layer chromatography could be a useful criterion in the classification of dermatophytes in place of morphological identification.

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Artefacts arising from the use of sucrose solutions in electrofocusing techniques

Electrofocusing, pioneered by VESTERBERG AND SVENSSON[1–4], is a relatively new technique for the separation of protein mixtures. It involves the concentration of the proteins at their isoelectric points along a pH gradient by means of high voltage. A feature of the method is the use of a density gradient along the buffer tube to avoid diffusion of the bands by thermal agitation. The most commonly used commercial instrument is similar to that of the original workers and is manufactured by L.K.B. Ltd., the density gradient being formed usually from sucrose solutions.

Using this instrument, it has been found that many samples of "Analar" sucrose contain impurities which focus along the pH gradient in an identical manner to

proteins. Although the precise behaviour varies from batch to batch, the observations recorded in Table I may be taken as typical.

TABLE I

SEPARATION OF BANDS FROM SUCROSE SOLUTIONS

Applied voltage	*pH gradient*	*Bands observed*
300	3–10	None
400	3–5 5–8 7–10	1 diffuse band near anode, after 12 h
600	3–5	4 discrete bands along tube after 36 h

Mixtures of purely synthetic origin have been suggested[5] also to obtain the density gradient, and it is significant that ethylene glycol–glycerol–water (50:25:25 v/v) does not give rise to any bands in a blank determination. A drawback of those solutions, however, is that they require the focusing tube to be refrigerated.

Although these artefacts have not been identified positively, the following suggest strongly that they are protein in nature:

1 They possess isoelectric points.

2 It is not unreasonable to expect protein to be present as an impurity in a product isolated from a vegetable source.

3 "Analar" batches of sucrose may contain up to 0.002 % N. Since 40 g of sugar are used per column run, up to 5 mg of protein could be present.

4 One manufacturer (Schwarz Bio Research Inc.) markets "ribonuclease free" sucrose, from which it may be deduced that protein is a normal impurity present in sugars.

Clearly, it is important to carry out a blank determination on the sucrose density gradient solution itself prior to any protein separation.

Finally, it should be pointed out that stringent precautions were taken to avoid bacterial contamination of the sucrose solutions and the distilled water used and it is concluded that the artefacts are present in the sucrose as received from the supplier.

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