at pH 5·0 run slowly through, when aminoacetone is retained and ALA runs out. After the column has been washed with water the aminoacetone is eluted with HCl. For subsequent chromatography the eluate is evaporated to dryness *in vacuo*, the aminoacetone hydrochloride extracted with absolute ethanol and the bulky precipitate of NaCl filtered off. The solution is then sufficiently salt-free for most purposes.

Paper chromatography. Chromatographic separation of ALA and aminoacetone can be achieved by using the following solvent system: ethanol–M-acetic acid–pyridine–water (95:10:3:3, by vol.) in which ALA and aminoacetone have R_F values of 0·15 and 0·3 respectively. In five acidic solvents tested, including butanol–acetic acid, ALA and aminoacetone had identical R_F values. In view of this, and particularly since alkaline solvents cannot be used, the two compounds could easily be confused. Both give the same colour reactions with ninhydrin (yellow, turning purple at room temperature). ALA and aminoacetone can be separated by paper electrophoresis at pH 4·0–6·5 and the two can also be distinguished chromatographically by first converting them into pyrroles (Mauzerall & Granick, 1956).

SUMMARY

Conditions for the determination of δ-aminolaevulic acid and aminoacetone by the picrate reaction have been investigated and a simple procedure has been developed for the estimation of 0·05–0·8 μmole of δ-aminolaevulic acid or 0·05–0·5 μmole of aminoacetone. Methods for separating the two compounds are also described.

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The Chromatography of L-Myosin on Diethylaminoethylcellulose

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The methods employed by most workers for the purification of L-myosin from extracts of whole muscle usually involve two main principles. These are the selective precipitation at ionic strength about 0·3 of any actomyosin which may be present and the removal of albumin and other protein impurities by repeated precipitation by dilution with water to an ionic strength at which the myosin itself is insoluble (Edsall, 1930; Bailey, 1942; Szent-Györgyi, 1945; Portzehl, Schramm & Weber, 1950; Mommaerts & Parrish, 1951; Perry, 1955). Salting-out procedures have also been employed, although when ammonium sulphate is used (Tsao, 1953) appreciable loss in adenosine triphosphatase activity occurs.

Even in the best preparations of L-myosin from rabbit skeletal muscle, identifiable impurities, such as 5′-adenylic deaminase (Summerson & Meister, 1944) and nucleic acid (Bailey, 1946; Lajtha, 1951), are known to be present. In addition, on electrophoretic and ultracentrifugal analyses of such preparations it is often possible to detect small amounts of substances which have not been definitely characterized (Mommaerts & Parrish, 1951; Tsao, 1953; Raeber, Schapira & Dreyfus, 1955).

In view of the ability of myosin to interact with a number of proteins and of the limitations inherent in the standard methods of establishing protein homogeneity when applied to a substance with a molecular asymmetry comparable with that of myosin, it is very difficult to decide by the usual criteria just how homogeneous the usual preparations of L-myosin are. Certainly on electrophoretic evidence alone some workers consider the standard L-myosin preparations to contain 10–15% of impurities (Mommaerts & Parrish, 1951; Tsao, 1953). This is perhaps not surprising in view of the relative lack of specificity of the methods employed in the isolation of myosin, for it is a somewhat labile protein and the more selective methods of preparation are not readily applied. The use of synthetic ion-exchange resins has been limited to the chromatography of the more robust proteins, and until now there has been no report of the

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successful study of myosin by this technique. Diethylaminoethylcellulose (Peterson & Sober, 1956), which has achieved considerable success as a relatively-mild chromatographic medium effective in the region pH 7–8 for the separation of proteins, offered some promise. This paper deals with its application to the chromatography of myosin. It forms part of a general study of the muscle proteins by ion-exchange chromatography (Perry & Zydowo, 1959*a*, *b*).

METHODS

Chromatography. Diethylaminoethylcellulose and carboxymethylcellulose were prepared by the methods of Peterson & Sober (1956) and chromatography was in general carried out as described by Perry & Zydowo (1959*a*, *b*). Myosin solutions were equilibrated against the appropriate buffer by dialysis, and centrifuged for 20 min. at 10 000 *g* before application to the column, which had also previously been fully equilibrated with buffer. Usually the $E_{1\,cm.}$ (280 mμ) of the myosin solutions was within the range 3–4; occasionally at pH 7·6 somewhat more concentrated solutions [$E_{1\,cm.}$ less than 8·0 at 280 mμ] were applied. It was not practicable to apply more concentrated myosin solutions with the grade of diethylaminoethylcellulose used, which was made from Solka-Floc BW. 200 (Brown Co., Boston, Mass., U.S.A.), for the rate of entry into the column became very slow and the flow tended to stop completely. All chromatographic procedures and preparations of protein solutions were carried out at 1–2°.

L-Myosin. This protein was prepared as described by Perry (1955). No significant fall in viscosity was obtained on addition of adenosine triphosphate (ATP) to this preparation, indicating the absence of actin.

L-Myosin solution could be further purified by chromatography at 0° on diethylaminoethylcellulose as follows. A solution of $E_{1\,cm.}$ 3–4 at 280 mμ was equilibrated by dialysis against 0·16M-KCl–20 mM-tris-chloride buffer (pH 8·1) and run into a column (15–20 cm. × 2 cm. diam.) of diethylaminoethylcellulose equilibrated against the same buffer (2–5 mg. of protein N/g. of diethylaminoethylcellulose). After application of the myosin the column was washed with the original buffer solution until E_{280} of the eluate was negligible. Myosin was then eluted by the direct application of 0·22M-KCl, 20 mM-tris–HCl (pH 8·1). It was precipitated from the eluate by dialysis against 0·04M-KCl.

Enzymic assays. Adenosine triphosphatase (ATPase) assays were carried out at 25° as described by Perry & Grey (1956). The final incubation mixture of total volume 2 ml. usually contained: 5 mM-ATP; 5 mM-$CaCl_2$; 50 mM-tris–HCl buffer (pH 7·6); 0·3 ml. of 0·25M-KCl, 5 mM-tris–HCl (pH 7·6), or eluted myosin in the same solution; KCl to bring the final concentration to 0·2M. In some cases, for more accurate determinations, assays were carried out at three different concentrations of enzyme from a given fraction and specific activities calculated from the straight-line graphs obtained by plotting phosphate liberated against amount of protein. Specific ATPase activities were expressed as μg. of phosphorus liberated in 5 min. by 1 ml. of protein solution of $E_{1\,cm.}$ 1·0 at 280 mμ.

5′-Adenylic deaminase activity was determined as described by Perry & Zydowo (1959*a*).

Ribose and phosphorus determinations. The total phosphorus content of myosin preparations was determined by the method of Fiske & Subbarow (1925). For nucleic acid ribose and phosphorus estimations myosin solutions were precipitated at 0° with an equal volume of 15% (w/w) trichloroacetic acid, and the residue was extracted as described by Perry & Zydowo (1959*b*). Estimations were made on the extract obtained with hot 10% (w/v) $HClO_4$.

pH *measurements.* pH was measured on the buffers diluted to the concentrations used with the Pye glass electrode. The electrode was standardized at pH 6·99 with Soloid standard buffer tablets (Burroughs Wellcome and Co.).

RESULTS

Ion-exchange chromatography of myosin on cellulose derivatives presents certain difficulties because of the limited range of pH and ionic strength over which this protein is stable and soluble. For example at pH 5 and below the ATPase activity falls off rapidly, and at pH 7·0 an ionic strength of about 0·25 is necessary to keep appreciable amounts of myosin in solution.

In previous work (Perry & Zydowo, 1959*a*, *b*) it was convenient to elute the muscle proteins from diethylaminoethylcellulose by increasing the salt concentration at a constant pH. At pH 7·6 and below it was not practical, however, to employ this procedure with myosin, for when the protein dissolved in 0·25M-KCl containing 5 mM- or 20 mM-tris–HCl buffer, pH 7·6, was applied to a column of diethylaminoethylcellulose it was not held, although it appeared to be somewhat retarded on passing through the column. At lower ionic strengths the solubility of myosin decreases, and preliminary experiments in which the myosin was applied in 0·23M-KCl, 5 mM-tris–HCl (pH 7·6) were discontinued as only part of the myosin passed through unheld. Part appeared to be held or precipitated on the cellulose and was subsequently not satisfactorily eluted when the KCl concentration reached values at which, if used for the initial application to the cellulose, the myosin would not have been held at all. In 0·25M-KCl, 5 mM-glyoxaline–HCl buffer (pH 7·1) myosin was likewise not held, but if it was applied in 0·25M-KCl, 5 mM-histidine–HCl (pH 6·7) only part of the protein passed through unheld and elution of this material was impracticably slow.

A single attempt was made to chromatograph myosin on carboxymethylcellulose at pH 4·3. In 25 mM-sodium acetate buffer at this pH myosin is soluble but its ATPase activity is much reduced. Apart from a small amount of protein which was not held when myosin dissolved in the buffer was applied to the carboxymethylcellulose column, the bulk of the protein was strongly held and not eluted by M-KCl at this pH.

When myosin in 0·25M-KCl, 5 mM-tris–HCl (pH 7·6) was applied to a column of diethylaminoethylcellulose, about 40 cm. by 1 cm. diameter, and subsequently eluted by further application of the same buffer, the appearance of the eluted peak is shown in Fig. 1. Usually the peak was somewhat irregular in form, its appearance suggesting that it was composite, and often discrete steps could be recognized on the trailing side. With shorter and wider columns the peak was more compact but the same features were usually present. The variation in appearance of the peak appeared to depend more on the sample of diethylaminoethylcellulose and the column made from it than on the myosin preparation itself. For example, the chromatograms illustrated in Figs. 1 and 5 were obtained with the same sample of myosin. Although similar features can be recognized in both, the resolution is better in the experiment illustrated in Fig. 1.

Where myosin was chromatographed into a broad irregular peak, the behaviour of fractions on rechromatographing was determined by their original position on the peak. Material from the front of the peak, e.g. from fraction *A* in Fig. 2, on re-running on the same column under the same conditions underwent little or no retardation. Myosin from the trailing edge of the peak (fraction *B*, Fig. 2) was retarded compared to fraction *A*, however, when chromatographed again under identical conditions. This experiment indicated that fractions with real differences in chromatographic behaviour were present.

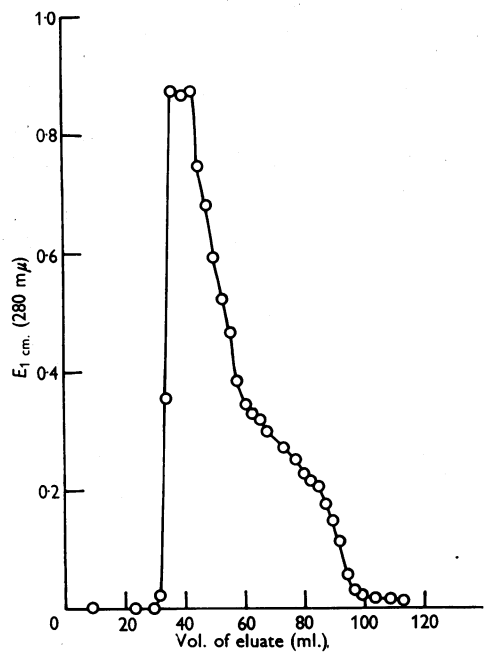

Fig. 1. Chromatography of L-myosin on diethylaminoethylcellulose. Protein solution (5 ml.; $E_{1\,cm.}$ 7·49 at 280 mμ) in 0·25M-KCl, 5 mM-tris–HCl (pH 7·5) was applied to a column 45·5 cm. × 1 cm. diameter and eluted with the same buffer.

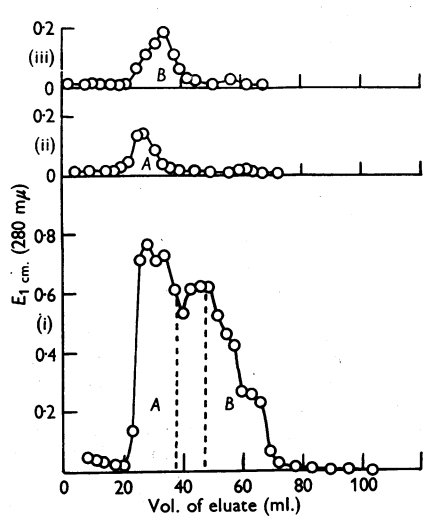

Fig. 2. Rechromatography of L-myosin eluted from diethylaminoethylcellulose. (i) 5 ml. of myosin ($E_{1\,cm.}$ 4·96 at 280 mμ) in 0·25M-KCl, 5 mM-tris–HCl (pH 7·6) was applied to a column 40 cm. × 1 cm. diameter, and eluted with the same buffer. Fractions *A* and *B* were concentrated by precipitation, by dialysis against 0·04M-KCl. (ii) 1·9 ml. of fraction *A* in 0·25M-KCl, 5 mM-tris–HCl (pH 7·6) was re-run on the column used for (i). (iii) 2·1 ml. of fraction *B* in 0·25M-KCl, 5 mM-tris–HCl (pH 7·6), was re-run on the same column as was used for (i) and (ii).

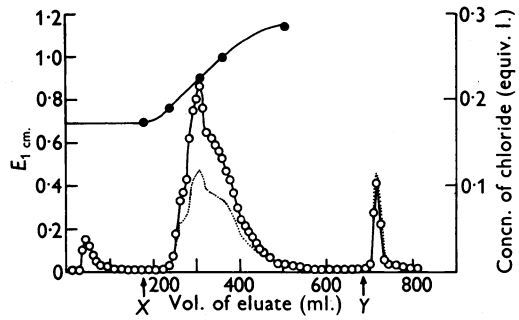

Fig. 3. Chromatography of L-myosin on diethylaminoethylcellulose. Protein solution (35 ml.; $E_{1\,cm.}$ 5·52 at 280 mμ) in 0·16M-KCl, 20 mM-tris–HCl (pH 8·2) was applied to a column 14 cm. × 2 cm. diameter. Elution by gradient to 0·3M-KCl, 20 mM-tris–HCl (pH 7·2) was applied at point *X* and step to 1·5M-KCl, 20 mM-tris–HCl (pH 8·2) at point *Y*. ○, $E_{280\,m\mu}$; ●, concentration of chloride; - - -, $E_{260\,m\mu}$.

By increasing the pH to 8·0–8·2 with 5 mM–20 mM-tris–HCl buffer satisfactory solutions of freshly prepared myosin ($E_{1\,cm.}$ 3·0–4·0 at 280 mμ) could be obtained in 0·16 M-KCl. Samples of myosin which had been stored 7–14 days were frequently less soluble in this solution than was freshly prepared material. Under these conditions myosin was held on the column and could be eluted when a gradient was applied (Fig. 3). On some occasions a small amount of material having a high 5′-adenylic deaminase activity, but without ATPase activity, passed through the column before the gradient was applied. More often, however, relatively small amounts of material with ATPase activity passed unheld through the column with the deaminase-rich material. The reason for this was not clear but it was apparently not due to overloading of the column. The peak with ATPase activity either appeared just behind that rich in 5′-adenylic deaminase activity or it was not distinguishable from it. The main peak which was obtained on application of a KCl gradient possessed ATPase activity and varied somewhat in shape according to the volume required for its elution. Its shape was usually similar to that illustrated in Fig. 3 and resembled the peak obtained when chromatography was carried out in 0·25 M-KCl, 5 mM-tris–HCl, pH 7·6. The peak, which was never symmetrical, occurred at a chloride concentration of 0·20–0·22 equiv./l.

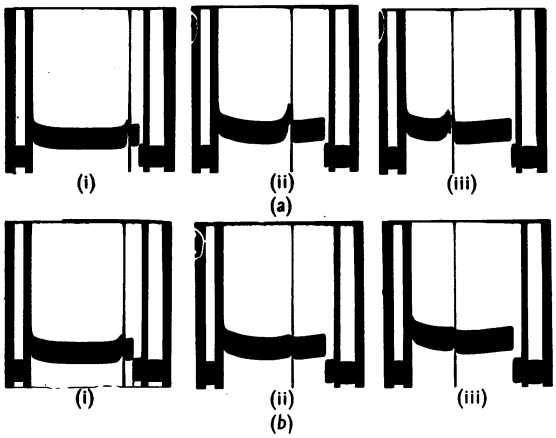

Fig. 4. Ultracentrifuge diagrams of myosin before and after chromatography on diethylaminoethylcellulose. Conditions were: 0·25 M-KCl, 0·05 M-potassium phosphate buffer (pH 7·2); $5{\cdot}98 \times 10^4$ rev./min.; 20°. (*a*) L-myosin was applied in 0·16 M-KCl, 20 mM-tris–HCl (pH 8·2) and eluted by application of gradient to 0·3 M-KCl; (i) 16 min., (ii) 64 min., (iii) 112 min. (*b*) The same sample of L-myosin was equilibrated with 0·16 M-KCl, 20 mM-tris–HCl (pH 8·2), but not chromatographed; (i) 16 min., (ii) 64 min., (iii) 112 min.

Myosin which had been chromatographed on diethylaminoethylcellulose retained its ability to combine with actin to form actomyosin, as indicated by a fall in viscosity on the addition of ATP to a solution in which both proteins were present (kindly carried out by Mr L. Leadbeater). The specific ATPase activity was appreciably increased and, in so far as the chromatographed protein was insoluble at pH 7·0 in 0·04 M-KCl and soluble in 0·5 M-KCl, the solubility characteristics were unchanged. On electrophoresis in 0·25 M-KCl, 50 mM-potassium phosphate buffer (pH 7·2) for 10 hr., no significant difference between untreated myosin and that which had been chromatographed at pH 7·6 or 8·2 could be detected. (At pH 8·2 electrophoresis was carried out on the main ATPase-rich peak.) At both pH values the myosin moved as a single peak which was somewhat asymmetric, and although it remained extremely sharp throughout a prolonged electrophoresis the descending boundary displayed minor irregularities which could be interpreted as evidence of heterogeneity.

In the ultracentrifuge the chromatographed myosin presented, in addition to the sharp characteristic myosin boundary, a slightly faster broader boundary which was not evident in control samples of unchromatographed myosin (Fig. 4).

Adenosine triphosphatase activity of myosin chromatographed on diethylaminoethylcellulose. The curve obtained by plotting the ATPase activity per ml. of the chromatographed myosin against the volume of eluent at both pH 7·6 and 8·1 followed in a general way a similar plot of absorption at 280 mμ. If, however, the activity was calculated as specific activity (see Methods) then the following results were obtained:

(1) The specific activity was not constant along the eluted peak. In general two main peaks could be seen, as illustrated in Fig. 5. The first peak of specific activity was close to or slightly in front of the main 280 mμ-absorbing peak, whereas the second usually occurred in the trailing edge. Occasionally the first peak of specific activity split into two, but the most persistent features were the fractions of lower specific activity obtained when about half, or slightly more than half, of the myosin had been eluted from the column. The data from a number of experiments are summarized in Table 1.

(2) In some preparations the specific activity at the front of the E_{280} peak was low, suggesting that inactive myosin or non-myosin material, which was not retarded by the cellulose, was present. This was probably due to the material without ATPase activity, but rich in 5′-adenylic deaminase activity, which was eluted as a separate peak with 0·16 M-KCl, 20 mM-tris–HCl (pH 8·2) (see above).

(3) With the exception of the small amount of material which was sometimes observed at the

front of the peak, the specific activity was greater at all other points along the peak at which it was measured than that of the original myosin (Table 1).

These observations were all made on myosin eluted at constant ionic strength, i.e. with 0·25 M-KCl, 5 mM-tris–HCl (pH 7·6). Limited studies on myosin applied to diethylaminoethylcellulose in 0·16 M-KCl, 20 mM-tris–HCl (pH 8·1), suggested that there was a similar distribution of specific ATPase activity along the myosin peak eluted on application of a gradient.

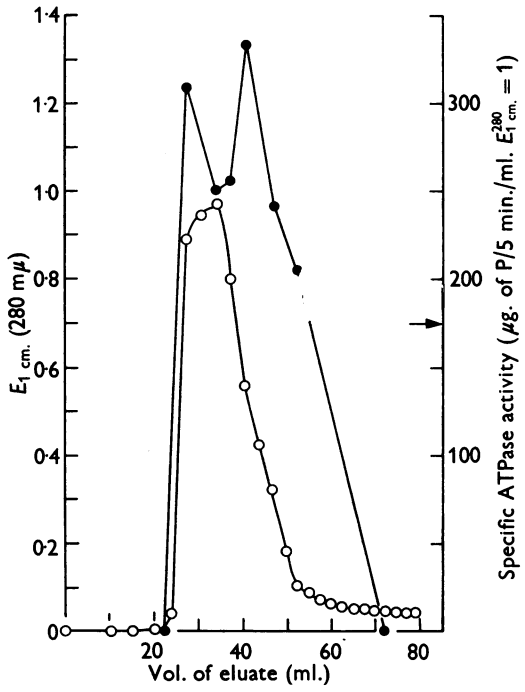

Fig. 5. Specific ATPase activity of L-myosin after chromatography on diethylaminoethylcellulose. Protein solution (4 ml.; $E_{1\,cm.}$ 7·46 at 280 mμ) in 0·25 M-KCl, 5 mM-tris–HCl (pH 7·6), was applied to a column 40 cm. × 1 cm. diameter and eluted with the same buffer. ○, $E_{280\,m\mu}$; ●, specific ATPase activity. The arrow on the specific activity ordinate indicates the activity of unchromatographed myosin.

The increase in specific activity related to E_{280} could not be explained by the preferential removal of ribonucleic acid (see below), which has a relatively greater $E_{1\,cm.}$ at 280 mμ than has protein. Comparison of the absorption at 280 mμ per mg. of protein nitrogen before and after chromatography on diethylaminoethylcellulose indicated a fall of less than 3 %. This finding, and control experiments in which ATPase activity was related directly to the nitrogen content of the fractions, clearly showed that after chromatography there was a significant increase in actual specific ATPase activity.

5′-Adenylic deaminase activity. The 5′-adenylic deaminase activity of the chromatographed myosin was much less systematically studied than was the ATPase activity. Preliminary experiments indicated that both halves of the main peak eluted at pH 7·6 possessed deaminase of activity comparable, although not identical, with that of the original myosin. Further work was not done at this pH, as from an earlier investigation (Perry & Zydowo, 1959*a*) it appeared that 5′-adenylic deaminase was probably eluted from diethylaminoethylcellulose at a lower ionic strength than that required to elute myosin. For this reason chromatography at pH 8·2, at which the myosin could be held on the diethylaminoethylcellulose, was considered more likely to achieve separation of the two enzymes. The small peak without ATPase activity, which was eluted by 0·16 M-KCl, 20 mM-tris–HCl buffer (pH 8·2), possessed deaminase activity 5–10 times that of the original myosin. When the material which passed unheld through the column before application of the gradient also possessed ATPase activity, the specific activity with regard to ATPase was usually lower than that of unchromatographed myosin, whereas the specific activity with regard to 5′-adenylic deaminase was appreciably higher. The main myosin peak obtained on

Table 1. *Specific adenosine triphosphatase activity of fractions of L-myosin eluted from diethylaminoethylcellulose*

Myosin was applied in 0·25 M-KCl, 5 mM-tris–HCl, pH 7·6, and eluted with same buffer, except in the case of Expt. 14 which was carried out in 0·25 M-KCl, 5 mM-glyoxaline–HCl buffer (pH 7·1). Specific activity was measured at 25° = μg. of phosphorus/5 min./1 ml. of protein, $E_{1\,cm.}$ 1·0 at 280 mμ.

Expt. no.	Myosin preparation	Age (days)	Specific ATPase activity: Unchromatographed	Chromatographed: 1st peak	Chromatographed: 2nd peak	Chromatographed: Minimum between peaks
8	A	11	179	232	294	188
13	B	13	161	303	229	198
14	B	16	175	255	235	218
15	B	22	176	309	335	252
19	C	4	186	235	222	210

Table 2. *Phosphorus and ribonucleic acid content of L-myosin preparations before and after chromatography on diethylaminoethylcellulose*

Results are expressed in μg./g. of myosin N. Those in columns 2 and 4 are obtained from the analysis of hot 10% $HClO_4$ extract of myosin preparations (see Methods). RNA phosphorus is obtained from the ribose figures assuming that 53·1% of total ribose is estimated under the condition of the orcinol reaction (Perry & Zydowo, 1959*b*).

		(1) Total P	(2) Ribose	(3) RNA-P	(4) Total P in $HClO_4$ extract
Expt. 1.	Before chromatography	1608	3375	1312	1111
	After elution by 0·25M-KCl, 20 mM-tris (pH 7·6)	104	280	109	53
Expt. 2.	Before chromatography	1091	2020	785	651
	After elution by KCl gradient in 20 mM-tris (pH 8·2)	153	49	19	45

subsequent application of a gradient had about one-third the deaminase activity of control unchromatographed samples. Thus chromatography on diethylaminoethylcellulose under these conditions partially removed the 5′-adenylic deaminase from L-myosin preparations.

Ribonucleic acid and phosphorus content of myosin. After complete elution of the myosin peak from diethylaminoethylcellulose columns to which the protein had been applied either in 0·25M-KCl, 20 mM-tris–HCl (pH 7·6) or in 0·16M-KCl, 20 mM-tris–HCl (pH 8·1), subsequent increase in the KCl concentration to 1·5M eluted a further small peak. This peak differed from the other material eluted from the column in that the ratio E_{260}/E_{280} was usually greater than 1 (Fig. 5). It was probable that this fraction contained ribonucleic acid (RNA), since the material absorbed strongly at a maximum of about 258 mμ, was non-dialysable and relatively rich in pentose and phosphorus compared with myosin. The presence of protein was evident from the general properties of this fraction; also it gave a positive biuret test and the minimum of the ultraviolet-absorption spectrum was at 240 mμ, rather than 230 mμ which is characteristic of pure RNA (Beaven, Holiday & Johnson, 1955). On dialysis against distilled water almost all the material absorbing at 280 and 260 mμ was precipitated. In this property the fraction differed from the nucleoprotein isolated from the myofibril (Perry & Zydowo, 1959*b*). Nevertheless the RNA-containing fraction from myosin probably contained this myofibrillar ribonucleoprotein, together with small amounts of myosin, whose presence was indicated by ATPase activity (usually less than 20% of that of myosin of comparable extinction at 280 mμ). Tropomyosin, which often contaminates myosin preparations, would also be expected to be eluted with this fraction unless special steps were taken to elute it from the column after removal of the myosin and before the fraction containing RNA is displaced (Perry & Zydowo, 1959*a*).

L-Myosin preparations used in this investigation contained as a persistent impurity 0·2–0·3% of RNA, values that are somewhat less than those reported by Mihalyi, Brodley & Knoller (1957). Myosin which had passed through a diethylaminoethylcellulose column, whether it was held or not, i.e. at pH 8·1 or 7·6, contained little or no RNA (see Table 2). It appeared therefore that the RNA-containing fraction eluted at higher concentrations of KCl represented almost all the RNA originally present in myosin preparations. The total phosphorus content of myosin was reduced by chromatography under these conditions to about 0·002%, a barely significant figure and equivalent to less than 5–10% of the original value.

The value for RNA phosphorus was calculated from the ribose figures assuming that 53·1% of the total ribose of the RNA, i.e. the value obtained for the myofibrillar nucleoprotein (Perry & Zydowo, 1959*b*), was estimated under conditions of the orcinol reaction. For the unchromatographed myosin these values were, however, higher than the estimated total phosphorus of the 10% $HClO_4$ extracts, suggesting that either the factor was too high or ribose other than that present in RNA was present in the 10% $HClO_4$ extracts. Further, as not all the total phosphorus was extracted from unchromatographed myosin by $HClO_4$, part may have been due to other substances, e.g. phospholipid. Much of this phosphorus insoluble in 10% $HClO_4$ was also removed by chromatography on diethylaminoethylcellulose.

DISCUSSION

The use of diethylaminoethylcellulose offers a means of applying chromatographic procedures for the study of myosin. Since the chromatographed myosin had a higher specific ATPase activity than the original preparation and retained its ability to form actomyosin, no loss in the biological activity of the protein was detectable. It is apparent that L-myosin prepared by conventional methods contains substances which do not possess ATPase activity. Chromatography provides a simple method

of removing at least one of these impurities, an RNA-containing fraction which is responsible for the bulk of the phosphorus present in unchromatographed myosin preparations. The RNA is associated with protein and part of this complex is probably identical with the ribonucleoprotein which can be isolated from the myofibril (Perry & Zydowo, 1959*a*, *b*). Traces of phosphorus remained associated with myosin after chromatography, but the amounts estimated were close to the limits of accuracy of the methods used. It is considered that this small amount of phosphorus is present as a contaminant and does not function as the prosthetic group of myosin.

The shape of the main peak which constitutes the bulk of the eluted material suggested heterogeneity under almost all the conditions of chromatography tried. Further evidence of heterogeneity is provided by the fairly regular pattern of variation of specific ATPase activity along the main peak of eluted material and by re-running fractions of chromatographed myosin on diethylaminoethylcellulose. When the retarded myosin fraction (fraction *B*, Fig. 2) was run alone its elution volume was lower than when the whole myosin was chromatographed. This may be due to displacement effects by the other myosin fractions. The simple increase of ATPase activity after chromatography could be explained by the active renewal either of some inhibitor or of enzymically inert material. At pH 7·6 usually 67–87% of the applied protein, as judged by absorption at 280 mμ, was recovered in the main eluted peak.

Myosin ATPase is known to be sensitive to variations in the ionic nature of the assay medium; such effects cannot be used to explain the variation in specific activity along the eluted peak, for care was taken to ensure that assays were carried out under standard conditions. One possible explanation is that there are several different forms of myosin, with different enzymic activity. These forms either could be present in the original myosin or could be produced by modification of the original protein as a consequence of chromatography on the diethylaminoethylcellulose. This investigation provides no clear evidence by which to decide between these possibilities. Changes in growth hormone during chromatography on diethylaminoethylcellulose have been reported by Ellis & Simpson (1956) and there is evidence from the present study of a difference in the sedimentation behaviour of chromatographed myosin compared with the untreated protein. This change in sedimentation pattern may represent partial aggregation of the myosin. It has been reported, however, that such changes occur spontaneously on storage of myosin (Holtzer, 1956), and it is not easy to relate them to the distribution of specific ATPase activity along the myosin peak. Further work is needed to interpret these changes in sedimentation pattern.

Another possible explanation is that normal preparations of L-myosin contain appreciable amounts of enzymically inactive material which is closely associated with the ATPase-containing protein and coprecipitated with it. The findings reported here could be explained if during elution from diethylaminoethylcellulose some separation of such enzymic and non-enzymic components occurred so that the component devoid of enzymic activity became concentrated mainly, although perhaps not entirely, about the centre or second half of the eluted material, the ATPase itself being eluted as a fairly broad peak over the whole range. Treatment of myosin with proteolytic enzymes of different specificities splits myosin into similar enzymic and non-enzymically active fragments, the H- and L-meromyosins (Szent-Györgyi, 1953; Gergely, Gouvea & Kariban, 1955). Several workers have considered the possibility of the composite nature of myosin (Tsao, 1953; Kominz, Hough, Symonds & Laki, 1954; Snellman, 1956; Kominz, Carroll, Smith & Mitchell, 1959). It is conceivable that the conventional preparations could consist of a firmly bound complex of two or more components, not involving covalent bonds, whose separation is facilitated by slight modification of the components, e.g. by proteolytic enzymes (cf. Middlebrook, 1958). Evidence which suggests that the meromyosins may lead an independent existence is supplied both from studies on their turnover *in vivo* (Velick, 1956) and from investigations with fluorescent antibodies (Holtzer & Marshall, 1958) on the localization of myosin and the meromyosins in the myofibril.

Whatever may be the explanation of the enzymic results, chromatography on diethylaminoethylcellulose offers an effective way of removing certain impurities in myosin and gives results which suggest that the conventional preparations of this protein may be complex systems. The findings of a number of physical and biochemical investigations on L-myosin may have to be reconsidered in the light of this apparent complexity. For example, if the ribonucleoprotein contaminant plays a part in protein synthesis in muscle, it has possibly a relatively high turnover rate, thus influencing appreciably the turnover values which have been obtained with conventional myosin preparations in which it is present as an impurity.

SUMMARY

1. The chromatographic behaviour of L-myosin on diethylaminoethylcellulose has been investigated at pH 7·6 and 8·2.

2. Evidence is presented for a regular variation in the specific adenosine triphosphatase activity of myosin fractions eluted from diethylaminoethylcellulose.

3. Partial separation of 5′-adenylic deaminase activity has been obtained by chromatography at pH 8·2.

4. Myosin has been prepared with less than 0·002% of phosphorus and virtually free from the ribonucleic acid invariably present in L-myosin preparations.

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Distribution and Activity of Monoamine Oxidase in Mouse Tissues

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During a recent study of the radioprotective action of 5-hydroxytryptamine in the mouse (Hope, 1959) we were impressed by its low toxicity in this species compared with the rat. The LD_{50} for 5-hydroxytryptamine administered intraperitoneally as the creatinine sulphate in the mouse was approximately 1 g./kg. body weight. Rapport & Virno (1952) found that the LD_{50} for this substance administered in the same way in rats was 50 mg./kg. body weight, i.e., it is 20 times as toxic to the rat as to the mouse.

Rapport & Virno (1952) investigated in a number of mammalian species the fall in oxygen consumption after the administration of 5-hydroxytryptamine. The rat was more sensitive in this respect than any other species examined. The authors suggested that the low amine oxidase activity of rat kidney was responsible.

In view of the now well-established fact that amine oxidase is an important catalyst of the destruction of 5-hydroxytryptamine in mammals (Blaschko, 1958) we decided to study the activity of the enzyme in mouse kidney and some other mouse tissues.

In this paper the rate of oxidation of 5-hydroxytryptamine has been compared with that of representatives from the main types of monoamine oxidase substrates. These included tryptamine, β-phenylethylamine, *iso*amylamine and benzylamine. Tyramine, although in some respects an unsatisfactory substrate of amine oxidase (Blaschko & Himms, 1954), was also included in order to