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The Hormonal Control of Sexual Development

Jean D. Wilson, Fredrick W. George, James E. Griffin

Summary. Male and female human embryos develop identically during the first phase of gestation. The indifferent gonads then differentiate into ovaries or testes and soon begin to secrete their characteristic hormones. If ovaries (or no gonads) are present the final phenotype is female; thus no gonadal hormones are required for female development during embryogenesis. Two hormones of the fetal testis—Müllerian regression hormone and testosterone—are responsible for the formation of the male phenotype. Analysis of fibroblasts from the skin of patients with abnormalities of sexual development due to single gene defects shows that testosterone is responsible for virilization of the male internal genital tract, that its derivative dihydrotestosterone causes development of the male external genitalia, and that both hormones act in the embryo by the same receptor mechanisms operative in postnatal life.

Human embryos of both sexes develop in an identical fashion for the first 2 months of gestation, and only thereafter do anatomical and physiological development diverge to result in the formation of the male and female phenotypes. The fundamental mechanism of sexual differentiation was elucidated between 1947 and 1952 by Alfred Jost (*1*). He established that the castrated mammalian embryo develops as a female. Male development is induced in the embryo only in the presence of specific hormonal signals arising from the fetal testis. According to the Jost formulation—now the central dogma of sexual development—sexual differentiation is a sequential, ordered, and relatively simple process. Chromosomal sex, established at the time of conception, directs the development of either ovaries or testes. If testes develop, their hormonal secretions elicit the development of the male secondary sex characteristics, collectively known as the male phenotype. If an ovary develops or if no gonad is present, anatomical development is female in character.

Stimulated by this paradigm, subsequent investigators have sought to identify the specific hormones that are secreted by the fetal testis and to elucidate the control mechanisms that regulate the rates of secretion of these hormones at the crucial moment in embryonic development. They have also attempted to characterize, at the molecular and genetic level, the mechanisms by which the testicular hormones act to induce the conversion of the sexually indifferent embryo into the male phenotype. As a consequence, the original formulation of Jost has been refined and expanded, and insight has been obtained into the pathogenesis of many derangements of sexual development in humans which result from single gene defects that impede either the formation or the cellular actions of the hormones of the fetal testis.

Other authors have described the chromosomal basis for sex determination (*2*) and the mechanism by which the X and Y chromosomes cause the differentiation of the gonad into a testis or ovary (*3*). In this article we describe current concepts of the processes by which the fetal gonads acquire the capacity to function as endocrine organs and of the mechanisms by which the endocrine secretions of the fetal testis modulate male development. We focus first on the anatomical events involved in the formation of the sexual phenotypes and then on the factors that mediate this development.

Formation of the Sexual Phenotypes

The temporal relation between the differentiation of the ovary and testis and the development of the sexual phenotypes in the human embryo is shown schematically in Fig. 1. The germ cells do not originate in the embryo itself but rather in the yolk sac (*4*). By about the stage at which the embryo reaches 10 to 20 millimeters in crown-to-rump length, the germ cells migrate to their ultimate destination in the genital ridges of the embryo. After this migration, the primitive gonads in male and female embryos appear identical, and each such gonad has three components: (i) the primordial germ cells, (ii) the mesenchyme of the genital ridge, and (iii) a covering layer of epithelium. Histological differentiation begins when the germ cells in the testis

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line up to form the spermatogenic cords. Shortly thereafter, Leydig (interstitial) cells appear in the connective tissue of the testis; these cells synthesize testosterone. In contrast to the early, rapid development of the testis, the primordial ovaries show little histological development until the second trimester, when the definitive ovarian follicles develop. Phenotypic development is largely accomplished in both male and female embryos when they are between 30 and 90 mm in crown-to-rump length. Thus, histologic differentiation of the testis precedes male phenotypic development, whereas histological differentiation of the ovary takes place after female phenotypic development is far advanced.

The anatomical events in the development of the male and female urogenital tracts are shown schematically in Fig. 2 (*5*, *6*). In brief, the primordial genital tract of both sexes has three components: (i) the gonads, (ii) two genital duct systems (Wolffian and Müllerian), and (iii) a common opening for the genital ducts and the urinary tract to the outside through the genital folds on the abdominal wall.

The process by which this primordium undergoes sexual differentiation is straightforward. The internal genital tracts in the two sexes develop from different duct systems, Wolffian and Müllerian (Fig. 2A). In the male the Wolffian ducts persist, and the Müllerian ducts regress. In the female the Müllerian duct system persists, and the Wolffian ducts degenerate. The external genitalia in the two sexes develop from common primordia, namely the genital tubercle, folds, and swellings (Fig. 2B).

Male development. The main components of the male urogenital tract are shown in Fig. 2A. Sperm are formed in the testis from which they are transported in a tube (the vas deferens) that empties into the urethra. Development (virilization) of the male urogenital tract begins shortly after the formation of the spermatogenic tubules of the testis. The initial event is the onset of regression of the Müllerian ducts, ultimately resulting in their disappearance, Müllerian duct regression is quickly followed by virilization of the Wolffian ducts. The upper portion of the Wolffian duct becomes connected to the testis to form the epididymis, the central portion develops a thick muscular coat to become the vas deferens, and the terminal portion gives rise to the ejaculatory duct and seminal vesicle. Simultaneously, the prostate gland arises from endodermal buds in the lining of the primitive urethra.

Development of the male penis and scrotum commences shortly after the onset of virilization of the Wolffian duct (Fig. 2B). The genital folds elongate and fuse to form the penis and male urethra. As a consequence of the fusion the urogenital swellings on each side of the

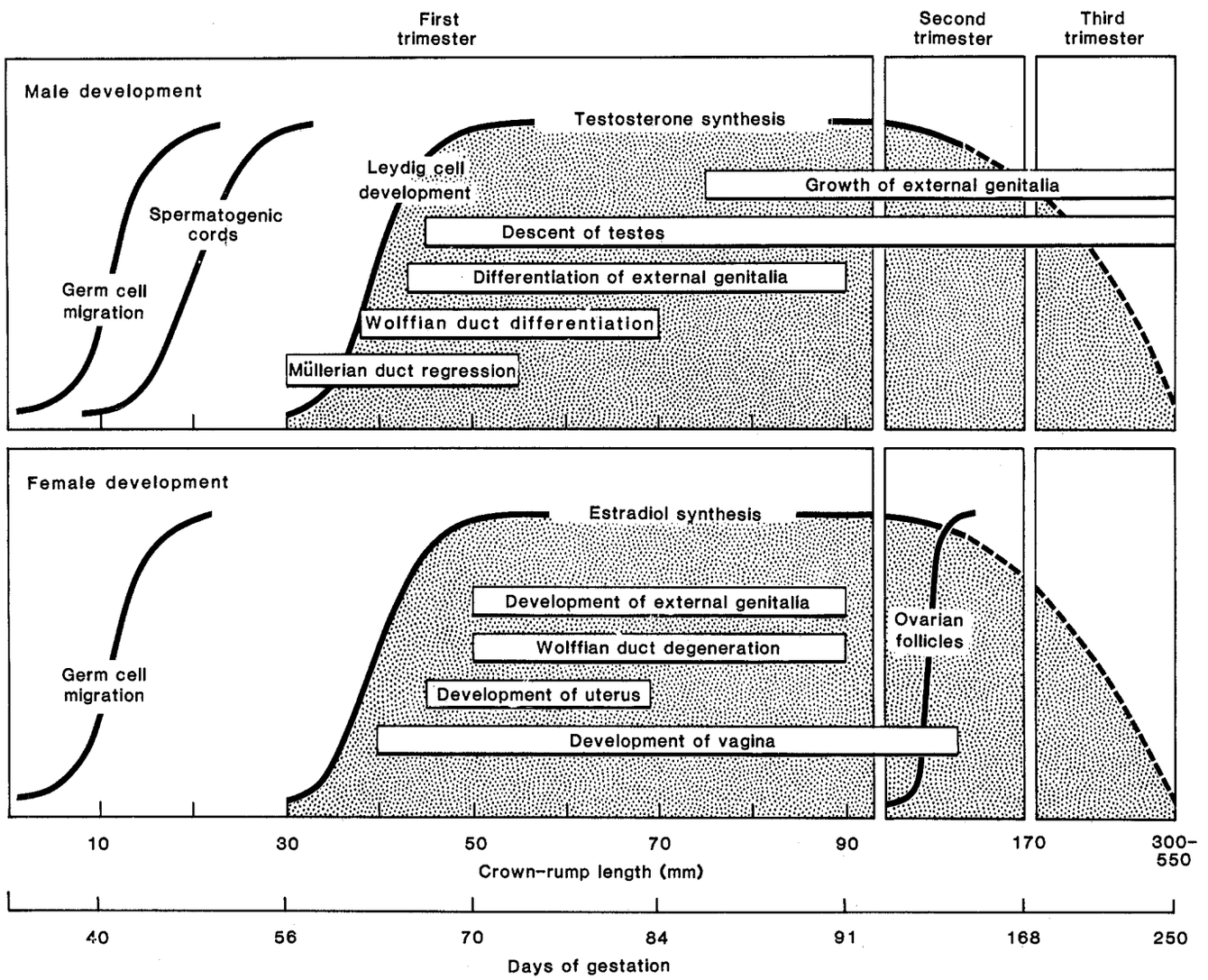

Fig. 1. Relation between differentiation of the gonads and the anatomical differentiation of the human male and female embryos. [Drawn from data in (*5*, *14*, *19*).]

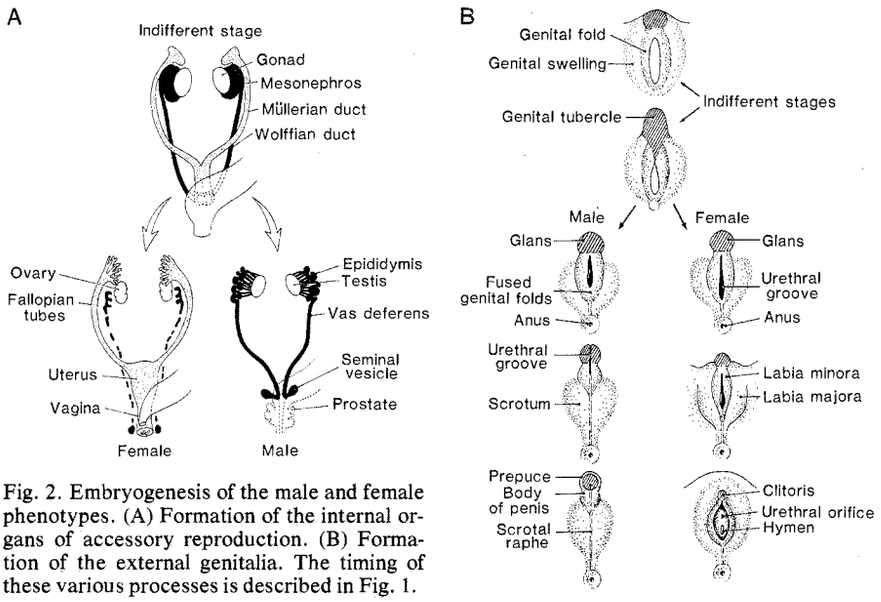

Fig. 2. Embryogenesis of the male and female phenotypes. (A) Formation of the internal organs of accessory reproduction. (B) Formation of the external genitalia. The timing of these various processes is described in Fig. 1.

urethral orifice form a bilobed scrotum that serves as the receptacle for descent of the testes.

Anatomical development of the male internal and external genitalia is accomplished largely by the end of the first third of gestation. In the last two trimesters two final aspects of male development, descent of the testes and growth of the genitalia, are completed. Descent of the testes in man takes place over a 7-month period, beginning about the sixth week and finishing in some instances after birth. When the formation of the male urethra is completed, no differential growth of the male external genitalia has occurred so that at this stage the size of the phallus does not differ in male and female embryos. Subsequently, growth in the male embryo commences in the external genitalia, the prostate, and the structures derived from the Wolffian duct and continues until shortly before birth.

Female development. In the female embryo the Müllerian ducts develop into the fallopian tubes and uterus and contribute to development of the vagina (Fig. 2A). The external genitalia in the female undergo little differentiation compared with the indifferent state (Fig. 2B). The genital tubercle becomes the clitoris, the adjacent genital swellings give rise to the labia majora, and the genital folds become the labia minora.

Breast development. Only one functional mammary bud develops on each side of the chest wall in the human embryo. (In many species multiple glands develop.) This bud is present in the 7-mm embryo and is well differentiated by the 40-mm stage. Little change occurs until mid-gestation when the nipple develops and secondary epithelial buds appear. Ductular proliferation ensues and by the time of birth 15 to 25 branches are present. In male embryos of some species the excretory ducts regress under the influence of testicular hormones, leaving the male breast as an isolated island in the subcutaneous tissue (*7*). Dimorphism of breast development has not been documented in the human embryo, and the breasts of boys and girls are identical prior to the onset of puberty (*8*).

Role of Testicular Secretions in Male Development

Jost established that the transformation of the indifferent urogenital tract and external genitalia into the male phenotype is determined by secretions of the fetal testis (*1*). The experimental basis for this thesis involved demonstration that removal of the gonads from embryos of either sex prior to the onset of phenotypic differentiation results in the development of a female phenotype. Thus, the male is the induced phenotype in that testicular secretions cause formation of the male urogenital tract whereas female differentiation is not dependent on the presence of an ovary.

Jost also deduced that two secretions from the fetal testis are essential for male development—Müllerian-inhibiting substance and androgen (*1*). Müllerian-inhibiting substance is an incompletely characterized protein hormone that acts in the male to cause regression of the Müllerian ducts. Müllerian duct regression commences shortly after differentiation of the spermatogenic tubules, and consequently the formation of the inhibiting substance constitutes the first endocrine function of the embryonic testis. The inhibiting substance is a glycoprotein of about 70,000 molecular weight and is formed by the spermatogenic tubules (*9–11*). The mechanism by which the substance acts is uncertain. However, the concept that Müllerian regression is an active process is supported by the existence of a human genetic disease (the persistent Müllerian duct syndrome) in which genetic and phenotypic men have fallopian tubes and a uterus together with male Wolffian duct structures (*12*). The disorder is inherited as a recessive trait, either autosomal or X-linked. The nature of the underlying defect is believed to be either a failure to produce the Müllerian-inhibiting substance or an inability of the tissue to respond to the hormone. A striking feature of the persistent Müllerian duct syndrome is failure of the initial phase of testicular descent—namely, transabdominal movement of the testis. This suggests that Müllerian-inhibiting substance plays a crucial role in the movement of the testis into the scrotum.

Jost deduced that the second developmental hormone of the fetal testis was a steroid. The principal steroid hormone formed by the testis in postnatal life is testosterone (Fig. 3). Testosterone is also the androgen (male hormone) formed in the fetal testis; in the rabbit and human embryo the fetal testis begins to synthesize testosterone shortly after the onset of histological differentiation of the tissue and at the same time that the Leydig cells of the testis appear (*13, 14*). Testosterone has at least two functions in the embryo. It has a local function in the testis where it promotes maturation of the spermatogenic tubules, and it is secreted into the fetal circulation where it plays an essential role in development of the male genital tract.

The essential role of testosterone in virilization of the male urogenital tract was deduced from embryological and endocrine studies. There is now ample genetic evidence substantiating this concept. In man, five separate genetic defects are known to cause inadequate testosterone synthesis and incomplete virilization of the male embryo during embryogenesis (*5, 15*). Each defect involves an enzyme (or enzyme complex) required for the conversion of cholesterol to testosterone (20,22-desmolase, 3β-hydroxysteroid dehydrogenase, 17-hydroxylase, 17,20-desmolase, or 17β-hydroxysteroid dehydrogenase). In each of these disorders the virilization of the male urogenital tract is incomplete; the degree of abnormality varies, depending

on the severity of the enzymatic deficiency. Some affected males develop as phenotypic women with complete failure of virilization of the Wolffian ducts, urogenital sinus, and external genitalia. At the other extreme affected men appear normal except for incomplete development of the penis in which the urethral orifice does not reach the end of the glans penis, creating a condition known as hypospadias. The fact that no fallopian tubes or uterus are present in such men indicates that regression of the Müllerian duct takes place normally during embryogenesis and that this testicular function is independent of testosterone biosynthesis.

What Turns on Testosterone Synthesis in the Fetal Testis?

To provide insight into the regulation of testosterone synthesis in the fetal testis, we used the rabbit embryo as an experimental model. The onset of virilization of the male genital tract in the rabbit embryo occurs between days 17 and 18 (approximately a day after the histological differentiation of the testis). During a 12-hour period between days 17 and 17.5 of gestation, testosterone synthesis begins. This synthesis is made possible by the appearance of 3β-hydroxysteroid dehydrogenase; in contrast, testosterone formation in the fetal ovary remains low (*16-18*) (Fig. 4A). Thus, the programming of testosterone formation is perfectly timed to supply the hormone for virilization of the male genital tract. Unexpectedly, however, we also found that the ovary begins to form potent estrogenic (female) hormones at exactly the same time as the onset of testosterone synthesis in the testis, indeed before histological differentiation of the ovary has been recognized (Fig. 4B). The onset of characteristic endocrine function also develops simultaneously in the testes and ovaries of the human embryo between 6 and 8 weeks of gestation (*19*). Thus, testes and ovaries acquire the capacity to secrete their characteristic hormones at the same time in embryonic development; in the male this coincides with the histological development of the Leydig cells and immediately precedes the onset of virilization of the genital tract.

The genetic determinants that cause the indifferent gonad to develop into an ovary or a testis influence the rates of only a limited number of reactions in the pathway of steroid hormone synthesis. The enzymatic profile that underlies the onset of the characteristic endocrine

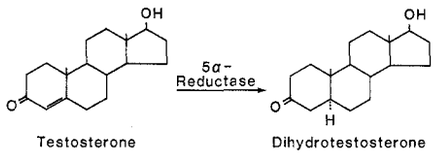

Fig. 3. The two androgenic hormones responsible for virilization of the male. Testosterone, the major androgen secreted by the testis, is converted in target tissues to dihydrotestosterone, which is the intracellular effector for some actions of the hormone.

function in the ovary and testis of the rabbit is depicted in Fig. 5. On day 18 of gestation (at a time when histological differentiation of the testis is advanced) only two differences in the steroid hormone-synthesizing machinery can be detected between ovary and testis. First, there is approximately 50 times as much 3β-hydroxysteroid dehydrogenase in testis as in ovary; this enzyme is rate-limiting in testosterone synthesis at this stage of development in the rabbit, and thus more testosterone is synthesized in the testis than in the ovary. Second, the fetal ovary has the capacity to convert the small amount of testosterone synthesized in the tissue into estradiol whereas the testis does not. All other enzymes in the pathway of steroid hormone synthesis from cholesterol are equal in ovary and testis at this time. Thus, differences in the rates of only a few enzymatic reactions in the gonads at a critical time in development can have profound consequences for the further differentiation of the individual. The actual rate-limiting enzyme in testosterone synthesis may be different in other species.

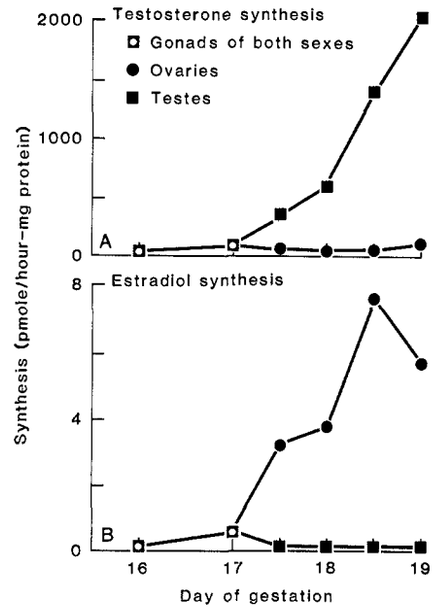

Fig. 4. Onset of endocrine function in the fetal testis and ovary of the rabbit embryo. Each gonad begins to synthesize its characteristic hormone at approximately the same time, beginning on day 17.5 (*17*).

The question as to whether the rates of formation of steroid hormones are themselves regulated at the onset by other hormones is not resolved. In the postnatal state (and late in embryogenesis) peptide hormones (gonadotropins) derived from the pituitary or placenta regulate the rates of estradiol and testosterone formation in the ovary and testis, primarily by increasing the side-chain cleavage of cholesterol to pregnenolone, thereby providing precursors for the formation of the hormones. In the rabbit the anterior pituitary differentiates at about the same time as the onset of testosterone synthesis in the fetal testis, suggesting the possibility that the pituitary may control testosterone synthesis (*20*). Alternatively, in other species, including the human, in which the placenta produces large amounts of gonadotropic hormones, testosterone production could be initiated by placental gonadotropins. A receptor for gonadotropin is demonstrable in rabbit testis at the time of Leydig cell development and the onset of testosterone synthesis (*16*). From its first appearance in the Leydig cell membrane, this receptor is functionally coupled to the side-chain cleavage process by which cholesterol is converted to pregnenolone (*21*).

Nevertheless, two types of experiments suggest that the onset of testosterone synthesis in the rabbit may be independent of the pituitary or other hormonal control. First, the endocrine differentiation of the gonads occurs in organ culture. That is, testes and ovaries of day 16 rabbit embryos synthesize testosterone or estradiol at the appropriate time when cultured for 2 to 4 days in synthetic media devoid of hormones (*22*). Second, gonadotropin does not appear to be required for testosterone formation in the fetal rabbit testis until late in embryogenesis when pregnenolone formation becomes rate-limiting in the enzymatic sequence of testosterone biosynthesis (*21*). These findings suggest that the differentiation of the gonads as endocrine organs is controlled by factors intrinsic to the gonads themselves. If this interpretation is correct, the onset of steroid hormone synthesis in the embryonic ovary and testis is independent or autonomous. As stated above, late in embryogenesis testosterone production in all species is gonadotropin-dependent, and consequently the late testosterone-mediated events in male development, such as growth of the male genitalia, are modulated indirectly by hormones from the

pituitary or placenta. The mechanisms by which the gonad is converted from an autonomous endocrine tissue to a gonadotropin-dependent tissue are not understood.

Mechanisms by Which Androgens Virilize the Male Embryo

The current concepts of how androgens exert their cellular actions within target tissues in postnatal life are summarized schematically in Fig. 6. Testosterone is the androgen secreted by the testis and the major androgen in plasma. Testosterone enters target tissues by a passive diffusion process. Inside the cell testosterone can be converted to dihydrotestosterone (Fig. 3) by the 5α-reductase enzyme. Either testosterone or dihydrotestosterone is then bound to the same high-affinity androgen receptor protein in the cytosol. The hormone-receptor complexes (*TR* and *DR* in Fig. 6) move from cytosol to the nucleus. Inside the nucleus the steroid-receptor complexes interact with acceptor sites on the chromosomes. The character of the acceptor sites within the nucleus (that is, whether protein or DNA) and their number are not resolved. The overall result of the nuclear interaction of the hormone-receptor complexes is to increase transcription of specific structural genes with the subsequent appearance of new messenger RNA and new proteins in the cytoplasm of the cell. The reason that testosterone mediates some androgen effects and dihydrotestosterone mediates others is not clear. It may involve some subtle difference in the affinity of the receptor for the androgen or some aspect of the metabolism of testosterone and its local concentration in the genital tract.

Because mammalian embryos are so small and there is little tissue available for study, few studies of the mechanism of androgen action have been performed in embryonic tissues. Consequently, the various proteins that participate in the intracellular action of steroid hormones in the embryonic tissues of the male urogenital tract have not been characterized directly. However, from studies in animals and humans with single gene mutations that cause resistance to androgen action and cause partial or complete failure of male development, it has been deduced that androgens act in the embryo by the same mechanisms as in the postnatal state (*23*). It is also established that the testosterone-receptor complex is responsible for virilization of the Wolffian duct, whereas the dihydrotestosterone-receptor complex induces virilization of the urogenital sinus and external genitalia during embryogenesis (Fig. 6).

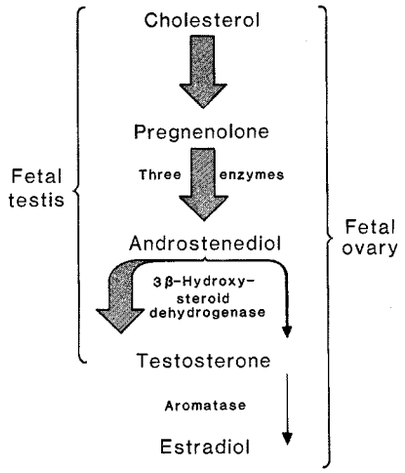

Fig. 5. Enzymatic profile of the pathway of steroid hormone synthesis in the ovary and testis of the rabbit embryo on day 18 of gestation. The complement of enzymes responsible for the conversion of cholesterol to male and female hormones differs only in two regards—the testis has more 3β-hydroxysteroid dehydrogenase than the ovary, and the ovary has the ability to convert testosterone to estrogen whereas the testis does not.

Three types of single gene mutations have been informative in establishing the validity of this scheme. Each type of mutation affects one of the three major processes in the pathway of normal androgen action, namely, the 5α-reductase enzyme, the androgen receptor, or the subsequent phases of androgen action. Each of these defects results in hereditary resistance to androgen action, leading to incomplete virilization during embryogenesis (and in subsequent life) despite the fact that testosterone formation and Müllerian duct regression are normal.

Role of testosterone and dihydrotestosterone. That testosterone is responsible for virilization of the Wolffian ducts and that dihydrotestosterone is responsible for virilization of the external genitalia was postulated on the basis of studies of androgen metabolism in embryos of several species (*14*, *24*). These results were substantiated by genetic studies of human patients with a rare form of abnormal sexual development now known as 5α-reductase deficiency (*25*, *26*). Affected individuals are genetic males with testes; they have predominantly female external genitalia but normal male Wolffian structures (epididymis, vas deferens, seminal vesicles, and ejaculatory ducts) that terminate in the vagina. This phenotype—male Wolffian ducts with female vagina and external genitalia—would be predicted if dihydrotestosterone formation is essential for virilization of the external genitalia but not of the Wolffian ducts. The disorder is due to the homozygous state for an autosomal recessive gene that causes abnormal sexual development only in males. Evidence that this disorder is due to a defect in the conversion of testosterone to dihydrotestosterone was first reported in 1974 (*25*, *26*), and the molecular characteristics of the mutant 5α-reductase enzyme from several families has subsequently been characterized. The enzyme appears to be profoundly deficient in some families, to be formed at a normal rate but with abnormal kinetic properties in others, and to be both deficient and kinetically abnormal in still others (*27*). No phenotypic difference has been identified between patients with abnormal enzyme and those with deficient enzyme activity.

Role of the androgen receptor. Several disorders of the androgen receptor cause abnormal sexual development (Fig. 6). The first to be characterized in molecular terms was the testicular feminization (*Tfm*) mutation in the mouse, an X-linked disorder in which affected males have testes and normal testosterone production but differentiate as phenotypic females with blind-ending vaginas (*28*). No Müllerian duct derivatives can be identified, indicating that the Müllerian regression function of the testis is intact. However, failure of all androgen-mediated aspects of male development in the Wolffian duct, urogenital sinus, and external genitalia occurs because affected animals are profoundly resistant to the action of their own and to exogenous androgen both during embryogenesis and in postnatal life. Dihydrotestosterone formation is normal, but the cytosolic androgen receptor protein (Fig. 6) is undetectable. Consequently, the hormone cannot reach the nucleus of the cell and interact with the chromosomes (*29*). Elucidation of the pathophysiology of this mutation documented the critical role for the androgen receptor in the normal embryonic action of androgen and established that the same receptor protein serves as the mediator of the actions of both testosterone and dihydrotestosterone.

Analysis of the genetic abnormalities of the androgen receptor in the human has provided additional insight into the role of the androgen receptor in embryonic virilization. One such disorder is the human form of testicular feminization. The clinical features are characteristic (*23*). A woman with normal breast development and normal external genitalia seeks medical advice because of a primary failure to menstruate. Axillary, facial, and pubic hair are absent or scanty. The vagina is short and blind-ending and may

be absent or rudimentary. All internal genitalia are absent except for testes, which are located in the abdomen, along the course of the inguinal canal, or in the labia majora. The karyotype is 46,XY; thus, the outwardly normal girl is actually a genetic male. Occasionally, patients come to attention before puberty because the testes in the inguinal canal are associated with femoral hernias.

Keenan and co-workers (*30*) demonstrated that the amount of specific high-affinity androgen receptor is diminished in fibroblasts cultured from the skin of some patients with testicular feminization. Recently, evidence was obtained for another abnormality of the androgen receptor in other patients with the disorder (*31*). In some patients, as in the mouse with testicular feminization, there is no functional receptor, whereas in others there is a qualitatively abnormal receptor. Since patients with the abnormal receptor can have as severe androgen resistance as those who fail to bind androgen it is presumed that the abnormal receptor cannot function within the nucleus. Thus, two different molecular abnormalities of the receptor can lead to testicular feminization in the human, absence or abnormality of the receptor.

Another type of mutation of the human androgen receptor appears to cause a defect in the function of the protein that is not as severe as that in testicular feminization. This disorder, termed the Reifenstein syndrome, also appears to be X-linked. Affected men can manifest a wide spectrum of abnormalities ranging from breast enlargement and absence of sperm production through more severe defects including abnormal development of the penis (hypospadias) and blind-ending vaginas (*32*). The common phenotype is a man with hypospadias, no sperm production, and breast enlargement. Levels of the androgen receptor in fibroblasts cultured from patients with the disorder are about 50 percent of normal (*23*). The partial (and variable) virilization that occurs in this disorder is probably mediated by the residual androgen receptor that may be deficient in amount or structure. The most subtle manifestation of this quantitative defect in the androgen receptor is infertility due to absence or profound deficiency of sperm in otherwise normal men (*23*); this disorder may prove to be the most common abnormality of the androgen receptor.

Thus, two broad classes of mutations can be identified for the diseases involving the 5α-reductase and the androgen receptor: one in which function has been abolished (enzyme activity or androgen binding is profoundly deficient) and another class in which enzymatic activity or binding is detectable but the function is qualitatively abnormal. This latter class of mutations is of particular importance since they imply that the identified abnormalities are in fact the primary result of alterations in the genes encoding these two molecules, thus ascribing an essential role to the 5α-reductase and the androgen receptor in normal male development.

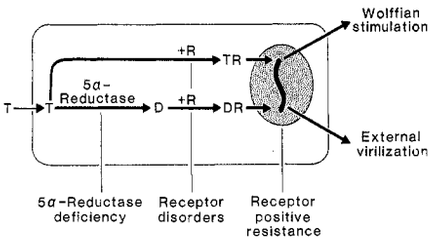

Fig. 6. Mechanism by which androgens act to virilize the male embryo. Abbreviations: *T*, testosterone; *D*, dihydrotestosterone; *R*, high-affinity androgen receptor protein; *TR* and *DR*, hormone-receptor complexes. Three types of mutations in this pathway have been particularly useful in proving the application of this model to the events in the male embryo, namely those that result in abnormalities in the 5α-reductase enzyme, disorders of the androgen receptor, and so-called receptor positive resistance.

Presumptive nuclear processing of the androgen receptor. A third category of androgen resistance was delineated by Amrhein and his colleagues (*33*) in a family with a phenotype similar to that in testicular feminization in which affected individuals had normal levels of 5α-reductase enzyme, normal amounts of androgen receptor, and normal nuclear localization of dihydrotestosterone in cultured fibroblasts (*33*). The patients described to date are 46,XY males with bilateral testes and normal male production of testosterone. The site of the molecular abnormality in these patients (Fig. 6) and the genetic nature of the underlying mutations are uncertain. Either subtle qualitative abnormalities of the receptor, such as inability to attach to the high-affinity acceptor sites within the nucleus, are present, or the defect may involve the intranuclear processing of the hormone-receptor complex. The latter possibility would imply the role of one or more additional proteins essential for the normal nuclear events in androgen action.

It should be emphasized that female embryos have the same androgen receptor system and the same ability to respond to androgens as male embryos. The difference between men and women resides only in the nature of the hormones that are produced. If a female embryo is exposed to androgens it will virilize like the male. Such androgen exposure can come from androgen ingestion by the mother, from maternal tumors, or from overproduction within the fetus. The most common cause of female virilization in the human is congenital adrenal hyperplasia due to an autosomal recessive mutation that results in a defect in the 21-hydroxylation reaction (*15*). In this disorder the synthesis of adrenal steroid (cortisol) is deficient, and late in gestation there is a compensatory increase in the synthesis of adrenal androgen, which produces virilization of the genitalia in affected females. Likewise, the administration of androgens to pregnant rats and mice results in female offspring with both male and female urogenital tracts (*34*).

Possible Role of Estrogens in Embryonic Development

In contrast to the situation in regard to testosterone synthesis and action, in which many single gene mutations have been characterized, to date no mutations have been identified that result in either deficient estrogen synthesis or resistance to estrogen action. Estrogen synthesis is activated temporarily in both male and female embryos before they become implanted in the wall of the uterus, and this estrogen may be essential for implantation and survival of the embryo (*35*). This implies that estrogen action is essential to life itself. If mutations prevent either the synthesis or response to estrogens they may block the implantation of the embryo and thus be lethal. In contrast, androgen action is not necessary for survival of the embryo.

Later in embryogenesis estradiol formation is initiated in the embryonic ovary before definitive histological differentiation of the ovary occurs, and it is possible that the histological differentiation of the tissue may be mediated in part by a local action of estradiol. Whether estrogen plays any other role in the development of either sex is uncertain. Embryogenesis normally takes place in a sea of hormones (steroid and nonsteroid) derived from the placenta, the maternal circulation, the fetal adrenal gland, the fetal testis, and possibly from the fetal ovary itself. It is not known whether these substances influence female phenotypic development, but it is possible that ovarian hormones are involved in the growth and maturation of the internal genitalia of the female during the latter phases of embryonic life, even if they are not required for their differentiation.

Discussion

The fundamental validity of the Jost formulation for sexual development is now amply documented. Chromosomal sex determines gonadal sex, and the gonads in turn determine the development of the sexual phenotypes through their function as endocrine organs. A striking feature of this model is its overall simplicity. As the result of a difference in the rate of one or a few enzymatic reactions in the gonads at a critical time in development, testosterone synthesis is activated in the fetal testis. This hormone in turn has profound developmental effects in the male that account in large part for the differences between men and women.

In at least two regards the Jost model must now be amplified. First, it is clear from the analysis of single gene mutations that genetic sex is more complex than can be explained by the constitution of the sex chromosomes alone. In the human at least one X-linked gene is essential for testicular development, and autosomal genes necessary for differentiation of the ovaries and testes have been identified in several species [for a review, see (*2, 3, 5*)]. Some of these genes influence migration of the primordial germ cells, others regulate the processing or function of differentiative antigens necessary for gonadal development, some code for the enzymes required for steroid hormone synthesis, and the function of the remainder is unknown. The important point is that gonadal development is determined by genes located on the autosomes as well as on the X and Y chromosomes.

Second, the process by which male phenotypic sex develops depends on the expression of several gene products necessary for androgen action. Thus, sexual development involves a series of sequential interactions between the genetic machinery and regulatory factors. We now have considerable insight into the nature of the various genes involved in this process. This is because aberrations at any stage of sexual development, whether due to environmental causes, chromosomal abnormalities, or single gene mutations, are expressed as a characteristic defect in sexual development. Investigation of the pathogenesis of such defects in man and animals has provided insight into the endocrine, molecular, and genetic determinants that regulate the normal process.

The analysis of the single gene mutations that produce abnormal sexual development in man and animals has been particularly informative in this regard. A minimum of 19 genes has been implicated in human sexual differentiation (*5, 36*). It should be emphasized that the process by which male development is imposed on the indifferent embryo requires the participation of many normal genes common to both the male and female embryo. The involvement of such a large number of genes does not imply a greater complexity for sexual differentiation than for other developmental processes but rather reflects the comparative ease with which mutant genes affecting the normal process of sexual development can be identified. Normal sexual development is essential to the survival of species but not to the life of individuals. In contrast, developmental defects in organ systems essential to life frequently cause abnormalities that result in abortion or early death. Therefore, individuals with even the most serious abnormalities of sexual development survive, usually come to the attention of physicians, and have been the subject of many detailed pathophysiological studies.

Some fundamental issues in the embryonic development of the genital tract are still poorly understood. One relates to the mechanism by which specific tissues develop the capacity early in embryogenesis to respond later in development to a hormonal stimulus. As the result of careful studies in the embryo, Cunha and his colleagues (*37*) have established that the capacity for tissues of the urogenital tract to respond to androgen is acquired early in embryogenesis; the connective tissue (mesenchyme) of the embryonic urogenital tract contains the receptor mechanism that regulates the epithelial response to androgen, namely the development and proliferation of the prostatic buds. How the appropriate signal is transferred from connective tissue to epithelium is unclear, and it is not known how connective tissue of the urogenital tract acquires this capacity. Another problem relates to the precise mechanisms by which the same hormonal signal is translated into different physiological effects in different tissues. For example, the diverse effects of androgen during embryogenesis include regression of the mammary duct (in some species), budding and proliferation in the urogenital sinus and Wolffian duct, fusion of cells in the urethral fold, and differential growth of the entire male genital tract late in embryogenesis. At present we have no insight into the mechanisms by which these apparently different functions are accomplished. Thus, these questions of embryogenesis will have to be resolved before it becomes possible to understand the entire program by which the myriad of genetic determinants and regulatory factors interact to cause the development of phenotypic sex.

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