D-Galactosamine Hepatotoxicity
II. Mechanism of Fatty Liver Production[1] (35837)

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(Introduced by H. J. Zimmerman)

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In a previous report from this laboratory (1) it was demonstrated that a single dose of D-galactosamine (1.5 g/kg of body wt) produced ultrastructural and biochemical evidence of hepatocellular necrosis and fatty liver in the rat. Hepatic triglycerides were increased threefold at 24 hr and a preliminary electron microscopic study disclosed lipid accumulation 15 hr and hepatocellular injury 6 hr following injection of D-galactosamine.

In a more extensive ultrastructural study (2) alterations of the endoplasmic reticulum and lipid accumulation were observed within 4 and 6 hr, respectively, of D-galactosamine administration. The recognition of ultrastructural changes in the organization of the rough endoplasmic reticulum suggested that the fatty liver produced by D-galactosamine might be related to a decrease in the secretion of lipoproteins secondary to inhibition of protein synthesis. The present study was designed to elucidate the pathogenesis of fatty liver in this experimental model by investigating the effects of D-galactosamine on protein synthesis and lipoprotein secretion.

Our results indicate that D-galactosamine produces an inhibition of protein synthesis, decreased secretion of beta-lipoproteins and impaired post-Triton WR 1339 hypertriglyceridemia. The accumulation of hepatic lipid induced by D-galactosamine was not modified by the administration of adenine, adenosine-5′-triphosphate (ATP), uridine, or its nucleotides.

Materials and Methods. D-Galactosamine HCl, D-glucosamine HCl, adenine sulfate, ATP, uridine, uridine-5′-triphosphate (UTP), uridine-5′-diphosphate (UDP), and uridine-5′-diphosphoglucose (UDPG) were purchased from Sigma Chemical Co., St. Louis, Mo. Triton WR 1339 was obtained from Winthrop Laboratories, N.Y. Uniformly ^{14}C labeled *l*-leucine (260 mCi/mmole) was purchased from New England Nuclear Corp., Boston, Mass. Sprague-Dawley female rats (180–200 g) were obtained from the Animal Research Center, Braintree, Mass.

D-Galactosamine HCl, dissolved in isotonic saline, was administered by intraperitoneal (ip) injection in a single, standard dose of 1.5 g/kg of body weight. Control rats received equimolar D-glucosamine in isotonic saline or equivalent volumes of isotonic saline by single ip injection. All animals were fasted throughout the experimental period but allowed free access to water. Groups of D-galactosamine treated rats were also injected subcutaneously or by ip injection with either 120 mg of adenine, 300 mg of ATP, 300 mg of uridine, 450 mg of UDP, 450 mg of UTP, or 450 mg of UDPG in equally divided doses at 0, 2, and 4 hr following D-galactosamine administration. Twenty-four hours after the first injection, the livers were excised and hepatic triglycerides were measured as previously described (1).

Triton WR 1339, in a dose of 100 mg/rat, in saline, or an equal volume of saline alone was injected into the saphenous vein of rats treated 6 or 20 hr earlier with the standard dose of D-galactosamine, D-glucosamine, or saline. Ninety minutes later the animals were bled from the abdominal aorta, and plasma was assayed for triglyceride by the method of

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Van Handel and Zilversmit (3).

Serum lipoprotein electrophoresis on cellulose polyacetate or paper was performed by the method of Beckering and Ellefson (4), and Frederickson *et al.* (5), 6 and 16 hr after administration of the standard ip dose of D-galactosamine or saline.

At 1, 3, or 16 hr after administration of D-galactosamine, D-glucosamine, or saline, 20 μCi of 1-^{14}C-leucine was given by ip injection. One hour later the livers were excised, homogenized in ice-cold 0.25 *M* sucrose, and the microsomal fraction was isolated by differential centrifugation as previously described (6). Aliquots (0.1 ml) of microsomal suspensions were applied to 3 MM Whatman filter paper discs, fixed in 10% trichloroacetic acid, and then extracted sequentially by the method of Mans and Novelli (7). Radioactivity was determined in a liquid scintillation spectrometer employing toluene based scintillation fluid. Microsomal protein was measured in additional aliquots by the method of Lowry *et al.* (8).

Results. As shown in Table I, hepatic triglycerides at 24 hr were increased more than 3-fold in D-galactosamine-treated animals when compared to saline or D-glucosamine-treated controls. The administration of adenine, ATP, uridine, uridine nucleotides, or UDPG to groups of rats had no inhibitory effect on the fatty liver induced by D-galactosamine. Although not shown, D-glucosamine had no effect on hepatic triglyceride accumulation when it was administered concomitantly with D-galactosamine in equimolar quantities.

TABLE I. Hepatic Triglycerides 24 hr after D-Galactosamine (1.5 g/kg) and the Effect of Additional Treatment.[a]

Treatment group	Additional agent[b] (mg)	Liver triglycerides
Saline	Saline	6.3 ± 1.0 (6)
D-Galactosamine	Saline	20.6 ± 2.9 (6)
D-Glucosamine	Saline	6.2 ± 0.8 (4)
D-Galactosamine	Adenine, 120	23.3 ± 2.4 (4)
	ATP, 300	31.5 ± 4.0 (4)
	Uridine, 300	33.6 ± 7.9 (4)
	UDP, 450	25.9 ± 3.4 (3)
	UTP, 450	22.9 ± 9.4 (3)
	UDPG, 450	24.7 ± 4.3 (3)

[a] Triglycerides (mg/g of wet wt of liver) are given as mean ± SE; number of animals in the group is given in parentheses.

[b] Administered subcutaneously or ip, in equally divided doses at 0, 2, and 4 hr after the initial treatment.

Plasma triglycerides, 90 min after intravenous saline, were similar at 6 and 20 hr after ip injection of D-galactosamine, D-glucosamine, or saline (Table II). The expected 90-min post-Triton hypertriglyceridemia observed in control rats was strikingly reduced at 20 hr in D-galactosamine-treated animals; however, no difference in post-Triton response was detected 6 hr after ip injection of D-galactosamine when compared to ip injected controls.

Electrophoresis of serum lipoproteins revealed a marked decrease in beta-lipoproteins 16 hr after ip D-galactosamine. In contrast, at 6 hr after D-galactosamine administration, the serum lipoprotein pattern on cellulose polyacetate or paper was indistinguishable from that observed in control groups.

As indicated in Table III, ^{14}C-*l*-leucine incorporation *in vivo* into microsomal protein was inhibited 40% at 4 hr and 57% at 17 hr following ip administration of D-galactosamine. No inhibition was demonstrable 2 hr after injection of D-galactosamine. ^{14}C-*l*-leucine incorporation in D-glucosamine- and saline-treated control animals were similar at each time period studied.

Discussion. Fatty liver has been produced by a variety of agents which result in a decreased hepatic ATP concentration, inhibition of protein synthesis, and impaired lipoprotein formation. Ethionine is a prototypic agent in this respect. The administration of ATP (9, 10), or an ATP precursor, such as adenine (11), completely protects against the ethionine-induced fatty liver in rats, and reverses the ethionine-induced inhibition of protein synthesis (10). In other models, *e.g.*, azaserine and carbon tetrachloride, administration of ATP also reverses the hepatic triglyceride accumulation. However, fatty liver production by these two models is not specifically dependent on inhibition of protein synthesis since ATP repletion did not

TABLE II. Effect of D-Galactosamine on Plasma Triglycerides in Rats Injected Intravenously with Saline or Triton WR 1339.[a]

Treatment group	Time (hr)[b]	Saline	Triton
Control[c]	6	24.1 ± 6.9 (3)	209.3 ± 21.4 (3)
D-Galactosamine	6	18.7 ± 1.2 (3)	169.5 ± 22.1 (3)
Control	20	24.5 ± 2.9 (4)	208.5 ± 8.0 (3)
D-Galactosamine	20	27.3 ± 5.6 (3)	90.2 ± 8.5 (4)

[a] Plasma triglycerides (mg/100 ml) are given as mean ± SE; number of animals in each group is given in parentheses.

[b] Elapsed since ip treatment.

[c] Animals injected ip with either D-glucosamine or saline.

alter the inhibition of protein synthesis evoked by these agents (10). Hepatic ATP, as well as uridine, uridine nucleotides, and UDPG levels are reduced in the D-galactosamine-treated rat (12). Although fatty liver is always produced by D-galactosamine (1), in the rat, the administration of adenine, or ATP or a variety of uridine derivatives, as shown here, did not prevent the development of lipid accumulation.

The post-Triton WR 1339 and lipoprotein electrophoresis data presented here suggest that hepatic secretion of lipoprotein is impaired during the period of hepatic triglyceride accumulation, although, unexpectedly, plasma triglycerides were not depressed. Protein synthesis *in vivo* is inhibited several hours before impaired hepatic lipoprotein release can be demonstrated. The mechanism of inhibition of protein synthesis by D-galactosamine was not studied in the present experiments. Monier and Wagle (13) have suggested that decreased availability *in vivo* of cofactors might be responsible since protein synthesis *in vitro* (in the presence of ATP, an ATP generating system, GTP and magnesium chloride), was intact in microsomes prepared from D-galactosamine-treated animals.

Although inhibition of synthesis of the protein moiety of lipoprotein may be the primary factor in the D-galactosamine-induced fatty liver, other mechanisms such as impaired lipoprotein assembly or secretion may be contributory. A defect in the secretion of lipoprotein has been postulated as the mechanism of the fatty liver induced by orotic acid. In this model an impairment of hepatic lipoprotein release has been demonstrated yet the synthesis of the apoprotein is not affected (14).

Judah and Nicholls (15) have recently reported that the intracellular hepatic concentration of potassium ions found in ethionine-treated animals is sufficiently low to result in a significant reduction in the rate of transport of serum albumin and lipoproteins across the hepatocyte plasma membrane. Thus the fatty liver induced by ethionine may reflect a generalized transport defect, as well as impaired synthesis of proteins and lipoproteins.

Evidence of a defect in the intrahepatic transport of albumin and lipoproteins, pre-

TABLE III. Incorporation *in Vivo* of L-^{14}C-Leucine into Protein by Microsomal Preparations from D-Galactosamine-treated and Control Rats.[a]

Time after treatment (hr)	Control[b]	D-Galactosamine	Inhibition (%)
2	2443 ± 53 (3)	2497 ± 134 (3)	0
4	2830 ± 374 (3)	1697 ± 198 (4)	40
17	1854 ± 99 (3)	812 ± 133 (2)	57

[a] Values (cpm/mg of microsomal protein determined 1 hr after injection of 20 μCi of ^{14}C-leucine) are given as mean ± SE; number of animals in each group is given in parentheses.

[b] Rats injected with saline or D-glucosamine (1.5 g/kg).

sumably due to alterations of membrane phospholipids, has also been reported by Oler and Lombardi (16) in their studies of choline deficiency in the rat.

Whether or not D-galactosamine administration produces a fatty liver only by inhibiting lipoprotein formation remains to be determined. The absence of a depression of plasma triglycerides during the period of fatty liver production suggests that other mechanisms, *e.g.*, increased mobilization of fatty acids, may contribute to the accumulation of hepatic triglycerides. The possible existence in this model of a defect in intrahepatic lipoprotein and albumin transport is currently under investigation.

Summary. The administration of a single dose of D-galactosamine (1.5 g/kg) results in hepatocellular necrosis and fatty liver in the rat. Hepatic triglyceride accumulation is not altered by administration of adenine, ATP, uridine, uridine nucleotides, or uridine diphosphoglucose.

Protein synthesis *in vivo* is impaired as early as 4 hr after administration of D-galactosamine. Subsequently the expected post-Triton WR 1339 hypertriglyceridemia is reduced and beta-lipoproteins are decreased by electrophoretic analysis. These data suggest that impaired hepatic lipoprotein release, presumably due to inhibition of synthesis of the protein moiety of lipoprotein, is the mechanism of the D-galactosamine-induced fatty liver.

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