Decrease of Coenzyme A Content in Fatty Liver.* (22265)

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Some years ago it was found that pantothenic acid deficiency produces fatty liver in dogs(1). This observation appeared of particular interest after the discovery of coenzyme A (CoA)(2) and the identification of pantothenic acid as precursor of CoA(3,4), this coenzyme having a pivotal position in degradation and synthesis of fatty acids and cholesterol(5-7). Since CoA represents the functional form of pantothenic acid in tissues, it appeared reasonable to suppose some relation between CoA-deficiency and formation of fatty liver. However, more recently it has been reported that, not only does fatty liver not occur in pantothenic acid-deficient rats but that these rats are resistant to liver fat deposition ordinarily caused by cholesterol-rich diet(8). The difference of results in the 2 species is probably due to the fact that adrenal changes produced by pantothenic acid deficiency are much more severe in rats than in dogs(*cf.* 9). Adrenal hypofunction has been found to occur in pantothenic acid-deficient rats, which, in some respects, react as though adrenalectomized(10). That adrenal glands play an important role in the formation of fatty liver is shown by the fact that adrenalectomy prevents deposition of fat in the liver due to different steatogenic treatments(11-16). It seemed to us worthwhile to investigate whether CoA is implicated in the pathological accumulation of fat in liver. This paper reports the results of the estimation of CoA in fatty liver produced with carbon tetrachloride.

Materials and methods. Adult female albino rats, weighing between 250-300 g, were used. Animals were kept on a standard diet of the following composition: glucose, 73%; casein, 18%; maize oil, 3%; cod liver oil, 2%; salts, 4%(17). Supplementary crystalline vitamins were added in the following quantities per 100 g of diet: thiamine, 400 μg; riboflavin, 800 μg; pyridoxine, 400 μg; niacin, 2000 μg; calcium pantothenate, 2000 μg and choline chloride, 100 mg. After 3 weeks on this diet, the animals were divided into 2 groups. Rats of the first group were used as normal controls and those of the second group were injected for 8 days with CCl_4 to produce fatty liver (0.2 ml/100 g body wt/day of 20% solution of CCl_4 in olive oil, subcutaneously). During treatment with CCl_4 the animals were fed with the aforementioned standard diet. Both normal and experimental rats were fasted for 12 hr before the experiment. They were killed by decapitation and livers were quickly removed. Portions of liver were taken for histological examination and for estimation of nitrogen and lipid contents. The remainder of hepatic tissue was immediately chilled and homogenized with water. The homogenate was rapidly boiled, centrifuged, filtered(18) and CoA estimated in the boiled extract by a modification(19) of the method of Kaplan and Lipmann(18). The tissue samples for histological study were placed in 10% formalin within a few minutes after death; paraffin sections were stained with hematoxylin-eosin and frozen sections were stained with Sudan III for fat and hematoxylin as a counterstain. Nitrogen was determined with Nessler's reagent after digestion of the samples with sulphuric acid containing copper selenite(20). Total lipids were determined by extraction of dry tissue with ether in Soxhlet apparatus and weighing of fatty residue after removal of solvent. Tissue was dried by heating in oven at 100°C until constant weight. Acetylating apoenzyme was prepared from pigeon livers according to Kaplan and Lipmann(18) but livers were frozen overnight and homogenized directly from the frozen state into cold acetone as suggested by Handschumacher, Mueller and Strong(19). Chromatographically pure 4-aminoazobenzene was used; the commercial product ob-

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TABLE I. Coenzyme A Content in Normal and Fatty Livers.*

	Total lipids, mg/mg liver N.	CoA, μg pantothenic acid bound as Co-A/mg liver N.
Normal rats	1.32 ± .07	2.2 ± .13
CCl_4-intoxicated rats	2.41 ± .11	1.54 ± .11
Difference of the means	1.09	.66
P†	<.01	<.01

* Figures are means of 8 experiments ± stand. error.

† Probability determined by *t* test.

tained from Eastman Kodak Co. was purified as indicated by Handschumacher *et al.*(19). Adenosine triphosphoric acid (barium salt) and a crude preparation of CoA were obtained from the Sigma Chemical Co. All the other chemicals were reagent grade.

Results. Microscopic examination confirmed that CCl_4-intoxicated rats developed typical fatty livers. Chemical analyses of livers revealed amounts of lipid which corresponded well with the microscopic picture. As to be expected, the fat content was markedly increased in livers of CCl_4-intoxicated animals (Table I). The results of CoA estimations in both normal and pathological livers are summarized in Table I. It is apparent that CoA content was significantly lowered in fatty livers as compared with normal controls (30% decrease).

Discussion. Results presented are sufficient evidence for a marked decrease of CoA content in fatty liver. However, because of the method used in estimation of CoA it is possible that the actual decrease of CoA in fatty liver was greater than we observed. It has been shown that pigeon liver extract of Kaplan and Lipmann(18), in the presence of adenosine triphosphate (ATP) is capable of synthesis of CoA from some fragments containing pantetheine, in particular from dephospho-CoA and from phosphopantetheine (21,22). The CoA-assay system used in our experiments included both ATP and the Kaplan-Lipmann extract of pigeon liver as an acetylating apoenzyme. Thus, values for CoA here reported represent the sum of preexisting complete CoA plus any CoA synthesized from smaller molecules during the assay. The possibility exists that the amount of precursors of CoA was increased in fatty liver because of an impaired formation of complete coenzyme. If this were true, the synthesis of CoA during the assay would erroneously raise the figures for fatty livers more than those for normal livers. The uncoupling of oxidative phosphorylation found in mitochondria from fatty livers(23) could account for the impairment of CoA synthesis and for the accumulation of the precursors. ATP, in fact, is involved in biosynthesis of CoA(24). It is also to be noted that mitochondria swollen *in vitro* release CoA into the suspension medium where the coenzyme is broken down to fragments containing pantetheine(25). This observation may be of interest because mitochondria from fatty liver closely resemble, in their morphology and enzymatic activities, osmotically damaged mitochondria(23). Although an impaired synthesis seems the most likely explanation for the decreased content of CoA in fatty liver, the possibility of an increased breakdown of the coenzyme cannot be ruled out. It also remains obscure whether the CoA-deficiency plays a pathogenic role in the formation of fatty liver or it only represents a consequence of cell damage. As far as concerns the possible participation of CoA-deficiency in the pathogenesis of fatty liver, a hypothesis can be put forward if only provisionally. The low level of CoA and ATP, which are necessary for activation of fatty acids, would lead to impaired utilization of lipids in the liver and, consequently, to pathological accumulation of fat. Of course, this pathological condition is more severe when fat mobilization from depots to the liver is accelerated. According to the results of previous work(*cf.* 26), an increased mobilization of fat from the peripheral tissues to the liver actually occurs during fatty liver formation.

Summary. Coenzyme A has been estimated in both normal and CCl_4-fatty livers. The level of coenzyme A is decreased in fatty livers as compared with normal controls. The possible role of this change in the formation of fatty liver is discussed.

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Determination of L-Glutamic Acid by Use of L-Glutamic Acid Decarboxylase from *Mycobacterium phlei*. (22266)

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Amino-acid decarboxylases of bacterial origin are now widely used for the quantitative estimation of certain amino-acids(1-7,9). Gale(1) suggested the use of *Clostridium welchii* SR-12 for the determination of glutamic acid. This organism releases CO_2 not only from L-glutamic acid, but from L-glutamine(3), and L-aspartic acid(5,6) as well. Additional tests and special conditions are necessary(3,5,6) to obtain separate values for each of these compounds. Glutamic acid decarboxylases obtained from different strains of *E. coli* were used by several investigators(2,7-9) for the assay of L-glutamic acid. In general, the *E. coli* preparations possessed in addition to L-glutamic acid decarboxylase, a glutaminase(7), or a L-lysine and L-arginine decarboxylases(2,8,9). Recently, Najjar and Fisher(9) reported that they were able to destroy the arginine and lysine decarboxylases without seriously affecting the L-glutamic acid decarboxylase.

In the present paper a description of the use of cell-free extracts from *Mycobacterium phlei* for the determination of L-glutamic acid will be given. Extracts from this organism decarboxylate quantitatively L-glutamic acid only and do not react with 18 other amino-acids tested. Furthermore, no glutaminase or glutamo-racemase activity could be detected under conditions optimal for the decarboxylase activity.

Methods. A strain of *Mycobacterium phlei* from the stock of this laboratory is