Myocardial fibre calcification

J McCLURE, AS PIETERSE, DJ POUNDER, PS SMITH

From the Division of Tissue Pathology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia

SUMMARY Three cases of myocardial fibre calcification found at post-mortem examination are described. In one case there was antemortem hypercalcaemia and hyperphosphataemia and the case was clearly an example of metastatic calcification. In the other two cases there was ischaemic myocardial necrosis and calcification was seen in fibres which were not overtly necrotic, but which were both in proximity to (the majority) and remote from the necrotic zones. Since renal failure with hyperphosphataemia was present in both cases, these were considered to be examples of augmented (by the hyperphosphataemia) dystrophic calcification. The histological, histochemical, and ultrastructural features were identical in the three cases. Hydroxyapatite formation was observed initially in mitochondria, followed by spillage of crystals into the cytosol and ultimately into the interstitium. It is suggested that the fundamental lesion is a dysfunction of the fibre membrane; the similarity of this reaction with the calcification seen in skeletal muscle fibres in various myopathies is noted and a unifying hypothesis of the mechanism of skeletal and cardiac muscle fibre calcification is thereby suggested.

Calcification confined to or mainly involving the myocardium is most often found in old ischaemic infarcts and is seen with sufficient frequency at necropsy as to be regarded as commonplace. By contrast calcification involving individual myocardial muscle fibres is rarely seen but may occasionally be observed following a wide variety of myocardial insults. Whilst the anatomical distribution of the myofibre involvement varies with the nature of the myocardial insult the morphology of the cellular calcification process appears remarkably uniform, suggesting a common mechanism of calcification. The Wrogemann-Pena¹ hypothesis that skeletal muscle fibre overload with calcium represents a general mechanism of fibre death has prompted us to study three cases of myocardial fibre calcification for morphological evidence of a common mechanism of skeletal muscle and myocardial fibre calcification.

Case reports

CASE 1

This was a 75-year-old woman who had been well until just over one year before her terminal admis-

sion when she had discovered a mass in the left breast but had not sought medical help. There was a large mass involving most of the left breast and extending into the left axilla. Trucut needle biopsy showed that this was an infiltrating ductal carcinoma. Bone scan suggested multiple skeletal metastases. A chest x-ray examination revealed a left basal effusion but no focal lung lesion. Biochemical testing showed a hypercalcaemia of 4.78 mmol/l (range 2.20-2.55 mmol/l) with a normal serum albumin concentration. Serum phosphate concentrations (1.58 mmol/l) were also raised (range 0.80-1.30 mmol/l). Her condition deteriorated rapidly and she died 15 days after admission.

At necropsy, metastases were found in the axillary lymph nodes, both adrenal glands and were extensive in the skull and vertebral column. The connective tissue stroma of the tumour showed calcification. Similarly, calcification of blood vessel walls (affecting both the internal elastic laminae and media) was noted in the kidneys, spleen, pancreas and adrenal capsules. There was also calcification of fibrotic foci in the thyroid and left lung. Renal tubular nephrocalcinosis was also seen. The parathyroids were macroscopically and microscopically normal.

The heart weighed 480 g and showed left ven-

Fig. 1 Calcification of the elastic lamina (EL) of a blood vessel and groups of myocardial fibres (CMF) in the case of metastatic calcification. von Kossa's stain $\times 100$

tricular hypertrophy. All the main branches of the coronary arteries were atheromatous with focal severe stenosis. Microscopically, there was some subendocardial scarring affecting the posterior wall of the left ventricle. There was no histological evidence of recent myocardial necrosis. Affecting the full thickness of the myocardium, there was dense basophilia of groups of myocardial fibres. These groups of fibres were randomly distributed bearing no particular relation to blood vessels. Nuclear and cytoplasmic details were obscured in the affected fibres but immediately adjacent fibres were of a normal appearance. A basophilic reaction was also observed in the subendocardial scar and in the elastic laminae of small arterial blood vessels.

Sections of involved myocardium (which had been fixed in 10% buffered formalin) were stained by von Kossa's stain (Fig. 1), chloranilic acid, alizarin red S and periodic acid-Schiff (PAS) techniques and the basophilic fibres gave positive reactions with these methods. The application of Perl's Prussian blue stain failed to reveal the presence of iron.

CASE 2

This was a 65-year-old man who was admitted to hospital with dyspnoea and right upper quadrant abdominal pain. He had had a proven myocardial infarction nine years previously and an episode of congestive cardiac failure six months before the present admission. His current dyspnoea had been getting progressively worse over a two week period. Clinical examination revealed biventricular failure and ECG showed premature ventricular contractions with short runs of ventricular tachycardia. The ventricular dysrhythmia settled and the biventricular failure responded to treatment. Within 24 hours his condition had improved. However, four days after admission, he suffered a cardiac arrest and resuscitation was not attempted. Biochemical tests during his period in hospital showed a serum calcium concentration of 2.51 mmol/l (range 2.20-2.55 mmol/l) with a normal serum albumin value of 42 g/l (range 34-45 g/l). However the serum phosphate concentration was 2.56 mmol/l (range 0.70-1.25 mmol/l) and there was evidence of renal failure

Fig. 2 Myocardial fibres (arrowed) showing a granular basophilia. Haematoxylin and eosin $\times 200$

—namely, urea 14.1 mmol/l (range 3.0–8.0 mmol/l) and creatinine 0.27 mmol/l (range 0.05–0.12 mmol/l). Chest x-ray examination revealed an enlarged heart with pulmonary changes consistent with congestive cardiac failure.

At necropsy, the pericardial sac contained 150 ml of blood-stained serous fluid. The heart was enlarged (580 g) mainly due to left ventricular hypertrophy and dilatation. There was severe coronary artery atherosclerosis but no recent occlusive thrombi. The parathyroids were normal and the kidneys were healthy.

Histologically, the wall of the left ventricle showed extensive recent subendocardial necrosis with foci of fibre swelling, loss of nuclei and cross-striations with increased eosinophilia. At the margins of these zones were prominent groups of myocardial fibres showing a basophilia which varied from granular to dense obscuring nuclear and cytoplasmic detail. Occasional foci of similar basophilic fibres (Fig. 2) were seen deep in the myocardium and surrounded by apparently normal fibres. Special stains were applied as in case 1 and positive reactions were again obtained with von Kossa's, chloranilic acid, alizarin red S and PAS techniques. The iron stain was negative. Calcification was not observed in any other organ.

CASE 3

This was a 70-year-old man admitted to hospital

with a two day history of urinary retention and suprapubic pain. He had had a suprapubic prostatectomy two years previously for a carcinoma of the prostate. He had been a diabetic for a number of years controlled by oral hypoglycaemic agents. The serum calcium concentration was 2.23 mmol/l (range 2.20–2.55 mmol/l) and the serum albumin 33 g/l (range 34–45 g/l). Serum phosphate concentrations were raised at 2.87 mmol/l (range 0.70–1.25 mmol/l) in the presence of renal failure—namely, urea 41.2 mmol/l (range 3.0–8.0 mmol/l) and creatinine 0.84 mmol/l (range 0.05–0.12 mmol/l). His condition declined inexorably and he died nine days after admission.

At post-mortem examination, the urinary bladder trigone was occupied by a large fungating, partly haemorrhagic, partly necrotic tumour which had arisen from the prostate and which had caused bilateral ureteric obstruction with mild hydronephrosis. No metastatic tumour was identified. The parathyroids were not enlarged.

The heart weighed 350 g and there was extensive calcific atheroma with severe stenosis of the main coronary arteries. Histologically, there was some subendocardial scarring with foci of dense fibrosis and foci of less mature granulation tissue. There were fairly extensive zones of recent subendocardial necrosis with fibre swelling, increased eosinophilia, loss of nuclei and loss of cross-striations. At the margins of these necrotic zones, there were groups

Fig. 3 Myocardial fibre (arrowed) showing a granular basophilia. Haematoxylin and eosin $\times 400$

of myocardial fibres showing a mainly granular basophilia (Fig. 3), only in some fibres was the reaction homogeneous. Similar but less frequent foci of fibre reaction were seen in other parts of the heart wall and not apparently related to foci of recent necrosis. Again special stains showed positive von Kossa, chloranilic acid, alizarin red S and PAS-positive reactions corresponding to the fibre basophilia. Iron stains were negative.

Ultrastructural studies

Tissue for electron microscopy was excised from

those areas in the paraffin blocks showing calcification by light microscopy. Paraffin wax was removed with xylol and the tissues rehydrated through graded alcohols to a cacodylated buffered sucrose wash solution. Normal postfixation in osmium tetroxide, dehydration in ethanol and embedding in Spurr's resin followed. Survey sections ($0.5 \mu\text{m}$) were cut and those showing calcification were thin sectioned for electron microscopy.

In the first case, examination of non-calcified foci showed typical longitudinal sections of cardiac muscle cells containing banded myofilaments and numerous mitochondria and the cytological details were of a high quality (Fig. 4). These cells were joined end to end by interdigitation in the normal manner and were occasionally separated laterally by a small amount of collagen and small blood vessels. Large dense lipofuscin granules occurred frequently in the cell cytoplasm between the myofilaments. The obviously calcified cells contained an electron opaque material (Fig. 5). This was of needle-shaped crystals identical in shape and size to hydroxyapatite. In these cells, the normal ultrastructure was obliterated by crystals which were associated with extracellular collagen. Other cells revealed hydroxyapatite accumulation within their mitochondria (Fig. 6). Individual mitochondria showed a variable degree of crystallisation and variable numbers of mitochondria were involved in different cells (Fig. 7). Other organelles were not crystallised but the myofilaments in these cells were generally blurred.

Fig. 4 Banded myofilaments in a myocardial fibre $\times 17\,600$

Fig. 5 Zone of calcification (C) in a myocardial fibre. The arrows indicate a focus of dense extracellular calcification. In the inset clusters of hydroxyapatite crystals are seen in association with collagen fibres. $\times 8500$ (inset $\times 5700$)

Identical changes were seen in material studied in a similar manner from the second and third cases.

Discussion

Both dystrophic and metastatic calcification may occur in the heart. Dystrophic calcification is a localised occurrence in the absence of a generalised disturbance in calcium metabolism; hence, it is important to consider local events in the tissue when endeavouring to elucidate the pathogenesis of the process. There is no general agreement as to the mechanisms involved in the mineralisation process of pathological dystrophic calcification. Various factors such as local tissue alkalinity, citric

acid—which has a high affinity for calcium and binds it in an ionic form,² alkaline phosphatase³ and acid mucopolysaccharides⁴ have all, at one time or another, been ascribed important roles in pathological calcification.

The majority of cases of cardiac calcification would seem to be dystrophic in type occurring most often in ventricular aneurysms after myocardial infarction.⁵ In these cases, calcification occurred in the fibrous connective tissue of the aneurysms and calcification of individual myofibres was not seen.

Metastatic calcification occurs when there is a movement of calcium salts from their usual sources in diet and bone towards the serum (causing a hypercalcaemia) and from the serum to the soft tissues.⁶

Fig. 6 Mitochondria (arrowed) containing hydroxyapatite crystals
× 15 200

Fig. 7 Hydroxyapatite crystals in association with mitochondria (arrowed). The dense dark bodies are lipofuscin (LF) granules.
× 35 600

Metastatic calcification is seen in disease processes associated with resorption of skeletal bone mineral and matrix. Such resorption may be local, as in

myelomatosis, metastatic tumours, osteomyelitis and leukaemias; or it may be a generalised abnormality as in resorption due to immobilisation of the

skeleton, hypervitaminosis D, hyperparathyroidism and chronic renal disease.⁶ Terman *et al*⁷ found metastatic cardiac calcification in 10 out of 26 patients on chronic dialysis. These patients had a proven hypercalcaemia. Early calcification in these cases was seen as a fine granular basophilia in small clusters of otherwise intact fibres. Later changes consisted of loss of myocardial fibres with fibrosis and the accumulation of large masses of calcified material. Parathyroid hyperplasia was noted in all these patients.

Calcification of cardiac muscle fibres has also been noted in patients with severe renal disease by Gore and Arons⁸ and after cardiovascular surgery.^{9,10} Both in association with renal disease and as a consequence of cardiovascular surgery, calcification appears to be initiated in the mitochondria of myofibres.^{11,12} Mitochondrial calcification would explain the fine granular basophilia reported by Terman *et al*⁷ in their cases. Therefore it would seem that initial mitochondrial calcification is part of the mechanism of myocardial calcification in instances of metastatic calcification (although it should be pointed out that in the case of Woodhouse and Burston,¹¹ hypercalcaemia was never shown).

Myocardial fibre calcification seen as a complication of cardiac surgery is regarded as a form of dystrophic calcification. D'Agostino and Chiga¹² reported two cases and unequivocally demonstrated that the fibre mitochondria were the principal site of mineralisation. Both patients had severe coronary atherosclerosis and healed myocardial infarcts but the calcified fibres were interspersed between histologically normal fibres and were not adjacent to the myocardial scars. D'Agostino and Chiga¹² suggested that calcium influx was triggered by an alteration in either mitochondrial membrane permeability or mitochondrial function secondary to myocardial hypoxia or trauma resulting from prolonged and difficult cardiovascular surgery. In these cases of myocardial fibre calcification complex systemic biochemical disturbances may occur and although hypercalcaemia may not be a feature other changes such as hyperphosphataemia may favour calcium salt deposition at sites of local tissue damage thereby augmenting the dystrophic calcification process.

The three cases reported here illustrate the point that myocardial fibre calcification may be a feature of both metastatic and augmented dystrophic ectopic cardiac calcification. In the first case, there was a hypercalcaemia due to skeletal carcinomatosis and groups of myocardial fibres throughout the wall of left ventricle exhibited calcification and were surrounded by groups of histologically normal fibres. Only calcified fibres showed evidence of cell degeneration suggesting that there was probably

no dystrophic element to the calcification process. However, in the presence of severe ischaemic heart disease it is not possible to exclude absolutely a dystrophic element. Calcification of a subendocardial scar and the walls of blood vessels both features of metastatic calcification were also noted in this case. Ultrastructural studies indicated that the granularity of the calcific reaction as seen by light microscopy was due to mineralisation of the mitochondria.

In the second and third cases, myocardial fibre calcification was seen in hearts which were also the site of recent ischaemic necrosis. Groups of calcified fibres were seen both in proximity to the zones of necrosis and remote from them, although the former was more frequently observed. The light microscopical pattern and staining reactions in these cases was identical to that of the first case except that calcification of scar tissue and blood vessel walls was not observed. The necrotic zones showed the features of a coagulation necrosis typical of that which occurs in an ischaemic infarct. The changes were not those of the myofibrillar degeneration described by Reichenbach and Benditt which occurs after cardiac surgery and which also exhibits granular mitochondrial calcification.

The second and third cases are examples of dystrophic calcification. However, although neither had evidence of hypercalcaemia and the parathyroids were normal, both had renal impairment with consequent hyperphosphataemia. Consequently the dystrophic calcification was augmented by the hyperphosphataemia. Therefore considering the calcification reactions exhibited by these three cases, one represents a metastatic reaction, the second and the third represent augmented dystrophic reactions. Morphologically, the reactions are identical and this suggests that in both the metastatic and augmented dystrophic situation there is a common mechanism of fibre calcification.

Support for this concept is found in the ultrastructural studies. In the three cases, material for study was recovered from paraffin-embedded tissue. The structural detail of non-calcified fibres was of high quality and this allowed valid interpretation of structures seen in the calcified zones. There seemed to be a progression in both the number and degree of mitochondrial mineralisation. At this stage the myofilament detail was blurred and this may indicate intracellular damage. There was ultimate obliteration of fibre detail by numerous crystals in the cytosol with spillage into interstitial connective tissue. This latter feature was seen by EM in all cases, although calcification of connective tissue was only observed by light microscopy in the first case. Therefore initial mitochondrial mineralisation followed by total fibre involvement and

extension to the interstitial tissues would seem to be the universal sequence of myocardial fibre calcification. These findings are in accord with those described by Woodhouse and Burston in their account of metastatic calcification of myocardial fibres.

Wrogemann and Pena¹ have provided an hypothesis which states that in myopathies caused by diverse factors such as genetic or nutritional deficiencies, immunological factors, infective agents and trophic influences, or a combination of these, there is a primary or secondary defect which results in an influx of calcium ions into the muscle fibre. This forces the mitochondria to maintain intracellular calcium homeostasis by sequestering the excess quantity of the ion. This is an energy-consuming process and when individual mitochondria are massively loaded with calcium, an irreversible defect in oxidative phosphorylation occurs. Thus less energy is available for pumping calcium out of the cell and the process of mitochondrial calcium overload will be accelerated. Ultimately, the mitochondria will no longer be able to sequester calcium. The cytoplasmic concentration will then rise. The particular calcium salt found will depend on the availability of appropriate anions.

An application of this hypothesis to cardiac muscle would provide an explanation of the changes seen in the current cases and would provide a unifying hypothesis of the mechanism of myocardial fibre calcification. The essential lesion would be a dysfunction of the cell membrane in a cell which was at least initially otherwise functioning normally. Since the extracellular space calcium concentration is normally two to four orders of magnitude higher than in the cytosol, any increase in the extracellular space calcium beyond a certain concentration would lead to a net influx of ion and set in train the calcification process detailed above and be the basis of metastatic myocardial fibre calcification. Similarly, selective damage to the membrane such as might occur in the proximity of an ischaemic infarct or after cardiac surgery or as a consequence of drug treatment would explain the phenomenon of dystrophic and augmented dystrophic myocardial fibre calcification.

Cells with membrane damage but functional mitochondria would accumulate calcium in their mitochondria and this process would be expected to be augmented in the presence of high concentrations of phosphate. Intramitochondrial calcium accumulation with mitochondrial disruption might

cause progression of a sublethal and potentially reversible cell membrane lesion to irreversible cell damage. Since mitochondria accumulate calcium and phosphate in molecular ratios approximating to those found in hydroxyapatite, the formation of hydroxyapatite crystals such as we have demonstrated is not entirely unexpected. This morphological observation of hydroxyapatite formation within mitochondria in cases of metastatic and augmented dystrophic calcification probably represents an uncommon morphological expression of a common biochemical mechanism of myocardial fibre death.

References

- 1 Wrogemann K, Pena SDJ. Mitochondrial calcium overload: a general mechanism for cell necrosis in muscle diseases. *Lancet* 1976;i:672-3.
- 2 Harrison HE, Harrison HC. Inhibition of urine secretion and the production of renal calcinosis in the rat by acetazolamide (Diamox) administration. *J Clin Invest* 1955;34:1662-70.
- 3 Hendrichsen T. Alkaline phosphatase and calcification in tuberculous lymph nodes. *Exp Cell Res* 1956;11:511-9.
- 4 Levine MD, Rubin PS, Follis RH, Howard JE. Histochemical studies on calcinosis universalis with respect to the possible relationships between normal and pathological calcification. In: Reifstein EC, ed. *Metabolic Interrelationships*. New York: Josiah Macy Foundation 1949.
- 5 Lasky II. Massive dystrophic calcification of myocardium. *Ann Intern Med* 1954;40:626-31.
- 6 Eisenstein R, Truehart RE, Hass GM. Pathogenesis of abnormal tissue calcifications. In: Sognnaes RF, ed. *Calcification in biological systems*. Washington: Amer Assoc Adv Sci 1960.
- 7 Terman DS, Alfrey AC, Hammond WS, Donndelinger T, Ogden DA, Holmes JH. Cardiac calcification in uraemia. A clinical, biochemical and pathological study. *Am J Med* 1971;50:744-55.
- 8 Gore I, Arons W. Calcification of the myocardium. Pathologic study of 13 cases. *Arch Pathol* 1949;48:1-12.
- 9 Hermann G, Haupt GJ, Birkhead NC. Rapid myocardial calcification after cardiac surgery. *JAMA* 1963;186:260-1.
- 10 Reichenbach DD, Benditt EP. Myofibrillar degeneration. A response of the myocardial cell to injury. *Arch Pathol* 1968;85:189-99.
- 11 Woodhouse MA, Burston J. Metastatic calcification of the myocardium. *J Pathol* 1969;97:733-6.
- 12 D'Agostino AN, Chiga M. Mitochondrial mineralisation in human myocardium. *Am J Clin Pathol* 1970;53:820-4.

Requests for reprints to: Dr J McClure, Division of Tissue Pathology, Institute of Medical and Veterinary Science, Box 14 Rundle Street Post Office, Adelaide, South Australia 5000.