TESTICULAR CARNITINE ACETYLTRANSFERASE ACTIVITY AND SERUM TESTOSTERONE LEVELS IN DEVELOPING AND CRYPTORCHID RATS

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Summary. Carnitine acetyltransferase activities have been determined in the testes of developing and cryptorchid rats. This enzyme appears to be associated with the primary spermatocytes and early spermatids of the rat testis since activities increased markedly when these cells were proliferating. The usefulness of carnitine acetyltransferase as a 'marker enzyme' in the spermatogenic process is re-emphasized. Serum testosterone levels in the same animals closely paralleled the increased enzymatic activity ($r = 0.59$; $P < 0.01$). The testes of rats made experimentally cryptorchid contained reduced activity of carnitine acetyltransferase. The enzyme activity of 1-day cryptorchid testes was reduced to approximately half, and the activity of 10-day cryptorchid testes was reduced to one-sixth that of normal adult testes.

INTRODUCTION

In a recent review, Bishop (1968) advanced the concept that analysis of enzyme patterns in different spermatogenic cell types would be useful in 'fingerprinting' non-morphological changes during cell differentiation or following cell loss. Among other germinal cell-related enzymes (see Gomes & VanDemark, 1974, for recent review), carnitine acetyltransferase (CAT) has been shown to be a useful enzyme for this purpose.

The presence of high levels of CAT in rat testis was first noted by Marquis & Fritz (1965a), who also reported that spermatozoa contained the highest CAT specific activity of the several tissues examined; these workers suggested that spermatozoa probably contribute a major portion to the total enzyme activity in the testis (Marquis & Fritz, 1965b). In the same study, 18-day cryptorchid testes contained CAT levels which were nearly as low as those found in pre-pubertal testes. It was noted that androgens were required to permit main-

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tenance of normal levels of carnitine in the epididymides of castrated rats, leading to the suggestion that carnitine and CAT in the testes and epididymides of rats are under androgenic control (Marquis & Fritz, 1965b).

Vernon, Go & Fritz (1971), using the sedimentation velocity technique of Lam, Furrer & Bruce (1970), were able to separate the germinal cells of the rat testis and compare enzyme levels in relatively homogeneous cell populations. Changing activities measured during development indicated that maximal CAT activity was found when primary spermatocytes, particularly diplotene primary spermatocytes, were present; spermatogonia were lowest in CAT activity. Vernon *et al.* (1971) also found a significant decrease in testicular CAT levels 14 days after hypophysectomy, with a continued decline until 35 days after removal of the pituitary.

The results of these studies pose at least two questions: (1) if the appearance of CAT is predominately associated with primary spermatocytes in the rat and if synthesis of the enzyme is androgen-dependent, are testicular CAT levels and testosterone production related; and (2) do levels of the enzyme decrease in cryptorchid rats because enzyme-containing cells are lost, or does the enzyme disappear because of an earlier effect of temperature within the germinal epithelium? These are the questions to which the present investigation is addressed.

MATERIALS AND METHODS

All rats used in this study were of the Wistar strain and were either purchased from the Lab Supply Co., Indianapolis, Indiana, or raised in the animal colony of the Animal Reproduction Teaching and Research Center, The Ohio State University. The animals were fed a diet of Purina Lab Chow and were allowed free access to a supply of fresh water. Animals were maintained at 24°C and were subjected to a regimen of 12 hr light/12 hr dark daily.

To study developmental changes in testicular CAT, twenty-one growing rats and ten normal adult rats, 120 to 180 days of age and weighing 350 to 400 g, were used. On each of 7 days, i.e. at 16, 23, 29, 33, 40, 50 and 61 days of age, the testes of three of the growing rats were surgically removed after blood samples had been taken by cardiac puncture. Samples were also collected after cardiac puncture from the ten adult rats.

In order to determine the effects of abdominal temperature on testosterone levels and CAT activity, forty rats were made artificially cryptorchid. The rats were anaesthetized with ether and the abdomen was opened through a mid-ventral incision. Both testes were retracted into the abdominal cavity and retained there with a loose ligature. At daily intervals thereafter for 10 days, four cryptorchid animals were killed and testicular tissue and blood samples were collected for analysis.

Samples from each testis were assayed (33°C) for carnitine acetyltransferase by the methods described by Marquis & Fritz (1965a). The remaining tissue was fixed in Bouin's solution and prepared for histological examination under the light microscope (Gomes, Hall, Jain & Boots, 1973).

Blood samples were allowed to clot and the serum was stored at -20°C

until it was prepared for assay. Testosterone concentrations were determined by the radioimmunoassay procedure of Gomes *et al.* (1973).

RESULTS

The developing rat

Testicular weight and histology. Mean testicular weights in the developing rat and in adults are given in Table 1. Initial weights of 0.08 ± 0.002 g (mean $\pm$ S.E.) increased to 1.68 ± 0.04 g in adult rats with a maximum growth rate occurring between 23 and 50 days of age. Weights were significantly smaller ($P < 0.01$) than those of adult rats until the age of 61 days. The sigmoid growth curve of the rat testis is illustrated in these data.

Histological evaluation of the seminiferous epithelium in 16-day-old rats showed only Sertoli cells and numerous spermatogonia. Few interstitial cells were visible in animals of this age group. In 29-day-old rat testes, numerous interstitial cells were present and the germinal epithelium had advanced to the stage of pachytene spermatocytes. The tubules of 61-day-old rat testes were nearly of adult size and contained all types of germinal cells, including spermatozoa.

Table 1. Changes in testicular weights, carnitine acetyltransferase activity and serum testosterone with age in the rat

Age (days)	No. of rats	Testis wt (g)	CAT activity (μ mol NADH produced/min/g tissue)	Serum testosterone (ng/ml)
16	3	$0.08 \pm 0.00^*$	$1.20 \pm 0.08^*$	$0.28 \pm 0.02^*$
23	3	$0.13 \pm 0.02^*$	$3.43 \pm 0.11^*$	$0.70 \pm 0.10^*$
29	3	$0.30 \pm 0.02^*$	$4.58 \pm 0.37^*$	$0.45 \pm 0.05^*$
33	3	$0.44 \pm 0.01^*$	$4.55 \pm 0.17^*$	$0.68 \pm 0.11^*$
40	3	$0.93 \pm 0.03^*$	6.24 ± 0.17	$1.30 \pm 0.15^*$
50	3	$1.40 \pm 0.02^*$	6.54 ± 0.16	$0.83 \pm 0.13^*$
61	3	1.60 ± 0.02	5.95 ± 0.08	1.63 ± 0.34
Adult	10	1.68 ± 0.04	6.21 ± 0.07	2.36 ± 0.41

Values are given as means $\pm$ S.E.

* Significantly different from adult values ($P < 0.01$).

Testicular carnitine acetyltransferase. As shown in Table 1, CAT activity in the developing rat testis was 1.20 ± 0.08 units at 16 days of age and increased to 6.21 ± 0.07 units in adult animals. The marked increase in activity at 23 and 29 days of age coincides with a rapid proliferation of the germinal epithelium, at a time when primary spermatocytes become prominent. Activities were significantly lower ($P < 0.01$) in the testes of rats at 16 to 33 days of age compared to those of adults.

Serum testosterone levels. The mean serum testosterone level determined by radioimmunoassay in normal adult rats was 2.36 ± 0.41 ng/ml. Low levels were found in rats which were 16 days old (Table 1) and elevated levels were found at a time when gonadotrophin titres were expected to increase, i.e. at 23 days. Rat serum from 16-day-old animals contained approximately 10% of the adult

testosterone level, after which levels increased as a function of age. These levels remained significantly lower than adult levels until the age of 61 days ($P < 0.01$).

The cryptorchid rat

Testicular weight and histology. As shown in Table 2, the weight of the testes of cryptorchid rats was higher than that of the controls 1 to 3 days after surgery. After Day 4, the testis weight decreased, the seminiferous tubular diameter appeared to decrease, sperm numbers were diminished and the appearance of multinucleated cells was evident. Similar but more dramatic changes occurred with prolonged periods of cryptorchidism, i.e. increased damage to the germinal epithelium, absence of spermatozoa and mature spermatids, and an increased number of multinucleated cells were evident in these testes.

Table 2. Changes in testicular weights, carnitine acetyltransferase activity and serum testosterone in rats made cryptorchid

Days following cryptorchidism	No. of rats	Testis wt (g)	CAT activity ($\mu\text{mol NADH produced/min/g tissue}$)	Serum testosterone (ng/ml)
1	4	2.25 ± 0.12	$3.24 \pm 0.22^*$	0.62 ± 0.12
2	4	$2.45 \pm 0.15^*$	$2.29 \pm 0.13^*$	1.75 ± 0.75
3	4	$2.47 \pm 0.10^*$	$2.03 \pm 0.12^*$	0.85 ± 0.15
4	4	1.63 ± 0.16	$1.84 \pm 0.13^*$	3.18 ± 0.65
5	4	$1.18 \pm 0.10^*$	$1.80 \pm 0.19^*$	1.78 ± 0.93
6	4	$1.20 \pm 0.09^*$	$1.08 \pm 0.18^*$	0.80 ± 0.05
7	4	1.49 ± 0.15	$1.46 \pm 0.18^*$	1.12 ± 0.93
8	4	$1.22 \pm 0.10^*$	$1.21 \pm 0.17^*$	1.48 ± 0.52
9	4	$1.05 \pm 0.10^*$	$1.18 \pm 0.14^*$	2.12 ± 1.28
10	4	$0.99 \pm 0.11^*$	$0.91 \pm 0.07^*$	2.45 ± 0.65
Control	10	1.68 ± 0.04	6.21 ± 0.07	2.36 ± 0.41

Values are given as means $\pm$ S.E.

* Significantly different from control values ($P < 0.01$).

Testicular carnitine acetyltransferase. Levels of CAT in control and cryptorchid rat testes are shown in Table 2. Control levels of 6.21 ± 0.07 units compare with considerably lower levels (0.91 ± 0.07 units) after 10 days of cryptorchidism. The activity of CAT was diminished as early as 1 day after surgery ($P < 0.01$) and continued to decline throughout the experimental period.

Serum testosterone. The extreme variability which existed in serum testosterone levels prevented the expression of any significant changes in these values. In general, hormone levels tended to be lower than those of the controls during the early period of cryptorchidism, then returned to normal by the end of the experimental period (Table 2).

DISCUSSION

The changes in CAT found in this study are qualitatively similar to those reported by Vernon *et al.* (1971), but the actual levels found are considerably lower. Although the same method was used, the earlier workers (Marquis & Fritz, 1965a) did not report the assay temperature so they and Vernon *et al.*

(1971) may have used a temperature different from the 33°C employed in this study. The different strains of rats used may also have contributed to the apparent inconsistency. In any event, the changes in CAT levels reported here support the contention of Vernon *et al.* (1971) that the enzyme is highly concentrated in primary spermatocytes; the greatest increases in CAT activity occurred between 20 and 30 days of age, when the primary spermatocytes were developing (Clermont, 1972). Unfortunately, the data from cryptorchid rats do little to help resolve this question since decreases in the enzyme were found before any cell type disappeared from the tubule. Primary spermatocytes are among the most heat-labile of the testicular cells (VanDemark & Free, 1970), so it is possible that CAT may disappear from the cells as they lose functional integrity but before they disappear from histological preparations. Alternatively, testicular CAT may, in itself, be heat-labile, in which case its loss might result in cell death. If lysosomes from heat-sensitive germinal cells are impaired early after the induction of cryptorchidism, as reported by Lee & Fritz (1972), it is possible that enzymes released from the lysosome may destroy CAT before the appearance of histological changes in the germinal epithelium. Further study is necessary to examine these alternatives.

Although the changes in serum testosterone in the developing rat closely paralleled the increases in CAT specific activity ($r = 0.59$, $P < 0.01$) shown in Table 1, it appears unlikely that cause and effect relationships can be inferred. It seems more likely that CAT and testosterone increase simultaneously, but independently, in the growing rat. The data in Table 2 further this suggestion, since no relationship between these two characteristics of the testis is seen.

Changes in serum testosterone found in developing rats are similar to those reported by Miyachi, Nieschlag & Lipsett (1973), but the actual levels found are considerably lower. The values reported by Miyachi *et al.* (1973) for adult Holtzman rats are also much higher than those reported elsewhere for adult Wistar rats (Gomes *et al.*, 1973) and Sprague-Dawley rats (Free & Tillson, 1973; Free, Jaffe, Jain & Gomes, 1973).

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