Effect of Cholic Acid Administration on Taurine Concentration of Rat Organs.* (22057)

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The administration of cholic acid to growing rats causes a suppression of growth if the diet is low in sulfur amino acids(1). This effect can be reversed by feeding cystine, methionine or cysteine, but not taurine. In studying experimental atherosclerosis, Fillios and Mann(2) observed that rats fed cholesterol did not develop hypercholesterolemia if methionine was fed at the same time. White (1) points out that the cysteine deficiency observed after feeding cholic acid is the result of a diversion of cysteine into detoxification reactions. This is a plausible explanation; however, cholic acid is known to form mostly taurocholic acid in the rat(3,4), and endogenous taurine is used up in this reaction. It has been observed that the taurine concentration of rat organs remains constant even when animals are starved(5). Thus taurine must be formed from cystine, cysteine and to a lesser extent from methionine to replace the taurine consumed in the formation of taurocholic acid.

In this paper, data are presented which support the idea that taurine is formed from cysteine (or cystine) even when these amino acids are not supplied in the diet. In such a case, taurine is formed from endogenous cysteine, thus causing a cysteine deficiency.

Methods. Rats of the Sprague-Dawley strain were allowed to gain weight on a diet of Purina chow. When the animals weighed 200-220 g, they were divided in groups of 6 and each animal placed in an individual cage. Food consumption was determined daily. The diets used were prepared from a basic protein-free food mixture† of the following composition:

	%
Cornstarch	70
Vegetable oil	24
Salt mixture U.S.P.I.	4
Cod liver oil	2
Vit. supplement‡	

With this mixture we prepared diets containing 8 and 12% casein. Cholic acid diet was prepared by incorporating in the protein-free diet 8.16 g per kilo of cholic acid. A second diet was prepared containing in addition to the cholic acid 6 g per kilo of taurine. The animals were fed these diets for 10 days. At the end of this period, they were sacrificed and free taurine measured by a method recently reported(5). Liver, kidney, spleen, heart and muscle were studied.

Results. The absence of protein or the presence of either 8 or 12% casein in the diet has no effect upon the free taurine concentration of the organs studied (Table I). In Table II values are given for the taurine concentration of 5 organs from rats fed a protein-free diet and supplemented with cholic acid and cholic acid plus taurine. The values reported in these tables are within the normal range of values which we have reported for fed rats(5). None of the differences shown are significant.

Discussion. The early work of Foster, Hooper and Whipple(6) has already demonstrated that in the formation of taurocholic acid the limiting dietary factor was cholic acid and not taurine. The amount of taurine lost as taurocholic acid is not known precisely. The taurine excretion in the urine amounts to 50 to 80 micromoles per 24 hours(5) (6.2-10.0 mg). It is difficult to assess how much taurocholic acid is formed when animals are fed extra cholic acid in the diet. We believe that sufficient quantities of taurocholic acid are formed to create a deficiency in cysteine/cystine when these are present in limited amounts. The fact that no reduction in the

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† This was obtained from Nutritional Biochemicals.

‡ Vitamin Fortification mixture from Nutritional Biochemicals.

TABLE I. Effect of Dietary Protein on Taurine Concentration of Organs (Female Rats). (Micromoles taurine/g fresh tissue.)

Diet casein, %	Avg wt loss, g	Liver	Kidney	Spleen	Heart	Muscle
12	11	2.0 ± .4	8.3 ± .4	11.5 ± .5	25.0 ± .9	8.4 ± .4
8	14	1.6 ± .1	7.6 ± .4	11.6 ± .5	25.5 ± 1.5	9.2 ± .8
0	30	1.5 ± .2	9.4 ± .2	12.6 ± .4	25.6 ± .4	9.2 ± .7

Values are averages from 6 animals ± stand. error of the mean.

TABLE II. Effect of Cholic Acid Administration on Taurine Content of Rat Organs. (Micromoles taurine/g fresh tissue.)

Diet*	Avg wt loss, g	Liver	Kidney	Spleen	Heart	Muscle
			Males			
Basic (B)	24	1.0 ± .1	6.0 ± .6	12.2 ± .7	21.6 ± 1.4	8.5 ± .7
(B) + Ch	33	1.1 ± .5	6.2 ± .3	13.2 ± .3	27.1 ± .7	11.6 ± 1.3
(B) + Ch + T	30	1.0 ± .2	8.9 ± .5	13.6 ± .7	27.6 ± .9	12.6 ± .7
			Females			
Basic (B)	20	2.6 ± .4	9.1 ± .9	12.5 ± .4	28.1 ± 1.3	9.6 ± .2
(B) + Ch	38	1.8 ± .2	7.9 ± .5	12.0 ± .2	25.5 ± 1.5	7.7 ± .7
(B) + Ch + T	40	1.9 ± .2	10.2 ± .4	13.8 ± .8	30.3 ± .6	10.1 ± .8

* Ch = Cholic acid; T = Taurine.
Values are averages from 6 animals ± stand. error of the mean.

taurine concentration was observed in any of the group studied indicates that taurine was probably formed. If we consider the urinary excretion of taurine alone, the amount of cysteine, or methionine used in 10 days for taurine formation would be the equivalent of 62 to 100 mg. This is a considerable amount. Taurine fed to the animal has no effect in relieving symptoms of cystine/cysteine deficiency. Fed taurine is known to be rapidly excreted(7,8) and it is also possible that the rates at which taurine and cholic acid enter the liver are different. In order for the two to combine, they must be present at the same time at the site of the condensing enzyme. This is not very probable since both compounds differ markedly in properties. In Table II, we show that the addition of an excess of taurine over cholic acid (2:1 on a molar basis) fails to alleviate the effect of cholic acid upon growth, or to cause an increase in the taurine concentration of any of the organs investigated. Thus we must assume that taurine is formed at a fairly constant rate, and that its formation is independent of the amount of taurine present in the diet. Moreover, it can be concluded from this work that dietary taurine is not utilized for taurocholic acid formation to any great extent.

Portmann and Mann(9) have recently reported that taurine-S^{35} administered to rats gives rise to labeled bile salts. The greater portion of taurine, however, was excreted in the urine.

Summary. 1. Taurine concentration of 5 organs was measured in animals fed a protein free diet and diets containing 8 and 12% casein. No changes in taurine concentration were observed in any of the groups. 2. Administration of cholic acid alone or cholic acid plus taurine to rats maintained on a protein free diet had no effect on the taurine concentration of the 5 organs studied.

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Metabolism of Reserpine-C^{14}. II. Species Differences as Studied *in vitro*. (22058)

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The crystalline alkaloid, reserpine, which is currently finding use in the treatment of hypertension(1,2,3) and psychiatric disorders (4,5,6), was identified in extracts from the roots of *Rauwolfia serpentina* Benth by Müller, Schlittler and Bein(7). In a report on the pharmacology of reserpine, Plummer *et al.*(8) mention the wide range in doses necessary to elicit the characteristic tranquilizing response in the various laboratory animals. Since Sheppard, Lucas and Tsien(9) showed that the rat is capable of oxidizing the 4-methoxy carbon of the 3,4,5-trimethoxybenzoic acid (TMBA) moiety to carbon dioxide as well as hydrolyzing reserpine to yield TMBA, it was thought that the ability to degrade reserpine might be related to dose requirements. To test this hypothesis, liver slices of several animal species were tested for their ability to metabolize reserpine. Part of this work was presented at a meeting of the American Chemical Society(10).

Experimental. In vitro: The livers of mature rats, mice, pigeons and guinea pigs were obtained immediately after death from animals sacrificed for the purpose; while those from dogs and rabbits were obtained by biopsy. The livers were sliced freehand and incubated with 200 μg of reserpine-C^{14}. The preparation of fractions as illustrated in Fig. 1, and the methods of counting and synthesis of reserpine labeled with C^{14} in the 4-methoxy carbon of the 3, 4, 5-TMBA moiety are the same as those previously described(9). Subcellular particles of guinea pig liver were prepared according to the method of Schneider (11). Each fraction was incubated with 100 μg of reserpine-C^{14} for 3 hours at 37°C in Krebs bicarbonate buffer. The reaction was stopped with acid and the tissue extracted as previously described(9). *In vivo*: Four male guinea pigs weighing about 400 g were injected with reserpine-C^{14} in vehicle C(9). One animal received 1.0 mg/kg intravenously while the others received 0.5 mg/kg. $C^{14}O_2$ collections were made and counted as previously described(9).

Paper chromatography and radioautography. Aliquots of fraction C and C_B were mounted on Whatman No. 1 filter paper. 40 μg of TMBA were then added to each spot and the sheets chromatographed by descending method with a solvent composed of 5 normal ammonium hydroxide: butanol: isoproponal (3:2:1). Under these conditions TMBA gives an R_F of about 0.61 and fluoresces a bluish purple when exposed to ultraviolet light. To locate the radioactive spots radioautographs were made using Kodak No-Screen X-ray Safety Film.

Results. The results of the *in vitro* studies with liver slices are summarized in Table I. The rat possesses the greatest ability for the oxidation of the 4-methoxy carbon of the 3, 4, 5-trimethoxybenzoic acid moiety to carbon dioxide. Only the pigeon and rabbit seemed to be devoid of this activity. That most of this process depends on oxygen is obvious from the drop in $C^{14}O_2$ recoveries that occurred when incubation was carried out under nitrogen. The rat liver slices, however, show little ability for splitting off TMBA. Surprisingly, the guinea pig liver slices show a very marked ability in this respect, hydrolyzing about 80 per cent of the reserpine in three hours. The mouse and rabbit show fair hy-