THE ELECTROPHORESIS OF SOME HUMAN MUSCLE PROTEINS ON STARCH GELS

B.P. Hughes

Institute of Neurology,
The National Hospital,
London, W.C.1.

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Introduction.

As a means of studying normal and diseased human muscle, starch gel electrophoresis has been used for fractionating the proteins soluble in solutions of low ionic strength. The present communication records some preliminary results of this work and deals only with normal muscle.

Materials and Methods.

Tissue samples: These were obtained by biopsy and immediately transferred to a container cooled in ice.

Protein extracts: As soon as possible after excision muscle samples were homogenized with phosphate buffer (Na_2HPO_4/NaH_2PO_4, pH 7.7 , I=0.07) using approximately 5 ml. of buffer per gram of fresh tissue. The homogenate was allowed to stand for 2 hours at 0° and then centrifuged at 6000 g. for 7 minutes at 0°. Where necessary, lipid material was removed by filtering the supernatant liquid through a plug of glass wool. The filtrate which was usually clear or only slightly turbid and contained about 30 mg. protein (Nx6.25) per gram of muscle, was concentrated approximately ten-fold by ultrafiltration through cellophane membranes under raised pressure of nitrogen. In some instances the protein extracts were dialysed against excess of the phosphate buffer before concentration and any material precipitating removed by further centrifugation. However, as there was no obvious

difference between the electrophoretic patterns of the dialysed and undialysed material, dialysis was omitted in order to reduce the time of manipulation.

Starch gel electrophoresis: This was carried out in a manner similar to that described by Smithies (1955), using commercially available hydrolysed starch (Connaught Research Laboratories, Toronto), but the same buffer was used for electrophoresis as was used for preparing the protein extracts. Electrophoresis was carried out for 17 - 18 hours at a current density of 10 m/a per cm. width of the gel corresponding approximately to a potential difference along the gel of 5 - 6 volts/cm. To avoid overheating the gel was sandwiched between hollow copper plates cooled by circulating water and the whole apparatus was operated in a cold room at 6°. Under these conditions a thermocouple inserted into the gel indicated a temperature of about 18°.

Protein staining: Two methods were used for staining the starch. Slices about 1.5 mm. thick were cut off the top or bottom of the gel and stained with naphthalene black 10B in 50% methanol as described by Smithies (1955); the remainder of the gel was stained and made transparent by the method of Fine and Waszczenko-Z (1958), except that glycerol was used to make the final preparation less brittle. The second method of demonstrating proteins on the gel has the advantage of giving a preparation which can be easily stored and allows very faint components to be demonstrated photographically.

Peroxidase activity: The sliced gels were stained with o-dianisidine by the method of Owen, Silberman and Got (1958).

Aldolase activity: Sections of the gel were frozen, thawed and starch free liquid obtained by supporting the thawed sections on sintered glass discs and collecting the filtrate which separated on low speed centrifugation. The filtrate was tested for activity by the method of Sibley and Lehninger (1949).

Results and Discussion.

Fig. 1 shows the distribution of protein staining material on a typical starch gel electropherogram. For the sake of convenience the bands invariably seen are numbered from 1 to 12 starting with the fastest moving band. There are also other faint components which cannot always be detected.

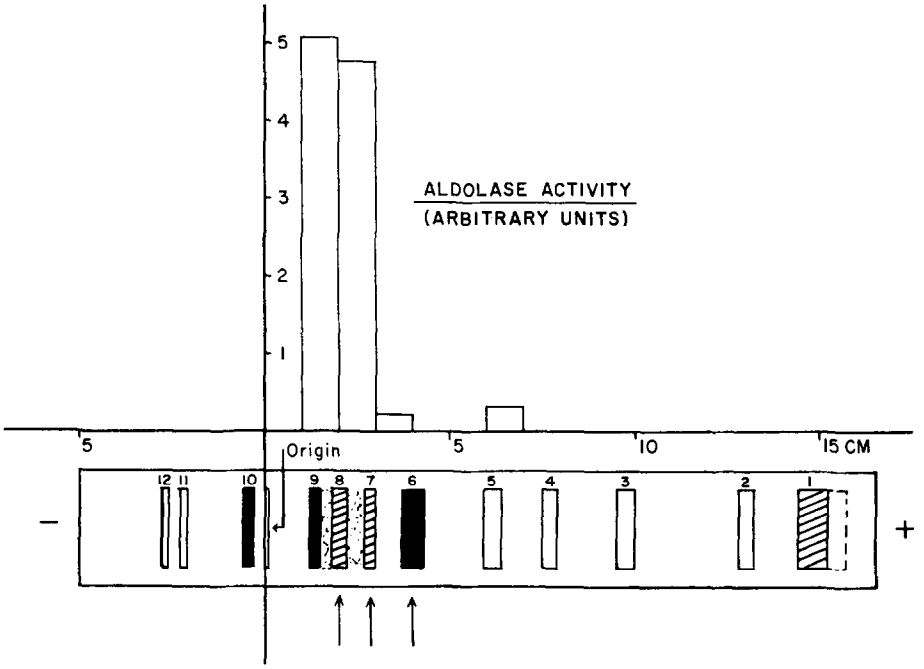

Fig. 1. Distribution of protein, peroxidase and aldolase activity on a starch gel electropherogram of human muscle extract (Phosphate pH 7.7, I=0.07). Arrows show bands with peroxidase activity; stippling indicates a background of unresolved protein staining material.

There is a general similarity between the distribution of proteins after electrophoresis on starch and that observed in free solution electrophoresis (Bosch, 1951; Haan, 1953) in that the bulk of the material is rather slow moving but is accompanied by a fast component. Relatively minor components of intermediate mobility are also present and on starch there are in addition components which move towards the cathode. Storage of muscle at -25° for periods of up to a month before

preparing a protein extract makes no qualitative difference to the observed pattern, nor were any quantitative changes obvious to visual inspection.

Although the nature of the proteins which comprise this pattern is largely unknown, some information has been obtained about a few of them. Bands 6, 7 and 8 show peroxidase activity. Band 7 has the mobility of normal haemoglobin and is sometimes almost absent; it presumably arises from contamination of human biopsy material with blood. Band 6 shows the strongest activity and it is assumed that myoglobin is associated with it. The cause of the peroxidase activity in band 8 is uncertain; compared with band 6 the activity is usually weak and may possibly be due to the presence of myoglobin II (Rossi-Fanelli and Antonino, 1957). Recently, Dreyfus and Schapira (1959) have also reported three bands with peroxidase activity in starch gel electropherograms of rat muscle extracts. Band 1 is of interest. In a few instances partial splitting into two components occurred and when this happened the slower component had the mobility of human plasma albumin. However, usually only one band with the mobility of plasma albumin can be seen. It is readily detected even when little haemoglobin is present and may represent in part a penetration of the plasma proteins into the extra-capillary space (Gitlin and Janeway, 1954). The probable presence of plasma proteins in muscle samples, even those taken in animal experiments where the animal was thoroughly exsanguinated, suggests that a considerable proportion of the fast electrophoretic component assumed by various authors (Jacob, 1947; Fischer, 1950; Bosch, 1951) to be the myoalbumin of Bate-Smith (1937) is in fact plasma albumin. Corsi and Buscaino (1959) have recently made a similar point in connection with extracts of human muscle. The faster component of band 1 may possibly be the true myoalbumin.

The area around bands 8 and 9 shows aldolase activity, although the maximum activity appears to be associated with band 9. A trace of

activity is also found in band 5.

The distribution of protein and of enzyme activity in buffer systems other than phosphate is somewhat different, and the possibility of using this fact to attain improved separation is currently being studied.

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