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AN X-RAY DIFFRACTION STUDY OF THE CROSS-CIRCULATED CANINE HEART

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SUMMARY

1. The equatorial X-ray diffraction pattern was recorded from a papillary muscle of a cross-circulated canine heart at different phases of the cardiac cycle. The intensity ratio of the 1, 0 and the 1, 1 reflexions ($I_{1,0}/I_{1,1}$) was 0·79 in the systolic phase and 1·19 in the diastolic phase.

2. Using the intensity ratio obtained, the approximate proportion of the myosin projections present in the vicinity of the thin filaments was calculated. This was 70–71% in the systolic phase and 51–52% in the diastolic phase of the total myosin projections.

3. The peak systolic tension was roughly proportional to the proportion of the projections present in the vicinity of the thin filaments during systole.

4. The projections which stayed in the vicinity of the thin filaments during diastole did not produce significant contractile force.

INTRODUCTION

The movements of the myosin projections in skeletal muscle have been studied using an X-ray diffraction technique (Huxley & Brown, 1967; Huxley, 1968; Haselgrove & Huxley, 1973). This technique was applied to heart muscle by Matsubara & Millman (1974) and led to the conclusion that the myosin projections are transferred from the vicinity of the thick filaments to that of the thin filaments when heart muscle goes into rigor. Recently Kamiyama, Matsubara & Suga (1976) recorded the equatorial X-ray patterns from perfused heart muscles undergoing cyclic contractions, and showed that the myosin projections are transferred from the thick to the thin filaments as the muscle shifts from the diastolic to the systolic state. However, the amount of the transfer they observed was

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rather small, and they attributed this to the fact that their preparation was in a hypodynamic state.

We present here the results of our recent study of the behaviour of the myosin projections in a cross-circulated heart muscle. The preparation used produced a much larger systolic tension than the previous preparation. We have found a larger amount of transfer taking place during the systolic phase.

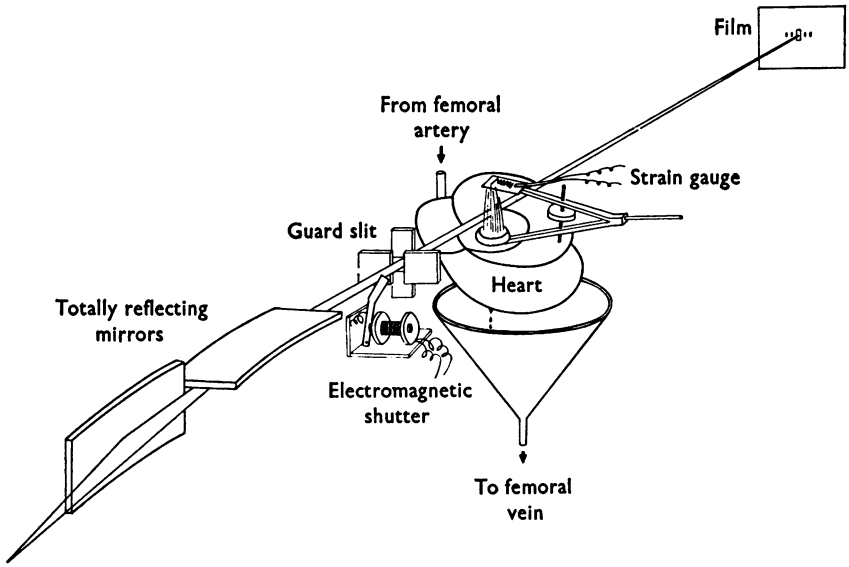

Fig. 1. Schematic diagram of the experimental set-up. X-rays were collimated by two bent mirrors. An electromagnetic shutter was placed between the second mirror and a guard slit, and was operated through a pulse generator. A papillary muscle was exposed in the right ventricle, and was held isometric between a strain gauge force transducer and a metal ring which had four spikes stuck into the ventricular septum. The coronary arterial inflow was supplied from the femoral artery of a donor dog and the coronary venous outflow was collected by a funnel and sent back to the femoral vein of the dog. The output of the force transducer was fed to an amplifier, which was connected with a recorder and the pulse generator for operating the shutter. The diffraction pattern was recorded on a film which was placed near the focus of the X-ray beam.

METHODS

Specimen preparation. In each experiment, two mongrel dogs of 6–12 kg body weight were used. The dogs were anaesthetized with an intravenous injection of a mixture of chloralose (60 mg/kg) and urethane (600 mg/kg).

An excised cross-circulated heart was prepared without interruption of coronary perfusion by the method described by Suga & Sagawa (1974); the coronary arterial inflow was supplied from the femoral artery of a donor dog, and coronary venous outflow was collected by a funnel and returned to the femoral vein of the donor

dog (Fig. 1). A part of the arterial tubing was immersed in a temperature controlled bath to keep the perfusing blood at 37° C. Perfusion pressure was above 75 mmHg.

A papillary muscle was exposed in the right ventricle through an incision in the free ventricular wall. A branch of the arterial tubing was used to drip arterial blood on the surface of the papillary muscle in order to prevent the surface from drying out. The muscle was held isometric using the method developed by Suga & Numao (1975) (Fig. 1). A metal ring encircling the muscle was pinned down on the ventricular septum and stitched to the tissue surrounding the ring. The tendinous end of the muscle was tied to a hook of a strain-gauge isometric force transducer. The muscle contracted with a regular sinus rhythm (Fig. 2).

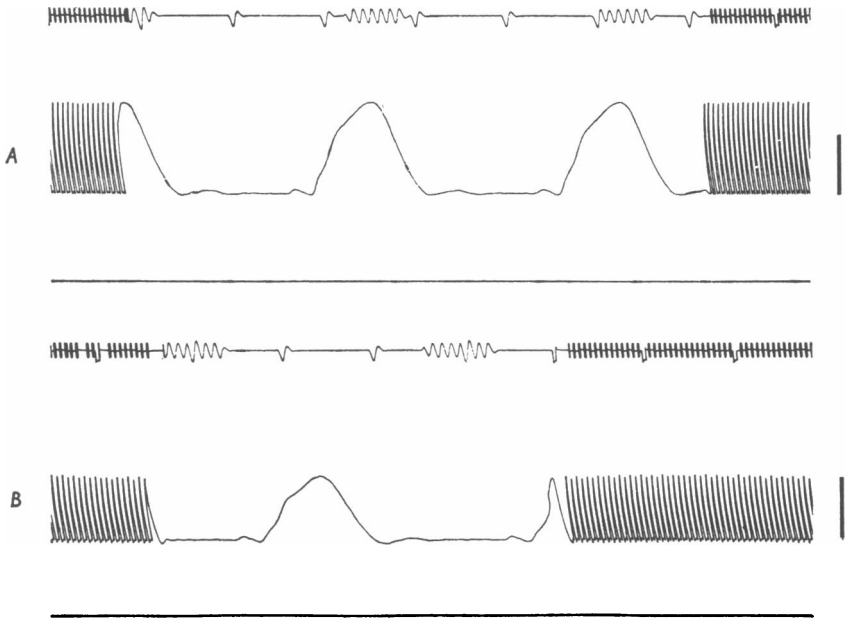

Fig. 2. Tension records during the systolic and the diastolic exposures of the same muscle. In the centre portion of each record, the paper speed of the recorder was increased by fifty-times. The wavy line (50 Hz) above each record indicates the period of X-ray exposure. The wavy lines appear as regularly spaced bars in the low-speed portions of each record. The interval between the time signals (above each tension record) indicates 10 sec in the low-speed portion, and 200 msec in the high-speed portion. The vertical bars at the right indicate 20 g. *A*, systolic exposure, the shutter was opened when the tension exceeded half to two thirds of the peak tension. The muscle was held at L_{max}. *B*, diastolic exposure, the shutter was opened when the tension was at the steady diastolic level. The peak tension was about 70% of that obtained during the systolic exposure, since the muscle was held at $0{\cdot}9L_{max}$ during the diastolic exposure.

At the beginning of each experiment, the muscle was stretched to the length (L_{max}) where the developed tension was greatest. The muscle was held at this length during the exposure for obtaining the systolic pattern. During the exposure for the diastolic pattern the muscle was held at a shorter length ($0{\cdot}9L_{max}$). The two

different muscle lengths are required for the comparison of the systolic and the diastolic patterns at approximately the same sarcomere length because the sarcomeres of an isometrically held papillary muscle shorten by 5–16 % at the peak of systole (Kreuger & Pollack, 1975; Julian & Sollins, 1975).

X-ray diffraction. A double-mirror Franks camera (Franks, 1955; Elliott & Worthington, 1963) was used. The X-ray source was a high-power rotating anode generator (Rigaku, type FR) operated at 40 kV with a tube current of 80 mA (nominal focal size 1 mm × 0·1 mm, viewed at an angle of 6°). The papillary muscle was placed behind a guard slit, and the equatorial diffraction pattern was recorded on X-ray film (Sakura N) with a specimen-to-film distance of 45 cm (Fig. 1). An electromagnetic shutter was placed between the second mirror and the guard slit. The shutter was coupled with the force transducer and passed the X-ray beam only in a specified phase of the cardiac cycle. To obtain the systolic pattern, the shutter was opened as the tension exceeded two thirds of the peak systolic tension and closed as the tension fell below half of the peak tension (Fig. 2*A*); we were not able to open and close the shutter at the same tension level since the operation of the shutter involved a certain delay. To obtain the diastolic pattern, the shutter was opened and closed while the tension was at the steady diastolic level (Fig. 2*B*). Each systolic or diastolic exposure lasted 80–140 msec, and approximately 10,000 contractions were needed for recording one pattern. Both the systolic and the diastolic patterns were obtained from each muscle.

The recorded pattern was densitometered, and the integrated intensities of the 1, 0 and the 1, 1 equatorial reflexions were measured to obtain the intensity ratio ($I_{1,0}/I_{1,1}$). Using the equatorial intensities (corrected for the Lorentz factor), the electron density distribution in the myofilament lattice projected onto the plane perpendicular to the muscle axis was calculated by Fourier synthesis on the assumption that the phases of the 1, 0 and the 1, 1 reflexions are 0°. From the density distribution obtained, the apparent mass of the thick and the thin filaments were calculated following the method described by Haselgrove & Huxley (1973).

RESULTS

The papillary muscle contracted with a regular sinus rhythm of 115 ± 14 beats/min (mean ± S.D. of an observation, $n = 6$), producing a peak systolic tension of 595 ± 84 g/cm^2 ($n = 6$); the cross-sectional area of the muscle was measured at the middle of the muscle. Both the systolic and diastolic patterns were recorded from each muscle; the order of the systolic and diastolic exposures was changed from muscle to muscle. At the end of each exposure, the peak systolic tension was 94 ± 8 % ($n = 12$) of the initial value.

Systolic and diastolic patterns

Fig. 3*A* shows a densitometer trace of the equatorial pattern recorded during the systolic phase. Two peaks were seen, and these could be indexed as the 1, 0 and the 1, 1 reflexions from the hexagonal array of the myofilaments (Huxley, 1953). The spacing between the 1, 0 lattice planes ($d_{1,0}$), calculated from the positions of the 1, 0 and the 1, 1 reflexions, was 376 ± 7 Å ($n = 6$). From this value, the sarcomere length (S) of the papillary muscle during contraction (at L_{max}) could be calculated using

the empirical equation relating $d_{1,0}$ to S ($S \times d_{1,0}^2 = 3{\cdot}0 \times 10^{-3}\ \mu m^3$, Matsubara & Millman, 1974). The empirical equation is based upon observations on resting heart muscle, but is expected to hold for contracting heart muscle by analogy to skeletal muscle in which the interfilament separation (at a fixed sarcomere length) does not change significantly on contraction (Haselgrove & Huxley, 1973). The sarcomere length thus

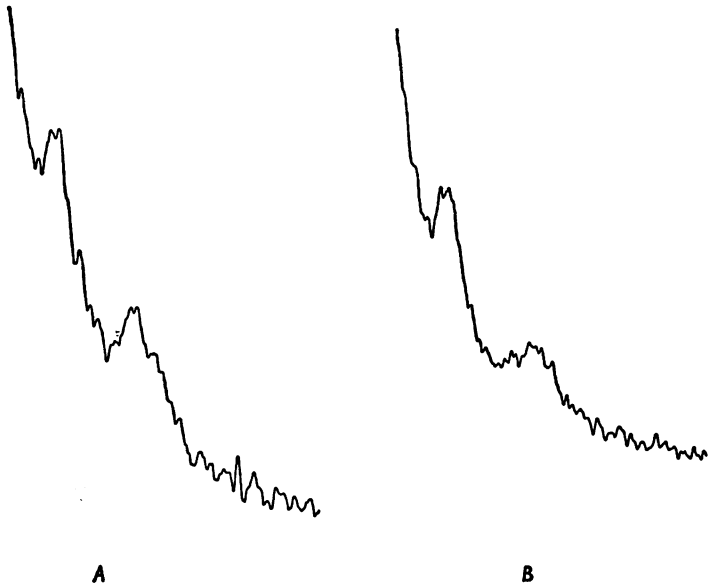

Fig. 3. Densitometer traces of the equatorial diffraction patterns recorded from the same muscle at different phases of the cardiac cycle. *A*, systolic phase; *B*, diastolic phase. In each trace, two peaks are seen; the one at the left is the 1, 0 reflexion, and the other is the 1, 1 reflexion. Note that the 1, 1 reflexion looks stronger in the systolic pattern than in the diastolic pattern, whereas the 1, 0 reflexion appears to be of about the same intensity in the two patterns.

obtained for the papillary muscle during contraction (at L_{max}) was 2·12 μm, close to the value obtained by the method of light diffraction (2·15 μm, Krueger & Pollack, 1975).

Fig. 3*B* shows the diastolic pattern recorded from the same muscle as used for Fig. 3*A*. The 1, 0 and the 1, 1 reflexions were also observed in the diastolic pattern. The $d_{1,0}$ calculated from the positions of these reflexions was 369 ± 6 Å ($n = 6$). The difference between the systolic and the diastolic values for $d_{1,0}$ was not statistically significant ($P > 0{\cdot}05$). This suggests that the sarcomere length during the diastolic exposure was not significantly different from that during the systolic exposure, justifying the procedure of holding the muscle at 0·9 L_{max} during the diastolic exposure.

Comparison of the systolic and the diastolic patterns (Fig. 3*A*, *B*) revealed that the integrated intensity of the 1, 1 reflexion was markedly reduced in the diastolic pattern. The intensity ratio ($I_{1,0}/I_{1,1}$) was $0{\cdot}79 \pm 0{\cdot}11$ ($n = 6$) in the systolic pattern, and $1{\cdot}19 \pm 0{\cdot}13$ ($n = 6$) in the diastolic pattern. The difference was statistically significant ($P < 0{\cdot}01$). Both the systolic and the diastolic ratios were smaller than the ratio reported for the heart muscle *not* undergoing cyclic contractions (3·07), but were greater than the ratio reported for the heart muscle in rigor (0·37) (Matsubara, Kamiyama & Suga, 1977).

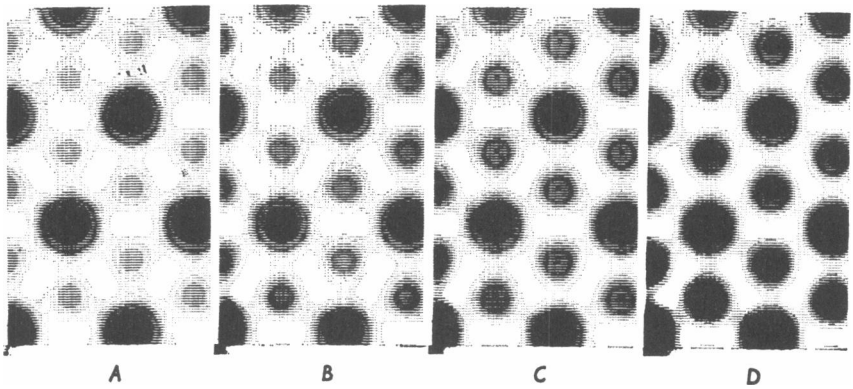

Fig. 4. Electron density distribution in the myofilament lattice of heart muscle projected onto the plane perpendicular to the muscle axis. *A*, quiescent state (i.e. not undergoing the cardiac cycle); *B*, diastolic state; *C*, systolic state; *D*, rigor. The density distributions for the diastolic and the systolic states were calculated from the intensities of the 1, 0 and the 1, 1 reflexions obtained in the present study, whereas the distributions for the quiescent and the rigor states were calculated from the data of Matsubara *et al.* (1977). The larger peaks in each diagram represent the thick filaments and the smaller peaks represent the thin filaments.

Fourier projections

The electron density distribution in the filament lattice projected on to the plane perpendicular to the muscle axis was calculated by Fourier synthesis of the equatorial intensities. The spatial resolution of the density map thus obtained was bound to be poor, since only the 1, 0 and the 1, 1 intensities were used in the calculation. However, the cross-sections of the thick and the thin filaments were clearly visible (Fig. 4) and the approximate mass of the material associated with each filament could be estimated from the map (Huxley, 1968; Haselgrove & Huxley, 1973). Fig. 4*B* and *C* are the density distributions for diastolic and systolic phases respectively. These were calculated from the present equatorial data. Fig. 4*A* and *D* are the density distributions for the heart muscle in

the 'quiescent' state (i.e. not undergoing cyclic contractions) and in rigor. They were calculated using the intensity ratios obtained previously by Matsubara *et al.* (1977).

Comparison of the four Fourier projections in Fig. 4 reveals that the mass of the material associated with the thin filaments increases as the state of the muscle shifted from one at the left (the quiescent state) towards the one at the right (rigor), and that this is accompanied by a decrease in the mass of the material associated with the thick filaments. Similar changes have been observed in skeletal muscle (Huxley, 1968; Haselgrove & Huxley, 1973) and heart muscle (Matsubara *et al.* 1977), and have been attributed to the transfer of myosin projections from the vicinity of the thick filaments to that of the thin filaments. By making the crude assumption that the mass of the material transferred from the thick to the thin filaments as the heart muscle shifts from the quiescent to the rigor state (i.e. the mass of the 'rigor transfer') represents the total mass of the myosin projections, we calculated the approximate proportion of the myosin projections present in the vicinity of the thin filaments in the diastolic and the systolic phases. The calculation indicated that approximately 51–52 % of the total myosin projections were in the vicinity of the thin filaments in the diastolic phase (Fig. 4*B*). The smaller value (51 %) was obtained when the lowest density in the Fourier map was chosen as the background for calculating the mass of the myofilaments, and the larger value (52 %) was obtained when the lowest density along the line connecting the centres of the thick and the thin filaments was chosen as the background (see Haselgrove & Huxley, 1973). Similarly, the proportion of the myosin projections present in the vicinity of the thin filaments in the systolic phase (Fig. 4*C*) was calculated to be 70–71 % of the total. Thus the present results indicated that more myosin projections were in the vicinity of the thin filaments in the systolic phase than in the diastolic phase.

Comparison with the hypodynamic preparation

In order to compare the systolic and diastolic tensions of the present preparation with those of the hypodynamic preparation used by Kamiyama *et al.* (1976) (see Introduction), the following experiment was performed. First, the systolic and diastolic tensions were recorded from a papillary muscle in a cross-circulated heart preparation identical to the present experiment. Then the papillary muscle, together with the ventricular septum, was isolated from the cross-circulated heart and perfused through the septal artery with Tyrode solution containing 1 % blood. During isolation the muscle was kept connected to the force transducer so that there was no change in the muscle length. The muscle was stimulated 12 times

per minute and the systolic and diastolic tensions were recorded. The peak developed tension during the systolic phase fell to 20–25 % of that of the cross-circulated preparation, but the steady level of the diastolic tension was not changed significantly.

DISCUSSION

We have shown that the intensity ratio of the 1, 0 to the 1, 1 equatorial reflexions changes between the systolic and the diastolic phases. Similar variations in the intensity ratio have been found in skeletal muscle (Huxley, 1968; Haselgrove & Huxley, 1973) and were interpreted as the result of radial movements of the myosin projections between the thick and the thin filaments. Recently an alternative interpretation, attributing the changes in the intensity ratio to azimuthal movements of the projections, has been proposed by Lymn (1975). However, we have chosen the former interpretation in analysing our data for the following reasons: (i) there is electron-microscopical evidence indicating the occurrence of radial movements of the projections (Huxley, 1968); (ii) according to Lymn's view, the myosin projections in resting muscle are stretched further away from the thick filaments, and when the muscle passes into rigor the projections move azimuthally to attach the thin filaments. This view has been criticized by Haselgrove, Stewart & Huxley (1976) who have shown that the myosin projections in resting muscle are more likely to be located in the vicinity of the thick filaments.

Our analysis indicated that the proportion of the myosin projections present in the vicinity of the thin filaments is greater in the systolic phase than in the diastolic phase. This allows us to postulate the net movement of the projections during each cardiac cycle; a certain proportion of the projections are transferred from the thick to the thin filaments as the muscle contracts, and in the opposite direction as the muscle relaxes.

The average proportion of the projections found in the vicinity of the thin filaments during the systolic phase (70–71 % of the total projections) was about 3·5 times greater than that reported for a hypodynamic preparation of the canine papillary muscle during systole (18–20 %, Matsubara *et al.* 1977). The fact that the systolic tension of the present preparation (595 g/cm^2) was 4·4 times greater than that of the hypodynamic preparation (135 g/cm^2) suggests that the systolic tension is roughly proportional to the proportion of the projections present in the vicinity of the thin filaments during systole.

About 51–52 % of the projections were found in the vicinity of the thin filaments during the diastolic phase. These projections, however, are not likely to produce significant contractile force. This was indicated by the comparison between the cross-circulated and the hypodynamic prepara-

tions. In the hypodynamic preparation, the myosin projections found in the vicinity of the thin filaments during the diastolic phase were 8–9% of the total projections (Matsubara *et al.* 1977). Since the diastolic tension of the cross-circulated preparation in which 51–52% of the projections were found in the vicinity of the thin filaments during diastole did not differ significantly from that of the hypodynamic preparation, we conclude that most of these projections are not producing significant contractile force.

In skeletal muscle, it has been known that the myosin projections do not resume the 'resting' position immediately after the end of contraction; the myosin layer line does not recover the 'resting' intensity until several seconds after the end of a short tetanus (Huxley, 1973). In the present experiment, the time available to the projections for returning to the thick filaments was only 200–300 msec (i.e. the duration of each diastolic phase). It is therefore probable that the projections did not have enough time to move back to the thick filaments before the onset of the next contraction.

If the duration of the diastolic phase is the major factor in determining the proportion of the projections staying near the filaments over the diastolic phase, then the proportion is expected to be a function of the heart rate. Further, we would expect the 'remaining' projections to behave differently from other projections during the initial phase of contraction and thus modify the amplitude and rate of change of the developed systolic tension. We conclude that heart rate dependent properties of the cardiac muscle such as the staircase phenomenon should be reviewed in this light.

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