Effect of Age of Rat on Choline Oxidation *in vitro*.* (21309)

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Kensler, Rudden, Shapiro and Langemann (1) reported that "choline oxidase" activity was low in the livers of young rats. These investigators did not distinguish between two of the enzymes involved in choline oxidation, choline dehydrogenase (CD) and betaine aldehyde dehydrogenase (BAD), nor was a study made of diphosphopyridine nucleotide (DPN), the coenzyme required by BAD and shown to stimulate CD activity(2,3). The work reported here was undertaken to determine which of these components was responsible for the low rate of choline oxidation by livers of young rats.

Methods. Rats of the Sprague-Dawley strain maintained on a dog chow ration were used in this study. The DPN content, choline dehydrogenase and betaine aldehyde dehydrogenase activities in the livers of these rats were determined at specified times after birth. Manometric technics were used to measure choline dehydrogenase and betaine aldehyde dehydrogenase activities. For the determination of choline dehydrogenase the method of Strength, Christensen and Daniel(3) was used with the addition of 2 μmol of DPN per flask. Betaine aldehyde dehydrogenase activity was measured by the method of Christensen(4), as follows: To the main compartment of the Warburg flask were added 1 ml of 0.1 M barbiturate buffer, pH 8.4 containing 0.1% nicotinamide; 3 μmol DPN; 18 μmol methylene blue; 14 μmol betaine aldehyde chloride; and enough water to bring the total volume to 2.6 ml. The center well contained 0.2 ml of 2 N sodium hydroxide. To the side arm of the flask was added 0.4 ml of a 30% liver homogenate in 0.1 M barbiturate buffer. After a 5-minute equilibration period at 37°C, the homogenate was tipped into the main compartment, the stopcocks closed, and the oxygen consumption measured at 10 and 20 minutes, with air as the gas phase. The time interval of 20 minutes was used to calculate oxygen uptake, since in this time the amount of betaine aldehyde formed from choline does not interfere appreciably with the determination of choline dehydrogenase activity(5). In every case, a correction was made for endogenous respiration. The choline dehydrogenase and betaine aldehyde dehydrogenase activities are expressed as microliters (μl) of oxygen uptake/hr/mg of nitrogen [$Q_{O_2}(N)$]. DPN was determined by the lactic apodehydrogenase-diaphorase-methylene blue method of Sumner and Krishnan(6) with modifications as described by Strength, Mondy, and Daniel(7). DPN concentration is expressed as μg/mg of nitrogen. For each age-group, livers of rats of both sexes selected from different litters were pooled. Before pooling, each liver was divided into two portions. One portion was dropped immediately into chilled phosphate buffer and used for the DPN and choline dehydrogenase activity determinations. The other portion was dropped into the chilled barbiturate buffer and used for the determination of the betaine aldehyde dehydrogenase activity.

Three lots of animals were used for each age group. The livers from 12 animals were pooled to make one lot for the 2-day-old animals. For the one-week-old group, 9 animals were used/lot, 5 animals/lot for the 2-week-old group, 3 animals/lot for the 3-week-old group, and 2 animals/lot were used for the remaining age groups. The 6 adult animals used were from the same stock colony

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TABLE I. DPN Content, Betaine Aldehyde Dehydrogenase Activity, and Choline Dehydrogenase Activity of Rat Liver as Affected by Age of Animal.*

Age of rat	DPN content, μg/mg N	BAD† activity, Qo_2 (N)§	CD‡ activity, Qo_2 (N)
2 days	5.5	97.4	32.1
1 wk	9.5	91.9	44.9
2	15.8	90.2	50.2
3	13.8	—	58.1
5	18.9	—	65.9
7	21.9	—	71.9
9	23.8	—	75.7
13	—	—	106.6
Adult‖	25.1	107.4	125.4

* Animals from mixed litters and of both sexes were sources of tissue. Each value is avg of 3 values obtained from 3 different lots of animals, with the exception of the 5 wk results for CD which represents 2 lots.

† BAD = betaine aldehyde dehydrogenase. DPN (3 μmol) was included in the test system. No value was obtainable after the 2-wk value because of mold contamination of the betaine aldehyde chloride supply. Adult values were determined at same time as those for one-wk-old group.

‡ CD = choline dehydrogenase. DPN (2 μmol) was included in the test system.

§ Qo_2 (N) values are μl oxygen uptake/hr/mg nitrogen based on oxygen uptake for first 20 min. These values have been corrected for endogenous respiration.

‖ Animals were 11 mo of age from the same colony, but not the same breeding as the young rats.

but not from the same breeding. They were 11 months old when sacrificed.

Results. The data obtained on the choline dehydrogenase and betaine aldehyde dehydrogenase activities and the DPN content of livers of rats from 2 days to 13 weeks of age are summarized in Table I.

The choline dehydrogenase activity and DPN content of rat liver were low at birth, approximately 25% of the adult values, and they increased gradually with the increasing age of the animal. The DPN concentration of the liver approached the adult level when the animals were 7 to 9 weeks of age. Choline dehydrogenase activity was 85% of the adult level when the animals were 13 weeks of age. The low choline dehydrogenase activity in young rats may be related to their high requirement for choline(8). The deviation from the trend of increasing DPN concentration with increasing age of the animal, which was observed in the three-week-old rats, may have been due to an adjustment of the animal to the change in diet at the time when the young began to consume the stock ration along with the milk of the dam.

The results obtained for betaine aldehyde dehydrogenase activity indicate that the rat at birth had a betaine aldehyde dehydrogenase activity in the liver that was approximately 90% that of the adult rat. There was no significant change in the activity of this enzyme during the first 2 weeks of life. There was mold contamination in the supply of betaine aldehyde chloride making it impossible to obtain values after those for the 2-week-old group. The adult values were determined at the same time as those for the one-week-old group.

The results of this study confirm those of Kensler *et al.*(1), which indicated that "choline oxidase" activity in the livers of young rats was considerably lower than that of the adult rat. The present work suggests that this low activity may be due to a low level of choline apodehydrogenase, since betaine aldehyde apodehydrogenase was not limiting. No conclusion can be drawn with respect to the adequacy of DPN for the reactions studied, since DPN was added to the test systems. Lower concentrations of DPN were observed in the livers of young rats, however, and the possibility cannot be excluded that this factor may also be limiting in the *in vivo* oxidation of choline to betaine in young rats.

Summary. (1) The DPN content and choline dehydrogenase activity were low in livers of young rats and increased with the age of the animal. (2) The DPN content approached the adult level when the animals were 9 weeks of age. (3) Choline dehydrogenase activity reached 85% of the adult level by the time the animals were 13 weeks old. (4) The betaine aldehyde dehydrogenase activity in livers of newborn rats was 90% of that found in adult animals. (5) It appears that the low rate of oxidation of choline encountered in young rats may be due to a lack of choline apodehydrogenase. (6) The low level of DPN found in the livers of young rats may also be a limiting factor in the *in vivo* oxidation of

choline to betaine.

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Loss of Pharmacologic Activity in X-Irradiated Epinephrine Solutions.* (21310)

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In the course of a study of the effects of x-irradiation of the isolated rabbit heart on its responses to epinephrine it was observed that the activity of the epinephrine solution itself, which was contained in the tubing immediately adjacent to the heart, was apparently being destroyed by the radiation. The present work was therefore carried out in order to study some of the quantitative aspects of the effect of x-rays on epinephrine solutions.

Earlier workers give conflicting reports regarding the action of x-rays on epinephrine solutions. Richter and Gerhartz(1) observed in such solutions a diminished pressor potency and a questionable decrease of blood sugar raising activity. On the other hand, Lüdin(2) saw no change in the action of x-irradiated epinephrine solutions on isolated strips of rabbit and guinea pig intestine, while Terata and Ito(3-5) reported that x-rays augmented the potency of the drug in the rabbit with respect to its inhibitory action on intestinal movements, its stimulatory action on the uterus, and its pressor effect. These latter workers noted, however, that very large doses of x-rays caused a diminution in the pressor activity of epinephrine. The discordant results reported could thus probably be readily explained by the different doses of radiation used. The actual doses employed by the earlier workers(1,2), cannot be determined in terms of modern standards. Terata and Ito(3-5) used amounts ranging from 0.25 to 5 skin erythema doses.

Materials and methods. A Keleket x-ray therapy machine was employed to deliver unfiltered radiation (250 KVP and 15 ma) to aqueous and 0.9% saline solutions of epinephrine. The solutions were placed in 25 ml portions in 1 oz polyethylene screw cap bottles and they were irradiated at a rate of approximately 470 r per minute. The bottles were arranged horizontally in a compact circle 28 cm from the target and were slowly rotated on a revolving platform while being exposed. Dogs were used for the bioassay of the pressor activity of some of the solutions by the method described in the U.S. Pharmacopeia XIV. Qualitative tests for epinephrine activity were made on the isolated perfused rabbit heart (Langendorf method) and on the isolated rabbit ileum suspended in Locke-Ringer solution. Estimations of the epinephrine content in irradiated solutions were also made by a modification of the fluorometric procedure of Lund (5). In this method epinephrine is oxidized to adrenochrome by manganese dioxide and further changed to fluorescent adrenolutine by sodium hydroxide. The amount of fluorescence can be determined photo-electrically and

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