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SEX STEROID HORMONES DURING MULTIPHASE PUBERTAL DEVELOPMENTS

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SUMMARY

Somatic changes during adolescence were related in this study to the breast or genitalia maturational ratings described by Tanner[1].

Urinary androsterone excretion in boys as well as in girls paralleled more the development of skeletal age as a maturational parameter than the chronological age of the adolescent individuals. Sex differences became obvious in relating the androsterone excretion in boys or girls to the skeletal maturity. A similar relationship between girls and boys to the skeletal development was encountered measuring testosterone excretion values.

Plasma estradiol and estrone concentrations in girls and plasma testosterone and dihydrotestosterone levels in boys exhibited close correlations to the pubertal developmental stages. Estradiol levels in boys were highly correlated to testosterone concentrations, indicating that perhaps a significant portion of the estrogen is derived from circulating androgens. 5α-androstane-3α, 17β-diol showed a rise during the maturational stages in boys. Changes in the relationship between dihydrotestosterone/testosterone and androstanediol/testosterone indicate that there is an increment of 5α-reductase activity at the beginning of puberty.

Longitudinal studies revealed parallelism between testosterone excretion in the urine and the plasma testosterone concentrations, showing the most marked increments between ages 12 and 14 years.

In the rat it was found that plasma testosterone levels rose dramatically after the 25th day of life, the surge in dihydrotestosterone occurred at the 26th day of life, but the peak is reached later. These changes were compared to LH and FSH levels. The rate of increment in plasma FSH concentrations is greatest between 16 and 20 days of age and followed by a spurt in the LH concentration after day 20 of age. The abrupt rise in plasma testosterone seen after day 26 is perhaps mediated by the sudden rise in the two gonadotropins. Changes due to castration and cryptorchidism are discussed.

The attainment of reproductive capacity is a complex process. The purpose of this report is to review the state of our present knowledge with respect to the following questions:

1. At what chronological age and sexual maturational stages do the adrenal and gonadal steroid hormones begin to rise toward adult levels?
2. What is the subsequent time course during puberty once the increase in the sex steroid hormone levels has begun?
3. How do the results of assays of the excreted and circulating steroid hormones compare?
4. Can the hormonal data obtained in experimental animals during pubertal changes be compared with those of children?

SOMATIC CHANGES DURING ADOLESCENCE

The development towards final maturity in a normal child, which proceeds through multi-phase stages, is generally divided into five broad groups[1]. Although various modifications of the Tanner scheme for somatic changes have been suggested by a number of investigators, in the present study we have throughout related our endocrinological findings with the breast or genitalia maturational ratings described by Tanner[1].

Recent investigations [2], on the somatic changes occurring during adolescence, have revealed that the relationship between various areas of development vary considerably. It is perhaps possible to note the existence of constancy of sequence within a given development but inconstancy between different areas of development. It is also possible that all children may not pass through all the individual stages of sexual ratings. For this reason investigators who want to relate their endocrinological findings should be careful in defining the ratings with which they have associated their findings. Because of the absence of constancy in the parallel development of the sexual characteristics it would be misleading just to say that hormonal findings have been associated with pubertal stages.

STEROID HORMONES DURING PUBERTAL DEVELOPMENT

Before we come to the question of the level of steroid hormones in body fluids of children during their adolescent growth, it should be made clear that the demonstration of a particular steroid in the body fluid, or its concentration does not really give any information about its secretion. Single determinations of plasma steroid hormone levels reflect only the momentary balance between the entry and removal of that substance from the blood pool. The rigorous criterion to prove the secretion of a steroid should be

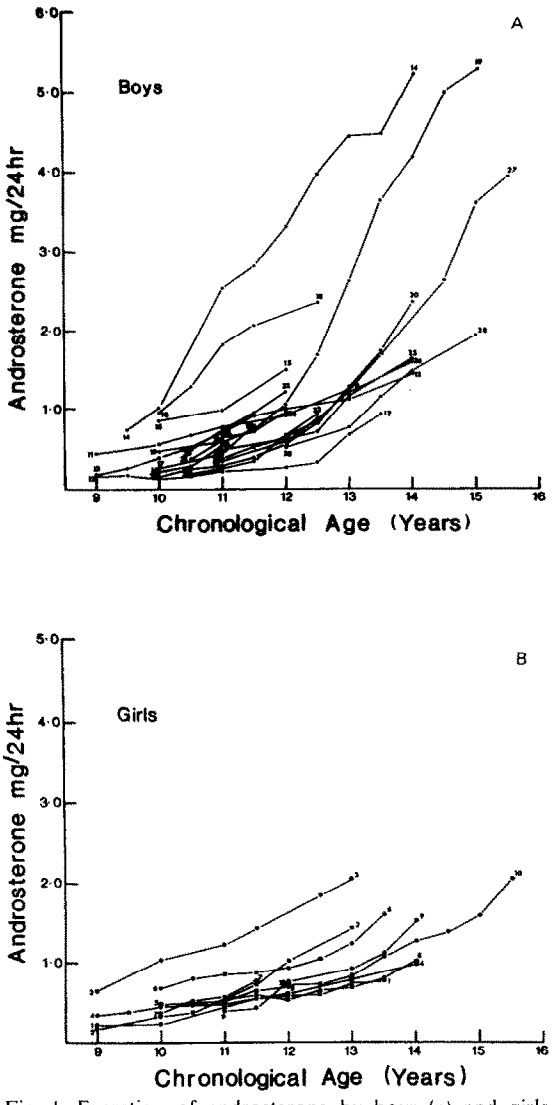

Fig. 1. Excretion of androsterone by boys (a) and girls (b) at successive intervals of chronological age. Numbers indicate different subjects.

to demonstrate that the venous effluent from the gland has a higher concentration of that particular steroid than has peripheral blood. Understandably, therefore, the complex problems associated with the secretion of steroid hormones in normally maturing children are handicapped, on ethical grounds. We have to be satisfied with the urinary excretion or plasma concentration levels of steroid hormones in normally developing children, although the latter is still hard to perform.

Urinary steroids

Figure 1a shows the excretion of androsterone in boys at successive chronological ages. Here, though most of the individuals give a regularly increasing curve, there is great variation among individuals. At age 11 years the values ranged from about 0·2 to 2·6 mg per 24 h. At age 14, the range was about 1·4 to 5·2 mg per 24 h. Figure 1b shows the excretion of this steroid by girls. Here the variation between individuals is not as prominent as seen in the boys. The girls' range at the age 14 is about 1·0 to 1·5 mg per 24 h. Some of the variation in excretion, is due to differences in the developmental age of the children. Some children are skeletally more mature than others at the same chronological age.

Figure 2a, therefore, demonstrates the same data plotted against skeletal age. The variation between individual boys is now considerably reduced. The high excretors being also early developers had their curves shifted toward the higher skeletal ages. Fig. 2b shows the excretion of androsterone in the girls plotted against skeletal age. One can see that among the girls also the high excretors had shifted over to the right as being early maturers.

One of the major findings in the urinary steroid studies published was the lack of any significant sex difference before the age of 12 to 13 years. Yet in the 11-deoxy-17-oxosteroid excretion of the adult there is a considerable difference, as men produced substantially more. Evidently, the boys' excretion continues to rise during the later part of their adolescent growth spurt, at a time when the girls' growth spurt

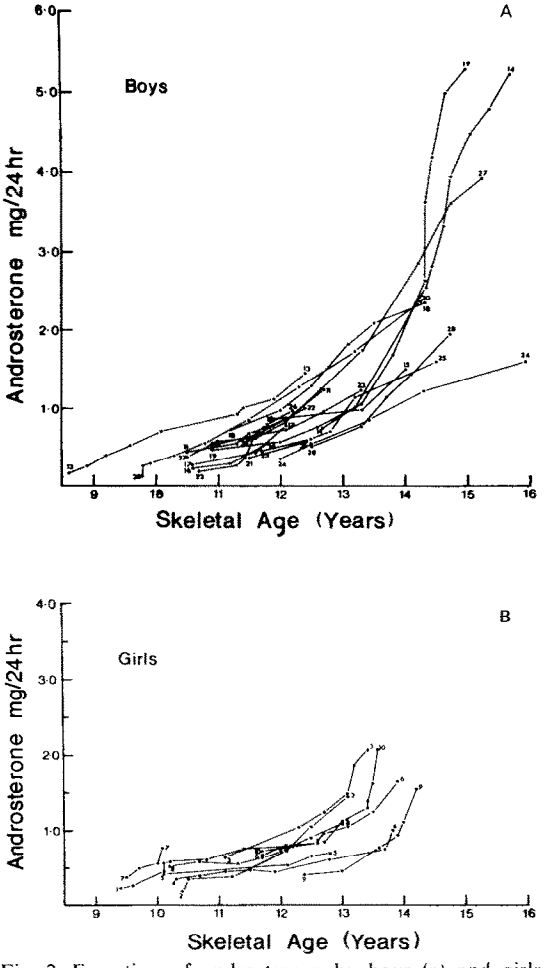

Fig. 2. Excretion of androsterone by boys (a) and girls (b) plotted against skeletal age. Numbers indicate different subjects.

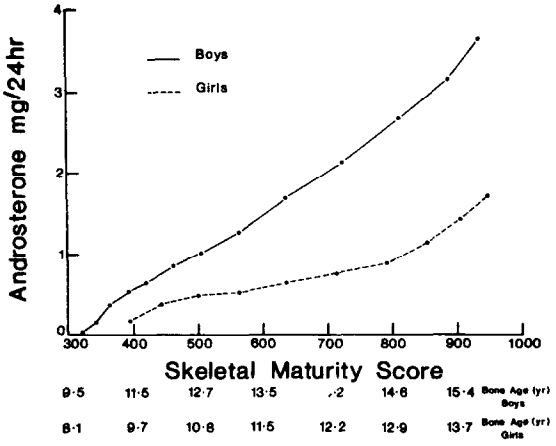

Fig. 3. Mean values for the excretion of androsterone for skeletal maturity score. Skeletal ages for boys and girls corresponding to skeletal maturity score are given in the bottom scale.

and their increase in 11-deoxy-17-oxosteroid excretion is over. But this is mainly in terms of chronological age, and perhaps physically and auxologically misleading. The boys' adolescent spurt occurs 2 years later than the girls. Thus a more informative comparison could be achieved, by plotting steroid excretion in relation to physiological or developmental age, as represented by skeletal maturity. In Fig. 3 the mean trend in the increment of androsterone excretion with skeletal maturity score is given. This is a measure common to both boys and girls of the degree to which skeletal maturity has been achieved. The score of 800, for example can be regarded as representing a skeleton that is 80% mature. Plotted this way, boys clearly demonstrate a higher rate of excretion of androsterone over girls for the same skeletal maturation. Plots of cortisol metabolites (not shown here) did not show a sex difference in the pattern of excretion.

In the current literature there are only a few reports on the excretion levels of testosterone by pre-school, pre-adolescent and adolescent children. Figure 4 shows the mean trends in the excretion of testosterone and epitestosterone by children during their adolescent growth. As there was no sex difference between 3 and 7 years, the results were pooled for both sexes for this range. The girls were more or less low excretors for testosterone, and the sex difference is apparent at age 11 years. The ratio of testosterone excretion value for adult males to pre-school boys is 50; for adult male to pre-adolescent boys is 9·4 and for adult male to adolescent boys 1·8. This ratio for adult female to pre-school, pre-adolescent and adolescent girls is 10, 2·9 and 1·1 respectively. In the excretion of epitestosterone there was no obvious difference between boys and girls nor any obvious relationship to skeletal age. Although no sex difference could be observed, it became apparent that boys had a higher mean rate of excretion than girls, when the excretion was related to maturity score.

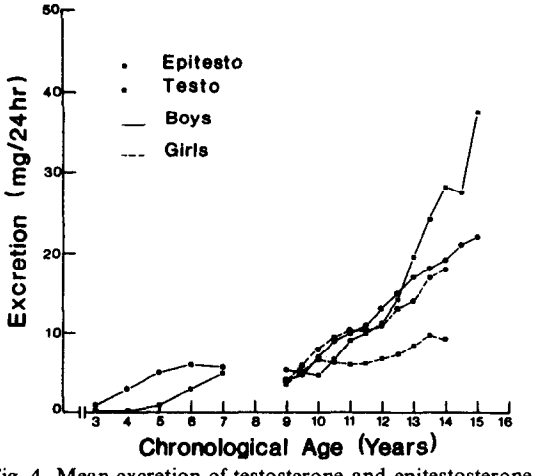

Fig. 4. Mean excretion of testosterone and epitestosterone by boys and girls at successive intervals of chronological age.

PLASMA STEROIDS

Plasma concentrations of the sex steroid hormones increase progressively from pre-pubertal to adult levels during sexual maturation. In our own series of investigations between individuals, plasma hormone concentrations at certain chronological ages were found to be considerably reduced when the same data were related to the pubertal developmental stages. In the majority of cases the concentrations of estradiol and estrone had a close association to the girls' sexual maturation. Similarly, plasma concentrations of testosterone and dihydrotestosterone had a high correlation coefficient with boys' pubertal developmental stages. Therefore in the following figures the values have been given as function of pubertal developmental stages.

Figure 5 shows the mean trend in the plasma levels of estrone and estradiol in boys and girls during sexual maturation. At pubertal stage 1 and 2 the results are homogeneous, with boys and girls showing barely any difference. At stage 5 the girls showed more scatter than the boys. The increase in the S.D. of the estrogen values with sexual maturation in girls has not been previously stressed. During pubertal stages 3 and 4 the S.D. for estrone and estradiol were 0·26 and 0·80 respectively, increasing to 0·82 and 1·49 in stage 5. This increase is perhaps reflecting the onset of cyclic activity. Figure 6 demonstrates the mean plasma concentration of testosterone and dihydrotestosterone as a function of pubertal development in boys and girls. The girls' plasma testosterone concentration tends to be low and steady. It appears that boys and girls have the same amount of testosterone during stages 1 and 2. The sex difference starts to be apparent at stage 3 with boys showing a steep increment during the latter stages.

The mean trend for dihydrotestosterone concentration in girls, on the other hand, surprisingly shows a higher increment than that seen for testosterone. Boys show a clear upward trend in concentration and the sex difference becomes discernible at stage

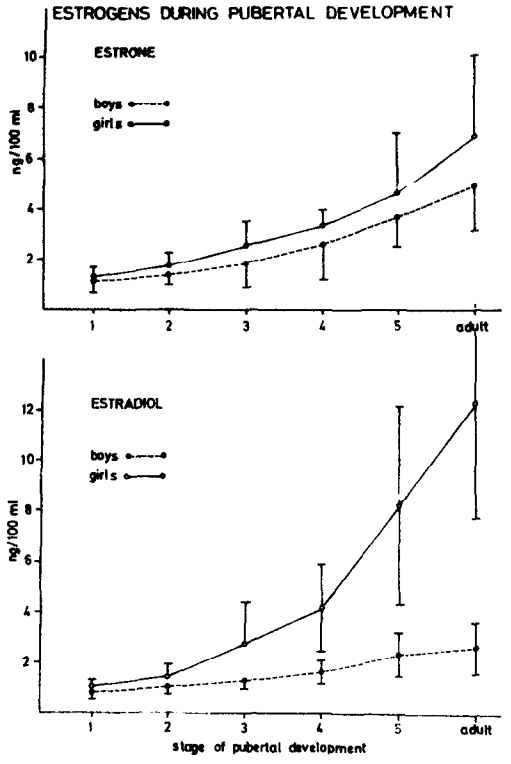

Fig. 5. Mean trends in plasma concentrations of oestrone and oestradiol in children in relation to pubertal developmental stages.

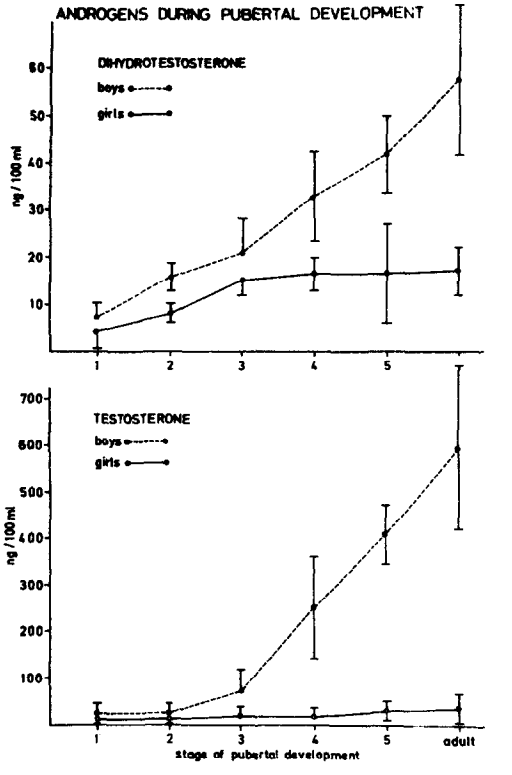

Fig. 6. Mean trends in plasma concentrations of dihydrotestosterone and testosterone in children in relation to pubertal developmental stages.

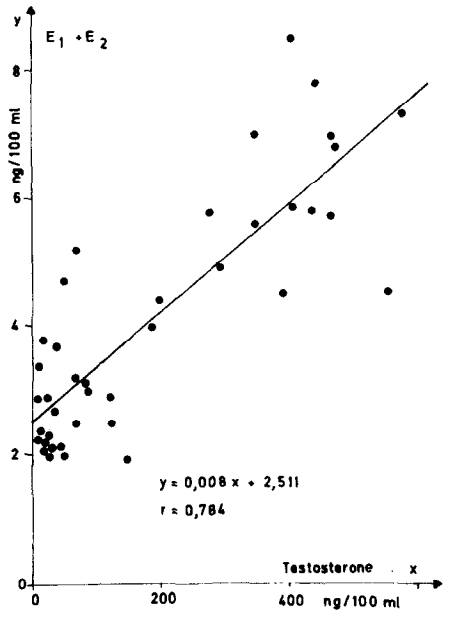

Fig. 7. Correlation between plasma oestrogens and testosterone in normal boys. The linear regression equation Y = 0·008x + 2·511, and the correlation coefficient (r) = 0·784.

4 rather at stage 3, as seen with testosterone. The majority of the fully matured boys (genitalia stage 5) shows plasma levels of testosterone and dihydrotestosterone below the adult male range. Similar observations were made earlier by us [3, 4] with urinary and plasma androgens, being estimated by different techniques. The current results indicate that the levels of the androgens in boys rise even after the fullest sexual maturation, perhaps to maintain and control adult sexual function.

Figure 7 demonstrates the relationship between testosterone and the two estrogens in the peripheral plasma of boys. The high correlation coefficient indicates that perhaps a significant portion of the estrogens is derived from the circulating androgens and not from the gonadal sources. For the girls however, no such relation was observed, inferring that their estrogens have a more independent origin.

In recent years another steroid metabolite, 5α-androstane-3α,17β-diol (androstanediol) has gained some importance in relation to the question of androgenic function. The 5α-reduction of testosterone to dihydrotestosterone has been recognized to be an important event in androgen action. Dihydrotestosterone after being reduced from testosterone is further metabolized in the target cells by conversion to androstanediol, the rate of which has been noted to be higher in men than in women [5]. We have developed [6] a radioimmunoassay method for the estimation of the circulating level of this substance in prepubertal and pubertal boys during their sexual maturation. From a level of 0·56 ng/100 ml in stage 1, the peripheral concentration of androstanediol leaps to a 13-fold higher concentration in stage 5. Figure 8 demonstrates that when the relationship between androstanediol and testosterone is examined

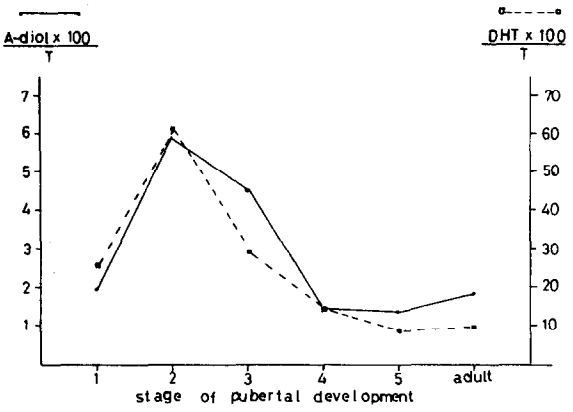

Fig. 8. The ratios of androstanediol/testosterone and dihydrotestosterone/testosterone are given as a function of pubertal developmental stages.

in the form of a ratio, a sharp rise is recorded from stage 1 to stage 2. This rise, however, declines sharply during the transition from stage 2 to 4 and reaches a plateau thereafter. The change in the relationship between dihydrotestosterone and testosterone parallels the androstanediol/testosterone curve, indicating that at the beginning of puberty there is an increment of 5α-reductase activity.

SEX STEROID BINDING PROTEIN

It has been currently established that a large fraction of the non-conjugated steroids is bound to plasma proteins and only a very small fraction remains unbound. This unbound fraction is considered to be the "biologically active" form of the hormone. The percentage of hormone bound to proteins is influenced by several factors; these are (a) the concentration of the steroid binding protein, (b) the number of binding sites on that protein, (c) the concentration of the steroid hormone and (d) the existing association constant between the protein and that particular steroid. Pearlman *et al.*[7], Mercier *et al.*[8] and Rosner and Deakins[9] demonstrated that in fact the concentration of the binding protein is the main factor responsible for the increased binding of testosterone observed in pregnant females, and in estrogen-treated males when compared to non-pregnant females and normal adult males respectively. Although Forest and Migeon[10] did not observe any influence of age or sex on the percentage binding of testosterone or androstenedione in a group of children (4 months to 12 years), August *et al.*[11] have correlated testosterone and testosterone binding affinity levels in the plasma of boys from the age 1 through 12 years, and found that the testosterone

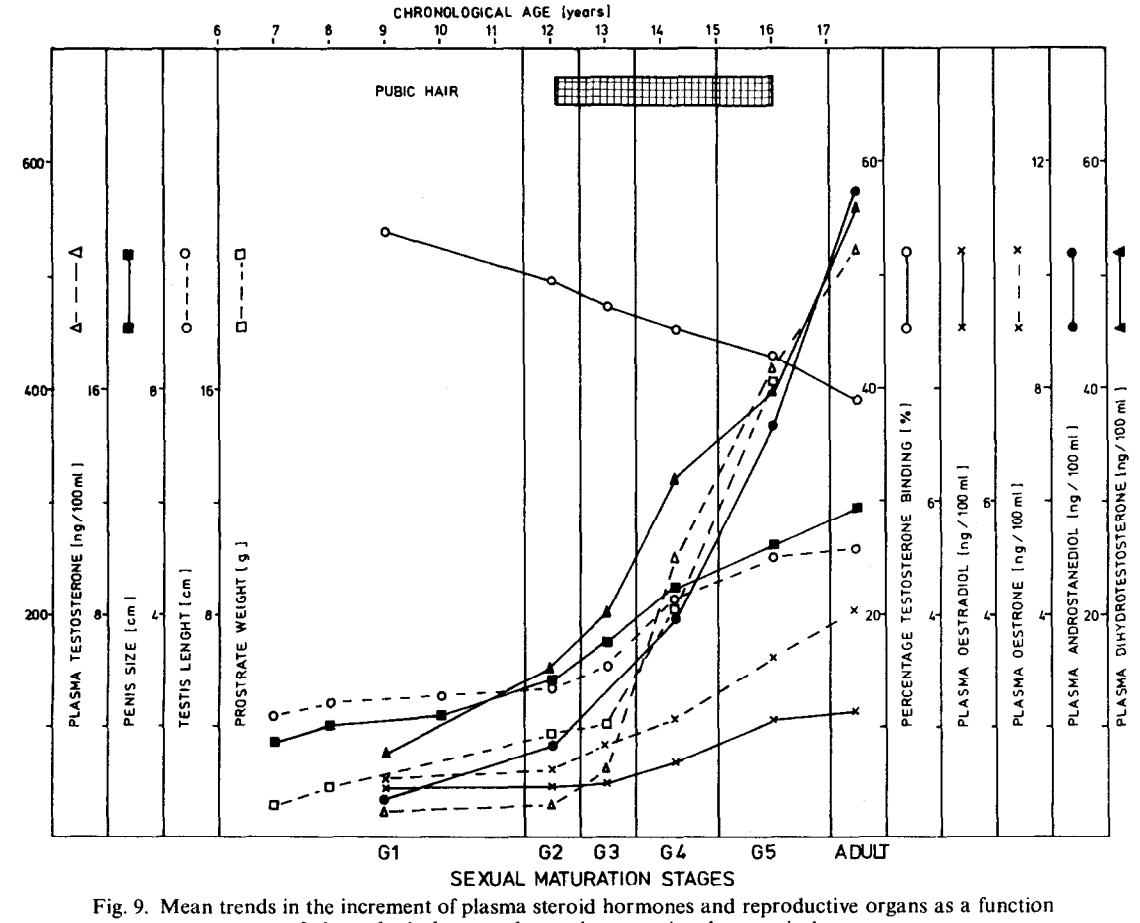

Fig. 9. Mean trends in the increment of plasma steroid hormones and reproductive organs as a function of chronological age and sexual maturational stages in boys.

binding affinity decreased with an increase in age. In our own investigations [12–14] we found that as early as stage 3 of pubertal development the difference regarding the percentage binding of testosterone between sexes was significant. When the value of normal adult females were compared to those of adult males and pre-adolescent children, it was found that adult males had minimum binding while the adult female had the maximum. The pre-adolescent children had values between the adult male and the adult female. It seems that in the boys the time interval between the development of secondary sexual characteristics and the initial testosterone increase together with the decrease in testosterone binding affinity may be related to the amount of unbound testosterone available.

MEAN TRENDS IN HORMONAL INCREMENTS

The accompanying two figures (Figs. 9 and 10) demonstrate a composite picture of the chronological age and the stages of sexual maturation in boys and girls where the plasma concentration of testosterone, dihydrotestosterone, estradiol, estrone and percentage binding of testosterone first exceed pre-pubertal levels. Along with these, the data for penis size, testes length and prostate weight are given (Fig. 11). Figure 12 illustrates ovarian weight and uterine weight with additional hormonal values. It can be seen that the mean chronological age at which testosterone, dihydrotestosterone and androstanediol begin to rise in boys is 13 years (stage 3) with the prostate weight, testis length and penis size also demonstrating simultaneous increments. The levels of plasma estrone and estradiol in boys do not show such comparable increments. Percentage binding of testosterone decreases at the same time towards the adult level. The corresponding age when estradiol shows signs of a marked increment in girls is before 12 years (stage 2), whereas estrone starts to rise at a mean age of 12·5 years (stage 3). Marked increments in the growth of the ovary and the uterus also synchronize at that stage of life. Thus the rise toward the adult levels of the androgens and the estrogens begins one to two years earlier in the girls. Appreciable sexual maturation in both sexes can be associated with the period when the plasma concentrations of the androgens in boys and the estrogens in girls have begun to rise rapidly towards the adult levels.

The changes in all the gonadal steroid levels accompanying the transition from late puberty (B5/G5) to adulthood are highly variable, and reflect partly the limitations of cross-sectional investigations. It is, therefore, most important to appreciate the fact that the real magnitude of a hormonal increment in

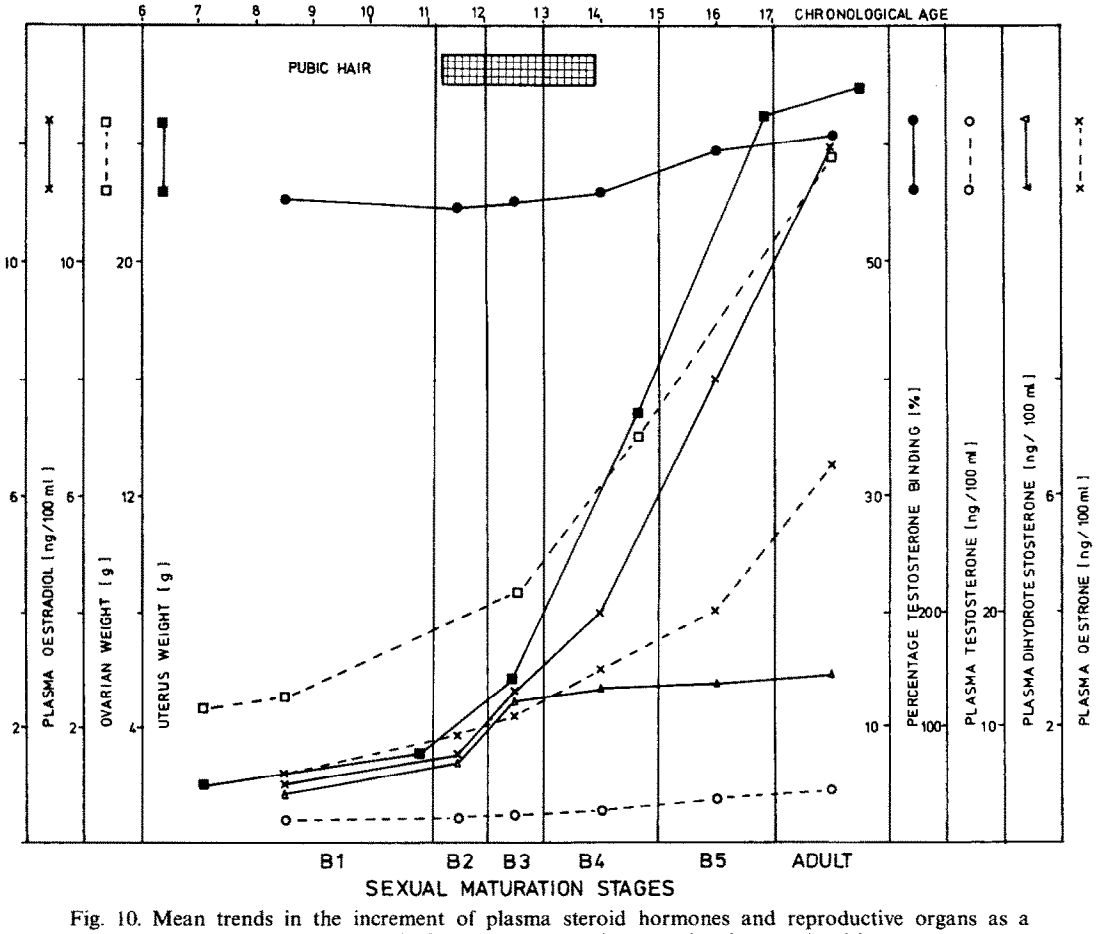

Fig. 10. Mean trends in the increment of plasma steroid hormones and reproductive organs as a function of chronological age and sexual maturational stages in girls.

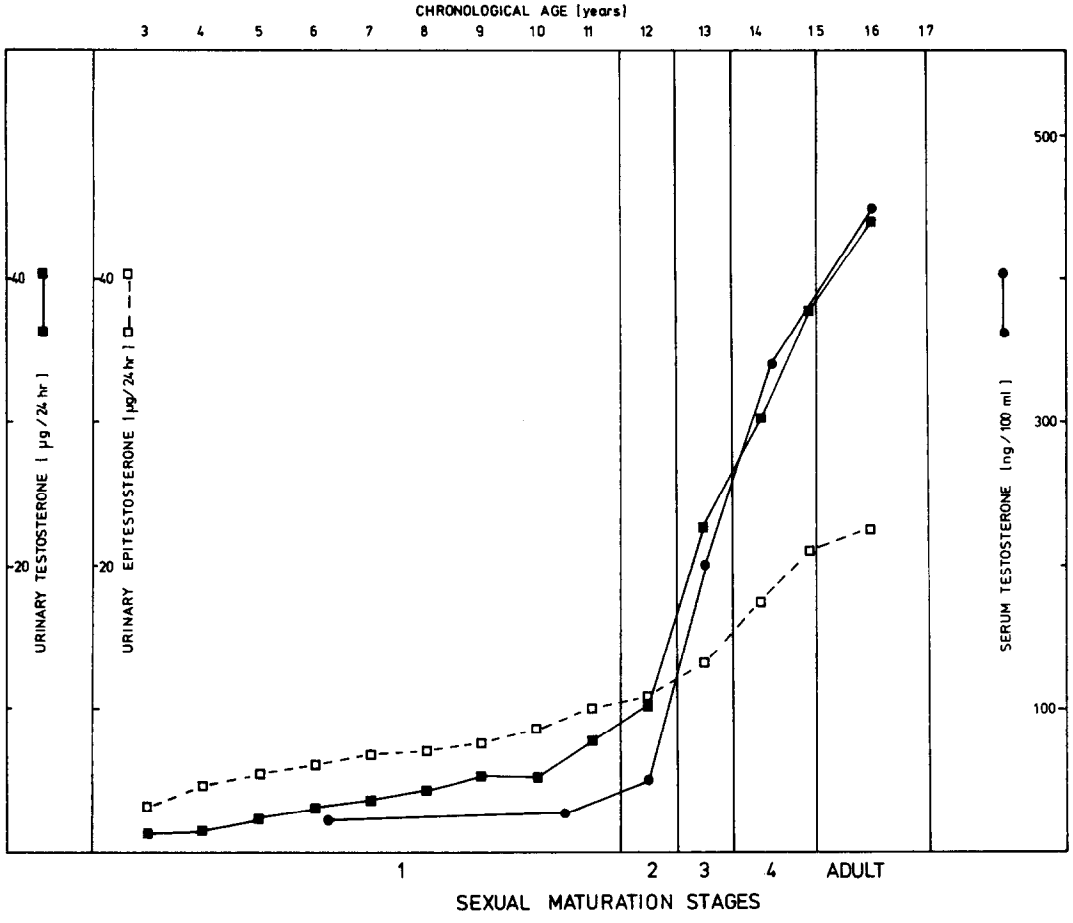

Fig. 11. Mean trend in the increment of urinary excretion and plasma concentration of testosterone in boys studied longitudinally as a function of chronological age and sexual maturational stages. Redrawn from the data of Gupta and Butler[15] and Faiman and Winter[16].

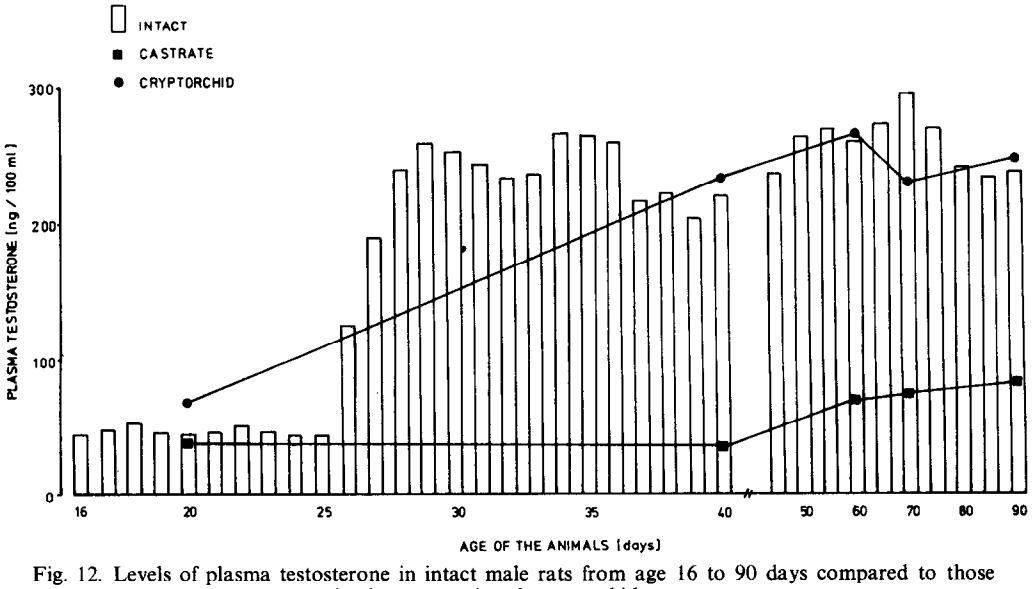

Fig. 12. Levels of plasma testosterone in intact male rats from age 16 to 90 days compared to those in the castrated and cryptorchid ones.

a given individual during his sexual maturation can only be known through a longitudinal study.

LONGITUDINAL INVESTIGATION

Figure 11 depicts two longitudinal investigations at present carried out independently in two different parts of the world. The first is on the excretion of urinary testosterone and epitestosterone[15] and the second is on the serum concentration of circulating testosterone[16] in sexually maturing boys. In spite of the enormous procedural differences, when the two sets of data are compared, a striking parallelism between the mean trends in the hormone excretion and hormone concentration emerge. Utilizing the same method of calculation for mean trends in a longitudinal investigation [17], these results illustrate that during pubertal stage 1, testosterone levels in blood and urine are low and the increments are gradual. The most marked increment in plasma concentration and urinary excretion occurs between ages 12 and 14 years. The excretion of epitestosterone, on the other hand, shows a gradual increase which is favourably associated with the increments in body size rather than the pubertal developmental stages.

GONADAL-STEROID-GONADOTROPIN RELATION IN SEXUALLY MATURING RATS

The production of the gonadal steroid hormones during sexual maturation is entirely under the control of the pituitary gland. Before puberty, when no mature Leydig cells are identifiable in the testes, levels of plasma LH are low. Rising levels of testosterone and LH correlate well with the pubertal developmental stages and Leydig cell maturation. In the next few figures the dynamic relationship between the gonadal steroids and gonadotropins in male rats has been shown. These figures demonstrate the circulating levels of gonadal steroids and gonadotropins measured daily from the age of 16 to 40 days, and then until 90 days with an interval of 5 days in the same animals. These figures give additionally the data on the circulating hormones observed in castrated and cryptorchid at various stages of sexual development. Figure 12 shows that the surge in testosterone level is dramatic in the male rat after the 25th day of life, and in 3 days a level is reached that is 5-fold higher than seen earlier. The peak value seen in the 70-day-old animal is only 111% of the plasma testosterone concentration seen at the 26th day. Figure 13 shows that the surge in the dihydrotestosterone value occurs at the 26th day, but the peak is reached later, ie, day 33, than that seen in the profile of testosterone. On the 38th day dihydrotestosterone reaches a new concentration level and then declines, to remain constant throughout adulthood. Figure 14 demonstrates the plasma profile of LH seen in the same animals. Plasma LH has a clear peak between the ages of 25 and 30 days after which it declines to rise again at age 70 days. Figure 15 shows the plasma profile of FSH in the intact male animals. Between 25 and 35 days of age, FSH values almost double, reaching a peak level at age 33 days. Thereafter, the levels fall at 40 days and decline further to a minimum value at age 70 days. Examination of the velocity of increment of plasma FSH concentration, reveals that it is highest between 16 and 35 days, and the sharp decline starts at age 35 days,

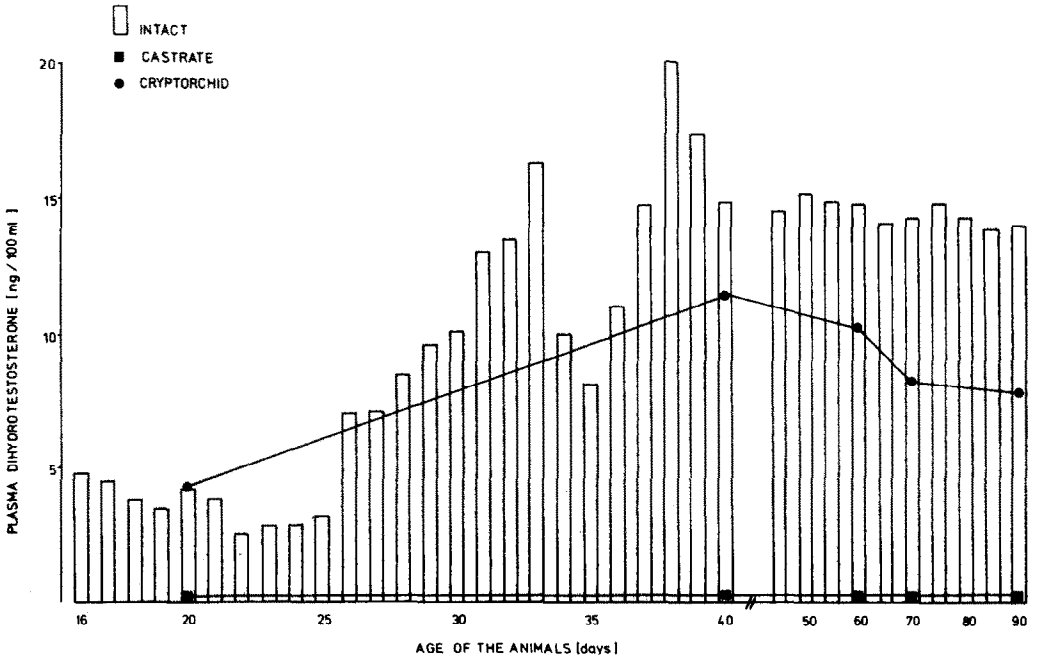

Fig. 13. Levels of plasma dihydrotestosterone in intact male rats from age 16 to 90 days compared to those in the castrated and cryptorchid ones.

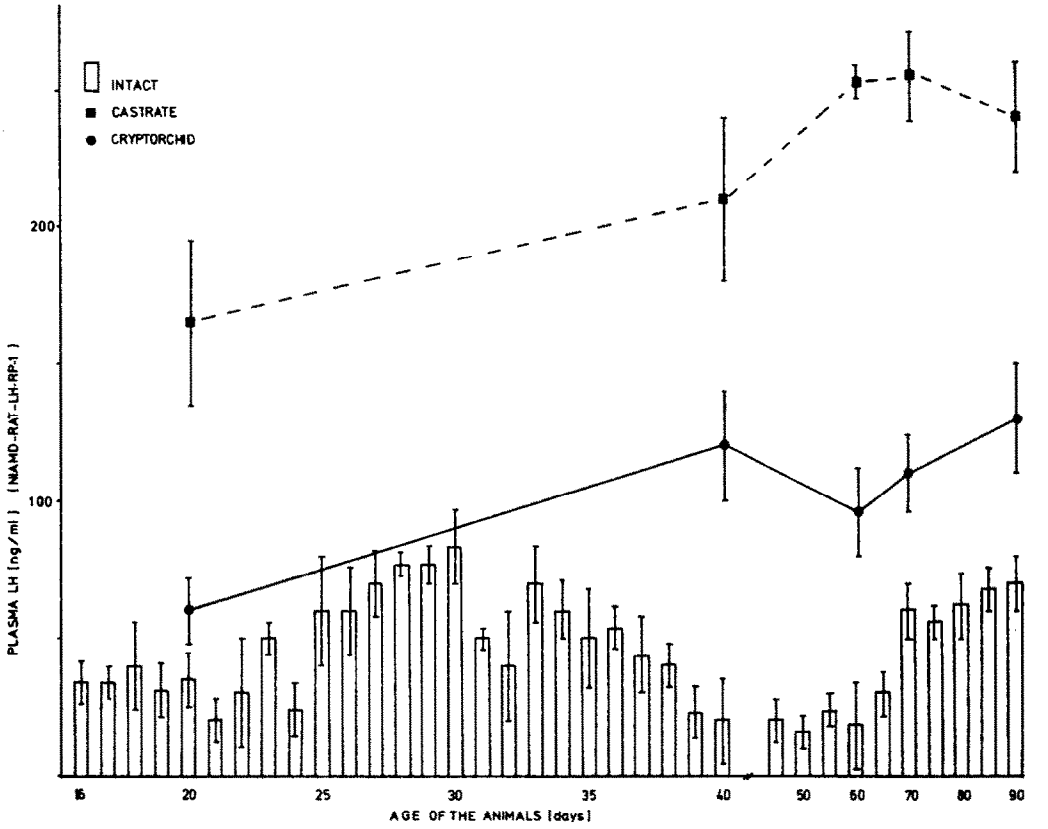

Fig. 14. Levels of plasma LH in intact male rats from age 16 to 90 days. The levels of LH in castrated and cryptorchid rats at various ages are also given for comparison.

ie, just before mature sperm appears in the tubules, and then remains steadily constant throughout the later phase of sexual development.

These cumulative experimental data illustrate that between 16 and 20 days of age the rate of increment in the plasma concentration of FSH is greater than at any other time, and is followed by a spurt in the LH concentration after the 20th day. The abrupt increase in plasma testosterone concentration seen between 25 and 30 days, is perhaps mediated by the

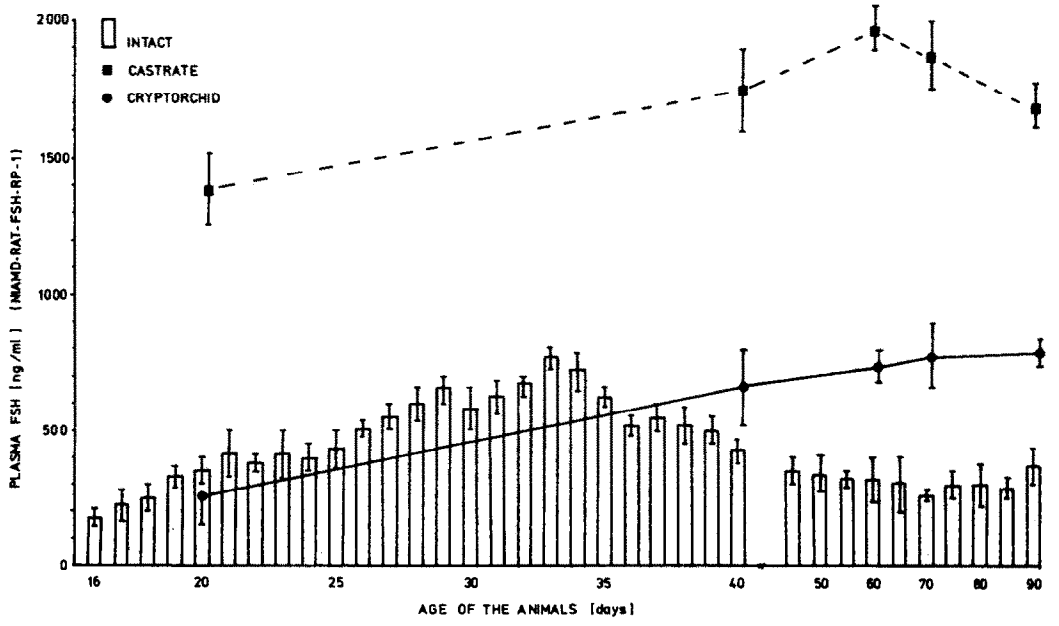

Fig. 15. Levels of plasma FSH in intact male rats from age 16 to 90 days. The levels of plasma FSH in castrated and cryptorchid rats at various ages are also given for comparison.

sudden rise in the rate of increase in the two gonadotropins. The higher gain in dihydrotestosterone is, however, a little delayed. These events of higher hormonal activities in the male rat are perhaps related to the initiation of the Leydig cell differentiation seen at about the same time[18, 19].

Following bilateral gonadectomy, plasma levels of LH and FSH increased within 4 days, indicating the existence of an intact feedback relation between the gonadal sex steroids and the pituitary gonadotropins, long before the onset of puberty. In contrast to the results seen in the orchidectomized animals, the experimentally cryptorchid groups present such evidence which demonstrates that the gonadal steroid-gonadotropin feedback cannot be the only factor in initiating puberty. The plasma FSH concentrations are not different from those of the age-matched controls at earlier stages of development. At 60 days of age, FSH concentration in the cryptorchid animals continue to rise, while falling in the intact rats. In contrast, LH levels in the cryptorchid animals are higher even in the earliest stage of sexual maturation, while this difference gradually increases in the later stages.

In contrast to the changes in the testosterone levels seen in the intact pre- and post-pubertal animals, we observed that FSH secretion correlates more uniformly with the dihydrotestosterone levels. It has also been recently noted [20–22], that dihydrotestosterone and androstanediol effectively inhibit the plasma FSH release in the intact and in the orchiectomized rat. Besides the androgenic inhibition of FSH secretion, it has been additionally postulated that the germinal epithelium exerts a negative feedback on FSH production [23]. When these postuations are taken together with the early observations on the apparent difference in the regulatory mechanism of FSH secretion, one can infer (on the basis of current data), that the steady levels of plasma FSH in the cryptorchid immature rats are the result of the balance of the FSH-inhibiting factor(s) in the immature gonad and do not reflect the change in dihydrotestosterone concentration. The higher magnitude of increment in FSH levels, seen in the later stages of maturation in the cryptorchid animals over the control animals is perhaps due to the destruction of the germinal epithelium in the cryptorchid group. The feedback inhibition provided by the still existing low levels of the circulating dihydrotestosterone prevents plasma FSH reaching the high levels of the castrated animals.

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