Albumen-gel as a Supporting Medium in Zone Electrophoresis

Electromigration of proteins in an albumen-gel could be followed for coloured proteins, detection of colourless proteins being otherwise impossible with the usual staining method because of the nature of the supporting medium. The difficulty could be overcome by using ^{131}I-labelled proteins and detecting them by autoradiography. The separatory power for serum proteins of albumen-gel electrophoresis was then investigated.

Albumen from eggs (dry fine powder, E. Merck AG, Darmstadt) at 13% concentration (w/v) was dissolved in a buffer containing 0.152 *M* tris-(hydroxymethyl)-aminomethane, 0.010 *M* citric acid and 0.015 *M* sodium azide (pH 8.9). After overnight standing at room temperature, a clear solution was obtained by filtration (Schleicher & Schüll N.595 filter paper) of the supernatant obtained by centrifugation of the original solution at 3000 g for about 30 min. 900 ml of the solution (the final protein concentration and pH were about 10.5% and 8.5 respectively) were carefully poured into a Pyrex tray with a glass plate (27.5 × 20.0 × 0.3 cm) at the bottom. The tray, covered with a heavy glass lid sealed with Parafilm, was kept at 85° for 45 h. After the gel had cooled, it was freed from the edges of the tray with a razor and then, by inserting a spatula between the glass plate and the bottom of the tray, it was removed while still firmly attached to the glass plate. It was transferred to a clean tray and submerged with tris buffer diluted 1:1 with water. The washing of the gel was continued for 7 days with daily changes of tris buffer. Most of the soluble proteins diffused from the gel in the first 48–72 h. Some of the fractions were undialysable and a study is in progress for their characterization.

The starch-gel electrophoretic patterns (SMITHIES' technique[1]) of the soluble albumen proteins prior to gel formation of the hen and human serum proteins are shown in Figure 1.

The albumen-gel could be stored for months at +2°, was white and had little mechanical strength.

The gel slab was prepared for electrophoresis by performing the following operations: (1) A very tense stainless steel wire (0.2 mm) was passed between the bottom of the gel and the glass plate, thus allowing a uniform gel contraction during the electrophoresis. (2) Excess liquid was eliminated by gently blotting the top of the gel with absorbent paper. At this stage the gel slab size was about 26.5 × 19.0 × 1.1 cm. (3) The protein solutions (70–80 mg/ml) were taken up in Ford's A 4 filter paper strips (about 0.065 ml per cm^2 of filter paper) and these were introduced into vertical slits in the gel.

Electrophoresis was carried out with the gel slab in the horizontal position, each bi-compartmented vessel containing borate buffer (0.30 *M* boric acid and 0.07 *M* sodium hydroxide). During electrophoresis the gel was protected from loss of water by evaporation and it was suitably cooled by means of a running tap-water system (BOCCI[2]). As the electrophoresis proceeded, a visible boundary moved through the gel towards the anode concomitantly reducing the thickness of the gel by about 1/3. Electrophoresis was considered complete in about 10 h when the boundary was 3–4 cm from the anodal end: the voltage gradient was about 5 V/cm (current of 160–190 mA). Thereafter the gel was left in methanol-distilled water-glacial acetic acid (50:50:10, v/v) for at least 24 h and was then easily sliced into two halves. Autoradiography

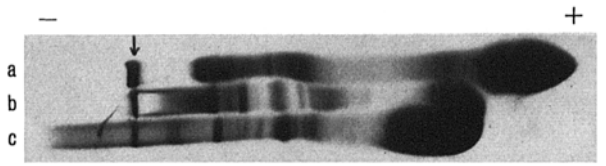

Fig. 1. Starch-gel electrophoretic patterns of (a) egg albumen soluble proteins, (b) serum proteins of laying-hen and (c) human serum proteins. The origin is indicated by the arrow.

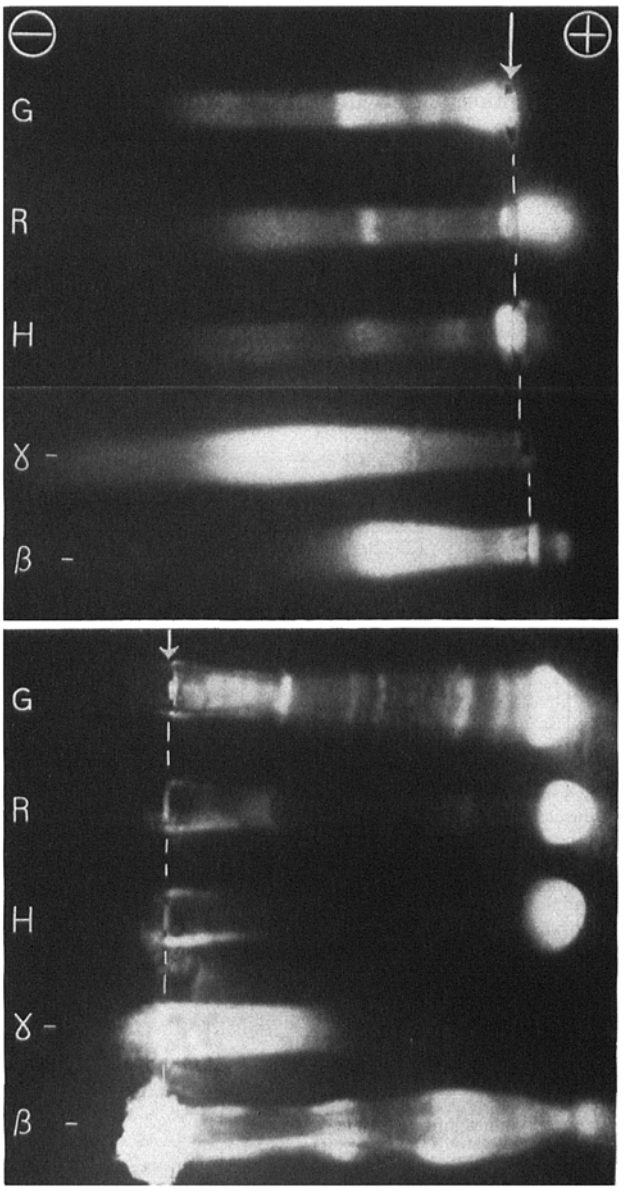

Fig. 2. Albumen gel (upper) and starch-gel (lower) autoradiographs of ^{131}I-labelled guinea-pig (G) serum, rabbit (R) serum, human (H) serum, human γ-globulin (γ-) and β-globulins (β-). The origin is indicated by the arrow; exposure time 3 days.

[1] O. SMITHIES, Biochem. J. *71*, 585 (1959).
[2] V. BOCCI, Science Tools *10*, to be published.

of the slices was carried out by sealing them inside polythene bags and placing their surfaces in contact with X-ray films (Kodirex).

Samples of guinea-pig, rabbit, and human serum and of human γ- and β-globulins were labelled with ^{131}I (McFarlane[3]) and were separated either by albumen-gel or by starch-gel electrophoresis (Poulik[4]). The autoradiographic patterns are shown in Figure 2. As a high electro-osmotic flow was present, the origin was set at 7–8 cm from the anodal end of the slab. The albumin was visible because of binding bromo-phenol blue and entered the gel on both sides although it moved very little from the origin. However, rabbit albumin was all on the anodal side. The γ-globulins moved far from the origin towards the cathode and showed a large range of electrophoretic mobilities. Transferrin was localized next to γ-globulin as a narrower band of characteristic salmon-pink colour. α-globulins were always on the cathodal side between albumin and β-globulin. Rabbit haemoglobin, without showing interactions with the stabilizing medium, could be located at about 4 cm from the origin towards the cathode. Results were easily reproducible. This investigation presents evidence that autoradiographic patterns of serum proteins obtained after separation on a proteic gel medium resembled those obtained in a starch-gel, the latter medium having, however, higher resolving power. The strong electro-osmotic flow did not diminish with the use of cellulose membrane barriers or by increasing the ionic strength of the buffer. Attempts to modify the pore size of the gel either by increasing the concentration of albumen proteins, or by separating the serum proteins in a gel slab previously having undergone a blank electrophoresis, did not change the electrophoretic pattern[5].

Riassunto. Un gel composto dalle numerose frazioni proteiche dell'albumina d'uovo fu preparato ed usato quale mezzo di supporto per la separazione elettroforetica di siero-proteine marcate con ^{131}I.

A causa di un notevole flusso elettro-osmotico la separazione proteica, dimostrabile mediante autoradiografia, si spostò verso la regione catodica mostrando tuttavia analogie con quella ottenibile su gel di amido.

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[3] A. S. McFarlane, Nature *182*, 53 (1958).
[4] M. D. Poulik, Nature *180*, 1477 (1957).
[5] Supported by a grant from the Consiglio Nazionale delle Ricerche, Roma.

PRO LABORATORIO

Laboratory Deep Culture of Micro-Organisms Using a Vibrating Stirrer

Deep-culture methods with forced aeration are frequently required in the laboratory in order to obtain the required amount of cell mass or metabolic products of aerobic organisms in one single batch, or to increase the rate of growth or metabolism, or to carry out scaling-up operations. A homogeneous culture (i.e. absence of cell clumps) and a reliable mechanical set-up for aeration and agitation will undoubtedly increase the reproducibility of the results.

The earlier methods for mass cultivation of aerobic micro-organisms consisted in forcing air through sintered glass into the substrate without using mechanical agitation. Satisfactory results on the laboratory scale could then be obtained for propagation of yeasts and bacteria where it is easy to obtain a high degree of homogeneity of the culture. Filamentous fungi, however, tend to agglomerate, and can form pellets of variable and not always reproducible size; furthermore, they can increase the viscosity of the culture very strongly and thus make a uniform oxygen transfer to the fungal hyphae impossible; clogging of the sintered glass by fungal growth is also a common observation. Using more or less strong agitation the first two disadvantages can generally be avoided, thus restoring homogeneity and reproducibility and maintaining the growth rate at a sufficiently high level. Agitation is generally done by a rotating stirrer fitted into the culture vessel, alternatively the vessel itself may be rotated. In a recent review[1] various types of apparatus have been described. If contaminations are completely to be avoided, technical complications arise which cannot be ignored even if a positive pressure is maintained inside the vessel.

In the present article a vibrating stirrer (Vibro Mixer) is described, of which the major advantages are the omission of rotating parts, a very fine distribution of air without using sintered glass, and easily adjustable degree of agitation. The apparatus has now been used for many years and has given interesting results in the study of growth kinetics of moulds, in the production of amylase by *Aspergillus oryzae* and for the oxidation of long-chain hydrocarbons by micro-organisms.

Description and Operation of the Apparatus[2]. Vertical vibrations are generated at the frequency of the alternating current of the mains and are of gradually variable amplitude with a maximum of about 3 mm. The stirrer consists generally of a circular plate fitted firmly to a shaft which is inserted into the holder of the vibration generator. A considerable degree of agitation can be imparted to the liquid with a large amplitude when position and size of the stirrer plate in relation to the vessel are suitably chosen (by trial and error). For aseptic work the stirrer is fitted through a tight sealing membrane (preferably of neoprene) mounted in a holder which can be inserted into a rubber bung in the neck of the culture vessel. The whole vessel can be sterilized with the stirrer *in situ*, disconnected from the vibrator.

[1] R. Elsworth, in *Progress in Industrial Microbiology* (Hockenhull, ed., 1960), vol. 3, p. 103.
[2] Full technical details of the presently described apparatus are given in the descriptions of the manufacturer: Chemie Apparatebau AG., Männedorf, Zürich (Switzerland).