EXPERIMENTAL DATA ON THE MECHANISM OF DEVELOPMENT OF ALCOHOLIC CARDIOMYOPATHY

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The world-wide increase in the number of patients with alcoholism [3], and also with its visceral manifestations observed in recent years has stimulated interest in the study of the pathogenesis of alcoholic lesions in the internal organs. Foremost among these is alcoholic cardiomyopathy (ACMP), which is one of the main causes of sudden death in people of working age [1]. However, the impossibility of simulating this disease in animals [7] and also the fact that ACMP does not arise in all persons who abuse alcohol, and not even in all severe chronic alcoholics, suggest the existence of a certain predisposition to ACMP, which may evidently be expressed as inactivity of enzymes of alcohol metabolism [9]. It has been shown experimentally [8] that if catalase is inhibited in rats receiving ethanol, changes develop in the myocardium that resemble those of ACMP in man [1].

This paper describes an attempt to verify these data and, if they were confirmed, to undertake more detailed morphological and functional study of the myocardial and liver tissue.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 160-180 g, divided into four groups. The rats of group 1 were given physiological saline (control 1), rats of group 2 received ethanol for 6 weeks and, at the same time, were given intraperitoneal injections of physiological saline (control 2), animals of group 3 were given sucrose of equal calorific value to ethanol but with catalase activity depressed by the specific inhibitor aminotriazole (AT; control 3). Rats of the 4th (experimental) group received alcohol for 6 days, accompanied by intraperitoneal injections of AT. Alcohol was given to the animals in a dose equivalent to 36% of the total calorie intake, namely 10-12 g pure ethanol/kg body weight/day. Each group contained three animals receiving physiological saline by intraperitoneal injection.

The rats were decapitated on the day after the last dose of alcohol. The heart was stopped with cold KCl solution. Tissue from the myocardium of the left ventricle and the liver was fixed and treated for electron microscopy as described previously [1]. Permeability of the sarcolemma was studied in some blocks of myocardium with the aid of colloidal lanthanum, and nickel was determined electron-histochemically as a marker of myocardial ischemia [4, 5]. The diameter of the cardiomyocytes (CMC) was measured in semithin sections stained with hematoxylin and eosin (in 50 nucleated cells for each animal). In the course of the experiment the animals' body weight was monitored. The weight of the heart was determined post mortem.

EXPERIMENTAL RESULTS

The study of semithin sections through the myocardium of the experimental rats revealed vacuolation of virtually all muscle fibers, the presence of hypercontraction bands in CMC, and curiously shaped nuclei of the muscle cells. Some cells exhibited myocytolysis. The small myocardial vessels were highly dilated and congested with blood and the space between the muscle cells and capillaries was widened. The changes described above were more marked in the subendocardial zones of the myocardium.

The electron-microscopic study of CMC revealed considerable changes in the contractile system of the cells: disturbance of correct orientation of the myofibrils and their lysis,

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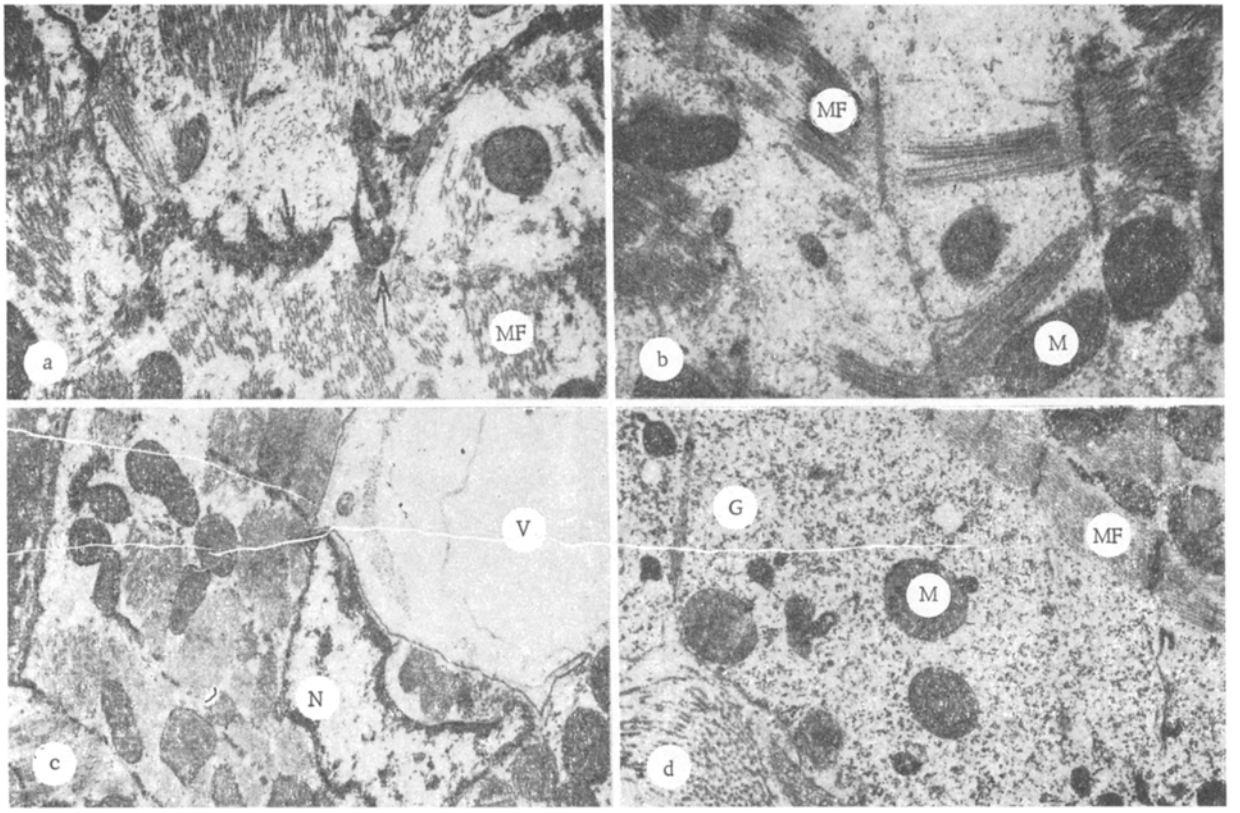

Fig. 1. Ultrastructure of CMC in experimental ACMP: *a*) lysis of myofibrils (MF) in region of intercalated disk (arrows). 8300 ×; b) CMC with considerable disturbance of myofibrillary apparatus. 10,000 ×; c) large vacuole (V) close to nucleus (N) of CMC. 6600 ×; d) marked accumulation of glycogen (G) at periphery of CMC. 10,000 ×; M) mitochondrion.

which were particularly marked close to the intercalated disks (Fig. 1a). Lysis of the myofibrils was considerable in some cells (Fig.1b). Dying CMC with virtually homogeneous contents were found.

In most CMC dilatation of the cisterns of the sarcoplasmic reticulum and the appearance of vacuoles filled with finely granular contents, among which remnants of organelles of the CMC were sometimes distinguishable, were observed. These vacuoles sometimes were several sarcomeres in length, and they often were discovered in the perinuclear space, where they appeared to compress the nuclei (Fig.1c).

In some cells lipid infiltration, hyperplasia of the mitochondria, and the appearance of secondary lysosomes were observed, while in some cells considerable quantities of glycogen accumulated (Fig. 1d). Destructive changes in the mitochondria were found in only some CMC. Large drops of fat were found occasionally in the widened interstitial space (Fig. 2a), the capillary endothelium in most vessels was flattened, the vessels were congested with blood (Fig. 2b), and a very small number of capillaries were empty and their lumen was virtually undetectable.

The histochemical reaction for Ni revealed the presence of foci of myocardial ischemia, which bordered on intact zones (Fig. 2c).

With the aid of colloidal La increased permeability of the sarcolemma for its particles was found; in some cases colloidal La also was found in the intracristal space of the mitochondria (Fig. 2d).

The study of the control material showed that the combination of changes described above was virtually never found in the animals of groups 2 and 3. Although in the rats of group 3 single vacuoles were observed in CMC, a very mild degree of destruction of individual mitochondria was rare. Colloidal lanthanum passed through the sarcolemma in a few isolated CMC.

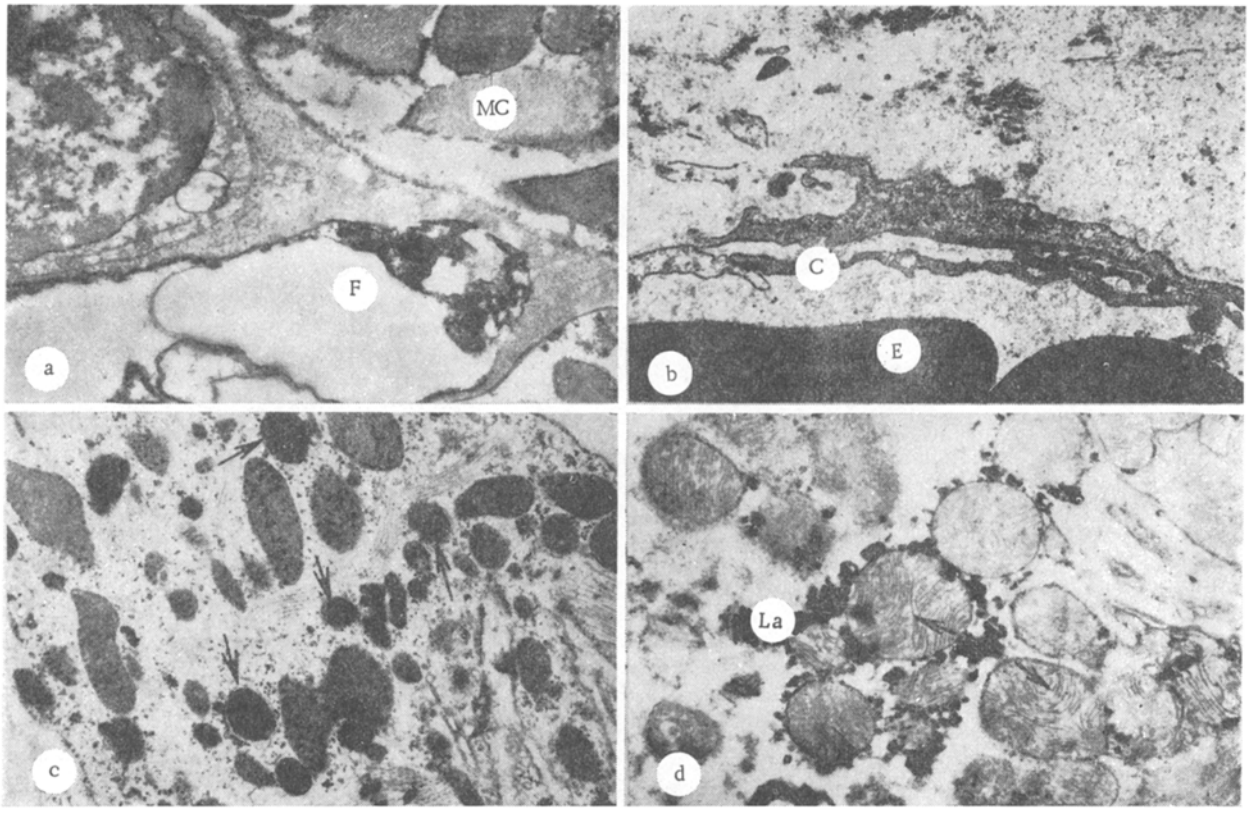

Fig. 2. Ultrastructure of interstices and some electron-histochemical parameters of myocardium in experimental ACMP. *a*) Appearance of fat (F) in interstices (MC — muscle cell). 10,000 ×; b) thinning of capillary wall (C), congestion of vessels (E — erythrocyte); c) accumulation of Ni of dimethylglyoxime complexes in CMC (arrows); d) increase in permeability of sarcolemma for colloidal La, particles of colloidal La found around mitochondria (La); arrows indicate accumulation of La in mitochondrial cristae.

TABLE 1. Weight of Heart and Diameter of CMC in Rats of Various Experimental Groups

Group of animals	Weight of heart, g	Diameter of CMC, μ
Control 1	1,01±0,01	18,43±0,54
Control 2	1,04±0,02	15,11±0,62
	$P<0,25$	$P<0,001$
Control 3	1,01±0,001	17,02±0,41
	$P>0,25$	$P<0,05$
Experimental	0,94±0,05	16,02±0,70
	$P<0,01$	$P<0,01$

In the animals of group 2 solitary lipid inclusions were found in the interstices and in individual cardiomyocytes, and permeability for colloidal lanthanum was identical with that in the experimental group. Just as in control 2, so also in the experimental group fatty degeneration of the liver was found.

During the experiment all animals of the control group gained in weight by 75% of their original weight. Rats of group 4 began to lag behind in weight after the 5th week of the experiment, and by the end of the experiment their gain in weight was 60% of its initial value. The weight of the heart in rats of the experimental group was less than in the control animals. The diameters of CMC in the rats of groups 2 and 4 were significantly less than in the rats of groups 1 and 3 (Table 1).

Most alcohol which enters the body is known to be oxidized in the liver. Three systems of alcohol oxidation are known: by means of the enzyme alcohol dehydrogenase, by means of the microsomal alcohol oxidation system, and by means of catalase.

In the myocardium there are only traces of alcohol dehydrogenase, and no microsomal alcohol oxidation system has been discovered. Thus catalase in fact plays the leading role in ethanol metabolism in the myocardium. It was shown previously that during systematic alcohol consumption, catalase activity in the heart increases [6]. Kino [8] suggested that this increase in activity is a protective mechanism against the action of alcohol on the myocardium.

In fact, when catalase was inhibited and ethanol given simultaneously to the experimental animals of group 4, changes virtually identical with those during ACMP in man were found [1]. The reaction for Ni revealed areas of myocardial ischemia, which also have been recorded in persons dying suddenly from ACMP. Kino [8] previously performed similar experiments. Although in his investigation no such marked disturbances of the myofibrillary apparatus were found as in the present study, some of the animals (9-10%) receiving ethanol died suddenly.

The presence of fatty degeneration of the liver in animals receiving ethanol confirms the view that alcohol has a direct toxic action on this organ. The action of ethanol on the myocardium, however, probably depends on many factors, including the frequency of ethanol administration [2] and, as the present investigation showed, the level of activity of enzymes involved in its metabolism.

LITERATURE CITED

1. A. M.Vikhert and V. G. Tsyplenkova, Arkh. Anat., No. 1, 14 (1984).
2. V. A. Kononyachenko, V. A. Frolov, V. E. Dvornikov, et al., Kardiologiya, No. 7, 102 (1983).
3. Yu. P. Lisitsin and N. Ya. Kopyt, Alcoholism [in Russian], Moscow (1983).
4. V. G. Sharov, R. B. Jennings, and H. K. Hawkins, Arkh. Patol., No. 10, 35 (1980).
5. I. Balogh, E. Somogyi, P. Sotonyi, et al., Z. Rechtsmed., 90, 7 (1983).
6. H. D. Fahimi, M. Kino, L. Hicks, et al., Am. J. Pathol., 96, 373 (1979).
7. J. L. Hall and D. T. Rowlands, Am. J. Pathol., 60, 153 (1970).
8. M. Kino, J. Molec. Cell. Cardiol., 13, 5 (1981).
9. A. F. Whereat and J. K. Perloff, Circulation, 47, 915 (1973).