# Alcohol-Induced Triglyceride Deposition in Liver Through Derangement of Fat Transport

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The hypothalamic-pituitary system controls the activity of a number of biochemical functions. One such function is fat transport, which ordinarily responds to the energy requirements of the body by mobilizing energy-rich fatty acids from adipose tissue and transporting them to various organs.

Many substances can interfere with the endocrine control of the fat transport system. Among these is alcohol. Our studies show that large doses of alcohol in rats can upset the pituitary control of fat transport. As a result excessive amounts of triglycerides are mobilized from adipose tissue as free fatty acids; these are carried by plasma to liver, reformed to triglycerides, and deposited as such in this organ.

Our studies on the effect of alcohol on fat transport have been facilitated by the development of a simple direct method for the estimation of liver triglycerides.[1] This method lacks the considerable error of other procedures in which the triglyceride value is calculated from total lipid, less the values for cholesterol, cholesterol esters and phospholipids.

## LIVER TRIGLYCERIDE

The effect of single doses of orally administered alcohol on the liver triglyceride content of female rats was determined. Eighteen hours after the administration of 4.8 gm./kg. of alcohol, the triglyceride level had risen almost threefold (Table 1). The level was maximal in fifteen to twenty hours and returned to normal in thirty to fifty hours. Pathologic examination disclosed little or no necrosis. Larger doses of alcohol produced a greater rise in triglycerides; a dose of 6 gm./kg. increased the triglycerides almost fivefold (Table 1). An increase in triglycerides after alcohol has also been reported by di Luzio,[2] and an increase in total lipids has been found by Mallov and Bloch.[3] We found no change in the liver phospholipids, a finding in agreement with that of di Luzio.[2]

The problem arises whether the fat deposition results from (1) a stimulatory effect of alcohol on fatty acid synthesis in the liver, as suggested by Lieber et al.[4]; (2) an increased rate of fatty acid formation by incorporation of alcohol or a two-carbon fragment that it forms[5]; (3) inhibition of fat metabolism in liver; or (4) mobilization of fatty acids from the triglycerides in adipose tissue.

Table 1
Liver Triglyceride Levels After Single Large Oral Doses of Alcohol in Female Rats

| Treatment | Liver Triglycerides (mg./gm. ± S.E.) |
|---|---|
| Saline (12.0 ml./kg. orally).... | 4.8 ± 0.25 (4) |
| Alcohol (4.8 gm./kg.)..... | 12.7 ± 1.7 (9) |
| Alcohol (6.0 gm./kg.)..... | 22.6 ± 2.9 (8) |

Note: Alcohol, in a 50 per cent solution by volume, was administered to NIH female Sprague-Dawley rats weighing 150 to 200 gm. that were killed eighteen hours later. Figures in parentheses represent number of animals.

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Presented at the Symposium on the Neurological and Hepatic Complications of Alcoholism, under the sponsorship of The National Vitamin Foundation, Inc., March 1, 1960.

TABLE II

The Linoleic Acid and Oleic Acid Content of Triglycerides Deposited in Liver and of Triglycerides in Adipose Tissue Eighteen Hours After Oral Administration of Alcohol (4.8 gm./kg.) to Rats

| Tissue | Treatment | No. of Rats | Liver Triglycerides (mg./gm. ± S.E.) | Linoleic Acid | | Oleic Acid | |
|---|---|---|---|---|---|---|---|
| | | | | In Total Liver Triglycerides (% ± S.E.) | In Deposited Liver Triglycerides (% ± S.E.) | In Total Liver Triglycerides (% ± S.E.) | In Deposited Liver Triglycerides (% ± S.E.) |
| Liver | Control | 6 | 6.5 ± 1.8 | 29.2 ± 1.2 | ... | 29.3 ± 1.3 | ... |
| Liver | Alcohol | 6 | 24.2 ± 5.7 | 25.8 ± 0.5 | 23.7 ± 1.1 | 34.6 ± 0.9 | 37.5 ± 1.7 |
| Adipose tissue | ... | 10 | ... | 21.2 ± 0.3 | ... | 38.1 ± 0.5 | ... |

NOTE: The linoleic acid in the triglycerides deposited in liver was calculated for each rat from the following equation:

$$y = \frac{AB - (6.5)(29.2)}{A - 6.5}$$

where A = total liver triglycerides in milligram per gram after alcohol; B = per cent linoleic acid in total liver triglycerides after alcohol; and y = per cent linoleic acid in deposited liver triglycerides.

A direct answer to this problem has been provided by the application of gas chromatography to the assay of linoleic acid in liver triglycerides. This unsaturated fatty acid is not synthesized by the rat, but is of dietary origin. The liver triglycerides formed by the action of the alcohol had virtually the same content of linoleic acid as the triglycerides of adipose tissue (Table II). This suggested that the triglycerides were formed from fatty acids mobilized from adipose tissue. As further evidence of this view was the finding that the oleic acid content of the triglycerides formed in liver was also almost identical with that of adipose tissue (Table II). Since the concentration of both acids in the liver reflects that in adipose tissue, little if any of the triglycerides deposited in liver could have been derived from an increased synthesis of fatty acids in this organ.

To verify the assumption that linoleic acid was not formed in the body in these experiments, adipose tissue was labeled by pretreatment of rats with $C^{14}$-acetate, twenty-four hours before giving the alcohol. All the fatty acids in liver triglycerides, except linoleic acid, were found to be highly labeled. This demonstrates the well known fact that this acid is not ordinarily synthesized in the rat, again indicating that the increased liver triglycerides must have been formed from fatty acids which had been mobilized from adipose tissue.

### THE PITUITARY

The pituitary has an important role in causing the rise in liver triglycerides, for these were not elevated by alcohol administered to hypophysectomized rats (Table III). These results are in accord with those of Mallov and Bloch[3] who reported that the admin-

TABLE III

Inability of Alcohol to Elevate Liver Triglyceride Levels in Hypophysectomized Rats

| Treatment | Liver Triglycerides | |
|---|---|---|
| | Intact Rats (mg./gm. ± S.E.) | Hypophysectomized Rats (mg./gm. ± S.E.) |
| Saline (8 ml./kg. orally) | 5.3 ± 1.2 (3) | 3.6 ± 0.6 (3) |
| Alcohol (3.4 gm./kg.) | 21.6 ± 2.2 (6) | 4.4 ± 0.9 (6) |

NOTE: Alcohol, in a 50 per cent solution by volume, was given orally to intact and hypophysectomized female Sprague-Dawley rats weighing 175 to 200 gm. (obtained from Hormone Assay Laboratories, Chicago); they were killed eleven hours later. Figures in parentheses represent number of animals.

TABLE IV
Prevention of Alcohol-Induced Triglyceride Deposition in Rat Liver by Adrenergic Blocking Agents

| Procedure* | Liver Triglycerides (mg./gm. ± S.E.) |
|---|---|
| Saline (12 ml./kg. orally) | 4.8 ± 0.23 (4) |
| Alcohol, 4.8 gm./kg. | 12.8 ± 0.30 (5) |
| Dibenamine and alcohol | 4.0 ± 0.06 (4) |
| Phenoxybenzamine and alcohol | 5.1 ± 0.14 (5) |
| Ergotamine and alcohol | 4.4 ± 0.05 (6) |

NOTE: Alcohol, in a 50 per cent solution by volume, was given to NIH female Sprague-Dawley rats weighing 150 to 200 gm. that were killed eighteen hours later. Figures in parentheses represent number of animals.

* Dibenamine hydrochloride (50 mg./kg.) was injected subcutaneously twenty-four and forty-eight hours before the alcohol was given. Phenoxybenzamine hydrochloride (10 mg./kg.) was injected subcutaneously twenty-four hours before the alcohol was given. Ergotamine tartrate (2.5 mg./kg.) was injected subcutaneously immediately before the alcohol was given.

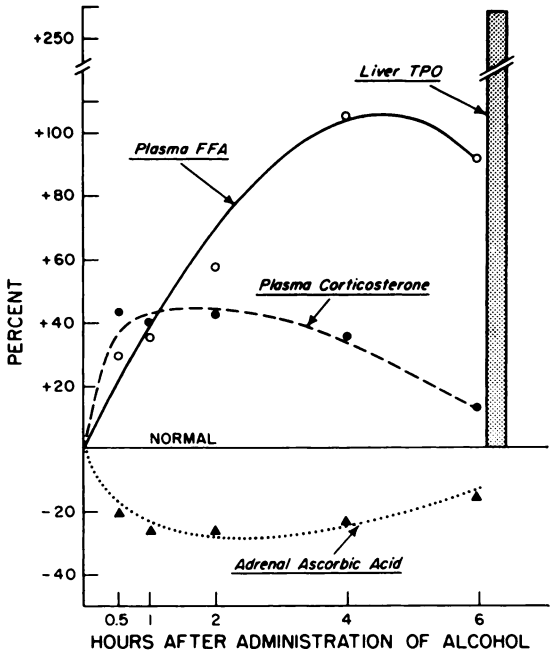


FIG. 1. Changes in plasma corticosterone, plasma free fatty acids (FFA), adrenal ascorbic acid, and liver tryptophane peroxidase after oral administration of alcohol, 4.8 gm./kg. as a 50 per cent solution, v./v. Each point is the mean of values obtained from twelve to fifteen rats of either sex. No sex difference was observed. Sprague-Dawley rats weighing 160 to 200 gm. were used.

istration of alcohol did not cause a rise in total lipids in hypophysectomized rats. This suggested that the triglyceride deposition was mediated through hormones released from the pituitary. We determined, therefore, the effect of alcohol on the pituitary-adrenal axis. It was found that alcohol depleted adrenal ascorbic acid, elevated the level of plasma corticosterone, increased the activity of liver tryptophane peroxidase, and elevated the level of plasma free fatty acids (FFA) (Fig. 1). Similar responses were elicited by exposure to certain stressful situations such as cold, intradermal formaldehyde and fear. The rise in FFA, incidentally, is another strong indication that the liver triglyceride deposition after alcohol is causally related to the mobilization of fatty acids from adipose tissue.

The administration of alcohol increased liver triglycerides to a much greater extent in female rats than in male rats. In preliminary studies, alcohol induced about the same pituitary responses in both sexes as shown by loss of adrenal ascorbic acid, rise in liver tryptophane peroxidase activity, and rises in plasma corticosterone and FFA. Now under study is the possibility that the difference between the sexes may concern liver enzymes that form triglycerides from fatty acids and glycerol.

The mechanism by which alcohol mobilizes fat is still unknown. Alcohol obviously causes the pituitary to liberate ACTH and perhaps other hormones important in mobilizing fat, but other factors are also involved. For example, pretreatment of rats with the adrenergic blocking agents, Dibenamine,® phenoxybenzamine (Dibenzyline®), or ergotamine, prevented the alcohol-induced deposition of fat in the liver (Table IV). The administration of Dibenamine also prevented the depletion of adrenal ascorbic acid and the rise in liver tryptophane peroxidase. Dibenamine may affect triglyceride deposition by blocking the action of norepinephrine at nerve endings which innervate fat cells or by preventing the release of pituitary hormones. These possibilities are under study.

The question arose whether the effect of alcohol on the pituitary was due to the pres-

ence of alcohol in the bloodstream or to a reflex action elicited by gastric irritation. Alcohol was therefore administered by slow intravenous infusion. Again a marked triglyceride deposition was observed, showing that alcohol does exert a systemic effect on the pituitary.

## COMMENTS

A number of unsolved problems are raised by these experiments which show that single doses of alcohol can produce triglyceride deposition in liver through derangement of the fat transport. By what mechanism does alcohol cause the pituitary to release ACTH and perhaps other hormones? How do adrenergic blocking agents prevent the fatty deposition, and does this have clinical implications in the treatment or prevention of Laennec's cirrhosis? Is the acute response to alcohol related to the chronic response?

We suggest that the chronic and acute conditions have something in common. For one thing, the dose of alcohol that produces fatty deposition in rats is close to that taken by a human subject who poisons himself with six double martinis. Since alcohol is metabolized more slowly in man than in the rat, it is possible that daily consumption of a large amount of alcohol can cause "reversible" fatty deposition to become "irreversible." In addition, there is said to be a twofold range in the rates at which different subjects metabolize alcohol, so that the daily dose required to elicit triglyceride deposition in the liver might be considerably smaller in some persons than in others.

## SUMMARY

Single large doses of alcohol in rats promote a pronounced rise in liver triglycerides which is maximal in fifteen to twenty hours and disappears in thirty to fifty hours. The proportion of linoleic acid in the triglyceride deposited in liver is virtually the same as in the triglycerides of adipose tissue. Since linoleic acid is not synthesized in the rat, the liver triglycerides must have been formed from fatty acids mobilized from adipose tissue.

The action of alcohol is apparently mediated through hormones released from the pituitary gland and can be explained in part through stimulation of the pituitary-adrenal axis. The deposition of triglycerides in liver is blocked by the administration of adrenergic blocking agents, suggesting that catecholamines are also involved in the mobilization of fat.

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