The Adenosinetriphosphatase Activity of Myofibrils Isolated from Skeletal Muscle

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Muscle has long been known as a source of many well characterized enzymes and proteins, but information about the localization of these substances within the cell is obtained mainly by inference and other indirect means. Direct investigation of the structural interrelationships of such constituents of muscle, most of which have been intensively studied in the purified form, should reveal considerable information about the working of the cell in general and the contractile process in particular. Muscle does not lend itself to the fractionation techniques which have been applied with considerable success to liver and kidney because the cells are not easily broken up by mechanical means, and the cytoplasm is extremely viscous owing to a high degree of structural organization. In consequence the procedures hitherto employed to isolate organized components from the muscle cell have been drastic. Behrens (1932) isolated nuclei from calf-heart muscle by dehydrating the tissue with organic solvents, grinding and finally centrifuging the powder in a benzene-chloroform mixture. The method of Stoneburg (1939) for isolating muscle nuclei, employing citric acid treatment followed by pepsin digestion in 1% hydrochloric acid, is hardly less drastic.

The main cytoplasmic constituent, the myofibrils, can be isolated in quantity by fixing the muscle in formalin for several days and then breaking up the tissue mechanically. Such preparations are useful for examination in the electron microscope (Hall, Jakus & Schmitt, 1946; Draper & Hodge, 1949), but are of limited value for more precise biochemical investigation. By weakening the muscle cell with trypsin at 0° and pH 7 Schick & Hass (1949) have indicated a useful method for obtaining myofibrils in amounts sufficient for biochemical study. These authors (1949) have shown that such myofibrils shorten in the presence of adenosinetriphosphate (ATP), and that when isolated from skeletal and heart muscle their solubility properties are in general similar to those of actomyosin (Ashley, Hass & Schick, 1950).

From the results of investigations on the adenosinetriphosphatase (ATPase) activity of extracted myosin and actomyosin, Mommaerts & Seraidarian (1947) and Braverman & Morgulis (1948) conclude that with the concentration of calcium and magnesium known to exist in muscle, the myosin present would split off phosphate from ATP at a rate which is about one-hundredth of the rate at which phosphate appears when muscle is contracting. The work described here was undertaken to investigate the ATP-splitting properties of the isolated myofibril and to see if it showed any marked differences in behaviour to the presumed myofibrillar component, actomyosin. A modified method of preparation was used as it soon became apparent that at 0° low concentrations of trypsin have a considerable effect on the myofibril. Even traces of the enzyme can attack both the myofibril and extracted myosin without destroying the ATPase activity, yet the physical properties of the myosin are considerably changed, particularly with respect to its interaction with actin. Gergely (1950) has recently reported independently a similar finding with myosin. A preliminary account of this work has been communicated to the Biochemical Society (Perry, 1950*a*).

EXPERIMENTAL

Methods

Preparation of myofibrils. The method is a modification of that used by Schick & Hass (1949). A 2–3 g. portion of psoas muscle from a freshly killed rabbit is dissected free from other tissues, allowed to stand for 30 min. at room temperature and frozen on the stage of a freezing microtome. The frozen tissue is sectioned into slices 25 μ. thick, which are rapidly transferred into 50 ml. of a well stirred, cold 0·08 M-borate buffer, pH 7·1. It is important that the borate solution is kept as cold as possible without freezing, and that the muscle slices are transferred to it whilst they are still in the frozen state to prevent the myofibrils from going into thaw rigor (Szent-Györgyi, 1949; Perry, 1950*b*). This shortening depends on the concentration of ATP in the thawed muscle and takes place very slowly or not at all at 0°. The effect can be eliminated by allowing the muscle to go into rigor mortis before freezing and sectioning. To the suspension of muscle cells 25 mg. of purified collagenase is added, and the enzyme is allowed to act at 0° for 3–7 hr. This period varies with the collagenase preparation and must be determined by experiment. The suspension is diluted with 150 ml. of the borate buffer and homogenized for 20 sec. in a chilled Waring blender. At this stage a drop of the suspension stained with methylene blue should show that practically all the cells have broken up into myofibrils. The myofibrils are now washed three times with borate buffer by centrifugation at 1500 *g*, these and subsequent operations being carried out at 0°. The first supernatant is usually slightly turbid, but later supernatants are clear. Large particles, synaeresed myofibrils, and cell fragments are now removed by resuspending in 100 ml. and centrifuging for 1 min. at 400 *g*. The material

which settles is discarded and the suspension centrifuged down again at 1500 *g*, resuspended in 100 ml. of borate and centrifuged for 1 min. at 400 *g*. The turbid supernatant is a very slowly settling suspension of myofibrils which usually contains 1–2 mg. protein/ml. As shown in Fig. 1 the suspension is quite homogeneous; the myofibrils are about 1 μ. wide, and occur singly and sometimes in bundles of two or three.

For preparations with trypsin the psoas slices were suspended in 200 ml. of ice-cold borate and 4 mg. of crystalline trypsin added. When microscopic examination indicated that the cells would readily rupture, usually after about 30 min., they were homogenized and the preparation carried out as for the method using collagenase.

Myosin and actomyosin. Myosin was prepared by the method of Bailey (1942). Actomyosin was the myosin B of Banga & Szent-Györgyi (1942) and was purified by three precipitations as in the method for myosin.

Adenosinetriphosphate. The Ba salt of ATP was prepared by a method similar to that of Needham (1942), and which included a final precipitation of the Ba salt with 50% (v/v) ethanol at pH 3. It was converted to the Na salt as recommended by Bailey (1942).

Adenosinetriphosphatase activity. ATPase determinations were carried out in 0·1 M-borate buffer, pH 6·9, and 0·1 M glycine buffer usually pH 8·6. In addition to activators ($CaCl_2$, $MgCl_2$, and KCl), 0·3 ml. ATP (2·0 mg. 10 min. hydrolysable P/ml.), and myofibril suspension, glass-distilled water was added to each enzyme test sample to bring the volume up to 2 ml. in all cases. After incubation, usually 15 min. at 25°, the reaction was stopped by 1·5 ml. of 10% trichloroacetic acid.

Collagenase. The preparation, kindly donated by Dr E. Bidwell of the Wellcome Research Foundation, was active against collagen, gelatin, and reticulin, but did not attack elastin. The dried powder contained 11·6 *Q* units of collagenase/mg. (Bidwell & van Heyningen, 1948), an active hyaluronidase and a small amount of lecithinase.

Trypsin. This preparation was the crystalline enzyme containing 50% $MgSO_4$, as supplied by the Armour Company.

Viscosity determinations. Determinations of viscosity and inhibition of actomyosin formation were carried out as described by Bailey & Perry (1947).

RESULTS

Trypsin-prepared myofibrils. Some difficulty was experienced in minimizing the action of trypsin on the myofibrils themselves yet at the same time allowing the enzyme to act long enough so that these structures could be readily liberated. It was found to be difficult also to remove the last traces of trypsin by washing three to four times on the centrifuge, with the result that within 2 or 3 days the myofibrils lost their morphological features. After longer storage at 0° the milky suspensions became almost clear, yet the adenosinetriphosphatase activity of the whole preparation did not fall; indeed in the early stages an increase was usually observed. The myofibrillar adenosinetriphosphatase is remarkably stable to trypsin, e.g. no loss in activity could be demonstrated when a preparation was treated at pH 7·1 and 0° for 106 min. with 0·015 mg. trypsin/ml.

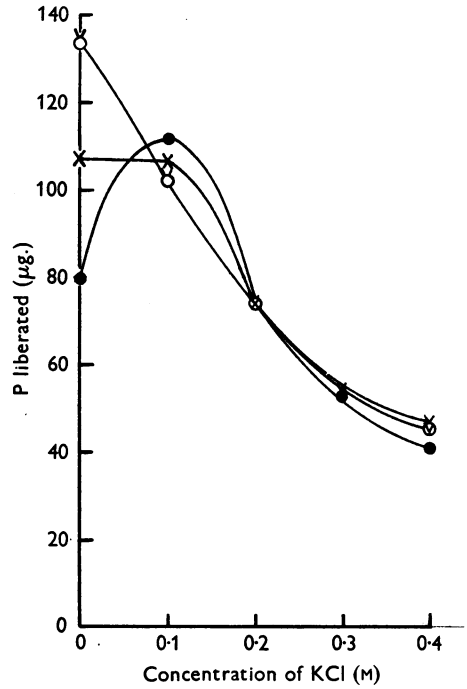

Fig. 2. The effect of KCl on the adenosinetriphosphatase activity of stored trypsin-prepared myofibrils. Enzymic activity measured with 0·005 M-$CaCl_2$ in glycine buffer, pH 8·6, at the following times after completion of the preparation: 1 hr., ●; 24 hr., ×; 48 hr., ○; 72 hr., ▼.

Table 1. *The adenosinetriphosphatase activity and soluble protein concentration of a trypsin-prepared myofibril suspension standing at* 0°

(Enzymic activity was measured in glycine buffer, pH 8·6, and 0·005 M-$CaCl_2$.)

Age of preparation (hr.)	Whole suspension: Protein concn. (mg./ml.)	Whole suspension: ATPase activity (μg. P/ml./15 min.)	Clear supernatant: Protein concn. (mg./ml.)	Clear supernatant: ATPase activity (μg. P/ml./15 min.)
1	1·51	243	0·12	Nil
24	1·51	275	0·50	Nil
48	1·51	280	0·65	29
72	1·51	277	0·83	64

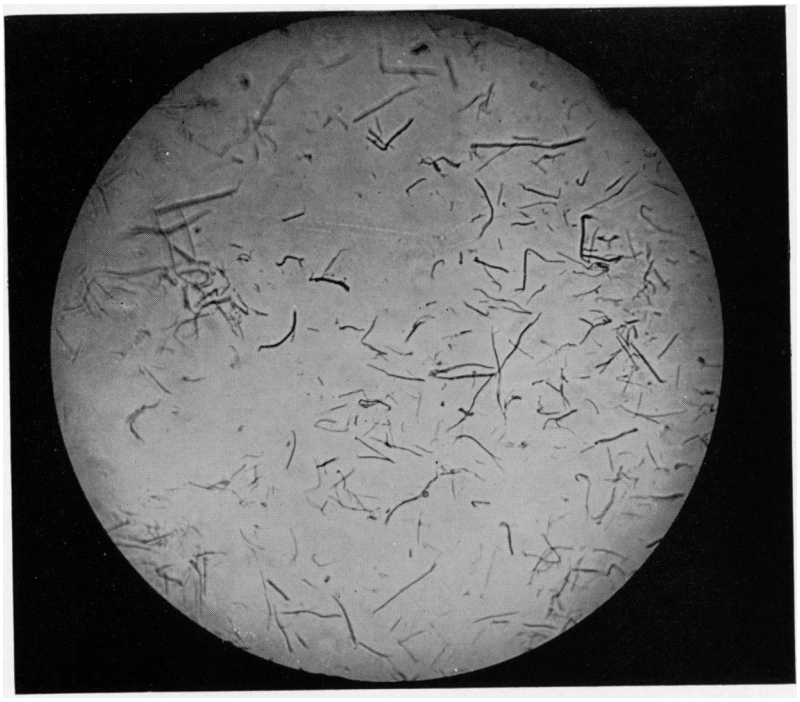

Fig. 1. Photomicrograph of collagenase-prepared myofibrils. Van Gieson stain. (× 280.)

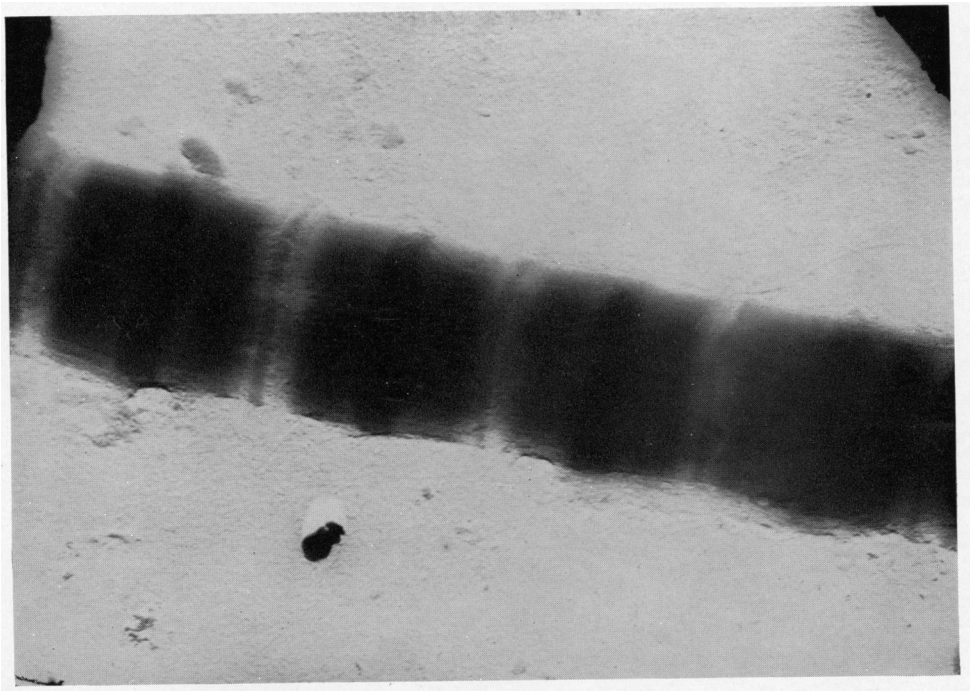

Fig. 4. Electron micrograph of collagenase-prepared myofibril. Fixed by 5 min. exposure to formalin vapour before drying. Gold-palladium shadowed. (× 15,400.)

On storing suspensions of trypsin-prepared myofibrils in 0·08M-borate at 0°, within 2–3 days about one-third of the total nitrogen appeared in the clear supernatant liquid obtained by centrifugation and could be precipitated by trichloroacetic acid. The adenosinetriphosphatase activity of the whole increased. Ultimately, as the amount of soluble nitrogen increased, adenosinetriphosphatase activity passed into solution (Table 1) until, after a week or more, practically all the adenosinetriphosphatase was dissolved and could not be separated by centrifugation at 20,000 *g* for 30 min. Overnight dialysis against 0·05M-potassium chloride at pH 6·5–7·0 did not precipitate the enzyme, nor could any adenosinetriphosphatase activity be detected in the dialysate. Fig. 2 shows that freshly prepared myofibril suspensions have maximum adenosinetriphosphatase activity in 0·1M-potassium chloride when activated by 0·005M-calcium chloride in glycine buffer at pH 8·6. After the suspension has stood at 0° the effect of potassium chloride changes, and within a few days all concentrations inhibit the adenosinetriphosphatase. In parallel with this changing effect of potassium chloride, the enzymic activity in the absence of the salt increased.

During the period in which the adenosinetriphosphatase of the myofibrils was maintained, the fall in viscosity produced by addition of adenosinetriphosphate to a 0·6M-potassium chloride extract of the myofibrils diminished until finally it was almost absent, indicating that there was very little actomyosin in the extract.

Action of trypsin on myosin. It was considered that the changes in the properties of the myofibrils were due to the continued action of slight traces of trypsin. To confirm this, the action of the crystalline enzyme on myosin was studied. A dilute myosin gel (1–2 mg. protein/ml.) in 0·05M-potassium chloride, treated with crystalline trypsin (0·0045 mg./ml.) at 0°, became almost completely clear after a few hours. The adenosinetriphosphatase activity (measured in 0·1M-potassium chloride, 0·005M-calcium chloride, and 0·1M-glycine buffer, pH 8·6) increased by 20–30%; considerably greater increases were obtained in the absence of potassium chloride, but the ability to form actomyosin dropped appreciably, as shown in Fig. 3, and after some days became almost negligible although the myosin was still enzymically active. At pH 8·6 increasing concentrations of potassium chloride inhibited the calcium-activated adenosinetriphosphatase of trypsin-treated myosin in an identical fashion to their effect on stored trypsin-prepared myofibrils. The effect of progressive tryptic action on the viscosity of myosin is indicated in Table 2, which gives the figures for actomyosin viscosity in the presence of adenosinetriphosphate. As a constant amount of actin is added under comparable conditions in each case to the trypsin-treated myosin, the fall in viscosity of the dissociated actomyosin must indicate a fall in the viscosity of the myosin with progressive tryptic action.

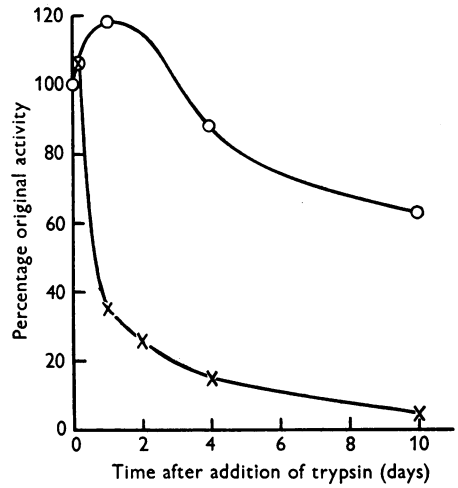

Fig. 3. The actomyosin-forming and the adenosinetriphosphatase activities of myosin treated with trypsin. Actomyosin-forming activity, ×; adenosinetriphosphatase activity, ○.

Table 2. *Effect of trypsin on the adenosinetriphosphatase and actomyosin-forming activities of myosin*

(The two centre columns show the specific viscosities at 0°, with and without 0·0004M-ATP, of the solutions obtained when samples of the trypsin-treated myosin were added to a constant amount of actin in each case. Enzymic activity was measured in glycine buffer, pH 8·6, containing 0·005M-$CaCl_2$.)

Time after addition of trypsin (hr.)	Specific viscosity of actomyosin		ATPase activity
	No ATP	ATP	(μg. P/ml./15 min.)
1·5	0·665	0·284	504
24	0·262	0·138	563
48	0·198	0·107	—
96	0·161	0·109	418
120	0·104	0·087	297
0 (control)	0·662	0·309	475

Collagenase-prepared myofibrils. Myofibrils prepared by this method show definite cross-striations which are visible without staining in the phase-contrast microscope, but are made more distinct with methylene blue. With van Gieson stain the A, I and Z bands can be very clearly distinguished. Electron microscope examination (Horne & Perry, 1951) reveals that the myofibrils have retained their fine structure (Fig. 4); filaments with the 400A. period are readily seen, and in general the picture is similar to that obtained by Draper & Hodge (1949) with myofibrils obtained from muscle fixed in

formalin. Examination of some fifteen preparations by this method indicated that they were quite homogeneous. The only recognizable contaminants seen were collagen-like filaments presumably of sarcolemmal origin, and quite infrequently very dense bodies considered from their size to be nuclei.

When 0·0004M-adenosinetriphosphate and 0·002M-magnesium chloride are added to the myofibrils suspended in borate buffer, the fibrils shorten and settle rapidly. Unlike threads made from extracted actomyosin which synearese isodimensionally under these conditions, myofibrils do not obviously get thinner, although the decrease in length may be considerable. Centrifugation at 1000 rev./min. for 20 sec. causes the adenosinetriphosphate-treated myofibrils to settle down in the tube, whereas the control myofibrils, treated with magnesium chloride only, require much longer to bring about the same effect. After prolonged centrifugation both settle down to approximately the same volume, suggesting that there is no great change in the water content.

Good preparations can be obtained from muscle which is allowed to go into rigor mortis by leaving overnight at room temperature. Like the normal preparations these myofibrils shorten in the presence of adenosinetriphosphate, whereas attempts to prepare myofibrils from muscle which had gone into thaw rigor gave rather granular, rapidly settling bodies, which were not obviously affected by adenosinetriphosphate.

The myofibrils keep their structure well in 0·08M-borate. After storage at 0° for 1–2 weeks striations are still visible, little aggregation is to be seen and no adenosinetriphosphatase activity can be detected in the clear supernatant liquid obtained when the suspension is centrifuged.

Adenosinetriphosphatase activity of collagenase-prepared myofibrils. If the assumption is made that the protein concentration of a myofibril suspension is six times the nitrogen concentration, the usual adenosinetriphosphatase activity, Q_p, obtained at 25° with calcium activation ranges from 400–600 for collagenase-prepared myofibrils. (For definition of Q_p see Bailey, 1942.) It is unlikely that all the myofibrillar protein is myosin and preliminary determinations suggest that this protein makes up about 65–75% of the total. Q_p values calculated on the basis of the amount of myosin present, and assuming a twofold increase in activity on raising the temperature from 25 to 37°, would compare quite favourably with those obtained by Bailey (1942) and Meyerhof & Polis (1947) for average Edsall myosin preparations. The slightly higher Q_p values obtained with the trypsin-prepared myofibrils are to be expected in the light of the results described earlier. It is of interest to note, in view of the emphasis in the literature given to the calcium-activated adenosinetriphosphatase of myosin, that Q_p values similar to those given with calcium can be obtained in the presence of low concentrations of magnesium in the absence of other salts.

Collagenase itself has no action on the calcium-activated adenosinetriphosphatase at pH 8·6, e.g. the adenosinetriphosphatase activity of collagenase-prepared myofibrils which had been treated for an additional 33 hr. with collagenase (5 hr. with 0·6 mg./ml. followed by 28 hr. with 0·5 mg./ml.) was found to be identical with the activity of a control sample of the same preparation kept under similar conditions without collagenase. The collagenase used contained an active hyaluronidase and a little lecithinase; it is to be expected that this latter enzyme would have some effect on any magnesium-activated adenosinetriphosphatase, other than actomyosin, present in the myofibril (Kielley & Meyerhof, 1948, 1950).

Activation of adenosinetriphosphatase by cations. The effect of increasing potassium chloride concentrations on the calcium-activated adenosinetriphosphatase of the myofibril is similar to that obtained with myosin and actomyosin (myosin B). Fig. 5 shows that as the preparations age the general form of the curve is the same, but it is to be noted that the inhibiting effect of potassium chloride concentrations greater than 0·1M is not so marked (cf. Fig. 2). Myosin B shows a similar effect.

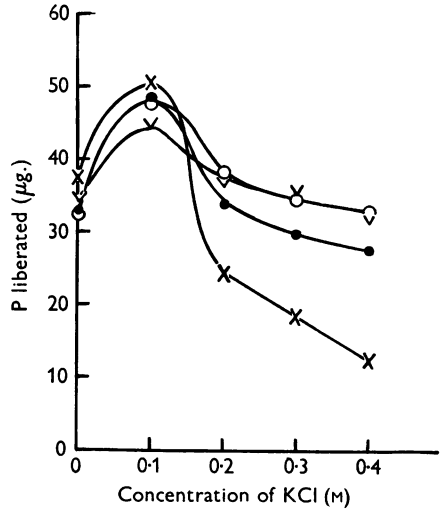

Fig. 5. The effect of KCl on the adenosinetriphosphatase activity of collagenase-prepared myofibrils stored at 0° in borate buffer, pH 7·1. Activity measured in glycine with 0·005M-$CaCl_2$ at the following times after completion of the preparation: 18 hr., ×; 7 days, ●; 11 days, ○; 18 days, v.

In the absence of cations other than those due to the adenosinetriphosphate and the buffer, maximum activation in glycine, pH 8·6, was reached at

0·01 M-calcium chloride, whereas under the same conditions 0·005 M-magnesium chloride produced an even higher rate of splitting at the maximum of the curve (Fig. 6). Comparable experiments with trypsin-prepared myofibrils indicated maximum calcium chloride activation at 0·002–0·003 M, whereas 0·005 M-magnesium chloride gave a flat maximum at 20–30 % of that obtained with calcium chloride.

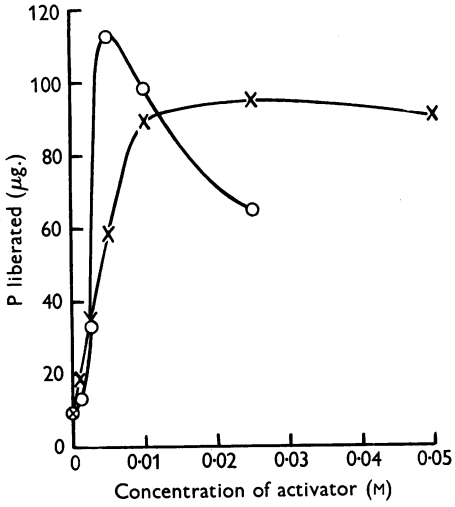

Fig. 6. Calcium and magnesium activation of the adenosinetriphosphatase activity of collagenase-prepared myofibrils in glycine buffer, pH 8·6. $CaCl_2$, ×; $MgCl_2$, ○.

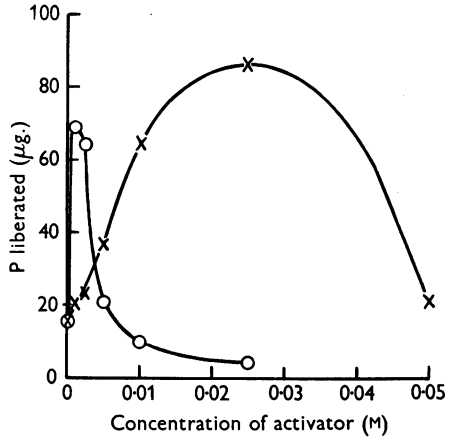

Fig. 7. Calcium and magnesium activation of the adenosinetriphosphatase activity of collagenase-prepared myofibrils in borate buffer, pH 6·9. $CaCl_2$, ×; $MgCl_2$, ○. The myofibril preparation was washed by centrifugation an additional three times.

The activation curves obtained in borate buffer, pH 6·9, are similar to those given with glycine. Fig. 7 shows that at a higher calcium chloride concentration, 0·025 M is required for maximum activation, and the peak with magnesium chloride is sharper and occurs at 0·001–0·002 M. Washing the myofibrils by centrifugation for a further three times did not alter the form of the activation curves at pH 6·9.

The magnesium-activated adenosinetriphosphatase was further investigated to decide whether it was a property of actomyosin or due to another independent enzyme. It is well established that the

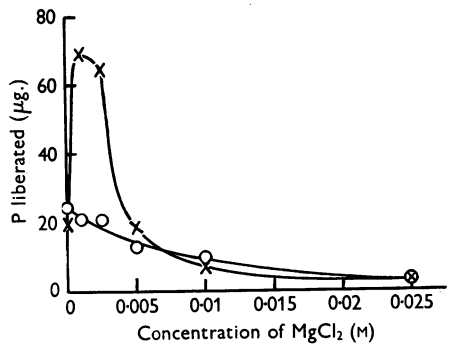

Fig. 8. The adenosinetriphosphatase activity in borate buffer, pH 6·9, with 0·001 M-$MgCl_2$, of actomyosin extracted from collagenase-prepared myofibrils and three times precipitated. In presence of 0·1 M-KCl, ○; no KCl, ×.

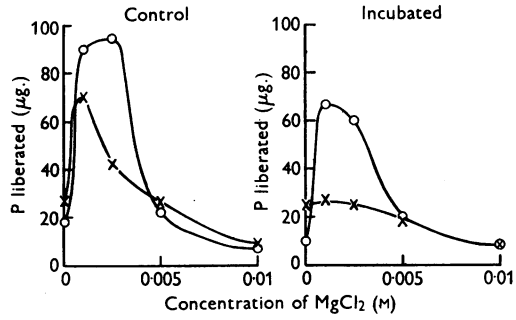

Fig. 9. The effect of incubation for 150 min. at 37° in borate buffer, pH 7·1, on the magnesium-activated adenosinetriphosphatase activity of collagenase-prepared myofibrils. Enzymic activity measured in borate buffer, pH 6·9. 0·1 M-KCl, ×; no KCl, ○.

adenosinetriphosphatase activity of myosin is increased by low concentrations of magnesium in the absence of other cations (cf. Fig. 8), whereas in the presence of potassium chloride magnesium inhibits (Banga & Szent-Györgyi, 1943; Mommaerts & Seraidarian, 1947). On the other hand, the adenosinetriphosphatase activity of myosin, in the absence of actin, is inhibited by magnesium irrespective of the potassium chloride concentration. In contrast to the behaviour of extracted actomyosin,

magnesium invariably produced an increase in the activity of freshly prepared collagenase myofibrils in the presence of 0·1 M-potassium chloride, but this effect tends to disappear on storing, in some cases after a day or two, at 0°. These stored preparations still showed a sharp peak in activity at 0·001 M-magnesium chloride in the absence of potassium chloride. Incubation of the myofibril suspension for 2–3 hr. at 37° had a similar effect to storing, as is shown in Fig. 9, although the latter results were not invariably obtained. Microscopic examination of the incubated myofibrils did not show any obvious morphological change.

Although these results seem to suggest the existence of a separate magnesium-activated adenosinetriphosphatase, it is considered that actomyosin is responsible for all the adenosinetriphosphatase activity, and that the state of organization of the actin and myosin in the myofibril is the important factor. The pH activity curves in Figs. 10 and 11 strongly suggest that one enzyme is responsible for the magnesium-activated adenosinetriphosphatase activity with and without potassium chloride, for with increasing pH, enzymic activities under these two conditions fell off together when the myofibrils were intact. If the myofibrils were first dissolved in M-potassium chloride and additions for the enzyme test arranged so that the final potassium chloride concentration was 0·1 M, 0·001 M-magnesium chloride inhibited; on the other hand, if the intact myofibrils were added directly to the enzyme tube already containing 0·1 M-potassium chloride, the same concentration of magnesium activated (Table 3). Fig. 13 shows that pH has little effect on the magnesium-activated adenosinetriphosphatase activity in the presence of 0·1 M-potassium chloride once the myofibrils have been treated with M-potassium chloride. It is difficult to explain the results on the basis that actomyosin is not as accessible to ions in the intact myofibril as in the dissolved and reprecipitated form, because the substrate readily reaches the former as judged by the rate at which adenosinetriphosphate is split, and both activation and inhibition effects are obtained with potassium chloride acting on the intact myofibril. The calcium-activated adenosinetriphosphatase activity measured in borate at pH 6·9 is not affected by dissolving the myofibrils in M-potassium chloride (Table 3).

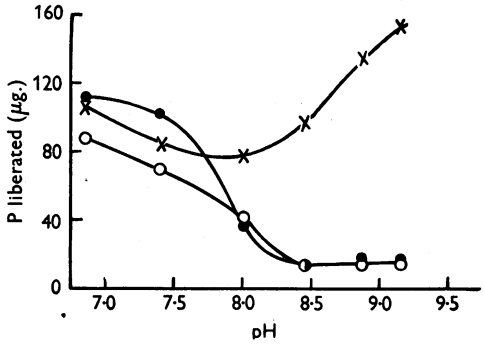

Fig. 10. The effect of pH on the adenosinetriphosphatase activity of intact collagenase-prepared myofibrils in borate buffer. 0·001 M-$MgCl_2$, ●; 0·001 M-$MgCl_2$, and 0·1 M-KCl, ○; 0·005 M-$CaCl_2$ and 0·1 M-KCl, ×.

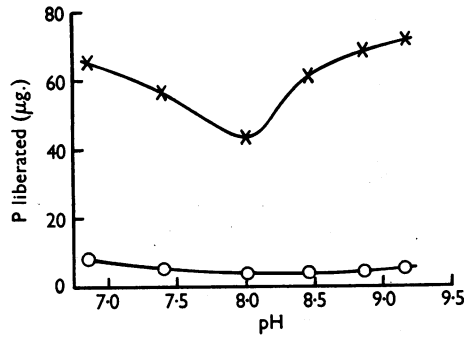

Fig. 11. The effect of pH on the adenosinetriphosphatase activity of collagenase-prepared myofibrils after initial treatment with M-KCl. Enzymic activity measured in borate buffer and 0·1 M-KCl. 0·001 M-$MgCl_2$, ○; 0·005 M-$CaCl_2$, ×.

Adenosinetriphosphatase activity other than that due to the myofibrils. To investigate adenosinetriphosphatase activity in the fraction dispersed in 0·08 M-borate buffer, pH 7·1, after centrifugation, a portion of the collagenase-treated suspension of muscle

Table 3. *Effect of cations on the adenosinetriphosphatase activity of collagenase prepared myofibrils before and after treatment with* M-KCl

(Enzyme activity was measured in borate buffer, pH 6·9.)

Final concentration of activator (M)			ATPase activity (μg. P liberated/ml. myofibrils/20 min.)	
KCl	$CaCl_2$	$MgCl_2$	Intact myofibrils	Myofibrils initially treated with M-KCl
0·1	Nil	Nil	85	88
0·1	Nil	0·001	232	49
0·1	0·005	Nil	308	318

slices was homogenized in the Waring blender, and centrifuged at 1500 *g* until no myofibrils could be detected in the supernatant. This supernatant was removed as completely as possible and the myofibrillar fraction resuspended in a volume of the borate buffer equal to that of the supernatant removed. The adenosinetriphosphatase activities of the supernatant and the resuspended myofibrillar fraction were compared under the following conditions. In borate buffer, pH 6·9, with (*a*) no activator; (*b*) 0·1 M-potassium chloride; (*c*) 0·001 M-magnesium chloride; (*d*) 0·1 M-potassium chloride and 0·001 M-magnesium chloride: and in glycine buffer, pH 8·6, with 0·1 M-potassium chloride and 0·005 M-calcium chloride. From the results it can be concluded that in no case does the myofibrillar fraction contribute less than 97% of the adenosinetriphosphatase-splitting activity of the whole homogenate obtained after collagenase treatment in 0·08 M-borate.

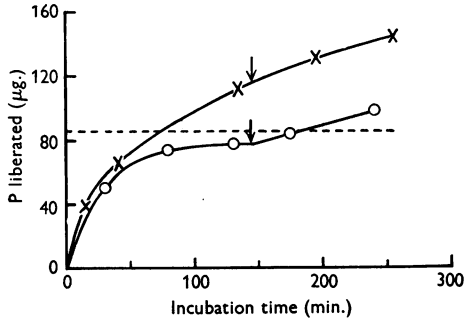

Fig. 12. The splitting of ATP by prolonged incubation with collagenase-prepared myofibrils. Incubation in glycine, pH 8·6, with 0·005 M-$CaCl_2$. The small arrows show the points at which additional amounts of the myofibril suspension were added, and the dotted line indicates 50% of the 10 min. hydrolysable P of the ATP. Normal preparation, ×; normal preparation after three additional washings with 0·08 M-borate, ○.

No magnesium-activated β-glycerophosphate-splitting activity at pH 6·9 or 8·6 could be demonstrated either in the supernatant from homogenized collagenase-treated muscle or by the final purified myofibril preparation.

Myokinase activity. On prolonged incubation at 25°, collagenase-prepared myofibrils split more than 50% of the adenosinetriphosphate acid-labile phosphate. Washing the myofibrils by centrifugation a further three times considerably reduced the myokinase activity, but still a little remained (Fig. 12). Trypsin-prepared myofibrils did not split more than 50% of the acid-labile phosphate after prolonged incubation and addition of fresh myofibril suspension, indicating the absence of myokinase. Presumably myokinase is more sensitive than myosin adenosinetriphosphatase to trypsin.

DISCUSSION

Myosin and adenosinetriphosphatase

The striking fact that myosin can be treated with trypsin until its gross physical properties are considerably changed, yet at the same time retain almost unimpaired the ability to split adenosinetriphosphate, suggests at first sight that adenosinetriphosphatase is not identical with myosin, the main structural component of the myofibril. On the other hand, however, the effects of a variety of sulphydryl reagents (Bailey & Perry, 1947), incubation at 37° and exposure to acid (Perry, 1947), on the adenosinetriphosphatase and actomyosin-forming activities of myosin suggest that these properties are closely related. These latter results are not in complete accord with those of Gilmour & Calaby (1949), who used ninhydrin to oxidize the myosin sulphydryl groups. The only specific criterion of actomyosin formation is the decrease in viscosity obtained when adenosinetriphosphate is added to the complex. Bearing this in mind it is difficult to interpret the experiments of Gilmour & Calaby, for in those cases in which the protein concentration was high enough to give an unambiguous viscosity decrease, addition of adenosinetriphosphate precipitated the mixture of actin and trypsin-treated myosin.

The results of Bailey and Perry can be reconciled with the findings reported here if it is assumed that trypsin breaks down the myosin molecule into smaller fragments which retain the active centres associated with adenosinetriphosphatase activity and actin interaction. These myosin fragments, however, would no longer be asymmetrical enough to produce, on interaction with actin, the gel structure of anastomosed protein filaments. This anastomosed structure is characteristic of normal actomyosin (Astbury, Perry, Reed & Spark, 1947) and can be broken down by adenosinetriphosphate with a pronounced change in viscosity. It should be emphasized that in the experiment with trypsin-treated myosin, a fall in viscosity on addition of adenosinetriphosphate is the only criterion used to indicate actomyosin formation. In the same way as myosin and G-actin are considered to give a complex, G-actomyosin (Szent-Györgyi, 1945), the viscosity of which is not affected by adenosinetriphosphate, trypsin-degraded myosin and F-actin could interact to give a similar complex.

Adenosinetriphosphate and the myofibril

So far as can be ascertained the myofibrils obtained by the collagenase method are little modified from the original intracellular structures. During the preparation they have been washed free of soluble sarcoplasmic protein but still show the characteristic structure assigned to the myofibril by

the work of Hall *et al.* (1946) and Draper & Hodge (1949). The point of action of the collagenase is not clear, but the function of both collagenase and trypsin treatments is to break down the interfibrillar links; possibly the Z bands which extend transversely across the myofibrils binding them together and to the sarcolemma (Draper & Hodge, 1949). It is of interest to note that the Z bands have in the past been suggested to be of a collagenous nature, although Barer (1948) reports that the sarcolemma is not attacked by collagenase. The possibility that a potent factor in breaking down the cell may be the hyaluronidase impurity in the collagenase preparation is being investigated.

The interaction of adenosinetriphosphate and the myofibril is of fundamental interest in the elucidation of the contractile process. Like actomyosin extracted from muscle by salt solutions, the intact myofibril shows the property of splitting phosphate from adenosinetriphosphate, the substance which is unique in causing the myofibril to shorten in a certain ionic medium. How related these properties are is not clear, but it may be significant that shortening is most marked at approximately the magnesium chloride concentration which gives optimum adenosinetriphosphatase activity.

In general it can be concluded that the myofibril splits adenosinetriphosphate at a rate to be expected from the amount of myosin that it contains. The fraction, insoluble in 0·08 M-borate, obtained after collagenase treatment of whole muscle, is responsible for 97 % of the adenosinetriphosphatase activity of the whole. Calculations of the type made by Mommaerts & Seraidarian (1947), on the basis of metal activation experiments with extracted myosin and actomyosin, are liable to be misleading as the work described here and that reported by Steinbach (1947) indicate. It must be concluded, however, if the figures obtained by Lundsgaard (1930) actually do represent phosphate liberated during contraction that there is a serious deficit between the adenosinetriphosphate-splitting power of the isolated myofibril and the amount of phosphate which appears during a short tetanus. Over a lower and more restricted concentration range, magnesium is almost as potent an activator for the myofibrillar adenosinetriphosphatase as is calcium, a fact which must be taken into account in theories of muscle contraction which involve the splitting of ATP.

For reasons given earlier, the magnesium-activated adenosinetriphosphatase of the myofibril is considered not to be a different enzyme, but merely part of the actomyosin complex. Preliminary results indicate that the Kielley-Meyerhof magnesium-activated adenosinetriphosphatase is destroyed during the preparation of the myofibrils, and the possibility that this enzyme is a myofibrillar component is being further investigated. If this enzyme is located in the myofibril, the adenosinetriphosphate-splitting potential of that structure would be considerably increased, but it seems more likely to be a sarcoplasmic component judging from the nature of the preparations and the manner in which they are obtained (Kielley & Meyerhof, 1950).

SUMMARY

1. Trypsin was found to be unsatisfactory for isolating myofibrils from rabbit psoas muscle, and a modified method employing collagenase is described.

2. At 0° low concentrations of trypsin degrade the myofibril and destroy the actomyosin-forming ability of extracted myosin, yet at the same time have little effect on the adenosinetriphosphatase activity.

3. Myofibrils isolated by the collagenase method show detailed fine structure in the electron microscope, and in the presence of adenosinetriphosphate shorten and retain considerable organization of structure.

4. The adenosinetriphosphatase activity of isolated myofibrils is activated by both calcium and magnesium. In the absence of other cations the extent of activation at pH 6·9 and 8·6 by 0·001–0·002 M-magnesium is of the same order as that obtained with optimum calcium concentrations.

5. Unlike extracted actomyosin, intact myofibrils have magnesium-activated adenosinetriphosphatase activity in the presence of 0·1 M-potassium chloride. Destruction of the organization by dissolving the myofibrils in 1·0 M-potassium chloride transforms the magnesium activation effect in 0·1 M-potassium chloride to one of inhibition. This change from activation to inhibition is frequently obtained on storing and also after incubation at 37°.

6. Normal preparations have myokinase activity which can be almost entirely removed by repeated washing at the centrifuge.

7. It is concluded that actomyosin alone is responsible for both the calcium and magnesium activated adenosinetriphosphatase activity of the myofibrils isolated by the collagenase method.

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On Ergothioneine in Blood and Diazo-reacting Substances in Maize

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Ergothioneine, discovered in ergot of rye (Tanret, 1909), was isolated from pig blood by Hunter & Eagles (1925, 1927) and identified as such by Newton, Benedict & Dakin (1926) and by Eagles & Johnson (1927). It was somewhat later prepared from pig blood by Benedict, Newton & Behre (1926). Hunter (1928) described for the substance a delicate and highly specific colour test by which ergothioneine appeared to be present in significant quantity in the blood corpuscles of several animal species. Gulland & Peters (1930) recorded the isolation of ergothioneine from pigeon blood, and Williamson & Meldrum (1932) its isolation from pig blood. Eagles & Vars (1928) brought forward some evidence that the presence of ergothioneine in the blood of the pig was traceable to the diet, and more specifically to some constituent of maize. There has been no confirmation of the work of Eagles & Vars, partly on account of the fact that there was no satisfactory method for the determination of ergothioneine in blood.

The observations of Lawson & Rimington (1947) on an antithyroid activity of ergothioneine revived interest in the substance, and Hunter (1949) has described a new fractionation procedure to obtain blood filtrates suitable for ergothioneine determination by the diazo method.

The amounts of ergothioneine found in human, pig and rat blood are shown in Table 1, and a glance at the mean values shows a wide variation associated apparently with geographical location and particularly apparent in the values shown for pig blood.

The values given for Toronto, are calculated from those published by Hunter (1928). In that paper ergothioneine was expressed as mg./100 ml. corpuscles. In Table 1 of the present paper those values have been halved to give corresponding approximate values for whole blood. It can be seen that pig blood in the Toronto area in 1928 had more than three times the mean ergothioneine content of pig blood in the London area in 1949–50; and at present pig blood in the Edmonton area has about twice the concentration of pig blood in the London area. The relationship between Edmonton (Canada) and London (England) at present is similar with respect to human and rat blood.

There can be little doubt that such variations in ergothioneine blood level are real. They have been

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