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FINE STRUCTURAL MORPHOLOGY AND ATP-ASE REACTION IN HEART MUSCLE AT VARIOUS SARCOMERE LENGTHS*

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With 12 Figures in the Text

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Electron microscopy of heart muscle of rat and dog (STENGER and SPIRO 1961) has revealed that the structure of the sarcomere is similar to that found in skeletal muscle. These authors therefore concluded that the structure of heart muscle sarcomeres fitted well in the filament model as suggested by HANSON and HUXLEY (1953, 1954, 1957, 1960) for skeletal muscle.

Selective extraction experiments on skeletal muscle (HANSON 1953, 1954; HUXLEY 1953, 1954, 1960; HASSELBACH 1953) have shown that l-Myosin is located in the A-band. On the strength of electron microscopical studies on muscle tissue previously treated with selective extraction, the localization of l-Myosin in the thick filaments was made probable (HANSON and HUXLEY 1953, 1954, 1960). Actin seems to be located in the thin filaments mainly in the I-zone. This substance is known in two forms: F(ibrillar) and G(lobular) Actin. There is no proof indicative of the presence of the G-form in the sarcomere.

From the work of SZENT-GYÖRGYI (1947) it has become clear that in vitro l-Myosin and Actin may combine to form Actomyosin, a substance which can have contractile characteristics. The location in the sarcomere of Actomyosin and other structural proteins, such as Tropomyosin, is not known.

Both l-Myosin and Actomyosin have an ATP-ase activity (HUXLEY 1960, SZENT-GYÖRGYI 1947, KIELLEY and MEYERHOF 1948, HODGE 1959), but as has been reported there are very marked differences in vitro between the rates of the ATP-splitting activity of l-Myosin and Actomyosin, dependent on the Mg^{++}/Ca^{++} ratio in the surrounding medium (HUXLEY 1960, p. 430). Tropomyosin and F-Actin have no demonstrable ATP-ase activity (HODGE 1959). The combination of F-Actin and G-Actin can have an ATP-ase activity under certain circumstances (MORALES and WATANABE 1961).

In a previous paper (DE BEYER, DE MAN, and PERSIJN 1962), we demonstrated histochemically with both light and electron microscopy the presence of a lead precipitate on the C_z-bands, cell membrane, intercalated disc and endothelial cells of mouse heart muscle after incubation with an appropriate medium, containing ATP. We tentatively suggested that this precipitate indicated the sites of activity of an ATP-ase. In the present paper we will try to specify some *characteristics of C_z-band ATP-ase with respect to its sensitivity to Mg^{++} and to*

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a sulphhydryl inhibiting agent such as parachlormercuribenzoate (PCMB). The sensitivity of this ATP-ase seems to be different from the ATP-ase on the cell membrane, intercalated disc, sarcoplasmic reticulum, and endothelial cells. The reversibility of the PCMB inhibition as a result of cysteine treatment (SINGER 1944; BARRON 1944, 1951; PADYKULA and HERMAN 1955; FREIMAN and KAPLAN 1960) will also be discussed. We will try to demonstrate *a possible identity of the ATP-splitting enzyme in the Z-line region with Actomyosin.* In relation to this problem we studied the ATP-ase reaction both light and electron microscopically at various sarcomere lengths, such as occur in hypercontracted and in stretched heart muscle.

Material and Methods

The experimental animals were F_1-Hybrids of C_{57}-black and O_{20} mice ranging in age from 3 to 4 months, with an average weight of 25 g. The mice received standard mouse food, wheat grains, and tap water ad lib.

a) In one series of experiments, mouse heart muscle tissue which was neither stretched nor brought in a condition of hypercontraction was used. Tissue was prepared according to procedures described below under I—IV.

b) For experiments on hypercontraction, one group of animals was injected under ether anesthesia intravenously in the inferior caval vein with a lethal dose (0.5 ml) of Strophantin in a concentration of 0.25 mg/ml.

In order to induce a hypercontraction of the heart muscle by giving an intravenous water load, and thus coincidentally eliminating a possible specific effect of Strophantin, another group of mice was injected intravenously in the inferior caval vein under ether anesthesia with a lethal dose (about 5 ml) of saline.

A third group of mice was injected subcutaneously with a lethal dose of digitalis (Cedilanide), thus eliminating an effect of the ether anesthesia.

Tissue was prepared according to procedures described below under I, II and IV.

c) For stretching experiments, the papillary muscle of a dog heart was found to be very suitable. Tissue slices from this muscle 1 cm in length, were taken immediately after death and then stretched to about $1^1/_2$ times their original length.

For stretching experiments with mouse heart muscle, it appeared to be technically extremely difficult to use the papillary muscle. In these cases, slices of the left ventricle, running from annulus to apex, were used. These slices with a length of about 8 mm and a diameter of about 1 to 2 mm, were similarly stretched to about $1^1/_2$ times their original length.

It must be kept in mind that in comparison with the papillary muscle of the dog, in which it may be assumed that practically all fibers run parallel to the long axis of the muscle, the fibers in the mouse ventricle have diverse directions. As a result, not all fibers will be stretched to the same extent in the stretched slices of the mouse ventricle.

Tissue in a stretched position was prepared according to the procedures described below under I, II and IV. Tissue preparation procedures were:

Preparation procedure I. For electron microscopy, tissue blocks of about 1 mm^3 were fixed for 1 hour in a cold (4° C), isotonic, 1% solution of OsO_4, buffered at a p_H of 7.2, with sucrose added to a final concentration of 4%. After rinsing in distilled water and dehydration in alcohol, the tissue blocks were embedded in methacrylate. Thin sections were made on Porter-Blum microtomes. The sections were collected on grids covered with a carbon film on collodion from which the collodion had been previously dissolved by means of amyl acetate.

Preparation procedure II. For the demonstration in the electron microscope of an ATP-ase activity in the presence of Mg^{++}, the method described by PERSIJN et al. (1961) was followed. The tissue blocks of about 1 mm^3 were prefixed for 3—4 minutes with a 1% OsO_4 solution in 0.05 M Tris-HCl buffer (p_H 7.3) containing 100 mg sucrose per ml. After decantation of the fixation fluid, the incubation mixture was added and changed several times during incubation (20—30 minutes at room temperature). The incubation medium contained: 0.05 M Tris-HCl buffer (p_H 7.3), 1.3×10^{-3} M ATP (Boehringer), 0.18 M potassium-sodium tartrate (Merck p. a.), 1×10^{-3} M magnesium chloride, and 5×10^{-3} M lead-nitrate (B.D.H. analar quality). The mixture was prepared in the sequence indicated. The mean final p_H

value was 6.8 ± 0.2. Sixty mg sucrose was added per ml because it had been found to improve the preservation of the fine structure. After incubation, the procedure described under I was followed.

Preparation procedure III. Parachlormercuribenzoate (PCMB) was used as a sulphhydryl inhibitor (SINGER 1944; BARRON 1944, 1951; PADYKULA and HERMAN 1955; FREIMAN and KAPLAN 1960). PCMB was added to the incubation fluid (II) to a final concentration of 0.003 M. In this concentration PCMB was completely soluble in the incubation fluid at p_H 6.8. The precipitate formed in the experiment on PCMB inhibition was compared with the precipitate formed in tissue blocks incubated at the same time and in the same medium without PCMB. After incubation, the procedure described under I was followed.

Preparation procedure IV. a) Tissue blocks for light microscopical histochemical study were frozen to about —20° C within 15 minutes after the death of the animal and sectioned in a cryostat. Mg^{++}-activated ATP-ase activity was demonstrated in the section according to a technique described by WACHSTEIN and MEISEL (1957, 1958). Running parallel to this, on other sections, the same technique was used but modified by WILLIGHAGEN (1961) in such a way that tissue sections were prefixed in a solution of 4% neutral formaldehyde, 6% Dextran (Mol. wt. 18000) and 2% calcium chloride for 10 minutes. As compared with the not prefixed sections this modification laeves the localization of the precipitate in the sections essentially unaltered. The prefixation eliminates a diffuse excess precipitate which can occur after longer incubation (40—50 minutes), making examination difficult. Before incubation, tissue sections were washed in distilled water. Incubation of unfixed sections was done for 15 minutes at 37° C. Prefixed sections were incubated for 45 minutes at 37° C.

In addition, in order to study the sensitivity to the presence of Mg^{++} in the incubation medium, we also used the same technique but omitting magnesium-chloride from the medium.

Experiments on ATP-ase activity were compared with controls in which non-prefixed sections were incubated in a medium which did not contain ATP but which was otherwise identical in composition.

b) In one series of experiments, PCMB was dissolved in the incubation medium in a concentration of 0.0025 M. Incubation for about 15 minutes for non-fixed, and 45 minutes for formaldehyde prefixed sections was done at a temperature of 37° C.

On other sections of the same material this incubation in a medium containing PCMB and ATP was followed by bringing the sections in a solution containing 0.0025 M cysteine in 0.05 M Tris-maleate buffer (p_H 7.2), after which an ATP-ase reaction as described under IVa was done. Both types of reaction were compared with an ATP-ase reaction on other sections of the same tissue. Except for the type of incubation medium used, the technique followed for the demonstration of a reversible inhibition of ATP-ase activity by PCMB was therefore essentially the same as described by PADYKULA and HERMAN (1955).

Results

I.

a) Morphology of heart muscle of mouse and dog at intermediate sarcomere length. In general, the sarcomere length we found in heart muscle was shorter than is generally described for skeletal muscle in similar states of contraction or stretch. If tissue blocks of mouse and dog heart muscle were fixed immediately after being taken out of the heart, a sarcomere length was found of about 1 μ (Fig. 1) which represents an intermediate condition between hypercontraction (Figs. 3 and 4) and the sarcomere length found after stretching (Figs. 5 and 6). (See paragraph on hypercontraction and stretching.) At this intermediate sarcomere length neither I-zones nor H-zones were clearly visible, while in some instances an M-line was vaguely indicated as an area of electron density in the centre of the sarcomere, consisting of a local thickening of the thick filaments. Both thick and thin filaments were visible throughout the entire sarcomere. As compared with the stretched heart muscle (see below), more vaguely defined, slightly

broadened electron-dispersing C_z-bandst ake the place of the more sharply defined Z-lines. The occurrence of slightly broadened Z-bands (C_z-bands) together with a shortening of the sarcomere as compared with the stretched muscle indicates that at what we call an intermediate sarcomere length a slight contraction exists. Our findings for other muscle fiber constituents, such as mitochondria, sarcoplasmic reticulum, cell membrane and intercalated disc resemble what has been extensively described in the literature (STENGER and SPIRO 1961; HUXLEY 1960, 1961; FAWCETT 1958, 1961).

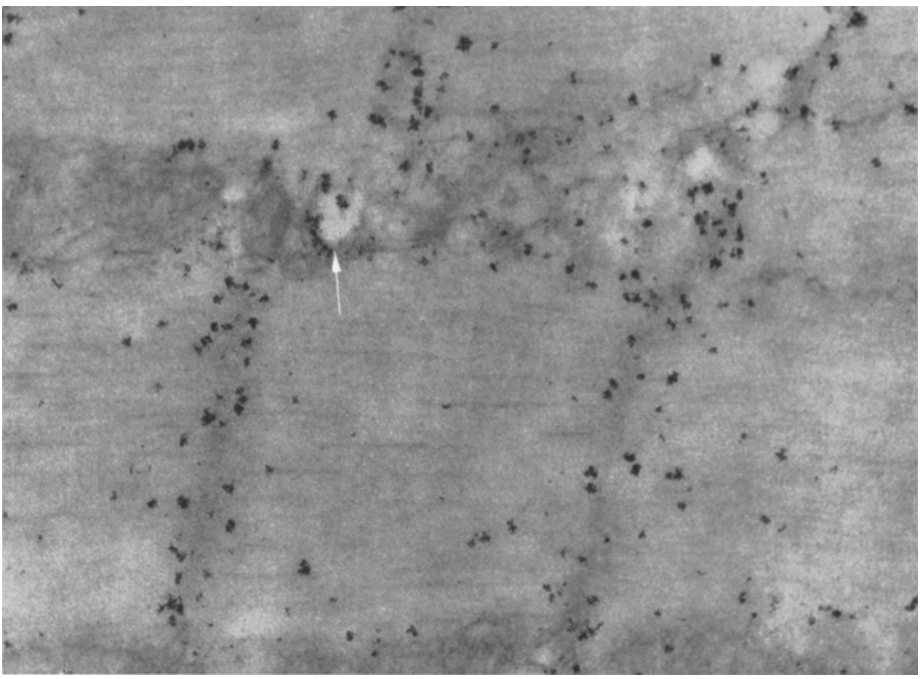


Fig. 1. Electron-micrograph of ATP-ase reaction of mouse heart muscle at intermediate sarcomere length. Precipitate in the C_z-bands. Vacuoles in the area between the fibrils, probably belonging to the sarcoplasmic reticulum, show some precipitate on the surrounding membrane (↑). Magn.: about ×50000

b) Morphology of hypercontracted mouse heart muscle. No differences in the morphological picture were found between the three groups of mice in which a hypercontraction of the heart muscle was induced by different means.

Heart muscle brought in a state of hypercontraction was characterized by a very marked shortening of the sarcomere length (to about 0.5 μ) (Figs. 3 and 4). In most instances, both thick and thin filaments, were visible in the centre of the shortened sarcomere (Fig. 4). However, occasionally sarcomeres were found in which no such filament structure was seen (Fig. 3). As compared with the C_z-bands in muscle tissue with an intermediate sarcomere length, the electron dispersing C_z-bands of hypercontraction (Hypercontraction bands) are markedly broadened. Although electron microscopically most of the muscle tissue seemed to be in a state of hypercontraction, occasionally a few areas were found in which sarcomeres with an intermediate sarcomere length occurred. In the contracted

areas the long axis of the mitochondria was in many instances found perpendicular to the direction of the fibrils. The cell membrane and the nuclear membrane showed a heavy folding.

c) Morphology of stretched heart muscle of dog and mouse. As compared with unstretched heart muscle tissue, the sarcomere length in stretched muscle of mouse (Fig. 5) and dog (Fig. 6) had markedly increased to about 2 μ. On both sides of a relatively sharply-defined Z-line and I-zone was visible, in which N-lines were frequently found. In the central part of the sarcomere, M-lines

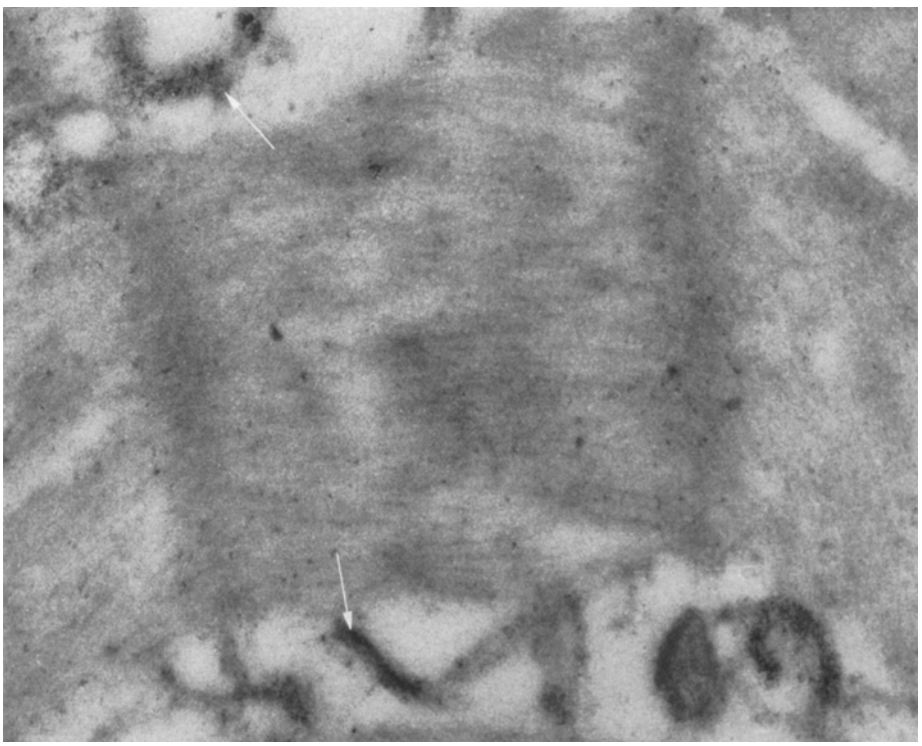


Fig. 2. Electron-micrograph of PCMB inhibition of C_z-band ATP-ase activity in mouse heart muscle at intermediate sarcomere lengths. A fine granular precipitate on parts of the sarcoplasmic reticulum (↑) indicates that the ATP-ase activity on these structures is much less inhibited than the C_z-band activity. Magn.: about × 50 000

surrounded by an H-zone were seen. Both thick and thin filaments were clearly visible in the A-zones, whereas in the I-zones a filamentous structure was much more difficult to distinguish. There were no indications that during the procedures we used, rupture of the filaments had occurred.

In the dog papillary muscle (Fig. 6), the subdivision of the sarcomere into zones and lines was more easily distinguishable than in the stretched left ventricle muscle of the mouse. In both mouse and dog heart muscle, the long axis of the mitochondria ran parallel to the long axis of the fibrils. The nuclear and cell membrane occasionally showed a slight folding, but in most instances they were perfectly flat.

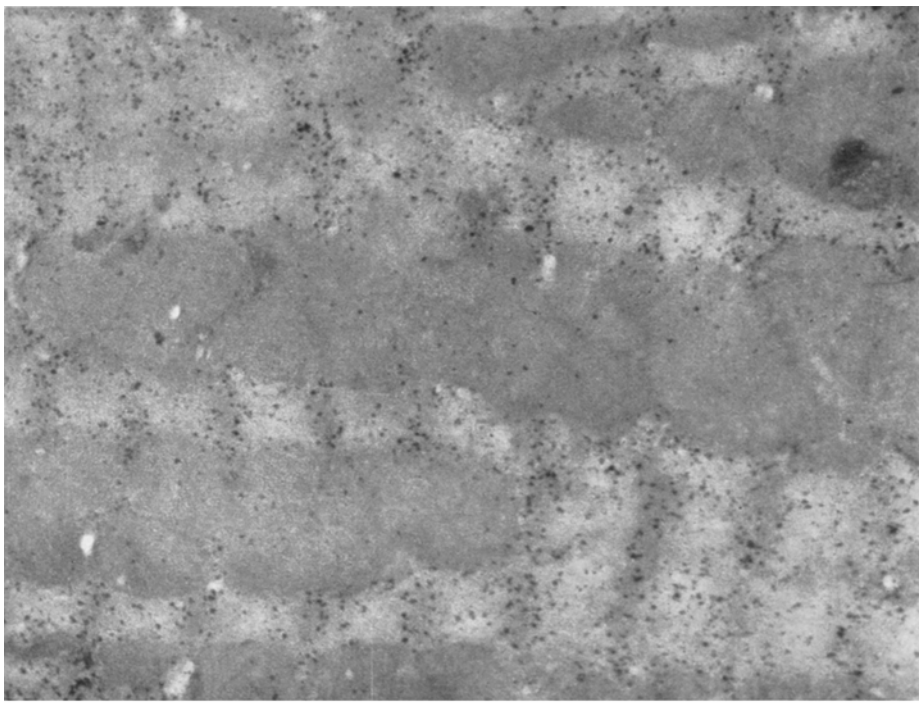


Fig. 3

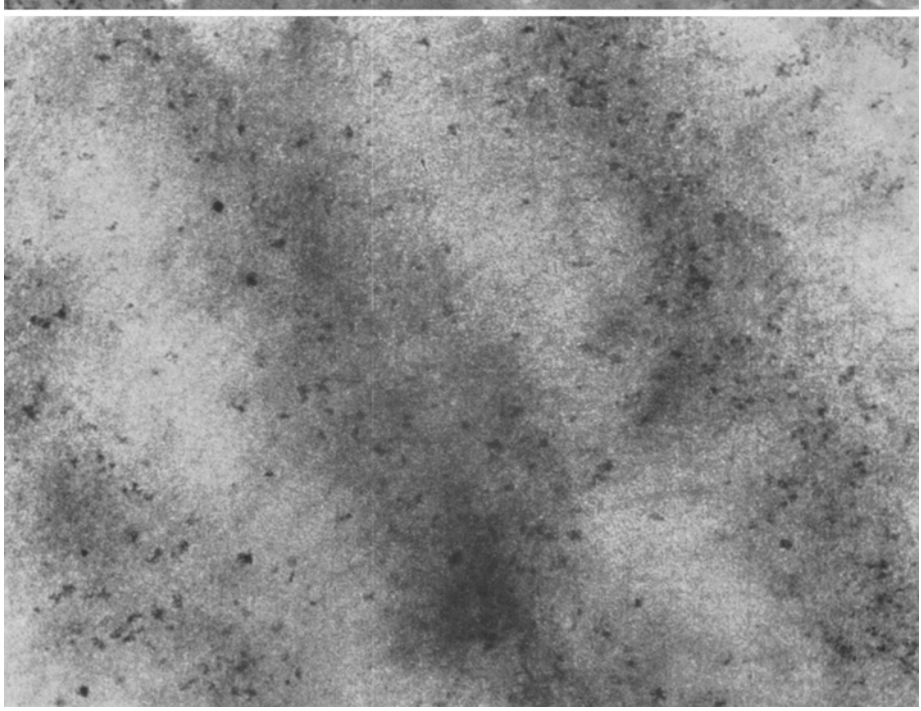


Fig. 4

Fig. 3. Electron-micrograph of ATP-ase reaction in hypercontracted mouse heart muscle. An intense formation of precipitate follows closely the broadening of the C_z-bands into hypercontraction bands. Magn.: about $\times 23\,000$

Fig. 4. id. Magn.: about $\times 77\,000$

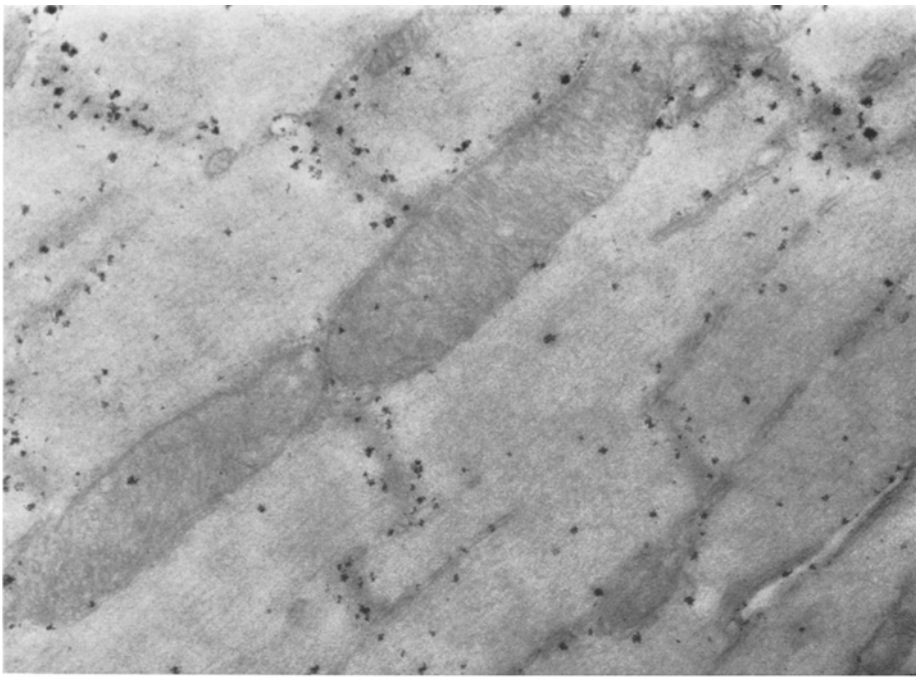


Fig. 5

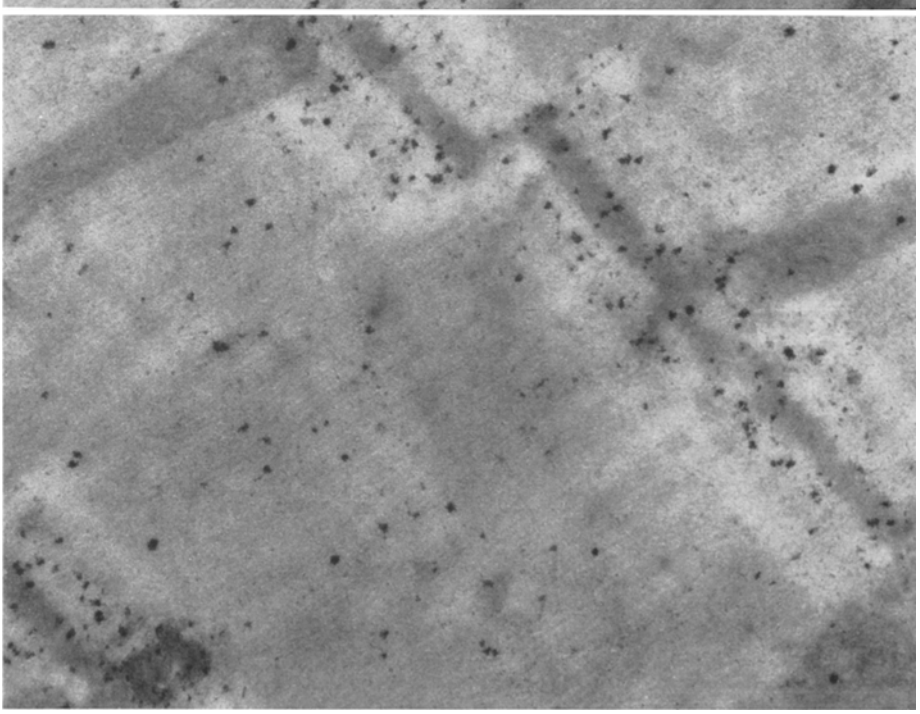


Fig. 6

Fig. 5. Electron-micrograph of ATP-ase reaction in stretched mouse heart muscle. Precipitate in the Z-line region. The formation of the precipitate seems to occur preferentially in the part of the I-zone lying closely adjacent to the Z-line, whereas practically no precipitate is found in the A-zone. Magn.: about ×59000

Fig. 6. Electron-micrograph of a stretched papillary muscle of a dog heart. Note that the Z-line region precipitate indicating the activity of ATP-ase is most intense in that part of the A-zone between N- and Z-lines. Magn.: about ×59000

Summarizing, we may say that at intermediate sarcomere length in mouse and dog heart muscle and in hypercontracted mouse heart muscle no I-zones or H-zones are visible, while an M-line is only vaguely indicated at intermediate sarcomere length. In the stretched dog and mouse heart muscle, I-zones and H-zones are visible, while N- and M-lines are sometimes clearly indicated, especially so in the dog heart muscle. As compared with muscle tissue at intermediate sarcomere length, the sarcomere in hypercontracted muscle is markedly shortened to a maximum of about 50%, whereas in stretched muscle tissue it can be lengthened to about two times the intermediate sarcomere length without signs indicative of rupture of the filaments. In comparison to the muscle tissue with intermediate sarcomere lengths, the Z-band in hypercontraction is markedly broadened, whereas in stretched tissue a relatively sharply defined, electron-dispersing line indicated the presence of a Z-line.

II. Experiments on the ATP-ase reaction

Our previous paper reported that a precipitate indicating an ATP-ase activity in the presence of Mg^{++} is electron microscopically visible on endothelial cells, cell membrane, intercalated disc and in the Z-line region. As can be seen in Fig. 1 some precipitate also seems to occur on the sarcoplasmic reticulum. In general, the localization of the precipitate corresponds with the light-microscopically visible precipitate (Figs. 1 and 7). In a paper by PERSIJN et al. (1961) and in our previous paper (DE BEYER, DE MAN and PERSIJN), the specificity of the reaction with respect to a possible splitting of ATP by nonspecific monophosphatases was discussed. In the latter paper we also mentioned our first experiment on the electron-microscopically visible inhibition of C_z-band ATP-ase with PCMB, as compared with cell membrane ATP-ase activity of the same muscle fiber. We have now extended these experiments and the micrographs in Figs. 7, 8, 9 and 10 show the specific inhibition of the cross-striational ATP-ase activity. This inhibition is abolished by subsequent treatment with cysteine. The enzyme activity on other structures such as blood vessels and cell membrane, seems to be relatively unaffected by PCMB. From an electron micrograph of a PCMB treated tissue block (Fig. 2) it appears that here the formation of precipitate on C_z-bands has decreased as compared with the sarcoplasmic reticulum on which the presence of precipitate is evident. The formation of precipitate on endothelial cells, cell membrane and intercalated disc was also less affected than the formation of C_z-bands precipitate. In accordance with the terminology of PADYKULA and HERMAN (1955) and FREIMAN et al. (1960) we may therefore regard most of the Z-line region precipitate as the result of the action of a "true" ATP-ase. As is illustrated by the micrographs (Figs. 11 and 12) only the cross-striational ATP-ase

Fig. 7. Light-micrograph of ATP-ase reaction in hypercontracted mouse heart muscle. Apart from the precipitate that is present at other sites, the precipitate on the cross-striations is clearly evident. Magn.: ×2900

Fig. 8. Light-micrograph of ATP-ase reaction in PCMB treated section. Note that cross-striational precipitate is practically absent. Magn.: ×2900

Fig. 9. Light-micrograph of ATP-ase reaction in PCMB treated section which treatment was followed by cysteine treatment. An intense precipitate on the cross-striations is found. Magn.: ×2900

Fig. 10. Control. The section was incubated in a medium in which no ATP was present. Magn.: ×2900

Fig. 7

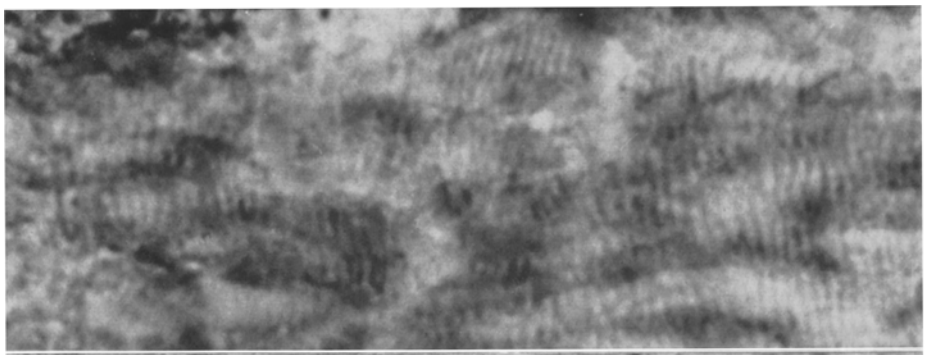


Fig. 8

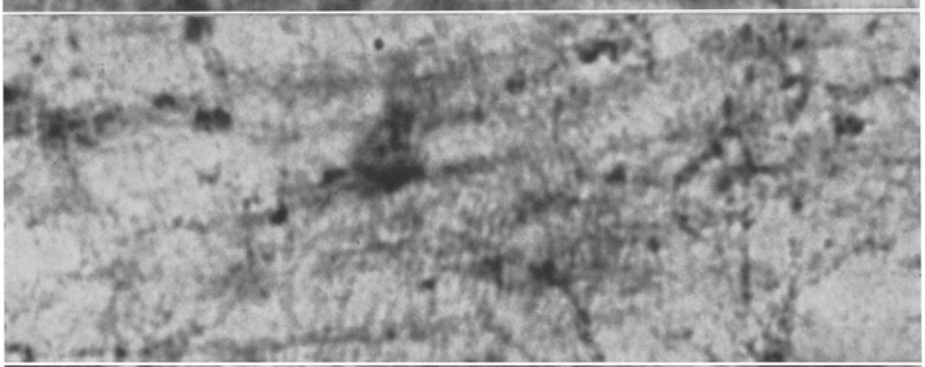
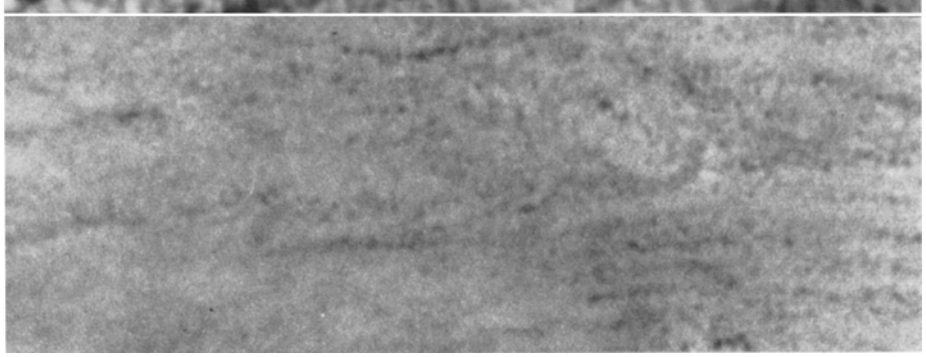
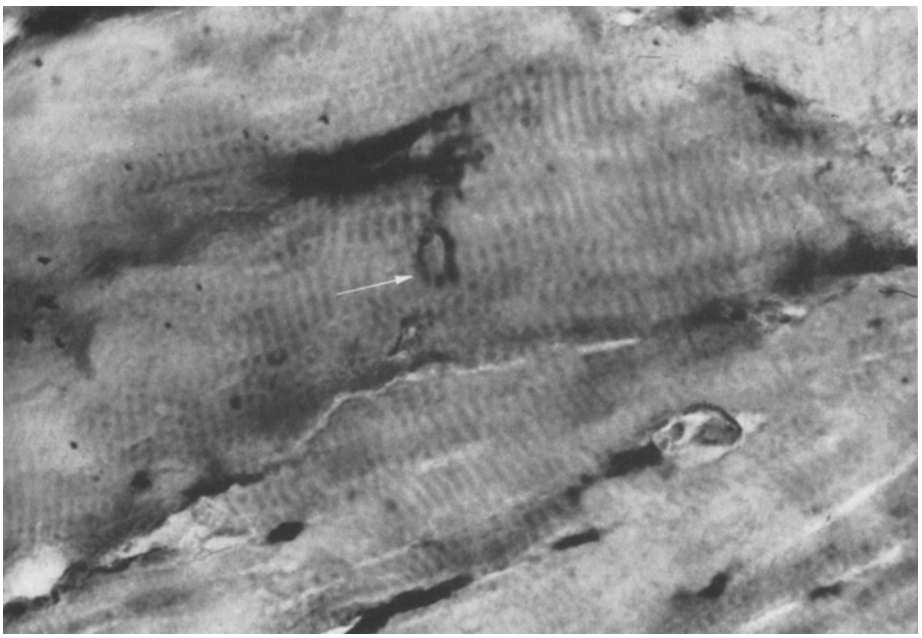


Fig. 9

Fig. 10



Figs. 7—10. Experiment on PCMB inhibition of ATP-ase activity and cysteine reactivation in adjacent sections of the same area of hypercontracted mouse heart muscle



Figs. 11 and 12. Experiment on the sensitivity to Mg^{++} activation of ATP-ase in adjacent sections of the same block of mouse heart muscle

Fig. 11. Mg^{++} present in the medium. Light micrograph which shows that precipitate is present on cross-striations, cell membrane, intercalated disc (↙) and endothelial cells. Magn.: ×2900

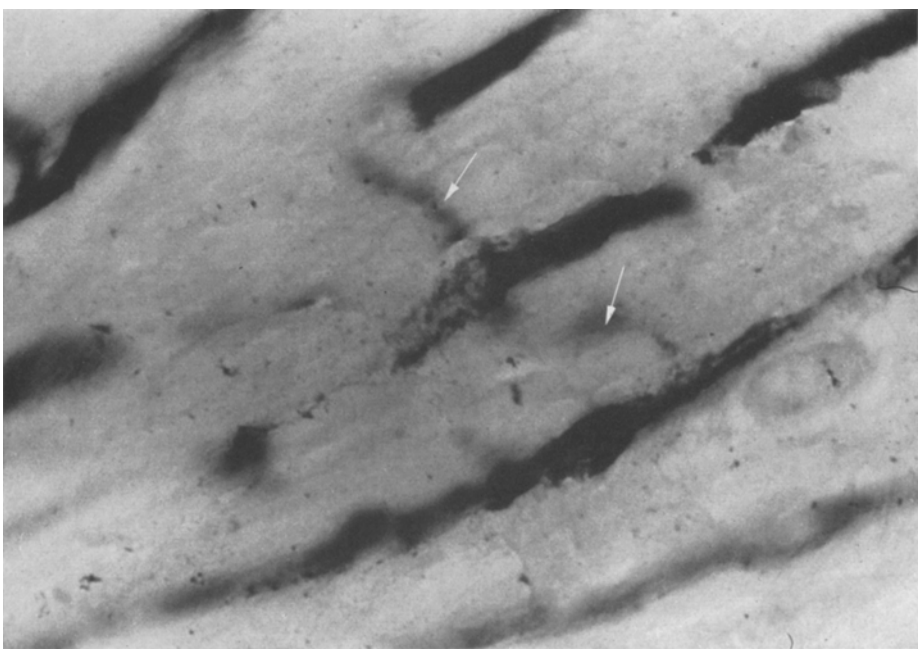


Fig. 12. Mg^{++} absent from incubation medium. Absence of cross-striational ATP-ase activity, while on the intercalated disc (↙), the cell membrane and on the endothelial cells the ATP-ase activity is still present. Magn.: ×2900

activity is strongly sensitive to the presence of Mg^{++}, since omission of $MgCl_2$ in the medium abolishes the reaction at these sites whereas the reaction at other sites, such as cell membrane intercalated disc and blood vessels, is unaffected.

Summarizing, we may say that an ATP-ase activity occurring in the presence of Mg^{++} in the Z-line region is sensitive to PCMB and can be reactivated by cysteine. This ATP-ase is strongly activated by Mg^{++}.

III. ATP-ase activity in heart muscle of mouse and dog at various sarcomere lengths

All the material studied showed a similar pattern of precipitate distribution indicating an ATP-ase activity. This consisted, both light and electron microscopically (Fig. 7 and Figs. 1, 3, 4, 5 and 6) of a precipitate on cross-striations, cell membrane, intercalated disc and on endothelial cells. Besides the heavy precipitate in the Z-line region, electron microscopy showed some precipitate on the sarcoplasmic reticulum and a few precipitate granules throughout the sarcomere and occasionally in some of the mitochondria.

Differences in the precipitate distribution between muscle tissue at various sarcomere lengths were seen exclusively in the Z-line regions. In muscle tissue with an intermediate sarcomere length, precipitate occurred diffusely throughout the slightly broadened electron-dispersing C_z-bands (Fig. 1). In hypercontracted muscle tissue, too, the precipitate occurs diffusely throughout the markedly broadened electron-dispersing hypercontraction bands (Figs. 3 and 4). Thus, in hypercontraction the spread of the precipitate followed the broadening of the C_z-bands into hypercontraction bands. The ATP-ase pattern showed no differences in the three groups of mice in which hypercontraction was brought about by various means. The stretched muscle showed no such distribution of a precipitate occurring diffusely throughout the Z-line. On the contrary, in this case most of the precipitate was found in the I-zone, in an area immediately adjacent to the Z-lines (Figs. 5 and 6), although in some instances a few precipitate granules were also found in the Z-lines. Whenever N-lines were clearly visible, which was especially the case in the stretched papillary muscle of the dog (Fig. 6), it seemed that the Z-line region precipitate was confined to the I-zone areas between N- and Z-lines.

Summarizing, we may say that exclusively in the Z-line region, the spread and localization of the precipitate formed as a result of an ATP-ase activity in the presence of Mg^{++} changes with alterations of the sarcomere length.

Discussion

The most interesting component of the ATP-splitting enzymes we found in the heart muscle seems to be the enzyme located in the Z-line regions. This enzyme can be differentiated from other ATP-splitting enzymes such as occur on the sarcoplasmic reticulum, the cell membrane, the intercalated disc and the endothelial cells on the basis of the following characteristics:

a) a high sensitivity to PCMB as a sulphhydryl inhibitor, an inhibition which can be reversed by cysteine,

b) a strong activation by Mg^{++},

c) the variability of the precipitate pattern in the Z-line region as related to the various sarcomere lengths: the precipitate follows closely the broadening of the C_z-bands in hypercontracted tissue. Furthermore, in the stretched muscle the precipitate is found mostly outside the Z-line in the part of the I-zone lying adjacent to the Z-line.

It appears from the evidence presented that the ATP-ase activity occurring in the Z-line region is attached to the structure of the sarcomere proper. With respect to this point, we must consider a possible ATP-splitting activity of the various structural proteins in the sarcomere. As is well known from the literature (HUXLEY 1960; SZENT-GYÖRGYI 1947; KIELLEY and MEYERHOF 1948; HODGE 1959), both l-Myosin and Actomyosin have an ATP-splitting activity which is strongly inhibited by PCMB and reactivated by cysteine (BARRON 1951; BÁRÁNY and BÁRÁNY 1959) and can therefore be regarded as "true" ATP-ase (PADYKULA and HERMAN 1955; FREIMAN and KAPLAN 1960). For Tropomyosin and F-Actin no such splitting activity is yet known (HODGE 1959). Combination of F- and G-Actin can have an ATP-splitting activity (MORALES and WATANABE 1961). It has not been proved, however, that such combinations of F- and G-Actin occur in the sarcomere.

There is evidence presented in the literature that l-Myosin occurs in the A-zone of the sarcomere (HUXLEY 1960). In this zone, however, we found much less precipitate resulting from the ATP-ase activity than in the Z-line region. In many instances the A-zone did not contain any precipitate.

The localization of Actomyosin in the muscle fiber is not very well known. Since we found an intense activity of ATP-ase in the Z-line region, either on the markedly broadened C_z-bands of hypercontraction or in the part of the I-zone lying adjacent to the Z-line in the stretched muscle, it seems possible that this ATP-ase activity indicates the presence of Actomyosin. However, we must still consider the hypothetical possibility that the enzyme activity is due to l-Myosin ATP-ase activity in the Z-line region, resulting from the possible presence of active groups on both ends of the l-Myosin containing thick filaments, while in the centre of these filaments the active groups might be masked. This assumption seems improbable, however, since in the stretched muscle (Figs 5 and 6) the precipitate is mostly located in the part of the I-zone closest to the Z-line, while, at the end of the thick filaments there is practically no precipitate. A second consideration with respect to this could be that it is known that l-Myosin ATP-ase at the p_H used by us is in vitro strongly inhibited by Mg^{++} (HUXLEY 1960, p. 430). The ATP-ase activity of Actomyosin under the same circumstances is markedly activated by Mg^{++} (KIELLEY and MEYERHOF 1948). These data indicate that the strongly Mg^{++} activated ATP-ase activity we demonstrated in the Z-line region is probably not the result of l-Myosin ATP-ase activity. The conditions we have chosen are favourable for the demonstration of Mg^{++}-activated Actomyosin ATP-ase.

The localization of the enzyme we found differs from the localization in the A-zone of a Ca^{++}-activated ATP-ase reported by TICE and BARRNETT (1960, 1962), which is probably due to l-Myosin. The localization in the A-zone of a Mg^{++}-activated ATP-ase they found, is much less suggestive.

In conclusion, it seems conceivable that the ATP-ase activity found by us in the Z-line region indicates the presence of Actomyosin.

Summary

The fine structural morphology and the ATP-ase activity of heart muscle at various sarcomere lengths was studied. ATP-ase activity was found on endothelial cells, cell membrane, intercalated disc, sarcoplasmic reticulum and in the Z-line region. It appeared that the activity of the ATP-splitting in the Z-line region was sensitive to PCMB and could be reactivated with cysteine. This points to an activity of "true" ATP-ase according to the terminology of PADYKULA et al. (1955) and FREIMAN et al. (1960). In addition, this cross striational enzyme was strongly activated by Mg^{++}.

At intermediate sarcomere lengths and in hypercontraction no I-zones or H-zones were visible, while at intermediate sarcomere lengths and M-line was faintly indicated. In the stretched muscle, both I- and H-zones and N- and M-lines were present. In comparison with muscle tissue with intermediate sarcomere lengths, the C_z-bands of hypercontraction had markedly broadened, whereas in stretched muscle a relatively sharply defined electron-dispersing line indicated the presence of a Z-line.

The localization and spread of the precipitate formed as a result of ATP-ase activity changes with alterations of the sarcomere lengths exclusively in the Z-line region.

The probable identity of the Z-line region ATP-ase with Actomyosin is discussed.

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