# HISTOCHEMICAL INVESTIGATION OF THE PROTEINS OF THE RAT LIVER IN ACUTE ALCOHOLIC INTOXICATION

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It has long been known that alcohol causes severe pathological changes in the liver. However, many facets of this process are still unknown and it is consequently impossible to build up a complete theory of the pathogenesis of the functional and organic disturbances which develop in the liver under the action of alcohol. Certain authors [3, 12, 13, 15] attribute the decisive role in the hepatic damage in alcoholic intoxication to such factors as disruptions of gastrointestinal function and nutritional disturbances; others [6, 9] assign the greatest importance to the direct action of alcohol on the hepatic tissue [1].

In recent years there has been a substantial number of biochemical investigations conducted to study the ways in which alcohol is metabolized and the influence of alcohol on the biochemical processes which occur in the organs of the body [12, 13]. Despite the precision and care with which this work was carried out, the data obtained did not make it possible to establish the microtopography of the biochemical changes in intact microstructures, a knowledge of which is very important for complete determination of the intimate mechanisms of the pathological processes under investigation. There are almost no works on the histochemistry of alcohol-induced damage to the liver (we know of only one such investigation, which was devoted to a study of certain enzymes and glycogen in the rat liver [18]).

We undertook a histochemical investigation of the action of acute alcoholic intoxication on hepatic tissue, since the dynamics of the initial biochemical changes can be best traced in an acute experiment. This work presents the results of an investigation of hepatic proteins, which were isolated by Danielli's tetrazone reaction, in acute alcoholic intoxication.

## EXPERIMENTAL METHOD

White rats (males) weighing from 170 to 220 g were divided into 3 groups. The first group comprised animals which received an ordinary diet (7 rats), the second comprised animals which received 50° ethyl alcohol intragastrically through a lavage tube in a dose of 1.2 ml of absolute alcohol per 100 g of body weight (37 rats), and the 3rd group comprised animals which received an equal volume of water rather than alcohol (8 rats). The animals in the 2nd and 3rd groups were preliminarily starved for 15-17 h. The rats were decapitated 1, 4, 24, and 48 h and 5, 8, 12, and 14 days after administration of the alcohol and 1, 4, and 24 h after administration of the water. Fragments of tissue from the left lobe of the liver were fixed in Carnot's solution. In order to avoid errors associated with varying section-processing conditions, fragments of liver tissue from rats of different groups were embedded in a single paraffin block (3-4 specimens per block). Specimens 4-5 $\mu$ thick were treated with tetrazone by Danielli's method. Additional were stained with Hematoxylin-eosin as a morphological control.

## EXPERIMENTAL RESULTS

The protein distribution in the structures of the hepatic cells exhibited a definite regularity in the livers of the animals of the 1st group.

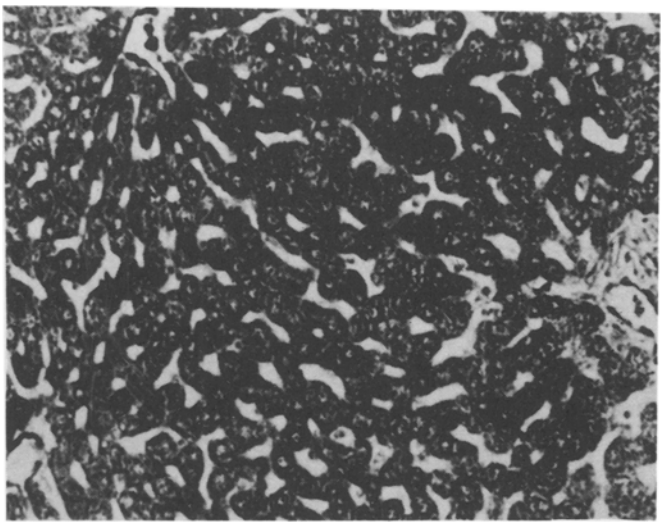

Fig. 1. Protein distribution in the hepatic lobes of rats which did not receive alcohol. Microphotograph. Danielli's tetrazone reaction. 10 × ocular, 20 × objective.

The greatest protein concentration was noted in the nuclear membrane, nucleoli, and large cytoplasmic inclusions, while the lowest concentration was observed in the hyaloplasm and karyoplasm. The small cytoplasmic inclusions and granules and the chromatin grains occupied an intermediate position with respect to protein content.

Our attention was struck by a slight difference in the distribution of cytoplasmic inclusions and granules within the hepatic lobe in individual animals. In certain cases the cells located near the center of the lobe contained small cytoplasmic inclusions arrayed rather densely, the spaces between them being filled with granules, while the cells toward the periphery of the lobe contained larger inclusions arrayed less densely and the quantity of granules was reduced. In other animals the distribution of cytoplasmic inclusions and granules was more or less uniform within the lobe (Figs. 1 and 2a).

The protein concentration in the epithelial cells of the interlobular biliary ducts was lower than in the hepatic cells. Within the epithelial cells the reaction was most intense in the nuclear membrane, nucleolus, chromatin grains, and cytoplasmic brush border and least intense in the karyoplasm. The protein concentration in the muscle layer of the walls of the interlobular vessels was higher than in the tissues of the biliary ducts; the greatest protein concentration was detected in the endothelial layer.

One hour after administration of the alcohol the cytoplasmic protein inclusions and granules were found to be displaced toward the periphery of the cell or nucleus; as a result substantial proteins of the cytoplasm did not contain inclusions and appeared "empty". The extent to which the cytoplasmic structures were stained did not decrease. These changes were observed in cells located along the periphery of the lobe.

After 4 h the cytoplasmic inclusions were more easily detectable, there were fewer inclusions, and the remainder of the cytoplasm was filled with fine granules in the majority of the animals (Fig. 2b). These changes were also more marked in cells located along the periphery of the lobes. Isolated transparent vacuoles appeared in the cytoplasm. It must be pointed out that these changes were not detected in all the animals. In three of the nine rats which received alcohol the character of the hepatic protein distribution and concentration was almost identical to that observed in the animals which received an ordinary diet.

After 24 h hepatic sections from the experimental animals appeared clearer when examined with the unaided eye. Microscopic examination of the hepatic cells revealed a profusion of vacuoles in the peripheral portions of the cytoplasm, increasing in number and size from the center of the lobe toward its periphery (Fig. 3). The fact that the specimens stained positively for fat indicated that they were lipid in character.* Only isolated cells, which

* A detailed description of the dynamics of fatty infiltration of the liver will be given in a subsequent work.

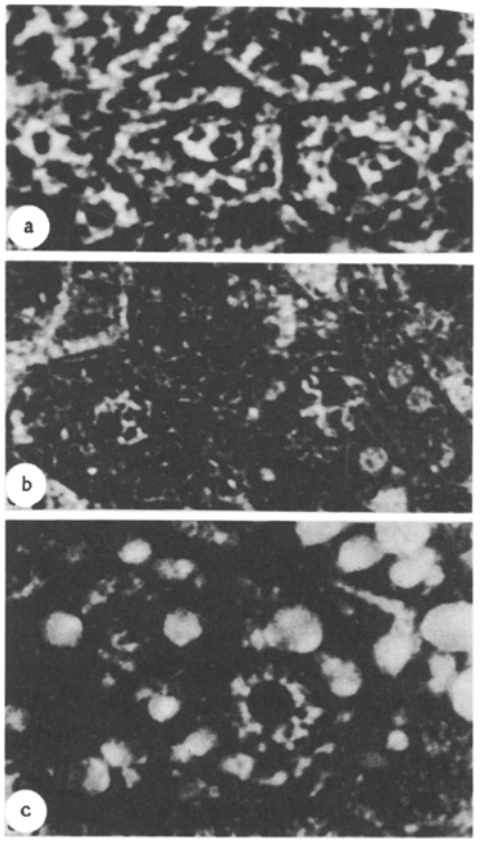


Fig. 2. Protein distribution in a hepatic cell from a rat in normal physiological condition (a) and 4 (d) and 24 (c) h after administration of alcohol. Microphotograph. Danielli's tetrazone reaction. 10 × ocular, 90 × objective (oil-immersion).

were located chiefly near the center of the lobe, did not contain vacuoles. The cytoplasmic proteins were detected in small granules filling the vacuole-free portions of the cytoplasm: there were almost no protein inclusions. The chromatin grains stained with an intensity equal to that of the cytoplasmic granules, while the nuclear membrane and nucleoli stained more darkly. Our attention was struck by the fact that the majority of the nuclei and nucleoli were enlarged (Fig. 2c) and that there were more nucleoli per nucleus. Many nuclei contained 3-4 nucleoli, as many as 7-9 in individual cases. A less marked increase in the intensity of the reaction was noted in the walls of the blood vessels and bile ducts.

After 48 h the cytoplasmic proteins of the hepatic cells were concentrated in a few inclusions and granules in the vicinity of the nucleus or along the periphery of the cell; as a result substantial areas of the cytoplasm appeared "empty." The nuclei were of ordinary size and rich in chromatin, while the number of nucleoli remained greater than normal in individual cases. All cellular structures were stained more intensely than in the preceeding subgroup, but the staining was still not as intense as in the animals which did not receive alcohol. These changes were most marked along the periphery of the lobe and were progressively attenuated toward the center. Increased mitotic activity in the hepatic cells was also characteristic of this group.

Gradual restoration of the character of hepatic protein distribution and content was subsequently observed, terminating toward the 14th day after administration of the alcohol. Increased mitotic activity was detected until the 8th day in individual cases. It must be noted that no relationship was established between the extent of the hepatic tissue damage and the severity of the clinical pattern of alcoholic intoxication in the majority of the experimental animals.

When the livers of the control animals were examined 1 h after administration of water changes similar to those detected after 1 h in the experiment with alcohol were observed in the distribution of cytoplasmic protein structures. However, these changes disappeared within 4 h.

The results of our investigations showed that a single administration of large doses of alcohol has a material influence on the protein distribution and content of the individual hepatic cells and the hepatic lobes as a whole. This is manifested primarily in a change in protein distribution and a decrease in protein content in the cytoplasm of the hepatic cells and a slight increase in the protein content of their nucleoli. The changes are more marked in the cells of the peripheral portions of the lobes.

Recent investigations have shown that the oxidative processes in the liver shift toward glycolysis under the influence of alcohol, while the rate of oxidation in the tricarboxylic cycle is reduced [10, 11, 16-18]. It has been established [17] that these disruptions are the earliest, preceding the disappearance of glycogen from the liver. Our joint investigation (with F. D. Lyubimova) of the change in hepatic glycogen content in rats in acute alcoholic intoxication [4] in turn indicates that the disappearance of glycogen from the liver occurs before changes develop in its protein components. There is thus a definite sequential character to the action of alcohol on hepatic biochemical processes.

These data enable us to assume that the shift in oxidation toward glycolysis which occurs in the hepatic tissues as a result of alcoholic intoxication leads both to increased glycogen consumption and, apparently, to a decrease in the total production of energy-rich substances necessary for the maintenance of normal protein synthesis: this also

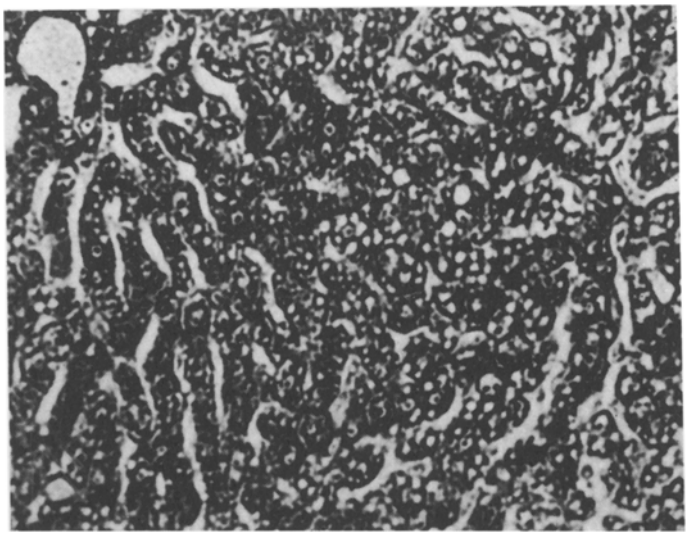

Fig. 3. Protein distribution in the hepatic lobe of a rat 24 h after administration of alcohol. Microphotograph. Danielli's tetrazone reaction. 10 × ocular, 20 × objective.

explains the decrease in the content of these substances in the cells of the hepatic parenchyma. It is also probable that some proteins are consumed to supply energy for the cell's needs after the glycogen disappears.

However, as our observations showed, these processes take place in the cytoplasm, the protein content of the nucleus not only failing to decrease, but even increasing somewhat.

How are we to explain this substantial difference between the reactions of the nucleus and cytoplasm? It has now been solidly established that the nucleus participates in the synthesis of cell proteins [2, 5, 7, 8]. At the same time, it has been demonstrated [14] that tagged carbon introduced into the body with alcohol accumulates rapidly in the cytoplasm of the hepatic cells and substantially more slowly in their nuclei; in the authors' opinion, this indicates that alcohol penetrates the cytoplasm as such, while only its carbon, already incorporated into the general metabolism of the body, enters the nucleus. Hence, it may be assumed that, on entering the cytoplasm, alcohol causes material changes in cytoplasmic metabolism, while its carbon, included in various metabolic products, may be incorporated into the nuclear components without having any substantial influence on the capacity of the nucleus to synthesize proteins. The disruption of cytoplasmic biochemical processes apparently retards protein transport from the nucleus and causes a detrimental accumulation of proteins therein.

These data, as well as the substantial predominance of these disturbances in cells located along the periphery of the hepatic lobes (the first to be "attacked" by alcohol) which we observed, enable us to assume that alcohol has a direct action on the hepatic cells; however, we cannot exlcude the possibility that its action is mediated through general regulatory mechanisms.

## SUMMARY

A histochemical study was made of the effect produced by acute alcoholic intoxication on proteins in the liver of rats. For this purpose 50° ethyl alcohol (1.2 ml of absolute alcohol per 100 g the animal's body weight) was introduced through a stomach tube. The animals were sacrificed in 1, 4, 24 h. and 5, 8, 12 days. The rats given the corresponding amount of water served as control. Sections were treated by Danielli's method with the use of tetrazone compound.

Following the 4th h after the alcohol administration the amount of proteins was progressively decreasing, while the hepatic cell cytoplasm became vacuolized; there was a rise in the nuclear protein content attended by an enlargement of nuclei as well as by an increase in the amount and size of nucleoli. The mentioned changes attained the peak 24-28 h following the alcohol intake. Later, the protein components of hepatic cells were gradually restoring, this being attended by an enhanced mitotic activity; the restoration was completed by the 8th-14th day. Possible mechanisms governing disturbance of the hepatic protein metabolism under the effect of alcohol are discussed.

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