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# EFFECT OF PRELIMINARY FASTING ON DEVELOPMENT OF ACUTE LIVER DAMAGE BY D-GALACTOSAMINE IN RATS

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Parenteral administration of D-galactosamine (GalN) leads to the rapid development of hepatocellular damage, resembling virus hepatitis in its morphological, biochemical, and clinical features [4]. Fasting for 24 h before administration of galactosamine has been shown to diminish considerably elevation of the serum AsAT and AlAT levels and to maintain the liver glycogen at high values [12].

The mechanisms of galactosamine detoxication have not been fully studied. There is no information on excretion of D-glucaric acid with the urine, which is increased when binding with glucaronic acid in the liver is intensified [3]. There is likewise no information on the role of another essential detoxicating mechanism of binding with reduced glutathione (GSH). The role of mixed function microsomal oxygenase (MFMO) in the hepatotoxicity of galactosamine has not been fully explained despite research in this direction [1, 6, 9, 15].

The aim of this investigation was to study the effect of a longer period of preliminary fasting on the development of acute GalN poisoning and also to study changes in some parameters of the detoxicating function of the liver.

## EXPERIMENTAL METHOD

Experiment were carried out on 48 male Wistar rats weighing 350-400 g. The animals were divided into four groups: two control groups consisted of intact rats which fasted for 24 and 72 h immediately before decapitation, and two experimental groups. Animals of the experimental groups received a single intraperitoneal injection of GalN in a dose of 1.855 mmole/kg body weight 24 h before decapitation. During this period the animals were given nothing to eat, and half of them fasted for a further period of 48 h before injection of GalN. Water was provided ad lib. None of the rats died. The rats were decapitated and equal volumes of serum from three animals were pooled for determination of AsAT and AlAT

TABLE 1. AsAT and AlAT Activity in Blood Serum

| Parameter | Control group | Animals | | |
|---|---|---|---|---|
| | | fasting | poisoned with GalN | fasting and poisoned with GalN |
| AsAT, mmoles/(min·liter) | 144 (126—150) | 168 (114—216) | 2100* (720—3720) | 2778* (1380—4080) |
| AlAT, mmoles/(min·liter) | 24 (12—36) | 60 (30—72) | 1200* (660—1800) | 2730* (840—4260) |

Legend. Here and in Table 2: four samples from three animals taken from all groups. Results shown as $\bar{x}$ and interval. *$p < 0.05$ compared with control group.

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TABLE 2. Parameters of Detoxicating Function of the Liver

| Parameter | Control group | Animals | | |
|---|---|---|---|---|
| | | fasting | poisoned with GalN | fasting and poisoned with GalN |
| Cytochrome P-450, mmoles/mg microsomal protein | 0,44 (0,40—0,50) | 0,33 (0,28—0,37) | 0,52 (0,41—0,57) | 0,49 (0,33—0,55) |
| Cytochrome $b_5$, mmoles/mg microsomal protein | 0,29 (0.22—0,33) | 0,24 (0,17—0,32) | 0,53* (0,38—0,63) | 0,45 (0,30—0,55) |
| GSH, mmoles/g | 3,0 (2,1—4,3) | 3,3 (2,8—4,1) | 3,5 (2,9—4,2) | 3,2 (2,9—3,8) |
| D-Glucaric acid in urine, mmoles D-glucaro-1,4-lactone/creatinine | 50,1 (35,7—77,1) | 19,1* (6,7—33,6) | 63,6 (40,0—90,0) | 42,0 (19,2—65,7) |

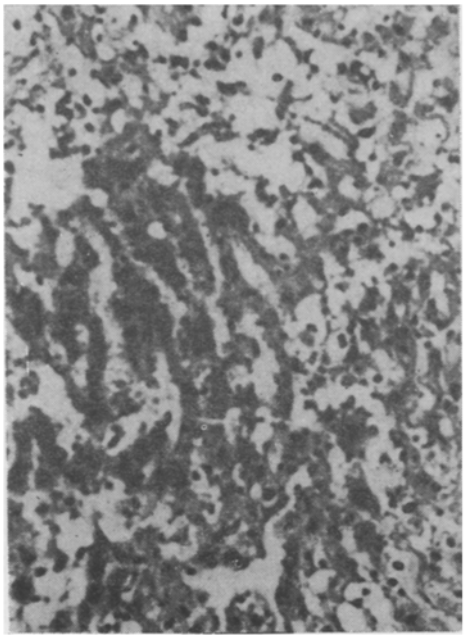

Fig. 1

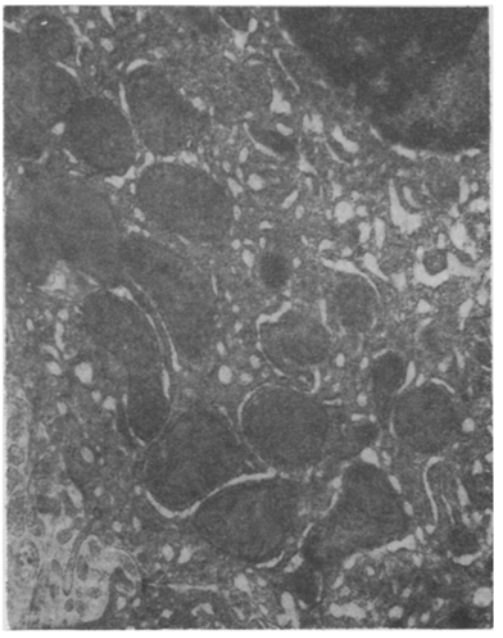

Fig. 2

Fig. 1. Necrotic changes in intermediae zone of liver lobule. He, 200

Fig. 2. Lipid drops. Mitochondria with altered shape and with irregularly arranged cristae. Concentric figures of curves smooth membranes, enclosing part of the cytoplasm, 18,000 ×.

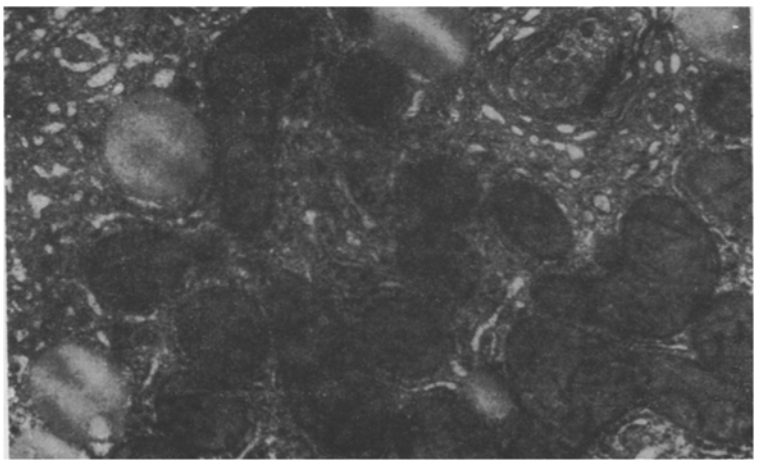

Fig. 3. Hypertrophy and hyperplasia of SER. 11,000 ×.

activity [14]. The microsomal fraction was isolated from liver homogenate [5] and used to determine cytochromes P-450 and $b_5$ [10, 11]. GSH was determined in the supernatant obtained by centrifuging the total liver homogenate at 1000 g for 20 min [2]. D-glucaric acid in the urine was determined quantitatively [8] in a pooled sample of urine from three animals. The results were subjected to statistical analysis by the Mann–Whitney test. Liver tissue was taken from eight animals of each group before perfusion for histological study, after staining with hemalum and eosin (HE). The liver of three animals of each group was investigated electron-microscopically.

## EXPERIMENTAL RESULTS

GalN gave rise to marked histological and electron-microscopic changes in agreement with results obtained by other workers [4]. Dystrophic changes, venous stasis, and inflammatory proliferation were observed in the periportal space and lobules. Focal centrilobular necrosis was found in some places. Preliminary fasting for 48 h aggrevated the picture of the morphological changes induced by GalN. Necrosis was observed most often in the intermediate zones of the lobule (Fig.1). Foci of necrosis crossing from one lobule into another also were found. Areas of inflammatory infiltration were more numerous. Mitochondrial polymorphism was more evident. The number of pycnotic nuclei, lipid droplets, and autophagolysosomes was increased (Fig. 2). Hypertrophy and hyperplasia of the smooth endoplasmic reticulum (SER) were found on a considerable scale (Fig. 3), even though fasting alone without the toxic agent led to hypotrophy and hypoplasia of SER.

Injection of GalN caused an excessive rise of AsAT and AlAT in the serum (Table 1). After preliminary fasting, these enzyme activities increased even more, but the differences were not statistically significant. Cytochrome P-450 and GSH in the liver and D-glucaric acid in the urine were unchanged by GalN (Table 2). Preliminary fasting caused no change in their values in animals receiving GalN. The cytochrome $b_5$ level rose after injection of GalN (Table 2).

A longer period of preliminary fasting had an unfavorable effect on the general development of GalN poisoning. Dystrophic, necrotic, and inflammatory changes were more marked and the ultrastructural disturbances more severe. Aminotransferase activity showed a tendency to increase still more. These results conflict with those obtained by Pickering [12], who found that preliminary fasting has a favorable effect. The disagreement can be explained both by the longer duration of fasting of the animals in the present investigation and by the fact that the experimental animals were older. Platt and co-workers [13] found that more adult rats are more susceptible to this form of poisoning.

GalN is known to become toxic only after metabolic conversion by enzymes of the galactose metabolic chain, leading to exhaustion of uridine derivatives [4]. It can be tentatively suggested that the longer period of fasting in the present experiments led to a greater fall of UTP and UDP levels, and this caused the more severe disturbances.

The results show that conjugation of GSH and glucouronide formation, assessed directly by determination of the GSH level in the liver and the D-glucaric acid level in the urine, do not play an essential role in the detoxication of GalN.

Elevation of the cytochrome $b_5$ level also was found by Shigeta [15] and Matyushin et al [1]. The protective action of preliminary administration of phenobarbital [6] and the changes in SER and cytochrome $b_5$ suggest that MFMO participates in the pathogenetic mechanisms of GalN poisoning, but its precise method of action is still unclear.

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# PRIMARY STRUCTURE OF HISTIDINE DECARBOXYLASE

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It is impossible to understand the mechanism of action of enzymes without a detailed study of their structural organization. It was for this reason, therefore, that we decided to investigate the primary structure of the enzyme histidine decarboxylase (HD) of Micrococcus sp. n., responsible for the catalytic decarboxylation of L-histidine, with the formation of the physiologically active substance, histamine [7].

The aim of this investigation was to summarize the results of a study of the primary structure of HD from Micrococcus sp. n. and the compare its primary structure with that of the HD from another bacterium, Lactobacillus 30a, as established by a group of American investigators [9, 12].

HD of Micrococcus sp. n. has mol. wt. of about 100 kD and consists of 3α- and 3β-polypeptide chains, differing in their molecular weight and amino-acid composition [10]. One difference between bacterial HD and pyridoxal-dependent HD of animal tissues is that the role of coenzyme in the former is played by the pyruvate residue, the carbonyl group of which takes part in the catalytic act like the carbonyl group of pyridoxal phosphate.

## EXPERIMENTAL METHOD

Pyruvate residues in the bacterial HD molecule are covalently bound with N-terminal amino acids of α-chains, and determination of the N-terminal sequence of amino acids in this chain therefore required the use of methods unblocking the α-amino group of the N-terminal amino acid: conversion of the pyruvate residue into alanine by reductive amination or its removal by the reaction with o-phenylenediamine [1]. Analysis of the N- and C-terminal amino acid sequences and also of amino-acid sequences in the various tryptic peptides of the α-chain (maleylated and nonmaleylated) was insufficient to establish its primary structure [8, 11]. Further structural analysis was undertaken of large fragments of the α-chain obtained by chemical cleavage of the protein molecules: cleavage beyond tryptophan residues (with iodosobenzoic acid), cleavage at cysteine residues (with nitrothiocyanobenzoic acid), and restricted acid hydrolysis (with acetic acid) [3-5]. On the basis of the results of these investigations, the primary structure of the α-chain could be identified and it could be shown to consist of 225 amino-acid residues.

The β-chain of HD consists of 79 amino-acid residues and differs from the α-chain is not containing proline, histidine, tryptophan, and cysteine. The primary structure of the β-chain was established from the results of analysis of amino-acid sequences: of tryptic peptides of maleylated and nonmaleylated protein, and of the N- and C-terminal regions of the chain [2, 11].

## EXPERIMENTAL RESULTS

The identified primary structures of the α- and β-chains together form the complete primary structure of HD of Micrococcus sp. n. (see Scheme 1).

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