Short Communications

Elution of proteins from starch gel into "Pevikon C-870"

In the last few years numerous techniques have been described for eluting proteins from starch gel[1–3]. The matter is also discussed by SMITHIES[4]. All methods need more or less complicated apparatus and have the undesirable feature of contaminating the proteins with large amounts of soluble starch. Chromatography of the eluate can eliminate soluble starch[5] but the procedure appears to be rather laborious.

The aim of this report is to present a method without these disadvantages. Serum proteins are separated by starch gel electrophoresis[6] and segments of gel containing the proteins to be eluted are inserted in corresponding spaces prepared in a PVK* block[7]. Correct PVK–starch gel–PVK junctions are obtained by gently pressing the PVK (lightly melted by adding drops of buffer) against the starch gel segment. Then by performing electrophoresis the proteins migrate into the PVK from which they are easily eluted[7]. The recovery of serum proteins including albumin, transferrin and Sα_2-globulin ranges between 93 and 99 % and up to 4 gel segments can be handled simultaneously.

An important point to study was the fate of the starch eluted from the gel segment during electrophoresis. Either segments (1–2 × 17 × 1.2 cm) or larger slabs of starch gel, with or without serum proteins, were inserted in PVK beds and electrophoresis carried out up to 23 hours. The PVK blocks were cut in segments of 1 cm each and the presence of soluble starch was investigated in the eluates either by addition of iodine or, for quantitative estimation, by the anthrone method[8]. 18.6 % of the eluted starch was dialysable. The peak of soluble starch was 2–4.5 cm cathodal to the gel segment in conditions in which the proteins moved 2–6 cm towards the anode. In the anodal PVK segments the reaction for starch was positive in the one in contact with the starch gel and only traces of sugars were detectable in the next two.

Thus by allowing the proteins to run in the PVK block about 5 cm ahead of the gel segments it is possible to eliminate carbohydrate contaminants completely. However, a protein fraction could become contaminated by starch from the preceding segment. This was eliminated by enveloping the gel with a cellophane sheet except on the anodal side. Subsequently only dialysable starch was found behind the gel. However, the simplest and most reliable way is obviously to insert the gel segments at great enough distances —about 15 cm—from each other.

In the experiment shown in Fig. 1 ^{131}I labelled[9] serum proteins were used. After autoradiographic localisation three segments of starch gel were cut and inserted in a

* PVK = Pevikon C-870.

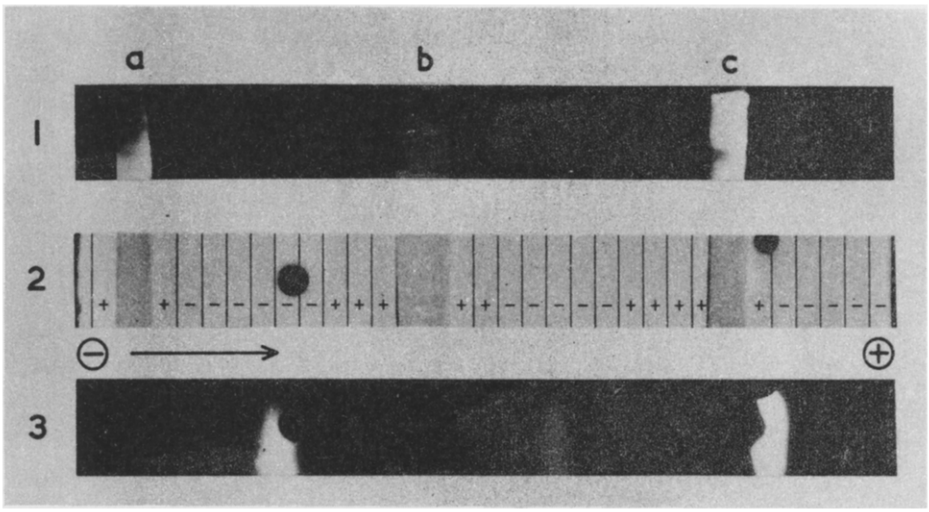

Fig. 1. (1) Autoradiography before electrophoresis: the ^{131}I proteins are in the starch gel segments. (a) albumin; (b) albumin dimer; (c) transferrin. (2) The arrangement of gel segments in the PVK bed photographed at the end of the run. The symbols + or — indicate the reaction for starch. (3) Autoradiography after electrophoresis: the ^{131}I proteins have migrated into the PVK.

PVK block (33.5 × 17 × 1.2 cm) equilibrated with borate buffer (boric acid 0.3 *M*, sodium hydroxide 0.075 *M*; pH 8.45). Electrophoresis was performed at +3°, for 10 hours employing a potential gradient of 3.5 V/cm. Radioactivities were shown to be quantitatively transferred from the starch gel into the PVK although transferrin only migrated a short distance from the gel segment.

If the segments of gel are inserted too near the anode it was noticed that migration of the proteins at a distance from the gel is prevented presumably by hydrodynamic flow.

Further studies of the purification of plasma proteins using this and other methods are in progress. I wish to acknowledge Dr. A. S. McFarlane's interest in this work.

National Institute for Medical Research,
Mill Hill, London V. Bocci
(Great Britain)

1 J. Moretti, G. Boussier and M. F. Jayle, *Bull. soc. chim. biol.*, 40 (1958) 59.
2 P. Jarrige and G. Lafoscade, *Bull. soc. chim. biol.*, 41 (1959) 1197.
3 A. H. Gordon, *Biochim. Biophys. Acta*, 42 (1960) 23.
4 O. Smithies, *Advances in Protein Chem.*, 14 (1959) 81.
5 C. De Paillerets, J. Moretti and M. F. Jayle, *Bull. soc. chim. biol.*, 41 (1959) 1285.
6 M. D. Poulik, *Nature*, 180 (1957) 1477.
7 H. J. Müller-Eberhard, *Scand. J. Clin. & Lab. Invest.*, 12 (1960) 33.
8 L. C. Mokrasch, *J. Biol. Chem.*, 208 (1954) 55.
9 A. S. McFarlane, *Nature*, 182 (1958) 53.

Received June 26th, 1961