demonstrable size or histologic changes in the liver prior to hepatectomy. Thus the mechanism of action of Dilantin in the stimulation of liver cell restitution is uncertain.

Previous attempts have been made to show the existence of serum mitotic stimulating or inhibiting substances which are modified after hepatectomy, resulting in the rapid restitution seen(6,7,8). The presently observed action of Dilantin could be related to these theories if Dilantin altered either the amount or form in which stimulating or inhibitory substances were present. Other authors, notably MacDonald(9) and Rogers, *et al.* (10) have failed to find any evidence of the presence of stimulating or inhibitory humoral factors directly governing hepatic regeneration.

In the present experiment there was no stimulatory effect of the drug on liver restitution when it was administered only after hepatectomy. Also, unpublished data from our laboratory have failed to show any increase in tensile strength of healing wounds after topical administration of Dilantin or systemic administration of the drug after experimental wound induction. Previous reports of the beneficial effects of Dilantin on wound healing have utilized preoperative treatment with the drug. It is apparent that Dilantin, under the conditions of this experiment may exert a proliferative effect on cells of tissue other than connective tissue.

Summary. Studies have been made on the effect of intraperitoneal injection of diphenylhydantoin sodium on liver restitution in the rat after partial hepatectomy. It was found that preoperative treatment of animals with 10 mg/day of the drug for 10 days followed by a continuation of therapy after hepatectomy resulted in a significantly greater rate of dry weight of liver restored per 100 g of body weight, from days 2 through 5 after hepatectomy. No effect of the drug was observed when administered only after operation. This experiment demonstrates that this proliferative effect is not limited to cells of connective tissue origin.

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Inhibition by Glucose of the Ethionine Induced Fatty Liver.* (27828)

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Ethionine (α-amino-γ-ethylmercapto-butyric acid) has been shown to produce a fatty liver in fasted intact female rats(1,2,3) and in fasted castrated male rats, while intact male rats and testosterone treated female rats were refractory(2). Both methionine and glucose administration prevent this effect(1, 3).

According to Recknagel and Lombardi(4) the fat accumulations in liver cells injured by different agents, among them ethionine(5), are characteristically accompanied in the earliest phases by decreased triglyceride levels in the plasma.

This investigation was carried out to compare triglyceride changes in liver tissue and

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plasma during the acute ethionine intoxication of fasted female rats with and without forced glucose ingestion.

Methods. Female albino rats (Wistar) weighing about 200 g were used. After 12 hours of fasting the following experimental procedures were initiated:

Group 1 received ethionine intraperitoneally, 100 mg/100 g of body weight. The ethionine was dissolved in warm water and neutralized with sodium bicarbonate to a final concentration of 25 mg/ml and was administered in 4 separated doses 2½ hours apart.

Group 2 received the same amount of ethionine as Group 1 and in addition had 1.53 molar glucose administered by stomach tube, 4 ml/100 g of body weight, in 4 separated doses 2½ hours apart at the same time the ethionine was given.

Group 3 received administrations of glucose identical to those of Group 2. In addition saline was given intraperitoneally, 4 ml/100 g body weight in the same schedule ethionine was administered to Groups 1 and 2.

Group 4 received only the intraperitoneal saline as described for Group 3. Twelve hours after beginning of the above treatment the animals were anesthetized by Nembutal and 2 ml of blood were withdrawn into sodium citrate from the abdominal aorta. The rats then were killed by exsanguination and livers and samples of the omentum were removed. Plasma was obtained from the citrated blood samples. Plasma and tissues were immediately used for lipid analysis.

Total lipids from liver and omentum were extracted according to the procedure of Folch *et al.*(6) and measured gravimetrically. Portions of lipids thus prepared were dissolved in chloroform and poured through a 2 g column of silicic acid-celite 545 (1:1) to separate the triglycerides and phospholipids according to Borgström(7). The chloroform eluate from the column was used for direct determination of triglycerides as described by van Handel and Zilversmit(8).

Plasma triglycerides were extracted with chloroform in the presence of Doucil(9) and measured(8).

Results. All results, except those obtained

TABLE I. Changes in Liver Weight, Total Liver Lipids, Liver Triglycerides and Plasma Triglycerides in Fasting Rats Fed Glucose, and/or Treated with Ethionine.

No. of rats	Treatment	Liver wt, g/100 g body wt	Total liver lipids, mg/100 g body wt		Liver triglycerides, mg/100 g body wt		Plasma triglycerides, mg/100 ml	
6	Saline	2.48 ± .12*	140.5 ± 7.4		32.5 ± 4.3		18.44 ± .21	
7	Saline & glucose	2.67 ± .10	120.4 ± 6.2		18.9 ± 3.5	2.44†	14.11 ± 1.04	3.79‡
5	Ethionine	2.65 ± .05	233.0 ± 10.5	7.39§	118.8 ± 10.7	8.00§	9.62 ± 1.39	3.48‡
6	Ethionine & glucose	2.60 ± .11	123.1 ± 8.0		14.6 ± 2.4	3.62‡	4.60 ± .63	20.91§

* Stand. error of mean.

Significant t value from Students test: † $p < .05$. ‡ $p < .01$. § $p < .001$.

from the omentum are given in Table I. The standard error of the mean is added to each value. The t value of the Students test is given only for those values which showed a significant difference from the control-group (saline treated) values. The difference was considered significant when calculation revealed a probability of change due to chance of less than 5% ($p<.05$). One asterisk (*) was added to the t value, if p was smaller than .05; 2 asterisks (**) with p smaller than .01, and 3 asterisks (***) with p less than .001.

At time of sacrifice, 12 hours after the first ethionine or saline administration, the average liver weight was essentially the same for all 4 groups of rats. However total liver lipids were almost doubled in the ethionine-treated rats. Administration of glucose prevented fat accumulation in the liver, as pointed out previously by Farber *et al.*(1).

Liver triglycerides increased about 4-fold after ethionine administration and accounted for more than 50% of total liver lipids, in comparison with the normal 20% in control animals. Ingestion of glucose not only prevented rise of triglycerides in liver tissue after ethionine administration, but the values of hepatic triglycerides after glucose ingestion, with and without ethionine, were even significantly lower than in fasting control animals. Plasma triglycerides decreased in animals treated with glucose alone and the most marked drop occurred in the animals which received both ethionine and glucose.

In the omental lipid and triglyceride concentrations the only statistically significant difference over the saline control group was an increase in triglyceride concentration in the glucose treated group from 67.93 ± 1.08 to 77.15 ± 1.75 mg/100 g *wet weight* (t 4.27**).

Discussion. Since the changes occurring 12 hours after ethionine treatment were investigated, the large increase in liver mass which is typical of the later stages (after 48 hours) with maximal histologic changes(3), was not detected. However signs of beginning fat accumulation were present. The changes of triglycerides in plasma and in liver tissue of ethionine treated rats were essentially the same as those observed by Lombardi and Recknagel(5), who have postulated that ethionine blocks the mechanism by which the triglycerides are transferred from the liver to the plasma, the net result being in the earliest phases of ethionine intoxication a decrease in plasma triglycerides and an increase in hepatic triglycerides. This is shown also by our data.

The feeding of glucose to untreated fasted rats lowered hepatic and plasma levels of triglycerides. This is consistent with the hypothesis that administration of glucose prevents the deposition of triglycerides in the liver of fasting animals, but does not enhance their release into the plasma. The finding of an increase of triglycerides in the omentum of glucose treated rats lends additional evidence to their hypothesis, though it must be stressed that these values represent only the concentration of triglycerides in this depot fatty tissue and not the total amount, which would be of greater significance.

The fact that glucose prevents development of a fatty liver by ethionine(1) was again confirmed. This cannot be explained by a neutralization of the ethionine action of blocking the release of triglycerides into the plasma, since in that case the plasma triglyceride level of ethionine plus glucose-treated rats should be normal or elevated. They are however even lower than in the group treated with ethionine alone. One must assume that, while ethionine still exercises its block on the triglyceride excretion from the liver, this is enhanced by a significantly lowered hepatic triglyceride level. This in turn is the consequence of inhibition by glucose of the fat mobilization from the periphery to the liver during fasting(10).

Summary. Data are presented which support the hypothesis of Recknagel and Lombardi(4,5) on the genesis of fatty livers in general and especially the triglyceride accumulation after ethionine, postulating that the release from the liver into the plasma is blocked. Our data also indicate that triglyceride increase in the liver depends on both blocking of the release from, and fat mobilization into the liver from the periphery (for instance during fasting). Glucose seems to

inhibit only the latter and by doing so prevents the increase of liver triglycerides by ethionine.

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Amino Acid Incorporation into Protein of Diabetic Rat Liver Slices After Acute Insulin Deprivation.* (27829)

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These experiments bear on the question whether insulin can act upon isolated liver. Earlier(1,2) it was found that insulin could stimulate amino acid incorporation into protein of liver slices from one type of mildly diabetic rat, *i.e.*, animals partially pancreatectomized 2-4 months previously. In the present series the effect of insulin on liver slices has been tested, using another type of diabetic liver donors, namely, alloxan-diabetic rats from which insulin had been withdrawn for various periods(3). Incorporation of leucine-1-C^{14} into protein was used as the test indicator of an insulin effect.

Materials and methods. Diabetes was produced in 24-hour fasting 150-250 g male Holtzman rats by intravenous injection of Eastman alloxan-monohydrate, 37 mg per kg; the rats were immediately returned to food and water. Insulin injection (subcutaneous) was started on the fourth day after alloxan. On 4th and 5th days the dose was 8 units regular insulin per rat in the morning and 8 units Lente Iletin (Insulin, Lilly, U-40) in the evening. On the 6th day and thereafter doses were reduced to 4 units each, morning and evening; regular insulin replaced Lente insulin on last 2 days before sacrifice, which was 15-18 days after alloxan. This regimen permitted the animals to maintain weight, with some nocturnal polyuria. All animals were fasted 24 hours before sacrifice, and used 3, 12, 24, or 48 hours after the last insulin injection. Blood sugars were determined at time of sacrifice(4).

Four liver slices (150-200 mg) were cut free-hand from the left lobe of each liver; one slice was placed in 1 ml Krebs-Henseleit bicarbonate buffer containing 1.6×10^5 cpm DL-leucine-1-C^{14} (Nuclear Chicago) at the tracer concentrations shown in Table I; the 2nd, 3rd, and 4th slices were put in 1-ml aliquots of the same medium containing glucose, insulin, or insulin plus glucose respectively, as shown in Table I. All slices were then shaken for 2 hours in a Dubnoff incubator at 37°C(2). Crude protein was isolated(2) and its radioactivity estimated with a D-47 Nuclear Chicago counter; all radioactivity values are corrected for self-absorption, but not for counter efficiency (35-40%).

Results. Liver slices from fasting diabetic rat donors incubated in Krebs solution containing leucine-1-C^{14} without added glucose

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