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Testosterone and Androstenedione Concentrations in Human Testis and Epididymis during the First Two Years of Life*

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ABSTRACT. Testosterone and androstenedione were measured in testicular and epididymal tissue of 37 previously healthy infants between 1 and 24 months of age who died suddenly. In half of the patients elevated plasma levels of cortisol and androstenedione suggested preterminal stress. Plasma testosterone levels, however, did not differ from those in healthy infants. Testicular testosterone concentrations were maximal in boys from 1–3 months of age (median, 36.6 ng/g; range, 7–380 ng/g) with peak values similar to those found in pubertal or even adult testes. Thereafter testicular testosterone concentrations decreased and after the age of 6 months all values were below 12.5 ng/g, which corresponds to the low normal range of older prepubertal boys. Plasma testosterone and testicular testosterone correlated significantly ($P < 0.001$). On average the testicular concentrations were 36.4 times higher than the corresponding plasma concentrations. Testicular androstenedione was low but correlated significantly with testicular testosterone ($P < 0.001$). Epididymal testosterone concentrations were surprisingly high (1–3 months: median, 10.3 ng/g; range, 4–42.7 ng/g) and averaged 30% of the testicular testosterone concentration. Thus, epididymal testosterone concentrations were significantly higher than the circulating plasma testosterone levels, indicating the capacity of the infant epididymis to accumulate androgens. These findings suggest that high local testosterone concentrations during early infancy are important not only for the testis itself but particularly for the developing epididymis. (*J Clin Endocrinol Metab* **57:** 311, 1983)

HEALTHY full term infants have transient activation of the hypothalamo-pituitary-gonadal axis leading to sex differences in the secretion of gonadotropins (1, 2) and sex hormones (3, 4). Forest *et al.* (3) were the first to demonstrate that in infant boys the characteristic rise in LH is associated with a transient but striking elevation of plasma testosterone. These findings have been confirmed by others (5–7) and it is assumed that the high circulating testosterone reflects transient activity of the infant testes. The significance, however, of this phenomenon is still unclear. It has been speculated that at this developmental stage high testosterone levels are necessary for the sex specific differentiation of the central nervous system (8), or, more recently, that high local testosterone concentrations are needed to promote maturation of the seminiferous tubules in the testes (9). To assess the hormonal environment of the developing seminiferous tubules we determined testosterone as well as androstenedione in the testes and epididymides of previously healthy infant boys who died suddenly. The use of autopsy material is justified by the study of Ruokonen *et al.* (10) who compared the testes of cadavers with those from orchiectomy and could not find differences in steroid composition, indicating that postmortem changes in testicular steroids take place slowly. The androgen content of testicular and epididymal tissue previously has not been measured in infants. Data are available only from fetal testes (11), from necropsy or biopsy specimens taken from prepubertal and pubertal boys with cryptorchidism (12), and from adults (10, 12–16).

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Subjects and Methods

Specimens

Testes and epididymides were obtained post mortem from 37 infant boys between 1 and 24 months of age who died of the sudden-infant-death syndrome or in an accident. In all body weight was within the normal range. Dystrophic boys and boys with undescended testes were excluded from the study. The time between death and the removal of the testes was less than 24 h. The right or the left testis was taken randomly. Testes and epididymides were cleaned of adhering tissue and were

separated from each other. The specimens were weighed, minced, and stored at −80 C. In each case heart blood was obtained for the determination of plasma steroids.

Steroid determinations

Before the steroid assay, the frozen tissue was homogenized with ice-cold distilled water. The volume of the water depended on tissue weight and varied from 1–5 ml. Homogenization was performed with the cell disruptor Sonifier B-10 (Branson Sonic Power Company, Danbury, CT). All manipulations were rapidly done in the cold to avoid metabolic changes. Testosterone and androstenedione from both plasma and tissue were determined by a modification of a radioimmunological method as previously described for estrogens (17). In brief, samples of plasma or tissue homogenates to which tritiated testosterone and androstenedione has been added as internal standards were extracted with ether. The dried extracts were chromatographed on Sephadex LH-20 with cyclohexane-chloroform-methanol (8:1:1) as the solvent. The androstenedione and testosterone fractions were collected separately. The recovery of both steroids was between 73% and 83% and was used to correct the final results. Quantitation of the steroids was done by RIAs using highly specific antisera which were raised by injecting rabbits with testosterone-C3-BSA and androstenedione-C6-BSA, respectively. Plasma cortisol determinations were performed according to the method of Pham-Huu-Trung *et al.* (18). The lower limits of detection were 3 ng/dl for androstenedione and testosterone and 0.3 μg/dl for cortisol. The interassay variability for a pooled plasma sample was 7.8% for testosterone ($\bar{X}$ = 89 ng/dl), 8.2% for androstenedione ($\bar{X}$ = 85 ng/dl), and 6.4% for cortisol ($\bar{X}$ = 11.2 μg/dl). When testosterone and androstenedione were determined in a testicular homogenate the interassay coefficient of variation in six consecutive assays was 9.6% and 10.9%, respectively.

Statistics

Data were expressed as median and range for each age group. Statistical comparisons between different age groups were performed using the nonparametric ranked analysis of variance procedure of Kruskal and Wallis (19). Interrelationships between corresponding steroid levels were examined by linear regression analysis. P values (two-sided tests) less than 0.05 were considered significant.

Results

The individual plasma concentrations of cortisol, androstenedione, and testosterone of the deceased infants are shown in Fig. 1. In approximately half of the deceased infants the cortisol levels exceeded the upper normal limits for age, indicating preterminal stress. Concomitantly plasma androstenedione was elevated resulting in a close correlation ($P < 0.001$) between cortisol and androstenedione in the same plasma samples. The plasma testosterone levels of deceased boys did not differ from those of healthy boys. There was no correlation between cortisol and testosterone nor between testosterone and androstenedione in plasma. The testicular weights (Fig. 2) showed a marked increase during the first 5 months of life. Thereafter our measurements suggest no further testicular growth. However, more data

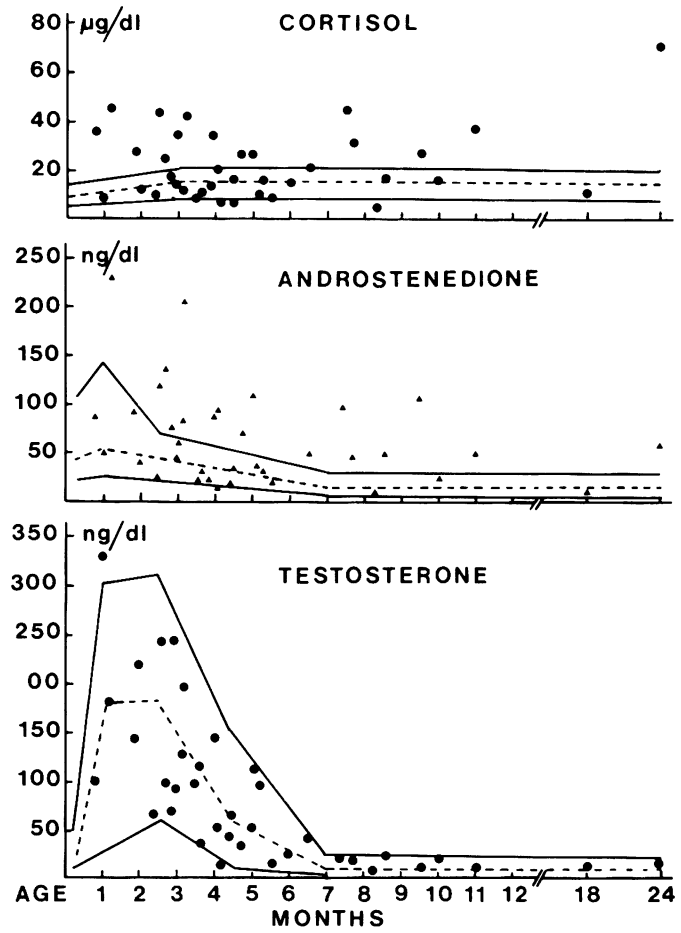

FIG. 1. The concentrations of cortisol, androstenedione, and testosterone in plasma of suddenly deceased infant boys. ——, ---, ranges and median values of normal male infants (7).

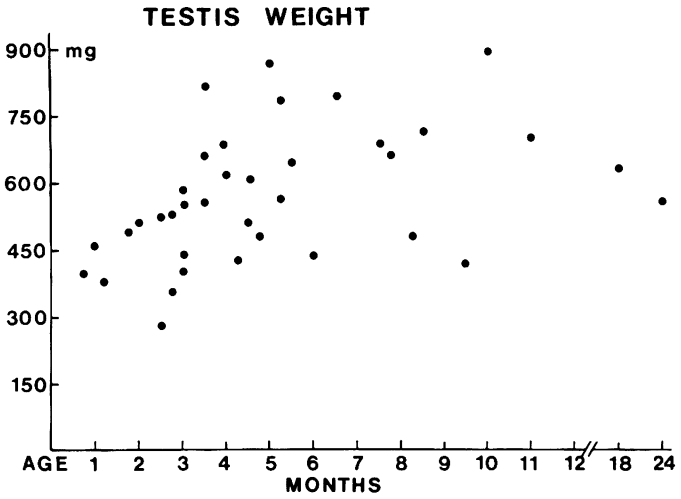

FIG. 2. Weight of a single testis during the first 2 yr of life.

points are needed to allow further conclusions.

Testicular and epididymal concentrations of testosterone and androstenedione expressed as nanograms per g wet tissue (ng/g) are shown in Fig. 3. Maximal testicular testosterone values were found in boys from 1–3 months of age. However, in the same age group low values were also found (median, 36.6 ng/g; range, 7–380 ng/g). In the age group 3–6 months the testosterone content (median, 14.7 ng/g; range, 2.9–60.2 ng/g) was significantly lower than in the younger infants ($P < 0.005$). After the age of 6 months until the end of the second year the values remained low (median, 4.4 ng/g; range, 1.3–12.5 ng/g) and were significantly different from those of 3- to 6-month-old boys ($P < 0.05$). Testosterone concentrations in plasma and testicular tissue were closely correlated ($P < 0.001$). During the first 2 yr of life on average 1 g testis contained 34.6 times more testosterone than 1 ml plasma. Testicular androstenedione concentrations were low. They were closely correlated with the testosterone concentrations in the same testis ($P < 0.001$). There was no correlation between androstenedione in plasma and testis.

Androgen concentrations in the epididymides (Fig. 3) were closely correlated with the testicular values (testosterone, $P < 0.001$; androstenedione, $P < 0.001$). They were highest in boys 1–3 months of age (testosterone median: 10.3 ng/g, range: 4–42.7 ng/g; androstenedione median: 2.1 ng/g, range: 0.8–12 ng/g) and lowest from 6–24 months of age (testosterone median: 2.4 ng/g, range: 0.5–6.8 ng/g; androstenedione median: 1.1 ng/g, range: 0.5–2.65 ng/g). The differences between these age groups were significant for both epididymal testosterone ($P < 0.001$) and epididymal androstenedione ($P < 0.01$). On an average 1 g epididymis contained 10 times more testosterone than 1 ml plasma.

Figure 4 compares the testicular testosterone concentrations which we observed in the infantile testes with values reported in the literature for the various periods of human development (11, 12).

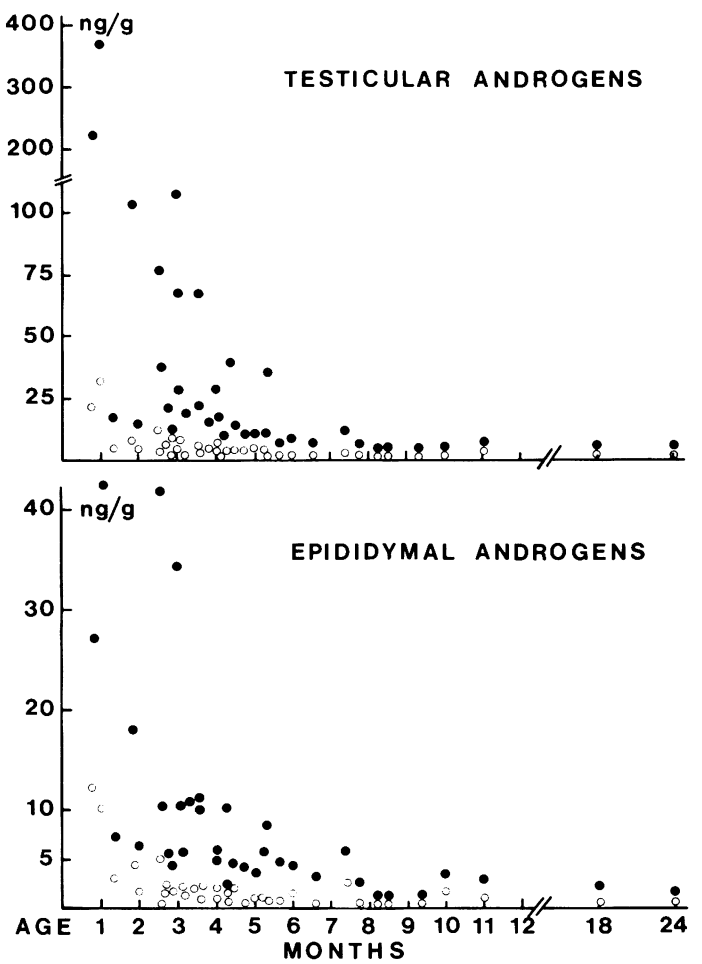

FIG. 3. The concentrations of testosterone (●) and androstenedione (○) in testes and epididymides of infant boys (nanogram per g wet tissue).

Discussion

The germinal epithelium of the testis and the epididymis are androgen dependent and androgens play an important role in the initiation and maintenance of spermatogenesis and sperm maturation (20, 21). In prepubertal boys spermatogenesis can be initiated when large amounts of androgens are produced by a tumor of the testis (22, 23). However, spermatogenetic development is only observed in the tumor-bearing testis and the most advanced tubules are found adjacent to the tumor. The epididymis of the affected testis may be indistinguishable from adult organs (22). This demonstrates the importance of high local hormone concentrations which have to be far above the circulating plasma concentrations in order to initiate spermatogenesis.

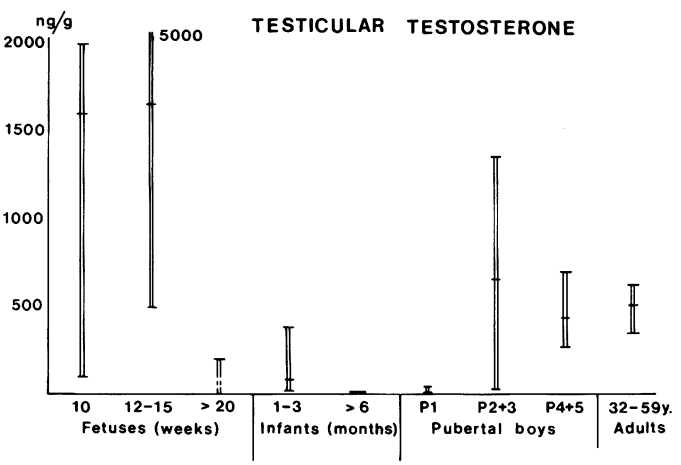

FIG. 4. Testicular testosterone concentrations (mean and range) at various stages of human sexual development. Our findings in infants are compared with the fetal data derived from Reyes *et al.* (11) and data from pubertal boys and men reported by Pasqualini *et al.* (12).

The aim of this study was to evaluate the hormonal environment of the infantile testicular seminiferous tubules and epididymal ducts during a time when physiologically high amounts of androgens appear in the peripheral blood but are apparently not yet needed for the production of fertile sperm. The plasma testosterone concentrations in the infants of this postmortem study were not different from those of normals and agreed well with the previously described rise and fall during infancy. Thus, we conclude that gonadal activity was largely unchanged before death in these subjects. The testosterone concentrations in the testes were much higher than in plasma. Like plasma testosterone testicular testosterone was maximally elevated during the first 3 months of life. The concentrations in plasma and testis were closely correlated, which demonstrates the role of the testes as the source of the early androgen surge in blood. Androstenedione plays a minor role in the testicular androgen stores but as the immediate precursor in testosterone biosynthesis it was consistently present in higher concentrations in the testes than in plasma.

The transient gonadal activation after birth leads to testicular testosterone concentrations in 1- to 3-month-old boys which are higher than those of older infants and prepubertal children. The peak concentrations correspond to values which are normal for pubertal or even adult testes (10, 12). However, the mean concentration of the early infant group remained significantly lower than the mean concentration in pubertal or adult testes.

In adults 1 g testis contains at least 100 times more testosterone than 1 ml plasma (12), whereas our results show that even during the infantile androgen surge testosterone concentrations in the testes were only 35 times higher as compared to plasma. Thus, testosterone seems to be released more rapidly into the circulation from the infant as compared to the adult testis. Therefore, the local testosterone concentrations which act on the seminiferous tubules in infants are lower than those resulting in complete spermatogenesis in the adult.

Epididymal androgen concentrations in the infants were surprisingly high. Whereas in adult men only 10% of the testicular testosterone is found in the epididymis (15) an average of 30% of testicular testosterone was found in the infantile epididymis. Thus, in infancy the testosterone content of 1 g epididymal tissue was 10 times higher than that of 1 ml plasma, which indicates the capacity of the epididymis to bind and accumulate testosterone even during a time when androgens are not yet necessary for sperm maturation. Further, epididymal testosterone content in infants was much higher than the androgen concentrations in the prostate, another target organ for androgens, during all the developmental periods of the human male from birth to old age (24). Therefore, high local testosterone concentrations during infancy seem to be important not only for the maturation of the testis but also for the development of the epididymis which in this period of life may be of particular significance for the descending testis (25).

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International Symposium "Immunology in Diabetes 1984"

An international symposium "Immunology in Diabetes '84" will be held March 15 to 17, 1984 in Rome, Italy. Topics to be covered include: immunology to Type I and other types of diabetes, immunology of transplants in diabetes, immunology of diabetic pregnancy, immunogenicity of insulins, immunopathology of diabetic complications, and the influence of diabetes on the immune system. The audience will be limited to 300 participants. The registration deadline is November 30, 1983. Participants interested in presenting short original communications should submit an abstract of 400 words or less by November 30, 1983.

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