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Ultrastructural Morphometric Study on the Rat Heart After Chronic Ethanol Feeding*

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Summary. Male Wistar rats were fed with ethanol for 33 weeks. Ethanol was administered in a liquid diet containing 33% of the calories as ethanol. In the control group ethanol was isocalorically replaced by glucose. Light and electron microscopic investigation of the hearts did not indicate significant structural abnormalities, in contrast to the results of some other groups. Nevertheless, morphometric analysis revealed considerable quantitative changes. The number of mitochondria was remarkably reduced and the volume of an average single mitochondrion nearly doubled, whereas the volume fractions of mitochondria and myofibrils were unaltered. The analysis of the mitochondrial subcompartments indicated a slight decrease of the surface area of inner mitochondrial membranes per unit volume of mitochondria and a slight increase of the volume fraction of the mitochondrial matrix space. Myocardial cell hypertrophy or atrophy were not observed. We believe that the mitochondrial changes are the expression of an impaired biogenesis of these organelles.

The increased number of capillaries in the ethanol-fed group is possibly caused by functional ethanol effects which may be partly similar to effects of chronic hypoxia.

Ethanol- or acetaldehyde-induced damage of heart mitochondria may play an important role in the pathogenesis of alcoholic cardiomyopathy.

Key words: Ethanol – Myocardium – Ultrastructure – Morphometry – Mitochondria.

Introduction

Alcoholic cardiomyopathy is a definite clinical entity (Bing 1978). The reversibility of the disease on alcohol withdrawal indicates that alcohol is involved

* Dedicated to Prof. Dr. G. Ule on the occasion of his 60th birthday

** More detailed discussion in T. Mattfeldt: Inauguraldissertation (in prep.)

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in the aetiology of this condition (Bing 1978; Schwartz et al. 1975; Demakis et al. 1974). The clinical, haemodynamic, and morphological findings in alcoholic cardiomyopathy are similar to those in "idiopathic" congestive cardiomyopathy.

The reasonable hypothesis that congestive cardiomyopathy occurs in predisposed persons who are subject to additional risks like chronic alcoholism has been discussed by several authors (Kuhn and Loogen 1978; Kübler et al. 1976). The results of experimental investigations are compatible with the latter assumption: chronic ethanol ingestion alone does not produce congestive heart failure (Fahimi et al. 1979). However, many physiological and biochemical effects of ethanol on the hearts of animals can be demonstrated experimentally including loss of contractility (Newman and Valicenti 1971; Ahmed et al. 1973; Wong 1973; Horwitz and Atkins 1974; Hirota et al. 1976; Child et al. 1979), changes in mitochondrial respiration and mitochondrial enzymes (Gvozdiák et al. 1976; Sarma et al. 1976; Alexander et al. 1977; Williams and Li 1977; Segel and Mason 1979), myocardial lipid metabolism (Regan et al. 1974; Alexander et al. 1977), protein synthesis (Schreiber et al. 1972; Schreiber 1974; Rawat 1979) and calcium transport (Bing et al. 1974; Retig et al. 1977).

Myocardial ultrastructure has been investigated in acute and chronic experimental ethanol ingestion and in alcoholic cardiomyopathy in man (Doerr and Rossner 1977); the effects of chronic ethanol feeding are controversial, however. Some investigators found alterations of mitochondria, sarcoplasmic reticulum and/or intercalated discs (Sohal and Burch 1968; Burch et al. 1971; Alexander et al. 1977), but Hall and Rowlands (1970) found nearly normal ultrastructure. Fahimi and coworkers (1979) described an increased number of microbodies in the rat heart, but additional structural alterations were absent, above all, the mitochondria appeared normal. The discrepancies between the results of different authors are probably caused by different experimental conditions.

By using a balanced, vitamin- and protein-rich diet, we tried to exclude disturbing factors such as malnutrition and avitaminosis which may play an important role in human alcoholic cardiomyopathy (Weiss and Wilkins 1937; Eliaser and Giansiracusa 1956). Fixation artifacts which could resemble pathological changes were hopefully prevented by vascular perfusion with the fixative.

Using light and electron microscopic morphometric methods we hoped to obtain quantitative structural data, which might elucidate the effects of ethanol on the heart and contribute to our understanding of the pathogenesis of alcoholic cardiomyopathy.

Materials and Methods

40 male Wistar rats (initial body weight: 220 g) were randomly divided into 2 groups of 20 animals each; one group was fed with ethanol, the other served as control. Both groups were fed with a liquid diet containing Stardit^R and ethanol or glucose, respectively. Stardit^R contains 68% of the calories as carbohydrates, 24% as proteins and 8% as lipids supplemented by vitamins and minerals. The ethanol doses were increased weekly, until after a period of 11 weeks a final level of 33% ethanol in the total caloric intake was attained. This dose was given over a period of 22 weeks, whereas in the control group ethanol was isocalorically replaced by glucose. In our experience the Wistar rat does not tolerate a higher rate of ethanol feeding without severe dystrophy.

The diet is similar to the liquid diet proposed by Lieber et al. (1965) for chronic ethanol feeding studies. The caloric proportion of ethanol in this diet is comparable to the amounts of ethanol consumed by chronic alcoholics (Neville et al. 1968). The daily caloric intake was monitored in both groups. All rats were caged individually. Body weights were determined weekly.

The viscera were fixed by retrograde vascular perfusion after catheterization of the abdominal aorta, as described previously (Mall et al. 1978). The vascular system was flushed with a Dextran 40 solution (Rheomacrodex®). and cacodylate buffer containing 3% glutaraldehyde was used for fixation. Slices of myocardial tissue were postfixed in OsO_4, dehydrated in ethanol and embedded in Araldite.

The left ventricular posterior papillary muscles were cut at an angle of 32.4° to the longitudinal axis for morphometric investigations. The anterior papillary muscles were dissected longitudinally and transversely. Furthermore, 5 blocks from the left chamber wall and 3 from the right were randomly selected. In addition to the hearts, the livers and the brains were prepared for microscopic investigations.

Morphometry

The morphometric procedure was modified in some respects when compared with the earlier reports from our laboratory (Mall et al. 1978; Mall et al. 1980).

At *stage 1* magnification we use now ultrathin silver-stained sections (Fig. 1) examined by light microscopy at a magnification of 1,050:1 rather than the electron micrographs at a magnification of 1,300:1 used previously. The following variables were measured at this stage: Volume densities of myocardial cells, interstitial space and capillaries and surface densities of myocardial cells and capillaries. These surfaces can be assumed to be oriented parallel to the longitudinal axis of the muscles. In the cross sections which we used the formula for calculating the surfaces $S_V = B_A$ is applied, where S_V respects the surface density and B_A the profile border length of section profiles per test area (Sitte 1967). B_A is estimated by well established morphometric methods (cf. Weibel 1969). One silver stained section per animal and two test areas per section were evaluated with a ZEISS – eyepiece containing a morphometric test grid with 100 test points and 10 test lines. The method now preferred is advantageous because light microscopy is not so time-consuming as electron microscopy.

At *stage 2*, electron microscopic magnification 6,200:1, the volume densities of mitochondria, myofibrils and sarcoplasmic matrix and the surface density of mitochondrial envelopes were measured. The sections cut at an angle of 32.4° to the longitudinal axis of papillary muscles were evaluated. This angle has been demonstrated to be optimal in the case of anisotropic surfaces, if the degree of anisotropy is not exactly known, as is the case with heart mitochondria (Mall et al. 1978).

The cristae mitochondriales were investigated on positive prints of stage two negatives (final magnification: 30,000:1), the values were corrected for the systematic error resulting from the loss of obliquely cut membranes (Mall et al. 1977; Mall et al. 1978).

Moreover, in the present investigation we analyzed mitochondrial subcompartments according to the method described by Smith and Page (1976): the volume of intercristal space per unit mitochondrial volume and the volume of mitochondrial matrix and cristae per unit mitochondrial volume was determined at a final magnification of 200,000:1.

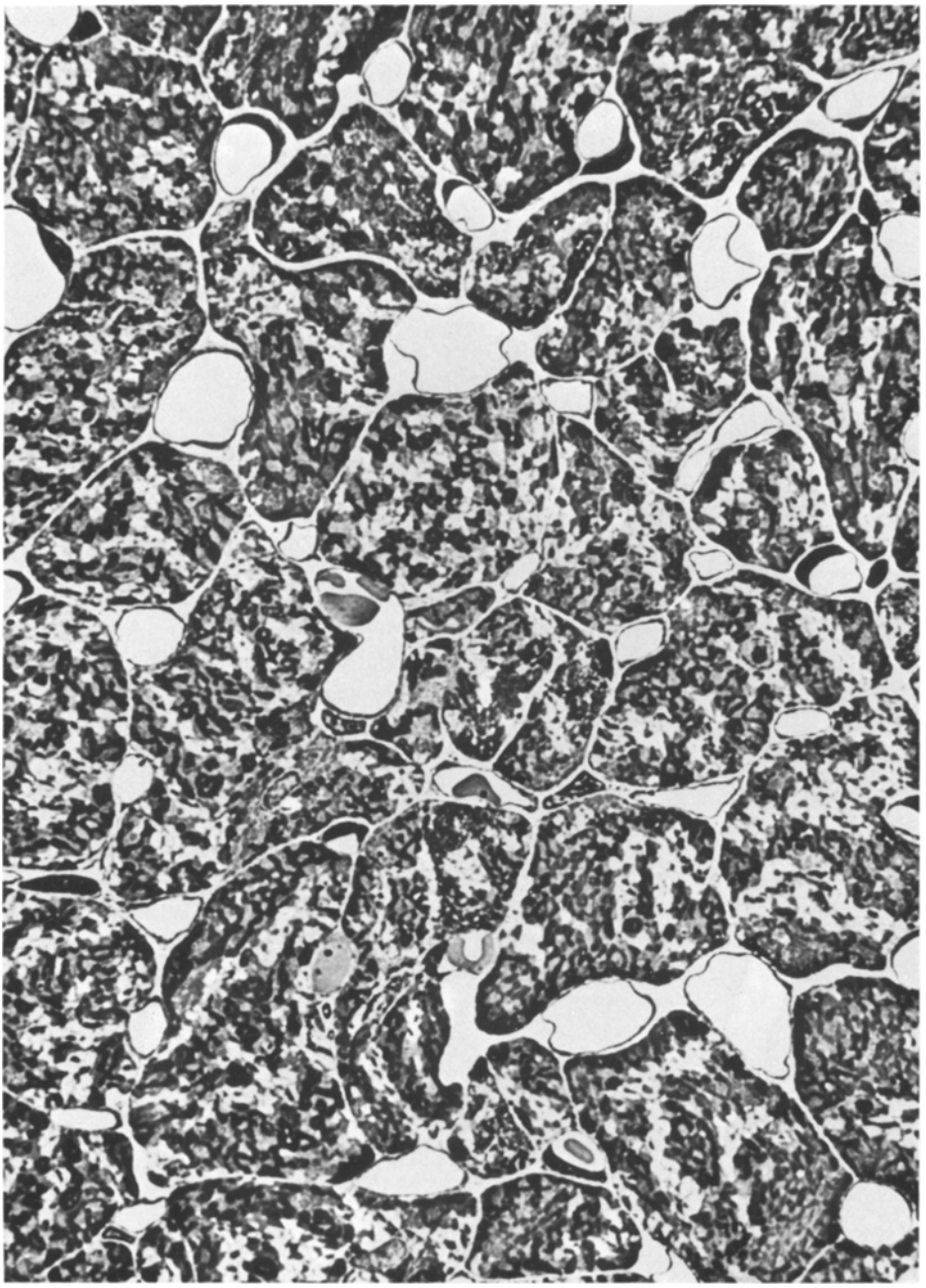

Fig. 1. Magnification 1,362:1. Light microscopy. Silver-stained ultrathin section for morphometric purposes. Cross section of a left ventricular papillary muscle (ethanol group). No apparent abnormalities of the myocardial tissue

The semiautomatic image analyzing system MOP AM 03[1] was used to obtain further information on myocardial mitochondria. The electron micrographs of stage two were evaluated to obtain the arithmetic means, the medians and the size distributions of the following mitochondrial variables: area, circumference, maximal diameters and the feret diameters of section profiles. The feret diameters were oriented parallel to the axis of sectioning (X diameters) and normal to this axis (Y diameters). All these parameters may yield valuable information about mitochondrial structural changes.

Results

The body weights of the ethanol-fed rats were significantly lower than the control group (268 g versus 324 g; $P < 0.001$, Students t test), whereas the average daily caloric intake was not different in the 2 groups (60 cal per day per rat). The weights of the hearts and the livers did not show significant alterations (Table 1). Relative organ weights were increased in the ethanol-fed group. Light and electron microscopic investigation of the hearts failed to reveal abnormalities in the left or right ventricular myocardium (Figs. 2–4). Investigation of the livers showed no striking structural alterations, in particular none of the ethanol-fed rats developed fatty changes of the liver or severe mitochondrial damage (Fig. 5). The study of the brains have been presented in a separate paper (Volk et al. 1979).

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Table 1. Effects of chronic ethanol ingestion on laboratory rats

	Control group		Ethanol group		Percent change in ethanol group	Level of statistical significance[a]
	mean	±S.E.	mean	±S.E.		
1. Drinking behaviour Food intake per animal (Calories/day)	59.83	±0.97	59.44	±0.65	− 0.65	N.S.
2. Body weights (g)						
Onset of experiment	224.42	±2.21	222.51	±2.22	− 0.85	N.S.
End of experiment	324.32	±4.43	267.87	±9.61	−17.41	$P < 0.001$
3. Organ weights (g)						
Left ventricle	0.8146	±0.0268	0.7611	±0.0287	− 6.57	N.S.
Right ventricle	0.1984	±0.0113	0.1851	±0.0119	− 6.70	N.S.
Liver	11.160	±0.637	11.310	±0.422	+ 1.34	N.S.

[a] A result was considered to be statistically significant if the probability of error, P, was smaller than 0.05

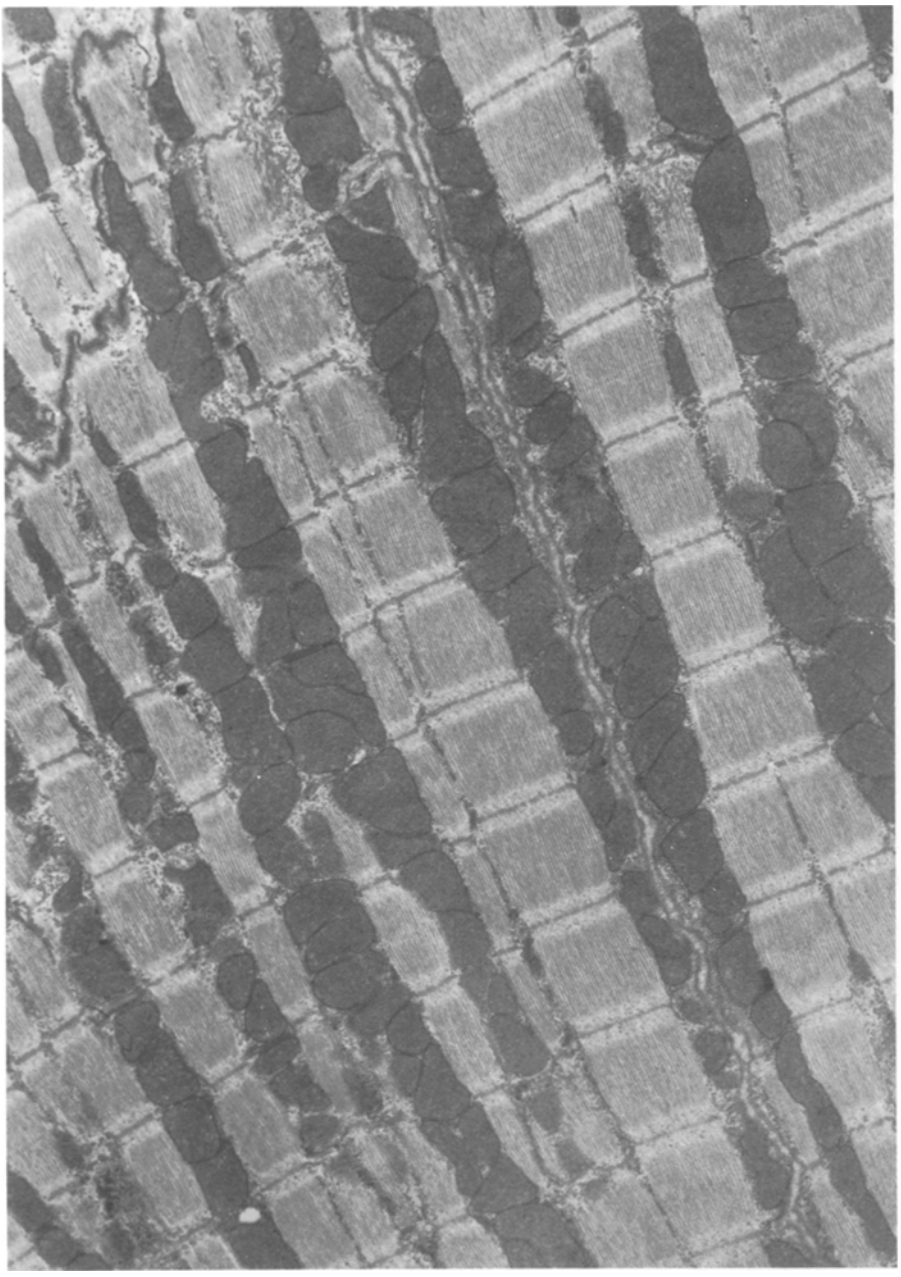

Fig. 2. Magnification 8,840:1. Longitudinal section of the left heart chamber of a chronically ethanol-fed rat. Normal appearance of the ultrastructure. No abnormalities of mitochondria and myofibrils. No dehiscene of the intercalated disc

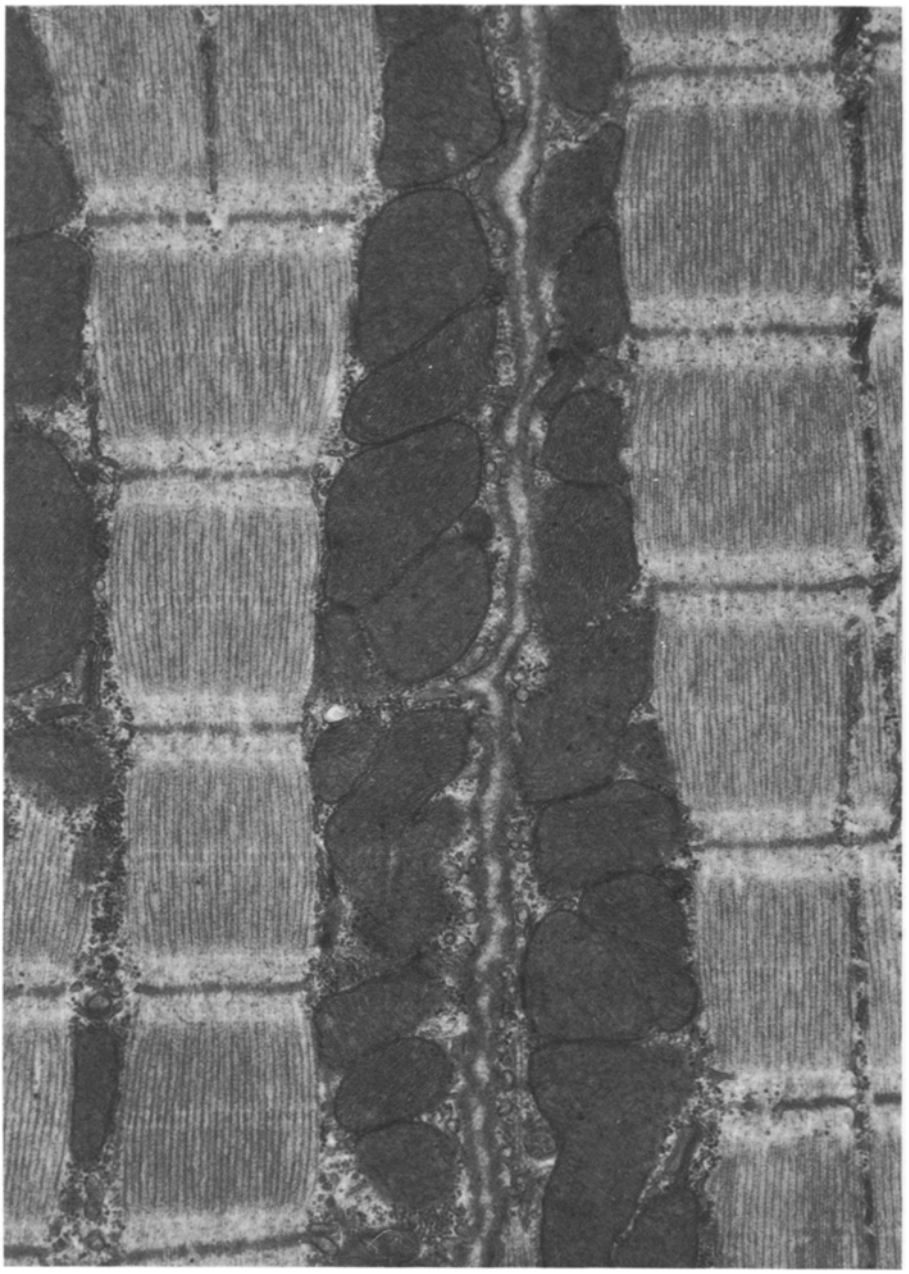

Fig. 3. Magnification 19,840:1. Normal density of the mitochondrial matrix indicates the absence of mitochondrial swelling processes. Cristae membranes without structural changes. No dilatation of T tubules and sarcoplasmic reticulum

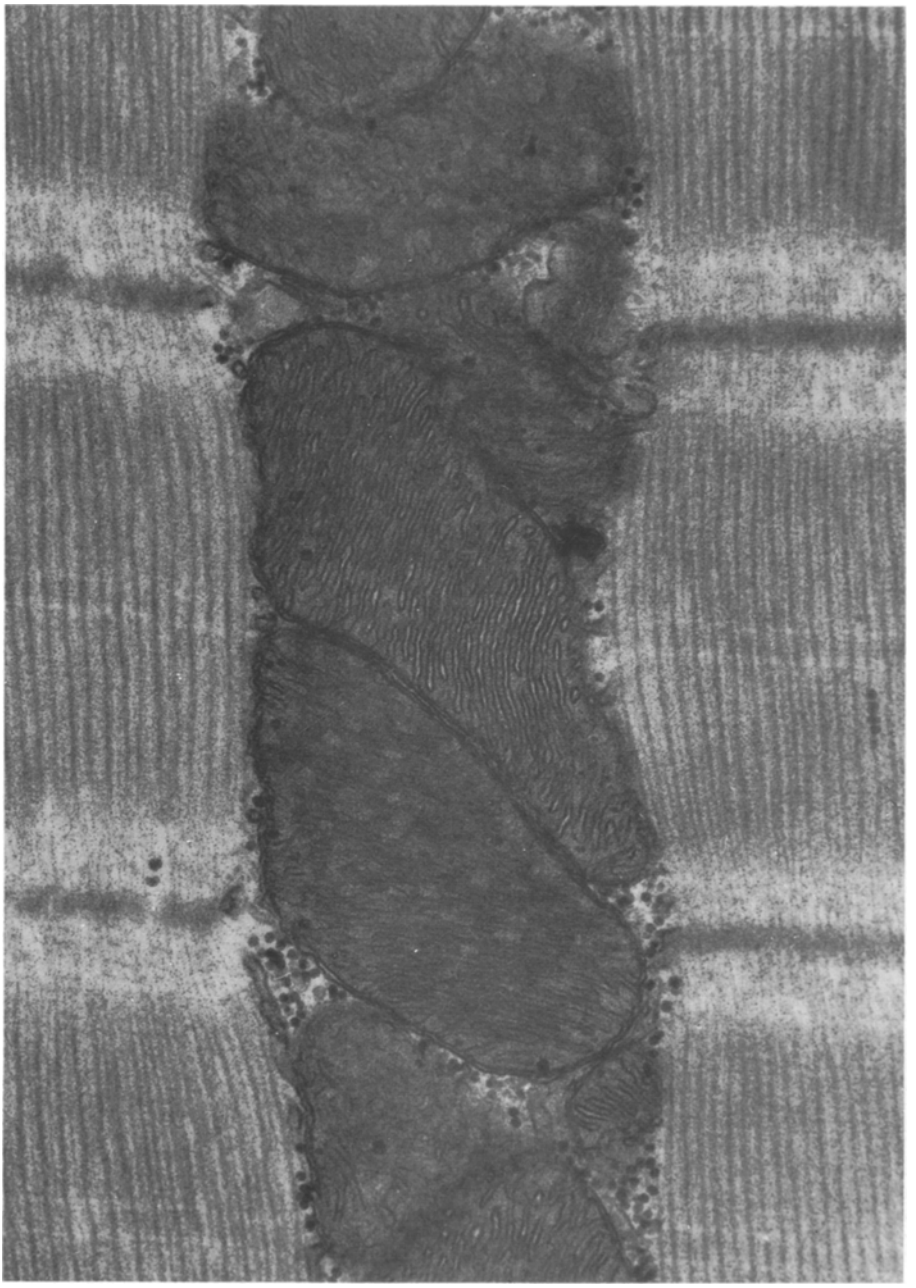

Fig. 4. Magnification 50,000:1. Normal structure of actin and myosin filaments. No membrane alterations

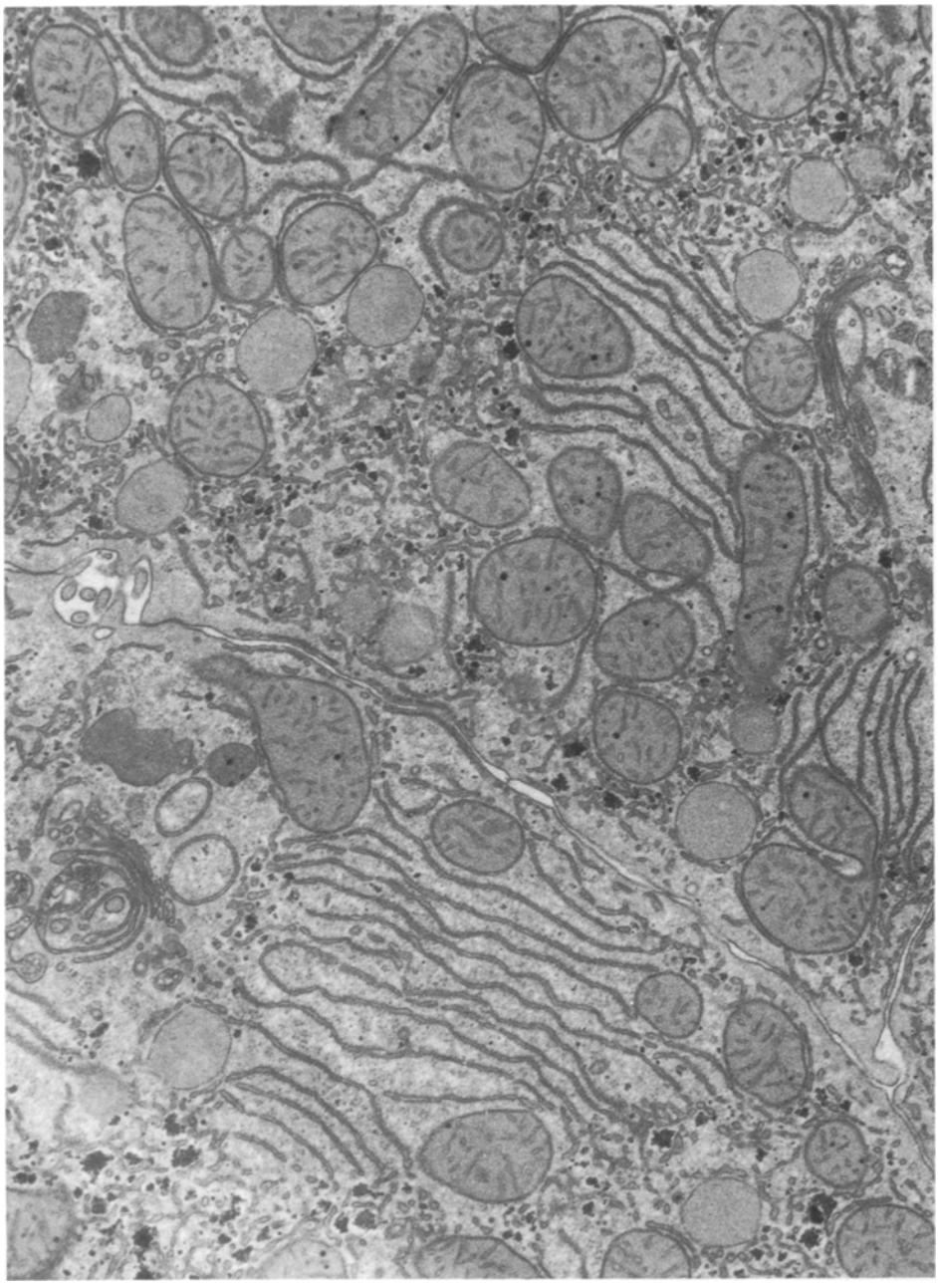

Fig. 5. Magnification 17,360:1. Ultrastructure of the hepatic parenchyma of an ethanol treated rat. Absence of apparent mitochondrial damage. No fatty changes

Table 2. Morphometric results obtained by light microscopy

Morphometric parameters Component parameter/ Reference parameter	Control group n=7		Ethanol group n=7		Level of statistical signifi-cance[a]
	mean	±S.E.	mean	±S.E.	
1. Volume densities (μ^3/μ^3)					
Myocardial cell volume/ Total tissue volume	0.8074	±0.0122	0.8463	±0.0068	$P<0.025$
Capillary volume/ Total tissue volume	0.1824	±0.0108	0.1443	±0.0066	$P<0.025$
Residual interstitial volume Total tissue volume	0.0102	±0.0022	0.0094	±0.0006	N.S.
2. Surface densities (μ^2/μ^3)					
Myocardial cell surface area/ Total tissue volume	0.1999	±0.0047	0.2083	±0.0094	N.S.
Capillary surface area/ Total tissue volume	0.0930	±0.0057	0.0922	±0.0039	N.S.
3. Surface-to-volume ratios (μ^2/μ^3)					
Myocardial cell surface area/ Myocardial cell volume	0.2481	±0.0079	0.2467	±0.0126	N.S.
Capillary surface area/ Capillary volume	0.5122	±0.0209	0.6404	±0.0156	$P<0.001$
4. Line densities (mm/mm^3)					
Length of capillaries/ Total tissue volume	3,171	±859	4,172	±501	$P<0.025$

[a] A result was considered to be statistically significant if the probability of error, P, was smaller than 0.05

The morphometric results are illustrated in Tables 2–4. The volume density of the capillaries decreased in the ethanol-fed group, whereas the myocardial cell density increased ($P<0.025$). The surface-to-volume ratio (S_V ratio) of capillaries was raised in the ethanol group ($P<0.001$), the S_V ratio of myocardial cells was unchanged. The capillary length density increased in the ethanol-fed group. The decreased surface area of the outer mitochondrial membranes per unit mitochondrial volume is the most remarkable result ($P<0.001$). The surface of inner mitochondrial membranes (+cristae mitochondriales) per mitochondrial unit volume was slightly decreased ($P<0.001$). The volume of the mitochondrial matrix per unit mitochondrial volume increased, whereas the intercristal space decreased ($P<0.01$). All mitochondrial variables obtained with the semiautomatic image analyzing system MOP AM 03 were significantly changed (Table 4). The size distribution of the mitochondrial section profile areas is evidently influenced by ethanol feeding, as shown by Fig. 6.

Discussion

The most surprising result of our study is the low level of unequivocal qualitative histological and ultrastructural alterations, findings which contradict the results

Table 3. Morphometric results obtained by electron microscopy

Morphometric variable Component variable/Reference variable	Control group n=7		Ethanol group n=7		Level of statistical signi-cance[a]
	mean	±S.E.	mean	±S.E.	
1. Volume densities of organelles (μ^3/μ^3)					
Myofibrillar volume/ Cytoplasmic volume	0.5538	±0.0091	0.5418	±0.0096	N.S.
Mitochondrial volume/ Cytoplasmic volume	0.3384	±0.0092	0.3545	±0.0098	N.S.
Sarcoplasmic matrix volume/Cytoplasmic volume	0.1078	±0.0033	0.1037	±0.0046	N.S.
2. Surface-to-volume ratios (μ^2/μ^3)					
Outer mitochondrial membrane surface area/Mitochondrial volume	8.92	±0.21	7.15	±0.13	$P<0.001$
Cristal surface area/Mitochondrial volume[b]	28.50	±0.49	26.53	±0.61	$P<0.05$
Total inner mitochondrial membrane surface area/Mitochondrial volume[b]	37.42	±0.56	33.68	±0.59	$P<0.001$
Total inner mitochondrial membrane surface area/Myofibrillar volume[b]	22.87	±0.94	22.04	±1.08	N.S.
3. Volume densities of mitochondrial subcompartments (μ^3/μ^3)					
Volume of mitochondrial matrix +cristae/Mitochondrial volume	0.7497	±0.0071	0.7846	±0.0079	$P<0.01$
Volume of intercristal space/ Mitochondrial volume	0.2503	±0.0071	0.2154	±0.0079	$P<0.01$
4. Numerical density of mitochondrial section profiles					
Number of mitochondrial profiles/ 1,000 μ^2 cytoplasmic area	990	±50	681	±26	$P<0.001$

[a] A result was considered to be statistically significant if the probability of error, P, was smaller than 0.05

[b] These results have been corrected for the loss of membrane images from oblique sectioning (Mall et al. 1977)

of some other groups. Segel et al. (1975) described swelling of mitochondria and tubuli as well as supercontractions of the myofibrils in chronic ethanol-treated rats. Rossi et al. (1976) found severe ethanol-induced alterations in rat hearts which were confirmed by light microscopic examination. Alexander et al. (1977) investigated hearts of chronically ethanol-fed mice and observed an increased number and size of mitochondria and megamitochondria. They also observed interstitial scarring. The intercalated discs were found to be affected in mice (Burch et al. 1971; Alexander et al. 1977) and in dogs (Ettinger et al. 1976). In contrast, Fahimi et al. (1979) and Hall and Rowlands (1970) observed only minimal ethanol effects on the heart in chronically ethanol fed rats.

Numerous ultrastructural changes have been reported in alcoholic cardiomyopathy in humans (Bulloch et al. 1972; Mösslacher et al. 1971; Hibbs et al.

Table 4. Dimensions of an average mitochondrial section profile

	Control group n=7		Ethanol group n=7		Percent change in ethanol group	Level of statistical significance[a]
	mean	±S.E.	mean	±S.E.		
Area (μ^2)	0.3265	±0.0054	0.4818	±0.0097	+47,57	$P<0.001$
Circumference (μ)	2.2581	±0.0223	2.7476	±0.0324	+21.68	$P<0.001$
Maximal diameter (μ)	0.7764	±0.0090	0.9709	±0.0133	+25.05	$P<0.001$
Feret X (μ)[b]	0.7491	±0.0090	0.9138	±0.0128	+21.99	$P<0.001$
Feret Y (μ)[c]	0.6312	±0.0069	0.7772	±0.0105	+23.13	$P<0.001$

[a] A result was considered to be statistically significant if the probability of error, P, was smaller than 0.05

[b] Feret X is the maximal diameter of a section profile parallel to the direction of the section plane (32.4° to the longitudinal axis)

[c] Feret Y is the maximal diameter of a section profile normal to Feret X.

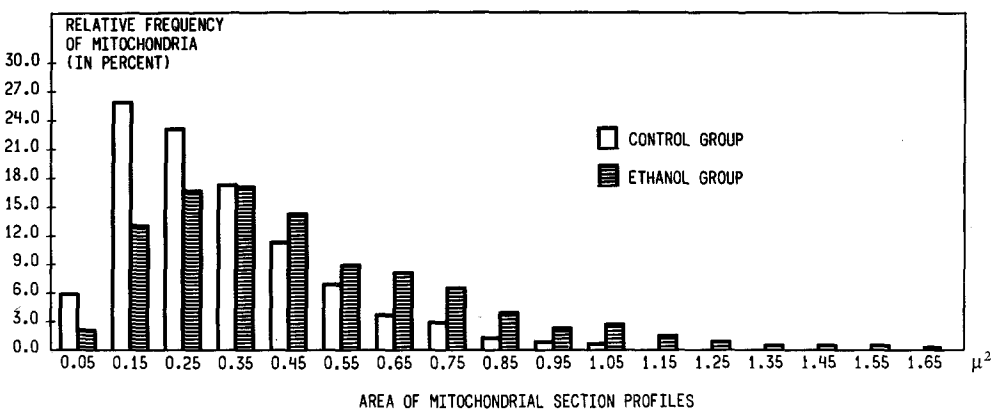

Fig. 6. The analysis of the mitochondrial size distribution in the heart reveals enlargement of mitochondria after ethanol feeding. The mitochondria of the ethanol-fed rats predominate in the higher size classes

1965; Doerr and Rossner 1977). Myocardial ultrastructural alterations have also been reported to follow acute ethanol intoxication (Lacerda et al. 1969; Klein and Harmjanz 1975).

We believe that these various discrepancies may be caused by the following factors:

1) Ethanol induced cardiac changes in animals are not a simple analog of alcoholic cardiomyopathy in man.

2) Sensitivity to ethanol may vary among different mammalian species.

3) The use of variable ethanol concentrations and variable diets by different investigators are an important factor in modifying results.

4) Different tissue preparation techniques may be the cause of incompatible ultrastructural results, a point supported by the observations of Fahimi and

coworkers (1979) and Hall and Rowlands (1970), who fixed the hearts by vascular perfusion and found very few abnormalities. It has been shown that immersion fixation can produce ultrastructural artifacts which are indistinguishable from pathological findings (Vodovar and Desnoyers 1975).

Morphometric analysis revealed considerable quantitative changes in cardiac histology and ultrastructure. Before discussing these results in detail the significance of the surface to volume ratios should be explained. In cylindrical structures the diameters d are derivable from the S_V ratios by the relation: $S_V = 4/d$ (Weibel 1969). The surfaces of capillaries and myocardial cells are similar to cylinder mantles. Thus, we derive the following diameter estimates: The diameters of the muscle fibers are estimated to be identical in both groups ($d = 16.1\ \mu$). The diameters of the capillaries amount to $6.2\ \mu$ in the ethanol-fed group and $7.8\ \mu$ in the control group. The morphometric data evaluated at stage 1 magnification (light microscopy) therefore lead to the unequivocal conclusion that all the quantitative changes are caused by a decrease of the capillary diameters in the ethanol-fed group and an increase of the number of capillaries, whereas the myocardial cells were unaffected at this stage.

The decreased mitochondrial S_V ratios indicate the occurence of enlarged mitochondria in the ethanol-fed group. This statement is substantiated by the following observations: The S_V ratio of particles clearly depends upon the size and shape of the particles. The values measured with the MOP AM 03 give evidence that the mitochondrial shapes were not significantly changed: the one-dimensional data, circumferences, maximal diameters and feret diameters of mitochondrial section profiles were raised by the same degree (1.2 times), and the two-dimensional value, the average profile section area were raised 1.4 times ($\approx 1.2^2$ times). Furthermore, the large difference between the two groups could not be explained by shape changes alone because bizarre-shaped mitochondria were not observed in any number.

The mean value of a single mitochondrion is calculated from number of mitochondria per unit volume of tissue (numerical density: N_V) and the volume of mitochondria per unit volume of tissue (volume density: V_V). The N_V value is derived from N_A (number of section profiles per unit area) and from the mean caliper diameter $\bar{D}$ of the particles using the formula $N_V \cdot \bar{D} = N_A$ (DeHoff and Rhines 1961). The S_V ratios are inversely proportional to the mean caliper diameters, if the shapes of the particles are constant. Thus, we estimate that the number of mitochondria is nearly halved and the volume of an average single mitochondrion is nearly doubled in the ethanol group (80% increase).

In contrast to these marked quantitative alterations the mitochondrial subcompartments are not changed to such an extent. From the values listed in table 3 we can calculated the geometry of standardized mitochondria as described by Reith (1977). Such mitochondria are thought to have parallel oriented cristae. The mean width of the intercristal space is calculated to be nearly the same in both groups (171 Å versus 163 Å) whereas the width of the matrix space between the cristae is enlarged to 485 Å in the ethanol-fed group compared with 396 Å in the control.

The unchanged volume densities of the myofibrils, mitochondria, and the sarcoplasmic matrix and the constant heart weights indicate that the absolute volumes of the myocardial cell organelles are not influenced by chronic ethanol

ingestion. This is entirely consistent with data which suggest that such changes follow cardiac hypertrophy or atrophy (Anversa et al. 1978; Smith and Page 1976).

The index: surface of inner mitochondrial membranes per myofibrillar volume is not significantly altered in the two groups (22.04 μ^2/μ^3 versus 22.87 μ^2/μ^3 in the control). This index was proposed by McCallister and Page (1973) for comparing the structures mainly involved in the ATP production and consumption and is possibly related to the myosin ATPase activity (Smith and Page 1976). The constancy of the index in our study may indicate an absence of severe metabolic disturbances in the heart. This is supported by the absence of apparent signs of cardiac failure, such as dilatation of the heart chambers or hepatic congestion.

The most striking result of our study is the decreased surface area of the outer mitochondrial membranes, which is accompanied by a reduced number of mitochondria and an almost doubling in volume of an average single mitochondrion.

It is important to emphasize that this phenomenon cannot be interpreted as the result of mitochondrial swelling, for in that case we should have observed a reduction of the cristal surface area to at least the same degree as the diminution of the outer mitochondrial membrane. The mitochondrial matrix should also appear more translucent under the electron microscope. However, we observed neither of these processes in our animals.

This peculiar mitochondrial quantitative reaction pattern has not been observed in the heart muscle in such an extreme form. Reith and Fuchs (1973) described enlarged mitochondria and a decreased number in hearts of riboflavin-deficient rats supplemented with riboflavin and triiodthyronine. The result was interpreted as the expression of altered mitochondrial biogenesis. Other experiments have failed to establish severe changes of the single volumes of cardiac mitochondria except in cases of mitochondrial swelling. However, many investigations have described a similar mitochondrial reaction in the liver. Rohr and Riede (1973) found decreased mitochondrial numbers per hepatocyte and increased single volumes of mitochondria in long term experiments using riboflavin deficiency, uremia, starvation, vitamin E deficiency and salicylate treatment. Chloramphenical, which inhibits the mitochondrial protein synthesis, produces megamitochondria in the liver (Albring et al. 1975).

Enlarged liver mitochondria were found in both chronic ethanol-treated animals (Kiessling 1968; Koch et al. 1978) and in human chronic alcoholics (Kiessling and Pilström 1971). Koch et al (1978) discuss the possibility that mitochondrial enlargement could be – analogous to the chloramphenicol effect – an expression of impaired mitochondrial protein synthesis. Burke and Rubin (1979) recently demonstrated in vitro effects of ethanol and of its primary metabolite acetaldehyde on the protein synthesis of liver mitochondria and on rats after ethanol feeding. They found that some subunits of mitochondrial membranes, namely subunits of cytochrome oxidase, cytochrome b and ATPase are more sensitive to the inhibition effect than other proteins. Thus, it is interesting that ethanol decreases the activities of respiratory chain enzymes in liver mitochondria (Rubin et al. 1970), especially coupling site I (Cederbaum et al.

1974). These effects may also be influenced by the primary metabolite of ethanol, acetaldehyde (Cederbaum et al. 1975)

Acetaldehyde is produced by alcoholdehydrogenase (ADH) and the microsomal ethanol-oxidizing system (MEOS) in the liver. Small traces of ADH were found in the heart, the occurence of MEOS in the heart is unknown (Forsyth et al. 1976). Fahimi et al. (1979) found small amounts of catalase in the heart. This is able to metabolize ethanol and its activity was elevated in chronically ethanol-fed rats. In any case, a considerable amount of acetaldehyde is transported from the liver to the heart via the circulating blood. This acetaldehyde is then oxidized by aldehyde dehydrogenase, an enzyme which occurs in the liver as well as in the heart (Forsyth et al. 1973).

Thus, the heart is exposed to both ethanol and acetaldehyde in vivo but can only metabolize acetaldehyde to a significant degree. In this context, it is interesting that in chronic alcoholics the blood levels of acetaldehyde following ethanol intoxication are higher than in normal subjects (Korsten et al. 1975).

Biochemical studies on heart mitochondria displayed both acute in vitro effects of ethanol and acetaldehyde (Segel and Mason, 1979) as well as alterations after chronic ethanol ingestion (Gvozdiák et al. 1976; Sarma et al. 1976; Alexander et al. 1977; Williams and Li 1977). Mitochondria isolated from hearts of chronic alcoholic animals have been shown to exhibit depressed respiratory activity (Pachinger et al. 1973; Segel et al. 1975). The acute effects (Segel and Mason 1979) consisted in depression of cardiac respiration, especially the mitochondrial respiratory control ratios.

Some studies have been published which concern the myocardial protein synthesis in relation to chronic alcoholism. Schreiber et al. (1972, 1974) have shown that acetaldehyde inhibits cardiac protein synthesis. Rawat (1979) found that chronic ethanol consumption resulted in a significant decrease in cardiac contents in total proteins and RNA, whereas the DNA content was unaltered.

Thus, we suggest that our results are the expression of an impaired synthesis of mitochondrial proteins, which is probably the cause of the enlargement of mitochondria and the reduction of the number of these organelles in our experiment. Furthermore, it is interesting that this process develops in a similar manner in both heart and liver. It should be noted that the enlargement of the mitochondria results in a continuous size distribution as Figure 6 demonstrates. In other words, the increased average mitochondrial volume is not caused by development of megamitochondria, but a slight enlargement of otherwise normal mitochondria which can be demonstrated only by quantitative analysis. This result is in accordance with the morphometric investigations of Kiessling and Pilström (1971) on chronically ethanol exposed liver. French (1979) suggested that the production of megamitochondria might be a dose-dependent effect, since all studies in which high levels of ethanol were administered (46% of the calories) showed this effect. In what respects the increased mitochondrial matrix space and the slight, but significant decrease of cristal surface are related to alterations of mitochondrial respiration or fatty acid metabolism, or how far they are also related to an impairment of protein synthesis is not conclusively established.

The increased number and the decreased diameters of capillaries in the ethanol-fed group are difficult to interpret. An increased number of capillaries

was observed in hearts following chronic hypoxia (Friedman et al. 1973). Alexander et al. (1977) discuss the possibility that ethanol causes hypoxia, since chronic ethanol administration and excessive acetate as substrate in hypoxia both lead to similar phenomena (triglyceride accumulation and maximal effects in the subendocardium) and a considerable amount of free acetate produced from hepatic ethanol oxidation occurs in the circulating blood. Moreover, they found that the activity of acetyl-CoA-synthetase, the first enzyme in acetate activation, was depressed in mice after 25 weeks of ethanol treatment. This indicates a diminished capacity of the heart to utilize acetate. It should be mentioned that this enzyme is localized in the outer mitochondrial membrane, in which we found the most profound alterations of our study. Thus, the biochemical investigations of Alexander and his coworkers as well as our morphometric results favor the hypothesis that similar structural and functional effects may occur in both chronic alcoholism and hypoxia.

A systematic ultrastructural study of the liver was not performed. An examination of representative samples showed, however, that striking ultrastructural alterations were absent (Fig. 5). This lack of severe liver damages in our rats indicates adequate nutrition rather than the lack of ethanol effects. Porta et al. (1967) demonstrated that the development of a fatty liver following ethanol ingestion is modified by the accompanying diet rather than by ethanol effects.

To summarize: the enlargement and the decreased number of heart mitochondria are probably the expression of an impaired synthesis of mitochondrial proteins. Biochemical studies (Schreiber et al. 1972; Rawat 1979) indicate that this effect is the result of acetaldehyde intoxication rather than a direct effect of ethanol. The quantitative reaction pattern of heart mitochondria is similar to the early ethanol-induced changes in the liver. This mitochondrial damage resulting from ethanol or acetaldehyde may play a role in the pathogenesis of alcoholic cardiomyopathy.

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