Fatty Liver Induced by Orotic Acid Feeding. (22005)

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In the course of a study of the effects of orotic acid, a normal component of milk, on the nutrition of young rats, it was observed that several animals developed fatty livers(1). The present study was undertaken to determine whether this effect can be consistently elicited. Since orotic acid is known to be the normal precursor of thymine and the methyl group of thymine is derived from the "C_1 pool"(2), it seemed possible that fatty livers induced by orotic acid derive from depletion of the available supply of methyl groups, analogous to the effects of nicotinamide(3) and glycocyamine(4). This possibility was tested by including various lipotropic agents in orotic acid containing diets.

Experimental. Male rats of a locally maintained strain(5), weighing 40-45 g, were placed on the various experimental diets, in groups of 10 rats each. The basal diet was as follows: casein 18%, salts(6) 5%, sucrose 75%, corn oil 2%. To each 2 kg batch of diet was added the following vitamin supplement: cod liver oil 10 g, thiamine hydrochloride 25 mg, inositol 500 mg, pyridoxine hydrochloride 25 mg, riboflavin 50 mg, calcium pantothenate 125 mg, niacin 500 mg, *p*-aminobenzoic acid 250 mg, methylnaphthoquinone 25 mg, biotin 2 mg. In addition, all diets contained 0.2% of sulfadiazine to limit the activity of the intestinal flora. The diets of various groups, as shown in Table I, contained also the following special supplements: folic acid 5 mg/2 kilo, vit. B_{12} 1 mg/2 kilo, methionine 0.8%, choline 0.3%, thymine 1%, uracil 1%. The animals were sacrificed after 28 days and liver fat was determined by a method described previously (7). The results are summarized in Table I.

Discussion. Although the mechanism remains obscure, it is apparent in Table I that the presence of orotic acid in the diet of the rat results in the accumulation of liver fat. Analysis of the lipid mixture from such livers revealed that the increase is almost entirely in the neutral fat component, much as in the fatty livers of choline-deficient animals. The fatty livers do not appear to relate to some general action of pyrimidines *per se,* since neither uracil nor thymine induced fat accumulation. The failure of folic acid, cobalamine, methionine and choline to prevent the liver fat accumulation induced by orotic acid feeding would appear to eliminate the possibility that orotic acid, by forcing thymine synthesis, creates so serious a drain on the "C_1" or methyl group supply that choline deficiency results. Indeed, the most heavily fatty livers observed in the entire series were

TABLE I. Effect of Orotic Acid and Lipotropic Factors on Liver Fat.

Orotic acid, %	Special supplement	Wt gain, g/day	Liver wt, g	Liver fat, % (wet basis)	
				Mean ± S.E.	Range
0	0	2.9	7.07	5.8 ± .8	4.3– 7.2
.2	0	2.8	6.02	9.2 ± 1.7	5.2–13.8
.5	0	2.5	7.94	10.9 ± 1.3	7.5–15.3
1.0	0	2.4	9.07	13.6 ± 1.4	8.3–17.0
0	Folic, B_{12}	3.0	6.50	5.9 ± .8	3.9– 9.7
.2	*Idem*	3.1	7.57	4.9 ± .6	3.6– 6.3
.5	"	3.1	9.70	9.7 ± 1.9	5.5–17.3
1.0	"	2.8	9.50	10.1 ± 2.3	5.8–14.8
1.0	Methionine	1.9	5.36	12.7 ± 1.6	7.6–16.1
1.0	Choline	2.4	8.82	16.8 ± 1.7	11.1–22.9
0	Thymine	2.6	6.73	5.5 ± .5	4.2– 6.2
1.0	"	1.8	7.24	13.4 ± 1.4	7.8–21.6
0	Uracil	2.8	7.23	4.8 ± .7	3.3– 6.5

those exhibited by rats receiving both orotic acid and choline. No alternate explanation of the effect of orotic acid on the neutral fat content of the rat liver is apparent at this time.

Summary. 1. Inclusion of orotic acid in the diet of young rats results in fatty liver formation. 2. Uracil and thymine, known to be synthesized from orotic acid, are without effect on liver fat content. 3. This effect of orotic acid is not counteracted by lipotropic factors such as folic acid, cobalamine, methionine or choline.

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Production of Type Specific Antisera to Poliomyelitis Viruses in Rabbits.* (22006)

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The objective of this study was the production for experimental purposes of anti-poliomyelitis sera of high titer, with a high degree of type-specificity. The report of Hirst and Gotlieb(1) of the production of highly specific antisera to influenza viruses by intravenous injection of rabbits suggested that this approach might well serve our purpose. Levinson *et al.*(2) have reported the production of antisera capable of neutralizing poliomyelitis viruses, by intravenous or intramuscular injection of rabbits. They refer to earlier experience of other investigators in producing such neutralizing antibody in the serum of non-susceptible animals.†

The results reported here consist of an extension of the findings of Levinson's group, by the use of smaller doses of virus as antigen given by the intravenous route. In addition, a direct effect of the antisera alone on kidney cell culture has been observed. The main objective was achieved by repeated intravenous injections of rabbits with small doses of each of two types of poliomyelitis viruses grown in tissue culture. The titer of antibody to the same type of virus as that used for antigen (homotypic) as well as to the other type of virus (heterotypic) have been determined by neutralization tests in tissue culture.

Materials and methods. Type 1-Brunhilde and Type 2-YSK poliomyelitis viruses (obtained from Dr. Joseph L. Melnick, Yale University) which had been grown in either monkey testicular or human uterine tissue culture were used as antigens to start immunization of rabbits and for a booster dose at 5 months. The 50% endpoints (negative logarithm) of the cytopathogenic action of the viruses in HeLa culture were approximately 5 and 4 respectively. For booster doses, at 6 months or more from the beginning of immunization, Type 1-Mahoney and Type 2-MEF1 viruses grown in monkey kidney culture (obtained from Connaught Laboratories, Toronto, Canada) were used. The 50% endpoints of viral action in monkey kidney culture were 6.4 and 5.3 respectively. Supernate from a lightly centrifuged culture served as

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† By personal communication, Dr. Hubert Malherbe in the laboratory of Dr. John F. Enders has also reported the production of neutralizing antisera to poliomyelitis viruses, by intramuscular injection of rabbits with virus incorporated in oily adjuvants.