Lack of Effect of Dietary Choline on Cholesterol Absorption in Rat.* (22603)

E. H. RICE, G. F. HUNGERFORD,† AND W. MARX.

Departments of Biochemistry and Nutrition, and Anatomy, School of Medicine, and the Laboratories of the Allan Hancock Foundation, University of Southern California, Los Angeles.

Two recent reports indicate that dietary choline influences plasma cholesterol levels in the rat. Weiss *et al.* found that rats fed a diet containing cholesterol and low in lipotropic factors exhibited plasma cholesterol levels markedly lower than animals fed the same diet supplemented with choline and inositol(1). Ridout *et al.* reported that dietary choline further accentuated the rise in plasma cholesterol occurring 2 to 4 hours after cholesterol feeding(2). These authors suggested that this effect of choline may be due to some action on cholesterol absorption or utilization.

Consideration of these findings has led to the working hypothesis that choline may stimulate cholesterol absorption, and the experiments here described were designed to test this hypothesis. The results indicate that under the conditions reported, choline had no significant effect along these lines.

Methods. A. *Direct determination of cholesterol absorption by thoracic lymph collection.* Male Long-Evans rats, weighing from 90 to 120 g, were fed synthetic diets deficient in, and supplemented with, choline (Diet A, Table I). After a minimum feeding period of 3 weeks, thoracic lymph ducts were cannulated by a modification of the technic of Bollman *et al.*(3). The rats were given 0.85% saline to drink in order to encourage lymph flow. After 24 hours, from 3 to 5 mg of cholesterol-4-C^{14}‡ dissolved in 0.5 to 0.7 ml of Wesson oil were administered by stomach tube to animals exhibiting satisfactory lymph flow, and lymph was then collected in graduated centrifuge tubes for one or 2 hour intervals over a period of 12 to 24 hours. Aliquots of the lymph were placed directly on tared aluminum counting dishes, dried under a heat lamp, and the radioactivity measured in a gas flow counter. The results were corrected for self-absorption. The cholesterol absorption was computed from the radioactivity recovered in the lymph at the end of twelve hours and expressed as percent of the radioactivity administered.

TABLE I. Composition of Diets.*

Ingredients	Diet A (%)	Diet B (%)
Casein	10	15
Sucrose	72	56
Cottonseed oil	9.5	20
Celluflour	2	4
Salt mixture†	5	4.5
L-Cystine	0.5	0.5
Inositol	1.0	0.1
Vitamins	‡	‡

* The supplemented diets contained the following amounts of choline chloride: Diet A, 1%; Diet B, 0.5%.

† Wesson modification of Osborne-Mendel salt mixture.

‡ Each kilogram of diet contained following vitamins: 10 mg each of thiamin hydrochloride, riboflavin, and pyridoxine hydrochloride; 60 mg calcium pantothenate, 60 mg niacin, 0.1 mg biotin, 400 mg para-aminobenzoic acid, 10 mg folic acid, 100 mg ascorbic acid, 5 mg menadione, 4000 USP units vit. A, 800 USP units vit. D, and 0.5 g mixed tocopherols. Water-soluble vitamins were mixed with sucrose, fat-soluble ones with cottonseed oil, before being added to the diet.

B. *Indirect determination of cholesterol absorption by analysis of gastro-intestinal contents.* Male weanling rats of the University of Southern California strain were fed diets deficient in, and supplemented with, choline (Diet B, Table I) for a period of 6 weeks. Following a 24-hour fast, 1.6 mg of cholesterol-4-C^{14} dissolved in 1 ml of Wesson oil were administered by stomach tube to each rat under light ether anesthesia. At the same time, the supplemented rats received, by the same route, 25 mg of choline chloride dissolved in 0.25 ml of water. After a 6 hour absorption period, the animals were sacrificed

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† Present address: Department of Anatomy, George Washington University, Washington, D. C.

‡ The cholesterol-4-C^{14} was prepared and purified as reported(4); it was homogeneous when analyzed by paper chromatography(5) and had a specific activity of 1.3 x 10^5 cpm/mg of C (gas flow counter).

TABLE II. Influence of Dietary Choline on Cholesterol Absorption as Determined Using Rats with Lymph Fistulae.

Choline supplementation	No. of rats	Radioactivity in lymph at 12 hr as % of radioactivity administered	
		Mean	Range
1% of diet	6	18.4	11.8–25.2
None	6	26.7	18.7–43.3

with ether; the livers were removed immediately and weighed.§ The gastrointestinal tract was excised and divided into stomach, upper intestine and lower intestine. The contents of each of these sections were collected separately by rinsing three times with 20 ml portions of hot saline. The washed walls of the intestine, the liver, and in some cases the carcass were also analyzed. Ethanol and potassium hydroxide were added to each sample to a final concentration of 50% (v/v) and 30% (w/v), respectively. The mixtures were heated for 2 hours to complete hydrolysis and then extracted 5 times with petroleum ether (Skelly B). The combined extracts of each sample were evaporated to dryness. The residues were dissolved in chloroform containing 75 mg of Wesson oil per ml of solvent. Duplicate aliquots were placed on tared counting dishes provided with lens paper to prevent creeping. After evaporation of the solvent, radioactivity was determined as above. Cholesterol absorption was computed by subtracting the radioactivity of the intestinal contents from the administered radioactivity and expressed as percent of the administered dose. In general, this difference agreed well with the sum of the radioactivities recovered from the liver, washed walls of the gut, and the carcass.

Results. The results of the first experiment (lymph collection) are presented in Table II. It can be seen that variation among individual animals was considerable. The values for the 2 groups overlap, and statistical analysis fails to show a significant effect of dietary choline. (P for difference of means: between 0.5 and 0.1). It is possible that these variations were in part a consequence of the use of surgery and restraining cages, both of which might affect absorption by way of the nervous and circulatory systems(6). The second experiment was undertaken in an attempt to eliminate these two possible sources of variability. No surgery was performed, and except for the slight trauma of intubating the radioactive cholesterol, the rats were not disturbed. In addition, weanling rats were used, and changes were made in the basic diet in order to produce a more severe choline deficiency, and, at the same time, promote better health and growth (Table I). The amount of choline in the supplemented diet was reduced, since the higher level fed in the first experiment appeared to cause anorexia. The results of the second experiment are given in Table III. The rats fed the deficient diet exhibited markedly enlarged, fatty livers (mean weight, as % of body weight: 6.68; control group: 3.27). One animal which died on the eighth day of the preliminary feeding period showed hemorrhagic kidneys. Both of these symptoms are typical of severe choline deficiency. As in the first experiment, cholesterol absorption was highly variable, and a statistical analysis of the values obtained indicates that

TABLE III. Influence of Dietary Choline on Cholesterol Absorption as Determined from Radioactivity of Gastrointestinal Contents of Rats Fed Cholesterol-4-C^{14}.

Choline supplementation	No. of rats	Mean radioactivity recovered at 6 hr as % of radioactivity administered*			
		GI contents	Washed intestinal wall	Liver	Carcass
.5% of diet	9	45.4 (29.2-62.4)	45.9 (36.1-53.4)	5.6 (.2-10.4)	.2† (.0-.4)
None	9	51.0 (32.2-79.8)	39.6 (21.4-57.4)	4.9 (1.4- 9.5)	.0† (.0-.0)

* Ranges are given in parentheses. † Two animals only.

§ Since, in the first experiment, most of the absorbed cholesterol did not reach the blood stream, and as, in the second experiment, the quantities of sterol administered and ingested with the diet were too low to significantly influence plasma cholesterol levels, the latter values were not determined.

there is no significant difference between the 2 groups of animals. (P for difference of means: between 0.4 and 0.5). It was concluded that, under the conditions of these experiments, choline had no significant effect on the intestinal absorption of cholesterol. These results appear to parallel findings of Tasker(7) and of Shoshkes *et al.*(8), who observed that choline did not influence the absorption of olive oil, and corn oil respectively, using technics similar to those reported in this paper.

Summary. The effect of dietary choline on the intestinal absorption of cholesterol was studied by 2 different technics. The results indicate that under the conditions of these experiments, choline did not influence cholesterol absorption.

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Effect of Exposure to a Cold Environment on Labeling of Phospholipide in Rat Liver Slices.* (22604)

DOROTHY KLINE,† CATHERINE MCPHERSON, E. T. PRITCHARD, AND R. J. ROSSITER.

Department of Biochemistry, University of Western Ontario, London, Canada.

If rat liver slices are allowed to respire in a suitably buffered medium containing acetate-1-C^{14}, the labeled carbon atom of the acetate is incorporated into the acetone-insoluble lipide, chiefly phospholipide(1,2). Recently Kline, DeLuca and Rossiter(3) reported that the labeling of the phospholipide of rat liver slices was decreased if the animal was exposed to a cold environment for either short (3 hr. at —5°C) or longer (12 days at 4-5°C) periods of time prior to killing.

In the present paper the effect of cold exposure on the subsequent labeling of the acetone-insoluble lipide of rat liver slices is described for a number of radioactive precursors. Inorganic P^{32}, acetate-1-C^{14}, glycerol-1-C^{14} and glycine-2-C^{14} were chosen for comparison, for it has been shown that each of these precursors labels a different portion of the phosphoglyceride molecule(4).

Methods. Male rats of the Sprague-Dawley strain (150-200 g) were maintained in an air-conditioned room at 22°C. In one series of experiments, groups of rats were transferred to a cold room maintained at 4-5°C for the 12 days preceding the experiment. In other experiments, groups of rats were transferred to a cold room maintained at —5°C for the 3 hours immediately prior to killing. In each experiment, groups of control animals maintained at 22°C were studied at the same time. All animals were fed *ad libitum* Master Fox Cubes (Toronto Elevators, Ltd.), containing approximately 53% carbohydrate, 3.5% fat and 20% protein.

Each animal was killed by decapitation. The liver was perfused rapidly *in situ* with cold (2-5°C) isotonic saline. Slices were prepared free-hand and approximately 500 mg sliced tissue was incubated for 4 hours at 37.5°C in a Warburg vessel containing Krebs-Ringer bicarbonate buffer. For the C^{14} ex-

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† Present address, Department of Biochemistry, University of Saskatchewan, Saskatoon.