A Modified Method of Filter Paper Electrophoresis

In the course of our work on the separation of protein fractions from wheat gluten by means of paper electrophoresis, it was found that good resolution of hitherto unresolved fractions could be achieved by paper electrophoresis using long paper strips and high voltages over an extended period of time. This method does not require an organic cooling liquid, such as monochlorobenzene[1]. It may be useful for the separation of enzyme mixtures, where contact with organic liquid may inactivate the enzymes.

In this method, the filter paper strip is wrapped, spiral fashion, around a glass tube, 22 in. long and $\frac{3}{4}$ in. external diameter, through which cold water is running (Fig. 1). To minimize evaporation from the strip, it is covered with a strip of thin 'Cellophane' of the same width. Humid conditions are maintained by enclosing a portion of the glass tube in a wooden box, which is lined with filter paper saturated with buffer. The ends of the filter paper, covered by 'Cellophane', pass in and out of the humid chamber by way of two narrow slits at the bottom, $\frac{3}{4}$ in. from each end. 'Cellophane' sleeves, attached to the chamber and terminating below the level of the buffer solutions, prevent the drying out of these portions of the strips.

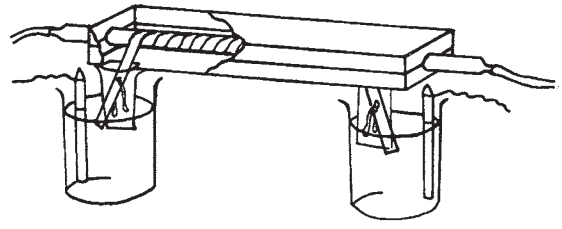

Fig. 1

Before use, the glass tube is treated with silicone grease. The filter paper strip is then passed through one sleeve, to which it is clamped with a paper clip, and wrapped tightly around the glass tube, passing out through the sleeve at the other end and clamped fast. To permit wrapping at an angle, the 'Cellophane' sleeves—through which the paper strips pass—are slit along the outside edge leaving a short uncut portion adjoining the box. At one end, together with the filter paper strip, a 'Cellophane' strip of the same width as the filter paper is clamped in. The point of application for the protein mixture is then marked on the paper and the buffer painted on with a brush. This procedure prevents tearing and bruising of the paper, which may occur when a buffer-moist, blotted strip is wrapped tightly around the glass tube. The substance is then applied to the marked line and the 'Cellophane' strip wrapped tightly so as to cover the whole length of the strip and to pass out through the second sleeve. The 'Cellophane' sleeves, filter paper and 'Cellophane' strip are then pierced and weighted with a piece of bent glass rod and the paper clips removed. The buffer vessels and the carbon electrodes are then placed in position below the chamber.

In our experiments, strips of Whatman 3*MM* paper, 60 cm. long and 2·5 cm. wide, were used. For a run of 24 hr. with a potential gradient of 9 V./cm., 2 × 2 litres of McIlvaine's buffer *p*H 2·07 and ionic strength of 0·025 were used. Unless high voltages are used (17 V./cm.), no condensation of water on the exposed surface of the glass tube takes place; but even where this happens, loss of substance through migration off the paper rarely occurs. The 'Cellophane' covering the strip prevents the redepositing of moisture on the filter paper. Protein spots obtained by this method are sharp and as well defined as spots obtained in electrophoresis between glass plates.

The full results of this investigation will be published elsewhere.

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[1] Consden, R., *Nature*, 170, 1069 (1952).

Isolation of Fungi from Hyphæ present in Soil

While it is known that fungi may exist in soil both as active mycelium and as dormant spores[1], a major problem in the study of soil fungi has been to discover which fungi in a soil are present as mycelium. Plating methods by which fungal colonies are isolated from soil[2] or from soil suspensions[3] on agar media suffer from the disadvantage that the unit of origin, spore or hypha, of the resulting colonies is not known. Direct microscopic examination, either of Rossi–Cholodny slides or soil films[4], has the complementary deficiency that although fungal hyphæ are seen, most are sterile and their identity cannot be investigated. I have devised a simple technique for the isolation of fungi directly from hyphæ present in soil. Essentially the method depends on the observation that when a soil suspension is prepared, many of the fungal hyphæ remain with the heavier soil particles of the residue. Removal of the fine suspended material from the residue also permits visual examination of the latter for the presence of individual hyphæ or hyphal masses, which may then be removed and grown on agar media.

A soil crumb of about 1·0–1·5 gm. is placed in a beaker part-filled with water and left to become saturated. After 4–5 min. the crumb is broken apart by filling the beaker with a rapid jet of tap water. The heavier soil particles are allowed to sediment for 1·0–1·5 min. and then most of the suspension is poured off. Further water is added, the heavier particles allowed to sediment and the suspension again removed. This procedure is continued until the liquid remains clear after standing for 1·0–1·5 min. The soil particles of the residue are then distributed in a small quantity of water among three sterile Petri plates and searched for the presence of fungal hyphæ using a binocular dissecting microscope (× 12·5 and × 25). Individual hyphæ or portions of hyphal masses are removed with fine forceps from among the soil particles and are placed in a drop of sterile water on a clean sterile isolation plate.

Since many fungal hyphæ isolated from soil are attached to mineral grains or to humus particles on or in which contaminating fungi and other microorganisms may be present, it is desirable to remove as many as possible of these attached particles. Many may be removed by gently tapping the hypha against the bottom of the plate; others cannot be removed without killing the hypha. Hyphal masses may also need to be teased out, since they usually consist of more than one species of fungus. When sufficient hyphæ (usually 20–50) are on the plate, it is poured with melted but cooled agar and the hyphæ