AGE EFFECTS ON LACTIC- AND MALIC-ACID DEHYDROGENASES IN C57BL/6 MOUSE TESTES[1]

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ABSTRACT

There is evidence that lactic-acid-dehydrogenase (LDH) and malic-acid-dehydrogenase (MDH) activities vary with age in several mammalian organs. This study shows that both enzymes in the testes of the C57BL/6 mouse exhibit age-related changes. These changes are most evident when the LDH/MDH ratio is considered. The LDH/MDH ratio decreases to two days before onset of spermatogenesis, remains unchanged to one day before weaning, increases to four or five days prior to breeding activity, and oscillates through the remainder of the 365 days studied. Although these are *in vitro* results, activity changes occur coincident with physiological changes in the testes.

INTRODUCTION

Although lactic-acid-dehydrogenase (LDH) isozymes of mammalian testes have been studied recently (Goldberg and Hawtrey, 1967), total LDH activity has not been studied extensively nor has malic-acid-dehydrogenase (MDH) activity. There is evidence that both these enzymes exhibit age-related changes in activity for a variety of mammalian organs (Ross and Ely, 1965; Flexner *et al.*, 1960; Roux and Tordet-Caridroit, 1965; Schmukler and Barrows, 1966). We have shown an age effect for both enzymes in the C57BL/6 mouse liver (Burich and Ewing, 1968).

The testes have been shown to contain a unique isozyme of LDH (Zinkham *et al.*, 1964; Goldberg and Hawtrey, 1967), which appears at 15 to 17 days postpartum (Goldberg and Hawtrey, 1967; also confirmed for C57BL/6 mice in our laboratory). This is also the time at which spermatocytes are first maturing from spermatogonia in the testes (Nebel *et al.*, 1961; Goldberg and Hawtrey, 1967). Sexual activity and breeding, as recorded in our laboratory, begin in the C57BL/6 mouse at about 45 to 50 days, with a reduction occurring at about one year.

This study was conducted to determine whether LDH and MDH activities were coincident with significant physiological changes of the testes and/or whole body, appearance of secondary sex characteristics, spermatogenesis, onset of breeding, and/or regression of sexual desire and/or capability. LDH was chosen because of current interest in LDH isozymes, especially LDH-X, and the observation that a total activity determination in testes had not been done. MDH was chosen because it is indicative of an aerobic metabolic pathway (Tricarboxycylic Acid Cycle) and can be assayed in a very similar manner.

MATERIALS AND METHODS

The C57BL/6 inbred strain of mouse was used in this study. Experimental animals were non-breeders raised in our laboratory from original stock obtained from Jackson Laboratory, Bar Harbor, Maine. Experimental animals were separated from the dam at weaning (21 days). All mice were killed between 7:15 A.M. and 9:15 A.M. by cervical dislocation. Testes were removed, placed on parafilm, and immediately stored on ice until homogenized.

Ten mice were used in each of the following age groups: 1, 3, 8, 13, 16, 18, 20, 22, 30, 40, 50, 60, 80, 110, 140, 170, 200, and 365 days. Littermate tissue was used at days 1 and 3, because a single testis or a pair of testes did not provide sufficient material for enzyme assay.

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LDH activity was measured spectrophotometrically by a method modified from Zinkham *et al.* (1964). The reaction mixture contained: 0.3 ml lactate solution (2.0 M solution in pH 9.00, 0.1 M Tris-HCl buffer), 0.2 ml nicotinamide adenine dinucleotide (NAD) solution (0.14 M solution in distilled water), and 2.49 ml buffer (pH 9.00, 0.1 M Tris-HCl). MDH activity was measured by a modification of the method of Wolfe and Neilands (1956). The MDH reaction mixture contained: 0.3 ml malate solution (0.5 M solution in pH 10.00, 0.1 M glycine-NaOH buffer), 0.3 ml NAD solution (0.014 M solution in distilled water), and 2.39 ml buffer (pH 10.00, 0.1 M glycine-NaOH). These reaction mixtures were made fresh daily from stock solutions and tested for stability and reproducibility using Enza-Trol (Dade Chemical Co.) as a standard.

A 3-percent (w/v) homogenate was made in cold Tris-citric acid buffer (pH 8.60, 0.05 M). The outer tunica albuginea layer and the epididymis were removed, except at ages one and three days, prior to weighing and homogenizing. In cases where only a portion of testis was needed, a center cross-sectional slice was used. The homogenate was centrifuged at about 3,000 g for 30 minutes, then the supernatant was removed and incubated at 25° C, as were the reaction mixtures, for 60 minutes prior to assay. A LDH and an MDH assay were performed on each sample. To each reaction mixture was added 0.01 ml of tissue extract. Each assay was followed at a wavelength of 340 mμ. The rate of NADH appearance was linear for five minutes under these conditions. The amount of NADH produced for each reaction after three minutes was determined using a NADH standard and Beer's Law. The activity was expressed in terms of NAD converted to NADH per gram testis per hour. The ratio LDH/MDH was computed directly from these units.

RESULTS AND DISCUSSION

Results for LDH, MDH, and the LDH/MDH ratio are shown in Table 1. A typical body-weight pattern was evident in animals used in this study. Analysis

TABLE 1

Testis Lactic- and Malic-Acid-Dehydrogenase Activity in C57BL/6 Mice

Age (Days)	Mean Value	Standard Error of Mean[a]		
	Mean Body Weight (Gm.)	LDH[b]	MDH[b]	LDH/MDH
1	1.50±.04	14.5±0.4	7.5±0.2	1.95±.10
3	2.12±.06	13.8±1.1	8.2±0.3	1.67±.09
8	5.20±.28	13.4±0.3	11.6±0.3	1.16±.03
13	7.65±.34	14.0±0.4	12.5±0.4	1.13±.03
16	8.32±.50	11.9±0.4	10.5±0.4	1.14±.02
18	8.83±.27	11.2±1.1	10.0±0.4	1.15±.02
20	10.15±.33	11.3±0.5	9.8±0.4	1.15±.02
22	8.66±.42	11.7±0.3	9.9±0.4	1.19±.03
30	13.34±.57	11.8±0.5	8.5±0.3	1.40±.04
40	19.26±.63	13.6±1.6	7.4±0.3	1.80±.11
50	20.61±.59	12.3±0.4	7.0±0.3	1.76±.03
60	23.54±.49	12.2±0.4	7.5±0.3	1.63±.03
80	22.21±.58	11.5±0.2	6.5±0.1	1.76±.02
110	25.88±.88	12.5±0.3	6.8±0.2	1.84±.02
140	26.31±.72	13.2±0.6	6.8±0.1	1.95±.08
170	27.34±.68	11.5±0.4	6.7±0.3	1.71±.04
200	29.93±.88	12.1±0.3	6.6±0.2	1.84±.03
365	28.90±.60	12.1±0.2	6.9±0.2	1.76±.03

[a] N = 10.

[b] Moles NAD × 10^{-4} converted/gram tissue/hour.

of variance showed that age was a significant ($P < .01$) factor influencing LDH, MDH, and LDH/MDH. Figures 1 and 2 depict the effect of age on LDH, MDH, and the ratio.

LDH activity decreased rather steadily until 21 days, followed by a general increase to 40 days, after which an oscillatory pattern developed. MDH exhibited a steady increase in activity to 13 days, after which it decreased and plateaued to 22 days. Activity then decreased to 50 days and plateaued at 80 days. The LDH/MDH ratio decreased sharply to 13 days, followed by little change through 20 days. The ratio then increased rapidly to 40 days, followed by an oscillatory pattern.

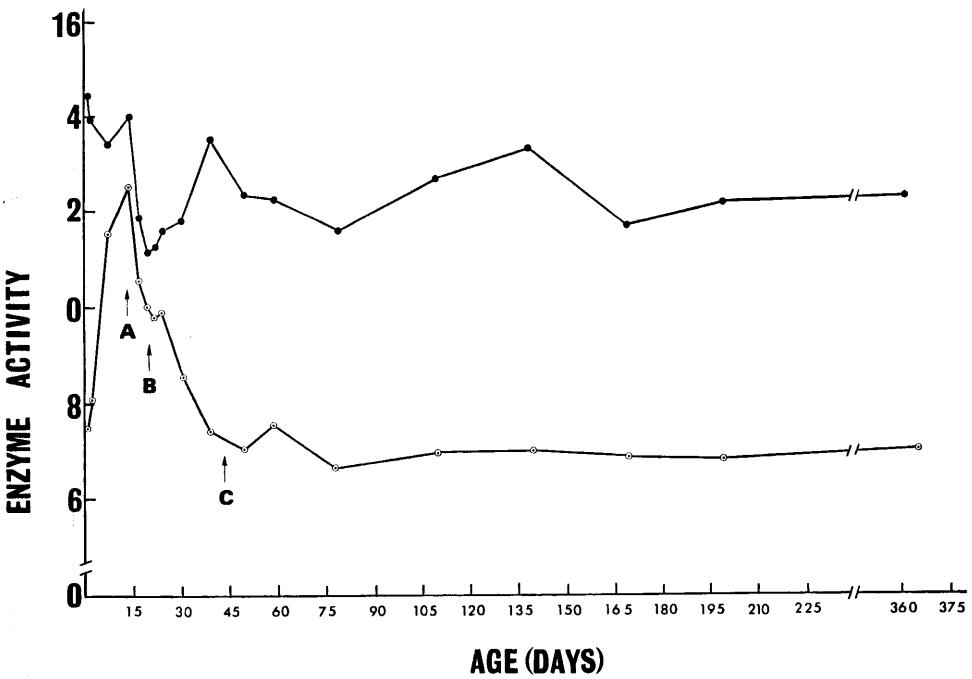

FIGURE 1. Lactic-acid-dehydrogenase (LDH) and malic-acid-dehydrogenase (MDH) activities (moles NAD$\times 10^{-4}$ converted/gram tissue/hour) in the C57BL/6 mouse testis at different ages. LDH represented by ——●—— and MDH represented by ——⊙——. A represents spermatocyte maturation; B represents weaning age; C represents sexual maturity.

Changes coincident with LDH and MDH activity were two physiological events involving the testes and weaning. Onset of spermatocyte maturation and the attainment of sexual maturity (A and C, respectively, fig. 1 and 2) appear as evident demarcations, especially in the LDH/MDH ratio. Weaning age (B, Figures 1 and 2) is a third clearly apparent demarcation shown in the ratio.

Maturation of spermatocytes is preceded by an increase in MDH activity and a decrease in LDH activity and in LDH/MDH ratio. The rather constant LDH/MDH ratio shown between spermatocyte maturation and weaning is coincident with a decrease in LDH and MDH activities. Between weaning and the onset of breeding potential (in our laboratory between 45–50 days and, in the case of many mice, as early as 42 or 43 days), MDH activity decreases and LDH activity increases, thus effecting a sharp increase in the LDH/MDH ratio. It has been suggested that a ratio of LDH to MDH might be a sensitive index to physiological

age (Fanestil and Barrows, 1965; Schmukler and Barrows, 1966; Burich and Ewing, 1968). Our data substantiate this proposal. Whereas LDH and MDH activity showed small changes, the LDH/MDH ratio clearly indicated a decrease to day 13 (two days prior to onset of spermatocyte maturation), a plateau to day 20 (one day prior to weaning), and an increase to day 40 (two to five days prior to breeding capability).

The changes with physiological events in the testes and attainment of sexual maturity are not surprising. However, the apparent effect of weaning on testicular enzyme-activity cannot be explained simply. Weaning age had no discernible effect on LDH and MDH activities in the C57BL/6 mouse liver (Burich and Ewing, 1968). We have initiated a study to determine if the effect we have shown is really a weaning-age effect.

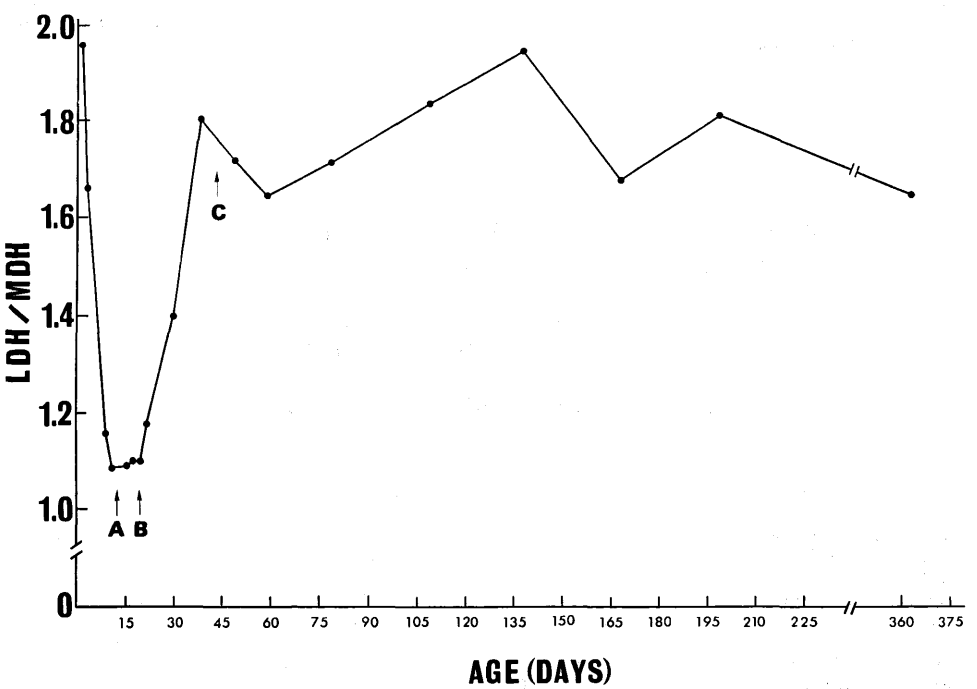

FIGURE 2. The ratio of LDH/MDH activities in the testis of different age groups of C57BL/6 mice. A represents spermatocyte maturation; B represents weaning age; C represents sexual maturity.

The implications of this study are not clearly evident. One might expect changes to occur as the testes mature, but how these changes are related to enzyme activity is not apparent. If LDH activity is indicative of an anaerobic carbohydrate metabolic pathway (glycolysis) and MDH activity is indicative of an aerobic carbohydrate pathway (TCA cycle), then the LDH/MDH ratio may represent the contribution of each of the tissue's carbohydrate metabolism or potential. This is speculative, but it is interesting to note how closely the LDH/MDH ratio parallels the definable parameters of spermatocyte maturation, weaning, and attainment of sexual maturity.

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