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Review

Keshan disease-an endemic cardiomyopathy in China

Keyou Ge, Anna Xue, Jin Bai, and Shuqin Wang
Institute of Health, Chinese Academy of Medical Sciences, 29 Nan Wei Road, Beijing, The People's Republic of China

Summary. Keshan disease, an endemic cardiomyopathy in China, was prevalent in rural areas located in a long belt region where the selenium content was low in foods. Intervention studies with well controled subjects revealed a prophylactic effect of sodium selenite. Multifocal necrosis and fibrous replacement of the myocardium characterized the histopathological features and myocytolysis was present in most cases. Ultrastructural observations on twelve postmortem cases revealed remarkable changes in many organelles of which mitochondria appeared to be most important in the development of myocardial lesions. Three kinds of specific granules, which have not been found in other reports on cardiomyopathies, were represented and other factors which might also contribute to the aetiology are discussed.

Key words: Keshan disease – Myocardial disease – Myocardial pathology – Myocardial ultrastructure – Selenium deficiency

Introduction

Keshan disease has been prevalent in China for more than hundred years. The first research work of epidemiological, clinical and pathological surveys of this disease was carried out in 1935, when there was a violent outbreak in Keshan county, Heilongjiang province, northern China, and the disease was named after the place (Apei 1937). Since then, cases have been reported from 206 counties located in 11 provinces and autonomous regions in the north (Sun et al. 1982) and also from many counties in Sichuan and Yunnan provinces in the south.

The epidemiological characteristics of Keshan disease show a regional distribution. The disease is distributed in a belt-like zone located between the coastal zone of southeastern China and the inland zone of northwestern China. The affected sites are usually focally distributed in hilly land of 1000–3000 meters above the sea level. The disease is seldom seen in high mountains or plains.

Offprint requests to: K. Ge at the above address

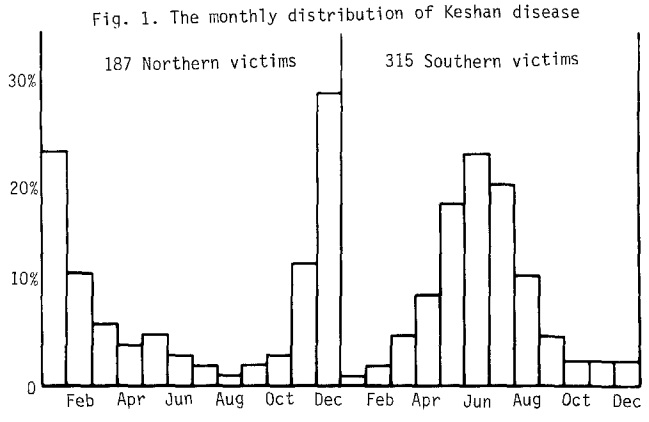


Fig. 1.

The susceptible population lives in rural areas and the disease occurs predominantly in peasants. Children below ten years of age are most susceptible. Of the 1,000 cases observed in Sichuan province (Chong et al. 1979) 984 were children of 2–10 years old. Women of child-bearing age were also involved in northern China. Migrants will not suffer from the disease until they have lived in the endemic area for at least three months.

The incidence varies greatly from year to year. According to Sun et al. (1982) there were more than 8,000 victims observed in each of the 3 peak years of 1959, 1964 and 1970, when the incidence was over 40 per 100,000 affected population, but it was much lower in other years. The incidence has sharply decreased to less than 5 per 100,000 and there were only about 1,000 new cases annually reported in recent years.

1,400 to more than 3,000 deaths occurred in each of the three peak years, more than five deaths per 100,000 population. From 1978 to 1980, however, only a little more than one hundred deaths were reported, less than one death per 100,000 populaton.

It is interesting to notice that the seasonal prevalence in northern China differs from that in southern. As showed in Fig. 1, 75% of the 187 cases observed in northern China occurred in November to next February (Gu et al. 1964), but 72% of the 315 southern cases were observed in May to August (unpublished).

Clinical manifestations

Keshan disease is classified into four types: acute, subacute, chronic and latent. There is no specific symptom or sign for identifying the disease.

Acute cases usually occur suddenly with acute heart function insufficiency such as cardiogenic shock, severe arrhythmia and pulmonary oedema. Obvious ST-T changes were also commonly seen as revealed by ECG.

Chronic cases may appear spontaneously or as the consequence of acute and subacute types. They usually showed moderate or severe heart enlargement with varying degrees of heart function insufficiency.

The clinical features of subacute cases are a combination of those of acute and chronic types. This form occurrs primarily in children. The onset was slower than in the acute patients but quicker than chronic cases, and most patients had facial oedema and a gallop rhythm.

Latent cases usually showed mild heart enlargement with normal heart function. ECG changes of right bundle branch block and ventricular extrasystoles may also present.

The role of selenium deficiency in Keshan disease

The aetiology of Keshan disease is not yet clear. Since 1935, many hypotheses have been proposed but none was well established. Experimental results (Su and Yu 1979) suggested that there might be some myocardial necrotizing factors and/or growth inhibiting factors in the water-soil elements in endemic areas. Wang et al. (1979) pointed out, based on the morphological and epidemiological studies that Keshan disease may be caused by some chemical agents acting upon the human body through foods and drinking water.

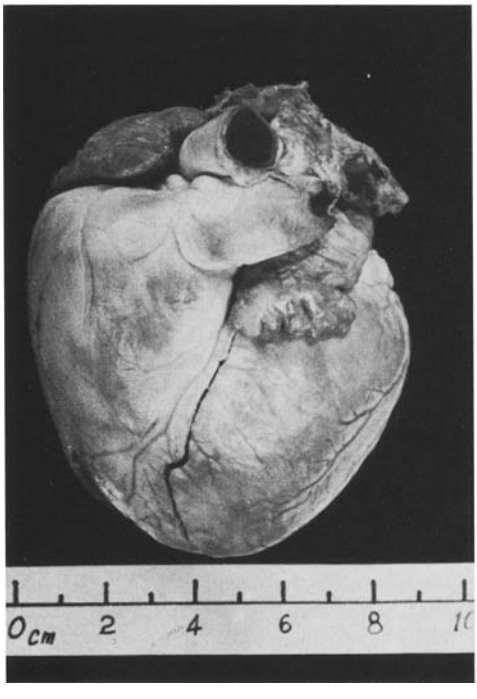
Work in our institute provided convincing evidence indicating an important role of selenium deficiency in the disease, which has been confirmed by others. The selenium content of about 5,000 samples of cereals and of hair collected from 196 sites throughout the country was estimated. Cereals produced in affected areas contain much less selenium when compared with the same species produced in nonaffected areas. The hair selenium content of the inhabitants in affected areas were below 0.12 ppm, while that of those in nonaffected areas were all above 0.20 ppm. The blood Se concentration, the glutathione peroxidase activity of whole blood and the quantity of Se excreted in urine of healthy subjects were lower in affected, compared with nonaffected, areas. These results demostrated the nutritional insufficiency of selenium in Keshan disease regions (Chen et al. 1980).

Intervention studies with sodium selenite among several thousand children in Sichuan province for four successive years revealed that Keshan disease is a selenium responsive disease (Chen et al. 1980). The incidence rate among Se-treated children was only 0–2.2 per 1,000, while it was 9.5–13.5 per 1,000 subjects in the placebo group. The difference is statistically significant.

In addition to the geochemical agents, biological factors also have been studied (Su et al. 1979). Experimental findings support the inference that selenium deficiency in combination with certain cardiotoxic agents, such as viral infection (Bai et al. 1980) or hypoxia (unpublished) might be responsible for the occurrence of the disease.

Pathological studies

Pathological observation on Keshan disease flourished in the 1950s along with the heavy prevalence of the disease in northern China. More than 3,600 autopsy cases had been collected by early sixties (Lu 1961). Different clinical-pathological types were included, but acute cases were predominant



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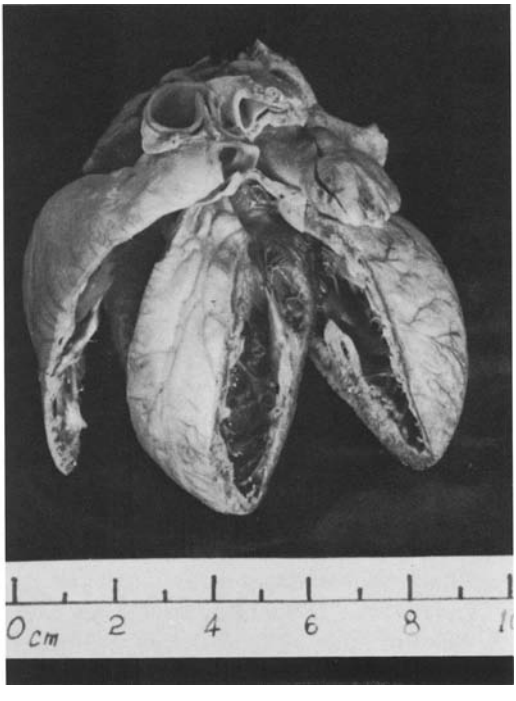

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Fig. 2. Subacute Keshan disease heart, dilatation of all chambers

Fig. 3. Subacute Keshan disease heart, distended cavities

in this group. The victims fell within a wide range of age but adult women were most commonly seen.

Later, an endemic cardiomyopathy which was prevalent in children in southern China was examined and identified as Keshan disease by a nation wide conference held in 1965. Since then, large amount of epidemiological, clinical and pathological data has been accumulated in the south.

Because of the fact that most of the work has not been published or published only in Chinese, attracting little attention in the world, a general histopathological description is given here.

Gross observations on the Keshan disease heart

The architecture of the heart varied with the different types of the disease. Chronic cases often showed a highly expanded, ball-shaped heart, it may appear mainly normal in acute cases. Myocardial hypertrophy was not so evident in comparison with the dilatation of the chambers. In the majority of subacute cases, especially in children, the heart usually showed a moderate enlargement with dilatation of ventricles and sometimes also of the atria (Fig. 2). Flattenning of the papillary muscle and columnae were commonly seen in the distended cavities but mural thrombi were observed rather infrequently (Fig. 3). Epicardial petechiae tended to occur around the coronary

sinus. Focal pericarditis was only observed in some specimens obtained from Tibet. Endocardium and coronary artery showed little abnormalities.

The myocardium was flabby and dull red in color with irregular yellowish or gray lesions on the cut surface. The lesions usually were small, could be recognized by a magnifying glass but patchy, infarct-like foci often associated with antemortem cardiogenic shock, were also observed in some acute cases.

Microscopic observations on the heart

Multifocal necrosis and fibrous replacement of myocardium was the principal pathological characteristic of Keshan disease. Because of the variety of size and distribution of the necrotic foci, the phases of the pathological process and the patterns of myocardial lesion, many and varied discriptions had been given to the pathological changes of Keshan disease heart and are summarized here.

a) Characteristics and distribution of necrotic foci. The focal lesions are located throughout the myocardium. As a rule, they are more frequently seen in the ventricular walls than in the atria and more on the left than the right side. The damage is usually heavier and older in the inner zone of the ventricular walls than in the outer zone. Four variations describe the distribution of the lesions.

(i) Small foci are seen scattered in the heart muscle. They are separated from each other in the early stage and not associated with the branching of the vascular system (Fig. 4). The small foci may expand and connect to each other to form a fibrous network with relatively intact myocardial fibers remaining in the meshes.

(ii) Focal condensation of many small lesions was encountered frequently. The cluster may connect with the terminals of an arterial branch. A large focus could result from the enlargement and merging of the small ones, however, there is always a certain amount of myocardial fibers visible within this focus (Fig. 5).

(iii) Myocardial necrosis develops along the branches of the artery. It is distinct from the unaffected myocardium. In the center of each focus there was an arteriole in which no morphological defects were revealed.

(iv) Patchy or zonal foci with severe degeneration of the parenchymal tissue was infrequently observed. Fibrosis takes place following the liquification and/or phagocytosis of the debris (Fig. 6).

b) Types of myocardial necrosis. Two different processes of myocardial necrosis were distinguishable in Keshan disease heart, one was myocytolysis, another, contraction band necrosis.

Myocytolysis is regarded as the representative change especially in subacute Keshan disease. It is initiated by the appearance of small vacuoles in the sarcoplasm. Disintergration of myofibrils and other elements take place in association with the enlargement and merger of the vacuoles. Con-

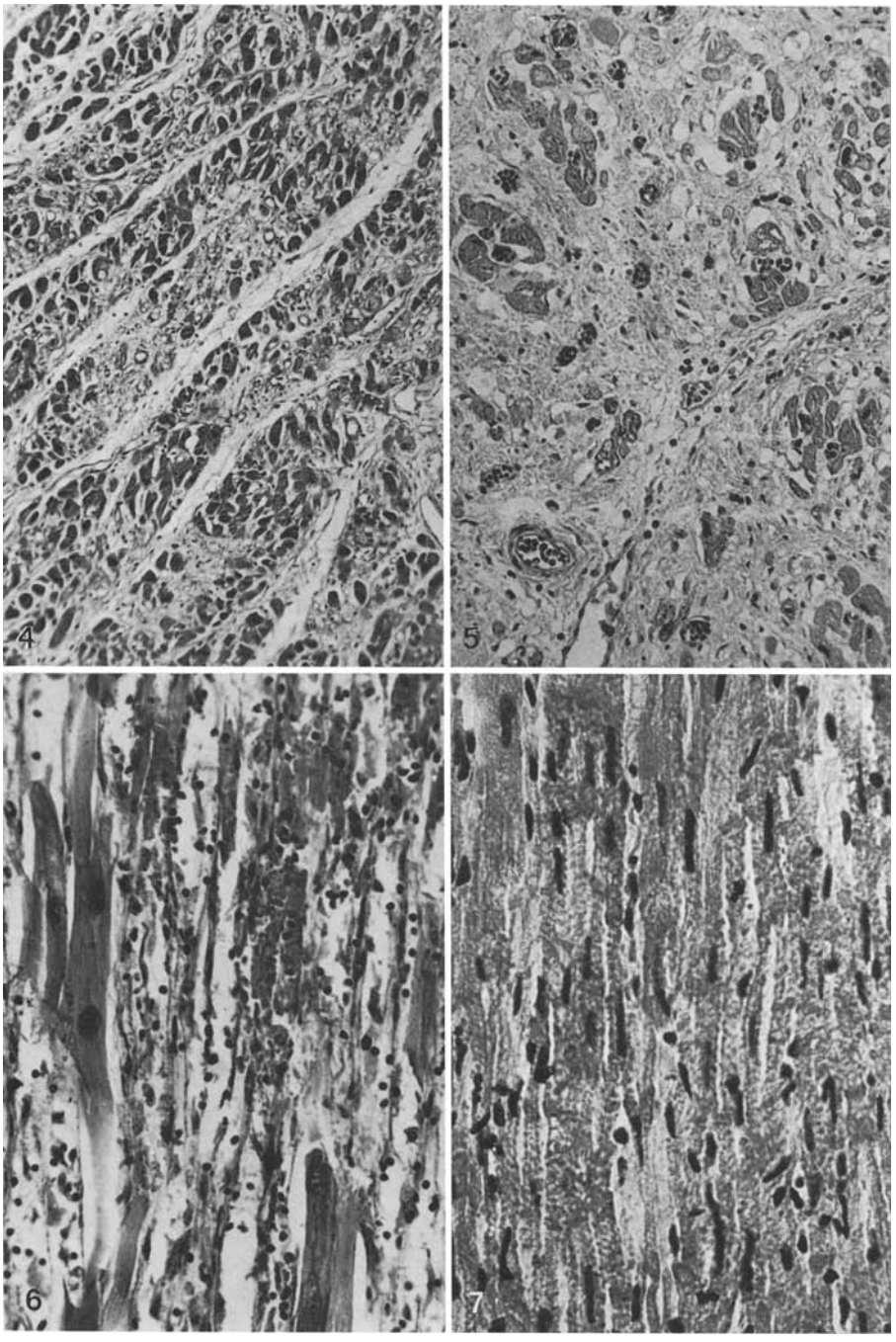

Fig. 4. Millary foci of myocaridal necrosis. HE ×88

Fig. 5. Fibrous network with myofibers in the meshes HE ×175

Fig. 6. Part of a patchy lesion, active phagocytosis HE ×350

Fig. 7. The early phase of contraction band necrosis HE ×350

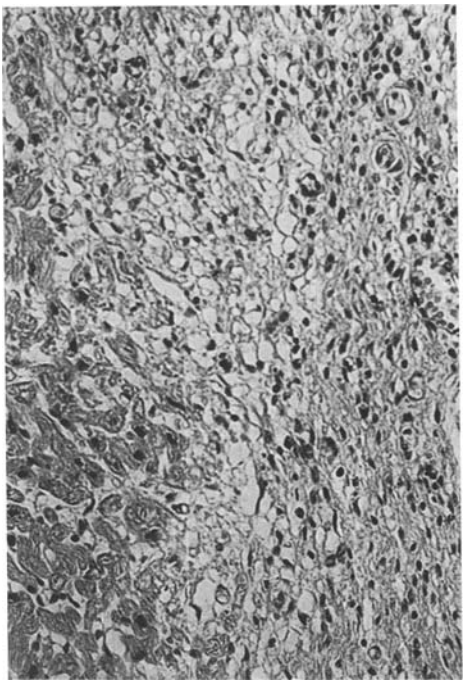

Fig. 8. Dense scar early fibrosis and fresh necrosis present simutaneously HE ×175

densation of the nuclei was also evident. Consequently, all the myo-elements disappeared and left only empty spaces outlined by the interstitial substances, and were finally replaced by fibrous tissue. It was obvious in some specimens that myocytolysis was expanding at the periphery while fibrosis was taking place in the center of a necrotic focus.

Contraction band necrosis provided a particular appearance. Instead of the regular striped appearance, coarse granules and transverse bands developed in the sarcoplasm of the affected fibers and it was associated with karyopyknosis and obscure sarcolemma (Fig. 7). Macrophages may follow to invade the damage fibers for removing the debris.

c) Lesions in various pathological phases. In the majority of cases of Keshan disease, the heart lesions seemed to be the result of an accumulation of damages reulted from multiple episodes. It was not rare to see that an old scar, early fibrosis and myocytolysis ran parallel in one section (Fig. 8). Simultaneous existance of lesions of different pathological phases has been regarded as an important characteristic for the diagnosis of Keshan disease. Some acute cases, however, may merely present irregular patches of contraction band necrosis. On the other hand, dense scar may be predominant, even exclusive in some chronic cases.

d) Inflamatory cell infiltration. Various kinds of inflamatory cells were mainly found within or around the necrotic foci, of these lymphocytes take

the principal part. Foci of lymphocytes were infrequently demonstrated in the endocardium or epicardium. A great number of macrophages may appear following acute myocardial necrosis. Eosinophilia may be prominent in rare cases.

Changes in other organs

Slight changes in skeletal muscle were revealed in the diaphragm of a small number of cases. They usually consisted of degeneration of a few muscle fibers. Inflamation may be evident around the lesions.

Varying degrees of hepatic congestion and fatty change were observed in most cases. Hepatic necrosis located at the inner zone of the lobuli was also revealed in certain advanced cases.

Mesenteric lymphadenosis was not rare in young victims. Enlargement of the germinal center of the cortical follicles, karyorrhexis and phagocytosis were the common findings.

Ultrastructural observations

Ultrastructural observations on Keshan disease were started in the last decade and limited material has been published. The description here is based on the examination in our laboratory, of 12 postmortem cases collected from Sichuan province, southern China. All cases were children of two to nine years old, of them two were diagnosed clinically as acute, nine as subacute and one as chronic types.

Myocardial tissue blocks were obtained from atria, ventricles, ventricular septa and papillary muscles of each case, and autopsy and sampling were performed within six hours following death. Specimens were processed as routine, sectioned with a LKB ultratomy and examined with a JEM electron microscope. The architecture of myocardiocytes was good enough for morphological evaluation though there were pictures indicating a slight autolysis.

Mitochondrial changes

Many organelles showed remarkable morphological changes, however, mitochondria appeared to be the most commonly and conspicuously affected. They were involved early and different features could be revealed in damaged fibers.

The affected mitochondria showed marked dilatation with rather clear matrix. They usually contained only a few crista or only some degenerated segments. Later, mitochondria may lose all their contents and appeared as inflated balloons outlined by the remaining outer membrane. The balloons may pack tight between the myofibrils and result in myofibrillar deformation. They may also break and merge into perinuclear space and contribute to myocytolysis described in conventional pathology. This change was obvious in all subacute cases.

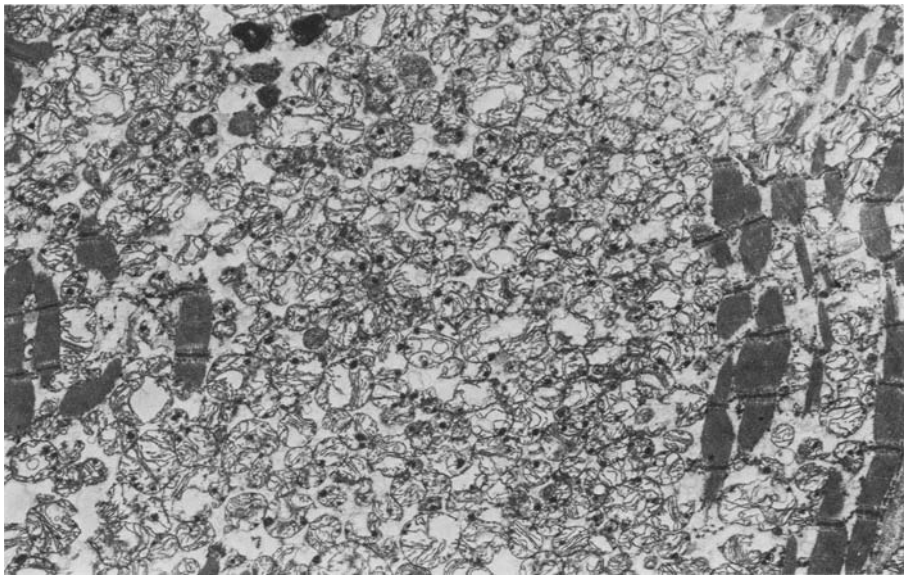

Fig. 9. Proliferation of mitochondria × 5,000

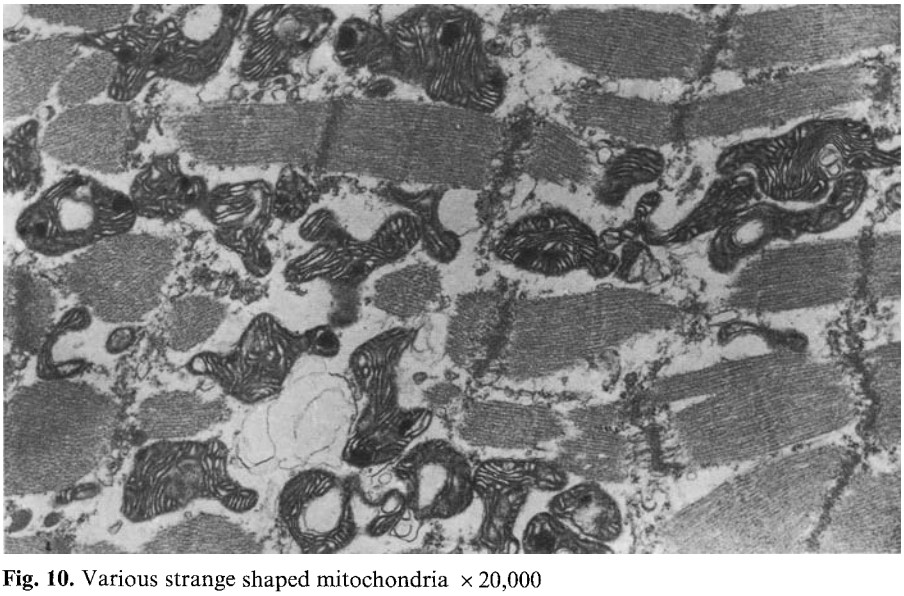

Fig. 10. Various strange shaped mitochondria × 20,000

Great numbers of mitochondria could be seen concentrating in the myoplasm and the myofibrils appeared fewer and thinner (Fig. 9). This change was observed mainly in advanced cases and believed to be a myocardial reaction to hypoxia.

Queer looking mitochondria were seen with various shapes in seven

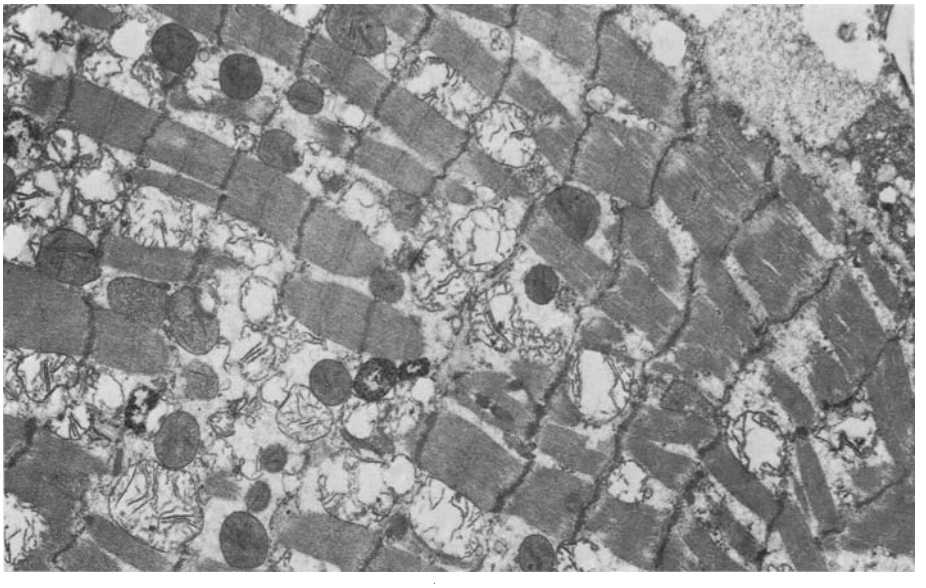

Fig. 11. Two kinds of mitochondria located closely × 10,000

of the 12 cases. They consisted of dense crista and very clear matrix, and always connected with some degenerative membranous remains (Fig. 10). This may suggest a re-organization of mitochondria after their partial destruction and the pale debris may represent the destroyed parts of the predecessors.

Two kinds of mitochondria with different characteristics were found in one myofiber. They often located closely together but appeared distinct from each other. One was larger, translucent with fewer crista, another was smaller, denser with rather concentrated crista. It was obvious in five of the 12 cases (Fig. 11).

Tri-membrane cristae were also present of dark and stiff appearence, seen in swollen mitochondria in all cases though rare in some. They consisted of three layer membranes, the new one usually occurring at the widened interspace of the cristal membranes. Sometimes, it also involves the peripheral membranes (Fig. 12). This tri-membrane structure can be induced by alterations of the biochemical environment of the experimental animal heart (Somogyi et al. 1973). Presumably, it might be also related to certain metablic disorders in Keshan disease.

In addition, calcification and fatty degeneration of mitochondria were infrequently demonstrated in some specimens.

Myofibrillar changes

In contrast to mitochondria, myofibrillar changes were observed predominantly in acute cases dying during the attack phase, when there were severe circulatory disorders.

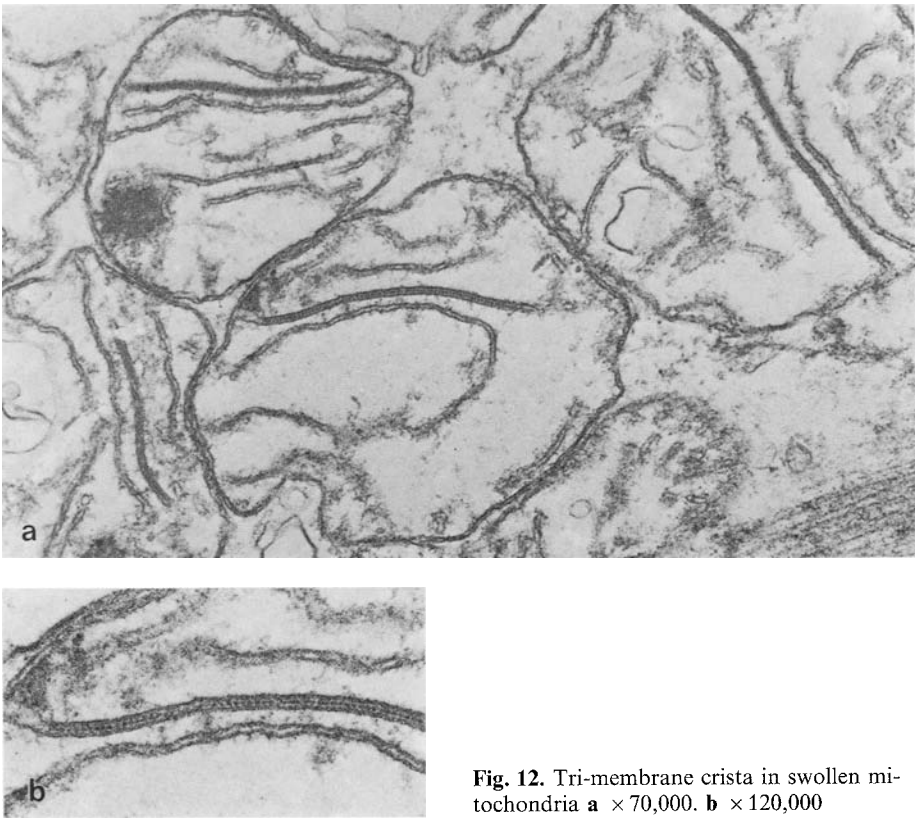

Fig. 12. Tri-membrane crista in swollen mitochondria **a** ×70,000. **b** ×120,000

Dense transverse bands were created by over-contraction of myofibrils. The configuration of sarcomers was totally disturbed and obscure Z band material was irregularly distributed in a big mass of transformed myofilaments. It was often built up nearby but detached from the interculated discs.

In severely damaged fibers the myofibrils were broken into pieces consisting of disorganized sarcomers. Small round mitochondria with a few osmiophilic granules inside, interspersed among the filament masses. Condensed nucleus and broken sarcolemma were present as well.

Changes in the intercalated discs

The intercalated disc displayed remarkable changes in the affected myofibers and the fascia adhaerens seemed to be the most vulnerable part. Increased tortuosity of this part and detachment of I band filaments from it were common sights. It may also separate into two layers and create vacuoles in between, or even break to destroy the partition of adjacent myofibers. Simultaneously, the nexus may became faint and elongated. However, the desmosomes appeared relatively intact, indicating the resistance of this structure.

Fig. 13. Granules related to myofibrils ×40,000

Changes in other organelles

Other organelles also showed abnormalities, such as deformation of the nucleus, dilation of the sarcoplasmic reticulum and increased numbers of lysosomes. These changes were either infrequently found in this group or, in our view, were not of much importance.

Specific granules

Three kinds of granules of unknown properties were demonstrated in some specimens.

(i) Granules related to myofibrils. The granules were round, of simillar density, with a diameter of 50 to 80 nm. As shown in Fig. 13, many of them may pack together in connection with a damaged myofibril, and some of them may condense to form a dense body. Sometimes, several granules were observed to cluster or line up in a split between the myofilaments and may appear not distinct from the adjacent filaments. These granules were occasionally found in six of the 12 cases. It suggests the possibilty that they were derived from the degeneration of myofilaments under certain conditions.

(ii) Granules related to mitochondria. Multiple granules were infrequently seen within mitochondria of which the structure was largely destroyed, and were also found occasionally in sarcoplasm near by the destroyed mitochondria. They were about 40 to 80 nm in diameter. Many of them appeared round, dense with a rough surface (Fig. 14). Some were irregular with uneven density and may connect to each other by fine threads.

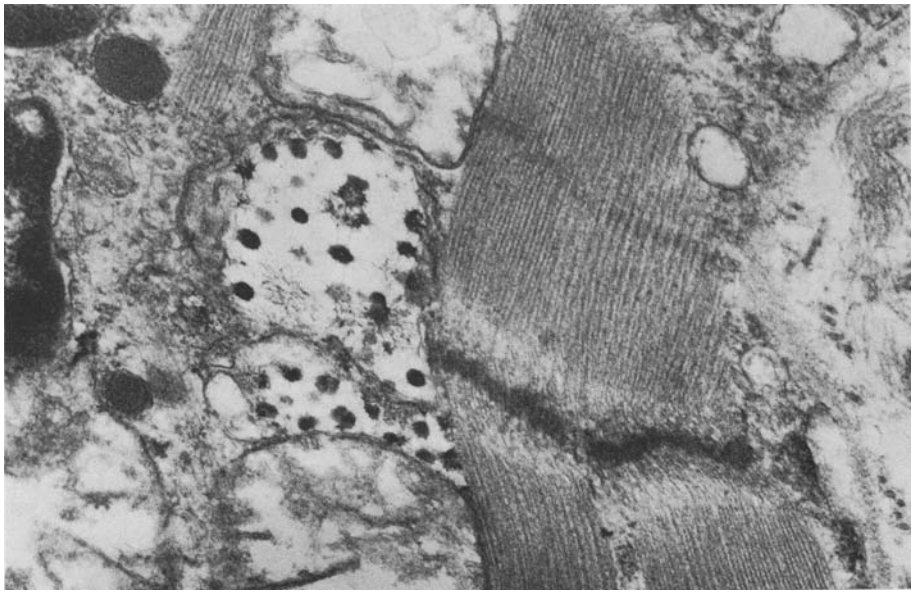

Fig. 14. Granules in destroyed mitochondria × 45,000

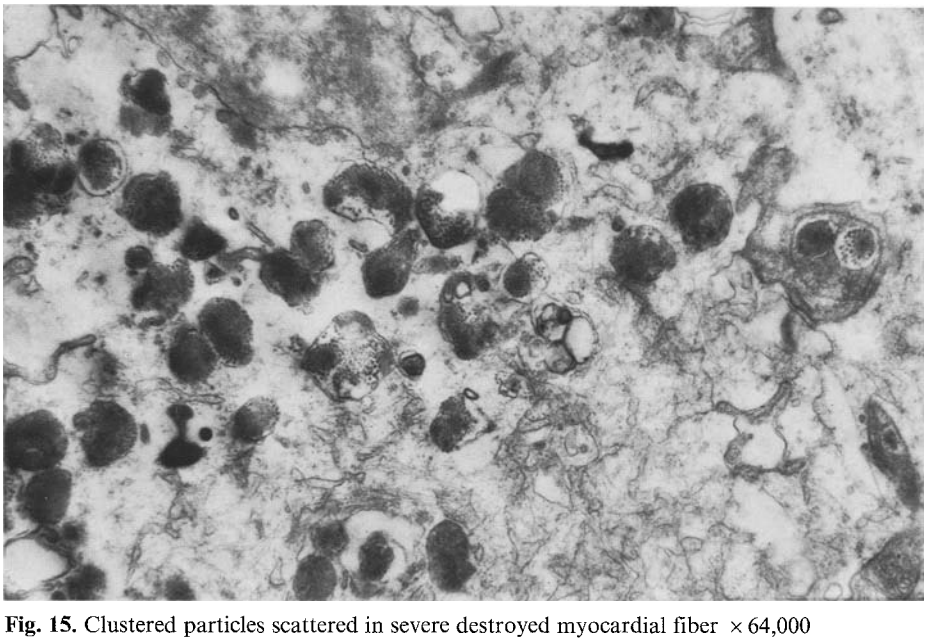

Fig. 15. Clustered particles scattered in severe destroyed myocardial fiber × 64,000

(iii) Clustered particles. Rather specific tiny particles were discovered in nine of 12 cases. They aggregated usually into clusters, but isolated ones were also evident. The configuration of myocardial fibers was subjected to severe disorder wherever these particles were present (Fig. 15). They were uniform in shape and size, about 20 to 27 nm in diameter. They existed

often in association with dense bodies or obscure membranes. Their nature is not at all clear.

Discussion

Keshan disease is the only known selenium responsive human disease. Multifocal myocardial necrosis and fibrous replacement is characteristic, although not specific for this disease. The histopathological picture differes from that of idiopathic congestive cardiomyopathy and that of viral myocarditis (Roberts and Ferrans 1975), but somewhat resembles the lesions of calves suffering from white muscle disease, a livestock disease related to selenium deficiency (unpublished). An occidental case of cardiomyopathy was reported by Johnson et al. (1981). The patient had experienced a long period of parenteral nutrition without selenium administration and his heart lesions resembled those of Keshan disease.

It is generally accepted that selenium deficiency alone may not induce severe heart damage in experimental animals. Presumably, in addition to selenium deficiency, other factors are required for the pathogenesis of Keshan disease. Slight depression of serum vitamin E concentration was revealed in endemic inhabitants, but there was no difference between that of patients and healthy individuals living in endemic areas (unpublished). Experimental viral infection with a coxsackie B4 strain which was isolated from blood sample of a subacute case, induced many more myocardial lesions in selenium deficient mice than it did in the selenium adequate ones (Bai et al. 1980). Many other factors are also being investigated.

Two types of myocardial necrosis were described in Keshan disease. Contraction band necrosis was obvious only in acute cases and was identified by myofibrilar degeneration (Reichenbach and Benditt 1968) through a cytolocalization procedure (Ge et al. 1980). Similar changes could be induced by temporary block of the coronary circulation (Buja et al. 1971; Ashraf and Sybers 1975). Therefore, it may be the consequence of circulatory disorders in Keshan disease.

Myocytolysis seems to be initiated by mitochondrial damage and results in the final disappearance of the myofibers. The multiple mitochondrial appearances may illustrate the reaction or compensatory response of this organelle to injury by pathogenic factors. It exists generally in the majority and is regarded as a representative lesion of Keshan disease.

The specific granules reported in this paper, to our knowledge, have not been seen in other cardiomyopathy or myocarditis. The two larger ones are believed to be derivatives of degenerated myofibrils and mitochondria respectively. The cluster of tiny particles is still a puzzle to us. It is unlikely a transformed proper components of myocardiocyte. Jezeguelet and Steiner (1966) demosntrated that coxsackie viruses may appear as clusters of round, dense particles during their duplication or when they were poorly developed. Although the particles do not resemble an ordinary virus, the possibility of these being atypical viral particles could not be ruled out.

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