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In vivo Effect of CoA and Other Factors on Lipide Phosphorylation.* (22946)

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Considerable progress has been made in possible pathways of phospholipide synthesis in liver for partial purified enzyme systems. Recent work has provided evidence that CoA (1), ATP(2,3), and maintenance of oxidative phosphorylation(4) are essential for phospholipide synthesis in liver mitochondria. Recently Kennedy has shown that liver mitochondria synthesize lecithins, involving phosphorylcholine as an intermediate and incorporate choline directly into the lipide molecule, requiring ATP and CTP(4,5). Phospholipide synthesis in the liver of animals maintained on stock diet as measured from P^{32} turnover studies is already at a maximum rate(6) and even under nutritional stress or liver cell death this is not altered. In choline deficiency the total liver lecithin content decreases and total lipide concentration increases(7) resulting in increased need in the liver cell for choline-containing phospholipides(8). Thus, compounds which are involved directly in phospholipide synthesis should affect the rate of turnover in fatty liver cell since there is an increased demand for lecithins. In view of these observations and the experimental work on partial purified enzyme systems, the *in vivo* effects of CoA, ATP, AMP, oxalacetate and CMP on lipide phosphorylation in liver of rats have been investigated in choline deficient animals.

Methods. Male albino rats of Sprague-Dawley strain weighing 100-110 g were divided into 5 series. The animals in Series I, II, and III were maintained on 5% Casein-5% Fat Diet(9), for 4 weeks. In Series IV, rats were maintained on pantothenic acid deficient diet(10) for 10 weeks. Control animals for this group received similar diet containing the vitamin. In each series, 4 to 6 rats were housed in individual cages with raised screen bottoms. Animals were fed *ad libitum*. Intraperitoneal injection of 4 μc of P^{32} as NaH_2PO_4 was administered one minute following injection of above compounds listed in Series I-IV. Six hours later the animals were killed by decapitation. The liver was removed, rapidly weighed and divided into 2 fractions. On one fraction acid-soluble phosphorus was removed by extracting with 10% TCA containing 0.4 M $MgCl_2$(11) and radioactivity and total P(12) were determined. The other fraction was covered with alcohol and lipides extracted with alcohol, alcohol-ether and purified with chloroform (13). On aliquots of the chloroform solution, radioactivity(13) and phosphorus(12) were determined. As a measure of phospholipide synthesis specific and relative specific activity (14) have been calculated. Twelve animals in Series V were maintained on Diet 26(9) for 8 weeks. At the time of dietary regime they were divided into 3 groups and intraperitoneal injection of compounds listed in Series II(5) was given. One minute later 4 μc of P^{32} was administered and in 6 hours the animals were killed by decapitation, liver was

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TABLE I. Effect of a Single Dose of Various Agents on Lipide Phosphorylation in Liver of Rats.

Series No.	Group No.	Materials admin.	No. of animals	Relative specific activity of phospholipide P
I	1		10	.154 ± .023
		CoA	17	.221 ± .030‡
	2		10	.162 ± .033
		AMP	15	.172 ± .022
	3		5	.155 ± .037
		AMP	5	.186 ± .018
		ATP	5	.162 ± .030
			4	.162 ± .028
		ATP*	10	.192 ± .024
	4		8	.180 ± .024
		Ox†	6	.213 ± .019‡
II	1		6	.160 ± .025
		Choline	7	.230 ± .027‡
		" , CoA	7	.217 ± .042‡
	2		5	.182 ± .020
		Choline	6	.171 ± .023
		" , Ox	4	.218 ± .050
	3		5	.200 ± .013
		Choline	5	.278 ± .026‡
		" , AMP	5	.257 ± .037
	4		9	.158 ± .030
		Choline	9	.164 ± .015
		" , AMP, Ox	9	.224 ± .025‡§
	5		18	.161 ± .018
		Choline	19	.202 ± .046‡
		" , Ox, AMP, CoA	20	.289 ± .018‡§
III	1		5	.124 ± .013
		Choline	4	.143 ± .019
		CMP	4	.167 ± .023‡
		" , choline	5	.189 ± .019‡§
		" , Ox	5	.161 ± .024
		" , " , choline	5	.179 ± .012‡§
	2		6	.166 ± .032
		CMP, CoA	5	.148 ± .022
		" , " , AMP, Ox, choline	4	.246 ± .052
		" , AMP, Ox, choline	3	.219 ± .011‖

All materials inj. as 10 mg/100 g body wt, except CoA and CMP 5 mg and oxalacetate 50 mg. Controls received equivalent amounts of saline.

No. preceded by ± are stand. dev.

* 100 mg/100 g body wt.

† Ox = Oxalacetate.

Test of significance was applied to difference between means of controls, saline (‡) or choline (§), and experimental values. The P probability for chance occurrence of this difference was <.01; ‖ indicates that this probability when compared to saline was <.05.

removed and minced in an iced beaker, diluted 10 fold with cold 0.88 M Sucrose. The mince was homogenized in Potter-Elvehjem type tissue grinder with teflon pestle for 4 minutes in ice bath and centrifuged in Serval Angle Centrifuge (Type PR-1), according to modification of procedure of Hogeboom *et al.*(15) and Griffiths and Pace(16) for separation of cellular fractions by differential centrifugation. Total lipides from cellular fractions were extracted with 95% ethanol for 6 hours in Soxhlet Continuous Extractor. The lipides were purified with chloroform (13). After evaporation of solvent, the residue was dissolved in methanol and the solution filtered and treated with MgO for separation of choline-containing from non-choline-containing phospholipides(17). Radioactivity and phosphorus were determined on aliquots of the solution before and after absorption on the MgO.

Results. The effects of CoA and other factors on lipide phosphorylation are shown in Tables I-III. To evaluate the statistical significance of the results, the *t* test of significance(18) was applied to the difference between means for controls receiving saline or choline and experimental values. It is apparent that administration of a single dose of CoA stimulates lipide phosphorylation as shown from data of Series I. This stimulating effect is still pronounced in those animals of Series IV which are pantothenic acid deficient where CoA synthesis is limited. This increase of lipide phosphorylation due to ad-

TABLE II. Effect of CoA and Pantothenic Acid on Lipide Phosphorylation in Pantothenic Acid Deficient Rats.

Series No.	Materials admin.	Exp. conditions	No. of animals	Relative specific activity
IV	NaCl	Controls	15	.127 ±.02
	"	Deficient	7	.153 ±.01
	CoA*	"	9	.180 ±.02‡§
	NaCl	Controls	17	.144 ±.02
	"	Deficient	10	.166 ±.025
	Ca pantothenate†	"	9	.156 ±.022

* 10 mg/100 g body wt.

† 2.65 mg/100 g body wt, or .013 mole of pantothenic acid.

The *t* test of significance was applied to difference between means of saline controls (‡), or saline deficient (§) and experimental values. P probability for chance occurrence of this difference was <.01.

No. preceded by ± are stand. dev.

TABLE III. Phospholipide Synthesis in Cellular Fractions of the Liver, 4 Samples/Group.

Series No.	Materials admin.	Specific activity of phospholipide P					
		Mitochondria		Nuclei		Homogenates	
		c.c.	n.c.c.	c.c.	n.c.c.	c.c.	n.c.c.
V	Saline	80.62	60.69	81.90	60.62	83.65	78.02
	Choline	85.95	37.96	90.80	41.26	92.31	94.30
	Choline, oxalacetate, AMP and CoA	111.34	64.80	101.73	57.58	107.54	72.53

c.c. = choline containing phospholipides. n.c.c. = non-choline containing phospholipides. All materials inj. as 10 mg/100 g body wt, except CoA 5 mg and oxalacetate 50 mg.

ministration of CoA is not the result of pantothenic acid content of CoA, for animals of Series V which received single injection of vitamin did not demonstrate any stimulation. This would be in agreement with the data of Chernick *et al.*(19) who have shown that CoA content of rat liver is not limited by availability of pantothenate and that injection of calcium pantothenate had no effect on liver CoA content.

Administration of AMP, or ATP had no effect on stimulation of lipide phosphorylation. This may be due to rapid hydrolysis of these compounds following injection. Oxalacetate administration stimulates lipide phosphorylation as shown from data of Series I. The dose of choline used to stimulate lipide phosphorylation which the animals received in Series II is considerably less than that previously reported(6). This dose was chosen so that the effect of other agents when used in conjunction with choline would be demonstrable. It is apparent from data of Series II that there is a more marked lipide phosphorylation when CoA, AMP and oxalacetate is mixed with choline than when choline or saline alone was administered. This mixture stimulates lipide phosphorylation in both choline and non-choline-containing phospholipides in the mitochondria and nuclei of the liver as shown from data in Table III. The greatest stimulation occurred in the lecithin fraction. It is apparent from data in Series III that CMP stimulates lipide phosphorylation. There is further stimulation of lipide phosphorylation when oxalacetate, AMP and choline is mixed with CMP. These results demonstrate clearly that CoA, oxalacetate and CMP stimulate lipide phosphorylation and give further *in vivo* support to their role in purified enzyme system(1,4,5).

Summary. 1. The *in vivo* effects of CoA, ATP, AMP, CMP and oxalacetate on lipide phosphorylation in the liver of rats have been investigated in choline deficient rats. 2. A single dose of coenzyme A stimulates phospholipide synthesis in choline and in pantothenic deficient animals; oxalacetate stimulates in choline deficient animals. 3. Administration of a single dose of a mixture of CoA, AMP, oxalacetate and choline stimulates lipide phosphorylation in both mitochondria and nuclei greater than choline or saline alone. The greatest stimulation occurred in the lecithin fraction. 4. Cytidine monophosphate stimulates lipide phosphorylation. There is further stimulation when CMP is administered with AMP, oxalacetate and choline.

The following abbreviation will be used in this paper: CoA = Coenzyme A; ATP = adenine triphosphate; CTP = cytidine-5′-triphosphate; AMP = adenosine-5′-phosphate; CMP = cytidine-5′-monophosphate; TCA = trichloroacetic acid.

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Ammonia Excretion and Renal Glutaminase Activity During Administration of Strong Acid and Buffer Acid.* (22947)

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Excretion of urinary ammonia rises during administration of strong acid loads as a consequence of increased ammonia production owing to activation of the ammonia-producing enzymes of the renal tubular cell(1,2,3) and accelerated ammonia transport because of enhanced diffusion of ammonia into an acid urine. Activation of renal glutaminase and other ammonia-producing enzymes by administration of a strong acid (NH_4Cl) could result from the impact of the acid load on intracellular buffers, effect of acid tubular fluid, or rapid removal of ammonia from the tubular cell.

Theoretically, a buffer acid load should have the same effect on the intracellular pH as a physiologically equivalent load of strong acid; in the kidneys, however, the presence of the buffer anion permits secretion of large amounts of H^+ without a drastic fall in urine pH. Ingestion of NaH_2PO_4, therefore, accelerates excretion of titratable acid without notably altering ammonia excretion(4,5). In the present study, therefore, the effects of physiologically equivalent loads of strong acids and buffer acids on ammonia excretion and renal glutaminase activity were compared to determine whether activation of glutaminase results from prerenal or renal effects of an acid load.

Procedure. Three groups of male Sprague-Dawley rats, weighing 200-300 g, were fed 10 cc of standard electrolyte deficient diet (SEDD) twice daily and distilled water *ad lib.* for 14 days (Table I). Group 1 received only the SEDD, Group II the SEDD plus 2 mM KCl and 3 mM NH_4Cl daily, Group III the SEDD plus 2 mM KCl and 3.75 mM NaH_2PO_4.† Daily 24 hour urine samples were collected under mineral oil using phenyl mercuric nitrate and toluene as preservatives. The ammonia of urine was determined by modification of the microdiffusion method of Conway(6). Urine pH was measured with a Beckman pH meter using external glass electrodes, and corrected to 37.5°C by a factor of 0.01 pH unit per degree. To determine titratable acid, urine was titrated to a corrected pH 7.4 with 0.1 N NaOH using Beckman automatic titrator. At the end of

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† Since the pK of NaH_2PO_4 is 6.8, the ratio $\frac{Na_2HPO_4}{NaH_2PO_4}$ at pH 7.4 is $\frac{4}{1}$. Therefore, 3.75 mM of NaH_2PO_4 would have the same impact on the buffer systems of the body as 3 mM NH_4Cl, since only 3 mM of the NaH_2PO_4 would be converted to Na_2HPO_4.