Metabolism of Choline and Related Compounds by Hepatic Tissue From Several Species Including Man.* (20882)

C. J. KENSLER† AND H. LANGEMANN.‡

From the Department of Pharmacology, Cornell University Medical College.

The enzymatic activity of the same tissue or organ is known to vary widely from species to species. Differences in choline oxidase activity of hepatic tissue are particularly marked(1,2). It is the purpose of the present report to record the variation among species of the activity of the enzyme systems involved in the oxidation of choline and succinate and the formation of methionine employing choline or betaine or dimethylthetin as methyl donors and homocysteine as the methyl acceptor. Data on the activity of fresh human liver are included. This study represents one phase of a more general study of the possible relationship between the production of liver tumors in rats by chronic choline deficiency and their production by the carcinogenic azo dyes(3).

Methods. Succinoxidase and choline oxidase activity were measured manometrically using the methods reported by Potter and Schneider(4) and Kensler and Langemann(5) respectively. Methionine formation was measured colorimetrically using the method of McCarthy and Sullivan(6) as modified by Dubnoff and Borsook(7). Methionine synthesis when choline or betaine were employed as methyl donors was carried out under conditions essentially the same as those used by Dubnoff(2) except for higher concentrations of choline (0.01 M) and betaine (0.02 M). The incubation period was one hour. Transmethylation from dimethylthetin was measured using 10 mg of tissue, a pre-incubation period of 60 minutes at 38°C, and a reaction time of 5 minutes, conditions which were found earlier(8) to give maximal values. Tributyrinase activity was measured manometrically using 1 mg (wet weight) or less of tissue per manometer. For ease of comparison among the different enzymatic activities measured, they are all expressed as Q values, *i.e.* mm^3 of gas consumed or produced (volumetric equivalent of the amount of methionine formed) per mg dry weight of tissue per hour. As dry weight-wet weight ratios were not determined for all species or samples, it has been arbitrarily assumed that the dry weight equals 30% in all cases. The samples of human liver were obtained by biopsy during abdominal operation, immediately immersed in ice cold Ringer-phosphate solution and then homogenized in glass homogenizers and the assays started within 30 minutes of removal. Microscopic sections were obtained of the liver in two cases and were morphologically normal. All 4 were cholecystectomy cases. We are indebted to Dr. T. H. Jukes of the Lederle Laboratories for the 4- to 6-week-old New Hampshire Red (NH) and hybrid (H) chicks used in this study. They were maintained on a casein base ration and were the completely supplemented controls used in a study of vit. B_{12} and folic acid deficiency(9). The rats and mice were on a dog pellet ration and the rabbits and guinea pigs received rabbit pellets (Rockland) and greens.

Results. The data obtained are summarized in Table I. Choline oxidase activity, in agreement with earlier reports(1,2) varies widely from species to species. Adult rat liver contains the greatest activity whereas guinea pig, rabbit and human liver contained little or no detectable activity. The low values reported for rabbit and human liver fall within the experimental error of the method. In agreement with Dubnoff(2) no methionine formation from choline and homocysteine was observed with guinea pig liver. Rabbit and

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† Sloan Foundation Scholar.

‡ Present address: Pharmakologisches Institut, Zurick, Switzerland.

TABLE I. Enzymatic Activity of Liver from Various Species.

Species	Strain or wt group	Choline oxidase, Q_{O_2}	Succin-oxidase, Q_{O_2}	Transmethylation from— Choline, Q meth	Betaine, Q meth	Dimethyl-thetin, Q meth
Rat	6–49 g	2 (16)*	65 (10)*	.04(10)*	.43(10)*	63(15)*
	50–85 g	3.2 (9)	78 (9)	.06(9)	.38(9)	68(8)
	85 g	6.5 (24)	78 (10)	.09(20)	.32(20)	69(19)
Mouse	C_3H	2.0 (4)	90 (4)	.20(4)	.60(4)	120(6)
	A	2.0 (5)	93 (5)	—	—	97(5)
	C	1.7 (3)	77 (3)	.11(2)	.52(3)	72(3)
Chicken	NH	1.9 (10)	50 (10)	.10(10)	.24(10)	10(10)
	H	2.5 (10)	55 (10)	.16(10)	.32(10)	19(10)
Dog		1.9 (2)	63 (2)	—	—	28(2)
Rabbit		.10(5)	37 (5)	0 (5)	.17(5)	30(5)
Man		.06(3)	5.9(4)	0 (3)	.26(3)	16(4)
Guinea pig		.0 (4)	54 (4)	0 (4)	.39(4)	32(4)

* No. of animals assayed.

human liver also produced no detectable methionine when choline was the methyl donor. Mouse, rat and chicken livers were active.

In contrast to the results obtained with choline, when betaine was the methyl donor methionine was synthesized by the livers of all species examined. Dimethylthetin (the sulfur analog of betaine) was much more effective than betaine as a methyl donor to form methionine from homocysteine in the livers from all species examined. It will be noted, however, that the relative effectiveness of dimethylthetin and betaine varies from species to species. This may indicate that two different enzymes are involved or that substrate affinity (specificity) of a single enzyme varies from species to species. Dubnoff and Borsook(7) concluded that 2 different enzymes were involved in rat liver on the basis of a selective loss of betaine transmethylase activity from tissue extracts transmethylating from both betaine and dimethylthetin. In their experiments, activity was measured under conditions which were nearly optimal for betaine but suboptimal for dimethylthetin (Q—1.5 as opposed to 69 in present study). Thus, a decrease in betaine activity would be readily detected whereas a similar decrease in dimethylthetin activity might be missed.

As is indicated in Table I, only 4 samples of human liver were obtained for analysis. All were from females between 35 and 59 years of age who had been under ether anesthesia for from 1 to 3.5 hours prior to the excision of the liver tissue. No obvious differences in enzymatic activity with respect to age or duration of anesthesia were noted. The Q value for choline oxidase varied from 0 to .1, that for succinoxidase from 2.1 to 10., that for methionine formation from betaine from .13 to .33, that for methionine formation from dimethylthetin from 11 to 20. No methionine formation was detected in any of the 3 livers tested with choline as the methyl donors. The succinoxidase activity values are surprisingly low but are in agreement with the low values reported by Waterlow(10) for succinoxidase and cytochrome oxidase activity in human liver. Tributyrinase activity was measured in 2 cases (Q = 1520, 1930) and found to be of the same order of magnitude as that found in the liver of rats and mice (Sherman strain rats Q = 1120: C_3H mice Q = 3235).

Discussion. On the basis of the limited data so far obtained on adult human liver tissue, it appears that enzymes are present which actively methylate homocysteine to form methionine when either betaine or dimethylthetin are the methyl donors. No methylation of homocysteine could be detected when choline was the methyl donor. This pattern of activity is thus similar to that observed in liver tissue from rabbits and guinea pigs. Choline oxidase activity is also low or

absent in these three species in contrast to the at least 15- to 60-fold higher levels observed in the other 5 species examined.

The bulk of the work that has been done on choline requirements and metabolism has been done on rats, mice and dogs(11,12). Comparatively little work has been done with guinea pigs or rabbits. Handler(13) has fed seven different choline deficient diets to guinea pigs and rats. All seven led to a marked increase in liver lipids in rats but only the peanut meal base ration which increased rat liver lipids to as high as 25% of the wet weight caused a slight increase in guinea pig liver lipid content. Choline administration was without effect on this increase in liver lipids in the guinea pig. Dubnoff(14) has reported that C^{14} methyl labelled groups of choline are found in the methyl groups of tissue proteins. Whether this represents transmethylation or oxidative demethylation and resynthesis to methyl groups has not been established. In the rabbit, Spellberg, Keeton and Ginsberg(15) reported the dietary production of cirrhosis which was not prevented by vit. B concentrates containing choline although Blumberg, MacKenzie and Seligson (16) have produced cirrhosis in young rabbits and prevented its development with large amounts of choline. Transmethylation from methionine to form choline has been found to occur in rabbits(17). In man, Simmonds and du Vigneaud(18) have shown that the methyl group of methionine is utilized to form choline and creatinine. No comparable studies have been made on the lability of the methyl group of choline in man or the rabbit. In view of our preliminary findings in regard to the *in vitro* failure to detect transmethylation from choline to homocysteine in human as well as guinea pig and rabbit liver, further study of this process in these species appears desirable.

The data confirm and extend the observations of Dubnoff(2) that transmethylation from choline to homocysteine is detectable *in vitro* only in those species which possess an active choline oxidase system. In the rat where a sufficient number of animals in different age groups have been examined, it appears that transmethylation from choline, as well as choline oxidase activity(19), increases with the growth of the animal. Transmethylase activity from betaine or dimethylthetin in the livers of the same animals does not show this change.

Summary. 1. Data on the activity of choline oxidase, succinoxidase, tributyrinase and the enzymes concerned in the formation of methionine from choline, betaine or dimethylthetin and homocysteine are presented for liver tissue from the rat, mouse, guinea pig, rabbit, chicken, dog and man. 2. Analysis of 3 samples of fresh human liver indicate that man resembles rabbit and guinea pig in that choline oxidase activity and capacity to utilize choline to methylate homocysteine are low or absent. 3. Livers of all species examined were capable of utilizing betaine and its sulfur analog dimethylthetin to methylate homocysteine to form methionine. 4. Succinoxidase activity of 4 samples of fresh human liver were markedly lower than that of other species examined. Tributyrinase activity of two samples examined was of the same order of magnitude as that found in livers from the rat and the mouse.

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Mouse-Strain Differences in Response to Radiation. (20883)

M. C. REINHARD, E. A. MIRAND, H. L. GOLTZ, AND J. G. HOFFMAN.

From Roswell Park Memorial Institute, Buffalo, N. Y.

In the course of an experiment(1) on the protective effect of hormones on the mortality of irradiated mice, there was observed a considerable difference in the radiation doses delivered to the heads of mice of 2 different strains necessary to elicit the same degree of mortality. The doses differed by more than a factor of 2 in the dba and Marsh-albino mice.

The difference in acute radiation mortality has now been examined in 2 additional mouse strains, making a total of 4. This is a report on the results to date, which were obtained with each strain represented by several hundred mice of either sex in the age range from 8 to 15 weeks. The entire experiment is being repeated with larger numbers of mice as well as with additional mouse strains.

Procedure. In order to establish the acute mortality curves, mice of the dba, Marsh, C57 black, and C3H strains were irradiated as follows: (a) whole body exposed, (b) only the head irradiated, and (c) the head shielded and the remainder of the body irradiated. All mice were irradiated at a distance of 30 cm to the midsection with an x-ray beam having a H.V.L. of 0.9-mm copper and a dose rate of 150 r per minute in air. The x-ray generator was monitored continuously for constancy of output. Doses reported herein are air doses. For whole-body radiation 10 mice were placed in a 6"-diameter Petri dish and covered with a perforated aluminum disc ½-mm thick. Since the space was limited, it was not necessary to immobilize the animals by anesthesia. Measurements of dosage when the dish was filled with purple-heart wood to simulate the setup when filled with mice showed that the radiation dose intensity decreased from 100% at the center to 91.8% at the outer periphery of the dish. Thus, the doses to the mice varied approximately 4% above and below the average dose in the dish. For irradiation of the head only the mice were held in position by a half cylinder of 1/16" lead which functioned both as an immobilizing device and as a radiation shield. Only the head was exposed. The mice and shield were fastened to a wooden board in 2 rows of 3 mice each, nose to nose, so that the heads of 6 mice could be irradiated simultaneously. Over the bodies of the mice covered with 1/16" lead 2 additional layers of 1/8" lead rubber were laid. Transmission of the x-rays through one layer of 1/8" lead rubber is 0.06%. The boundary between the irradiated head and shielded body was defined by the edge of the lead-rubber shield, which ran across the cervical vertebrae. Measurements of radiation dose in this setup were made with the mice simulated by purple-heart wood mock-ups. At the center of the mouse body under the shield the dose due to scattered x-rays was 4% of the dose delivered to the exposed head. For body irradiation without head the above procedure was modified by using a semicylinder of ½-mm-thick aluminum instead of lead to restrain the mice. The heads were shielded by 1/8" lead. The edge of the lead defined the boundary between head and body and ran across the cervical vertebrae. Dosage measurements with the purple-heart wood mock-ups showed that the