Arch. Biol. Med. Exp. 22: 1-13 (1989)
Printed in Chile

Disorders of gonadal development in humans

Desórdenes del desarrollo gonadal en humanos

JOE LEIGH SIMPSON

Department of Obstetrics and Gynecology, Health Science Center, University of Tennessee, Memphis, TN38103, USA.

In humans, genes controlling sex differentiation must be determined by deductive reasoning. In the future the molecular basis of genetic sex determinants should become evident, permitting new experimental approaches. However, at present such investigation is still not possible. In humans we must survey the disorders of sex differentiation in order to elicit clues into the control of normal sex reproduction.

In this communication we shall summarize current knowledge concerning genetic control of human sex differentiation. We shall focus upon selected disorders that allow localization of gonadal determinants to specific regions on the X chromosome, on the Y chromosome, and on autosomes. We shall emphasize disorders of complete or nearly complete gonadal failure. Discussed elsewhere are several related conditions, namely Klinefelter syndrome, male pseudohermaphroditism, and female pseudohermaphroditism (Simpson, 1976, 1982, 1985a).

I
Y CHROMOSOME AND TESTICULAR DIFFERENTIATION

The Y chromosome has long been known to direct testicular differentiation. In the past 10-15 years, analysis of individuals with structural abnormalities of the Y has localized the Y-testicular determinant(s) to a specific region.

A. *Localization of Y-Testicular Determinants*

Several 46,X,i(Yq) individuals have been reported (See Davis, 1981). All are female in appearance and all have bilateral streak gonads. Such cases permit the deduction that testicular determinants are located on the Y short arm (Simpson, 1976). Coupled with knowledge that males with ring or small metacentric Y chromosomes have testes (German, Simpson, McLeMore, 1973), the Y-testicular determinant(s) can be localized near the centromere on Yp (Fig. 1).

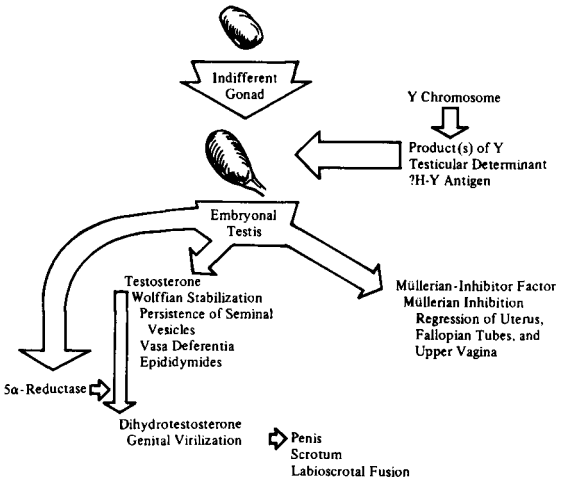

Fig. 1: Human testicular determinants are located near the centromere in the non-fluorescent region. From Simpson (1976).

We shall repeatedly observe in this communication that an intact Y does not assure normal testicular differentiation. A variety of other loci must remain intact. At this point one particular set of observations should be noted. In mice, Washburn and Eicher (1983) have shown that a Y chromosome capable of directing testes differentiation in some strains will fail to do so when the Y is transferred to certain other mouse strains. The latter strains possess a dominant mutant gene on chromosome No. 17. In those strains with the mutant No. 17, only the Y specific for that

strain can direct normal testicular differentiation. We can conclude that autosomal loci are integral for testicular development, that the effect of the Y-testicular determinants is subject to regulation, and that the Y-testicular determinants do not necessarily act identically within a given species.

B. *Evidence for H-Y Antigen Being Integral for Testicular Differentiation*

The manner by which the testicular determinant(s) acts is not totally understood, but the consensus is that a cell surface antigen, H-Y antigen, is integrally involved in testicular differentiation (Wachtel, 1983). Both circumstantial as well as direct evidence suggests that H-Y antigen can direct testicular differentiation. The following circumstantial data, referenced elsewhere by Wachtel (1983), are consistent with this hypothesis: 1) H-Y is evolutionarily conservative, in all species being present in that sex containing the heterogametic sex chromosome (Y or W); 2) one locus essential for H-Y expression is near or identical to the locus for the testicular determinant(s); 3) H-Y is present in XY humans and mice with androgen insensitivity, indicating that H-Y is not merely induced by androgens, 4) H-Y continues to be expressed after H-Y cells are transferred to a female (XX) host; 5) H-Y is present in approximately 50% of mouse blastocysts, a stage prior to organ differentiation and, hence, prior to testicular differentiation; 6) sex-reversed (46,XX) males and true hermaphrodites have H-Y antigen; and 7) H-Y antigen is present in the testicular but not the ovarian portion of ovotestes. In summary, H-Y is in general considered present in individuals with testes, but not in those lacking testes.

Direct evidence also exists. Reaggregation of neonatal rodent testes disassociated by Moscona-type disruption produces tubular-like (male) structures (Ohno *et al.*, 1978, Zenzes *et al.*, 1978a). However, H-Y antisera causes testicular cells to reaggregate into follicle-like (female) fashion. Moreover, H-Y antigen recovered from cultured Sertoli cells directs disassociated ovarian tissue into tubular-like structures (Zenzes *et al.*, 1978b).

C. *H-Y is Not the Gene Product of the Y-Testicular Determinant*

Even if H-Y is integral for testicular differentiation, the phenomenon cannot be ascribed solely to H-Y antigen. Not only shall we see that most females with XY gonadal dysgenesis (Section IV) are H-Y positive (Wolf, 1979, Wolf *et al.*, 1980a), but 45,X and 46,X,i(Xq) individuals are H-Y positive (Wolf *et al.*, 1980b; Wolf, 1981) as well. Conversely, presence of ovaries in an XY phenotypic female who had an additional band on Yp has been observed (Bernstein *et al.*, 1980). A potential explanation is that the additional band suppressed expression of H-Y.

It is thus clear that the Y-testicular determinant cannot correspond to the structural locus for H-Y, for 45,X individuals obviously lack a Y. To be sure, H-Y titers in 45,X or 46,X,i(Xq) may be reduced. However, this merely suggests a threshold below which H-Y cannot direct the indifferent gonad into a testis. If H-Y indeed directs testicular differentiation, it follows that the Y-testicular determinant must act in regulatory fashion.

If not located on the Y, the H-Y structural locus must be either autosomal or X-linked. Its precise location remains uncertain, but several observations are noteworthy. *First,* the X contains at least one locus that when mutant (i.e., XY gonadal dysgenesis) precludes testicular differentiation. It would be logical to wonder whether this mutant involves the locus whose wild type ordinarily directs male development. However, most cases of XY gonadal dysgenesis are H-Y positive, indicating that the mechanism of gene action need not involve an abnormality of H-Y synthesis. (H-Y receptors could be absent or abnormal). Irrespective, if H-Y is the key agent in testicular differentiation, the XY gonadal dysgenesis locus and the H-Y structural locus cannot be one and the same.

One unifying scheme assumes an autosomal location for the H-Y structural locus. Perhaps the Y contains a regulatory locus capable of suppressing second locus on Xp. The Xp locus would otherwise

suppress the structural autosomal locus for H-Y. Alternatively, the H-Y structural locus could be on the X. Interestingly, we have shown that there may exist not only an X-linked, H-Y antigen positive form of XY gonadal dysgenesis but potentially also an autosomal recessive form (i.e., genetic heterogeneity) (Simpson *et al.*, 1981). If the latter were H-Y antigen negative, the mutant might correspond to the structural locus for H-Y.

D. *Evidence that H-Y is Not Integral for Testicular Differentiation*

McLaren and co-workers (1984) and Kiel-Metzger *et al.* (1985) do not believe that H-Y is pivotal to male differentiation. The former group not only found H-Y expression in some phenotypic female mice carrying the Sxr (Sex-reversal) (X/X^{Sxr}) mutant (E. Simpson *et al.*, 1984), but male (testes) differentiation in X/X^{Sxr} mice lacking H-Y (McLaren *et al.*, 1984). For this reason, various investigators have turned to the possibility that certain Y-specific DNA sequences are pivotal. Of course, DNA sequences would be expected to control the Y-testicular determinant necessary for H-Y expression. However, the implication of "Y-specific sequences" in the present context is that such sequences bear no direct or indirect relationship to H-Y.

Using conventional molecular methods, a host of DNA clones derived from the human Y have been isolated. Of 26 such clones isolated by Bishop *et al.* (1984b), 9 contained no homologous sequences elsewhere. The other clones showed homology to autosomes or to the X chromosome. In fact, X-Y homology is now well accepted (Page *et al.*, 1983, 1984), and considered the basis for X-Y pairing during meiosis (Bishop *et al.*, 1984b). Moreover, in 1973 the author and colleagues (German *et al.*, 1973) proposed that statural determinants existed on Xp and Yp, their pairing the basis of X-Y association. Exchange of X and Y segments may lead to perturbations of sex determinants, XX males and XX true hermaphrodites representing clinical consequences of such an exchange (Sections VI and VII).

At any rate, identification of Y-specific clones led to the logical hyphothesis that one of these DNA sequences might well direct testicular differentiation. Unfortunately, Y-specific and many other probes usually signify only repetitive DNA; unique sequence DNA would more logically be pivotal. Nonetheless, intriguing information exists. Experience with one particular clone illustrates well the dilemma.

A DNA sequence termed *Bkm* (banded krait minor satellite DNA) is highly conserved. It is present in heterogametic (female) snakes and heterogametic (male) mice. The same sequence is present in sex-reversed XX (Sxr) mice. For these reasons, one would expect *Bkm* to be a good candidate for the testicular determinant. However, *Bkm* hybridizes not to human Y chromosome DNA, but rather to the X and to No. 6. (In the mouse, *Bkm* hybridizes both to the Y and to No. 17, as reported by Kiel-Metzger and Erickson, 1984). The ostensible conclusion is that the *Bkm* sequences are not on the Y, and thus no longer a candidate for the primary Y-testicular determinant. On the other hand, it is possible that *Bkm* in humans has too few copies (single copy) to permit hybridization, whereas in mice multiple copies readily display hybridization. Alternatively, *Bkm* sequences could still be pivotal to testicular determination even though located on an autosome. Recall those studies showing that a locus on murine No. 17 alters expression of the Y (Washburn and Eicher, 1983). Interestingly, the mouse 17 is probably homologous to the human 6, both chromosomes containing the MHC locus.

E. *Conclusion Concerning Control of Testicular Differentiation*

The Y chromosome obviously remains pivotal to male differentiation, but little else is absolutely certain. I personally still favor an integral role for H-Y, but contradictory data of McLaren *et al.* (1984) deserve attention. That the Y contains a regulatory but not a structural locus seems probable, but not assured. A

Y-regulatory role must exist if *H-Y* or *Bkm* is essential, but not necessarily if another mechanism or DNA sequence is essential.

Irrespective, after having differentiated from the indifferent gonad, the developing testes secrete two hormones (Fig. 2). First, fetal Leydig cells produce an androgen, probably testosterone. This hormone stabilizes the Wolffian ducts to permit differentiation of vasa deferentia, epididymides and seminal vesicles. After conversion by 5 α-reductase to dihydrotestosterone, the external genitalia are virilized. These actions can be mimicked by the administration of testosterone to female or castrated male embryos. This is demonstrated clinically by existence of teratogenic forms of female pseudohermaphroditism. Second, fetal Sertoli cells produce a second hormone, a non-steroid hormone of high molecular weight that diffuses locally to cause regression of Mullerian derivatives (uterus and Fallopian tubes). The action of this hormone cannot be duplicated by any known compound. In the absence of the hormones described above, the external genitalia develop along female lines; the Mullerian ducts develop into a uterus and Fallopian tubes; and the Wolffian ducts regress. Again, these changes occur in 46,XX embryos and in castrated 46,XY embryos.

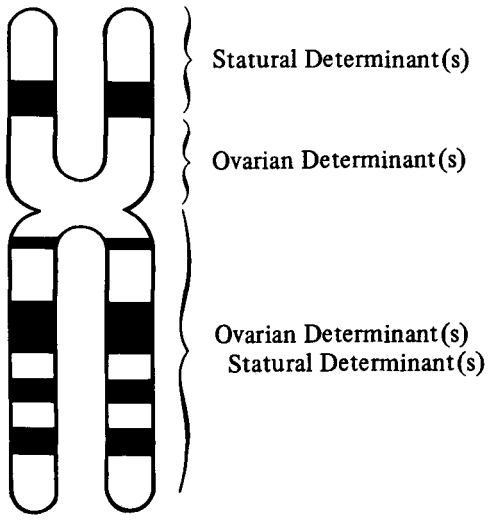

Fig. 2: Probable location of the X chromosome of determinants necessary for ovarian-maintenance and for sature. From Simpson and LeBeau (1981).

II
X CHROMOSOME AND OVARIAN DEVELOPMENT

In the absence of a Y chromosome, or more specifically in the absence of H-Y antigen, the indifferent gonad develops into an ovary. This occurs in either 46,XX or 45,X embryos. If two intact X chromosomes are not present, most ovarian follicles degenerate by the time of birth.

We shall now review the various complements associated with ovarian failure. Comparing phenotypes associated with specific X abnormalities will not only show the basis for the above statements, but allow us to determine locations of determinants necessary for oocyte maintenance. By way of definition, this author applies the term "gonadal dysgenesis" to any individual with streak gonads. The term "Turner stigmata" is reserved for individuals with short stature and certain other somatic anomalies (Simpson, 1976).

The complement most frequently associated with gonadal dysgenesis is 45,X. Approximately 50% of all patients with gonadal dysgenesis have a 45,X complement; 25% have sex chromosomal mosaicism without a structural abnormality (e. g., 45,X/46,XX); the remainder have a structurally abnormal X or Y or, more often, no detectable chromosomal abnormality.

A. *45,X*

1. *Embryonic Lethality and its Cellular Explanation*

Approximately 50% of spontaneous abortions occurring during the first three months of gestation are associated with a chromosomal abnormality; 20% of chromosomally abnormal abortuses are 45,X (Boué *et al.*, 1978). Of all pregnancies, 12-15% terminate in first trimester spontaneous abortions; thus, 1-2% of all conceptions must be 45,X. Because the incidence of 45,X at birth is only about one per 10,000 females, over 99% of all 45,X embryos must be aborted. Intrauterine growth retardation is characteristic of the rare surviving 45,X neonate.

The high embryonic lethality, intrauterine growth retardation, and postnatal short stature could all be explained by a prolonged 45,X cell cycle. Our group believes that cell cycle time (CGT) is prolonged in 45,X. In 1981 we showed that CGT was 22-24 hrs in adult 45,X fibroblasts, compared to 18-19 hrs in adult 46,XX fibroblasts (Simpson & LeBeau, 1981).

More recently, we have shown that 45,X cells are at a comparative disadvantage compared to 46,XX cells (Verp *et al.*, 1985). In artificial 45,X/46,XX mixtures, the frequency of the normal diploid line increases at the expense of the monosomic line. Prolonged CGT in 45,X could offer an attractive pathogenic explanation for several related phenomena-embryonic lethality, low birth weight, short stature. Even somatic anomalies could be explained because decreased cell number is ultimately the mechanism of teratogenesis.

2. *Gonadal Development and Somatic Anomalies*

In the small percentage of 45,X individuals who survive until adulthood, the normal gonad is usually replaced by a white fibrous streak, 2-3 cm long and about 0.5 cm wide. The streak is located in the position ordinarily occupied by the ovary. This streak gonad is characterized histologically by interlacing waves of dense fibrous stroma, devoid of oocytes but otherwise indistinguishable from normal ovarian stroma. Absence of oocytes in monosomy X is the result of increased oocyte atresia, not failure of germ cell formation. Indeed, 45,X embryos and 45,X neonates have germ cell (Jirasek, 1976). Inasmuch as germ cells are present in 45,X embryos, it is not too surprising that occasional (3-5%) 45,X individuals menstruate spontaneously. Indeed, fertile 45,X individuals have been reported (See Simpson, 1981).

Secondary sexual development does not, however, usually occur in 45,X individuals. Estrogen and androgen levels are decreased; FSH and LH are increased. Pubic and axillary hair fail to develop in normal quantity. Although well differentiated, external genitalia, vagina, and Mullerian derivatives (uterus) remain small. 45,X individuals are usually short (mean height 141 cm) and show certain somatic anomalies. The most common anomalies are epicanthal folds, high-arched palate, low nuchal hair line, webbed neck, shield chest, coarctation of aorta, ventricular septal defect, renal anomalies, pigmented nevi, lymphedema, nail hypoplasia, and cubitus valgus. No feature is pathognomonic, but in aggregate they form a spectrum of anomalies more likely to occur in 45,X individuals than in other individuals.

Because X chromosomes in excess of one are inactivated (Lyon hypothesis), it is actually less obvious than one might suspect that 45,X individuals should manifest developmental abnormalities. Indeed, relatively normal ovarian development occurs in 39,X mice, as well as in most other monosomy X mammals. There are data supporting several related explanations for these findings: 1) Most plausible is the hypothesis that some loci on the human heterochromatic (inactive) X are not truly inactivated, apparently unlike the situation in mice. For example, the locus for steroid sulfatase, which is located on Xp21 → pter, is, not inactivated (Mohandes *et al.*, 1979). It would not be surprising if ovarian determinants likewise escaped X-inactivation. 2) Moreover, X-inactivation never occurs in human oocytes (Gartler *et al.*, 1972). Females heterozygous for G6PD synthesize both alleles in oocytes. 3) Theoretically, X-inactivation might occur only after some crucial time of indifferentiation, beyond which only a single euchromatic (active) X is necessary for continued oogenesis. 4) Finally, all or part of the heterochromatic X could become *re*activated.

Finally, the parental origin of 45,X is of genetic interest. Monosomy X (45,X) may originate during oogenesis, during spermatogenesis, or after fertilization. In humans 70% of liveborn 45,X individuals have lost a *paternal* sex chromosome (Sanger *et al.*, 1977). In neither 45,X abortuses nor liveborns is the mean maternal age increased (Kajii & Ohama,

1979), an observation consistent with paternal origin for monosomy X. Murine monosomy (39,X) also results from loss of a paternal sex chromosome (Russell, 1962) at the time of fertilization.

B. *45,X/46,XX and Related Mosaicisms*

45,X/46,XX individuals have fewer anomalies than 45,X individuals. In one survey by the author, 12% of 45,X/46,XX individuals menstruated, compared to only 3% of 45,X individuals (Simpson, 1975). The mean adult height is greater in 45,X/46,XX (146 cm) than in 45,X individuals (142 cm). More mosaic (25%) than non-mosaic (5%) patients reach adult heights greater than 152 cm. Somatic anomalies are less likely to occur in 45,X/46,XX than in 45,X patients.

More recently, we are questioning whether even the 12% menstruation frequency is not underestimated. All 45,X/46,XX cases ascertained in our unit, which is based in a maternity hospital, have menstruated (Simpson *et al.*, 1986). We suspect that biases of ascertainment are influencing the perception of the phenotype, with prognosis for 45,X/46,XX better than initially suspected.

C. *Deletion of the X Short Arm [del (Xp)]*

A deletion of the X short arm may or may not cause gonadal dysgenesis, short stature and other features of the Turner stigmata. The phenotype depends upon the amount of Xp that is deficient.

Spontaneous menstruation, albeit usually leading to secondary amenorrhea, has occurred in almost 40% of reported 46,X, del(X) (p11) individual (Fig. 3). Almost all 46,X,(del(X) (p21) individuals menstruate (Fraccaro *et al.*, 1977). Elsewhere I provide complete references (Simpson, 1985a). These data not only indicate that ovarian tissue persists more often in del(Xp) individuals than in 45,X individuals, but also that ovarian failure occurs only if both the proximal and terminal portions of Xp are deleted. On the other hand, the mean adult height is 140 cm in 46,X,

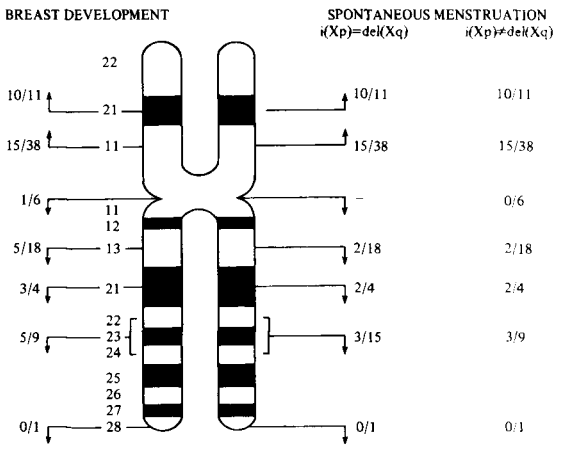

Fig. 3: Frequencies of spontaneous menstruation and breast development in X-deletions. Two tabulations are prepared, depending upon whether 46,X,i(Xp) truly exists or whether reported cases are actually 46,X,del(X) (q24). Modified from Simpson and LeBeau (1981).

del(X) (p11) and 146 cm in 46,X,del(X) (p21 or 22) (Simpson & LeBeau, 1981). Inasmuch as 46,X,del(x) (p21) individuals are short yet show normal ovarian function, ovarian determinants and statural determinants must be located in different regions of Xp; statural determinants are more distal (Fig. 4). My conclusions are similar in this respect to those of others (Goldman *et al.*, 1982).

Analogous to the situation with respect to Y-specific DNA probes, there is as yet no evidence that any given X-specific probe bears special relationship to X-ovarian or X-structural determinants.

D. *Isochromosome for the X Long Arm [i(Xq)]*

Almost all 46,X,i(Xq) patients have streak gonads, short stature, and features of the Turner stigmata. In addition to having a duplication of Xq, i(Xq) subjects differ from del(X) (p11) subjects because not only the terminal portion but almost all of Xp is deleted. This difference could indicate that gonadal determinants are present at several different locations on Xp. A locus on Xp might be deleted in (Xq), yet retained near the centromere in 46,X,del(X) (p11). Duplication of Xq (i.e. 46,X,i(Xq)) does not compensate for deficiency of Xp; thus, gonadal deter-

minants on Xq and Xp must have different functions. Whether duplication of Xq *per se* produces abnormalities is unknown.

E. *Deletion of the X Long Arm [del(Xq)]*

Many patients with a deletion of the X long arm never menstruate, having streak gonads (Fig. 3). That X autosomal translocations involving the region Xq13 → 26 usually are associated with sterility (Mattei *et al.*, 1981; Madan, 1983) indicates either that important ovarian determinants exist in this region or that the entire region must remain unperturbed ("critical") for ovarian function. Since menstruation and breast development have on occasion been observed in individuals with del(Xq) or X/autosome translocation involving Xq, I believe that the critical region hypothesis needs revision. Perhaps several loci can affect ovarian function in additive fashion. If so, their precise location remains uncertain because the presence or absence of gonadal function cannot be correlated readily with the amount of Xq that is absent.

It originally appeared that deletion of Xq did not result in short stature (Ferguson-Smith *et al.*, 1964; Simpson, 1975). Our more recent tabulations show decreased mean height (152 cm) in del(X) (q22) and (q24) (Simpson & LeBeau, 1981). These data suggest the existence of a statural determinant of Xq (Fig. 4).

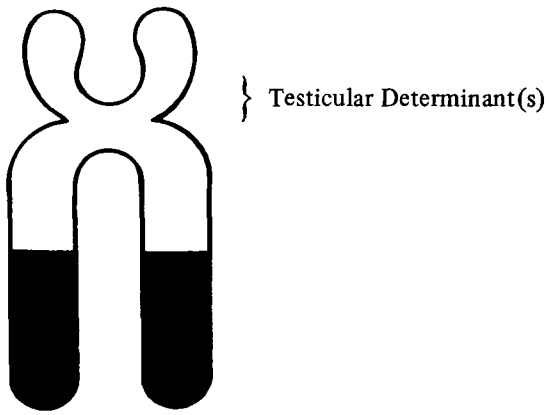

Fig. 4: Schematic diagram illustrating male sex differentiation. From Simpson (1980).

F. *Other X Abnormalities*

Centric fragments and ring [r(X)] chromosomes are often mitotically unstable; thus, phenotypic-karyotypic correlations are hazardous because monosomic lines are frequently associated. However, some individuals with ring (X) chromosomes menstruate and show normal breast development. This further confirms that not all gonadal determinants are telomeric, as postulated with Figure 4.

III
AUTOSOMAL GENES AND OVARIAN DEVELOPMENT

In addition to X-determinants, it can be deduced that autosomal genes are also pivotal for ovarian development.

A. *Gonadal Dysgenesis in 46,XX Individuals*

Decourt *et al.* (1960) reported the first individual with gonadal dysgenesis and an apparently normal female (46,XX) complement. By 1971 Simpson *et al.* (1971a) accumulated 61 individuals with XX gonadal dysgenesis and concluded that the disorder was inherited in autosomal recessive fashion.

External genitalia and streak gonads in XX gonadal dysgenesis are indistinguishable from those in gonadal dysgenesis due to a sex chromosomal abnormality. Likewise, the endocrine profiles do not differ. However, individuals with XX gonadal dysgenesis are usually normal in stature. Several pathogenic mechanisms can be postulated, but firm data have not been gathered. Phenocopies for XX gonadal dysgenesis are also well recognized-autoimmune disease, irradiation, hemorrhage, and mumps.

Both XX gonadal dysgenesis and neurosensory deafness have occurred in multiple sibs in several families (Pallister & Opitz, 1979); occurrence of deaf but fertile male sibs confirms autosomal inheritance. The coexistence of gonadal and auditory anomalies probably indicates a syndrome distinct from XX gonadal dysgenesis without deafness (i.e., genetic heterogeneity).

Indeed, further evidence for genetic heterogeneity can be cited. In at least four other families (see Simpson, 1985a), unique patterns of somatic anomalies indicate the existence of mutant genes distinct from those already discussed.

B. *Polycystic Ovarian Disease*

Polycystic ovarian disease is a relatively common disorder characterized by obesity, oligomenorrhea, and hirsutism. Physicians appreciate the varied traits shown by affected individuals, who often require ovulation inducing agents to achieve pregnancy. The disorder is characterized by increased LF/FSH ratio, elevated androstenedione and increased peripheral conversion of androstenedione to estrone. Heritable tendencies clearly exist, but their nature is uncertain. Autosomal dominant or possibly X-linked dominant inheritance has been proposed (Cohen *et al.*, 1975). A single gene may not exist, but heritable tendencies probably indicating autosomal loci are clear (Simpson, 1985b).

IV
AUTOSOMAL AND X-LINKED GENES NECESSARY FOR TESTICULAR DEVELOPMENT

Analogous to genes integral to ovarian development, we can deduce that genes other than those on the Y are integral to testicular development. In Section I we have already seen that normal testicular differentiation might well even be directed by an X-linked or autosomal locus. In fact, if H-Y is integral, it is essential that such a locus exists. A few illustrative disorders will suffice to show the necessity for genes beyond those on the Y.

A. *Gonadal Dysgenesis in 46,XY Individuals*

Gonadal dysgenesis can occur in 46,XY individuals. In XY gonadal dysgenesis, affected individuals are phenotypic females who show sexual infantilism and bilateral streak gonads. The gonads may undergo neoplastic transformation (20-30% prevalence) (Simpson & Photopulos, 1976). It is well accepted that at least one form of XY gonadal dysgenesis results from an X-linked recessive or male-limited autosomal dominant gene (Stenberg *et al.*, 1968; Simpson *et al.*, 1981). An autosomal recessive form may exist as well (Simpson *et al.*, 1981).

H-Y antigen studies in affected individuals were awaited with great expectation, especially after it became known that an analogous disorder in Swedish wood lemmings (*Myopus schistocolor*) was the result of an X-linked mutant that apparently suppressed H-Y antigen (Wachtel *et al.*, 1976). Unexpectedly, some humans with XY gonadal dysgenesis showed H-Y antigen, whereas others did not (Wolf, 1981). Neoplasia appears to occur more commonly in H-Y positive forms (Wolf, 1981; Mann *et al.*, 1983). In addition to showing genetic heterogeneity, these data raise the possibility that embryonic gonads in XY gonadal dysgenesis may sometimes be testes and sometimes ovaries. Histologic support for this hypothesis also exists. In particular, oocytes were detected in a human neonate with XY gonadal dysgenesis (Cussen & MacMahon, 1979). Perhaps such cases will all prove H-Y negative and at low risk for neoplasia.

Further evidence for genetic heterogeneity lies in existence of the syndrome of H-Y-negative XY gonadal dysgenesis and long-limbed campomelic dwarfism (Bricarelli *et al.*, 1981). Again, gonads may even resemble ovaries (sex reversal) (Schimke, 1979). In addition, Brosnan *et al.* (1980), reported a distinctive syndrome in which 46,XY "female" sibs displayed streak gonads, unusual facies, cardiac anomalies, renal anomalies, ectodermal abnormalities like scalp defects, and mental retardation. XY gonadal dysgenesis has been associated with yet other multiple malformation patterns, namely one characterized by facial asymmetry, prominent forehead, cleft palate, ventricular septal defect, and skeletal abnormalities.

B. *Agonadia*

In agonadia 46,XY individuals show absent or abnormal external genitalia and ru-

dimentary Mullerian or Wolffian derivatives. External genitalia usually consist of 1) a phallus about the size of a clitoris, 2) nearly complete fusion of the labioscrotal folds, and 3) often a persistent urogenital sinus. By definition, gonads cannot be detected (agonadia). Likewise, neither normal Mullerian derivatives nor normal Wolffian derivatives are present; however, structures resembling a rudimentary Fallopian tube, an epioophoron, or an epididymis may be detected along the lateral pelvic wall. Somatic anomalies are common-craniofacial anomalies, vertebral anomalies, and mental retardation.

Any explanation for agonadia must explain not only the absence of gonads, but also abnormal external genitalia and lack of normal internal ducts. At least two explanations seem reasonable: 1) fetal testes functioned sufficiently long to inhibit Mullerian development, yet not sufficiently long to complete male differentiation, or 2) the entire gonadal, ductal and genital systems developed abnormally, as result of either defective anlage, defective connective tissue, or action of a teratogen. The frequent coexistence of somatic anomalies supports existence of either a teratogen or defective connective tissue. Affected sibs have been reported; thus, a genetic etiology should be considered, possibly one affecting connective tissue. H-Y antigen is present (Schulte, 1979), suggesting that pathogenesis does not involve an abnormality of this system.

C. *Leydig Cell Agenesis*

A few 46,XY patients have been reported to have complete absence of Leydig cells, precluding embryonic virilization (Berthezene *et al.*, 1976). They showed normal female external genitalia or posterior labial fusion, no uterus and bilateral testes devoid of Leydig cells. Lack of a uterus distinguishes these patients from XY gonadal dysgenesis. Presence of testes excludes agonadia. Lack of breast development excludes complete androgen insensitivity (testicular feminization).

D. *Syndrome of Rudimentary Testes*

Bergada *et al.* (1962) reported four unrelated males who, despite well-formed testes less than 1 cm in greatest diameter, had small penises. Their testes consisted of small tubules containing Sertoli cells, a few Leydig cells, and an occasional spermatogonium. Wolffian derivatives were present; Mullerian derivatives were absent. The pathogenesis is unclear. However, testes were presumably normal during early embryogenesis, only later decreasing in size. Najjar *et al.* (1974) reported five affected sibs.

E. *Anorchia*

Males with anorchia have unambiguous male external genitalia, normal Wolffian derivatives, no Mullerian derivatives, and no detectable testes. Vasa deferentia terminate blindly. Unilateral anorchia is not extraordinarily rare, but bilateral anorchia is. Even in bilateral anorchia the penis is well-differentiated. Paghogenesis presumably involves atrophy of fetal testes after occurrence of genital virilization during weeks 8-11 of embryonic development.

Heritable tendencies exist, although the occurrence of monozygotic twins discordant for anorchia suggests that genetic factors are not paramount in all cases (Simpson *et al.*, 1971b).

F. *Mutant Genes Affecting Spermatogenesis and Spermiogenesis*

In plants and lower animals many aspects of meiosis are known to be under genetic control. Especially well documented is the existence of genes affecting spermiogenesis in bulls. Similar genus surely exist in humans, and their mutation should deleteriously affect reproduction. Indeed, in some families genes interfering with male meiosis lead to infertility (Chaganti *et al.*, 1980). Carson *et al.* (1983) studied a male whose spermatozoa showed a 4C DNA count; two sibs were similarly affected, indicating autosomal recessive inheritance. Additional evidence for meiotic mutants causing human infertility will doubtless be uncovered.

V.
KLINEFELTER SYNDROME

Reviewed elsewhere by the author in detail (Simpson, 1976), these well-known disorders are characterized by seminiferous tubule dysgenesis. 47,XXY individuals always show this trait, and its associated sterility and hypoandrogenism. Somatic anomalies may or may not be present. However, 48,XXXY and 49,XXXXY individuals inevitably show not only sterility but also mental retardation and other somatic anomalies.

These disorders make it clear that testicular development is adversely affected in males with more than one X chromosome. The actual pathogenesis is unclear. Probably seminiferous tubule dysgenesis is caused by either: 1) altered cellular milieu secondary to added chromatin, or 2) loci that are not inactivated, therefore causing imbalance. If the latter were responsible, monogenic factors would seem unlikely to be involved. Indeed, the consistency with which seminiferous dysgenesis exists excludes usual genetic hypotheses.

VI
TRUE HERMAPHRODITISM

True hermaphrodites possess both ovarian and testicular tissue. Most true hermaphrodites are 46,XX; however, a minority are 46,XX/46,XY, 46,XX/47,XXYY, 46,XY, or other complements (Van Niekerk & Retief, 1981; Simpson & Sarto, 1978).

Gonads may consist of one ovary and one testis, or more often, one or more ovotestes. In 80% of ovotestes the testicular and ovarian components are juxtaposed end-to-end. One unexplained observation is that a testis or an ovotestis is more likely to be present on the right than the left. Interestingly, in birds the right gonad is known to develop preferentially into a testis. In human true hermaphrodites spermatozoa are rarely present; however, apparently normal oocytes are often present, even in ovotestes. Presumably this difference relates to the intraabdominal position of the testes. A uterus is usually present, albeit often bicornuate or unicornuate.

About two thirds of true hermaphrodites are raised as males, although external genitalia may be ambiguous or predominantly female. Paradoxically, breast development usually occurs at puberty, despite predominantly male external genitalia; virilization usually does not. Most true hermaphrodites with a uterus menstruate, and some 46,XX true hermaphrodites have become pregnant (see Simpson, 1981).

The etiology of true hermaphroditism is heterogeneous. Most 46,XX/46,XY cases probably result from chimerism. However, experimental production of XX/XY mouse chimeras does not always result in true hermaphroditism, nor do all 46,XX/46,XY humans have true hermaphroditism. The presence of testicular tissue in 46,XX true hermaphrodites is perplexing because testicular development has occurred in the ostensible absence of the Y chromosome. Reasonable hypotheses include: 1) translocation of the Y-testicular determinant(s) to an X or to an autosome, 2) undetected mosaicism or chimerism, and 3) sex-reversal genes. The first or possibly the second hypothesis seems most likely in view of H-Y antigen being present in almost all 46,XX true hermaphrodites (Wachtel, 1983). Especially impressive is the presence of H-Y antigen in each of two 46,XX true hermaphrodite sibs (Fraccaro *et al.*, 1979).

VII
46,XX MALES (SEX-REVERSAL)

46,XX (sex-reversed) males are phenotypic males with bilateral testes. Affected individuals have small testes and show signs of androgen deficiency similar to 47,XXY Klinefelter syndrome. The penis and scrotum are small but usually well differentiated; Wolffian derivatives are normal. Seminiferous tubules are decreased in number and in size, Leydig cells are hyperplastic, and spermatogonia are not usually present.

As in 46,XX true hermaphrodites, testes in 46,XX males develop contrary to expect-

ations that a Y chromosome is required for testicular differentiation. Again, 46,XX males show H-Y antigen (Wachtel, 1983); thus, X-Y or Y-autosome translocation seems the likely etiology. This is highly consistent with molecular studies indicating: 1) homology between the X and Y (Bishop *et al.* 1984a), 2) hybridization of Y-specific probes in human XX males (Guellan *et al.*, 1984), 3) paternal origin of one X shown by X-restriction fragment length polymorphisms (Page & De la Chapelle, 1984).

Familial aggregates of either 46,XX males alone, or 46,XX males and 46,XX true hermaphrodites, have been reported. In one well-studied kindred, mothers of affected cousins were H-Y positive (Wachtel *et al.*, 1981). One possible explanation is the existence of a Y-X or Y-autosome translocation involving portions of H-Y genes that are ordinarily present in multiple copies. Unaffected females might transmit a translocated portion of the Y, too small to confer maleness but sufficiently large to behave in recessive sex-reversal fashion if a spouse were heterozygous for a similar mutant (Wachtel *et al.*, 1981). Familial aggregates and data consistent with this hypothesis have been made in XX true hermaphrodite dogs (Selden *et al.*, 1978). In addition, sex-reversed goats (Polled), and mice (Sxr), disorders homologous to human sex-reversal, are generally accepted as showing H-Y antigen (Wachtel, 1983). However, we have already noted (Section I) the claim that male Sxr mice (X/X^{Sxr}) may be H-Y negative (McLaren *et al.*, 1984). Assay vicissitudes could explain this discrepancy.

VIII
CONCLUSION

The data we have considered makes it obvious that genetic control of human sex differentiation is complex, and only beginning to be elucidated. Genes or, more precisely, at least determinants, exist on the X, on the Y, and on autosomes. All must interact appropriately for normal gonadal development. Such observations imply that the mechanisms responsible for sex determination in mammals (i.e., X-Y) and in dipteran species (i.e., X-autosomal balance) may not be so disparate as usually assumed.

Finally, we have not even considered the many different genes that must remain intact for external and internal genital development. Chromosomal factors are not paramount, but both monogenic (mendelian) as well as polygenic factors have been identified readily by ourselves and by others.

REFERENCES

1. BISHOP, C.; GUELLAEN, G.; GELDWORTH, D.; FELLOWS, M.; WEISSENBACH, J. (1948a) Extensive sequence homologies between Y and other human chromosome. *J. Mol. Biol. 173:* 403-417.
2. BISHOP, C.E.; GUELLAEN, G.; GELDWERTH, D.; VOSS, R.; FELLOWS, M.; WEISSENBACH, J. (1984b) Single-copy DNA sequences specific for the human Y chromosome. *Nature 309:* 253-255.
3. BERGADA, C.; CLEVELAND, W.W.; JONES, H.W.; WILKINS, L. (1962) Variants of embryonic testicular dysgenesis: Bilateral anorchia and the syndrome of rudimentary testes. *Acta Endocrinol. Copenhagen 40:* 521-536.
4. BERNSTEIN, R.; JENKINS, T.; DAWSON, B.; WAGNER, J.; DEWALD, G.; KOO, G.C.; WACHTEL, S.S. (1980) Female phenotype and multiple abnormalities in sibs with a Y chromosome and partial X chromosome duplications: H-Y antigen and Xg blood group findings. *J. Med. Genet. 17:* 291-300.
5. BERTHEZENE, F.; FOREST, M.G.; GRIMAUD, J.A.; CLANSTRAT, B.; MORNEX, R. (1976) Leydig cell agenesis: A cause of male pseudohermaphroditism. *N. Engl. J. Med. 295:* 969-972.
6. BOUE, J.; BOUE, A.; LAZAR, P. (1975) Retrospective and prospective epidemiological studies of 1,500 karyotyped spontaneous humans abortions. *Teratology 12:* 11-26.
7. BRICARELLI, F.D.; FRACCARO, M.; LINDSTEN, J.; MULLER, U.; BAGGIO, P.; CARBONE, L.D.L.; HJERPE, A.; LINDGREN, F.; MAYEROVA, A.; RINGERTZ, H.; RITZEN, E.M.; ROVETTA, D.C.; SICCHERO, C.; WOLF, U. (1981) Sex-reversed XY females with campomelic dysplasia are H-Y negative. *Hum. Genet. 57:* 15-22.
8. BROSNAN, P.G.; LEWANDOWSKI, R.C.; TOGURI, A.G.; PAYER, A.F.; MEYER, W.J. (1980) A new familial syndrome of 46,XY gonadal dysgenesis with anomalies of ectodermal and mesodermal structural. *J. Pediatr. 97:* 586-590.
9. CARSON, S.A.; RAO, R. (1983) Mutant gene causing large sperm heads with multiple tails. Abstracts, 30th Annual Meeting, *Society for Gynecologic Investigation,* p. 27.
10. CHAGANTI, R.S.K.; JHANWAR, S.C.; EHRENBARD, L.T.; KOURIDES, I.A.; WILLIAMS, J.J. (1980) Genetically determined asynapsis, spermatogenic degeneration and infertility in men. *Am. J. Hum. Genet. 32:* 833-848.
11. COHEN, P.N.; GIVENS, J.R.; WISER, W.L.; WILROY, R.S.; SUMMITT, R.L.; COLEMAN, S.A.; ANDERSON, R.N. (1975) Polycystic ovarian dis-

ease, maturation arrest of spermatogenesis and Klinefelter's syndrome in siblings of a family with familial hirsutism. *Fertil. Steril. 26:* 1228-1238.
12. CUSSEN, L.J.; MACMAHON, R.A. (1979) Germ cells and ova in dysgenetic gonads of a 46,XY female dizygotic twin. *Arch. J. Dis. Child. 133:* 373-375.
13. DAVIS, R.M. (1981) Localization of male determining factors in men: A thorough review of structural anomalies of the Y chromosome. *J. Med. Genet. 18:* 161-195.
14. DECOURT, J.; MICHAUD, J.P.; DELZANT, G. (1960) Syndrome de Turner avec caryotype feminin normal. *Rev. Fr. Endocrinol. Clin. Nutr. Metab. 1:* 321-331.
15. FERGUSON-SMITH, M.A.; ALEXANDER, D.S.; BOWEN, P.; GOODMAN, R.M.; KAUFMAN, B.N.; JONES, H.W. Jr.; HELLER, R.H. (1964) Clinical and cytogenetical studies in female gonadal dysgenesis and their bearing on the cause of Turner's syndrome. *Cytogenetics 2:* 142-155.
16. FRACCARO, M.; MARASCHIO, P.; PASQUALI, F.; SCAPPATICCI, S. (1977) Women heterozygous for deficiency of the (p21 → pter) region of the X chromosome are fertile. *Hum. Genet. 39:* 283-291.
17. FRACCARO, M.; TIEPOLO, L.; ZUFFARDIO-CHIUMELLO, G.; DINATALE, B.; GARGANTINI, L.; WOLF, U. (1979) Familial XX true hermaphroditism and the H-Y antigen. *Hum. Genet. 48:* 45-52.
18. GARTLER, S.M.; LISKAY, R.M.; CAMPBELL, B.K.; SPARKES, R.; GANT, N. (1972) Evidence of two functional X chromosomes in human ococytes. *Cell. Differ. 1:* 215-218.
19. GERMAN, J.; SIMPSON, J.L.; MCLEMORE, G.A. (1973) Abnormalities of human sex chromosomes. I. A ring Y without mosaicism. *Ann. Genet. 16:* 225-231.
20. GUELLAEN, C.; CASANOVA, M.; BISHOP, C.; GELWERTH, D.; ANDRE, G.; FELLOWS, M.; WEISSENBACH, J. (1984) Human XX males with Y single copy. DNA fragments. *Nature 307:* 172-173.
21. GOLDMAN, B.; POLANI, P.E.; DAKER, M.G.; ANGELL, R.R.. (1982) Clinical and cytogenetic aspects of X-chromosomal deletions. *Clin. Genet. 21:* 36-52.
22. JIRASEK, J. (1976) Principles of reproductive embryology. In *Disorders of Sexual Differentiation: Etiology and Clinical Delineation,* ed. J.L. Simpson. New York: Academic Press, pp. 51-110.
23. KAJII, T.; OHAMA, K. (1979) Inverse maternal age effect in monosomy X. *Hum. Genet. 51:* 147-151.
24. KIEL-METZGER, K.; ERICKSON, R.P. (1984) Regional localization of sex-specific Bkm-related sequences on proximal chromosome 17 of mice. *Nature 310:* 575-581.
25. KIEL-METZGER, K.; WARREN, G.; WILSON, G.; ERICKSON, R.P. (1985) Evidence that the human Y chromosome does not contain clustered DNA sequences (BKM) associated with heterogametic sex determination in other vertebrates. *N. Engl. J. Med. 313:* 242-245.
26. MADAN, K. (1983) Balanced structural changes involving the human X: Effect on sexual phenotype. *Hum. Genet. 63:* 216-221.
27. MANN, J.R.; CORKERY, J.J.; FISHER, H.J.W.; CAMERON, A.H.; MAYEROVA, A.; WOLF, U.; KENNAUGH, A.A.; WOOLLEY, V. (1983) The X linked recessive form of XY gonadal dysgenesis with high incidence of gonadal cell tumors: Clinical and genetic studies. *J. Med. Genet. 20:* 264-270.
28. MATTEI, M.; MATTEI, J.F.; VIDAL, I.; GIRAUD, F. (1981) Structural anomalies of the X chromosome and inactivation center. *Hum. Genet. 56:* 401-408.
29. MOHANDES, T.; SHAPIRO, L.J.; SPARKERS, R.S.; SPARKES, M.C. (1979) Regional assignment of the steroid sulfatase-X-linked ichthyosis locus: Implications for a noninactivated region on the short arm of human X-chromosome. *Proc. Natl. Acad. Sci. USA 76:* 5779-5783.
30. MCLAREN, A.; SIMPSON, E.; TOMONARI, K.; CHANDLER, P.; HOGG, H. (1984) Male sexual differentiation in mice lacking H-Y antigen. *Nature 312:* 552-555.
31. NAJJAR, S.S.; TAKLA, R.J.; NASSAR, V.H. (1974) The syndrome of rudimentary testes: Occurrence in five siblings. *Pediatrics 84:* 119-122.
32. PAGE, D.; WYMAN, A.; BOTSTEIN, D. (1983) Homologous single-copy sequences on the human X and Y chromosomes. In *Recombinant DNA Applications to Human Disease.* Banbury Report Nº 14, eds. C.T. Caskey, R.L. White. Cold Spring Harbor Laboratory, pp. 267-273.
33. PAGE, D.; DE LA CHAPELLE, A. (1984) The parenteral origin of X chromosome in XX males determined using restriction fragment length polymorphisms. *Am. J. Hum. Genet. 35:* 565-575.
34. PALLISTER, P.D.; OPITZ, J.M. (1979) The Perrault syndrome: Autosomal recessive ovarian dysgenesis with facultative, non-sex-limited sensorineural deafness. *Am. J. Med. Genet. 4:* 239-246.
35. RUSSELL, L.B. (1962) Chromosome aberrations in experimental mammals. *Prog. Med. Genet. 2:* 230-294.
36. SANGER, R.; TIPPETT, P.; GAVIN, J.; TEESDALE, P.; DANIELS, G.L. (1977) Xg groups and sex chromosome abnormalities in people of northern European ancestry: An addendum. *J. Med. Genet. 14:* 210-211.
37. SARTO, G.E.; SIMPSON, J.L. (1978) Abnormalities of the Mullerian and Wolffian duct systems. *Birth Defects 14 (6c):* 37-54.
38. SCHIMKE, R.N. (1979) XY sex-reversal campomelia possibly and X-linked disorder? *Clin. Genet. 16:* 62-63.
39. SCHULTE, M.J. (1979) Positive H-Y antigen testing in a case of XY gonadal absence syndrome. *Clin. Genet. 16:* 438-440.
40. SELDEN, J.R.; WACHTEL, S.S.; KOO, G.C.; HASKINS, M.E.; PATTERSON, D.F. (1978) Genetic basis of XX male syndrome and XX true hermaphroditism: Evidence in the dog. *Science 201:* 644-646.
41. SIMPSON, E.; MCLAREN, A.; CHANDLER, P.; TOMONARI, K. (1984) Expression of H-Y antigen by female mice carrying Sxr. *Transplantation 37:* 17-21.
42. SIMPSON, J.L. (1975) Gonadal dysgenesis and abnormalities of the human sex chromosomes: Current status of phenotypic-karyotypic correlations. *Birth Defects 11 (4):* 113-159.
43. SIMPSON, J.L. (1976) *Disorders of Sexual Differentiation: Etiology and Clinical Delineation.* New York: Academic Press.
44. SIMPSON, J.L. (1980) Genetics of human reproduction. In *Human Reproduction,* ed. E.S.E. Hafez. Hagerstown: Harper & Row, 2nd ed., pp. 395-411.
45. SIMPSON, J.L. (1981) Pregnancies in women with chromosomal abnormalities. In *Genetic Diseases in Pregnancy,* ed. J.D. Schulman, J.L. Simpson. New York: Acadmic Press, pp. 440-471.

46. SIMPSON, J.L. (1982) Genetic disorders of gonadal development in humans. In *Genetic Control of Gamete Production and Function,* eds. P.G. Crosignani, B.L. Rubin. London: Academic Press, pp. 199-228.
47. SIMPSON, J.L. (1985a) Ovarian failure (Gonadal dysgenesis). In *Progress in Infertility,* eds. J. Behrman, G. Patton, 3rd. ed., in press.
48. SIMPSON, J.L. (1985b) Genes causing female infertility. *Fertil Steril,* in press.
49. SIMPSON, J.L.; LEBEAU, M.M. (1981) Gonadal and statural determinants on the X chromosome and their relationship to *in vitro* studies showing prolonged cell cycles in 45,X; 46,X,del(X) (p11); 46,X,del(X) (q13); and 46,X,del(X) (q22) fibroblasts. *Amer. J. Obstet. Gynecol. 141:* 930-938.
50. SIMPSON, J.L.; PHOTOPULOS, G. (1976) The relationship of neoplasia to disorders of abnormal sexual differentiation. *Birth Defects 12 (1):* 15-50.
51. SIMPSON, J.L.; BLAGOWIDOW, N.; MARTIN, A.O. (1981) XY gonadal dysgenesis: Genetic heterogeneity based upon clinical observations, H-Y antigen status and segregation analysis. *Hum. Genet. 58:* 91-97.
52. SIMPSON, J.L.; BOMBARD, A.T.; MARTIN, A.O.; ELIAS, S. (1986) What are the clinical features of 45,X/46,XX mosaicism? In preparation.
53. SIMPSON, J.L.; CHRISTAKOS, A.C.; HORWITH, M.; SILVERMAN, F.S. (1971a) Gonadal dysgenesis in individuals with apparently normal chromosomal complements: Tabulation of cases and compilation of genetic data. *Birth Defects 7 (6):* 215-228.
54. SIMPSON, J.L.; HORWITH, M.; MORILLO-CUCCI, G.; MCGOVERN, J.H.; LEVINE, M.L.; GERMAN, J. (1971b). Bilateral anorchia: Discordance in monozygotic twins. *Birth Defects, Orig. Artic. Ser. 7 (6):* 196-200.
55. STERNBERG, W.H.; BARCLAY, D.L.; KLOPFER, H.W. (1968) Familial XY gonadal dysgenesis. *N. Engl. J. Med. 278:* 695-700.
56. VAN NIEKERK, W.A.; RETIEF, A.E. (1981) The gonads of human true hermaphrodites. *Hum. Genet. 58:* 117-122.
57. VERP, M.S.; ROSINSKY, B.; LEBEAU, M.M.; MARTIN, A.O.; KAPLAN, R.; WALLERMARK, C.-B.; SIMPSON, J.L. (1985) Selective growth disadvantage of fibroblasts with 45,X or X-structural abnormalities. Abstracts, *Amer. J. Hum. Genet.*
58. WACHTEL, S.S. (1983) *H-Y Antigen and the Biology of Sex Determination.* New York: Academic Press.
59. WACHTEL, S.S.; BASRUR, P.; KOO, G.C. (1981) Recessive male-determining genes. *Cell. 15:* 279-281.
60. WACHTEL, S.S.; KOO, C.; OHNO, S.; GROPP, A.; DEV, V.G.; TANTRAVAHI, R.; MILLER, D.A.; MILLER, O.J. (1976) H-Y antigen and the origin of XY female wood lemmings (*Myopus schistocolor*). *Nature 264:* 638-639.
61. WASHBURN, L.L.; EICHER, E.M. (1983) Sex reversal in XY mice caused by dominant mutation on chromosome 17. *Nature 303:* 338-340.
62. WOLF, U. (1979) XY gonadal dysgenesis and the H-Y antigen. *Hum. Genet. 47:* 269-277.
63. WOLF, U. (1981) Genetic aspects of H-Y antigen. *Hum. Genet. 58:* 25-28.
64. WOLF, U.; FRACCARO, M.; MAYEROVA, A.; HECHT, T.; MARASCHIO, P.; HAMEISTER, H. (1980a) A gene controlling H-Y antigen on the X chromosome. *Hum. Genet. 54:* 149-154.
65. WOLF, U.; FRACCARO, M.; MAYEROVA, A.; HECHT, T.; ZUFFARDI, O.; HAMEISTER, H. (1980b) Turner syndrome patients are H-Y positive. *Hum. Genet. 54:* 315-318.
66. ZENZES, M.T.; WOLF, U.; ENGEL, W. (1978a) Organization *in vitro* of ovarian cells into testicular structures. *Hum. Genet. 44:* 333-338.
67. ZENZES, M.T.; WOLF, U.; GUNTHER, E.; ENGEL, W. (1978b) Studies on the function of H-Y antigen. Dissociation and reorganization experiments on rat gonadal cells. *Cytogenet. Cell. Genet. 20:* 365-372.