THE MECHANISM OF INHIBITION OF LIPOPROTEIN SYNTHESIS BY OROTIC ACID*

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We have recently described the presence of an apoprotein in plasma which combines with lipid in the liver to form plasma lipoproteins (Roheim et al., 1965). This has led us to suggest that the synthesis of plasma lipoproteins may be a stepwise process in which synthesis of the protein portion is the first step with subsequent steps being the coupling of the protein to lipid to form lipoprotein and the release of the newly formed lipoproteins into the plasma. Interference with any of these steps in the formation of plasma lipoproteins could lead to accumulation of fat in the liver and depression of the concentration of plasma lipoproteins. Such changes do occur after the administration of puromycin which inhibits the synthesis of the protein portion of the lipoproteins (Robinson and Seakins, 1962). Administration of orotic acid to rats also results in accumulation of fat in the liver (Standerfer and Handler, 1955) and depression of plasma lipoprotein concentrations (Windmueller, 1963). However, these animals grow and reproduce normally (Creasy et al., 1961) and this has suggested that orotic acid administration might have a specific effect on lipoprotein synthesis without overall inhibition of pro-

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tein synthesis as occurs following administration of puromycin. Further evidence of the unique effect of orotic acid administration was obtained by electron microscopy which showed that the endoplasmic reticulum breaks down into individual vesicles containing lipid droplets much smaller than those seen after puromycin administration (Novikoff et al., 1964). The present studies have been carried out to determine which of the proposed steps in lipoprotein synthesis is affected by orotic acid feeding.

In the first series of experiments the effect of feeding orotic acid on the synthesis of plasma lipoproteins was determined. The data in Table I show that the incorporation of amino acids into

TABLE I

Incorporation of ^{14}C-labeled amino acids into plasma proteins of normal and orotic acid-fed rats

	Normal		Orotic Acid-fed	
	Total ^{14}C	Specific Activity	Total ^{14}C	Specific Activity
	dpm	dpm/mg	dpm	dpm/mg
Plasma	49000	860	43800	830
Lipoprotein				
VLD	523	6900	18	2100
LD	475	3000	145	2500
HD	1300	2200	540	2000

One group of 4 rats was fed a diet containing 1% orotic acid for 9 days. Another group of 4 rats received the same diet without orotic acid. The rats were injected intravenously with 2.8 μc ^{14}C per 100 gm body weight as chlorella protein hydrolysate (New England Nuclear Corp). The animals were killed after 100 minutes and the serum from each group was pooled. The plasma lipoproteins were separated by ultracentrifugation (Havel et al, 1955) into the d<1.019 (VLD), d 1.019-1.063 (LD) and d 1.063-1.21 (HD). Radioactivity of the proteins was determined by liquid scintillation counting after dispersion in Cabosil (Haft, et al 1961). Protein was determined by the method of Lowry (1951). Total ^{14}C represents dpm in the amount of protein present in 1.0 ml of plasma.

total plasma proteins of rats was not significantly altered by administration of orotic acid. However, the incorporation into the lipoprotein fractions was depressed. This was most marked in the very low density (VLD) lipoprotein fraction in which the incorporation of amino acids was 3.5% of that of the control group. Incorporation of amino acids into low density (LD) and high density (HD) lipoproteins of the animals fed orotic acid was 30% and 42% of that of the control group. The specific activity of the VLD lipoproteins in the orotic acid-fed rats was also markedly depressed, while the specific activities of the other lipoprotein fractions decreased only slightly. The specific activities of the remaining plasma proteins and of the liver proteins were not effected by orotic acid feeding. This inhibition of amino acid incorporation into plasma lipoproteins could have been the result of either the inhibition of the synthesis of the protein portion or of the inhibition of the subsequent steps in lipoprotein formation.

The experiments shown in Table II were carried out to distinguish between these possibilities. We have previously shown that perfusion of livers from cholesterol-fed rats with ^{14}C-labeled d>1.21 proteins (free of lipoproteins) results in incorporation of the labeled apoprotein present in this fraction into VLD lipoproteins of the plasma (Table II, Expt. 1). When livers from cholesterol-fed rats were perfused with labeled d>1.21 proteins prepared from rats fed orotic acid (Table II, Expt. 2), there was similar incorporation of labeled protein into the VLD lipoproteins. This experiment indicates that the orotic acid treated rats <u>did</u> produce the apoprotein. However, when the fatty liver from an orotic acid-fed rat was perfused with ^{14}C-labeled d>1.21 proteins prepared from normal rat plasma (Table II, Expt. 3), there was a profound decrease in the amount of lipoprotein recovered in the perfusate and, therefore, a decrease in total incorporation of apoprotein into lipoprotein. This suggests that in the orotic acid-fed animal the formation of plasma lipoproteins from apoprotein is inhibited.

TABLE II

Incorporation of ^{14}C-labeled d>1.21 proteins into VLD lipoproteins during perfusions of isolated rat livers.

	Expt. 1	Expt. 2	Expt. 3
Diet			
Liver Donor	Cholesterol	Cholesterol	Orotic Acid
Plasma Donor	Normal	Orotic Acid	Normal
Specific Activity (dpm/mg)	5820	5900	4200
Protein	1.62	2.17	.23
Total Incorporation (dpm)	9400	12800	990

Rats were fed diets containing 2% cholesterol and 20% olive oil mixed with Rockland Farm Mouse Pellets for 7 days or orotic acid as described in Table I. Isolated rat livers were perfused (Miller et al., 1951) with labeled d>1.21 protein solution to which was added washed rat erythrocytes, 200 mg of glucose and 20 mg of heparin. The labeled d>1.21 protein solution was prepared by administration of ^{14}C-labeled chlorella protein hydrolysate to the plasma donor rats with subsequent isolation of the d>1.21 plasma proteins by ultracentrifugation. Total ^{14}C in the d>1.21 protein added was 2.5×10^6 dpm in Expt. 1, 6.9×10^6 dpm in Expt. 2, and 8.7×10^6 dpm in Expt. 3. The plasma lipoproteins were separated and determined as in Table I.

Further evidence of the inability of the liver from an orotic acid-fed rat to incorporate the plasma apoprotein into lipoprotein is shown in Fig. 1. In this experiment a liver from an orotic acid-fed rat was perfused with d>1.21 protein solution. No lipoprotein was released. However, when the liver was replaced during the perfusion by a liver from a cholesterol-fed rat, lipoproteins rapidly appeared in the perfusate. In this experiment it is apparent that the liver from the orotic acid-fed rat did not remove or inactivate the apoprotein since the release of lipoproteins in the presence of the cholesterol-fed liver was similar to that seen when fresh d>1.21 proteins were used.

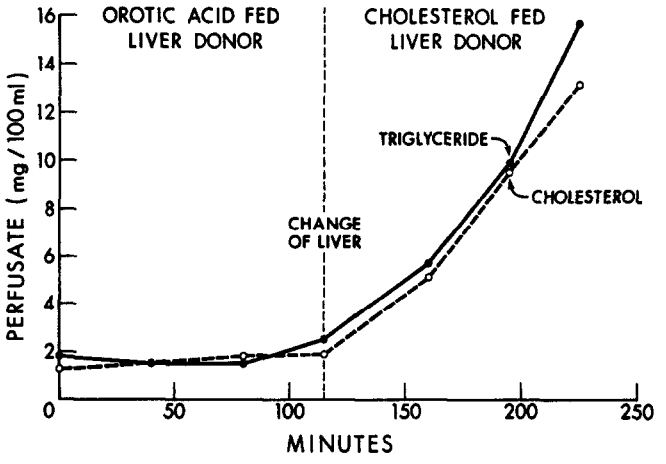

Fig. 1-

Plasma cholesterol and triglyceride concentrations (Abell, et al 1951; Van Handel, 1961) during liver perfusion with d>1.21 proteins. During the first 115 minutes the liver from an orotic acid-fed rat was perfused. The liver was then replaced with the liver from a cholesterol-fed rat and perfusion was continued for 115 minutes.

These experiments show that orotic acid specifically depresses the formation of VLD lipoproteins without overall inhibition of protein synthesis. Furthermore, they provide evidence which demonstrates that synthesis of the protein portion of the lipoproteins is unaffected by orotic acid feeding. The failure of livers from orotic acid-fed rats to utilize apoprotein to form lipoprotein supports the hypothesis that there may be one or more steps in lipoprotein formation subsequent to the synthesis of the protein and that one of these steps is inhibited by the administration of orotic acid.

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