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.

TABLE I. Effect of Exposure to a Cold Environment (12 Days at 4-5°C) on Subsequent Labeling of Acetone-Insoluble Lipides (Phospholipide) of Rat Liver Slices.

Exp. No.	Precursor	Specific activity (c.p.m./mg lipide) Control (22°C)	Cold (4-5°C)	Decrease (%)	P
1.	Acetate-1-C^{14}	5190 ± 560* (4)†	910 ± 120 (6)	82	<.001
2.	Inorganic P^{32}	150‡± 6 (7)	140‡± 9 (6)	7	>.5

* Mean ± stand. error of mean. † No. of animals. ‡ Counts/min./μg P.

periments 3 μmoles labeled substrate containing 1.3 μc radioactivity was added to the medium. For the P^{32} experiments 24 μc carrier-free inorganic P^{32} was added. At the end of the incubation period the tissue was homogenized and the lipides extracted. The phospholipides were precipitated and the radioactivity determined as described by Kline and DeLuca(2) for the C^{14} experiments and as described by Strickland(5) for the P^{32} experiments. Details of the preparation of the tissue, incubation, extraction and purification of the phospholipide, and counting procedures have been published(2).

Results. Table I shows that exposing rats to an environmental temperature of 4-5°C for 12 days caused a great reduction in the labeling of the acetone-insoluble lipide when acetate-1-C^{14} was the precursor, thus confirming previous findings(3). Table I also shows that a similar cold exposure had no significant effect on the labeling of phospholipide from inorganic P^{32}. When the rats were exposed to —5°C for the 3 hours immediately preceding the experiment, there was a decrease in the labeling of the acetone-insoluble lipide from acetate-1-C^{14}, again confirming previous findings (Table II)(3). However, when the radioactivity was derived from glycerol-1-C^{14}, glycine-2-C^{14}, (Exp. 3) or inorganic P^{32} (Exp. 4), there was no such significant decrease.

Discussion. The results indicate that previous exposure of rats to a cold environment causes a decrease in the subsequent labeling of the acetone-insoluble lipide of liver slices when acetate-1-C^{14} is the source of the radioactivity, but no significant change when the radioactivity is derived from glycerol-1-C^{14}, glycine-2-C^{14} or inorganic P^{32}.

Pihl and Bloch(1) showed that the fatty acid portion of the acetone-insoluble lipide was labeled when acetate-1-C^{14} was the precursor. Hydrolysis of the phospholipide of rat liver slices has confirmed this finding and has also shown that, with this precursor, the glycerol and the nitrogen-containing moieties of the phosphoglyceride molecule are not labeled to any significant extent(4). On the other hand, hydrolysis experiments, using the chromatographic procedures of Dawson(6), have shown that glycerol-1-C^{14} labels the

TABLE II. Effect of Exposure to a Cold Environment (3 Hr at -5°C) on Subsequent Labeling of Acetone-Insoluble Lipides (Phospholipides) of Rat Liver Slices.

Exp. No.	Precursor	Specific activity (c.p.m./mg lipide) Control (22°C)	Cold (-5°C)	Decrease (%)	P
3.	Acetate-1-C^{14}	3130 ± 380* (6)†	1610 ± 350 (6)	49	<.02
	Glycerol-1-C^{14}	1310 ± 130 (6)	1240 ± 160 (6)	5	>.7
	Glycine-2-C^{14}	1910 ± 120 (6)	1830 ± 60 (6)	4	>.5
4.	Inorganic P^{32}	140‡± 9 (6)	130‡± 4 (6)	7	>.05

* Mean ± stand. error of mean. † No. of animals. ‡ Counts/min./μg P.

glycerol portion of the phosphoglyceride molecule, but neither the base nor the fatty acid(4). Moreover, glycine-2-C^{14} labels the base (particularly the serine of phosphatidyl serine and the ethanolamine of phosphatidyl ethanolamine), but does not label to any significant degree the glycerol or the fatty acid portion of the molecule(4). It would thus appear that previous exposures of rats to a cold environment causes a decrease in the subsequent turnover of the fatty acid portion of the phospholipide in respiring liver slices, with no change in the turnover of other portions of the molecule. The finding that, with acetate-1-C^{14} as precursor, cold exposure causes a decrease in the labeling of the fatty acid portion of the phospholipides of rat liver slices, taken in conjunction with the report of Masoro, Cohen and Panagos(7) that under similar conditions there is a decrease in the labeling of the total fatty acid, might suggest that after cold exposure there is some decrease in the ability of the liver slice to metabolize acetate. However, the observations that cold exposure causes no significant decrease in the incorporation of labeled acetate into cholesterol(3,7), or in the oxidation of labeled acetate to CO_2(7) indicates that any change in acetate metabolism must be subsequent to its activation to form acetyl-CoA.

Summary. In rat liver slices respiring in a buffered medium containing suitable radioactive precursors, the acetone-insoluble lipide (phospholipide) is labeled metabolically. Exposure of the animals to an environmental temperature of 4-5°C for the 12 days prior to the experiment caused a decrease in the subsequent labeling of the phospholipide from acetate-1-C^{14}, but no significant change when inorganic P^{32} was the source of the radioactivity. Exposure of the rats to —5°C for the 3 hours immediately prior to the experiment caused a decrease in the labeling of the phospholipide when the radioactivity was derived from acetate-1-C^{14}, but no change when it was derived from glycerol-1-C^{14}, glycine-2-C^{14} or inorganic P^{32}.

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Effects of 6-Mercaptopurine on Respiration, Anaerobic Glycolysis and Succino-Dehydrogenase Activity in Normal and Tumor Tissues.* (22605)

ENRICO MIHICH,† DONALD A. CLARKE, AND FREDERICK S. PHILIPS.

Institute of Pharmacology, University of Milan, Italy, and Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.

It has been reported that 6-mercaptopurine (6-MP) produces a characteristically prolonged inhibition of the Crocker mouse Sarcoma 180 (S-180). Immediately after treatment with effective doses the majority of tumors fail to "take" when reinoculated into

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† Visiting Research Fellow under a Sloan Stipend for Advanced Study.