Summary. Antibody formation, which is inhibited by 500 r of total body x-irradiation into the rat, can be restored partially by spleen homogenates injected soon after irradiation. No striking difference in the degree of recovery is observed when spleen cells from rats stimulated earlier with a different antigen are used in place of "normal" spleen cells.

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Comparative Lipotropic and Lipide Phosphorylating Effects of Choline, Betaine, and Inositol.* (23573)

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It has been concluded that lipotropic action is more specific than stimulation of lipide phosphorylation since evidence showed that those substances which exert a marked lipotropic action also stimulate the formation of phospholipides, but that other substances stimulating phosphatide turnover may not necessarily cause a lipotropic effect(1). The lipotropic effect of choline, betaine, and inositol under specific dietary regimes is well known(2). However, the present study is, to our knowledge, the first to compare the lipotropic and phospholipide turnover actions of these compounds under identical experimental conditions. These conditions include the feeding of low protein-low fat and low protein-high fat diets to the experimental animals.

Methods. Male albino rats of Sprague-Dawley strain weighing 141 ± 43 g were divided into 2 groups. *Group I:* Ninety-nine rats, 26 of which served as controls, were maintained on a 5% casein-5% fat diet(3) or a 5% casein-32% fat diet(4) for a duration of 3 weeks. The animals were fed *ad libitum*. At the end of the dietary regime, the rats were stomach-tubed with a single dose of lipotropic agent (0.4 mM in 1 ml H_2O). All animals were then injected intraperitoneally with 1 ml of physiological saline containing 4 μc of P^{32} as NaH_2PO_4. Six hours later the animals were killed by decapitation. The liver was removed, rapidly weighed, and

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TABLE I. Effect of a Single Dose* of Choline, Betaine or Inositol on Total Liver Lipides and Lipide Phosphorylation.

Lipotropic agent	No. of rats	Total lipides, g	Relative specific activity
	5% casein—5% fat diet		
None	11	.84 ± .37	.186 ± .038
Choline	11	.59 ± .15	.282 ± .050†
Betaine	11	.59 ± .28	.230 ± .073‡
Inositol	10	.76 ± .38	.193 ± .034‡
	5% casein—32% fat diet		
None	15	2.06 ± .74	.255 ± .028
Choline	12	1.68 ± .96	.374 ± .057†
Betaine	14	1.44 ± .67	.284 ± .057
Inositol	15	1.61 ± .66	.251 ± .049

* 0.4 mM administered by stomach tube. No. preceded by ± are stand. dev. Test of significance was applied to difference between means of controls, saline, and experimental values. The P probability for chance occurrence of this difference was:
† <.01; ‡ <.05.

divided into 2 fractions. From one fraction, acid soluble phosphorus was removed by extracting with 10% TCA containing 0.4 M $MgCl_2$(5), and radioactivity and total P(6) were determined. The other fraction was covered with alcohol, and the lipides extracted with alcohol-ether and purified with chloroform(7). Radioactivity(7), phosphorus(6), and the weight of total lipides were determined on aliquots of the chloroform solution. As a measure of phospholipide turnover, specific and relative specific activities(8) were calculated. *Group II:* 59 additional animals were used to study the lipotropic actions of choline, betaine, and inositol when 100 mg/rat of each of these compounds was supplemented daily to a 5% casein-5% fat and to a 5% casein-32% fat ration(3,4) for 2 weeks. Fifteen of the rats served as controls. At the end of the dietary regime, all animals were injected intraperitoneally with P^{32}, and after 6 hours the animals were sacrificed by decapitation. The liver, after being rapidly removed and weighed, was divided into an acid soluble and phospholipide fraction for analyses as described above.

Results. The effects of single doses of choline, betaine and inositol are summarized in Table I. To evaluate the statistical significance of the results, the t test of significance (9) was applied to the difference between the means for control and experimental values. Choline exerted a pronounced increase in phosphatide turnover in animals maintained on either diet, whereas equal molar doses of betaine and inositol demonstrated an increase in the rate of phospholipide synthesis in rats receiving the 5% casein-5% fat diet. This increase was considerably lower than that observed for choline under these experimental conditions. It is also apparent that the phospholipide turnover rates in animals receiving the 32% fat diet was higher than that of those receiving the lower percentage fat diet. Although there was a decrease in liver fat in all the treated animals of Group I, the reduction was not significant over the control rats.

Only the total lipide data (Table II) from Group II are reported, since dietary experiments of this nature (in which smaller amounts of the active substances are ingested in divided doses) tend to show little change and considerable variation in the rate of synthesis, even though a slight change in the rate may cause a significant difference in tissue composition over a period of time(3). Choline and betaine produced an equally effective lipotropic response at the dietary dose levels used when supplemented to either diet. However, inositol produced this response only in the low protein-low fat diet.

Discussion. These results are in agreement with the evidence presented by workers who have conducted separate studies of these com-

TABLE II. Effect of Dietary* Supplements of Choline, Betaine or Inositol on Total Liver Lipides.

Lipotropic agent	No. of rats	Total lipides, g
	5% casein—5% fat	
None	7	.49 ± .13
Choline	7	.32 ± .08†
Betaine	7	.33 ± .05†
Inositol	6	.33 ± .09‡
	5% casein—32% fat	
None	8	.90 ± .65
Choline	8	.28 ± .05†
Betaine	8	.32 ± .07‡
Inositol	8	.75 ± .38

* 100 mg/rat/day. No. preceded by ± are stand. dev. Test of significance was applied to difference between means of controls and experimental values. The P probability for chance occurrence of this difference was:
† <.02; ‡ <.05.

pounds(2). Our study supports the concept that lipotropic action is more specific than that of lipide phosphorylation. Under those dietary conditions which led to a marked reduction in total liver lipides by a lipotropic agent, a corresponding increase in phospholipide synthesis was observed when the agents were administered as "acute" massive doses by stomach tube. Inositol, on the other hand, failed to exert any lipotropic response supplemented to the high fat diet and the administration of the "acute" single dose to animals on this diet caused no increase of the phospholipide turnover rate.

The equal effectiveness of all 3 agents in reducing the total lipides on the low casein-low fat diet is thought, on the basis of the recent work by Young *et al.*(10), to be due to the high dietary dose levels employed. At lower doses, however, these investigators reported the betaine to choline ratio necessary to produce a given fat level to be 3:1.

The order of effectiveness of single doses of the agents, in regard to lipide phosphorylation, under either dietary condition employed was the same, *i.e.*, choline > betaine > inositol. This greater stimulation by choline might well be expected, since choline itself is a part of the lecithin molecule. The results indicate that the transmethylation mechanism involved when a single dose of betaine is administered, is not nearly so effective as the administration of an equal molar dose of choline, even though the number of methyl groups available is the same. A reason for the low stimulation of phospholipide synthesis by inositol may have been the fact that inositol phosphatides represent a small percentage of the total liver phospholipides(11). Therefore, any increase in this portion would correspondingly have a small effect on the overall phospholipide turnover rate as measured by our methods.

Summary. The lipotropic and lipide phosphorylating effects of choline, betaine, and inositol were compared in rats maintained on low protein-low fat or low protein-high fat diets. All 3 agents (100 mg supplements/rat/day) were equally effective in causing a significant reduction of total lipides over controls in animals maintained on the low protein-low fat diet. Only choline and betaine supplements caused a substantial reduction of total liver fat in rats maintained on the high fat diet. Phospholipide synthesis was increased when choline, betaine, or inositol was administered as a single dose (0.4 mM) to animals maintained on the low protein-low fat diet. The order of effectiveness upon turnover rate was choline > betaine > inositol. Both choline and betaine, when administered as single doses, stimulated lipide phosphorylation in rats on the high fat diet, but inositol did not.

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