# ULTRASTRUCTURAL-MORPHOMETRIC STUDIES ON HUMAN HEART WITH ALCOHOLIC CARDIOMYOPATHY AND CHRONIC ISCHEMIC HEART DISEASE IN CASES WITH SUDDEN DEATH

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Sudden death is most commonly caused by alcoholic cardiomyopathy (ACM) and chronic ischemic heart disease. ACM is defined as a chronic heart failure which can be related to longlasting alcohol abuse when there are no any other diseases with a known etiology. Ultrastructural findings of ACM based on autopsy material were first reported by R. G. Hibbs et al. (14) in 1965. The implementation and pathologic-anatomic evaluation of myocardial biopsies in various heart diseases allows the research and differentiation between autolytic and disease-specific ultrastructural changes of heart muscle cells.

The strongly raising alcohol consumption in GDR, in the Soviet Union, in France during the last decades (1, 2 et al.) causes higher morbidity rates of chronic alcoholism (6—10 per mile in the SU) and, especially, of ACM, too. Between 8 and 15 per cent of chronic alcoholism patients die of functional heart disorders, 10 per cent (including a lot of young patients) die suddenly from an acute heart failure. The simultaneously high mortality rate of ischemic heart disease (IHD) in GDR (18,53 per mile) requires further research of pathogenesis of heart damage in these diseases to learn more about the clinics and practice. In this relation the results of experimental investigations which have revealed a slow autolysis of heart muscle cells of middle regions of left ventricle are considered.

The purpose of our present work is:

a) to study heart muscle cells ultrastructure in cases of sudden death (so-called heart death);

b) to summarize these changes divided into ACM and IHD according to the etiology and to express them in size and number; to confirm or exclude the subjective evaluations of changes of number and size of cell organells and intracellular structures;

c) by using statistical comparative methods and electronic data processing to try to prove certain differences between both groups of diseases.

## Material and methods

The study was carried out on myocardium samples from 3 males who had suddenly died and showed the morphologic, resp. histologic picture of ACM and IHD. Anamnestic data demonstrated alcohol consumption at least 80—100 g daily for 5—10 years. Myocardial samples from males who had died in a road accident and (in one case) of fulminant pulmonary thromboembolism were studied as controls. In general, the autopsy was performed 40 till 2 hours after death. The material was taken from the middle parts of the side wall of left ventricle.

Then it was postfixed in 2,5 per cent buffer solution of $OsO_4$ for 1 hour at 4 °C, complete darkness. Dehydration was made in rising alcohol concentrations and propylen oxide followed by embedding in araldehyde. Ultrathin sections were made by using the LKB-Microtome and observations were done on electron microscope JEM-100 B. Micrograms were taken with primary magnification of 10 000 and 27 000 times and then the end magnification reached photographically the value of 20 000, resp. 53 000 times. The estimation of size rates was carried out on a simple quadratic raster with network period of 10 and 9 mm (original size) correspoding to 0,45 and 0,1875 in end magnifitions after the common point and section-point counting method. The calculation of morphometric-stereologic parameters and its statistical analysis was done by using an EDP-programme.

## Results and discussion

### Mitochondria

Mitochondrial volume density is higher in both diseases than that in the controls (see fig. 1). The increase of mitochondrial relative total volume reaches 53 per cent in ACM and 62 per cent in IHD. The differences between patients and
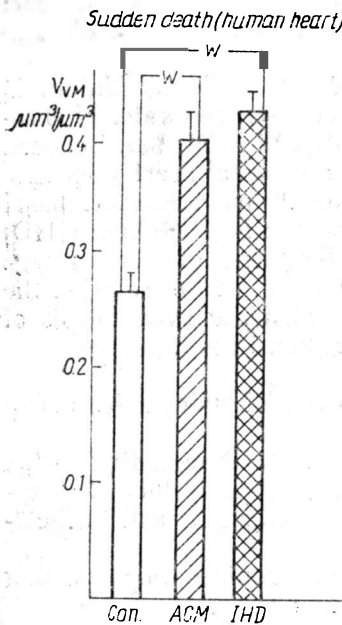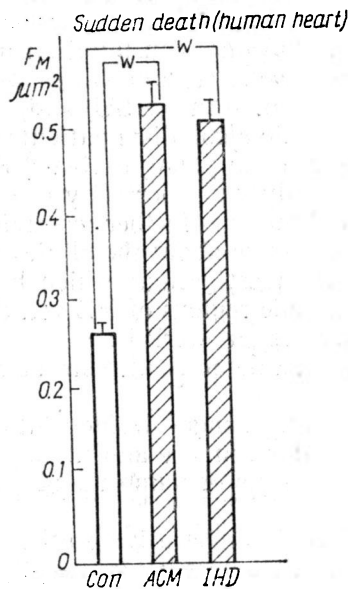


Fig. 1.                    Fig. 2.

controls concerning these parameters were proved statistically after Wilcoxon's test. The mean scratch surface of the mitochondria increases statistically significant with 96 per cent in ACM and with 90 per cent in IHD patients (fig. 2).

The specific surface density of the outer membrane of the mitochondria is with 30 per cent lower in ACM and with 34 per cent lower in IHD patients as compared with that of controls. There are significant differences between the group

of IHD and of controls (fig. 3). Mitochondria count decreases in both patients' groups as estimated per unit surfaces (with 19 per cent for ACM, and 16 per cent for IHD patients — fig. 4). The t-test shows a significant difference between patients and controls.
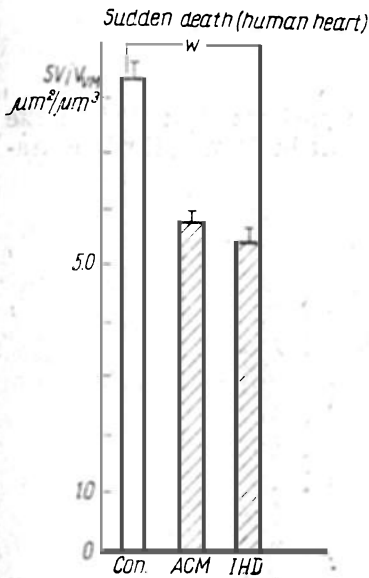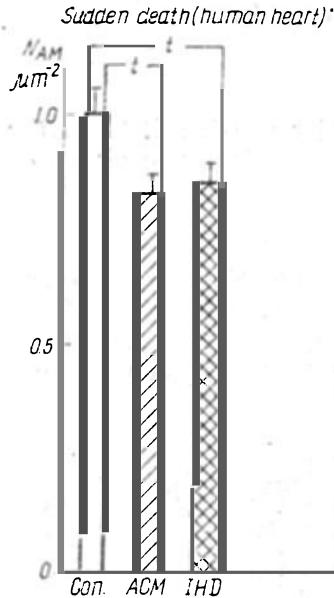


Fig. 3.



Fig. 4.

## Myofibrills

The volume density of the contractile substance demonstrates a decrease with 23 per cent in ACM patients and with 38 per cent in IHD ones in comparison with that of the controls. This difference was statistically significant according to Wilcoxon's test. The volume density of Z-band shows a diminution with 14 per cent in ACM patients and with 33 per cent (significantly — t-test) in IHD ones. The specific surface density of Z-band is higher with 31 per cent in ACM cases and with 15 per cent in IHD ones as compared with the control rates. The mean scratch surface of this structure is smaller with 24 per cent in ACM patients and with 21 per cent in IHD ones when it is compared with that of the controls. By using t-test the difference between mean rates of ACM and control group was found out to be statistically reliable. The same finding is established concerning the mean Z-band volume and the mean scratch surface. Z-band number per unit surface and unit volume of heart muscle cells decreases rather slightly in ACM patients that is, however, statistically reliable in comparison with the control rates. Concerning IHD patients there is a decrease of Z-band number per unit surface with 15 per cent and of number of this structure per unit volume with 12 per cent.

## Mitochondria — myofibrills — relation

The mitochondria-myofibrills ratio is replaced in favour of mitochondria in both groups. It increases with 135 per cent in ACM patients and with 281 per cent in IHD ones which is statistically significant as proved by Wilcoxon's test, when the control rates are considered.

## t - Tubules

The volume density of t-tubuli is smaller in the patients than that in the controls. The decrease of this parameter with 60 per cent in ACM patients is sta-
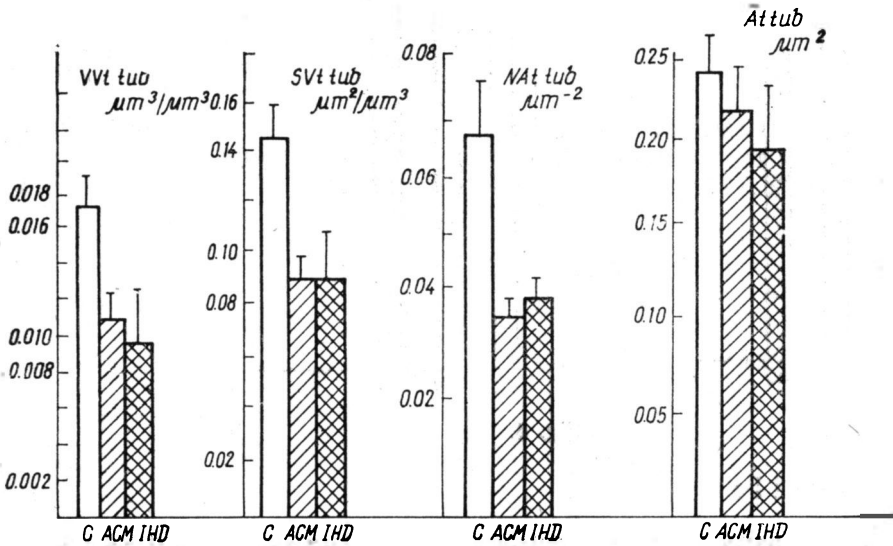


Fig. 5.

tistically reliable (t-test — fig. 5). However, the diminution of relative total volume of t-tubuli with 63 per cent in IHD patients is statistically insignificant. The surface density of t-tubuli is lower with 40 per cent in ACM patients and with 41 per cent in IHD ones as compared with that of the controls (fig. 5). where only the difference between ACM and control rates is statistically reliable (t-test). The number of t-tubuli per unit surface is smaller in patients than in controls (respectively with 50 per cent for ACM and 49 per cent for IHD ones) and it is statistically significant when both patients' groups are considered (t-test and Wilcoxon's test). The mean scratch surface of t-tubuli is smaller with 15 per cent in ACM patients and with 18 per cent in IHD patients in comparison with that of the controls (fig. 5). This difference is also statistically reliable according to Wilcoxon's test.

Sarcoplasmatic reticulum

Heart muscle cells show a decrease of volume density with 26 per cent in ACM patients and with 24 per cent in IHD ones as compared with that of the controls (fig. 6). The number of scratches of the longitudinal system of sarcoplasmatic re-
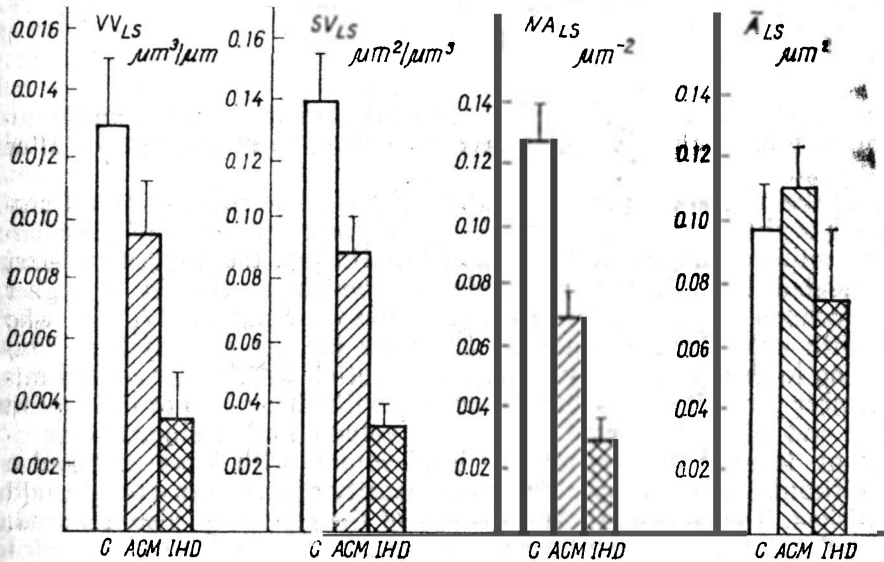


Fig. 6

ticulum per unit surface of heart muscle cell is smaller with 49 per cent in ACM cases and with 81 per cent in IHD ones than that of the control persons (fig. 6). This difference is statistically significant (Wilcoxon's test). The mean scratch surface of sarcoplasmatic reticulum is smaller with 9 per cent in ACM patients and with 21 per cent in IHD ones in comparison with that of the controls (fig. 6). The surface density of sarcoplasmatic reticulum is smaller with 36 per cent in ACM cases and with 76 per cent in IHD ones than that of the controls (fig. 6). The specific surface density of the longitudinal system of sarcoplasmatic reticulum has a higher value concerning ACM patients with 26 per cent and a lower one with IHD ones with 18 per cent as compared with that of the controls. There is a statistically significant difference between IHD and control group (Wilcoxon's test).

Lysosomes

The volume density is higher with 191 per cent in ACM patients and with 387 per cent in IHD ones in comparison with that of the controls. Lysosome number per unit surface is higher with 51 per cent in ACM cases and with 36 per cent in IHD ones than that of the controls. The number density of lysosomes is higher with 29 per cent in ACM patients but lower with 18 per cent in IHD ones than that of the controls. The mean scratch surface of lysosomes is higher with 165 per cent in ACM cases and with 301 per cent in IHD ones as compared with that of the controls. Therefore, the mean lysosome volume is higher with 283 per cent

in ACM patients and with 618 per cent in IHD ones than that of the controls. The surface density of lysosomes is higher with 126 per cent in ACM patients and with 201 per cent in IHD ones than that of the controls. The specific surface density of lysosomes is higher with 26 per cent in ACM patients but lower with 18 per cent in IHD ones than that of the controls. There is a statistically significant difference between IHD and control group (Wilcoxon's test).

Fatty  contents

All the parameters of intracellular fatty contents, volume, surface and number density of fatty drops show a higher fatty contents of heart muscle cells after alcohol exposition.

Concerning the alcoholic cardiomyopathy the results of ultrastructural studies on cardiomyocytes demonstrate some changes of the composition of the chondrioma. Single mitochondria are clearly enlarged although the number of heart muscle cells related to unit volume diminishes which results in a higher relative total volume rates. Similar data are already reported (17, 26), who experienced with rats given ethanol for 33 weeks (33 per cent of food contents). According to these authors there was a diminution with 45 per cent of mitochondria number per unit surface but an increase with 48 per cent of their mean scratch surface and with 80 per cent — of single volume while a slightly expressed insignificant increase of their relative total volume with 5 per cent and a slight but significant decrease of specific surface density of cristae (p <0,05) could be also established. The specific surface density of the cristae is also reduced. In these authors' opinion these results could be explained with the lack of mitochondrial biogenesis due to selectively suppressed mitochondrial protein synthesis. This hypothesis is confirmed by other authors (8) who induced by chloramphenicol, a liver mitochondrial protein synthesis inhibitor, megamitochondria in rats. I. P. Burke et al. (12) proved that alcohol and acetaldehyde inhibits mitochondrial protein synthesis as it was previously reported for acetaldehyde only (23, 24). The total protein content of the heart diminishes after chronic alcohol exposition (19). C. S. Alexander et al. (7) described the presence of enlarged and giant mitochondria (11—13 μ m or 5—6 sarcomere lenghts). In their opinion these mitochondria originate by mitochondrial fusion. By using semiquantitative methods the authors established an increase of mitochondria number which is opposed to our own results presented. However, they used mice in their experiments given ethanol for 15 and 25 weeks (36 per cent of given food calories). C. S. Alexander (6) established a mitochondrial increase in both size and number on biopsy material from humans chronically exposed to ethanol. Concerning ACM similar ultrastructural findings were also reported (11, 14, 18). However, there are data available that there is rarefaction and oedema of mitochondria combined with loss of cristae after chronic alcohol exposition (3, 9, 10, 13, 15, 26). It can be concluded that there is a functional change of the chondrioma such as diminution of energy consumption, limitation of respiratory function and calcium consumption and binding capacity both. The respiratory control index (RCI) which is a measure of coupling between oxidation and phosphorylation (16) as well as mitochondrial oxygen consumption is decreased (26).

The changes of the contractile substance of cardiomyocytes can be an expression of beginning myocardial hypertrophy or of selective lesion of the contractile substance itself by the toxic influence of alcohol, resp. of its metabolite acetaldehyde. Some authors (3, 9, 22) found out a destruction of contractile elements

of cardiomyocytes after chronic ethanol exposition. An other explanation of these finding could be the ischemic lesion of the contractile substance due to microcirculatory disorders of myocardium. They appear as results of capillary changes (diminution of relative total volume and increase of specific surface density, i. e. reduction in size — 17) and of small arteries through a perivascular oedema. On the other hand, oxygen needs and consumption of cardiomyocytes increases, by means of catecholamine emptying due to chronic alcoholism which results in creatin phosphate and ATP diminution (3) and, respectively, in an ischemic situation for myocardium in the begin of anaerobic glycolysis. The loss of potassium ions (20) can cause a hypokaliemia and by this an additional reason for damage of the contractile substance after chronic ethanol exposition. The relative total volume of the intramyofibrillar space (mitochondria, sarcoplasmatic reticulum, lipids and sarcoplasma, without t-tubuli) is totally low-gradually with 15 per cent enhanced. There is an increase of the distance between myofibrills in chronically alcohol exposed patients (6). t-tubuli diminution in size and number could be due to a hypertrophy of cardiomyocytes in ACM patients. L. Mall et al. (18) established an unchanged cell size after chronic alcohol addition in rat hearts while C. S. Alexander (6) found out a myocardial hypertrophy in heart biopsies in patients with chronic alcohol exposition. This discrepancy could be explained with the focus-like character of these changes.

Concerning the sarcoplasmatic reticulum our findings are rather surprizing, indeed. A rare appearance of these cell organells in cardiomyocytes after chronic ethanol exposition was also established by C. S. Alexander (6) in human biopsy material. Contrary to our results, a vacuolization and oedema, i. e. an enlargement of the sarcoplasmatic reticulum is reported by many authors (3, 6, 9). However, our results correlate better with these showing a diminution of speed and capacity of calcium binding and consumption both (9, 10, 21, 25) as well as of contractility (5), respectively of contractility reserve after angiotensin administration of the myocardium (20). Therefore, they are a better morphologic equivalent for diminution of the capacity of calcium transporting systems and of the myocardial contractility. It is evident that they can be considered to be a subcellular basis of ACM.

Concerning lysosomes similar findings were reported by other authors (9, 22). However, C. S. Alexander (6) established a diminished number of lysosomes in heart biopsies of patients with chronic alcohol exposition. Concerning the intracellular fatty contents the results are rather synonymous. There is a distinct increase of the number, size, and relative total volume of fatty drops similar to the findings reported by other authors (6, 9). T. J. Regan et al. (20) found out a decreased consumption of free fatty acids as well as an enhance of myocardial extraction triglycerides.

It can be generally concluded that the results from the ultrastructural-morphometric studies reveal similar changes of cardiomyocyte ultrastructure in both diseases.

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## УЛЬТРАСТРУКТУРНЫЕ И МОРФОМЕТРИЧЕСКИЕ ИССЛЕДОВАНИЯ СЕРДЕЦ ЛЮДЕЙ С АЛКОГОЛЬНОЙ КАРДИОМИОПАТИЕЙ И ХРОНИЧЕСКОЙ ИШЕМИЧЕСКОЙ БОЛЕЗНЬЮ В СЛУЧАЯХ ВНЕЗАПНОЙ СМЕРТИ

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Р Е З Ю М Е

Проведено исследование патологоанатомического материала внезапно умерших с катамнестической и морфологической картиной алкогольной кардиомиопатии и хронической ишемической болезнью сердца. Результаты ультраструктурных и морфометрических исследований показывают, что при алкогольной кардиомиопатии обнаруживаются четкие изменения хондриомита: уменьшение числа гребней, нарастание отдельных митохондрий и их относительного объема в миоцитах; в контрактильной субстанции — уменьшение числа и величины z-полос; в t-тубулах — уменьшение числа черт, их величины и объема; в саркоплазматической сетке — уменьшение числа черт и поверхностной и объемной плотности. При этих заболеваниях устанавливаются одинаковые ультраструктурные изменения миоцитов сердца. Некоторые различия морфометрического характера связаны с гипертрофией сердечной мышцы, с нарастанием миоцитов при ишемической болезни сердца и с увеличиванием количества жиров при алкогольной кардиомиопатии.