# ROD-SHAPED INCLUSIONS IN THE MYOCARDIUM AND BRAIN OF EXPERIMENTALLY ALCOHOLIZED RATS

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UDC 616.89-008.441.13-092.9-07:[616.831+616.12]-091

KEY WORDS: alcoholic intoxication; cytoplasmic inclusions; alcoholic hyalin.

Intracellular inclusions in chronic alcoholism were described a long time ago. They were first described in the liver, in the cells of which Mallory [11] first found hyalin-like masses, subsequently known as Mallory's bodies or alcoholic hyalin. The most reliable method of detecting alcoholic hyalin at the present time is by electron microscopy [6, 12].

Data on the nature and morphological variants of hepatocellular hyalin have been adequately described in a number of publications [2-6]. However, the problem of the origin of alcoholic hyalin has not yet been solved. So far alcoholic hyalin has been found only in the liver cells of patients, and most workers consider that the presence of Mallory's bodies is pathognomic of alcoholic hepatitis. In 1974, Hojima and Ogawa [10] first observed rod-shaped eosinophilic inclusions in large neurons in the caudate nucleus of patients with chronic alcoholism. By their staining properties, they were identified as Mallory's bodies in alcoholic hepatitis, and they were therefore called hyalin-like bodies. The same kind of inclusions in alcoholic encephalopathy were subsequently found by Akima et al. [8]. Kawano and Horoupian [9] described arrow-shaped inclusions in cells of the caudate nucleus of patients with various diseases (Alzheimer's disease, progressive hepatic failure of nonalcoholic etiology, metastases of breast cancer in the liver, etc.), including in patients with chronic alcoholism. The authors cited consider that the inclusions they observed do not resemble Mallory's bodies but are products of destruction or age changes in cells and are not characteristic of the changes in alcohol addiction.

The absence of unanimity in the interpretation of the data on these intracellular inclusions in the CNS and in other organs in chronic alcoholism is due to the small number of these observations and makes accumulation of further facts and special investigations in this field essential.

All the hyalin-like or arrow-like inclusions described above, incidentally were found during the investigation of autopsy or biopsy material, and no such discoveries in the course of experimental research have been reported in the accessible literature.

This paper describes an attempt to identify the above-mentioned inclusions in experiments on chronically alcoholized animals.

## EXPERIMENTAL METHOD

Rats weighing 150-200 g were subjected to chronic alcoholization by the inhalation method [7]. To reproduce chronic alcoholic intoxication the animals were kept in an airtight chamber into which 96% ethanol was sprayed in a concentration of 50-200 mg per liter of air. Rats of two groups were kept in the chamber for 12 h daily for 14 and 98 days respectively. The blood ethanol concentration of the animals of both groups averaged 5-6%. A clinical withdrawal syndrome developed in the rats 6-12 h after the 1st inhalation, whether administration lasted 14 or 98 days. The animals were decapitated 1 day after the last inhalation.

Pieces of myocardium from the left ventricle and pieces from the parietal and frontal cortex for electron-microscopic investigation were fixed by the usual methods, embedded in a mixture of Epon and Araldite, and examined in the UÉMV-100L electron microscope.

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0007-4888/85/0007-0959$09.50 

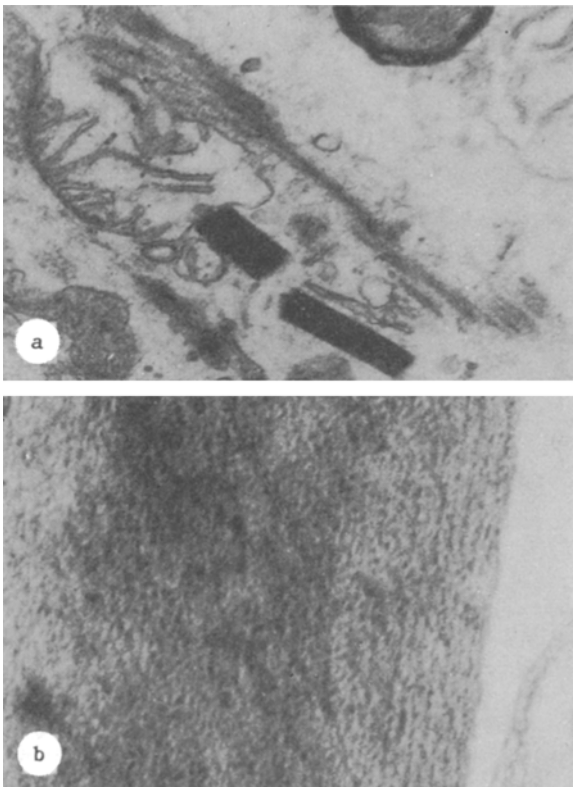


Fig. 1. Inclusions in areas of destruction of a cardiomyocyte: a) type I inclusions; b) fibrils in type I inclusions. Magnification 65,000×.

## EXPERIMENTAL RESULTS

Changes in the myocardial cells were found, in the form of swelling and marked destruction of the mitochondria, widening of the sarcoplasmic reticulum, distinct injury to the contractile system, sequestration of the sarcoplasm, karyolysis, disappearance of glycogen granules and ribosomes, accumulation of lipid inclusions, and diffuse microfocal cardiosclerosis. Pericellular and perivascular edema, swelling of astrocytes, and dystrophic and necrobiotic changes in the neurons, or even their destruction, were observed in the nerve cells of the brain [1].

Rod-shaped and arrow-shaped inclusions were observed in three of 20 animals: in cerebral cortical and myocardial cells in two rats, and in the myocardial cells alone in one rat.

These inclusions were of two types. The type I inclusions were found both in the brain and in the myocardium (mainly intracellularly) in areas of most severe dystrophy and partial destruction of the cells, and they resembled columns or rectangles, not surrounded by a membrane, and without tapering to a point (Fig. 1a). The length of these inclusions varied from 500 to 3000 nm and their width from 200 to 1530 nm. The inclusions were characterized by high electron density and consisted of quite uniform homogeneous material, in which areas of granular material were sometimes observed. However, in some of them, with magnifications of 50,000-80,000, areas of parallel fibrils could be observed (Fig. 1b). As a rule these inclusions were found closer to the cell membrane and they were not connected with the organelles. Only sometimes were they closely applied to the mitochondria, but no connection between them could be noted. In the myocardium, incidentlly, inclusions of this type were more numerous than in the brain.

Type II inclusions were found only in the brain; moreover, in regions of most marked dystrophy or even destruction of the nerve cells, in the latter case they were found extracellularly. The inclusions were similar to the arrow-shaped inclusions described previously [9]. They had the appearance of straight rods, not covered by membranes, with tapering ends about 6200-6600 nm long and 1100-1200 nm wide, consisting of electron-dense granular material with an area of rarefaction in the center (Fig. 2).

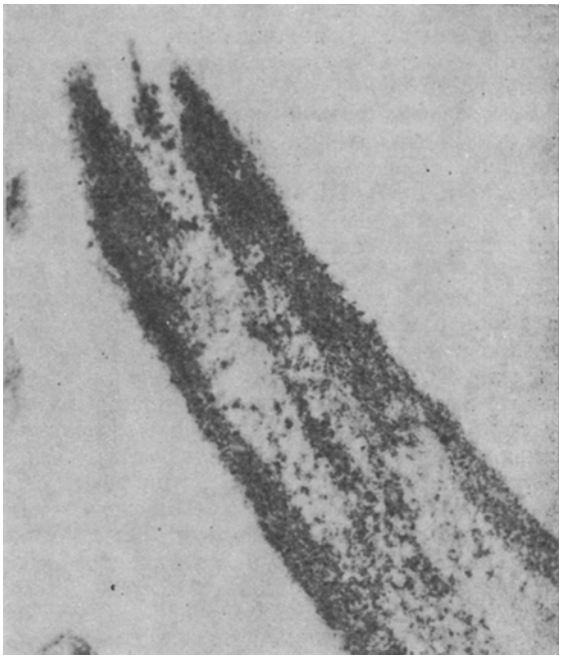

Fig. 2. Type II inclusion in rat brain. Magnification 30,000×.

Only type I inclusions were observed in the myocardium and brain of two animals; type I inclusions were found in the myocardium of one rat and inclusions of types I and II in its cerebral cortex.

No concrete conclusions can yet be drawn from these results on the nature and origin of these inclusions. However, the areas of fibrillary structure observed in the type I inclusions closely resemble the type I of alcoholic hyalin described in the liver [6]. Type II inclusions do not coincide in shape and external appearance with the granular variant of alcoholic hyalin described by the same workers, but they also consist of granular material. The fact that these inclusions are found only in sites of maximal cell damage suggests that the maintenance of a continuously high blood ethanol level in the experimental animals causes marked morphological changes which may amount to destruction of the cells, and as a result of this, to the formation of the above-mentioned inclusions. Some workers interpret these inclusions as the result of a sudden disturbance of cellular metabolism, and of injury to and death of the cells [9].

A state of physical dependence on ethanol obtained in this experiment, and the discovery of inclusions which previously have been observed only in human subjects, combined to make these experimental results comparable with clinical observations arising in the study of the morphology of alcoholic intoxication and alcoholism.

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