CONCENTRATION OF FREE AND ESTERIFIED CHOLESTEROL IN THE TESTES OF IMMATURE AND ADULT MICE

A. BARTKE

*Worcester Foundation for Experimental Biology,
Shrewsbury, Massachusetts 01545, U.S.A.*

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In the mouse, LH depletes cholesterol esters while prolactin promotes their accumulation in the testes (Bartke, 1969, 1971). In prepuberal mice, the concentration of total cholesterol in the testes has been reported to decrease (Simmons, 1969) and to increase with age (Ichihara, 1969). After weaning, the concentration of total cholesterol has been reported to remain approximately the same until the age of 90 days (Simmons, 1969) or to increase more than two-fold between the ages of 42 and 49 days (Ichihara, 1969). No information is available on the amounts of free and esterified cholesterol in the testes of immature mice. The concentration of esterified and free cholesterol in the testes of mice between the ages of 5 and 120 days has, therefore, been studied.

Random-bred mice derived from crossing YS/ChWf, YS/ChWf-dw and Dw/Wf strains were mated after 2 months of age and, thereafter, kept in pairs. The litters were weaned at the age of 21 to 23 days. Animals were killed by cervical dislocation and the concentration of free and esterified cholesterol was measured as previously described (Bartke, 1969). In mice less than 3 weeks old, the testes from two to eight animals were used for each determination. For statistical calculations and presentation, the results were pooled into nine groups. Within each group, there were no differences in the concentration of esterified or free cholesterol due to age.

As seen from the data presented in Table 1, the concentration of cholesterol esters increased gradually from 5 to 19 days of age, fell slightly ($P < 0.05$) after weaning and then rose abruptly ($P < 0.01$) to reach maximum values in mice 31 to 44 days old. Thereafter, it gradually declined. The concentration of free cholesterol apparently increased until the animals were 12 days old, then decreased and remained low until 44 days of age. It was increased ($P < 0.01$) in young adult males (50- to 83-day-old) and again lower ($P < 0.01$) in mice 105 to 120 days of age. In the animals studied, regardless of age, a majority of cholesterol (74 to 89%) was present in the free form. Consequently, the changes in the concentration of total cholesterol, with few exceptions, followed the changes in the concentration of free cholesterol.

The percentage of esterified cholesterol increased in suckling mice between 7 and 19 days of age ($P < 0.01$), remained unchanged for several days after weaning and then rose to 25.9% in 31- to 44-day-old animals ($P < 0.01$). It

TABLE 1
CONCENTRATION OF ESTERIFIED AND FREE CHOLESTEROL IN THE TESTES OF MICE AT DIFFERENT AGES

Age (days)	No. of mice	No. of samples	Body wt (g) (Mean $\pm$ S.E.)	Testes wt (mg) (Mean $\pm$ S.E.)	Concentration of cholesterol (mg/g)			% of esterified cholesterol (Mean $\pm$ S.E.)
					Esterified (Mean $\pm$ S.E.)	Free (Mean $\pm$ S.E.)	Total (Mean $\pm$ S.E.)	
5 to 6	8	1	3.6 $\pm$ 0.2	6 $\pm$ 0.3	0.18	1.30	1.48	12.3
7 to 9	8	2	6.4 $\pm$ 0.2	12 $\pm$ 0.9	0.20 $\pm$ 0.02 <i>P</i> < 0.01	1.60 $\pm$ 0.04	1.80 $\pm$ 0.06 <i>P</i> < 0.05	11.2 $\pm$ 0.6 <i>P</i> < 0.01
10 to 12	19	4	6.7 $\pm$ 0.1	14 $\pm$ 0.4	0.30 $\pm$ 0.01	1.90 $\pm$ 0.07	2.19 $\pm$ 0.08	13.7 $\pm$ 0.2 <i>P</i> < 0.02
16 to 19	11	3	9.0 $\pm$ 0.3	41 $\pm$ 1.7	0.33 $\pm$ 0.03 <i>P</i> < 0.05	1.79 $\pm$ 0.12 <i>P</i> < 0.05	2.12 $\pm$ 0.14 <i>P</i> < 0.02	15.6 $\pm$ 0.6
24 to 27	9	9	14.8 $\pm$ 0.6	103 $\pm$ 3.8	0.28 $\pm$ 0.01 <i>P</i> < 0.01	1.54 $\pm$ 0.04	1.82 $\pm$ 0.04 <i>P</i> < 0.01	15.4 $\pm$ 0.6 <i>P</i> < 0.01
31 to 44	14	14	20.9 $\pm$ 0.5	171 $\pm$ 10.0	0.53 $\pm$ 0.02 <i>P</i> < 0.01	1.50 $\pm$ 0.02 <i>P</i> < 0.01	2.03 $\pm$ 0.03	25.9 $\pm$ 0.8 <i>P</i> < 0.01
50 to 69	14	14	23.1 $\pm$ 0.8	214 $\pm$ 6.3	0.38 $\pm$ 0.02	1.62 $\pm$ 0.02	2.01 $\pm$ 0.03	19.3 $\pm$ 0.6 <i>P</i> < 0.05
70 to 83	13	13	23.4 $\pm$ 0.6	229 $\pm$ 10.0	0.34 $\pm$ 0.02	1.62 $\pm$ 0.02 <i>P</i> < 0.01	1.96 $\pm$ 0.03 <i>P</i> < 0.01	17.1 $\pm$ 0.8
105 to 120	8	8	29.1 $\pm$ 1.7	279 $\pm$ 19.4	0.29 $\pm$ 0.03	1.48 $\pm$ 0.03	1.78 $\pm$ 0.05	16.4 $\pm$ 1.3

P—significance of the difference between the adjacent means.

decreased ($P < 0.01$) between 44 and 50 days of age and, thereafter, continued to decline.

The results of this study differ from the data of Simmons (1969) and Ichihara (1969). This may be due to differences in the method of cholesterol extraction and quantification or to the use of a different stock of mice.

In the animals studied, the concentration of both free and esterified cholesterol tended to increase from the age of 5 days to weaning. After weaning, the concentration of free cholesterol decreased slightly and showed relatively small (although significant) fluctuations during the remainder of the period studied. The concentration of esterified cholesterol, however, reached peak values shortly before animals became sexually mature and, thereafter, decreased with age.

The rate of androgen production in mice of different ages was never directly measured. However, from the data on other species, it can be assumed that the production of testicular androgens declines, or perhaps ceases, soon after birth (Eguchi & Morikawa, 1968) and then, after a few days, begins to increase and reaches maximal values as the animal attains sexual maturity (Frasier, Gafford & Horton, 1969; Knorr, Vanha-Pertulla & Lipsett, 1970). In adult males, it probably declines slowly with age (Sciarra & Leone, 1970). The changes in the concentration of esterified cholesterol appeared to follow these suspected changes in the rate of androgen production. This observation is consistent with the earlier suggestion that this fraction of testicular cholesterol is utilized in steroidogenesis (Bartke, 1971). Recent studies by Moyle & Armstrong (1970) on the mouse Leydig cell tumours also indicate that esterified cholesterol may be utilized for the biosynthesis of testosterone.

The explanation for the dramatically elevated levels of esterified cholesterol during 3 weeks preceding puberty (i.e. the age of 50 to 60 days) is not immediately obvious. It could, however, be related to the acceleration of androgen production which may be assumed to occur at this time. In our animals, seminal vesicles were growing slowly before weaning and at the age of 24 to 27 days weighed 9.9 ± 1.8 mg. However, in 31- to 36-day-old mice they weighed 23.9 ± 2.4 mg or nearly half the seminal vesicle weight in adult males of this stock.

The concentration of cholesterol esters in the testes of intact, hypophysectomized and hereditary dwarf mice can be increased by prolactin (Bartke, 1969, 1971). In adult dwarf mice from the same stock, which have a hereditary prolactin deficiency, only 10% of testicular cholesterol is esterified (Bartke, 1969, 1971). In the male rat, the synthesis of prolactin *in vitro* was reported to be low before the age of 37 days, to increase appreciably during the following 4 weeks and to remain constant after the age of 65 days (Yamamoto, Taylor & Cole, 1970). These observations, together with the results of the present study, make it tempting to speculate that several weeks before the male reaches puberty, the production of pituitary prolactin increases, causing accumulation of cholesterol esters in the testes and thus provides a necessary pool of precursor for the increase in production of testicular androgens.

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