of the glass around the fragment was covered with a mixture of agar and antiserum against all serum proteins. Electrophoresis was carried out by Laurell's method [9].

The agar plate was washed in 5% NaCl for 72 h. It was then dried, stained with Coomassie R-250, and rinsed with ethanol.

"Rockets" with a maximum at different distances from the meniscus end of the fragment can be seen in Fig. 1. Their positions correspond to the depths of CT and, consequently, to different sedimentation coefficients. This fact suggests that the further development of this method may lead to wide investigation of proteins.

It must be pointed out that at the moment of electrophoresis in the "sluice," each geometric point belonging to a line running along the "sluice" wall (i.e., along the molding cylinder), protein molecules from the layers of the solution are incorporated into the cor-

responding point of the agar layer of the "sluice." This process is continuous in the vertical direction, and it is not interrupted by arbitrarily chosen stepwise fractions. The advantages of continuous immunosedimentation analysis are similar to the advantages of immunoelectrophoresis by the method of Grabar and Williams [8] compared with separate analysis [9, 11] of individual stepwise fractions. In general, any two-dimensional fractionation — whether two-dimensional electrophoresis [10] or two-dimensional chromatography [2] — cannot be replaced simply by rechromatography, i.e, by chromatography (in a new system of solvents) of the separate fractions collected at the first stage. The new technique also can be used to investigate proteins with a very large molecular weight, for they are fractionated in an aqueous medium and not in gel.

The method is suitable for analysis of different protein mixtures; it has passed its test for analytical work on neonatal serum for investigation of the immune status.

By contrast to electrophoresis in polyacrylamide gel [10] in sodium dodecylsulfate, the method described above can be used to characterize, not protein subunits, but native, undegraded protein molecules.

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DISTORTIONS OF RESPIRATORY PARAMETERS DURING MUSCULAR WORK IN INVESTIGATIONS USING MASKS

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The study of external respiration during muscular activity is usually based on the spirographic principle. This technique requires a mouthpiece or a mask worn on the face, and the use of these appliances increases the dead space and the resistance to respiration. These two factors may distort parameters of respiration. The use of a mask is known to reduce the respiration rate [6, 7]. It has been suggested that the tidal volume (TV) is increased at the same time, but this has not been verified because of the lack of any maskless method of determining the pulmonary ventilation [9]. Changes in TV have been estimated principally purely on the basis of changes in the degree of resistance to respiration when using a mask [1-3, 8].

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