An Orotic Acid-induced, Adenine-reversed Inhibition of Hepatic Lipoprotein Secretion in the Rat

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The induction of a severe fatty liver in rats by the addition of orotic acid to their purified diets was first reported by Standerfer and Handler (1). Creasey, Hankin, and Handschumacher (2) found that the liver fat was largely triglyceride and observed that its accumulation was both prevented and reversed by supplementing the orotic acid diets further with adenine (3). Marked alterations in the concentration of various acid-soluble nucleotides of liver also result from the ingestion of orotic acid, with a 400% elevation of uridine nucleotides and a 50% depression in adenine nucleotides being the most prominent (4). The mechanism by which dietary orotic acid and adenine alter lipid metabolism is unknown and is the subject of this investigation.

Several experimental approaches were used and the following parameters were studied: (a) the effect of altering dietary protein and fat levels on the action of orotic acid; (b) the fatty acid composition of accumulated liver lipids of rats fed a fat-free orotic acid diet; (c) the effect of orotic acid on lipogenesis and fat transport as measured by the incorporation in vivo of tritium from labeled body water into the fatty acids of various body compartments; (d) the effect of dietary orotic acid and adenine on the lipid, glucose, and protein content of plasma; and (e) the effect of dietary orotic acid on the hyperlipemia induced by the intravenous injection of a nonionic surface-active agent. From these studies it is concluded that the fatty liver induced by orotic acid results from a severe inhibition of hepatic lipoprotein secretion, and that the addition of adenine to the diet rapidly reverses this inhibition.

EXPERIMENTAL PROCEDURE

Animals and Diets

The rats used in all the studies, unless otherwise indicated, were males (60 to 120 g) of the Osborne-Mendel or Sprague-Dawley strains, caged individually in wire-bottomed cages and supplied with food and water ad libitum. Before testing of the effects of orotic acid or other dietary supplements, all animals were first fed a basal diet for an adjustment period of 7 to 10 days. The purified synthetic basal diets had the following composition: Diet W-1, as described by Standerfer and Handler (1) and containing 18% casein, 2% corn oil, and 72.8% sucrose; Diet W-2, the same as W-1, with the casein level increased to 40% at the expense of sucrose; Diet W-3, the same as W-1, with 38% hydrogenated vegetable fat (Crisco) added at the expense of sucrose; Diet W-5, the same as W-1, minus the corn oil, with the casein level increased to 22% and with the addition of 0.3% L-cystine; and Diet R-1-FF, a fat-free but otherwise complete purified diet containing 20% casein and 68.5% glucose monohydrate (Diet R-1 (5), with the corn oil replaced by glucose).

Materials

Orotic acid was purchased from General Biochemicals, Inc., adenine sulfate from Eastman Organic Chemicals, and tritiated water (100 mc per ml) from New England Nuclear Corporation. Triton WR-1339 (oxyethylated tert-octylphenol formaldehyde polymer), a nonionic surface-active agent, was obtained from Winthrop Laboratories.

Determination of Plasma and Liver Lипids

Rats were killed under ethyl ether anesthesia by exsanguination from the abdominal aorta with a syringe rinsed with heparin (0.1% in 0.95% NaCl). Livers were removed and kept frozen until analyzed. Plasma was collected by centrifugation, and the total lipids were extracted and washed by the procedure of Albrink (6). Total lipid extracts of liver were prepared and washed according to Falch, Loes, and Shane Stanley (7). Total lipid in liver extracts was determined gravimetrically after removal of the solvent under reduced pressure over CaCl2. Lipid phosphorus was determined according to Fiske and SubbaRow (8), and 1 mole of lipid phosphorus was assumed to be equivalent to 1 mole of phospholipid. Cholesterol was determined by the method of Pearson, Stern, and McGavack (9), and triglycerides by slight modifications of the direct method of Moore (10) after the separation of phospholipids on silicic acid (6). Total fatty acids in the lipid extracts were assayed by titration, after saponification, acidification, and extraction into n-hexane. Fatty acids in lipid extracts were converted to their respective methyl esters and determined by gas-liquid chromatography as described by Bieri and Andrews (11).

Incorporation of Tritium into Tissue Fatty Acids in Vivo

Labeling Body Water—The procedure was adapted from that described by Fain and Wilhelmi (12). Rats (approximately 100 g), fed as indicated in the figure legends, were placed in individual wire mesh cages under a fume hood and, after a 2-day period of adaptation, each animal was given a subcutaneous injection of a priming dose of 0.95 mc of 3H2O diluted to 0.2 ml with water. Food was supplied ad libitum, as was drinking water enriched with sufficient 3H2O to maintain a nearly constant specific activity of the body water over the experimental period (12).
Isolation of Tissue Fatty Acids—At death, 8 to 72 hours after the injection of tritium, the animals were anesthetized with ether and 3 to 4 ml of blood were drawn from the abdominal aorta. The blood was allowed to clot, and the serum was collected by centrifugation. Serum (1 or 2 ml) was saponified by the addition of 10 ml of alcoholic KOH (75 ml of saturated aqueous KOH made to 1000 ml with 95% ethanol). Likewise, the weighed liver, the small intestine with attached mesentery, and the pooled epididymal fat pads were each placed in 10 ml of alcoholic KOH. Most of the intestinal contents were gently squeezed out before the saponification. The spleen, stomach, cecum, and large intestine were discarded and the rest of the carcass was placed in 125 ml of 95% ethanol containing 20 g of KOH.

The samples were saponified by heating at 70-75° for 8 hours with occasional shaking. After the addition of sufficient water to adjust the alcohol content to about 50%, an aliquot of each reaction mixture was twice extracted with 2 to 4 volumes of petroleum ether (boiling range, 30-60°) and the extracts were discarded. After acidification with 10 N H₂SO₄, the fatty acids of the samples were extracted into petroleum ether and an aliquot of the extracts was transferred to 50-ml glass-stoppered centrifuge tubes, where the solvent was removed at 45° under a stream of nitrogen. The fatty acids were taken up in 15 ml of toluene.

Determination and Counting of Fatty Acids—The toluene solutions were dried and decolorized by the addition of 3 g of anhydrous Na₂SO₄ and 60 mg of activated charcoal (Norit), and after a brief centrifugation, a 10-ml portion of the clear supernatant fluid was counted in a liquid scintillation counter (12). The number of disintegrations per minute of each sample was determined after the addition of an internal standard. The fatty acid content of the toluene solutions was determined by titration (12). Recovery of palmitic acid added to tissue samples before saponification and carried through the isolation procedure was 96 ± 3%.

Tritium Content of Body Water—A 0.1-ml aliquot of serum obtained from each animal at death was deproteinized (13), and a 25-μl aliquot of the protein-free supernatant was added to 10 ml of liquid scintillation mixture (14) in a 20 ml counting vial. As before, an internal standard was added to permit the conversion of counts to disintegrations. For the purpose of calculation, the water content of serum was taken as 93% (15). When the concentration of tritium in the drinking water (millicuries per 100 ml) was twice the concentration of tritium injected as a priming dose (millicuries per 100 g of body weight), the average deviation in the concentration of tritium in the body water over the 72-hour experimental period was less than 2%.

Calculations—The tritium concentration of a sample is expressed as the specific activity, defined as disintegrations per minute per mmole of fatty acid divided by disintegrations per minute per mmole of body water.

The total tritium content of the fatty acids in a tissue is expressed as the relative total activity, calculated by multiplying the relative specific activity by number of millimoles of fatty acid.

By these calculations, the specific activity and total activity of the fatty acids of each animal are reported as a function of the specific activity of the body water of that animal; therefore, the relative specific and relative total activity values of all the animals can be strictly compared although there were small variations in body water specific activities.

RESULTS

Effect of Diet Modifications on Orotic Acid-induced Fatty Liver—In previous work on the induction of fatty livers in the rat with orotic acid, the diet most commonly used contained 2% fat and 18% casein as the protein (1-3). Table I summarizes results obtained with this diet (W-1) and with others of varying fat and protein content. The most severe fatty livers and those highest in cholesterol content were produced with the fat-free diet (W-5); the least severe fatty livers resulted from using the high fat diet (W-3). The concentration of total liver phospholipid was relatively constant and unaffected by any of the dietary modifications.

Fat-free basal diets were used in all subsequent experiments to maximize the effects of added orotic acid and to simplify interpretation of the data.

Fatty Acid Composition of Accumulated Liver Fat—Table II presents the quantity of each fatty acid found in the total liver fat from rats fed a basal fat-free diet (W-5) with and without 1%

### Table I

<table>
<thead>
<tr>
<th>Diet</th>
<th>Liver weight</th>
<th>Total lipid</th>
<th>Phospholipid</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% body wt.</td>
<td>%</td>
<td>μmoles/g</td>
<td></td>
</tr>
<tr>
<td>W-5 (22% protein, fat-free)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Orotic acid</td>
<td>5.6 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>36.8 ± 1.0</td>
<td>6.9 ± 0.2</td>
</tr>
<tr>
<td>+ Orotic acid</td>
<td>8.8 ± 0.3</td>
<td>33.1 ± 2.2</td>
<td>27.6 ± 0.5</td>
<td>28.7 ± 1.6</td>
</tr>
<tr>
<td>W-1 (18% protein, 2% fat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Orotic acid</td>
<td>4.8 ± 0.3</td>
<td>4.5 ± 0.2</td>
<td>41.2 ± 1.1</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>+ Orotic acid</td>
<td>6.5 ± 0.1</td>
<td>25.4 ± 4.0</td>
<td>40.0 ± 0.9</td>
<td>15.6 ± 2.8</td>
</tr>
<tr>
<td>W-3 (18% protein, 40% fat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Orotic acid</td>
<td>4.1 ± 0.1</td>
<td>7.7 ± 2.0</td>
<td>37.4 ± 0.8</td>
<td>7.7 ± 0.5</td>
</tr>
<tr>
<td>+ Orotic acid</td>
<td>5.8 ± 0.3</td>
<td>19.9 ± 2.4</td>
<td>36.5 ± 1.0</td>
<td>13.7 ± 1.2</td>
</tr>
<tr>
<td>W-2 (40% protein, 2% fat)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Orotic acid</td>
<td>4.9 ± 0.1</td>
<td>4.4 ± 0.2</td>
<td>39.4 ± 0.9</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>+ Orotic acid</td>
<td>8.2 ± 0.6</td>
<td>27.1 ± 2.0</td>
<td>33.7 ± 0.6</td>
<td>14.4 ± 0.8</td>
</tr>
</tbody>
</table>
The amount of a fatty acid in the accumulated liver fat (Column 4) is the difference between the content of that fatty acid in the fatty liver (Column 3) and in the normal liver (Column 2). The carcasses, mesenteric fat, and epididymal fat pads were from the same animals as the fatty livers. The carcass included the whole animal minus liver, spleen, stomach, and intestinal tract with attached mesentery. Tissues were saponified and the fatty acids extracted as described for the tritium incorporation studies under "Experimental Procedure." Each number is the average for four animals.

### Table II

**Fatty acid composition of accumulated liver fat and extrahepatic lipids from rats fed 1% orotic acid for 14 days**

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmoles/liter</td>
<td>µmoles/liter</td>
<td>% total</td>
<td>% total</td>
<td>µmoles/liter</td>
<td>µmoles/liter</td>
<td>µmoles/liter</td>
</tr>
<tr>
<td>Lauric</td>
<td>9</td>
<td>101</td>
<td>92</td>
<td>1.2</td>
<td>2.2</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Myristic</td>
<td>4</td>
<td>34</td>
<td>30</td>
<td>0.4</td>
<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Palmitic</td>
<td>302</td>
<td>2531</td>
<td>2229</td>
<td>23.3</td>
<td>34.1</td>
<td>33.8</td>
<td>27.2</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>75</td>
<td>962</td>
<td>887</td>
<td>11.7</td>
<td>10.8</td>
<td>10.6</td>
<td>13.5</td>
</tr>
<tr>
<td>Stearic</td>
<td>168</td>
<td>219</td>
<td>110</td>
<td>1.4</td>
<td>5.4</td>
<td>7.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Oleic</td>
<td>276</td>
<td>4420</td>
<td>415</td>
<td>84.4</td>
<td>49.9</td>
<td>48.8</td>
<td>42.9</td>
</tr>
<tr>
<td>Linoleic</td>
<td>15</td>
<td>110</td>
<td>95</td>
<td>1.2</td>
<td>2.6</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Linolenic</td>
<td>36</td>
<td>25</td>
<td>25</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>Trace</td>
</tr>
<tr>
<td>Eicosatrienoic</td>
<td>36</td>
<td>25</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Eicosatetraenoic</td>
<td>37</td>
<td></td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>862</td>
<td>8427</td>
<td>7613</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Fig. 1. Incorporation in vivo into fatty acids of tritium from labeled body water of constant specific activity.**

- ●, rats fed Basal Diet W-5; ○, rats fed Diet W-5 + 1% orotic acid (OA) for 4 days before the body water was labeled with tritium. The body water of all animals was enriched with $^3$H$_2$O at zero time (8 to 9 a.m.). The curves represent data from four experiments involving a total of 60 animals, with 4 to 11 animals per point. Relative total activity (RTA) is a measure of the total tritium found in the fatty acids of the respective body compartment (see "Experimental Procedure" for details).

Orotic acid. The accumulated liver fat, determined by difference, had nearly the same composition as mesenteric or epididymal adipose tissue and as the pooled carcass lipids. Ninety-five percent of the fatty acids in this accumulated liver fat were accountable as palmitic, palmitoleic, and oleic acids, and the figure was only slightly lower for the other tissues. The liver fat was somewhat higher in oleic and somewhat lower in stearic acid than the other tissues.

Also included in this study, but not in Table II, was a group of rats fed the basal diet supplemented with 1% orotic acid plus 0.25% adenine sulfate (Group C) and a group fed the basal diet with only the adenine supplement (Group D). There was no fat accumulation in the livers of Group C or Group D, confirming the action of adenine reported by Handschumacher et al. (3). The liver fatty acid composition of Groups C and D was identical with that shown in Table II, Column 2, for rats fed the basal diet only. The composition of carcass lipids and of mesenteric and epididymal adipose tissue was not altered by any of the dietary supplements; thus the compositions shown in Columns 6, 7, and 8 of the table apply as well to rats in Groups C and D and to those fed the basal diet only.

**Lipogenesis as Measured by Tritium Incorporation into Fatty Acids**—Fig. 1 shows the total incorporation of tritium from labeled body water into the fatty acids of various tissues as a function of time and orotic acid addition to the diet. The tritium content of liver fat in the control rats reached a maximum within 24 to 36 hours, whereas that of orotic acid-fed rats was still increasing after 72 hours (Fig. 1A), as was the amount of liver fat (see Table III). The data in Fig. 1, B to D, reveal that orotic acid-fed rats incorporate more tritium into the fatty acids not only of the liver but of all tissues examined. The whole rat, for example (Fig. 1D), accumulated nearly twice as much fatty acid tritium when 1% orotic acid was present in the diet.  

Fig. 2 gives the specific activities of tissue fatty acids in this experiment. The specific activity of liver fatty acids of the control animals rose quickly to a value near the maximum in 24 to 36 hours (Fig. 2A), confirming the rapid turnover of the fatty acids of this tissue (19). The liver fatty acids in these control rats reached a maximal relative specific activity of 4.25, which means that they contained 4.25 times as much tritium per atom as that found in the body water. During the first 8 hours (8 a.m. to 4 p.m.), the food intake was very low, which could explain the low rate of lipogenesis. In a control experiment with rats fasted for 24 hours before tritium administration, there was almost no incorporation into fatty acids of any tissues during the subsequent 24 hours during which the fast was continued. Similar results have been reported for the incorporation of acetate-14C (17) and deuterium (18).
Fig. 2. Specific activity of fatty acids tritiated in vivo from body water of constant specific activity. Symbols are described in the legend for Fig. 1. Relative specific activity (RSA) = disintegrations per minute per mmole of fatty acid divided by disintegrations per minute per mmole of body water. OA, orotic acid.

mole as did the body water, or that the equivalent of 8.5 g atoms of body water hydrogen had been incorporated per mole of fatty acid. Since the liver fatty acids contained an average of 31.7 hydrogen atoms per molecule, 27% of the fatty acid hydrogen atoms were derived from body water.

As in the normal livers, a portion of the fatty acids of livers from orotic acid-fed rats became quickly labeled during the first 24 hours, but the over-all specific activity was less, as a result of dilution by the larger pool of pre-existing fatty acids (see Table III). Even after 24 hours, the specific activity approached only slowly that of the control livers.

In the orotic acid-fed rats as well as in the controls, the relative specific activities of total serum and liver fatty acids were approximately equal (Fig. 2, A to B). As will be seen from Table IV, the amount of tritium in the serum of the orotic acid-fed rats was very low, as was the amount of circulating lipid (Table III).

The relative specific activity of the fatty acids of extrahepatic tissues in the control rats tends to reach a plateau at much lower values than liver fatty acids (Fig. 2, C to E), indicating the presence of pools of relatively inert fat which equilibrate slowly with newly made fat. This is seen most clearly in the epididymal adipose tissue (Fig. 2E), where the maximal relative specific activity obtained was only 0.7. When orotic acid was present in the diet, however, the relative specific activity for these extrahaepatic tissues were consistently higher than in the normal animal and they were still increasing during the 48- to 72-hour period.

Table III shows the total fatty acid content of the liver, the carcass, and the serum of the animals used in the tritium incorporation study. The livers of the orotic acid-fed rats were somewhat fatty at the start of the study, as indicated by the finding of twice the normal amount of liver fat as early as 8 hours after tritium administration, and liver fat accumulation continued over the subsequent 72-hour interval. The carcass fat content was quite variable, and no consistent difference between the two groups was apparent. The dietary orotic acid produced a striking fall in total serum fatty acids.

The data in Fig. 1, showing the total incorporation of tritium, as well as data on the serum content of fatty acid tritium, were recalculated to show the percentage distribution of the tritiated fatty acids among the various body compartments at each time interval after tritium administration (Table IV). In the controls without the orotic acid supplement, the percentage of total body tritiated fatty acids found in liver decreased from 12% to less than 7% between 8 and 72 hours, whereas there was a proportionate increase in the percentage found in the carcass, from 82 to 88%. The proportion found in the intestine plus mesentery was relatively constant at 5 to 6%. Thus, in the normal animal, there is a net flux of synthesized fatty acids from the liver to extrahaepatic tissues. In the orotic acid-fed rat, however, a larger proportion of the newly synthesized fatty acids are uncorrected for an isotope effect which probably favors the incorporation of protium over the heavier isotopes. When deuterium and tritium were made equally available in the body water of the rat, there was a preferential incorporation into fatty acids of deuterium by a factor of 1.19.

Table III

<table>
<thead>
<tr>
<th>Hours following tritium administration</th>
<th>Liver</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>-OA</td>
<td>+OA</td>
<td>-OA</td>
</tr>
<tr>
<td>µmoles/g</td>
<td>µmoles/g</td>
<td>µmoles/ml</td>
</tr>
<tr>
<td>8</td>
<td>99</td>
<td>220</td>
</tr>
<tr>
<td>24</td>
<td>85</td>
<td>200</td>
</tr>
<tr>
<td>48</td>
<td>114</td>
<td>412</td>
</tr>
<tr>
<td>72</td>
<td>114</td>
<td>586</td>
</tr>
</tbody>
</table>

Table IV

Percentage distribution of total fatty acid tritium among various body compartments

For experimental conditions, see the legend to Fig. 1. For each time period, the sum of fatty acid tritium in the four compartments is 100%. Orotic acid (OA) was added where indicated at a level of 1% of the diet.

<table>
<thead>
<tr>
<th>Hours following tritium administration</th>
<th>Tritated fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>-OA</td>
<td>+OA</td>
</tr>
<tr>
<td>µmoles/g</td>
<td>µmoles/g</td>
</tr>
<tr>
<td>8</td>
<td>11.9</td>
</tr>
<tr>
<td>24</td>
<td>9.7</td>
</tr>
<tr>
<td>48</td>
<td>7.9</td>
</tr>
<tr>
<td>72</td>
<td>6.8</td>
</tr>
</tbody>
</table>

* The serum content of tritiated fatty acids was not determined in animals killed 8 hours after tritium administration; therefore the total of the fatty acid tritium in the other three compartments was taken as 100% at this time period. In view of the small amount of circulating labeled fatty acid, its omission from the calculations would have little effect on the values determined for the other compartments.
acids was found in the liver and this proportion did not diminish with time, nor did the proportion found in the carcass increase. The very low proportion of new fatty acid found circulating in the serum of the erotic acid-fed rats, as compared to the controls, should also be noted.

**Plasma and Liver Lipids after Feeding of Orotic Acid and Adenine**—The low levels of serum lipids found in erotic acid-fed controls, should also be noted. The very low proportion of new fatty acid found circulating in the serum of the erotic acid-fed rats, as compared to the controls, did not diminish with time, nor did the proportion found in the carcass increase. Circulating lipids reached a minimum after 4 days of erotic acid feeding. After a week, the subsequent addition of adenine had no effect on it of adding adenine to the diet. A preliminary report of some of these observations has appeared (22). Fig. 3 shows the progressive decline in the levels of circulating triglycerides, phospholipids, and cholesterol which was already apparent within 16 hours after 1% erotic acid was added to the diet. Analyses on Days 0 and 2 established base-line values for animals and each point represents the average of four to seven animals. Weanling male rats were fed the basal diet (R-1-FF) for 7 days before the times shown above. Analyses on Days 0 and 2 established base-line values for animals on this diet. From Day 2, as indicated by the arrow, 1% erotic acid was added to the basal diet; 7 days later, 0.25% adenine sulfate was added in addition to the erotic acid. Food was supplied ad libitum and groups of animals were killed between 8 and 10 a.m. on the indicated days. The results are from two experiments and each point represents the average of four to seven animals.

**TABLE IV**

<table>
<thead>
<tr>
<th>Additions to basal diet (W-5)</th>
<th>Plasma component</th>
<th>mg/ml</th>
<th>mg/μg</th>
<th>mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Glucose</td>
<td>1.50</td>
<td>0.35</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td>Nonesterified fatty acids</td>
<td>0.35 (4)</td>
<td>0.35 (10)</td>
<td>36.7 (2)</td>
</tr>
<tr>
<td>1% Orotic acid (4 days)</td>
<td>Glucose</td>
<td>1.41</td>
<td>0.37</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>Nonesterified fatty acids</td>
<td>0.37 (3)</td>
<td>0.33 (10)</td>
<td>36.7 (2)</td>
</tr>
<tr>
<td>1% Orotic acid (21 days)</td>
<td>Glucose</td>
<td>1.20</td>
<td>0.33</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>Nonesterified fatty acids</td>
<td>0.33 (4)</td>
<td>0.33 (10)</td>
<td>35.0 (4)</td>
</tr>
</tbody>
</table>

Fig. 5. The effect of dietary orotic acid (OA) and adenine (AD) on hepatic phospholipids and cholesterol. •, phospholipids; ○, cholesterol. Experimental conditions were the same as in Fig. 3. The dashed line indicates the hepatic cholesterol content of rats that were given the orotic acid supplement from Day 2 but received no additional adenine supplement on Days 9 to 16.

**TABLE V**

Effect of dietary orotic acid on plasma concentrations of glucose, nonesterified fatty acids, and total protein.

<table>
<thead>
<tr>
<th>Additions to basal diet (W-5)</th>
<th>Plasma component</th>
<th>mg/ml</th>
<th>mg/μg</th>
<th>mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Glucose</td>
<td>1.50</td>
<td>0.35</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td>Nonesterified fatty acids</td>
<td>0.35 (4)</td>
<td>0.35 (10)</td>
<td>36.7 (2)</td>
</tr>
<tr>
<td>1% Orotic acid (4 days)</td>
<td>Glucose</td>
<td>1.41</td>
<td>0.37</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>Nonesterified fatty acids</td>
<td>0.37 (3)</td>
<td>0.33 (10)</td>
<td>36.7 (2)</td>
</tr>
<tr>
<td>1% Orotic acid (21 days)</td>
<td>Glucose</td>
<td>1.20</td>
<td>0.33</td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>Nonesterified fatty acids</td>
<td>0.33 (4)</td>
<td>0.33 (10)</td>
<td>35.0 (4)</td>
</tr>
</tbody>
</table>

**Fig. 3.** The depression of plasma lipids by dietary orotic acid (OA) and its reversal by adenine (AD). ○, triglycerides; ●, phospholipids; Δ, cholesterol. Weanling male rats were fed the basal diet (R-1-FF) for 7 days before the times shown above. Analyses on Days 0 and 2 established base-line values for animals on this diet. From Day 2, as indicated by the arrow, 1% erotic acid was added to the basal diet; 7 days later, 0.25% adenine sulfate was added in addition to the erotic acid. Food was supplied ad libitum and groups of animals were killed between 8 and 10 a.m. on the indicated days. The results are from two experiments and each point represents the average of four to seven animals.

**Fig. 4.** The effect of dietary orotic acid (OA) and adenine (AD) on the total fatty acids of plasma and liver. ○, liver; ●, plasma. Experimental conditions were the same as in Fig. 3. The dashed lines are for rats that were given the orotic acid supplement from Day 2 but received no additional adenine supplement on Days 9 to 16.

**Fig. 5.** The effect of dietary orotic acid (OA) and adenine (AD) on hepatic phospholipids and cholesterol. •, phospholipids; ○, cholesterol. Experimental conditions were the same as in Fig. 3. The dashed line indicates the hepatic cholesterol content of rats that were given the orotic acid supplement from Day 2 but received no additional adenine supplement on Days 9 to 16.
triglycerides in the control rats was 10 times faster than in those
acid was fed in the diet. Over the 3-hour period, accumulation of
in such plasma lipid accumulation which resulted when erotic
entering the circulation. Fig. 6 shows the very marked reduction
which may be a necessary prerequisite for such removal (27).
the circulation (26), possibly by inhibiting the lipase activity
venously, interferes with the normal removal of glycerides from
plasma concentrations of glucose, nonesterified fatty acids, or
mental Procedure”). Each point represents three to four animals.
feeding, but there was a longer delay after starting the adenine
supplement before liver cholesterol began to decline.
There was little or no effect of erotic acid feeding on the
plasma concentrations of glucose, nonesterified fatty acids, or
total protein (Table V).
Accumulation of Plasma Lipids after Triton WR-1339 Injection
Evidence is available that Triton, administered intra-
venously, interferes with the normal removal of glycerides from
the circulation (26), possibly by inhibiting the lipase activity
which may be a necessary prerequisite for such removal (27).
When removal from plasma is inhibited, the rate of plasma lipid
accumulation becomes a reflection of the rate at which lipid is
entering the circulation. Fig. 6 shows the very marked reduction
in such plasma lipid accumulation which resulted when erotic
acid was fed in the diet. Over the 3-hour period, accumulation of
triglycerides in the control rats was 10 times faster than in those
ingesting 1% erotic acid.

DISCUSSION
The mechanism by which dietary orotic acid induces a fatty
liver in the rat appears to involve a defect in the synthesis or
secretion of lipoproteins by this organ. Three types of evidence
are offered. (a) Hepatic fat accumulation is preceded by a
progressive decline in the levels of plasma triglyceride, cholest-
terol, and phospholipid, and the onset of the accumulation
coincides in time with the decline of circulating lipids to a mini-
mal level. The reversal of the hepatic accumulation by dietary
adenine is preceded by a return of circulating lipids to normal or
above normal levels. Several lines of evidence have led to the
conclusion that the liver is the major and possibly the only
source of endogenous plasma glycerides (28-30). This conclu-
sion receives further support from the results of the present
study, in which the specific radioactivities of hepatic and serum
lipids tritiated in vivo were found to be similar (Fig. 2). Further-
more, it seems generally accepted that lipids circulate in the
plasma as lipoproteins, which, in the absence of exogenous fat,
are synthesized and secreted by the liver (31). After rats had
been fed orotic acid for 10 days, examination of their plasma
lipoproteins in the ultracentrifuge showed a reduction of the
low density fraction (d < 1.063) to 10% and the high density
fraction (d > 1.063) to 40% of normal (22). (b) There is very
little plasma lipid accumulation when orotic acid-fed rats are
fed a fat-free diet (Table II) and from their high i4C content
of erotic acid synthesis in the rat, derived from their high 14C content
of fatty acids in their body lipids (32). Furthermore, orotic acid
may induce an expansion of the metabolically active

Fig. 6. Accumulation of plasma lipids after the intravenous
injection of a nonionic surface-active agent (Triton WR-1339).
*, rats fed basal diet R-I-FF; O, rats fed the basal diet + 1%
ortotic acid (OA) for 6 days before the Triton injection. At zero
time (8:30 to 10:00 a.m.), each rat (100 to 120 g) received an in-
jection via a tail vein of 0.5 ml of a 10% solution of Triton WR-1339
in 0.95% aqueous NaCl. After Triton administration, groups of
rats were killed and plasma lipids were analyzed (see “Experi-
mental Procedure”). Each point represents three to four animals.
fatty acid compartments in these tissues; there was no marked or consistent increase in the size of the total extrahepatic fatty acid pool (Table III). Such an expansion could result from a stimulation of fatty acid synthesis in these tissues, which in turn may result from the low levels of circulating lipid by the same mechanism that depresses fatty acid synthesis when fat intake and, consequently, the amount of circulating fat are high. Or possibly a feedback mechanism is operating to reduce the flow of fatty acids from other tissues into a liver which is already accumulating lipid. If the outflow of fatty acids from extrahepatic tissues is indeed diminished, it could also result from a direct effect of orotic acid on these tissues. Contrary to the present results, Creasey et al. (2), using acetate-1-\textsuperscript{14}C incorporation over a 6-hour interval as a measure of lipogenesis, found a higher specific activity in the extrahepatic tissues of control rats than in those which had been on a 1% orotic acid diet for 10 days. Because the effect of orotic acid on the size of the acetate pool is unknown, these results are difficult to interpret. Furthermore, after 10 days on a 1% orotic acid diet, liver fat accumulation is nearly complete (2) and the balance between the various lipid compartments within the animal has apparently reached some new equilibrium. Animals studied after only 4 days on orotic acid, as in the present study, are undergoing the marked alterations which lead to this new equilibrium.

Although it seems clear that in the rat the ingestion of orotic acid greatly diminishes the rate of lipoprotein synthesis or secretion by the liver, or both, the mechanism of this effect and its possible relationship to normal hepatic fat metabolism remain to be established. Hankin (35) found no accumulation of lipoproteins in the livers of orotic acid-fed rats which were accumulating large amounts of liver fat. This suggests that lipoprotein synthesis is inhibited. Orotic acid feeding has been shown to reduce the levels of liver acid-soluble adenine nucleotides (4). Furthermore, the fall in liver adenine nucleotides and their return to normal levels upon dietary adenine supplementation correlate very well with the concomitant fall in circulating lipid concentration and its return to normal. Therefore, it is possible that an orotic acid-induced adenine nucleotide deficiency in liver results in an inability to produce lipoproteins. The administration of ethionine to female rats also depresses liver ATP levels (36), inhibits protein synthesis (cf. (37)), depresses serum lipids (38), and results in liver lipid accumulation (39). All these effects are reversed by adenine supplementation (40). Thus, depressed liver adenine nucleotide levels are a common feature of fatty livers induced by orotic acid and ethionine. Also of interest is the observation of Henderson (41) that injection of 4-aminopyrazolopyrimidine, a specific adenine antagonist, produces a depression of plasma lipids and a fatty liver in mice. The normal synthesis or secretion of liver lipoproteins may be sensitive, directly or indirectly, to the availability of some adenine derivative.

**SUMMARY**

Weanling male rats were fed purified diets containing 1% orotic acid, a pyrimidine precursor. A large accumulation of liver triglycerides and cholesterol occurred with diets varying in fat content from 0 to 40% and in protein from 18 to 40%. Liver fat accumulation was greatest when a fat-free diet was used. The addition of 0.25% adenine sulfate to all of the diets prevented the fatty liver. With a fat-free diet containing orotic acid, 95% of the fatty acids accumulating in the liver were accountable as palmitic, palmitoleic, and oleic acids.

The rate of lipogenesis and the distribution of synthesized fatty acids in the body were determined at intervals over a 72-hour period by measuring the incorporation of tritium from labeled body water into the fatty acids. Supplementation of the diet with orotic acid caused a large increase in the incorporation of tritium into the fatty acids of liver as well as of other tissues, but reduced to 10% of normal the amount of labeled fatty acids appearing in the plasma. The specific activity of the accumulating liver fat characterized it as being newly synthesized. The specific activity of fatty acids in extrahepatic tissues was higher when orotic acid was fed.

Plasma concentrations of triglycerides, cholesterol, and phospholipid fell within 16 hours after addition of orotic acid to the diet, and minimal levels, which were 10 to 15% of normal, were reached within 4 days, at which time liver accumulation of fat began. After the subsequent addition of adenine to the diet, the levels of circulating lipid quickly returned to normal and an unloading of liver fat followed. Plasma concentrations of glucose, nonesterified fatty acids, and total protein were unaltered by feeding of orotic acid.

Orotic acid feeding prevented the accumulation of plasma triglyceride which followed the intravenous injection of Triton WR-1339, a nonionic surface-active agent.

It is concluded that orotic acid feeding inhibits the synthesis or secretion of lipoproteins by the liver; consequently, fatty acids, largely synthesized by the liver, accumulate as triglycerides. Adenine supplementation reverses this inhibition.

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**REFERENCES**

An Orotic Acid-induced, Adenine-reversed Inhibition of Hepatic Lipoprotein Secretion in the Rat
H. G. Windmueller

J. Biol. Chem. 1964, 239:530-537.

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