ethylene, the overgrowth observed on these spherulites is in accordance with the conclusion that the molecules of polyethylene are tangentially arrayed in the spherulites.

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Use of Pectin in Gel Electrophoresis

Electrophoresis of protein mixtures in agar gels has been described by Gordon *et al.*[1]. It has been developed by Grabar and Williams and used in their immuno-electrophoretic method[2]. The latter, thanks to the specificity and sensitivity of immunochemical reactions, permits the detection of the presence even of the smallest amounts of components in a protein mixture and permits defining them by their electrophoretic mobilities. The agar gels can be dried and stained[3], as in paper electrophoresis. However, the use of agar has one disadvantage: the samples are usually introduced in the gel, mixed with melted agar, thus being heated to 40°–45° C. Thus there is a constant danger of denaturing certain more sensitive protein components. A possible interaction of agar with some of the substances analysed cannot be excluded. Finally, it was of interest to find a gel from which the electrophoretically separated components could be extracted and estimated quantitatively.

Among a number of different substances yielding gels which have been examined by us, pectin seems to present certain advantages over agar. It can be gelled enzymatically at room temperature by pectin-methyl-esterase in the presence of Ca^{++}, the separation of proteins carried out electrophoretically, then either stained or cut out and the proteins released enzymatically by hydrolysis of the gel with pectinase. None of these steps affects the proteins. The latter can be estimated by one of the usual methods (biuret, protein nitrogen, etc.). The use of pectin furthermore has the advantage that the gel is prepared at room temperature and that the gelification can be more easily controlled. We found that the concentration of the pectin gel which gives very satisfactory results is 0·2–0·34 per cent, against that of 1·5–2 per cent of the agar. Thus electrophoresis takes place in 99·8 and 99·66 per cent buffer respectively, a condition approaching that of free electrophoresis.

Our method for preparing the gel is as follows. Immediately before use, to 15 ml. of a 2 per cent pectin-stock solution are added: 10 ml. of 0·65 *M* calcium chloride, 1 ml. 0·1 per cent commercial pectin esterase and 24 ml. double-distilled water, carefully mixed and finally diluted with an equal volume of veronal buffer *p*H 8·2 and ionic strength 0·60. The final ionic strength of the solution is 0·03. In recent runs we found that the mixture of borate and veronal buffers, as described by Consden and Powell[4], also gives very good separations.

The pectin–enzyme mixture is poured over a 13 × 18 cm. Eastman Kodak cover-glass, surrounded by a 0·5-cm. high wall made of heavy-duty aluminium foil. At both ends of the plates, filter paper strips (Whatman No. 1) are placed. The troughs in the middle of the plate, in which the serum samples are placed, are made in a manner similar to those of agar, except that we use as moulds small iron bars (approximately 10 × 5 × 2 mm.), which after the gelation are removed by a small, but powerful, magnet. The empty trough fills in a short time with buffer and to this buffer 0·1 ml. of the sample to be analysed is added, thus yielding a final dilution 1 : 10 (the volume of the trough is about 1 ml.). The plate is then placed on an electrophoresis apparatus, the filter paper immersed in the buffer chambers in the usual way, and the current, 170–200 volts giving 18 m.amp., switched on. After 4–5 hr. the run is completed, filter paper strips removed, the gel is fixed by leaving it for 30 min. in a mixture of alcohol–acetic acid (250 ml. of 40 per cent v/v ethyl alcohol + 3 ml. glacial acetic acid) and finally stained in amido-schwarz for 6 hr. After this period, the excess of dye is washed out of the plate with 60 per cent alcohol–acetic acid mixture (250 ml. alcohol + 3 ml. glacial acetic acid) until the plate is well differentiated. A typical result of such a run with normal human serum is given in Fig. 1.

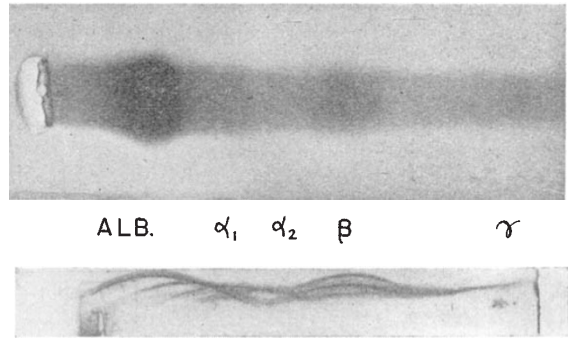

Fig. 1

Our present work consists of the quantitative estimation of these electrophoretically separated proteins, and the application of pectin gels to immuno-electrophoretic analysis (Fig. 1).

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