DEPRESSION OF PLASMA LIPIDS IN THE RAT BY OROTIC ACID AND ITS REVERSAL BY ADENINE

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Orotic acid (OA), an intermediate in pyrimidine biosynthesis, induces a severe fatty liver in rats when added to purified diets for 7-14 days (Standerfer and Handler, 1955). The addition of adenine to such antilipotropic diets prevents and also reverses liver fat accumulation (Handschumacher et al., 1960). The mechanism by which these compounds alter lipid metabolism is unknown. Creasy et al.(1961), after feeding 1% OA for 10 days, found plasma lipid concentrations in rats which were only about 20% of normal. They tentatively concluded that the fall in circulating lipid concentration accompanies the appearance of the fatty liver but does not precede it.

In this laboratory, during studies on lipogenesis, it was found that in rats ingesting OA for 5 days plasma lipids were routinely very low. Therefore, the time course of this depression and the effect on it of adenine were more systematically investigated.

Weanling male Sprague-Dawley rats were fed a fat-free but otherwise complete synthetic basal diet containing 20% casein and 68.5% glucose monohydrate (diet R-1, with corn oil replaced by glucose) (Williams, 1961). After one week, groups of rats were sacrificed for plasma lipid determinations at the intervals shown in Figures 1 and 2. Values on days 0 and 2 established the baseline. At sacrifice, the rats were anesthetized with

ether and bled from the abdominal aorta with a syringe rinsed with heparin. Plasma was collected by centrifugation and the total lipid extracted and washed with water (Albrink, 1959). Total lipid extracts of liver were prepared and washed according to Folch et al. (1957). Lipid phosphorus was determined according to Fiske and SubbaRow (1925), cholesterol by the method of Pearson et al. (1953), and triglycerides by a modification of the direct procedure of Moore (1962) after the separation of phospholipids on silicic acid (Albrink, 1959). Total fatty acids in the lipid extracts were assayed by titration, after saponification, acidification, and extraction into n-hexane.

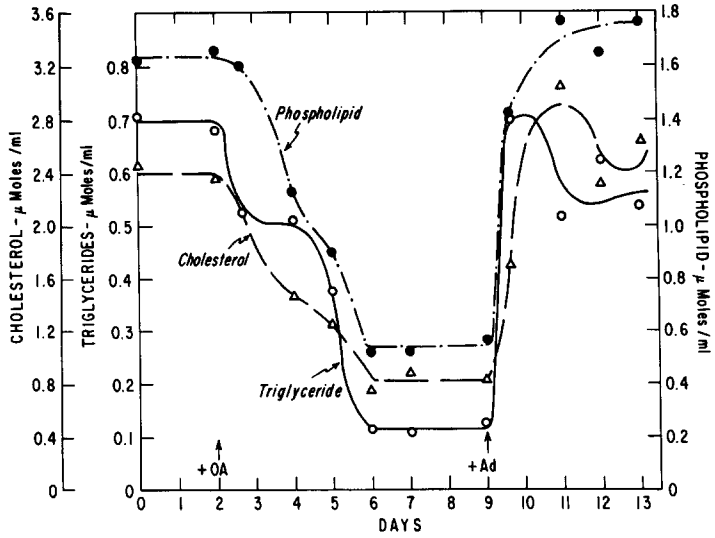

Fig. 1. Effect of Dietary Orotic Acid (OA) and Adenine on Plasma Lipids. Animals were fed the basal diet for one week prior to day 0. OA (1%) and adenine sulfate (Ad) (0.25%) were added to the basal diet on the day indicated by the arrow. Each point is the average of 3-4 animals.

From Figure 1 it is seen that plasma lipids, and particularly triglycerides, were depressed as early as 16 hours after OA was introduced into the diet. A minimum plateau was reached in 4 days, at which time the liver rapidly began to accumulate fat (Figure 2). The addition of adenine resulted in the restoration of nearly normal plasma lipid concentrations within 16 hours (Figure 1). Within 48 hours after the adenine

administration the liver had begun to lose its accumulated fat (Figure 2), which is mostly triglyceride but includes up to five times normal amounts of cholesterol.

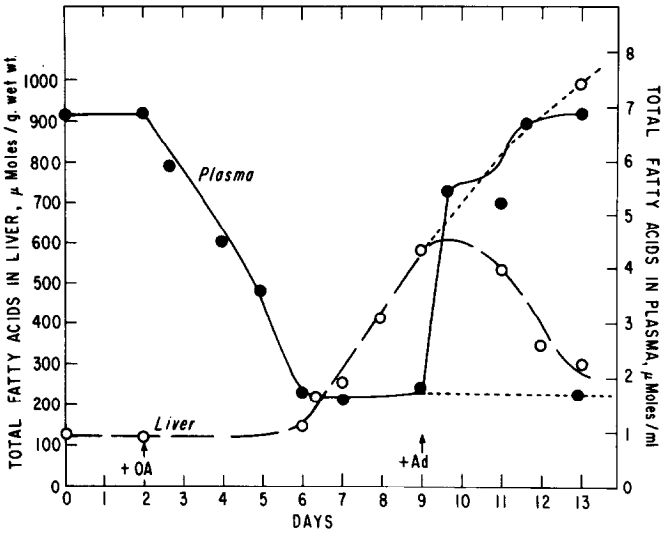

Fig. 2. Effect of Dietary Orotic Acid and Adenine on Total Liver and Plasma Fatty Acids (see legend Fig. 1; dashed lines are for animals not receiving the adenine supplement).

Table 1 shows that it was the low density lipoprotein fraction which almost disappeared from the plasma of OA-fed rats. The high-density fraction was reduced about 60%. These data also show that supplements of OA plus adenine or of adenine alone resulted in a normal lipoprotein distribution. None of the dietary supplements listed in this table modified the plasma concentrations of unesterified fatty acids or of glucose.

The liver is recognized as the major source of endogenous plasma triglycerides (Stein and Shapiro, 1959) (Byers and Friedman, 1960). This fact and the demonstration that depressed plasma lipid concentrations precede liver lipid accumulation suggest that the etiology of OA-induced fatty liver is related to a defect in the synthesis or secretion of the triglyceride-rich low-density lipoproteins by this organ. This defect may be mediated through a depressed level of liver acid-soluble adenine

Table 1

Effect of Dietary Orotic Acid and Adenine on Rat Plasma Lipoproteins*

Additions to basal diet	Density < 1.063		Density > 1.063	
	Cholesterol	Phospholipid	Cholesterol	Phospholipid
	(μmoles/ml plasma x 10^2)			
None	47	24	132	121
0.25% adenine sulfate	62	30	121	105
1% orotic acid	5	2	54	52
1% orotic acid + 0.25% adenine sulfate	54	35	150	107

* Each value was obtained by analysis of the pooled plasma of six rats (av. wt. 170 g.) fed the respective diets for 9 days. High and low density lipoproteins were obtained by ultracentrifugation for 16 hours, according to the procedure of Havel et al. (1955). The author is grateful to Mr. Oscar Young for these analyses.

nucleotides, a condition which is produced by OA feeding (von Euler et al., 1962) (Marchetti et al., 1962). In this connection, the following reports may be pertinent. An adenine antagonist, 4-aminopyrazolopyrimidine, will suppress plasma lipids and produce fatty livers in mice (Henderson, 1963). Also, adenine supplements reverse the ethionine-induced depression of liver acid-soluble adenine nucleotides (Shull, 1962) and may prevent ethionine-induced accumulation of liver fat (Farber and Castillo, 1963). Thus the normal assembly or secretion of lipoproteins by the liver may be sensitive, directly or indirectly, to the availability of some adenine derivative.

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ERRATA

Biochem. Biophys. Res. Commun. 11, 249 (1963), in the communication "The Carbohydrate-Protein Linkage in Ovomucoid" by Rex Montgomery and Ya-Chen Wu:

Page 253, line 3, "carbohydrate recovery representing 10% of that in ovomucoid." should read:

"carbohydrate recovery representing 16% of that in ovomucoid."

Page 253, line 8, "in the case of α_1-glycoprotein" should read:

"is also possible in the case of α_1-glycoprotein"