Absence of Fatty Livers in Rhesus Monkeys Fed Orotic Acid

ABSTRACT
Pairs of rhesus monkeys were fed for 10 wk a basal diet containing 1% orotic acid or 10% nonfat milk powders. Amounts of total lipids in the liver and hepatic morphology were normal after 10 wk indicating that orotic acid in the diet did not induce fatty livers in rhesus monkeys.

INTRODUCTION
Fatty livers are induced in rats fed orotic acid in the diet (6, 14, 19, 20, 22). The formation of fatty livers in rats induced by orotic acid is believed due to impaired hepatic assembly and/or secretion of very low density lipoproteins (VLDL) (19) resulting in hepatic triglyceride accumulation with concomitant depression of serum lipids (including cholesterol) (14, 22).

Although orotic acid and skim milk powders which contain large amounts of orotic acid cause fatty livers in rats (16), orotic acid does not appear to do so in chickens, mice, dogs, hamsters, pigs, guinea pigs, and monkeys (2, 3, 21). It is not known what effect, if any, orotic acid has on lipids of human liver. However, Portman (17) has suggested that rhesus monkeys are similar to man in their response to changes in diet with resultant changes in blood lipids, indicating rhesus monkeys would be ideal primates for studying effects of orotic acid on blood and liver lipids. Also, orotic acid given to humans at relatively high doses has a slight but significant hypocholesterolemic effect (9).

Two liters per day of skim milk (8) or fermented milk (12) consumed by human subjects exerts a definite hypocholesterolemia. Consequently, orotic acid, skim milk, or fermented milk in the diet of rhesus monkeys may be hypocholesterolemic, but at the same time 1% orotic acid in the diet may induce a fatty liver. This paper reports a preliminary study of effects of orotic acid and milk powders in the diet on blood and liver lipids of rhesus monkeys.

MATERIALS AND METHODS
Six rhesus monkeys (four males and two females), approximately 3 yr old, were fed a semipurified basal diet for monkeys for 2 wk. The basal diet was (% by weight): ground wheat (30.0), alfalfa meal (2.0), yellow corn (36.1), soybean meal (12.0), sucrose (10.0), corn gluten meal (5.0), animal fat (1.0), brewers dried grain (1.0), calcium carbonate (1.5), calcium phosphate dibasic (1.0), trace mineral salts (.3), and fat and water soluble vitamins (.1). After 2-wk adaptation to the basal diet, monkeys were divided into three pairs and were fed the following experimental diets for 10 wk:

- Pair 1, basal diet
- Pair 2, 1% orotic acid in basal diet
- Pair 3, 10% nonfat milk powders in basal diet

When the diet contained 10% milk powders, the yellow corn and the soybean meal components each were decreased by 5%. Pair 3 received the diet containing 10% nonfat dry milk for the first 6 wk. The next 4 wk they were fed the diet containing 10% nonfat dry yogurt to which lactose was added to compensate for that amount utilized during fermentation. Since skim milk was reported to be hypocholesterolemic in humans (8), nonfat dry milk was fed at 10% to observe any hypocholesterolemic effect that it might have in monkeys. However, no hypocholesterolemic effect over that of the basal diet was observed after
the monkeys had consumed the nonfat dry milk diet for 6 wk so they were changed to a nonfat dried yogurt diet to determine if fermented milk had any hypocholesterolemic effect as reported for humans (7, 12, 13, 18). Orotic acid was purchased from Aldrich Chemical Co., Milwaukee, WI; nonfat dry milk was from Carnation Co., Los Angeles, CA; nonfat dry yogurt was prepared in the University of Wisconsin dairy plant. Lactose was assayed by the method of Lawrence (11), and orotic acid was determined by the procedure of Okonkwo and Kinsella (15). Food intake was constant throughout the experiment (200 g/monkey per day). Body weight, cholesterol, and triglycerides of serum of each animal were measured weekly. There were no obvious differences in body weight gains. All blood samples were from animals fasted overnight. At the termination of the experiment, livers were biopsied for chemical and morphological evaluation. Cholesterol and triglycerides in blood serum were determined by the Bio-Dynamics Unitest System Kit (Bio-Dynamics/bmc, Indianapolis, IN); total liver lipid was determined by the method of Folch et al. as given by Christie (1).

RESULTS AND DISCUSSION

Because of a scarcity in the supply of rhesus monkeys for these experiments, there were insufficient numbers of animals in the experiment to allow statistical analyses of the results. However, there was a decline in serum cholesterol in all three pairs of monkeys over the 10 wk. The average decreases as percent of initial serum cholesterol were 20, 16, and 22 for pairs 1, 2, and 3. Thus, skim milk, yogurt, and orotic acid in the diet did not possess hypocholesterolemic effect above that of the basal diet. This may reflect the hypocholesterolemic effect of the soya proteins (4) in the diet. However, there was definitely no hypercholesterolemic response to the caseins in the milk-based diets as reported by Hamilton and Carroll (5). There were no definitive trends in serum triglycerides, with those samples from animals on the basal diet being slightly less at the end of the experiment.

In contrast to the results for rats (2, 6, 14, 19, 20, 22), orotic acid at 1% of the diet did not cause fatty livers in rhesus monkeys after 10 wk on the diet as judged by assay of the total liver lipid (Table 1) and microscopic examination of liver biopsy slices. Hepatic morphology was normal for all animals. Total lipid content of the liver varied from 5.1 to 7.6% of wet weight. Kerr et al. (10) have reported that total lipids comprise 8.13 ± 0.17% of the wet weight of liver in adult rhesus monkeys; however, juvenile monkeys, as in this experiment, accumulate less lipid in the liver than adults. Preliminary results by Valli et al. (21) reported that orotic acid at 4% in the diet did not induce fatty livers in an unspecified strain of monkey after 20 days of feeding. There is a possibility that our semipurified diet contained enough adenine to reverse any orotic acid-induced accumulation of hepatic tri-

<table>
<thead>
<tr>
<th>Pair no.</th>
<th>Diet</th>
<th>Orotic acid in the diet</th>
<th>Animals</th>
<th>Total lipids in wet liver&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Basal</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
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<tr>
<td>2</td>
<td>Basal + orotic acid</td>
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<td>Male</td>
<td>6.12</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Female</td>
<td>7.56</td>
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<tr>
<td>3</td>
<td>Milk powders&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Male</td>
<td>6.24</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>7.07</td>
</tr>
</tbody>
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<sup>a</sup>Assayed after 10 wk on indicated diets.

<sup>b</sup>Monkeys consumed the diet containing 10% nonfat dry milk during weeks 0 to 6 and 10% nonfat dry yogurt during weeks 6 to 10.
glycerides. Adenine sulfate, as low as .25% of the diet, reversed orotic acid-induced fatty liver in rats (6, 22). Milk contains virtually no adenine, but whole grain which was used to prepare the basal diet might contain moderate quantities of purines. Further extension of this work should exclude adenine containing components from the basal diet, or it should be demonstrated that this diet induces fatty livers in rats.

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REFERENCES