the assay mice. Thus plasma obtained from animals administered comparable large doses of estrogen does not contain residual biologically active estrogen (3). Nor does such plasma depress erythropoiesis in normal mice or antagonize the erythropoietic action of exogenous ESF (3).

Studies are in progress to determine more specifically by chemical methods, whether the serum proteins (particularly beta globulin) demonstrated by us (Wong *et al.*, unpublished) to serve as substrates for the REF are reduced in concentration by the dosages of estradiol employed in this study. In this regard, estrogen has been reported to depress some phases of protein metabolism in liver, e.g., the glutamic-oxaloacetic and glutamic-pyruvic transaminase systems (18).

Summary. Previous observations that testosterone incrases production of the ESF and the REF are confirmed and extended. The combination of testosterone and hypoxia act synergistically in augmenting plasma ESF and REF activities. The depressive effect of estradiol on ESF production is not accompanied by a decrease in REF activity. However, the serum from estradiol-treated rats exhibits a reduced capacity to serve as a substrate for the REF in the generation of the ESF.

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Protection by Orotic Acid Against the Renal Necrosis and Fatty Liver of Choline Deficiency* (33447)

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Choline deficiency produces fatty infiltration of the liver in rats (1). Recently, Porta, Manning, and Hartroft reported that feeding orotic acid hinders hepatic fat accumulation in choline deficiency (2). This observation is unexpected, because orotic acid feeding itself causes fatty infiltration of the liver (3, 4), an effect that can be prevented by simultaneous administration of adenine sulfate (4, 5).

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TABLE I. Hepatic Triglyceride Concentrations after 24 Hours of Feeding.

Dietary group[a]	Weight (g)		Food intake (g)	Hepatic triglycerides[b] (μmoles/g wet wt. liver)
	Initial	Gain		
Choline-supplemented	64.5	5.0	8.9	7.3 ± 1.7
Choline-supplemented + 1% orotic acid	64.3	5.2	9.0	7.5 ± 2.9
Choline-deficient	63.7	5.0	8.8	22.4 ± 3.0[c]
Choline-deficient + 0.25% adenine sulfate	63.8	5.2	8.7	20.9 ± 4.2[c]
Choline-deficient + 1% orotic acid	64.2	5.1	9.0	13.6 ± 2.5[d]
Choline-deficient + 1% orotic acid and 0.25% adenine sulfate	64.0	5.2	8.8	12.3 ± 4.9[d]

[a] Each group consisted of 6 animals.
[b] Mean ± SD.
[c] $p < .001$ compared with choline-supplemented group.
[d] $p < .01$ compared with choline-deficient group.

It is not clear whether the beneficial action of orotic acid in choline deficiency is due to a decreased need for choline or to some other mechanism. If orotic acid reduces the requirement for choline, it might be expected to also protect against the acute hemorrhagic necrosis that choline deficiency produces in the kidney of weanling rats (6, 7). The present study confirms the discovery of Porta, Manning, and Hartroft (2), and indicates that orotic acid does indeed inhibit the renal necrosis of choline deficiency. In addition, the ability of adenine sulfate to modify the protective action of orotic acid was studied.

Materials and Methods. The basal choline-deficient diet consisted of casein 6%, alcohol-extracted peanut meal 25%, sucrose 43%, lard 20%, salt mix (Hawk-Oser no. 3) 5% and vitamin mix 1%. The latter provided, per 100 g of diet, 0.2 mg thiamine hydrochloride, 0.4 mg riboflavin, 0.2 mg pyridoxine hydrochloride, 2.0 mg nicotinamide, 0.5 mg menadione, 1.0 mg calcium pantothenate, 20.0 mg inositol, 5.0 mg α-tocopherol, 25 IU vitamin D, and 500 IU vitamin A.

To assess the effect of orotic acid and of adenine sulfate on hepatic fat accumulation in choline deficiency, male weanling Sprague-Dawley rats were fed the basal diet supplemented with 0.5% choline chloride for 1 week, then were divided into 6 groups of 6 rats each, closely matched for weight. One group remained on the choline-supplemented diet, while the others were placed on the following diets respectively: choline-supplemented + 1% orotic acid; choline-deficient; choline-deficient + 0.25% adenine sulfate; choline-deficient + 1% orotic acid; and choline-deficient + 1% orotic acid and 0.25% adenine sulfate. All rats were individually caged and allowed food and water *ad libitum*. The animals were killed after 24 hr on the experimental diets, and their hepatic triglycerides were measured by the method of Butler *et al.* (8).

The influence of orotic acid, with and without adenine sulfate, on the renal lesion of choline deficiency was studied in two experiments. In the first, male Sprague-Dawley rats weighing 58–75 g were placed on either the basal choline-deficient diet + 1% orotic acid, the basal diet alone, or the basal diet + 1% orotic acid and 0.25% adenine sulfate. The last two groups of animals were pair-fed with the first and were closely matched for weight. All rats were individually caged and allowed water *ad libitum*. Surviving animals were sacrificed at 21 days and their kidneys examined microscopically for evidence of healing renal necrosis (6). Since these rats had been prefed the choline-supplemented diet for 1 week after weaning, a second experiment was performed in which the animals were not prefed, and weighed only 35–47 g; experimental procedure was otherwise the same.

Results. Hepatic triglyceride concentrations are shown in Table I. In 24 hr, choline deficiency caused a three-fold increase in triglyceride levels. Orotic acid feeding in the absence of choline deficiency did not influ-

TABLE II. Incidence of Hemorrhagic Renal Necrosis.

Dietary group	No. of rats	Weight (g) Initial	Gain, first 5 days	Mortality[a]	Incidence of renal necrosis Acute	Healing	Total
Experiment 1							
Choline-deficient	9	66.4	16.3	2 (22%)	2 (22%)	6 (67%)	8 (89%)
Choline-deficient + 1% orotic acid	19	67.1	16.0	1 (5%)	1 (5%)	6 (32%)	7 (37%)[b]
Choline-deficient + 1% orotic acid and 0.25% adenine sulfate	19	65.8	14.6	4 (21%)	4 (21%)	9 (47%)	13 (68%)
Experiment 2							
Choline-deficient	20	40.3	16.9	8 (40%)	8 (40%)	9 (45%)	17 (85%)
Choline-deficient + 1% orotic acid	20	41.1	16.7	5 (25%)	5 (25%)	4 (20%)	9 (45%)[b]
Choline-deficient + 1% orotic acid and 0.25% adenine sulfate	20	40.9	14.2	6 (30%)	6 (30%)	6 (30%)	12 (60%)
Total							
Choline-deficient	29	—	—	10 (34%)	10 (34%)	15 (52%)	25 (86%)
Choline-deficient + 1% orotic acid	39	—	—	6 (15%)	6 (15%)	10 (26%)	16 (41%)[c]
Choline-deficient + 1% orotic acid and 0.25% adenine sulfate	39	—	—	10 (26%)	10 (26%)	15 (38%)	25 (64%)

[a] All fatalities were associated with acute renal necrosis.
[b] $p < .05$ compared with choline-deficient group.
[c] $p < .001$ compared with choline-deficient group.

ence triglyceride concentrations in this short a period of time, confirming previous findings (5). Despite this, orotic acid prevented the accumulation of triglycerides in choline-deficient animals by almost 50%. Adenine sulfate did not influence either the degree of lipid accumulation in choline deficiency or the protective effect of orotic acid.

As shown in Table II, orotic acid provided partial protection against hemorrhagic renal necrosis, since in each experiment it lowered both the incidence of the lesion and the mortality rate. Although the change in mortality was not significant, the over-all decrease in the incidence of renal necrosis from 86% to 41% was highly significant ($p < .001$). Adenine sulfate appeared to partially nullify the protective action of orotic acid, but this effect was not significant. No morphologic differences were apparent among the renal lesions in the three groups of animals, during either the acute or healing phase. All deaths occurred between days 6 and 10. For the first 5 days, weight gain was less in the animals given adenine sulfate than in the other two groups, despite the use of pair-feeding.

Discussion. These studies indicate that 1% dietary orotic acid provides protection against both the fatty infiltration of the liver and the hemorrhagic renal necrosis produced by choline deficiency. Its beneficial effect on the liver within 24 hr is of interest, since in the absence of choline deficiency, orotic acid does not alter hepatic lipids for several days (5). Also, dietary adenine sulfate did not influence its protective effect on the liver, even though the delayed fat accumulation that orotic acid produces is completely prevented by adenine sulfate (4, 5).

The beneficial effect of orotic acid on both the liver and kidney lesions suggests that it may decrease the over-all need for choline. Dietary artifact cannot explain the findings in the liver, since the various groups of animals had similar weight gains and food intakes. Similarly, the protection against renal necrosis was not merely a pseudolipotropic effect of reduced food intake (9), because

pair-feeding was used and weight gain before necrosis developed was alike in animals fed the choline-deficient diet alone and those given orotic acid in addition. However, since impaired growth decreases the frequency of renal necrosis (7), the lesser weight gain of animals given adenine sulfate could have obscured an ability to significantly nullify the protective action of orotic acid.

The mechanism by which orotic acid may lower the requirement for choline is obscure. Since phospholipid abnormalities may be of pathogenetic importance in both the fatty infiltration of the liver (10) and the renal necrosis (11) of choline deficiency, perhaps orotic acid influences phospholipid metabolism. Radioactivity can be recovered in hepatic cytidine nucleotides after administration of orotate-6-^{14}C to rats (12); it is conceivable, therefore, that orotic acid stimulates the cytidine-diphosphate-choline pathway of lecithin biosynthesis (13). The fact that hepatic levels of cytidine-diphosphate-choline are not depressed in choline deficiency (14) does not rule out this possibility.

Further study is needed to clarify the metabolic relationships between choline and orotic acid.

Summary. Addition of 1% orotic acid to a choline-deficient diet lowered the incidence of hemorrhagic renal necrosis in young rats from 86% to 41%, and within 24 hr prevented the accumulation of hepatic triglycerides by almost 50%. Simultaneous supplementation of the diet with 0.25% adenine sulfate did not influence these protective effects of orotic acid in choline deficiency.

It is suggested that orotic acid may lower the requirement of the body for choline through a metabolic interaction.

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Exocrine Function of the Chick Pancreas as Affected by Dietary Soybean Meal and Carbohydrate* (33448)

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Chicks develop an enlarged pancreas when fed unheated soybean meal. Autoclaving the meal destroys or inactivates the heat-labile component(s) which cause this effect on the pancreas (5). Recent histological studies in our laboratory (24) have confirmed that this enlargement is due to hyperplasia of the pan-

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