pneumocytes of types both 1 and 2 was reduced, whereas large numbers of polysomes and ribosomes and an endoplasmic reticulum were present. Monocytes, eosinophils, and lymphocytes constantly appeared in the capillaries, especially from the 5th-7th day after infection, evidence of definite allergization of the animals. From the 5th day after infection the formation of collagen fibers was intensified and sclerosis was most marked on the 14th-21st days. It is interesting to note that protein metabolism and protein synthesis were intensified in all cell components of the air-blood barrier, evidently as a result of the action of the virus, as shown by an increase in the number of polysomes and cisterns of the endoplasmic reticulum, and separation of the intermembranous nuclear space, involved in the structure of the endoplasmic reticulum, into separate layers. At the same time, the type 2 pneumocytes contained few osmiophilic bodies, and this state of affairs may have led to a decrease in the production of surfactant and the onset of atelectasis. After the 21st day of experimental infection, no changes were observed in the respiratory organs.

LITERATURE CITED

1. L. O. Vishnevetskaya, Vopr. Okhr. Mat., No. 11, 50 (1964).
2. Z. I. Gavrilov, N. N. Dodonova, and E. S. Voronin, Respiratory Virus Infections [in Russian], Moscow (1963), pp. 176-177.
3. R. S. Dreizin and V. M. Zhdanov, Adenovirus Infections [in Russian], Moscow (1962), p. 287.
4. M. A. Kostyukov, "Adenovirus infection in monkeys," Dissertation for the Degree of Candidate of Medical Sciences, Moscow (1965).
5. O. T. Oganesyan, "Study of the role of maternal adenovirus and influenzal infection in fetal and neonatal pathology," Dissertation for the Degree of Candidate of Medical Sciences, Moscow (1967).
6. A. V. Tsinzerling, E. V. Polonskaya, A. P. Tarasova, et al., Arkh. Patol., No. 10, 21 (1965).
7. R. W. Atchison, B. C. Lasto, and W. M. Hammon, Virology, 29, 353 (1966).
8. A. O. Betts, A. R. Jennings, P. Lamont, et al., Nature, 193, 45 (1962).
9. G. S. Boyer, F. W. Denny, and H. S. Ginsberg, J. Exp. Med., 109, 85 (1959).
10. H. Handa, H. Shiroki, and H. Shomigo, Replication of Mammalian Parvoviruses, New York (1978), pp. 77-86.
11. R. N. Hull, J. R. Minner, and C. C. Mascoli, Am. J. Hyg., 68, 31 (1958).
12. H. G. Pereira and B. Kelly, Nature, 180, 615 (1957).

STEREOLOGIC ANALYSIS OF THE MYOCARDIUM IN ALCOHOLIC CARDIOMYOPATHY

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Acute alcoholic poisoning and long-term alcohol consumption lead to a disturbance of structural protein synthesis [15], inhibition of biosynthesis and the respiratory function of cardiomyocyte mitochondria [14], and reduced contractility of the myocardium as a whole [12], but do not themselves induce the formation of alcoholic cardiomyopathy [11]. An essential, but as yet incompletely studied role in the pathogenesis of human alcoholic cardiomyopathy is played by vitamin deficiency (mainly thiamine) and changes in protein metabolism. Hence it follows that in order to create an adequate model of experimental alcoholic cardiomyopathy,

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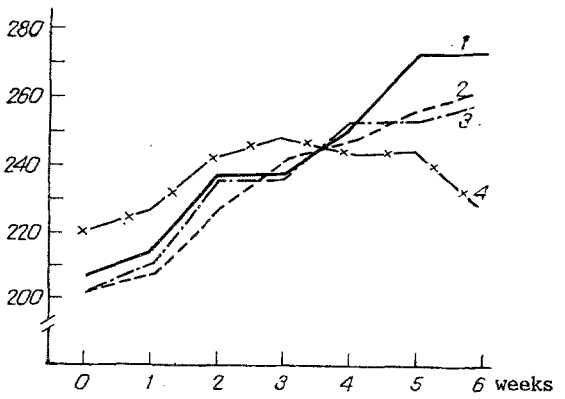

Fig. 1. Effect of ethanol, vitamin B_1 deficiency, and a combination of both on time course of rats' body weight. 1-4) Groups of animals (explanation in text).

the combined influence of ethanol and certain metabolic disturbances on the animals' heart is essential. Determination of the morphological features and the importance of disturbance of protein synthesis in the formation of cardiomyopathies of varied genesis [5] showed that the most informative methods of studying the heart in such situations are morphometry and stereologic analysis of the tissue organization of the myocardium. These techniques have been used to study the early stage of formation of cardiomyopathy in alcoholic poisoning, against a background of vitamin B_1 deficiency.

The aim of the present investigation was to compare the isolated and combined action of ethanol and a thiamine-deficient diet on the albino rat myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on 24 mature male Wistar rats weighing 200.2-228.2 g. During the experiment the animals were kept under standard animal house conditions and were divided into four groups. Group 1 (control) consisted of 5 intact rats receiving a balanced laboratory diet. The 7 animals of group 2 received the same diet but, instead of drinking water, they had free access to 20% ethanol solution, sweetened with 5% of sucrose. Rats of groups 3 and 4 (5 and 7 animals respectively) received a diet balanced for protein, carbohydrates, fats, salts, and vitamins [2], calculated so that each animal received daily 3 g of casein, 1 g of sunflower oil, 15 g of polished rise, and 830 mg of a mixture of all necessary salts and vitamins except vitamin B_1; the rats of group 4 received ethanol solution ad lib. instead of water to drink. The animals were weighed daily in the morning before taking food.

In accordance with the aims of the investigation the duration of the experiment was limited to 6 weeks, during which ultrastructural signs of alcoholic injury to the cardiomyocytes [10] and of vitamin B_1 deficiency were formed in animals kept on the corresponding diet [1]. The animals were taken from the experimental by decapitation under superficial chloroform anesthesia; the final body weight and weight of the myocardium of the heart ventricles were determined during decapitation.

The hearts were fixed in cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 8.0. The papillary muscles were postfixed with 1% osmium tetroxide solution in the same buffer with pH 7.4, and blocks were cut for electron microscopy [9]. Half of each heart, divided from base to apex in the frontal plane, was embedded in paraffin wax. Histological survey sections were stained with hematoxylin and eosin and by a combined histochemical method: with colloidal iron — PAS — hematoxylin. Stereologic analysis of the tissue organization of the myocardium was carried out on histological sections [3, 6]: the relative volume (bulk density) of the muscle fibers blood vessels, connective tissue, and the intermuscular tissue fluid and the surface density of the muscle fibers were determined. On the basis of these data the absolute weight of the structural components of the myocardium which were studied and the total surface area of the muscle fibers were calculated. The material was studied with the NU2 universal biological microscope (Carl Zeiss, East Germany) and the Tesla BS-500 electron microscope (Czechoslovakia), with accelerating voltage of 60 kV.

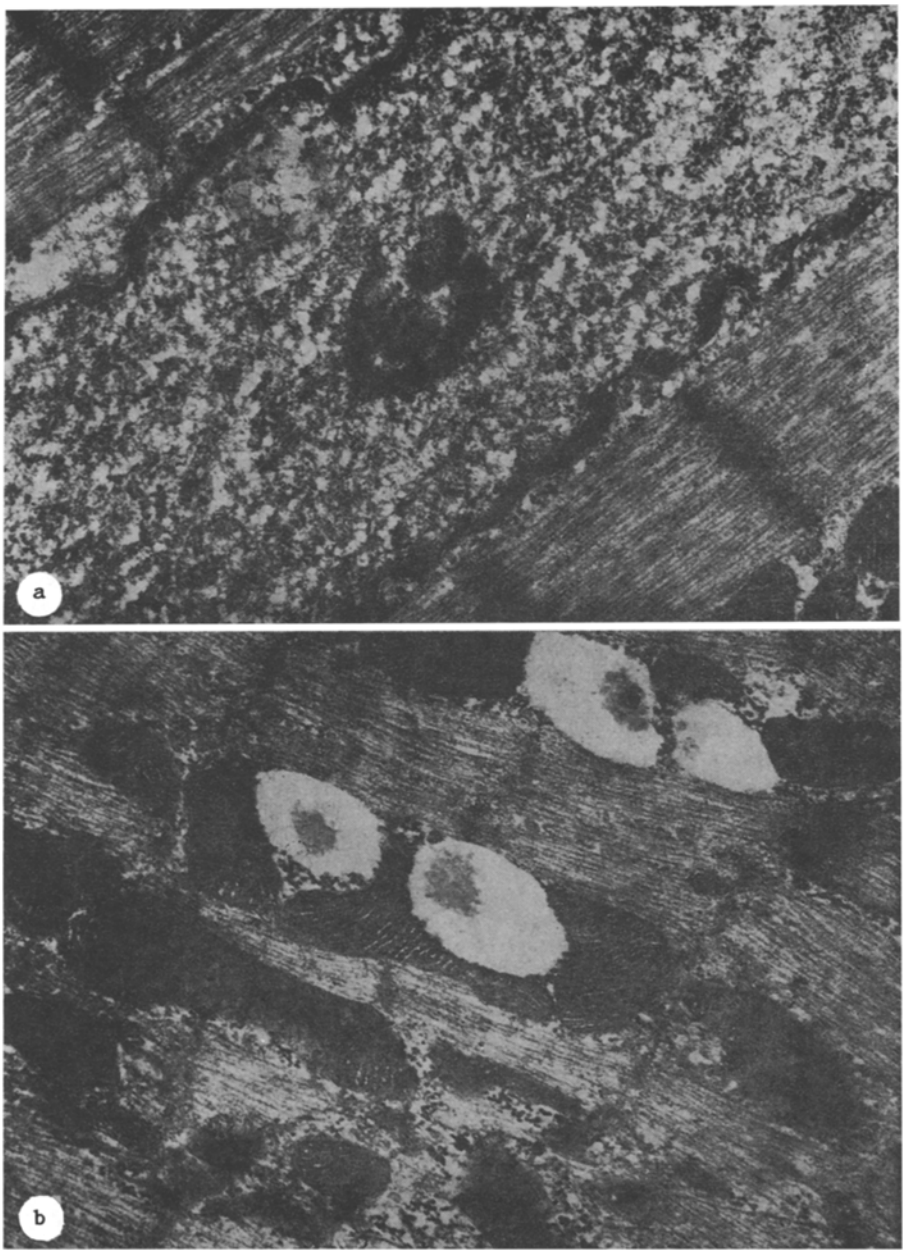

Fig. 2. Ultrastructural changes in cardiomyocytes in rats with alcohol poisoning. 30,000 ×. a) Fatty infiltration of cytoplasm due to isolated action of ethanol, b) structure of nucleus and nucleolus of cardiomyocyte during ethanol consumption by animal on thiamine-deficient diet.

EXPERIMENTAL RESULTS

The time course of the increase in final weight of the heart of the rats of groups 1, 2, and 3 during 6 weeks of observation was found to be similar, but no increase in body weight was observed in the rats receiving a thiamine-deficient diet and ethanol (group 4; Fig. 1). At autopsy no signs of circulation disturbance in the form of edema or stasis could be found in the parenchymatous organs.

In histological preparations the myocardium of the control and experimental animals had the usual structure. In group 1 the content of granular glycogen in the cardiomyocytes varied from animal to animal, but in the experimental rats of the remaining groups glycogen granules were not found in the cardiomyocytes. The content of glycosaminoglycans and collagen fibers in the myocardium of all the rats was identical and did not exceed the normal limits.

TABLE 1. Results of Stereologic Analysis of Myocardium of Rats Receiving a Combination of Ethanol and a Vitamin B_1-Deficient Diet (M ± m)

Parameter	Group of animals						
	1- (n=5)	2- (n=7)	$p_{1-2}<$	3- (n=5)	$p_{2-3}<$	4- (n=7)	$p_{3-4}<$
Body weight, g							
initially	206,7±8,7	200,2±14,1	—	202,1±2,5	—	228,2±10,5	—
finally	277,4±15,6	264,2±20,2	—	254,8±7,4	—	226,2±13,4	0,05
Weight of heart, mg	695,6±41,8	749,8±64,9	—	693,2±14,6	—	598,4±22,4	—
Bulk density							
muscle fibers	0,658±0,022	0,658±0,006	—	0,584±0,013	0,05	0,584±0,011	0,05
capillaries	0,240±0,019	0,148±0,008	0,01	0,203±0,009	—	0,196±0,008	—
connective tissue	0,046±0,002	0,109±0,008	0,01	0,067±0,008	0,05	0,090±0,007	—
tissue fluid	0,061±0,006	0,089±0,006	0,01	0,151±0,003	0,01	0,167±0,025	0,01
Surface density of muscle fibers (mm^{-1})	141,2±1,1	141,3±4,4	—	146,3±2,1	—	142,7±3,1	—
Absolute total weight, mg							
muscle fibers	462,5±38,7	494,3±43,3	—	403,6±6,9	—	349,1±16,3	0,05
capillaries	164,4±12,9	108,9±8,7	0,01	134,6±15,3	—	116,7±5,4	0,05
connective tissue	32,3±2,3	85,2±11,9	0,01	47,3±6,5	—	53,6±4,4	0,01
tissue fluid	40,4±1,9	64,7±5,7	0,01	104,7±3,7	0,01	103,2±17,3	0,01
Total surface area of muscle fibers, $mm^2 \cdot 10^3$	98,3±6,1	104,8±8,4	—	101,4±2,6	—	85,7±4,3	—

Legend. n) Number of animals.

An ultrastructural survey of the cardiomyocytes showed that the general structure and mutual arrangement of the intracellular organelles were the same in the muscle cells of all groups. Lipid inclusions were seen in the cytoplasm of the cardiomyocytes of the rats of group 2, and glycogen was present in the form of β-particles (Fig. 2a); in the rats of groups 3 and 4, however, there were chains of small β-particles. Secondary lyosomes and foci of degradation were frequently seen. Appreciable changes, most marked in rats receiving alcohol (groups 2 and 4), were found in the nuclei and nucleoli of individual cardiomyocytes. These changes included loss of tone of the nuclear membrane and fragmentation of the nucleolonema (Fig. 2b). Areas of lysis and regeneration of myofilaments on ribosomes were seen more often than in the control on the myofibrils.

Analysis of the morphometric and stereologic parameters (Table 1) showed that the bulk density of the capillaries was significantly reduced (by 38.4%) in the rats receiving a combination of ethanol and a balanced diet, and, consequently, their absolute total weight was reduced. These changes were linked with a decrease in capillary diameter [13]. Changes in the opposite direction took place in the connective-tissue stroma of the myocardium — its bulk density was increased by 134%.

According to the data of light microscopy of survey sections the increase in the relative volume and absolute total weight of connective tissue was due mainly to an increase in the number and size of the fibroblasts, which had no marked secretory activity. Proliferation of fibroblasts is one of the early signs of a lowering of the level of metabolic and synthetic processes in the cardiomyocytes [5]. Disturbances of synthesis of structural proteins in the cardiomyocytes of the rats of group 2 evidently did not reach the degree at which they were reflected in the bulk and surface density of the muscle fibers, although signs of their plastic insufficiency, present to a moderate degree, were found on electron microscopy.

Rats with vitamin B_1 deficiency, unlike the controls, showed a tendency ($p < 0.05$) for the bulk density of the muscle fibers to be reduced, but for this parameter for connective tissue to be increased, although this was not reflected significantly in their absolute parameters. Thiamine deficiency in the diet did not affect the bulk density of the myocardial capillaries.

The combined effect of ethanol and vitamin B_1 deficiency caused the most marked changes in the rat myocardium compared with the control. The weight of the ventricular myocardium of the heart was significantly reduced, and although the decrease was only 14%, it was evidently reduced much more in reality, because the initial body weight and, correspondingly, the weight of the heart, of the rats of group 4 was initially greater.

It will be clear from Table 1 that the bulk density of the muscle fibers was reduced proportionally to the reduction of weight of the heart. Judging by the level of statistical significance of the differences ($p < 0.05$) the values of the bulk density and absolute total weight of muscle tissue in the heart showed a marked tendency to decline. This conclusion is

based on the results of stereologic analysis of another component of the myocardium, namely capillaries. The relative volume of connective tissue was increased statistically significantly (by 94.4%).

Comparison of the results of examination of the survey sections in the light microscope and also the results of electron microscopy with those of the morphometric and stereologic study of the tissue organization of the myocardium enables a number of general conclusions to be drawn.

Initial ultrastructural features of the decline of protein synthesis in the cardiomyocytes develop in response to the isolated action of ethanol and B_1 deficiency. The strengthening of these features, including a decrease in the absolute total weight of the muscle tissue in the myocardium during the combined effect of ethanol and vitamin B_1 deficiency is evidence of the additive action of thiamine on cardiomyocytes, the mechanism of which requires special investigations for its elucidation.

Proliferation of stromal cells, manifested most clearly in the myocardium of the experimental rats of groups 2 and 4, and on the basis of the concept of the leading role of the functional state of elements of the parenchymatous organs in the regulation of parenchymal-stromal inter-relations [8], can be interpreted as a morphological criterion of the decline of structural metabolism in the cardiomyocytes [7]. This conclusion is confirmed by another sign of latent heart failure, namely interstitial edema of the myocardium.

Reduction in size of the capillaries is not characteristic of plastic cardiac insufficiency [7], and for that reason the appearance of such changes in the myocardium of rats consuming ethanol can be confidently ascribed to this direct toxic effect.

It was shown on the basis of biochemical, physiological, and ultrastructural studies that ethanol has a direct harmful action on myocardial muscle tissue [12, 14, 15]. The results of this investigation showed that a differential morphological assessment can be made of the contribution of the cardiotoxic influences of ethanol and vitamin B_1 deficiency to the development of the initial stages of alcoholic cardiomyopathy.

LITERATURE CITED

1. M. I. Smirnov (ed.), Vitamins [in Russian], Moscow (1974).
2. N. V. Kozlyakov, G. M. Erastov, A. P. Ryzhov, and Yu. I. Lifshits, A Manual on Feeding of Laboratory Animals, Experimental Birds, and Producers [in Russian], Moscow (1968).
3. L. M. Nepomnyashchikh, E. L. Lushnikova, L. V. Kolesnikova, et al., Morphometric and Stereologic Analysis of the Myocardium: Tissue and Ultrastructural Organization: Technical Recommendations on Morphology [in Russian], Novosibirsk (1984).
4. L. M. Nepomnyashchikh, Pathological Anatomy and Ultrastructure of the Heart (Combined Morphological Investigation of the General Pathological Process in the Myocardium) [in Russian], Novosibirsk (1981).
5. L. M. Nepomnyashchikh, D. E. Semenov, V. P. Tumanov, and Yu. G. Tsellarius, Byull. Éksp. Biol. Med., No. 9, 25 (1983).
6. L. M. Nepomnyashchikh, E. L. Lushnikova, and G. I. Nepomnyashchikh, Morphometry and Stereology of Hypertrophy of the Heart [in Russian], Novosibirsk (1986).
7. L. A. Semenova, L. M. Nepomnyashchikh, and D. E. Semenov, Morphology of Plastic Insufficiency of Heart Muscle Cells [in Russian], Novosibirsk (1985).
8. Yu. G. Tsellarius and L. A. Semenova, Histopathology of Focal Metabolic Lesions of the Myocardium [in Russian], Novosibirsk (1972).
9. Yu. G. Tsellarius, L. A. Semenova, and L. M. Nepomnyashchikh, Focal Lesions and Infarction of the Myocardium. Light, Polarization, and Electron Microscopy. Novosibirsk (1980).
10. G. E. Burch, H. Z. Colcolough, J. M. Harb, and C. Y. Tsui, Am. J. Cardiol., 27, 522 (1971).
11. H. D. Fahimi, L. Kino, L. Hicks, et al., Am. J. Pathol., 96, 373 (1979).
12. Y. Hirota, O. H. Z. Bing, and W. H. Abelman, J. Mol. Cell. Cardiol., 8, 727 (1976).
13. G. Mall, T. Mattfeldt, and B. Volk, Virchoes' Arch. Abt. A, Pathol. Anat., 389, 59 (1980).
14. T. Mattfeldt, G. Mall, and B. Volk, J. Cell. Cardiol., 12, 1311 (1980).
15. M. A. Rotschild, S. S. Schreiber, and M. Oratz, Clin. Toxicol., 8, 349 (1975).