Development of the Reproductive Organs

BERNARD GONDOS, M.D.

Department of Pathology,
University of Connecticut,
Farmington, CT 06032

ABSTRACT

Understanding of the development of the reproductive organs is essential to the evaluation of abnormalities in sexual differentiation. Recent advances resulting from application of genetic, biochemical, and ultrastructural techniques have helped to clarify the mechanisms regulating gonadal and reproductive tract development. The present review considers the major processes of sexual differentiation, development of the female reproductive system and development of the male reproductive system with emphasis on current understanding of basic regulatory mechanisms involved in normal and abnormal development of the reproductive organs.

Introduction

The reproductive organs in each sex consist of gonads, an internal duct system, and external genitalia. During development this basic organ system is adapted to the different functional needs of the two sexes. Development of the reproductive organs involves complex interaction of genetic, biochemical, morphologic, and hormonal factors. This intricate coordination leads to the establishment of structural and functional adaptations required for gamete maturation, sex hormone secretion, and reproduction.

The reproductive system is the principal site of divergent differentiation in the two sexes, resulting in the formation of separate male and female reproductive structures and distinctive types and patterns of hormonal activity. Early in development, however, there is an indifferent stage in which the gonadal anlagen and other structural primordia appear identical in male and female embryos. The process of sexual differentiation is the initial event imposed on these primitive structures. Subsequently, the male and female systems develop along separate pathways, but clearcut homologies remain as a result of their common origin.

Understanding of the development of the reproductive organs requires consideration of three main processes: (1) establishment of sexual differentiation; (2) development of the female reproductive system; and (3) development of the male reproductive system. The purpose of the present report is to provide an overview of current knowledge in these areas. Particular emphasis is given to recent advances in understanding of major regulatory aspects.
Sexual Differentiation

The development of the reproductive organs proceeds from an indifferent stage in which gonadal sex is not yet determined and the internal duct systems of mesonephric (Wolffian) and paramesonephric (Müllerian) origin are both present (table I). Genetically, however, the sex of the embryo is determined at the time of fertilization, depending on the presence or absence of a Y chromosome.

Sex Chromosome Expression

The precise mechanism of sex chromosome expression is not clear. Under normal circumstances, there is direct correlation of the presence of a Y chromosome and male phenotypic differentiation, as has been shown by procedures for Y chromosome fluorescence. However, analysis of various types of clinical disorders has demonstrated that genes essential for normal male development are also located on the X chromosome and genes involved in the development of both male and female phenotypes are found on autosomes.40

Recently it has been proposed that H-Y antigen, a histocompatibility antigen linked to a gene on the Y chromosome, may be the key sex-determining factor.38 In various types of intersex conditions, the presence of H-Y antigen has correlated more closely with testicular differentiation than the presence of a Y chromosome suggesting an inductive effect on the indifferent gonad. However, there have been sufficient problems in reproducibly characterizing this association in certain conditions to cast some doubt on its significance.15

A finding that has been well established is that sex chromatin bodies representing inactivated X chromosomes are present in all individuals with more than one such structure.24 The inactivation occurs early in embryonic development prior to the establishment of gonadal sex differentiation. Identification of sex chromatin bodies is useful both in determining normal genotypic sex and in characterizing various types of abnormalities. However, structural abnormalities of the X chromosome may escape detection by this method and therefore karyotype analysis is needed to indicate structural features.

Further understanding of the site and action of sex-determining genes will depend on refinements in genetic, biochemical and immunologic techniques.32 At present, the variety of abnormalities in sex differentiation related to structural and functional chromosome defects only underscores the need for additional information to clarify the mechanisms involved in sex chromosome expression.

Gonadal Differentiation

The genital ridges arise as thickenings on the medial aspects of the coelomic cavity adjacent to the mesonephros. At four to five weeks gestational age, gonadal outgrowths composed of coelomic epithelium and underlying mesenchyme gradually develop projecting into the future peritoneal cavity. Initially, the gonadal primordia are composed only of epithelial and mesenchymal cells of mesodermal origin. Soon, primitive germ cells, large spherical to ovoid cells,
begin to appear in the gonad. These cells are morphologically characteristic and histochemically distinctive because of their high alkaline phosphatase and glycogen content. The germ cells have been shown to originate extragonadally in the wall of the yolk sac from where they migrate to the developing gonads.\(^4^3\)

The gonads of the two sexes remain morphologically indistinguishable until six weeks of age, when the first evidence of testicular differentiation is seen. Aggregates of germ cells and somatic cells bounded by a basal lamina, representing the primitive spermatogenic cords, begin to form. In addition, a distinct tunica albuginea appears beneath the coelomic (surface) epithelium. The presumptive ovary shows no specific changes at this time and retains an undifferentiated appearance until the onset of meiosis at the end of the first trimester.

The mechanisms responsible for gonadal sex differentiation are unknown. Since it is characteristic of mammalian species that testicular differentiation precedes that of the ovary, a specific inductive factor in the male would appear likely. However, the identity of such a factor, or factors, remains elusive.

**Differentiation of Reproductive Tracts**

Two sets of paired ducts, the Wolffian and Müllerian ducts, are responsible for giving rise to major portions of the male and female reproductive tracts. In the male, the Wolffian ducts undergo extensive development to form the internal genital structures while the Müllerian ducts regress. In the female, only portions of the mesonephric or Wolffian duct system persist while the Müllerian ducts undergo profound developmental changes to give rise to principal reproductive structures.

Initially, the mesonephric ducts appear as elongated structures in the nephrogenic cord extending caudally to the cloaca. Connections are established between the cranial portions of the ducts and the gonadal outgrowths through mesonephric tubules. The latter join with cords of cells from the gonads comprising the rete system.

The Müllerian ducts arise as invaginations of the coelomic epithelium at five weeks gestational age. They extend caudally lateral and parallel to the mesonephric ducts. At their caudal ends, they cross the mesonephric ducts ventrally to fuse in the urogenital septum. The fused portion eventually comes in contact with the urogenital sinus to form the Müllerian tubercle.

The external genitalia take their origin from protuberances of the cloacal membrane in association with the urogenital sinus. The genital tubercle is located anteriorly flanked on either side by the genital swellings. At about five weeks of age, a urethral plate develops from the cloacal epithelium in association with the developing phallic tubercle and subsequently develops an opening, the urethral groove. The latter forms in two stages, the deeper part being endodermal in origin and the superficial part communicating with the urogenital orifice of ectodermal origin. Up to 10 to 11 weeks, the external genitalia in the two sexes are essentially similar.

**Female Reproductive System**

Major events affecting the function of the female reproductive organs occur during fetal development. These include differentiation and growth of the ovaries, formation of the tubal and uterine structures from the Müllerian ducts, establishment of the cervicovaginal canal by contributions from the Müllerian ducts, and urogenital sinus and formation of the female external genitalia. These events are generally paralleled by corresponding changes in the male reproductive system. However, a notable difference is
that oogenesis is initiated early in fetal development, while male germ cells do not enter spermatogenesis until the time of puberty. The early maturation of the ovarian germ cells indicates the importance of changes occurring in the fetal ovary for later reproductive function.

**Ovarian Development**

The basic structural and functional unit of the adult ovary is the follicle, consisting of an oocyte surrounded by granulosa and theca cells. During early development, referred to as the prefollicular stage, the ovarian cortex is characterized by germ cells and granulosa cells arranged in cords and sheets without specific organization. This arrangement persists until mid-gestation when follicle formation begins.

The first distinctive change in the fetal ovary is the onset of meiosis, which occurs at 11 to 12 weeks gestation. This is preceded by a period of differentiation of primitive germ cells into groups of actively dividing cells, the oogonia. The mitotic divisions of oogonia are associated with incomplete separation at telophase, leaving the daughter cells connected by intercellular bridges. This pattern recurs in subsequent divisions, so that groups of germ cells remain interconnected and, therefore, behave in a synchronous manner.

Following a series of mitotic divisions, there is progressive entry into meiosis by the cell groups beginning in the innermost cortex and gradually extending to the periphery. The cells, now designated as oocytes, pass through the various stages of the first meiotic prophase and by late gestation all of the oocytes have advanced to the diplotene stage. Differentiation is then arrested and does not resume until the time of ovulation.

An important unresolved problem in reproductive biology concerns the mechanisms responsible for the initiation of meiosis early in ovarian development, while this process is inhibited in the developing testis. Recently, it has been demonstrated that a meiosis-inducing substance (MIS) and a meiosis-preventing substance (MPS) produced by cells derived from mesonephric structures adjacent to the gonad are involved in regulating ovarian and testicular germ cell differentiation. The balance between these two substances varies in the two sexes at different stages of development with MIS predominating in the fetal ovary and MPS in the fetal testis. Further work on factors involved in meiotic regulation represents an area of active current investigation.

Although significant changes affecting germ cell differentiation occur in the fetal ovary, hormonal activity appears to be limited during this time. According to the early studies of Jost, ovarian hormone production is not required for the differentiation of the female reproductive tract while in contrast endocrine activity in the fetal testis plays a major role in organizing the differentiation of the male reproductive system. This concept has been generally borne out by ultrastructural studies which have shown no specific changes in fetal granulosa cells associated with steroid hormone secretion, such as are seen in fetal Leydig cells. Furthermore, theca cells, which play an essential role in steroid synthesis in the adult ovary, do not appear until late in gestation and retain a relatively undifferentiated appearance during the developmental period.

Nevertheless, some lines of evidence suggest a possible role for steroid hormones in early differentiation. Interstitial cells with characteristic ultrastructural features of steroid-secreting cells are present in the fetal ovary during the prefollicular period. Biochemical studies have shown that human fetal ovarian tissue is capable of converting testosterone to estradiol at this time. Moreover,
acquisition of the capacity for key enzymatic conversions involved in steroidogenesis takes place at the same time in the fetal ovary and testis. Ovarian steroidogenic activity, however, remains at a relatively low level during fetal development, and a specific role for estrogens has not been defined.

In regard to possible effects of fetal gonadotropins, pituitary gonadotropin production begins as early as 10 weeks with peak levels occurring at mid-gestation. It is of interest that pituitary follicle-stimulating hormone (FSH) is higher in female than in male fetuses at mid-gestation. However, while gonadotropins have a major influence on follicular development in the adult ovary, evidence for a similar function in the fetus is lacking.

Once follicle formation begins at 18 to 20 weeks gestation, the process continues throughout the remainder of fetal development. By the late fetal and early neonatal period all of the surviving oocytes have become surrounded by adjacent granulosa cells, and oocyte and follicle growth are well established. Many oocytes undergo degeneration prior to incorporation in follicles. Nevertheless, a pool of several hundred thousand follicles is present in the newborn ovary.

Follicle growth takes place throughout infancy and childhood. During this time, a gradual rise in estrogen and gonadotropin levels occurs. There is increasing asymmetry in the two ovaries which are filled with follicles of varying size, including preantral and antral follicles. However, it is only at the time of puberty that the cyclic changes involving ovulation and luteinization are initiated. The precise mechanisms responsible for the initiation of these major events remain to be determined, but recent studies suggest that changing levels of estradiol and luteinizing hormone (LH) are of critical importance.

**Uterotubal Structures**

The tubes and uterus are derived from the paired Müllerian ducts. Each duct is composed of a cranial longitudinal portion, an intermediate transverse portion, and a caudal longitudinal portion. The first two become the tubes while the fused caudal segments give rise to the uterovaginal canal. The latter contributes to the vagina as well as the uterus, but the vaginal epithelium is principally of extramüllerian origin.

The uterine portion of the uterovaginal canal becomes separated from the vaginal portion at mid-gestation. A slight curvature develops at the junction between the future cervical and fundal parts of the uterus at this time with a second more pronounced curvature between the cervix and vagina. The endometrial and endocervical linings derive from the Müllerian duct epithelium, while the stromal and muscular layers are formed from the mesenchyme surrounding the ducts. The basic structure of the uterus is established early in development. However, the cervical portion is larger than the body throughout fetal development and the fundus to cervix proportion characteristic of the adult appears only after the early postnatal period.

Stimulation of Müllerian duct development has not been shown to be under hormonal control. However, clinical evidence of uterine anomalies after prenatal exposure to diethylstilbestrol (DES) and experimental studies in animals treated with DES or estradiol during early development suggest that estrogens may be involved. An interesting observation in this regard is that differentiation of the uterine epithelium is controlled by the associated mesenchyme. Therefore, hormonal effects on early uterine development would have to be mediated through the Müllerian duct mesenchyme.

The proliferation of the Müllerian
ducts is accompanied by a corresponding degeneration of the mesonephric ducts. The mesonephric tubules associated with the cranial portion of the mesonephric duct generally remain to form connections with the rete ovarii. These structures persist throughout adult life as the epoophoron. The caudal part of the duct may also persist as Gartner’s duct.

**Cervix and Vagina**

While the vagina and ectocervix (portio vaginalis) develop from the Müllerian duct system as the caudal portion of the uterovaginal canal, their epithelial lining is derived in large part from the endodermal urogenital sinus and the ectodermal cloaca. Since the Müllerian ducts are of mesodermal origin, it is evident that all three germ layers are involved in cervicovaginal differentiation. Recent reviews on the development of the vagina indicate that there is still not general agreement regarding the relative contributions of these different sources.25,40

During the early development of the uterovaginal canal, a cord-like separation, the vaginal plate, forms between its lower portion and the cranial portion of the urogenital sinus. At this time, the developing vagina has a cuboidal lining. Subsequently, after fusion between the two structures occurs, stratified squamous epithelium from the urogenital sinus extends upward to line the vagina and ectocervix. This process begins in the late first and early second trimester but is not completed until late in fetal development. It is during this period that changes in maternal hormone exposure, such as DES administration, may be particularly important in affecting epithelial differentiation in the vagina and cervix.31

**External Genitalia**

After its establishment, the urogenital sinus is divided into an upper pelvic portion and a lower phallic portion related to the base of the genital tubercle and separated from the ectodermal cloaca by the urogenital membrane.

Epithelial buds arise from the primitive urethra and the adjacent pelvic part of the urogenital sinus at about 12 to 14 weeks gestation to give rise, respectively, to the urethral and paraurethral glands of Skene. The two sets of glands together constitute the female homologue of the prostate.

During approximately the same period, the genital tubercle becomes bent caudally and can be recognized as the clitoris. The lateral portions of the genital swellings enlarge to form the labia majora, while the urethral folds flanking the urogenital orifice persist as the labia minora. Bartholin’s glands and the lesser vestibular glands arise from the vestibule into which the vagina and urethra open. The general pattern of differentiation of the external genitalia is well established by mid-gestation.

The extent to which hormonal factors influence these changes is unclear. Information from animal studies indicates that removal of the fetal ovaries does not affect the differentiation of the female genitalia, implying that ovarian hormones are not required.22 However, from clinical observations it is known that high levels of maternal steroid hormones which cross the placental barrier will cause abnormal development of the external genitalia.

**Male Reproductive System**

Since both male and female reproductive systems develop from similar origins, parallel patterns of differentiation are evident. However, there are two
striking differences related to the major functions of gamete production and sex hormone secretion. Development of testicular germ cells is characterized by a delay in maturation so that spermatogenesis is not initiated until the time of puberty, long after the corresponding onset of oogenesis in the ovary. In regard to hormone production, the fetal testis is extremely active, in contrast to the relatively inactive fetal ovary, and secretes large amounts of testosterone which are essential for the formation of the male reproductive system. These differences may explain why most developmental defects of the female reproductive system involve chromosomal abnormalities affecting germ cell differentiation, while defects in male reproductive tract development are primarily a result of effects on testosterone production and metabolism.

**Testicular Development**

The basic structural unit of the adult testis is the seminiferous tubule containing large numbers of germ cells involved in spermatogenesis and Sertoli cells. The fetal and postnatal period of testicular development prior to the onset of spermatogenesis is referred to as the prespermatogenic stage. Limited numbers of germ cells of relatively undifferentiated type are present throughout this period.

Initially, groups of primitive germ cells and differentiating Sertoli cells become surrounded by a basal lamina within the fetal testis. An early plate-like arrangement of the cellular aggregates is demonstrated by stereological techniques such as scanning electron microscopy. At this time the germ cells, referred to as gonocytes, are randomly distributed among the more numerous Sertoli cells. Subsequently the plates are transformed into cell cords, the seminiferous cords, and the gonocytes begin to move to the periphery where they become situated along the basal lamina. These cells, the prespermatogonia, are now located in the position of spermatogenic stem cells, i.e. spermatogonia, but they still retain the general ultrastructural appearance of precursor cells. Animal studies have confirmed that, although large numbers of early germ cells degenerate, the surviving fetal gonocytes and prespermatogonia are the source of the definitive germ cells.

The onset of spermatogenesis at puberty begins after the differentiation of prespermatogonia into spermatogonia, which then undergo a series of mitotic divisions. The latter are characterized by incomplete cytokinesis, as in the oogonia of the ovary, resulting in the formation of interconnected rows of germ cells. The groups of spermatogonia differentiate into spermatocytes, which undergo two successive meiotic divisions, followed by the formation of spermatids and spermatozoa. Important events associated with the initial spermatogenic cycle are the development of a lumen within the seminiferous tubules and the formation of a blood-testis barrier by occlusive Sertoli cell junctions.

The stimulus for the initiation of spermatogenesis is not definitely known, but several lines of evidence point to testosterone as the key factor. For example, in precocious puberty associated with elevated testosterone levels, testicular biopsy characteristically shows premature spermatogenesis. Normally, the initiation of spermatogenesis is associated with a rise in testosterone from prepubertal baseline levels. It is likely that this is a result of maturation of the hypothalamic-pituitary axis and specifically changes in LH activity in response to gonadotropin releasing hormone stimulation.
Endocrine activity in the developing testis has been carefully studied, and it is now clear that there are two main types of hormones produced by the fetal testis: testosterone, which is required for differentiation of Wolffian duct structures and development of the external genitalia; and a nonsteroidal hormone, known as Müllerian inhibiting substance or anti-Müllerian hormone (AMH), which is responsible for regression of the Müllerian ducts (table II).

Secretion of anti-Müllerian hormone by the fetal testis is a relatively recently described phenomenon. This finding has helped to clarify the regression of the Müllerian ducts in the male fetus as well as certain related clinical abnormalities. Anti-Müllerian hormone has been found to be a glycoprotein produced by the fetal Sertoli cells beginning shortly after testicular differentiation. A monoclonal antibody to AMH is now available enabling further studies in this area. Such studies may help to explain the mechanisms by which the Sertoli cells become active in producing this substance so early in development.

Testosterone production by the fetal testis is associated with the differentiation of Leydig cells in the testicular interstitium. Leydig cells begin to appear at eight weeks gestation, are particularly prominent during the third and fourth months, and then undergo involutional changes at mid-gestation. A corresponding pattern of testosterone synthesis by the testis is evident, with activity first noted at eight weeks, peak levels at 12 to 13 weeks and a steady decline after 17 weeks. Relatively low levels of testosterone are produced in the second half of gestation and postnatally up to the time of puberty, except for a transient rise in the neonatal period.

During the latter part of gestation, the testis completes its descent into the scrotum. At approximately the seventh month, the processus vaginalis grows rapidly and the inguinal canal increases in diameter. Descent occurs as a result of degeneration of the portion of the gubernaculum in contact with the epididymis and testis allowing both to move through the dilated inguinal canal into the scrotum. Numerous studies on experimental cryptorchidism in animals and observations on clinical material have been evaluated in an attempt to define the precise functions of androgens and gonadotropins in testicular descent, but a clear picture is yet to emerge and consequently there is still considerable controversy on this subject.

**INTERNAL GENITAL STRUCTURES**

Establishment of male internal reproductive structures involves the regression of the Müllerian ducts and differentiation of the Wolffian duct system.

The first of these events, corresponding to the early secretion of AMH, is the regression of the paramesonephric ducts. Beginning at seven to eight weeks, the ducts undergo degenerative changes and lose their communications with the coelomic cavity. The cranial portion of each duct persists to form the appendix testis, but the remainder disappears entirely with the possible exception of contributions from the caudal end to the prostatic utricle.

The Wolffian ducts begin to grow and differentiate at nine to 10 weeks. The cranial part of each duct becomes con-

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<td><strong>Fetal Testicular Hormones</strong></td>
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<td><strong>Testosterone</strong></td>
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<td>Peak secretion during 3rd and 4th months</td>
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<td>Stimulates Wolffian duct differentiation</td>
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<td><strong>Anti-Müllerian hormone</strong></td>
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connected to the testicular cords through some of the mesonephric tubules, which give rise to the efferent ductules, and the rete testis. The adjacent portion of the duct undergoes extensive lengthening and convolution to form the epididymis. Later the distal segment develops a thick muscular layer and becomes the ductus or vas deferens. Close to the junction with the urogenital sinus, a glandular diverticulum subsequently arises to form the seminal vesicle. The part of each mesonephric duct between the seminal vesicle and the urethra becomes the ejaculatory duct.

The primary role of testosterone in mediating these events is now well established on the basis of experimental studies and clinical observations. The latter include cases of defective testosterone synthesis and abnormalities in androgen receptor function. In either instance, failure of normal differentiation of Wolffian duct structures occurs, indicating the dependence of male reproductive tract formation on testosterone action.

**External Genitalia**

As in the female, the external genitalia in the male arise in relation to the phallic portion of the definitive urogenital sinus. The prostate gland also develops in conjunction with the urogenital sinus including contributions from the primitive urethra and the pelvic portion of the urogenital sinus.

Mesenchymal influences have been shown to have an important inductive effect on the differentiation of the prostatic epithelium. Endodermal buds arise from the urethra at 11 to 12 weeks gestation and extend into the surrounding mesenchyme which differentiates into the fibromuscular component of the gland. The epithelial buds which grow out from the urethra are arranged into five groups, anterior, middle, dorsal, and two lateral, in accordance with the major lobes of the adult prostate.

At the end of the third month, the genital tubercle becomes elongated and is transformed into the cylindrical phallus. The genital swellings develop into the scrotal folds which become rounded, migrate caudally and fuse below the base of the penis. The definitive urethral groove extends forward on the ventral aspect of the phallus so that the lining of the penile urethra is derived principally from the endodermal urogenital sinus. Fusion of the urethral folds results in the formation of the perineal raphe.

Subsequently, there is a proliferation of ectodermal surface epithelium into the glans penis to meet the distal extremity of the urethral plate. The prepuce starts developing at about the same time from structures related to the urethral folds and the bulbourethral glands arise from a portion of the penile urethra as endodermal derivatives. Connective tissue surrounding the urethra becomes condensed to form the corpus cavernosum in which numerous convoluted blood vessels with arteriovenous anastomoses arise.

Development of the prostate and external genitalia from the urogenital sinus has been shown by Wilson and colleagues to be under the direct influence of dihydrotestosterone. Thus, while testosterone produced by the fetal testis is responsible for the differentiation of the epididymis, vas deferens and seminal vesicles from the Wolffian ducts, it is the reduced form of the hormone, DHT, which controls the differentiation of structures derived from the urogenital sinus, genital tubercle and genital swellings. This has been demonstrated both in animal experiments and in clinical studies on several pedigrees of families with 5α-reductase deficiency. Development of the male reproductive tract is evidently influenced by a number of gene-regulated enzymatic conversions in
the steroidogenic pathway related to testosterone and dihydrotestosterone formation. Specific genetic defects have been identified at several different levels in this process to account for various forms of abnormal male reproductive tract differentiation.9

Summary

Development of the male and female reproductive systems involves complex interactions at various levels leading to the formation of functioning gonads, reproductive ducts and external genitalia. Fundamental anatomic and histologic aspects of reproductive tract differentiation have been well established, but it is only in recent years with improvements in genetic, biochemical and morphologic techniques that significant progress in understanding basic regulatory mechanisms has occurred. Investigations utilizing these modern methods have enabled clarification of the normal process of sexual differentiation and of various developmental abnormalities.

At the present time, a considerable amount of information is available on structural and hormonal aspects of reproductive tract differentiation. Much of this new information is relevant to the diagnosis of different clinical disorders, and it is therefore important that laboratory scientists be aware of recent developments. Further advances useful in the evaluation of reproductive tract abnormalities are likely to be forthcoming in the future as a result of ongoing studies on reproductive tract differentiation and associated abnormalities.

References

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