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Cytochrome-c-Oxidase Deficient Cardiomyocytes in the Human Heart —An Age-Related Phenomenon

A Histochemical Ultracytochemical Study

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Cytochrome-c-oxidase, the terminal enzyme of the respiratory chain, was studied in 140 hearts from men obtained at autopsy revealing randomly distributed cardiomyocytes without enzyme activity. The expression of the defect was independent of an underlying heart disease and was observed both in normal hearts and in hearts with hypertrophy and/or coronary arteriosclerosis. In contrast, age was a discriminating factor: The defects occurred sporadically in the second decade, but were regularly present from the sixth decade on. Also, the number of defects/sq cm (defect density) increased with age from 2 to 3 in the second and third decade, to about 50 defects in advanced age. Irrespective of the defect density, the enzyme defect always affected isolated cardiomyocytes and ended abruptly at the intercalated disc of neighboring heart muscle cells, as revealed by ultracytochemistry. The results indicate that cytochrome-c-oxidase deficient heart muscle cells represent a degenerative lesion associated with cellular ageing and may be involved in the reduction of myocardial contractile ability in senescence. (Am J Pathol 1989, 134:1167–1173)

Cytochrome-c-oxidase is the terminal enzyme of the respiratory chain. It is essential for respiratory function because it irreversibly transfers electrons of the chain to molecular oxygen.[1]

In recent years, since the report of van Biervliet et al.,[2] it has become apparent that deficiency of cytochrome-c-oxidase is one of the most common defects of the respiratory chain and is associated with various benign and fatal disorders.[3–7] Especially chronic progressive external ophthalmoplegia (CPEO), a special entity of mitochondrial myopathies,[8–10] is associated with benign cytochrome-c-oxidase deficiency, affecting a relatively minor portion of skeletal muscle fibres.[11–20]

We recently demonstrated similar defects in the heart muscle of a patient with Kearns-Sayre syndrome,[17] a special variant of CPEO with an early onset characterized by, among other features, paralysis of the external ocular muscle, progressive proximal myopathy, pigmentary degeneration of the retina, and cardiac disturbances, especially of the conductive system.

The pathogenesis of cytochrome-c-oxidase deficiency and especially of the focal defects in CPEO, however, is still poorly understood. Keeping in mind that disturbances of mitochondrial function may be an important factor of cellular aging in senescence,[21–23] one attractive hypothesis could be that cytochrome-c-oxidase deficiency in CPEO is also related to cellular aging. This assumption is corroborated in the present study, in which an age-associated occurrence of cytochrome-c-oxidase deficient cardiomyocytes is described in both normal and diseased hearts. Cytochrome-c-oxidase deficiency in Kearns-Sayre syndrome and in CPEO might therefore develop in the course of accelerated cell aging.

Material and Methods

The study group consisted of 140 men up to 97 years of age who died before or shortly after hospitalization. Patients suffering from malignant disease or receiving chemotherapy are not included. In 80% of the cases sudden death occurred either by central nervous dysregulation (52%) due to traumatic brain damage caused by accidents, subdural hematoma, sinus venous thrombosis,

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Table 1. *Hearts with Cytochrome-c-Oxidase Deficient Cardiomyocytes: Age Dependency of Defect Expression*

Age (m)	0–7	14–20	21–30	31–40	41–50	51–60	61–70	71–80	81–97	Σ
Total	0/7	5/10	13/18	14/17	29/31	14/14	6/6	16/16	21/21	118/140
Normal hearts	0/7	5/10	10/14	11/13	8/9	5/5	3/3	6/6	2/2	50/69
Hypertrophy	–	–	3/4	3/4	9/10	3/3	1/1	5/5	–	24/27
Coronary sclerosis	–	–	–	–	4/4	3/3	–	3/3	9/9	19/19
Hypertrophy coronary sclerosis	–	–	–	–	8/8	3/3	2/2	2/2	10/10	25/25

and infarction, or by cardiovascular failure (28%) due to coronary heart disease, pulmonary embolism, or acute blood loss. In the remaining cases, causes of death were bronchopneumonia, cerebral palsy, drowning, and intoxications, generally in combination with cardiovascular disease. Autopsy specimens from the left heart ventricle were deep frozen in liquid nitrogen for enzyme histochemical studies between 5 and 96 hours after death (generally between 15 and 30 hours).

Cytochrome-c-oxidase activity was determined histochemically on frozen sections, as described previously,[24] without the addition of hydrogen peroxide. From every heart, one section (minimum area, 0.8 sq cm; maximum area, 2 sq cm; average area, 1.3 sq cm) was studied for the presence of enzyme deficient cardiomyocytes (×25). To quantify the results, the number of defects/sq cm (defect density) was determined in defined rectangular areas. For statistical analysis, the test of Kruskal and Wallis was applied (with the support of Prof. D. Hölzel, Institut für medizinische Informationsverarbeitung, Statistik und Biomathematik, Universität München). In addition, in five selected cases, cytochrome-c-oxidase was studied at the electron microscopical level. The histochemical activity of succinate dehydrogenase was determined according to Lojda et al.[25]

Results

Sixty-nine hearts revealed no pathomorphologic alterations. In 27 hearts, heart hypertrophy without coronary arteriosclerosis was present (heart weight up to 510 g). Nineteen hearts showed coronary arteriosclerosis without heart hypertrophy (heart weights below 350 g for men, 330 g for women) and 25 hearts exhibited both heart hypertrophy and coronary arteriosclerosis (Table 1).

In 118 of the 140 heart specimens, defects of cytochrome-c-oxidase activity were detected (Table 1); the defects being present in 50 of 69 normal hearts and in 68 of 71 hearts with pathomorphologic alterations. In the second decade of life, the defects occurred sporadically. From the sixth decade on, however, the defects were regularly observed in every case.

Similarly, as the number of hearts with enzyme defects increased with age, the number of defects/sq cm heart (defect density) also showed an age-dependent increase. The median value of defect density rose from 2 to 3 in the second and third decade up to 50 defects/sq cm in advanced age (Table 2), especially in the seventh decade and later. The increase of defect density was expressed both in hearts with and without pathomorphologic alterations approximately to the same degree.

Statistical analysis by the test of Kruskal and Wallis for ranks variance analysis (H-Test) revealed a highly significant age-dependent increase of defect density (H-value, 34.93). Furthermore, significant results were obtained between the lower age groups (14 to 30, 30 to 50 years) and the advanced age group (51 to 97 years). No significant differences existed between normal hearts and pathologic hearts in the various age groups. There was no correlation between the rate of defect expression and the time elapsed after death. The highest defect density (158/sq cm) occurred in the advanced age group 9 hours post mortem.

Conversely, only 28 defects/sq cm were observed in another case of the same age group despite an elapse of 72 h.p.m. After 4 days of autolysis, 33 defects/sq cm were noted in the heart of a septuagenerian. There was no appreciable difference in the time elapsed in the study group below and the one above 50 years of age, most hearts being studied between 15 and 30 h.p.m. After this period of autolysis the maximal defect density was 8 defects/sq cm in the lower age group and 131 defects/sq cm in the higher age group. Even after 85 h.p.m. the defect density did not rise above 2/sq cm in the lower age group.

Table 2. *Cytochrome-c-Oxidase Deficient Cardiomyocytes: Defect Density**

Age	14–30	31–50	51–97
Total	2.4	4.4	50
	0.4–5.3	1–13.3	2–158
Normal hearts	2.2	5	52
	0.4–5.3	1–12.5	17–133
Hypertrophy	3	4.5	51.3
	0.8–4	2–10	9–66
Coronary sclerosis	–	2.1	52
		1–9.5	8–158
Hypertrophy/ coronary sclerosis	–	4	36
		2.5–13.3	2.6–158

* Defects/sq cm (median, minimum, maximum).

Defects of succinate dehydrogenase were visualized to a far lower degree than those of cytochrome-c-oxidase. In the hearts with coronary arteriosclerosis or heart hypertrophy, the defects occurred most often (maximal defect density 14/sq cm).

Morphology of Cytochrome-c-Oxidase Deficient Heart Muscle Cells

Typically, loss of enzyme activity was always confined to single, randomly distributed cardiomyocytes. (Figure 1A). Occasionally, the defect was expressed in two to three neighboring heart muscle cells (Figure 1B). However, a defect of more than one muscle cell in a row was not observed. In most of the heart muscle cells there was a complete lack of enzyme activity; occasionally, however, some residual activity was present. As shown by ultramicroscopy, the defect always involved all the mitochondria of a cell and ended abruptly at the intercalated discs of the neighboring cardiomyocytes (Figure 2A). The ultrastructure of the affected heart muscle cells (studied in five cases) was inconspicuous, as far as it may be judged from autopsy tissue. No focal loss of myofibrils and no morphologically obvious increased content of mitochondria or of lipofuscin was seen. Occasionally some degree of cellular atrophy was observed (Figure 2B). Neither at electron microscopy nor at light microscopy were there signs of ischemic cell damage. There was no loss of cross-striation and no cellular infiltrate.

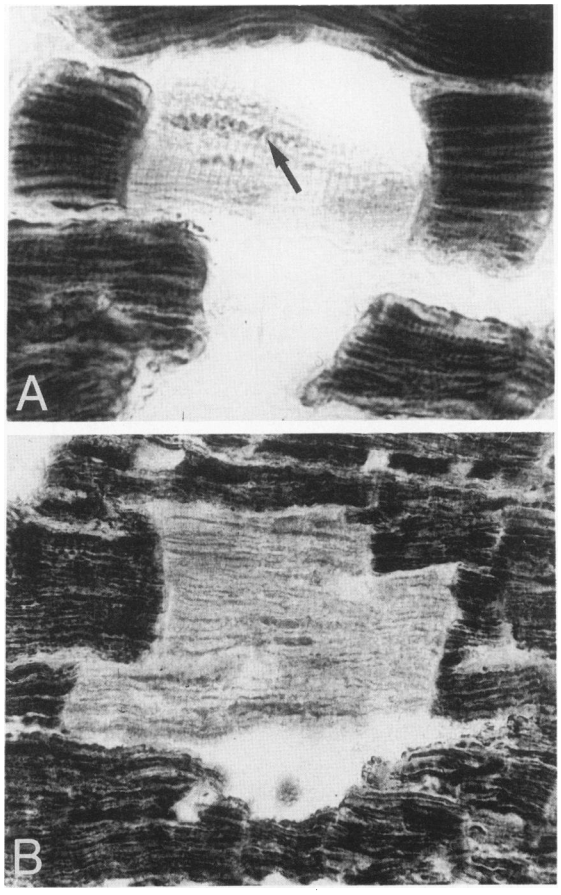

Figure 1. *Cytochrome-c-oxidase, enzyme histochemistry. Normal aged heart (without coronary sclerosis, hypertrophy).* **A:** *An isolated cardiomyocyte lacks enzyme activity (↑ lipofuscin granules).* **B:** *Neighboring cytochrome-c-oxidase-deficient heart muscle cells with some residual enzyme activity (A, ×1600; B, ×960).*

Discussion

Mammalian cytochrome-c-oxidase is a complex enzyme, composed of 13 subunits.[26,27] The larger subunits (I to III) are encoded on the mitochondrial genome and synthesized in the mitochondria itself. They contain the four redox centers (2 hem groups and 2 copper ions) and are essential for the catalytic function of the enzyme. The 10 smaller subunits (IV to VIII) are transcribed from the nucleus and are supposed to exert a regulatory influence on the enzyme function.[1,28]

Deficiency of Cytochrome-c-Oxidase

Deficiency of cytochrome-c-oxidase is a characteristic finding of primary mitochondrial disorders, especially of mitochondrial myopathies and encephalomyopathies, often with severe enzyme deficiency and a fatal outcome in early childhood. In CPEO, a typical mitochondrial myopathy of later onset, focal cytochrome-c-oxidase deficiency in single but not all fibers is now well established.

We recently observed similar defects in the heart of a 26-year-old patient with Kearns-Sayre syndrome, a variant of CPEO.[17] The detection of randomly distributed cytochrome-c-oxidase deficient cardiomyocytes, especially in the heart of elderly men without any signs of a mitochondrial cytopathy, is the significant finding of the present investigation. The expression of the enzyme defect was not dependent on preexisting pathologic alterations of the heart and therefore was present both in structurally normal hearts and in hearts with coronary sclerosis or heart hypertrophy. Furthermore, the defects were not limited to the advanced age groups, but were already present, although to a lesser degree, in younger patients. Therefore, these results are most consistent with an age-dependent process. Neither heart hypertrophy, coronary sclerosis, nor both variables in conjunction had a statistically significant amplification effect on the defect expression rate. Nevertheless, such an effect cannot be ruled out absolutely, more so as the highest defect density (158/sq cm), was noted in the group of coronary arteriosclerosis. With

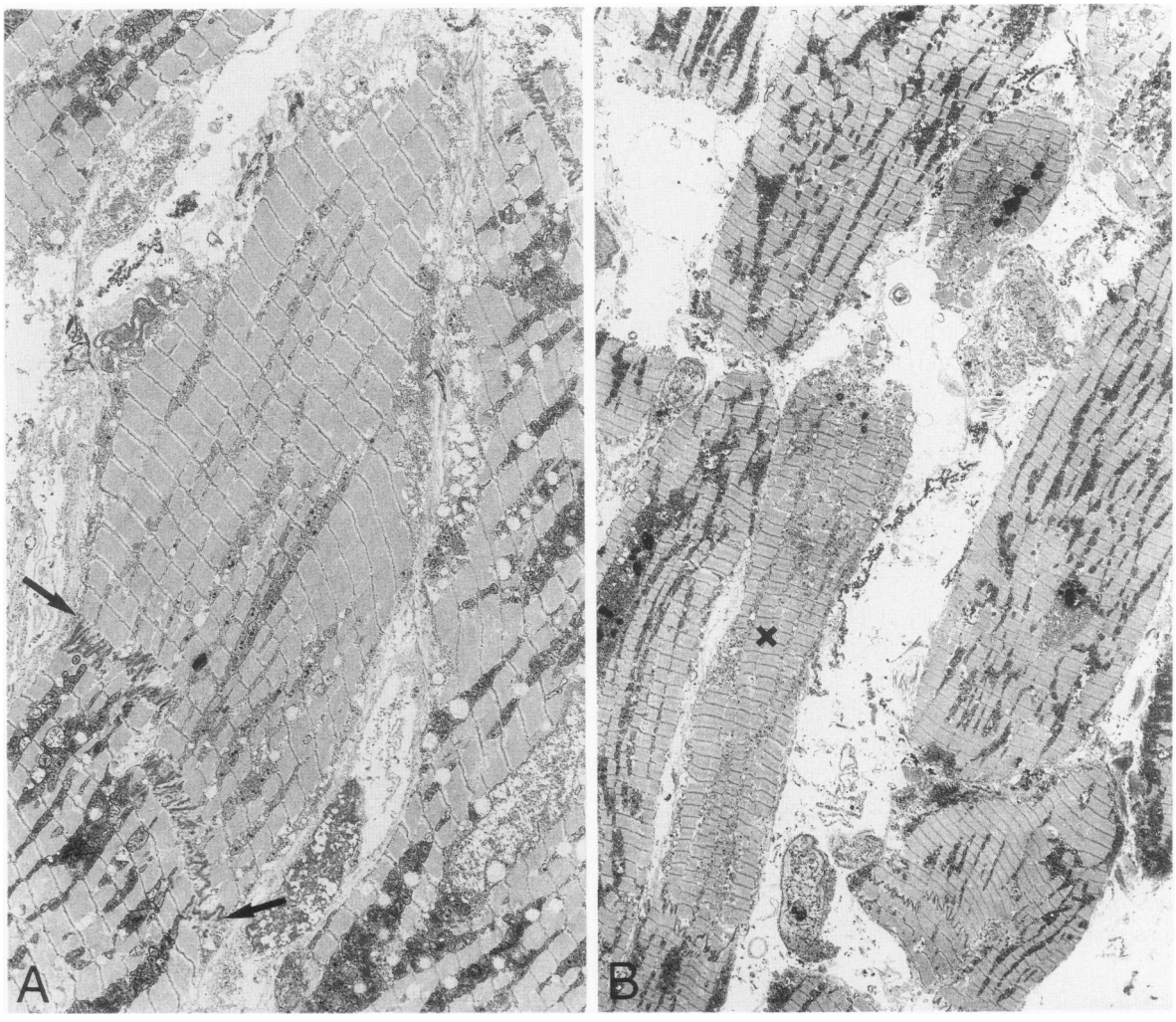

Figure 2. *Cytochrome-c-oxidase, ultracytochemistry.* **A**: *Enzyme-deficient heart muscle cell with inconspicuous ultrastructure. The enzyme defect is strictly limited to one cardiomyocyte ending exactly at the intercalated disc (↑) of the neighboring heart muscle cell.* **B**: *Enzyme deficient cardiomyocyte with cellular atrophy (x) (A, ×2000; B, ×1150).*

respect to the high range of individual values, only a longitudinal study design (which for ethical reasons remains hypothetical) could clarify this point.

The peculiarity of the alteration is illustrated by the strict limitation of the defect to single cardiomyocytes, with the defect always ending abruptly at the intercalated discs. Furthermore, there was no obvious alteration of the ultrastructure of the defective cardiomyocytes with respect to the content of mitochondria, myofibrils or lipofuscin, as far as may be judged from autopsy tissue and without morphometric analysis.

Specificity of the Lesion

With respect to the generally normal structure, nonspecific degenerative influences as in heart hypertrophy or cardiomyopathies[29,30] are of no obvious importance for the manifestation of the enzyme defect. Furthermore, various observations indicate that the described defects of cytochrome-c-oxidase are specific and cannot be attributed to autolytic lesions, which do occur after death.

The results demonstrate that the number of defects do not increase with, and are therefore independent of, the time elapsed since death. This is also illustrated by a case of Kearns-Sayre syndrome in a young man with a mitochondrial cardiomyopathy seen already after 3 h.p.m.[17] and a rather high defect density of 90/sq cm. In the rat heart enzyme, histochemically detectable activity of cytochrome-c-oxidase remains unaltered up to 4 days after death.[31] Biochemical enzyme studies performed in rat liver mitochondria aged *in vitro* for 120 hours reveal only minor losses of the enzyme's activity.[32] Moreover, cytochrome-c-oxidase, along with succinate dehydrogenase, is the most stable complex of the respiratory chain,

showing no early loss of activity in the ischaemic heart.[33,34] In addition, post mortem enzyme defects caused by autolysis are known to occur as patchy defects[31] and not as unicellular lesions. Also, no correlation existed between the cause of death and the rate of defect expression. There were, for example, no statistically significant differences between the defect density in old men with normal hearts dying of noncardiac cause and men dying because of coronary arteriosclerosis.

Alteration of Heart Function and Structure During Aging

Aging of the heart starts in approximately the third decade of life. The aging process involves a decrease of the maximal heart rate and the ejection volume, including the ejection fraction. The heart aging process also involves an increase of the end diastolic filling pressure and volume, an increase of heart wall stiffness (reduced compliance), a prolongation of contraction and relaxation time, a retarded sequestration of calcium in the sarcoplasmic reticulum, a decrease of catecholamine sensitivity and conduction velocity, as well as alterations of the iso-enzyme pattern of myosin ATPase.[35-43]

Morphologically, aging is accompanied by the accumulation of lipofuscin, originating from lipid peroxidation and polymerisation of membrane phospholipids derived from mitochondria, for example. In old animals, a reduced content of ribosomes as well as an increased content of lipids, glycogen, lysosomes, or of autophagocytic vacuoles has been described. Mitochondria may show myeline figures as well as vacuolization of their matrix, and structural alterations of the cristae have been recorded.[44,45] Quantitative data of mitochondria reveal conflicting results.[44] On the whole, however, there appears to be a tendency toward an increased number of mitochondria with a reduced volume, thus leaving the volume fraction/cell of mitochondria rather constant in the aging cardiomyocyte.

Functional alterations of mitochondria, including loss of respiratory control, a decrease of P/O ratio, and a 30% reduction of the activity of adenine nucleotide translocase, are known to occur in aged mitochondria.[46,47] Furthermore, a decrease of cytochrome-c-oxidase activity has been reported in aging hearts of rats[48,49] and in other organs,[50] as well as in cultures of podospora anserina.[51] On the whole, however, conflicting data exist concerning the activity of respiratory enzymes during senescence.[50,52]

Concerning the pathogenesis of the unicellular defects of cytochrome-c-oxidase described here, the random distribution of the deficient heart muscle cells is most consistent with a stochastic process.[53] In this respect above all, free radical injury of membranes, enzyme proteins, and probably of mitochondrial DNA itself (by superoxide and hydroxyl radicals that may be generated in the respiratory chain, for example)[21,22,54-61] are of special interest. The exact mechanism, however, remains to be investigated. Preliminary immunohistochemical data indicate that the loss of enzyme activity is accompanied by a reduced content of enzyme protein.

As for the significance of the defects, they may be associated with age-related loss of cardiac muscle cells[62-65] and the reduction of myocardial contractile ability in senescence. Furthermore, the presence of a rather high degree of such defects in the heart of a young man with a mitochondrial myocardiomyopathy[17] in the course of CPEO might indicate that premature cell aging is of pathogenetic importance in this special disorder.

This hypothesis is substantiated further by the fact that defects, similar to those in CPEO but to a lesser degree, also occur in the skeletal muscle and additionally in the diaphragm of men without mitochondrial or muscular diseases.[66] This situation, therefore, is similar to the presence of senile plaques even in the normal brain and their increased manifestation in age-associated Alzheimer dementia.

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