D-Galactosamine Hepatitis
I. Hepatocellular Injury and Fatty Liver Following A Single Dose (35648)

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(Introduced by H. J. Zimmerman)

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Although injury resulting in extensive hepatocellular necrosis can be produced in animals with a variety of viral agents, the histological and biochemical features of experimental viral hepatitis are not completely analogous to those observed in viral hepatitis in man (1). This lack of histological similarity has, therefore, limited the usefulness of animal models to aid us in understanding the pathophysiology of hepatic injury and disturbed liver function in the human disease.

In a recent study, Keppler *et al.* (2) reported the production of hepatocellular necrosis in rat liver following multiple injections, over a 24-hr period, of the amino sugar, D-galactosamine. The histological features of D-galactosamine hepatitis as described by these authors suggested a striking similarity to the histopathological changes in acute human viral hepatitis. The present study confirms the hepatotoxicity of D-galactosamine for rat liver and indicates, moreover, that a single dose (1.5 g/kg of body wt) is effective in producing widespread ultrastructural and biochemical evidence of hepatocellular necrosis commencing within 6 hr of administration of the drug. Our biochemical and ultrastructural data have failed, however, to confirm the original impression that hepatitis produced by D-galactosamine is similar to viral hepatitis. The demonstration of triglyceride accumulation in the liver and hyperplasia of the smooth endoplasmic reticulum accompanying hepatic cell necrosis suggests that the lesion induced by D-galactosamine more closely resembles that produced by chemical toxins such as CCl_4 (3) than that associated with human or experimental hepatitis virus infections.

Materials and Methods. Female rats (CD strain), weighing between 180 and 200 g, were obtained from Charles River Breeding Laboratories. D-galactosamine HCl, D-glucosamine HCl, and 2-deoxygalactose were obtained from Pierce Chemical Co., Rockford, Ill., and Sigma Chemical Co., St. Louis, Mo. D-Galactosamine HCl, dissolved in isotonic saline, was administered by ip injection in a single dose of 1.5 g/kg of body weight. Control rats received either equivalent volumes of isotonic saline or equimolar D-glucosamine or 2-deoxygalactose in isotonic saline by single ip injection. Following administration of the test drugs or saline, all animals were fasted but allowed free access to water.

At intervals between 2 and 24 hr, the animals were sacrificed by exsanguination from the abdominal aorta. Serum was assayed in duplicate for glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) by the spectrophotometric methods of Karmen (4), Wroblewski and LaDue (5), and enzyme activity was expressed as units per milliliter per minute. Hepatic lipids were extracted by the method of Folch *et al.* (6) and lipid classes were fractionated by thin-layer chromatography on silicic acid plates. Triglyceride content was measured by the method of Van Handel and Zilversmit (7). Values obtained are expressed as milligrams of triglyceride per gram of wet weight of liver.

Liver tissue was rapidly excised; and portions were prepared for light microscopy by

TABLE I. Serum Enzymes in Rats Injected with D-Galactosamine.[a]

Time after injection (hr)	SGOT[b]		SGPT[b]	
	Control	D-Galactosamine	Control	D-Galactosamine
2	62.0 ± 7.4 (4)	59.5 ± 6.2 (4)	19.5 ± 1.9 (4)	17.8 ± 1.8 (4)
3	74.7 ± 3.2 (3)	88.6 ± 12.1 (5)	19.0 ± 0.6 (3)	35.0 ± 3.5 (5)
6	72.8 ± 5.3 (6)	423.3 ± 131.9 (12)	17.0 ± 2.8 (6)	165.0 ± 35.1 (12)
24	60.9 ± 5.3 (10)	3470.0 ± 919.5 (10)	11.3 ± 1.6 (10)	1049.5 ± 295.0 (10)

[a] Female Charles River CD strain rats, 180–200 g, were fasted for 1–4 hr and then injected ip with a single dose of D-galactosamine, 1.5 g/kg of body weight.

[b] SGOT and SGPT (glutamic oxaloacetic transaminase and glutamic pyruvic transaminase) (units/ml/min) (4, 5). Results are mean values ± SE with the number of animals in each group in parentheses. Control animals received an ip injection of normal saline.

fixation in 10% buffered formalin. For electron microscopy, liver specimens were fixed in 2% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.5, for 2 hr at 4°, followed by postfixation in phosphate-buffered 1% osmium tetroxide for 1 hr at 4°. After fixation, the tissue was dehydrated in graded alcohols and embedded in epoxy resins using conventional techniques (8). Ultrathin sections were examined with an RCA-EMU 4A electron microscope.

Results. Serum enzymes after D-*galactosamine.* SGOT and SGPT were assayed at 2, 3, 6, and 24 hr after the ip injection of D-galactosamine, D-glucosamine, 2-deoxygalactose or isotonic saline. A progressive increase in the serum levels of both enzymes was evident only in those animals injected with D-galactosamine (Table I). As early as 6 hr after injection of D-galactosamine, there was a significant increase in SGOT and SGPT compared with controls; and even at 3 hr the SGPT was twice normal. A striking enzyme elevation was apparent at 24 hr in the animals who had received D-galactosamine whereas there was no increase in the serum level of either enzyme in rats injected with D-glucosamine or 2-deoxygalactose.

Light microscopy. The elevation of SGOT and SGPT suggested enzyme release from damaged liver cells. Light microscopic examination of hepatic tissue disclosed hepatocellular injury within 4 hr of a single injection of D-galactosamine. At this time, the injury consisted of single cell necrosis evidenced by acidophilic degeneration of the cytoplasm and the occasional presence of balloon cells. These changes were more evident at 6 hr at which time foci of polymorphonuclear inflammatory cell infiltration were present in areas of obvious hepatocellular necrosis. By 10 hr, there was widespread histological evidence of hepatic cell necrosis and inflammation. The changes were similar to those described by Keppler *et al.* (2) at 25.5 hr. The hepatocytes showed a striking variation in size and staining qualities. Balloon cells and acidophilic degeneration were seen frequently and acidophilic bodies in various stages of formation were evident. Hepatic sinusoids were narrowed by cytoplasmic debris, acidophilic bodies, inflammatory cells, and hyperplastic endothelial cells. In focal areas, liver cells were completely replaced by an inflammatory infiltrate consisting of polymorphonuclear leukocytes, mononuclear cells, and occasional eosinophils. By 15 hr the histological changes were more extensive, now involving most cells. In addition to the evidence of hepatocellular necrosis and inflammation, many cells now contained conspicuous cytoplasmic vacuoles of varying size suggesting the accumulation of lipid (Fig. 1). Sections, at 20 and 24 hr showed a further increase of the cytoplasmic vacuolization and the cellular necrosis and inflammation. Even at 24 hr the inflammatory response was focal, infiltrating areas in which complete necrosis of hepatocytes had occurred. The injection of D-glucosamine or 2-deoxygalactose did not result in any obvious histological changes in the liver at 24 hr. Light microscopic examination of other tissues, specifically small intestine, cardiac and skeletal muscle, disclosed no evi-

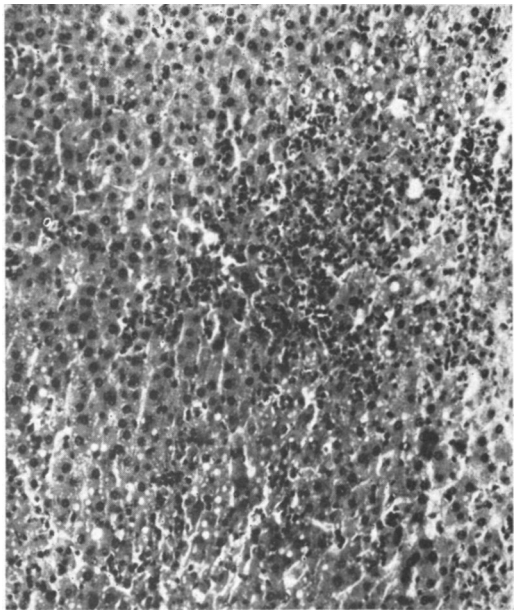

FIG. 1. Focal hepatocellular necrosis and inflammation 15 hr after a single ip injection of D-galactosamine. Note the small vacuoles in the cytoplasm of many cells indicating the presence of lipid. Hematoxylin and eosin ×150.

dence of injury following D-galactosamine administration.

Hepatic triglycerides in D-galactosamine hepatitis. The microscopic observation of cytoplasmic vacuoles in the histological sections from livers of D-galactosamine-treated rats indicated that the toxicity of the amino sugar probably resulted in an accumulation of hepatic lipids. The quantitative analysis of liver triglycerides indicated a marked increase 24 hr after the injection of D-galactosamine (Table II). Mean values for hepatic triglycerides at 24 hr in the D-galactosamine livers were 14.6 mg/g of wet weight compared with 3.3 mg/g in controls. Hepatic triglycerides were not elevated at 6 hr in the D-galactosamine-treated animals nor were they abnormal at 24 hr in rats injected with either D-glucosamine or 2-deoxygalactose.

TABLE II. Hepatic Triglycerides in Rats Injected with D-Galactosamine.[a]

Time (hr)	Control	D-Galactosamine
6	4.2 ± 0.3 (6)	3.8 ± 0.4 (6)
24	3.3 ± 0.7 (8)	14.6 ± 1.2 (10)

[a] Triglycerides (mg/g of wet wt of liver). Values are means ± SE with the number of animals in parentheses. Experimental procedure is indicated in Table I.

Electron microscopy. The ultrastructural aspects of D-galactosamine hepatitis were studied at intervals between 6 and 24 hr following the ip injection of a single dose of the amino sugar. At 6 hr many hepatocytes showed ultrastructural evidence of hepatocellular injury which was manifest by the formation of focal areas of cytoplasmic degeneration (Fig. 2). These areas consisted of membrane-bound autophagic vacuoles containing remnants of damaged mitochondria, glycogen, fibrillar and granular cellular debris. At 24 hr, in addition to extensive cytoplasmic necrosis, there was obvious evidence of lipid accumulation (Fig. 3). Many cells were filled with lipid droplets of varying size including small membrane-bound liposomes and lipid particles of much larger size which were formed by the coalescence of smaller droplets (Fig. 3). By 24 hr the focal cytoplasmic injury seen earlier had now progressed to include large portions of the hepatic

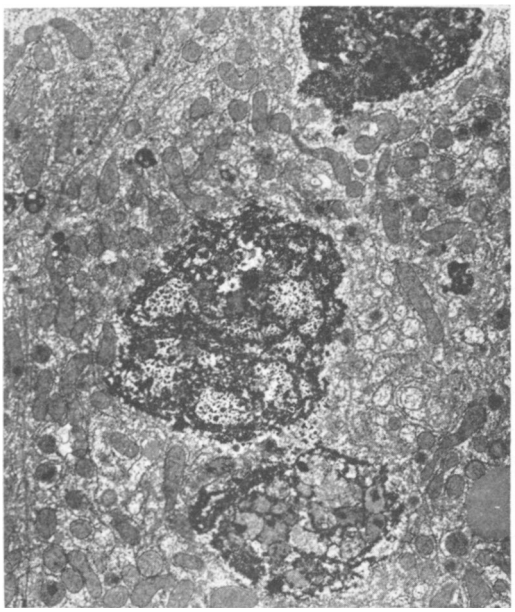

FIG. 2. Electron micrograph of the liver 6 hr after the injection of D-galactosamine. Focal injury consisting of membrane-bound areas of cytoplasmic degeneration is present. Note the necrotic mitochondria and presence of glycogen particles. ×5000.

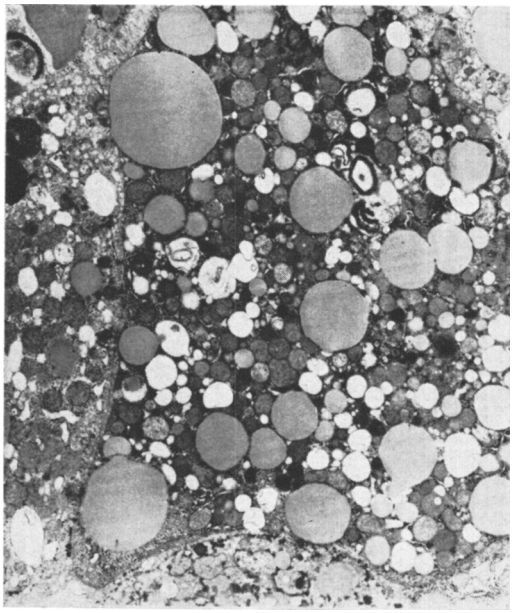

FIG. 3. Electron micrograph 15 hr after D-galactosamine demonstrating the marked accumulation of lipid droplets. Note the variation in size of lipid droplets. ×5360.

lobules which were now completely necrotic and filled with a mixture of amorphous cytoplasmic debris and subcellular organelles in varying stages of degeneration (Fig. 4). Among the prominent changes were degranulation of the rough endoplasmic reticulum resulting in the accumulation of large numbers of free ribosomes; dilatation of the rough endoplasmic reticulum and hyperplasia of the smooth endoplasmic reticulum; marked depletion of glycogen and a variety of mitochondrial abnormalities. A detailed report of the sequential ultrastructural changes following a single ip injection of D-galactosamine is in preparation (9).

Discussion. The present experiments confirm previous reports of Keppler *et al.* (2) and Medline *et al.* (10) of the hepatocellular toxicity of the amino sugar, D-galactosamine. In earlier studies, a total dose of 1.5 g/kg of body weight was administered as 6 multiple injections over 24 hr. Modification of this schedule to a single ip injection of the total calculated dose enabled us to discern definite ultrastructural and biochemical evidence of hepatotoxicity within 6 hr.

Keppler and co-workers stressed the striking similarity between D-galactosamine hepatitis and the histological changes in human viral hepatitis. Light microscopic examination of the liver in our experiments indicated that this histological similarity was more apparent than real, since vacuolization of the cytoplasm suggesting lipid accumulation was evident as early as 15 hr after injection. The presence of increased lipid within hepatocytes was confirmed both by electron microscopy and by chemical determinations of hepatic triglyceride content. Thus, the one histopathological feature, *i.e.*, absence of fat, which is often used to distinguish viral hepatitis from other forms of hepatocellular injury, was not present in D-galactosamine hepatitis. The combination of hepatic triglyceride accumulation in association with widespread hepatocellular necrosis and inflammation indicates that the D-galactosamine hepatitis more closely resembles that induced by chemical toxins such as CCl_4 than viral hepatitis.

The ultrastructural features of D-galactosamine hepatitis at 24 hr after a single injection were quite similar to those described by Medline *et al.* (10) after 6 injections over a 24-hr period. The marked glycogen depletion, hyperplastic smooth endoplasmic reticulum, mitochondrial abnormalities, and lipid accumulation are features of D-galactosamine injury which are different than the ultrastructural changes usually described in viral hepatitis (11). Of particular interest is the ultrastructural evidence of hepatocellular damage within 6 hr of administration of D-galactosamine. Focal cytoplasmic degeneration at 6 hr progressed to involve the entire cell and by 24 hr all cells showed evidence of either marked lipid accumulation or necrosis.

As is still true for CCl_4 and other hepatotoxins (12), the actual mechanism of liver cell injury induced by D-galactosamine is not known. The presence of a mixed type of injury (lipid accumulation and cellular necrosis) indicates that the specific injurious processes will be difficult to elucidate. Thus, for example, there is still controversy regarding the mode of action of those agents which produce pure defects in lipoprotein transport in the absence of overt toxic

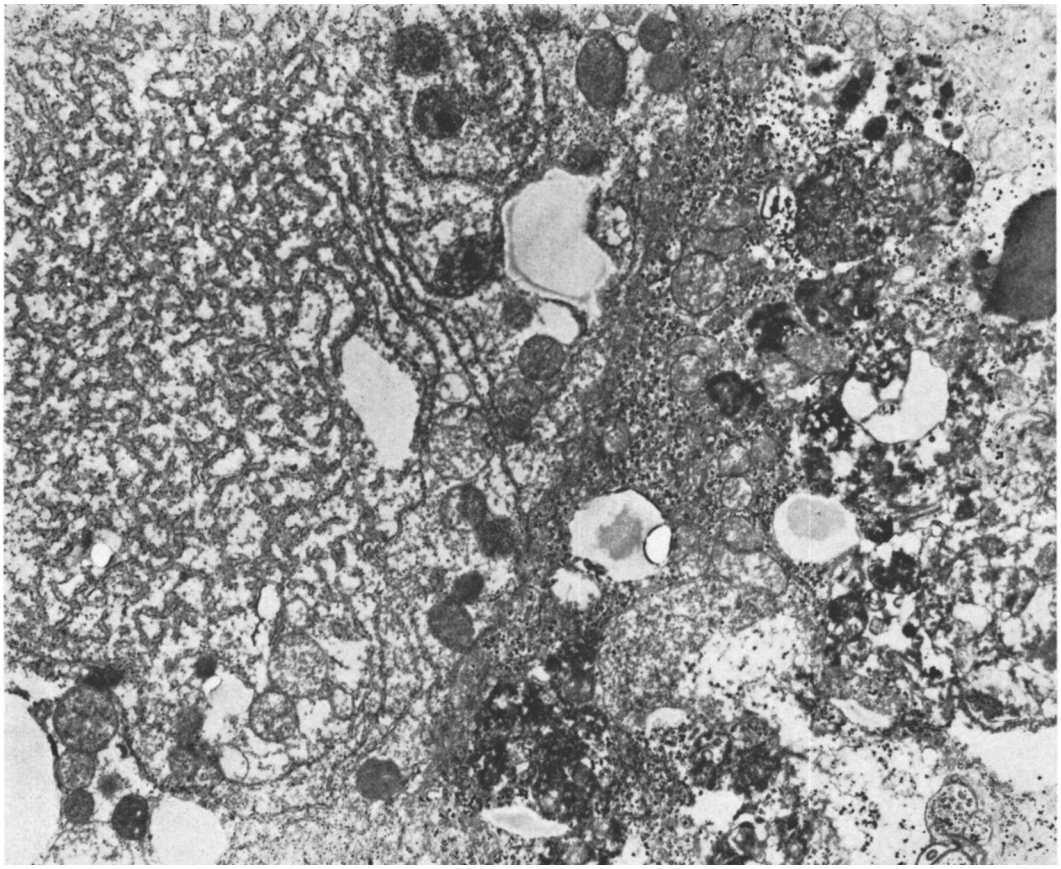

FIG. 4. Electron micrograph 24 hr after D-galactosamine. Degranulation and fragmentation of the rough endoplasmic reticulum is evident at the left of the micrograph. Note the fragmented cytoplasm containing subcellular organelles in varying stages of degeneration, free ribosomes and amorphous debris. ×12,800.

changes. Recent studies indicate that the fatty liver induced by ethionine may not be entirely due to inhibition of protein synthesis with resultant defects in lipoprotein formation but may reflect a more generalized transport defect. Thus, Judah and Nicolls (13) attribute the defect in lipoprotein secretion to a low intracellular potassium concentration and have produced evidence that low K^+ ion level can interfere with lipoprotein transport.

Although D-galactosamine is a constituent of glycoproteins and polysaccharides, the presence of the free amino sugar within tissues is not known (14). Preliminary evidence suggests that galactosamine or a metabolic derivative may interfere with the biosynthesis of glycogen. Following the administration of D-galactosamine, liver glycogen at 25.5 hr is reduced to about 3% of normal (2). The hepatic concentration of glucose-6-phosphate, free glucose, and uridine-diphosphoglucose, uridine and adenine nucleotides, and ATP are also reduced following D-galactosamine treatment (2). The decrease in uridine-diphosphoglucose suggests a defect in the biosynthesis of glycogen since in the liver glycogen synthesis is mainly through the uridine-diphosphoglucose pathway; whereas glycogen breakdown is through the phosphorylase pathway which would not be directly effected by the decrease in uridine nucleotides.

Perfusion of isolated rat liver with D-galactosamine results in a decrease in hepatic uridine nucleotide levels, presumably due to trapping of uridine phosphates by formation

of UDP-amino sugar (15). Keppler and Decker (16) found that galactosamine administration led to an accumulation of galactosamine-1-phosphate with subsequent inhibition of hepatic galactosamine metabolism.

The accumulation of galactosamine-1-phosphate, and the presence of fatty infiltration of the liver recalls the accumulation of galactose-1-phosphate and fatty infiltration in the human disease galactosemia. The mechanism whereby the accumulation of galactose or galactosamine metabolites results in hepatic cell injury and fatty infiltration has not yet been elucidated.

Bekesi *et al.* (17) studied the effects of a number of amino sugars on the incorporation of ^{14}C-labeled lysine, uridine, and thymidine into sarcoma 180 ascites cells *in vitro*. D-Galactosamine, as well as D-glucosamine, D-mannosamine, and D-mannose were the only compounds showing inhibition of protein, RNA, and DNA synthesis. In the present study, D-glucosamine administration *in vivo* had no effect on serum enzyme levels or histological features of the liver. Thus, the relevance of *in vitro* studies to *in vivo* effects of amino sugars is not clear.

Since fatty liver has been produced by a number of chemical agents which result in diminished hepatic ATP concentration, and administration of ATP or adenine can prevent the development of fatty liver in some experimental models (18), the reduction of hepatic ATP in the galactosamine-treated animal may play a role in the development of fatty liver described here. Whether or not D-galactosamine treatment results in impaired lipoprotein synthesis or release has yet to be determined.

Summary. The ip administration of 1.5 g/kg of D-galactosamine HCl in a single injection resulted in profound histological and biochemical evidence of hepatocellular injury.

Electron microscopy disclosed focal cytoplasmic degeneration 6 hr after injection and this was paralleled by a progressive increase in the serum levels of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase. Progressive cellular injury was accompanied by lipid accumulation, hyperplasia of the smooth endoplasmic reticulum, and organelle injury involving almost every cell. Although liver triglyceride levels were normal 6 hr following D-galactosamine administration, at 24 hr significant hepatic triglyceride accumulation was demonstrated. Serum enzyme levels, histologic features of the liver, and hepatic triglyceride content remained normal in animals treated with equimolar D-glucosamine HCl and 2-deoxygalactose.

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