glutarate) are decarboxylated synergistically in this system. The details of the mechanism by which this is effected have not, however, been elucidated.

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REFERENCES

Crawhall, J. C. & Watts, R. W. E. (1960). *Biochem. J.* **78**, 3P.
Crawhall, J. C. & Watts, R. W. E. (1961). *Abstr. Commun. 5th int. Congr. Biochem., Moscow*, section 23, p. 465. Communication no. 23.30.1322.
Fleming, L. W. & Crosbie, G. W. (1960*a*). *Biochem. J.* **77**, 23P.
Fleming, L. W. & Crosbie, G. W. (1960*b*). *Biochem. biophys. Acta*, **43**, 139.
Francis, G. E., Mulligan, W. M. & Wormall, A. (1959). *Isotopic Tracers*, 2nd ed., p. 484. London: Athlone Press.
Hogeboom, G. H., Schneider, W. C. & Striebich, M. J. (1952). *J. biol. Chem.* **196**, 111.
Liang, C.-C. (1962*a*). *Biochem. J.* **82**, 429.
Liang, C.-C. (1962*b*). *Biochem. J.* **83**, 101.
Nakada, H. I. & Sund, L. P. (1958). *J. biol. Chem.* **233**, 8.
Nakada, H. I. & Weinhouse, S. (1953*a*). *Arch. Biochem. Biophys.* **42**, 257.
Nakada, H. I. & Weinhouse, S. (1953*b*). *J. biol. Chem.* **204**, 831.
Neuberger, A. (1961). *Biochem. J.* **78**, 1.
Quayle, J. R. & Taylor, G. A. (1961). *Biochem. J.* **78**, 611.
Radin, N. S. & Metzler, D. E. (1955). *Biochem. Prep.* **4**, 60.
Ratner, S., Nocoto, V. & Green, D. E. (1944). *J. biol. Chem.* **152**, 119.
Richardson, K. E. & Tolbert, N. E. (1961). *J. biol. Chem.* **236**, 1280.
Richert, D. A., Amberg, R. & Wilson, M. (1962). *J. biol. Chem.* **237**, 99.
Sanadi, W. R. & Bennett, M. J. (1960). *Biochem. biophys. Acta*, **39**, 367.
Schneider, W. C. (1948). *J. biol. Chem.* **176**, 259.
Tabor, H. & Mehler, A. H. (1955). *J. biol. Chem.* **210**, 559.
Watts, R. W. E. & Crawhall, J. C. (1959). *Biochem. J.* **73**, 277.
Weinhouse, S. (1955). In *Symp. on Amino Acid Metabolism*, p. 637. Ed. by McElroy, A. D. & Glass, H. B. Baltimore: Johns Hopkins Press.
Weinhouse, S. & Friedmann, B. (1951). *J. biol. Chem.* **191**, 707.
Weinhouse, S. & Friedmann, B. (1952). *J. biol. Chem.* **197**, 733.
Weissbach, A. & Sprinson, D. B. (1953). *J. biol. Chem.* **203**, 1023.
Wild, F. (1953). *Estimation of Organic Compounds*. Cambridge University Press.
Wilson, W. E. & Koeppe, R. E. (1961). *J. biol. Chem.* **236**, 365.

Biochem. J. (1962) **85**, 171

A Chromatographic and Electrophoretic Study of Sarcoplasm from Adult- and Foetal-Rabbit Muscles

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Most of the studies which have so far been carried out on the proteins of foetal muscle have been concerned primarily with the development of the proteins of the contractile system, the myofibril. The protein system of the soluble cytoplasm of muscle, the sarcoplasm, is complex and, although this fraction does not appear to play a direct role in contraction, its composition reflects certain aspects of the metabolic activity of the tissue. As it contains the enzymes of the glycolytic and other systems of special importance to muscle as well as those of a more general significance (see Perry, 1956, 1960*a*, for reviews), sarcoplasm has frequently been used as the starting material for enzyme preparations. Nevertheless, relatively little systematic attempt has been made to develop methods for the fractionation and identification of the individual components which could be of value in comparative studies. Boundary-electrophoretic investigations (Jacob, 1947; Dubuisson, 1950) have indicated the presence of 10–12 distinct migrating boundaries with which certain enzymes have been identified by indirect evidence. Further work by others has shown that the electrophoretic pattern of sarcoplasm from a given muscle is distinctive for the species (see Perry, 1960*a*, for references) and in a given species for the particular muscle from which it is derived (Crepax, 1952).

The present paper presents the results of a study of the fractionation of sarcoplasm by chromato-

graphy on diethylaminoethylcellulose and by starch-gel electrophoresis, and the identification of certain proteins within these fractions. Comparison of the sarcoplasms of adult- and foetal-rabbit muscle by these techniques indicates marked differences in composition at these two stages of development. This work forms part of a general study of the development of muscle proteins in foetal tissue and some aspects of it have been reported in a preliminary communication (Hartshorne & Perry, 1961).

METHODS

Chromatography. Diethylaminoethylcellulose was prepared by the method of Peterson & Sober (1956) and chromatography was carried out at 1–2° as described by Perry (1960*b*) and Mueller & Perry (1961). Usually 50–75 mg. of protein was applied to 1 g. of diethylaminoethylcellulose in columns 2 cm. in diameter.

Sarcoplasm. Adult rabbits were killed by decapitation, and the muscles from the back and hind legs were immediately dissected out, chilled in ice and minced after being carefully blotted on filter paper. Foetuses were removed immediately after the death of the mother, decapitated and chilled in ice before the back and leg muscles were dissected out. The mince, or muscle fragments with foetal tissue, was homogenized in a small Waring Blendor-type of homogenizer with 3 vol. of 0·05 M-KCl–20 mM-tris–HCl, pH 7·6, and the homogenate centrifuged for 20 min. at 10 000*g*. The sedimented material was discarded and the supernatant then centrifuged for 1 hr. at 100 000*g* to remove the sarcoplasmic reticulum and other formed elements. The clear supernatant was concentrated by ultrafiltration and dialysed against the appropriate buffer before chromatography or electrophoresis. Ultrafiltration was carried out as follows. A length of Visking dialysis tubing ($\frac{8}{32}$ in. diam.) was attached to the stem of a dropping funnel, the free end of the dialysis tubing knotted and the protein solution introduced through the dropping funnel. The funnel was inserted into a Buchner flask with a ground-glass joint, the pressure in the flask reduced to 12–20 mm. Hg and the apparatus left until the required concentration had taken place. All manipulations were carried out at 1–2°.

Perfusion experiments. Veterinary Nembutal was injected into the marginal artery of the rabbit's ear until surgical anaesthesia was attained. Approximately 1000 units of heparin in 1 ml. were also injected to prevent blood from clotting in the capillaries. Cannulas with attached tubes were inserted into the descending aorta just above the iliac bifurcation and into the vena cava. Perfusion was then started with 0·9% NaCl from a reservoir maintained at a height to give sufficient pressure to ensure a steady flow of 0·9% NaCl through the hind limbs of the animal. Perfusion was continued until at least 100 ml. of colourless effluent emerged; usually approximately 400 ml. of 0·9% NaCl was required in all. After perfusion the clearly distinguishable red and white muscles of the hind limbs were dissected out and sarcoplasm was prepared separately from each type.

For perfusion of the heart a fluid-filled cannula was inserted into the aorta with the outflow towards the heart. Perfusion with 0·9% NaCl was started and the pulmonary artery quickly cut to permit outflow of the effluent. While perfusion was continued the pulmonary and systemic veins were ligated to prevent an inflow of blood. Perfusion was continued until the effluent was colourless.

Starch-gel electrophoresis. The procedure employed was that of Smithies (1955), with 20–24 mM-boric acid–NaOH, pH 9·1, the precise concentration of borate being that recommended by the manufacturers (Connaught Medical Research Laboratories) of the starch used. Sarcoplasm samples were adjusted to give E_{280} about 40 and applied on filter paper to a slit in the gel. Electrophoresis was carried out at 1–2° with a gradient of 6 v/cm. For normal staining the gel was sliced in half, immersed for 5 min. in a saturated solution of Naphthalene Black in ethanol–acetic acid–water (5:1:4, by vol.) and then washed in the same solvent.

For enzymic determinations the gel was sliced into three in a plane parallel to that of the supporting tray. The two outside slices were stained in the normal way and the inside slice was used for enzymic assays. The approximate position of the enzyme was first determined by cutting 1 cm. slices at right angles to the direction of electrophoresis. For aldolase assay the slices were homogenized in 2 ml. of 0·5 M-tris–HCl, pH 8·6. For the assays of creatine phosphokinase and 5′-adenylic acid deaminase the buffer used for elution was that used for the respective enzymic assay, and the elution of protein was facilitated by freezing the homogenate in acetone–solid CO_2. After thawing the starch was removed either by filtration or by centrifuging and the clear extract used for assay. For more precise fixation of the enzymic activity the area of the gel shown to be active by the preliminary experiment was cut into 2 mm. slices, elution was carried out with 0·5 ml. of buffer and the assays were repeated.

Enzymic assays. Aldolase was assayed as described by Sibley & Lehninger (1949), 5′-adenylic acid deaminase as described by Perry & Zydowo (1959) and creatine phosphokinase by the method of Chappell & Perry (1954).

Measurements of pH were carried out as described by Mueller & Perry (1961).

RESULTS

Chromatography and starch-gel electrophoresis of sarcoplasm of adult skeletal muscle

Perry & Zydowo (1959) reported that the bulk of the total protein of sarcoplasm prepared from rabbit skeletal muscle was not held on diethylaminoethylcellulose at pH 7·6 at an ionic strength of about 0·12. Further investigation at this pH showed that when the ionic strength was reduced to 0·016 (20 mM-tris–hydrochloric acid, pH 7·6) 80% of the protein still passed through the column unheld (Fig. 1). Nevertheless, some fractionation occurred at this ionic strength for a retarded asymmetric peak followed the main fraction which passed through the column under these conditions. The retarded peak was slightly brownish red and in the later fractions the extinctions at 260 mμ were greater than those at 280 mμ. Subsequent stepwise application of 0·5 M-potassium chloride–

20 mM-tris–hydrochloric acid, pH 7·6, eluted the remainder of the protein in which were detected small amounts of another brownish-red component (Fig. 1). No significant amount of material absorbing at 280 or 260 mμ was eluted by the application of a further step to 2M-potassium chloride–20 mM-tris–hydrochloric acid, pH 7·6.

Greatly improved resolution was obtained by carrying out the fractionation on diethylaminoethylcellulose equilibrated in the first instance with 40 mM-glycine–sodium hydroxide, pH 9·3 (cf. Marcaud, 1961). Under these conditions approximately 20% of the total protein passed through the column unheld (fraction 1) and the remainder of the protein could be eluted in five further major fractions (fractions 2–6) by stepwise increase of the ionic strength (Fig. 2). The final fraction eluted at 0·5M-potassium chloride–40 mM-glycine–sodium hydroxide, pH 9·3, differed from the fractions eluted at lower ionic strengths in possessing an extinction higher at 260 mμ than at 280 mμ.

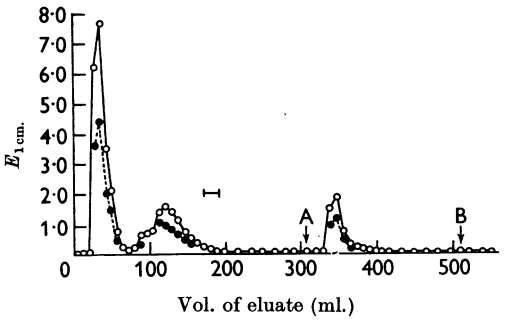


Fig. 1. Chromatography of sarcoplasm from adult-rabbit skeletal muscle on diethylaminoethylcellulose at pH 7·6. The protein solution (30 ml.; $E_{1\,cm.}$ 14·55 at 280 mμ) in 20 mM-tris–HCl, pH 7·6, was applied to a column 2 cm. diam. × 22 cm. The step to 0·5M-KCl–20 mM-tris–HCl was at A and the step to 2·0M-KCl–20 mM-tris–HCl was at B. ○, E at 280 mμ; ●, E at 260 mμ. Here and in Figs. 2 and 5, ⊢⊣ indicates the region of elution of coloured components.

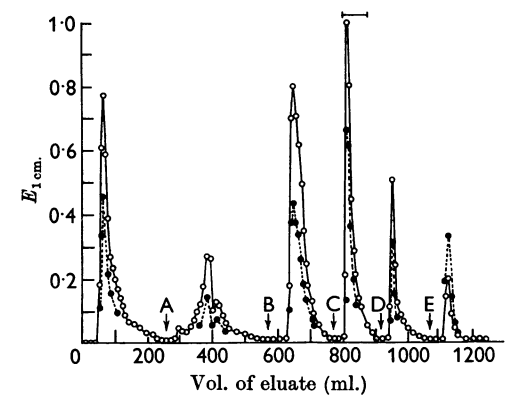


Fig. 2. Chromatography of sarcoplasm from adult-rabbit skeletal muscle on diethylaminoethylcellulose at pH 9·3. The protein solution (15 ml.; $E_{1\,cm.}$ 14·42 at 280 mμ) in 40 mM-glycine–NaOH, pH 9·3, was applied to a column 2 cm. diam. × 16 cm. The buffer concentration was kept constant throughout and the ionic strength increased by the stepwise addition of 0·01M-KCl, 0·03M-KCl, 0·1M-KCl, 0·2M-KCl and 0·5M-KCl at A, B, C, D and E respectively. The fractions were numbered 1–6 in order of elution. ○, E at 280 mμ; ●, E at 260 mμ.

Starch-gel electrophoresis of whole sarcoplasm from the white skeletal muscle obtained either from the longissimus dorsi or from the hind-leg muscles produced a characteristic pattern (Fig. 3; Plate 1). Although the general picture was consistent, slight variations in resolution were apparent in some experiments, but under the best conditions with concentrated sarcoplasm at least 16 bands could be recognized (Fig. 3). A special feature of white-muscle sarcoplasm was the group of distinct bands which moved towards the cathode. These bands stained particularly intensely with the dye, and, although the extent of endosmosis in these experiments could not be accurately assessed, test runs with formaldehyde (Smithies, 1959) suggested that at least the faster of the components moving towards the cathode possessed a positive charge under the conditions of electrophoresis.

Starch-gel electrophoresis of the fractions of sarcoplasm eluted from the diethylaminoethylcellulose indicated, as might be expected, that the more positively charged components were eluted at lower ionic strengths (Plate 1). At pH 7·6, however, chromatography produced a relatively limited separation of the electrophoretic compo-

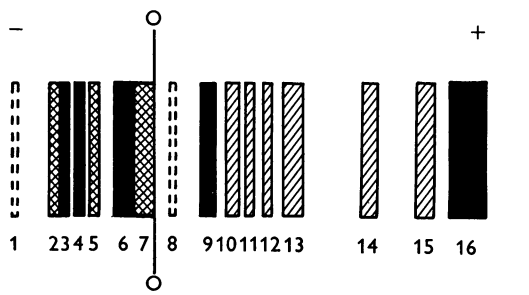


Fig. 3. Diagrammatic representation of the bands obtained on starch-gel electrophoresis of sarcoplasm from unperfused adult-rabbit skeletal muscle in 22 mM-borate, pH 9·1. The bands indicated by broken lines were only observed when the sarcoplasm was subjected to electrophoresis at concentrations which gave E_{280} 60 or more. The very dense bands are indicated in black; the intensity of staining in the other bands is roughly indicated by the hatching, cross-hatching indicating more intense staining than does single-hatching. ○, Origin.

nents. Thus the components moving towards the cathode were concentrated at the front of the material eluted from the column by 20 mM-tris–hydrochloric acid, pH 7·6, and the fastest band moving towards the anode was concentrated in the material eluted by a step to 0·5 M-potassium chloride–20 mM-tris–hydrochloric acid, pH 7·6. The improved fractionation obtained on chromatography at pH 9·3 was apparent on starch-gel electrophoresis of the fractions (Plate 1). The material not held by 40 mM-glycine–sodium hydroxide, pH 9·3, consisted almost entirely of the material moving towards the cathode, and the band moving fastest towards the cathode was concentrated in the front of the first peak eluted by this buffer (Fig. 2, Plate 1).

Identification of certain of the electrophoretic components. The fastest component migrating towards the anode on starch-gel electrophoresis had a velocity identical with that of rabbit serum albumin, although it usually moved as a more compact band than the latter. The possibility that the component represented by this band was not of sarcoplasmic origin but arose from the blood in the vessels present in the original muscle mince was investigated by perfusing the hind legs of the rabbit. Sarcoplasm was then prepared from red and white muscles which had been carefully dissected from the perfused limbs and homogenized separately. In the sarcoplasm from both muscle types the 'albumin' band was present with little apparent diminution of intensity compared with that from the unperfused control limb. In sarcoplasm from foetal muscle (see below) several faint bands which moved faster than the albumin were observed.

Mixed-skeletal-muscle sarcoplasm usually gave two red bands, which were presumed to correspond to concentrations of haemoglobin and myoglobin for they were the only bands which stained blue under the conditions of the benzidine test (Smithies, 1959). Both coloured components were eluted in the step to 0·1 M-potassium chloride–40 mM-glycine–sodium hydroxide, pH 9·3 (Fig. 2). Haemoglobin was confirmed as moving in the region of band no. 13 by comparison with the electrophoretic behaviour of rabbit haemoglobin prepared by the lysis of washed rabbit erythrocytes. The identification of the haemochromogens was further aided by comparison of the electrophoretic pattern obtained from the sarcoplasm of red and white muscle isolated from the perfused back legs of the rabbit. Sarcoplasm obtained from the two types of muscle was distinguished by the fact that band no. 15 (Fig. 3) moving behind the 'albumin' was very much more intense in the red-muscle sarcoplasm. This band was presumed to be myoglobin and usually could be recognized as a very faint band in sarcoplasm prepared from perfused white skeletal muscle and from rabbit mixed skeletal muscle, which contains a relatively small proportion of red muscle.

Aldolase activity was associated with the peaks eluted by 40 mM-glycine–sodium hydroxide, pH 9·3, and by 10 mM-potassium chloride–40 mM-glycine–sodium hydroxide, pH 9·3. Although the bulk of the activity was confined to these fractions, a small amount of the enzyme was also eluted in the subsequent step, i.e. to 30 mM-potassium chloride–40 mM-glycine–sodium hydroxide, pH 9·3. These observations suggested that it was present as a component migrating towards the cathode and enzymic assay on the material eluted from the starch gel indicated clearly that the enzyme was highly concentrated in the region of band no. 6 (Fig. 4). By a similar study creatine phosphokinase was shown to be localized in the region between the origin and band no. 9 (Fig. 4).

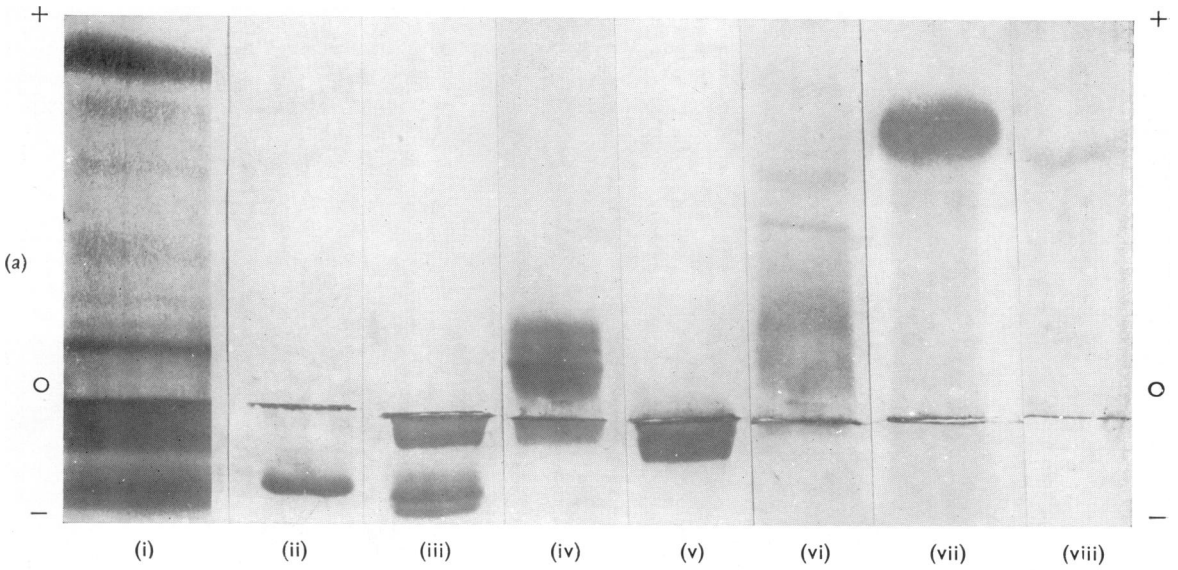
5′-Adenylic acid-deaminase activity was confined to the peak (fraction 5) eluted by the step to 0·2 M-potassium chloride–40 mM-glycine–sodium hydroxide, pH 9·3, but limited studies indicated that this enzyme could not be consistently eluted from the starch for assay to determine its distribution on electrophoresis. In ultraviolet light (366 mμ) marked yellow fluorescence was localized at band no. 6 suggesting a concentration of nicotinamide nucleotide coenzymes at this point.

Chromatography and starch-gel electrophoresis of sarcoplasm of foetal skeletal muscle

Study of the sarcoplasm prepared from the back and leg muscles of foetuses ranging from 24 to 31 days old revealed marked differences from the corresponding fraction obtained from adult tissue. The fraction of sarcoplasm eluted from the column by 20 mM-tris–hydrochloric acid, pH 7·6, rose from about 16% for a 26-day-old foetus to about 30% of the total protein eluted for a 30-day-old foetus. At the same time the fraction eluted between 20 mM-tris–hydrochloric acid and 0·5 M-potassium chloride–20 mM-tris–hydrochloric acid,

EXPLANATION OF PLATE 1

Plate 1. Starch-gel-electrophoresis patterns of rabbit sarcoplasm in 22 mM-borate, pH 9·1. (*a*) Fractionation of adult-mixed-skeletal-muscle sarcoplasm on diethylaminoethylcellulose at pH 9·3: (i) whole sarcoplasm; (ii) front of peak eluted by 40 mM-glycine–NaOH, pH 9·3; (iii) fraction 1; (iv) fraction 2; (v) fraction 3; (vi) fraction 4; (vii) fraction 5; (viii) fraction 6. Fractions 1–6 are as explained in Fig. 2. (*b*) Comparison of sarcoplasms from different muscles: (i) perfused adult red skeletal muscle; (ii) perfused adult white skeletal muscle; (iii) rabbit serum; (iv) perfused adult heart muscle; (v) mixed back and leg muscle of 24-day-old foetus.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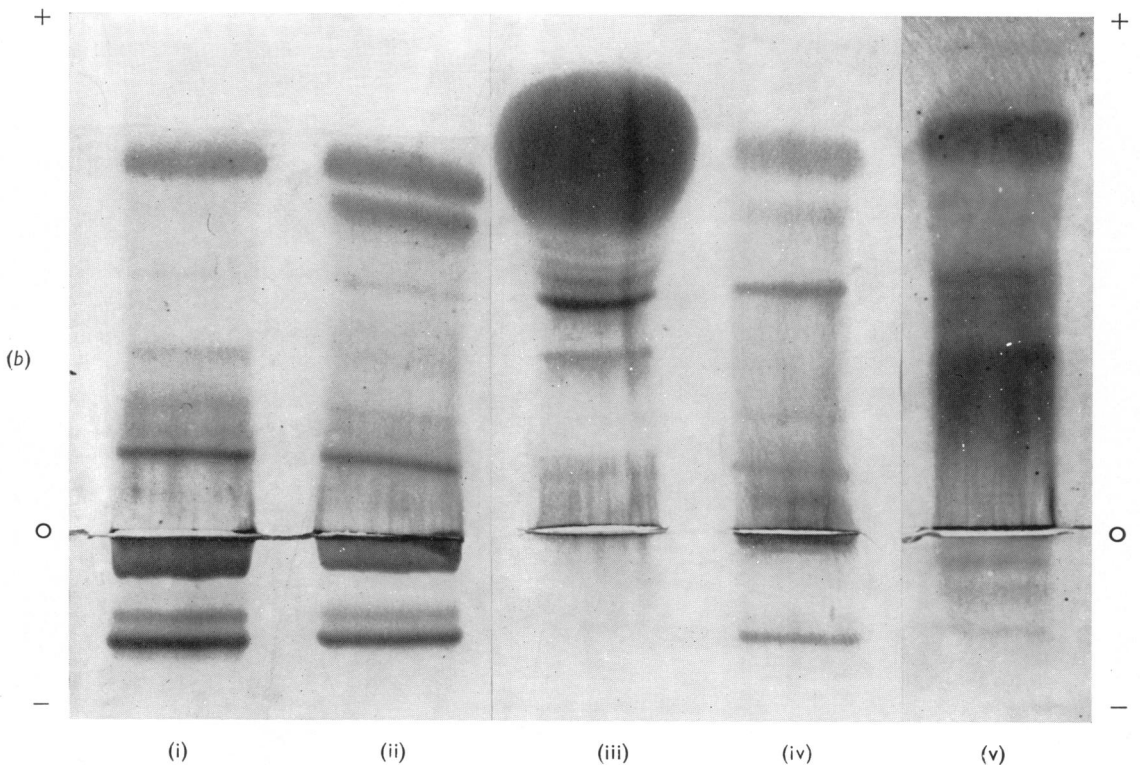
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pH 7·6, was correspondingly greater compared with that obtained from adult skeletal muscle (Fig. 5). This difference in the chromatographic behaviour of the sarcoplasmic proteins of foetal muscle was reflected in the starch-gel electrophoretic pattern obtained. In general fewer bands could be clearly identified, the components migrating towards the cathode being faint compared with those obtained with adult skeletal muscle, where these bands were the most densely staining of all those which could be identified on electrophoresis. In foetal extracts bands corresponding to 8–13 were not very distinct, as these areas were characterized by a more streaky appearance; the band in position 13 was often double and faint bands in front of the 'albumin' band (possibly prealbumins) could frequently be recognized (Plate 1).

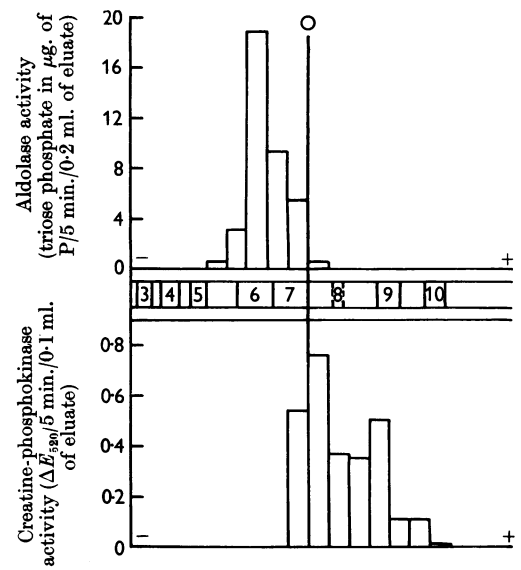

Fig. 4. Localization of aldolase and creatine-phosphokinase activities after starch-gel electrophoresis of adult-rabbit-skeletal-muscle sarcoplasm. The blocks represent the total activity in the protein eluted from 2 mm. slices of gel. The upper and lower nomograms represent aldolase and creatine phosphokinase respectively. The vertical line at ○ indicates the point of application of protein on the starch gel before electrophoresis, and the numbers 3–10 correspond to the band numbers explained in Fig. 3.

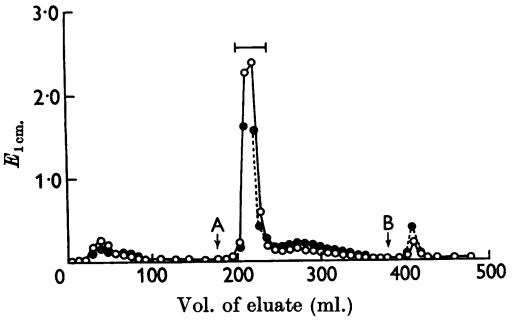

Fig. 5. Chromatography of sarcoplasm from foetal-rabbit muscle on diethylaminoethylcellulose at pH 7·6. The protein solution (20 ml.; $E_{1\,cm.}$ 4·45 at 280 mμ) in 20 mM-tris–HCl, pH 7·6, was applied to a column 2 cm. diam. × 13 cm. The steps to 0·5 M-KCl–20 mM-tris–HCl, pH 7·6, and 2·0 M-KCl–20 mM-tris–HCl, pH 7·6, were applied at points A and B respectively. ○, E at 280 mμ; ●, E at 260 mμ.

Chromatography and starch-gel electrophoresis of sarcoplasm of adult heart and red skeletal muscle

Sarcoplasm from adult heart muscle showed some similarities to the foetal-skeletal-muscle preparations in that the bands moving towards the cathode were significantly lower in intensity compared with those present in adult-white-skeletal-muscle sarcoplasm (Plate 1). Also the same bands in adult-red-skeletal-muscle sarcoplasm were less intense than those observed in equivalent white muscle, although generally more intense than the same bands observed in foetal-skeletal-muscle and adult-cardiac-muscle preparations. One faint band possessing the highest mobility towards the cathode was often observed in heart-muscle sarcoplasm applied at the normal concentration. A band in a similar position (band no. 1) was also observed in adult-skeletal-muscle sarcoplasm when applied to the starch gel at high concentrations (E_{280} greater than 40).

Faint bands moving faster than serum albumin, 'prealbumins', were also apparent in heart-muscle sarcoplasm. Perfusion diminished the band of mobility identical with that of serum albumin in heart sarcoplasm but did not eliminate it.

For band no. 6, the diminished intensity was confirmed as being due in part at least to a real difference in aldolase concentration by enzyme assays carried out on whole sarcoplasmic extracts. Adult-white-skeletal-muscle sarcoplasm possessed approximately ten times the aldolase activity of sarcoplasm isolated from the heart muscle of the same animal. Likewise the aldolase activity of foetal-skeletal-muscle sarcoplasm was more comparable with that of the adult heart muscle and did not rise to normal adult-skeletal-muscle levels until some time after parturition (Table 1).

DISCUSSION

Features of sarcoplasm which are not apparent in serum are the components which move towards the cathode when electrophoresis is carried out at pH 9·1. Although the movement of some of these bands migrating towards the cathode may be due to endosmosis it is very likely that those proteins

Table 1. *Aldolase activity of sarcoplasm isolated from rabbit muscle*

Experimental details are given in the text. The S.E.M. values are given where assays were carried out on several animals, the numbers of which are given in parentheses.

Source of sarcoplasm	Age	Aldolase activity (triose phosphate in μg. of P/5 min./mg. of N)
Mixed back and leg muscles	Adult*	586±47·1 (4)
Heart muscle	Adult*	52·8±3·23 (4)
Back muscle	26-day-old foetus	35·5
Back muscle	26-day-old foetus	38·8
Back muscle	28-day-old foetus	53·9
Back muscle	30-day-old foetus	65·9
Back muscle	31-day-old foetus	80·4

* Animals older than 6 months.

responsible for the bands moving fastest towards the cathode possess a net positive charge in borate buffer at this pH. Indeed, aldolase, which has been demonstrated as moving in the position of no. 6 band, is known to possess an isoelectric point in the alkaline range which is dependent on the buffer present (Velick, 1949; Rutter, 1961).

The present investigation does not permit a final conclusion about the origin of the fast band no. 16 moving towards the anode. This component is probably identical with myoalbumin (Bate-Smith, 1930), which a number of workers have considered to be responsible for the component of highest mobility observed on the electrophoresis of sarcoplasm. Certainly this component moves with a mobility very similar to that of serum albumin on starch-gel electrophoresis. So far as can be judged from its appearance before and after perfusion of skeletal muscle, the serum albumin in the vascular bed of the muscle makes relatively little contribution to this band. Perfusion of the heart clearly reduces the 'albumin' band but, nevertheless, it remains one of the most striking features of heart-muscle sarcoplasm [Plate 1: (*b*) (iv)].

Perfusion would not be expected to remove the serum albumin present in the extracellular spaces of the tissue, which, according to Gitlin, Nakasato & Richardson (1955), in rabbit skeletal muscle is about equal in total amount to that in the vascular system. In view of the evidence for the entry of serum albumin into the cells of many tissues (Coon & Kaplan, 1950; Gitlin, Landing & Whipple, 1953) it would not be unreasonable, however, to expect some intracellular as well as extracellular serum albumin to contribute to band no. 16. The question whether intra- and extra-cellular serum albumin can account for all of the protein in the band or whether in addition another distinct protein, myoalbumin, is present in adult-skeletal-muscle sarcoplasm cannot be decided from this study.

The most striking feature observed when the sarcoplasms of adult and foetal skeletal muscle are compared is the great relative increase in the more positively charged components which occurs after birth. These components, one of which is the enzyme aldolase, occur in the protein fraction more readily eluted from diethylaminoethylcellulose, and their contribution to the total sarcoplasmic protein increases from about 25–30 % in late foetal life to 80 % in the adult tissue. In both adult and foetal skeletal muscle the bulk of the proteins of sarcoplasm has albumin-like properties, but as development occurs it changes from being predominantly acidic to become more basic in character.

In assessing the true significance of these values the relative contribution to the sarcoplasmic fraction made by the serum proteins occluded in blood vessels and extracellular space in foetal and adult skeletal muscle must be evaluated. For adult skeletal muscle it can be estimated, from the data of Gitlin *et al.* (1955) and by assuming an extracellular space (excluding vascular space) of 15 %, that at most about 10–15 % of the total protein of sarcoplasm prepared from unperfused muscle could arise from serum proteins present in the blood vessels and extracellular spaces. About 80 % of rabbit serum protein is held on diethylaminoethylcellulose columns in 20 mM-tris–hydrochloric acid, pH 7·6 (D. J. Hartshorne & S. V. Perry, unpublished work).

To explain the chromatographic and aldolase-assay results obtained with foetal-skeletal-muscle sarcoplasm entirely on the basis of increased contamination with serum proteins of extracellular origin would, however, be unrealistic, as it is estimated that such an interpretation would demand that 80–90 % of the foetal-muscle-sarcoplasmic-protein fraction consisted of serum proteins. Further, the electrophoretic pattern of foetal-muscle sarcoplasm is different from that of rabbit serum. Similar arguments apply also to heart muscle, where the contribution of serum proteins to the sarcoplasm can be more definitely estimated to be completely inadequate to explain the low aldolase activity simply on the basis of dilution by an increased amount of serum protein in the tissue compared with skeletal muscle.

The aldolase concentration in sarcoplasm from foetal skeletal muscle is low, like that of adult heart muscle, although the enzymic activity rises as the foetus develops. The general conclusion of this comparison is that at least for the components moving towards the cathode foetal-muscle sarcoplasm is more comparable with that of adult heart muscle than that of white skeletal muscle. The sarcoplasm of red skeletal muscle appears to be intermediate between these two types. The high concentration of aldolase in the adult-skeletal-muscle sarcoplasm might be expected in view of the well-developed ability of this tissue to function anaerobically. These facts together with our findings suggest that after birth aldolase and other proteins of a high isoelectric point increase rapidly in amount in response to the increased activity of the skeletal muscle.

SUMMARY

1. Rabbit skeletal muscle has been fractionated by chromatography on diethylaminoethylcellulose at pH 7·6 and 9·3.

2. On starch-gel electrophoresis of adult-rabbit-skeletal muscle at least 15 migrating bands have been recognized and some of them identified with known proteins.

3. The more positively charged protein components which are readily eluted from diethylaminoethylcellulose are relatively much decreased in sarcoplasm isolated from foetal-rabbit skeletal muscle and from adult heart muscle compared with the amounts present in the corresponding fraction from adult skeletal muscle.

4. The level of aldolase activity in foetal-skeletal-muscle sarcoplasm is comparable with that in adult-heart-muscle sarcoplasm but much lower than that found in adult-rabbit-skeletal muscle sarcoplasm.

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REFERENCES

Bate-Smith, E. C. (1930). *Proc. Roy. Soc.* B, **105**, 579.
Chappell, J. B. & Perry, S. V. (1954). *Biochem. J.* **57**, 421.
Coon, A. H. & Kaplan, M. H. (1950). *J. exp. Med.* **91**, 1.
Crepax, P. (1952). *Biochim. biophys. Acta*, **9**, 385.
Dubuisson, M. (1950). *Biol. Rev.* **25**, 46.
Gitlin, D., Landing, B. H. & Whipple, A. (1953). *J. exp. Med.* **97**, 163.
Gitlin, D., Nakasato, D. & Richardson, W. R. (1955). *J. clin. Invest.* **34**, 935.
Hartshorne, D. J. & Perry, S. V. (1961). *Biochem. J.* **80**, 9P.
Jacob, J. J. C. (1947). *Biochem. J.* **41**, 83.
Marcaud, L. (1961). *Abstr. 5th int. Congr. Biochem., Moscow*, 201.
Mueller, H. & Perry, S. V. (1961). *Biochem. J.* **80**, 217.
Perry, S. V. (1956). *Physiol. Rev.* **36**, 1.
Perry, S. V. (1960*a*). In *Comparative Biochemistry*, vol. 2, p. 245. Ed. by Florkin, M. & Mason, H. S. New York: Academic Press Inc.
Perry, S. V. (1960*b*). *Biochem. J.* **74**, 94.
Perry, S. V. & Zydowo, M. (1959). *Biochem. J.* **71**, 220.
Peterson, E. A. & Sober, H. A. (1956). *J. Amer. chem. Soc.* **78**, 751.
Rutter, W. J. (1961). In *The Enzymes*, vol. 5, p. 341. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York: Academic Press Inc.
Sibley, J. A. & Lehninger, A. L. (1949). *J. biol. Chem.* **177**, 859.
Smithies, O. (1955). *Biochem. J.* **61**, 629.
Smithies, O. (1959). *Advanc. Protein Chem.* **16**, 76.
Velick, S. F. (1949). *J. phys. Colloid Chem.* **53**, 135.

Biochem. J. (1962) **85**, 177

Protein Synthesis in Mitochondria

3. THE CONTROLLED DISRUPTION AND SUBFRACTIONATION OF MITOCHONDRIA LABELLED *IN VITRO* WITH RADIOACTIVE VALINE*

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In previous publications from this Laboratory the conditions for incorporation of radioactive amino acids into the protein of isolated rat-liver mitochondria were examined (Reis, Coote & Work, 1959; Roodyn, Reis & Work, 1961*a*) and negligible rates of incorporation *in vitro* were reported for the soluble proteins in the mitochondrial fraction, including catalase, malic dehydrogenase and cytochrome *c* (Roodyn, Suttie & Work, 1962). The

* Part 2: Roodyn, Suttie & Work (1962).