H-Y ANTIGENS

PRIMARY SEX DETERMINATION

H-Y Antigen and the Development of the Mammalian Testis

STEPHEN S. WACHTEL

Tereisias, the blind prophet of ancient Greece, is remembered for his prophecy concerning Thebes. The city had been struck by a plague, and Tereisias declared that the plague would linger until Oedipus the king made penitence for his notorious indiscretion. Hymie Gordon of the Mayo Clinic tells the following story about Tereisias, "perhaps the most remarkable of all cases of sex change" (1):

As a youth, Tereisias was neither blindman nor prophet. According to Apollodoros the Athenian, Tereisias had encountered a pair of snakes coupling on the island of Cyllene. In violation of Olympian law, he killed the female, and as punishment, was himself transformed into a female. Tereisias lived as a female for seven years, at the end of which she (he) again spotted a pair of snakes coupling. This time Tereisias killed the male and this time his manhood was restored.

One night the Olympian gods Zeus and Hera were debating: who had the greater pleasure in sexual intercourse, the male or female? Hera maintained that it was the male. Zeus said it was the female. So they consulted Tereisias, as the only person who had enjoyed love's pleasure from both perspectives. Tereisias said:

> If the sum of love's pleasure adds up to ten,
> Nine parts go to women, only one to men.

Hera was infuriated and had Tereisias blinded. But Zeus was pleased with his answer and gave him the gifts of prophecy and long life.

If Tereisias' conversion was thorough, his gonads must have changed from testicles to ovaries and back again to testicles. Recently, Susumu Ohno has induced ovarian organization in testicular cells and testicular organization in ovarian cells (2). The events leading up to this extraordinary accomplishment are perhaps less fabulous than the story of Tereisias, but they are no less engaging.

H-Y antigen

Beginnings. Our modern story started in 1955 when Ernst Eichwald and Clarence Silmser, then working at the Montana Deaconess Hospital, observed rejection of male-to-female skin grafts in the highly inbred C57BL/6 strain of laboratory mouse (3). Skin grafts exchanged in the other three sex combinations (male-to-male, female-to-female, female-to-male) were accepted uniformly. Since the main difference between male graft donor and female recipient in this combination is the Y chromosome of the male, rejection was attributed to presence of a male-specific cell surface component determined by a gene on the Y chromosome. The gene was called the H-Y (histocompatibility-Y) gene and the cell surface component was called H-Y antigen.

Serology. H-Y antigen is demonstrable serologically. In 1971, Ellen Goldberg (4) discovered that serum from male-grafted females kills mouse sperm in the presence of rabbit complement (dead cells were identified visually by uptake of trypan blue dye), and in 1972 Margrit Scheid (5) developed a cytotoxicity test for H-Y using epidermal cells as targets. There are other

This work supported in part by grants from the Rockefeller Foundation and the NIH: AI 11982, CA 08748, HD 00171, HD 10065.

Address reprint requests to Stephen S. Wachtel, PhD, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

Table 1. Phylogenetic conservation of H-Y antigen. Representative vertebrate species showing distribution of sex-specific H-Y antigen identified by serum from male-sensitized female mice

Class	Species	Sex chromosomes		H-Y antigen found in
		Female	Male	
Mammals	Mouse	XX	XY	Male
	Human	XX	XY	Male
Birds	Chicken	ZW	ZZ	Female
Amphibians	Clawed frog	ZW	ZZ	Female
	Leopard frog	XX	XY	Male
Bony fish	Platyfish	WX, WY, XX	XY, YY	Male YY Male XY?
	Medaka	XX	XY	Male

kinds of serologic assays for H-Y antigen. These involve 1) tactics in which target cells are labeled with visual markers such as tobacco mosaic virus or sheep red blood cells (6–9), or fluorescent markers (10,11), and 2) hemagglutination (12).

For ease of preparation and consistency of results, our laboratory has emphasized the sperm cytotoxicity test. Specificity in this test is demonstrated by serologic absorption. H-Y antisera (from C57BL/6 females exposed to serial injections of C57BL/6 male spleen cells) are selected and pooled, and the pools are diluted and divided into aliquots. One aliquot is unabsorbed, one is absorbed with female cells ($H\text{-}Y^-$), and one is absorbed with male cells ($H\text{-}Y^+$). The absorbing cells (e.g. spleen cells) are suspended in the aliquots for about 30 minutes and the aliquots are reacted with the target cells (sperm). If the absorbing cells contain H-Y antigen on their surfaces, they specifically remove ("absorb") H-Y antibodies from the serum, and the serum now loses its ability to kill mouse sperm (or male epidermal) cells. For reasons that are not clear, only sperm and male epidermal cells of the mouse—and certain tumor cells of humans (11)—are killed by H-Y antiserum in the cytotoxicity test. H-Y antigen is, nevertheless, demonstrable on all male tissues by the technique of absorption.

Conservation of H-Y antigen. In 1959, a male-specific histocompatibility antigen was discovered in the rat (13); in 1962, one was discovered in platyfish (14); and in 1965, one in the rabbit (15). A female-specific histocompatibility antigen was described in the domestic chicken in 1967 (16). (It will be recalled that in chickens, it is the female who is the heterogametic [ZW] sex.) At about the same time that Goldberg developed her sperm cytotoxicity test (4), Silvers and Yang (17) noted that female mice could be immunized against intrastrain male skin grafts by injections of cells from male but not from female rats. This indicated homology or cross-reactivity of male antigens of mouse and rat, and the question was raised whether a similar phenomenon might be demonstrated serologically. In fact, cells of the male rat readily absorbed H-Y antibodies from mouse H-Y antiserum. But cross-reactivity was not limited to H-Y of the rat. H-Y antibodies of the mouse were absorbed by cells from males of every mammalian species tested, including guinea pig, rabbit, and human (18). Subsequently H-Y was detected in females of the domestic chicken; in males of the male heterogametic leopard frog, *Rana pipiens*; and in females of the female heterogametic South African clawed frog, *Xenopus laevis* (19). We have now detected H-Y in fish (20) (Table 1), and there is even some indication that the molecule may occur in prevertebrate species (21).

Widespread phylogenetic occurrence suggested conservation of a vital function, but as transplantation biologists, we could not immediately appreciate the potential significance of our own observations. We turned to Susumu Ohno as an expert in matters of sex, and the result was a joint communiqué suggesting that phylogenetically conserved H-Y antigen is the product of the mammalian testis-determining gene, and that H-W antigen may be the corresponding product of ovary-determining genes in the female heterogametic species (22).

Role of H-Y in primary sex determination. Sex determination may be viewed as comprising sequential processes: 1) establishment of genetic sex at fertilization, 2) translation of genetic sex into gonadal sex (primary sex determination), and 3) translation of gonadal sex into body sex (secondary sex determination) (23). According to this scheme, the indifferent gonad develops

Table 2. Examples of H-Y antigen expression in cases of aberrant sexual differentiation

Species	Sex phenotype	Gonads	Sex chromosomes	H-Y
Mouse	Female (*Tfm*)	Testes	XY	+
	Male (*Sxr*)	Small testes	XX	+
Human	Female (*Tfm*)	Testes	XY	+
	Male	Small testes	XX	+
	Ambiguous	Left ovary, right ovotestis	XX*	+
	Female	Streaks	XY*	+
	Female	Dysgenetic ovaries	Xp + Y*	−
Wood lemming	Female	Ovaries	XY	−
Dog	Female (enlarged clitoris)	Bilateral ovotestes	XX†	+
	Male	Small testes	XX†	+
Goat	Female (enlarged clitoris)	Bilateral testes	XX	+

* See text for discussion of cases.
† Both dogs carried apparent Y-to-autosome translocation; see text.

as a testis in mammalian embryos with the XY sex chromosome constitution and as an ovary in mammalian embryos with the XX sex chromosome constitution. Further (secondary) sex differentiation is mediated by testosterone secreted by the newly formed testis. In the absence of testosterone (23), or in cases of testosterone insensitivity, the embryo becomes a female despite presence of the Y chromosome and bilateral testicles (reviewed in reference 24). Evidently then, the developmental role of the Y chromosome is limited to the induction of testicular organogenesis. It follows that the sex-determining role of Y chromosome determined H-Y antigen should also be limited to the induction of testicular organogenesis. However, this makes a specific prediction, viz: that testicular differentiation invariably should be associated with presence of H-Y antigen regardless of apparent karyotype or secondary sex phenotype. Accordingly we set about to test our hypothesis by studying expression of H-Y antigen in a variety of animal and human subjects whose gonadal sex did not correspond with their phenotypic or chromosomal sex. Following is a summary of our experience.

Testing the hypothesis

Male pseudohermaphroditism. Male pseudohermaphroditism may be described as a condition in which genetic males with testes develop either partially or "completely" as phenotypic females (24). As such, the condition represents a failure of secondary sex differentiation resulting generally from errors of testosterone synthesis or function. A classic example of male pseudohermaphroditism is the "testicular feminization syndrome" (25). Affected individuals have the normal male karyotype (46,XY in man); under the influence of the Y chromosome, the gonads develop as testes. The testes secrete testosterone, but the fetal tissues cannot respond, due to mutation or deficiency of the nuclear cytosol androgen receptor (26,27), and further development is female; uterus, tubes, and cephalad portion of the vagina are absent, however, evidently suppressed by mullerian inhibition factor, another secretion of the fetal testis (28).

Testicular feminization syndrome has been described in laboratory rodents and cattle. Since it is due to mutation of a gene on the conservative X chromosome, the gene may be widespread among mammals. We have studied the tissues of six human females with the testicular feminization syndrome. All were H-Y$^+$ (29); and in an earlier study H-Y was reported in X^{Tfm}Y female mice (30).

XX male syndrome. A female sex chromosome constitution (46,XX) is found in one of every 30,000 human males (31). We have studied blood cells and skin fibroblasts from fourteen human XX males. H-Y was detected in all cases. In addition, we have detected H-Y antigen in XX males of the mouse (32), dog (33), and goat (34) (Table 2 and see below). (We assume that expression of H-Y antigen in the absence of a detectable Y chromosome indicates presence in the genome of cryptic, or translocated, Y chromosomal genes. Alterna-

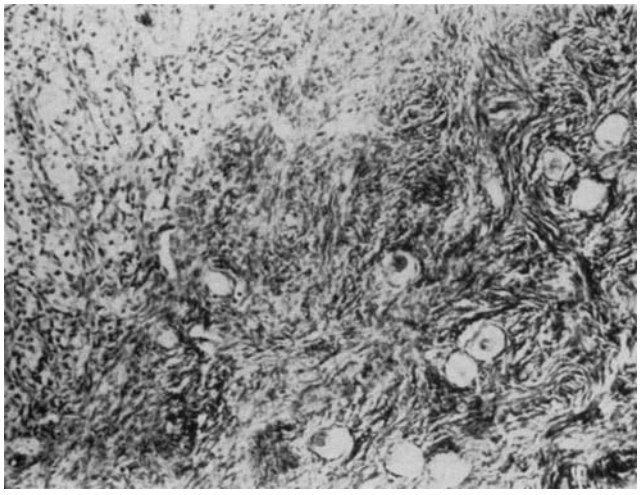

Figure 1. Cross-section from right ovotestis of 2-year-old human 46,XX true hermaphrodite with contralateral ovary. Note polarization of testicular and ovarian architectures and absence of germ cells in seminiferous tubules (upper left).

tively, expression of H-Y in XX males could signal mutational acquisition of Y chromosomal function by autosomal or X-linked genes. See reference 35 for discussion of Y-linkage versus X-linkage of the H-Y structural locus.)

XX true hermaphroditism. If H-Y antigen is the inducer of the mammalian testis, then the molecule should be found not only in XX males, but also in XX true hermaphrodites, i.e. individuals possessing both testicular and ovarian tissue. Of seven human XX true hermaphrodites tested in our laboratory, all were H-Y$^+$. It is perhaps worth mentioning that a statistical analysis of data from studies of three of these patients disclosed reduced expression of H-Y antigen on their somatic cells (36). If this is a general phenomenon, hermaphroditic differentiation in XX subjects bearing Y chromosomal genes (XX^Y), could represent abnormal display of H-Y antigen in all tissues, including the gonad.

Figure 1 shows part of the right ovotestis removed from a 2-year-old child with ambiguous external genitalia, 46,XX karyotype, H-Y$^+$ cellular phenotype (in blood leukocytes), and male testosterone levels (37). The striking polarization of testicular and ovarian architecture is characteristic of the ovotestis and signifies mosaicism of factors promoting development of testis versus ovary in the gonadal primordium. Indeed, in a more recent study (38), cells cultured from the testicular portion of a scrotal ovotestis (from a 46,XX "male") were typed H-Y$^+$, but cells cultured from the ovarian portion were typed H-Y$^-$ (Figure 2).

Fertile XY females of the wood lemming. In the Scandinavian wood lemming, *Myopus schisticolor*, there is a skewed sex ratio with a preponderance of females. Almost half of the females have a male sex chromosome constitution (XY), yet they are fertile and anatomically indistinguishable from their XX sisters (39). There is one other difference between XX and XY female wood lemmings: XY females bear only daughters. Evidently XY females produce only X-bearing eggs. They do not transmit the Y. Hence the sex reversed condition cannot be due to a defective Y chromosome. In fact the XY female wood lemming condition is inherited as an X-linked trait, and this suggests that a gene on the X

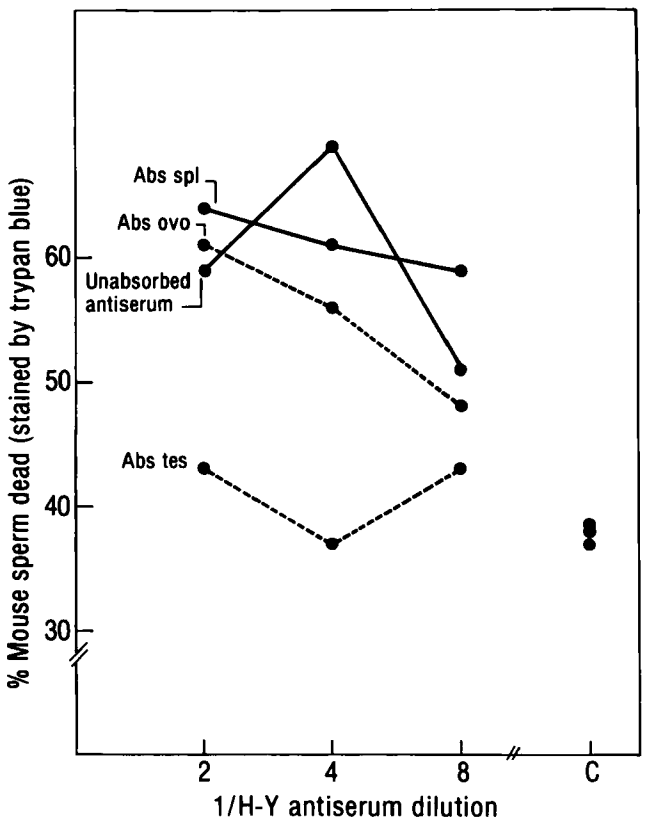

Figure 2. H-Y antigen mosaicism in the ovotestis. Cells cultured from the ovarian portion of scrotal ovotestis from human XX true hermaphrodite absorbed significantly less H-Y antibody than was absorbed by cells cultured from the testicular portion. **Abs spl** denotes absorption of H-Y antiserum with cells from spleen of female mouse (H-Y$^-$ control). **Abs ovo** and **Abs tes** denote absorption with fibroblasts cultured from ovarian and testicular portions, respectively, of the 46,XX ovotestis. **C** (control) values represent background above which sperm cell death is attributable to direct cytotoxic action of antibody and complement. (Reprinted by permission from The New England Journal of Medicine 300:745–749, 1979; see reference 38.)

chromosome can suppress the male-determining portion of the Y in this species at least (40). (See discussion below for description of a similar X-linked gene in humans.)

In collaboration with Professor Alfred Gropp of Lubeck, we studied a number of wood lemmings. All males were typed H-Y$^+$. All females were typed H-Y$^-$ including those with the XY sex chromosome constitution (41).

Genetics of H-Y antigen expression and function

Most of the following studies were initiated to evaluate the original proposition that H-Y induces the mammalian testis. They have served well in this regard. In addition, they have provided new and valuable insights into the genetics of primary sex determination.

Location of H-Y genes. Our detection of excess H-Y in the cells of human males with two Y chromosomes (XXYY and XYY) indicates that a genetic locus for H-Y antigen expression is situated on the human Y (42). By correlating presence of H-Y with presence of particular portions of the Y chromosome in seventeen patients exhibiting structural abnormalities of the Y, Gloria Koo of our laboratory has now obtained evidence that H-Y genes are located on the short arm, Yp, near the centromere. A locus on the long arm (Yq) could not be ruled out in one case (43). These findings agree with an earlier survey showing that male-determining genes occur on the short and long arms of the human Y chromosome, near the centromere in both cases (44), although they do not tell us whether the genes are regulatory or structural.

Genetic basis of XX male syndrome and XX true hermaphroditism: evidence in the dog. While studying a prominent family of American cocker spaniels, Jules Selden (33) recently discovered bilateral ovotestes in the *mother* of a male pup with unambiguous but small testes. Both dogs had a female karyotype (78,XX) and both were H-Y$^+$ in serologic tests. But there was an indication of excess H-Y antigen in the tissues of the 78,XY father of the true hermaphrodite (the grandfather of the XX male; see Figure 3). Subsequent studies revealed the possibility of a Y-to-autosome translocation in the tissues of all three dogs. So in addition to his normal Y chromosome, the father carried a Y-autosome translocation, and this is what he transmitted to his true hermaphrodite daughter, and she to her XX male pup.

These findings are remarkable for several reasons: 1) they provide a rare example of a mammalian true hermaphrodite functioning as a fertile female; two such human cases are known (45,46) but both required surgical correction of external genitalia and cesarean section; 2) they support our earlier observation that excess H-Y antigen on the plasma membrane is correlated with excess Y chromosomal material in the nucleus; 3) they suggest that XX male syndrome and XX true hermaphroditism represent alternative manifestations of the same developmental anomaly, i.e. that both conditions are caused by abnormal transmission of H-Y genes; and 4) they implicate Y-to-autosome translocation as a sex-reversing factor in mammals generally. The last point is worth emphasizing because the autosomal dominant gene, Sxr, specifies H-Y antigen and testicular differentiation in the mouse, but there is scant evidence for Y-to-autosome translocation in XX males sex-reversed by this gene, or in XY males carrying it (47). Of course, if H-Y is coded by a locus on the Y chromosome, then mere expression of H-Y may be taken as evidence for the presence of at least a portion of the Y, regardless of what the karyotype may appear to be.

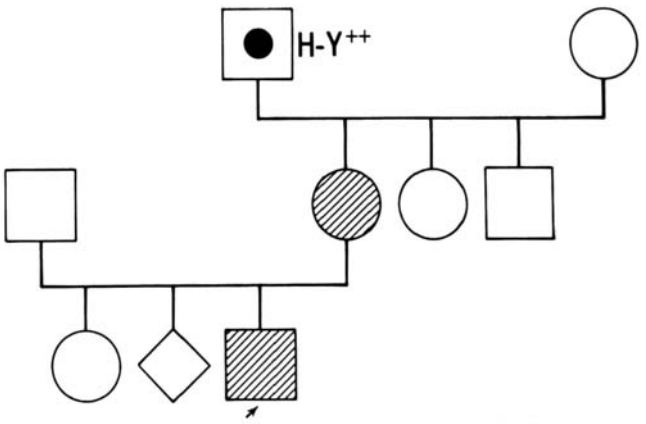

Figure 3. Abnormal transmission of testis-determining H-Y genes in a family of cocker spaniel dogs. XX male propositus (**arrow**) is represented as a shaded square, XX true hermaphrodite mother as a shaded circle, and father of true hermaphrodite (XY) as a square with dark circle. No data are available concerning the dead pup (diamond). Father of the XX male and littermate sister of XX true hermaphrodite were not available for study. The other three family members are apparently normal. **H-Y^{++}** denotes excess H-Y antigen. (Selden JS: The intersex dog: classification, clinical presentation, and etiology. The Compendium on Continuing Education for the Small Animal Practitioner 1:435–441, 1979. Reprinted by permission.)

Recessive male-determining genes. Among goats there is an autosomal gene called "polled" (*P*). Goats carrying a single copy of this gene (*P*/+) are born with-

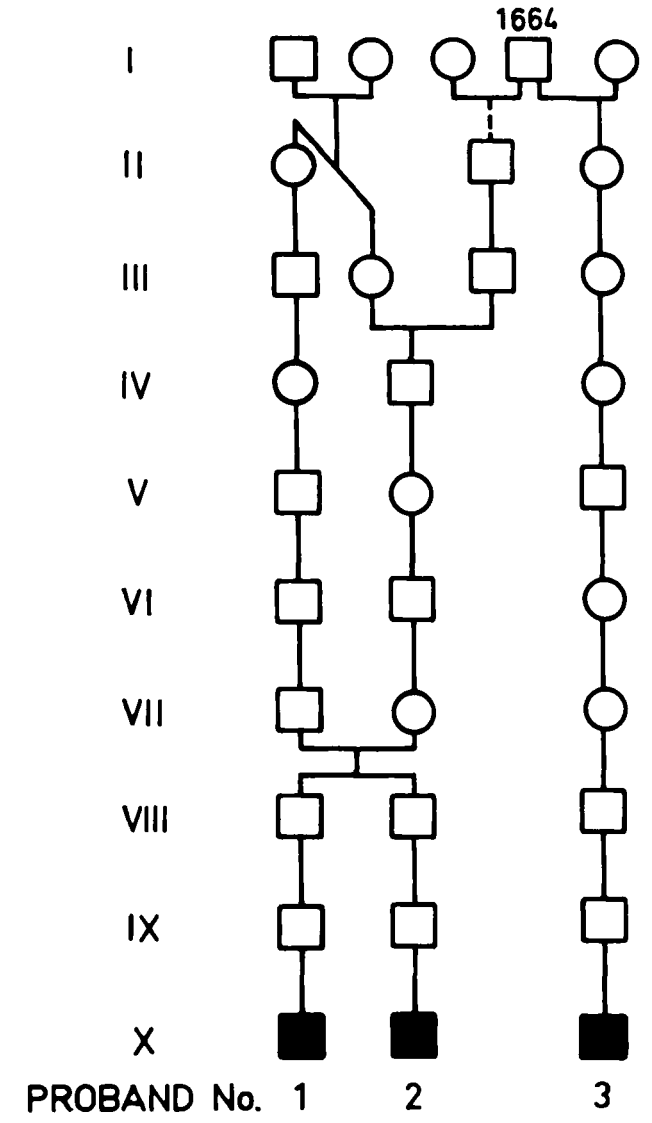

Figure 4. Recessive male-determining genes. Simplified paternal pedigree shows three 46,XX males in one family. Probands 1 and 2 are second cousins; proband 3 is related to probands 1 and 2 through several generations dating back to common ancestor born in 1664. Mothers of probands 3 and 2 are also related (not depicted). After de la Chapelle et al (51).

out horns, but they are normal sexually. However, XX goats that are homozygous for polled (*P/P*) have testes or ovotestes. In other words a locus closely associated with *P* acts as an autosomal recessive testis determinant; two copies of this locus can generate XX males and XX true hermaphrodites (48–50). After ascertaining that H-Y antigen is present in normal males (+/+) and absent in normal females (+/+), we studied a group of polled goats that included two unrelated XX intersex kids (*P/P*). Not unexpectedly both were H-Y$^+$ (34).

A similar situation may occur in humans. In Finland, de la Chapelle discovered three XX males with a common ancestor born in 1664. In view of the rarity of the 46,XX male syndrome, it seems reasonable to assume a common etiology for three cases within the same pedigree. Recessive male-determining genes are implicated as follows:

XX males 1 and 2 are second cousins related through their paternal grandfather (Figure 4). The fact is that the grandfather did not transmit his X chromosome to the fathers of these two XX males, so sex-reversal in this family could not be due to X-linked genes. It must be due to an autosomal locus. Thus it must be

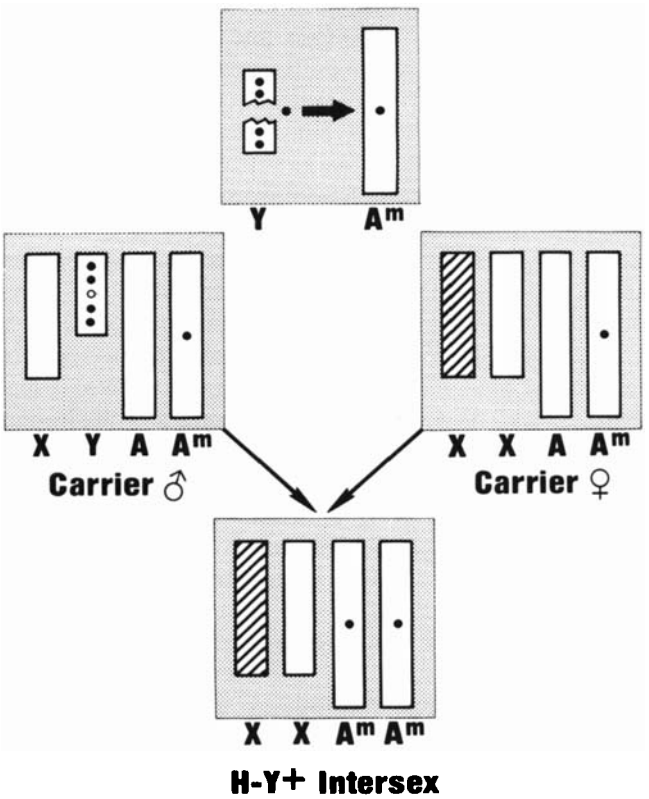

Figure 5. Hypothetical origin of recessive testis-determining H-Y genes. Assuming a system of multiple testis-determining H-Y genes on the Y chromosome, translocation of a sub-critical portion (e.g. 20%) gives rise to mutant autosome A^m, unable by itself to induce testicular differentiation. Presence of A^m does not preclude development of fertile ovary in carrier female. However, critical accumulation of H-Y genes generates synthesis of threshold amount of H-Y antigen in A^{m}A^m homozygote causing testicular differentiation in XX "sex-reversed" male. From Wachtel et al (34); copyright 1978 by The MIT Press.

due to an autosomal *recessive* locus (as in the polled goats). If it were a dominant gene, we should expect other affected males, and there are none. Moreover XX male 3 is related to XX males 1 and 2 through ten generations involving five female ancestors. They could not have been female had they carried a dominant testis-determinant. All three XX males were typed H-Y$^+$ in our serologic assays. In this case, however, there was evidence for a modicum of H-Y antigen expression in the mothers (51).

So we have examples of "recessive" inheritance of H-Y and testis-determining genes in goats and in humans. But we have indicated that H-Y antigen expression and testicular differentiation in XX subjects represent translocation of Y chromosomal genes. How could Y-to-autosome translocation generate a *dominant* mode of XX sex reversal, as in Sxr mice, Selden's dogs, and some human families (52), and a *recessive* mode of XX sex reversal as in the polled goats and de la Chapelle's human family?

It has been argued that the heteromorphic X and Y sex chromosomes of mammals arose from a homomorphic and essentially homologous pair of autosomes, and that differentiation was accomplished at the expense of the Y, which underwent genetic degeneration to become a specialized testis-inducer, while the X remained invariant (53). What if the mammalian Y chromosomal testis-determining gene came to exist in multiple copies as a result of this degeneration and specialization (53)? And what if there were a critical number of genes coding for a critical lower threshold of H-Y antigen, below which testicular differentiation could not be sustained? Then Y-to-autosome translocation could generate either dominant or recessive modes of testis-determination depending on the particular quantity of genes transferred (Figure 5).

XY gonadal dysgenesis: an X-linked regulator. Earlier we indicated that a gene on the X chromosome could suppress testis-determining elements of the Y chromosome, giving rise thereby to fertile XY females in the wood lemming. What would be the effects of a similar gene in humans? Whereas XO rodents become fertile females, XO human embryos initially develop ovaries but the ovaries degenerate. They are represented around the time of birth by undifferentiated gonads containing ovarian stroma but devoid of follicles (gonadal dysgenesis) (54). With respect to the number of X chromosomes that are present, XY embryos resemble XO embryos. Thus mutational suppression of the testis-determining segment of the Y in a human XY embryo should give rise to an H-Y$^-$ phenotypic female with dysgenetic "streak" gonads.

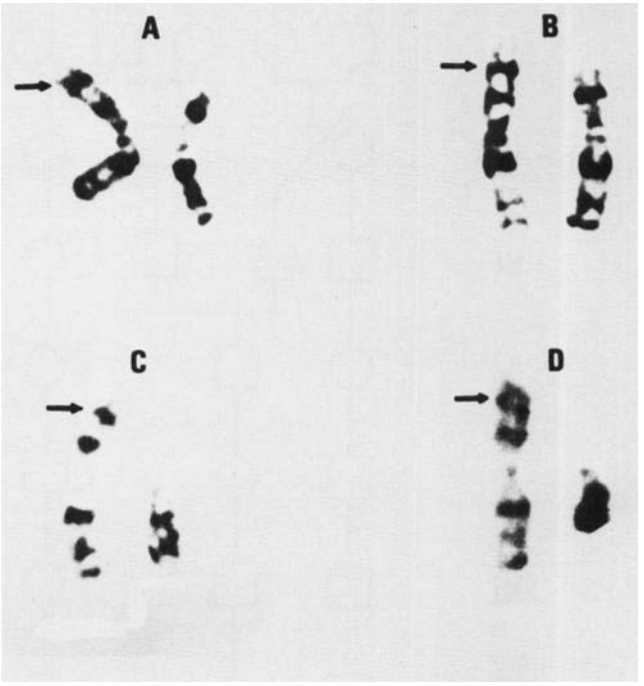

Figure 6. Abnormal X chromosome in 46,XY gonadal dysgenesis. **Arrows** mark extra band on short arm of X chromosomes (Xp$^+$) in (**A**) normal XXp$^+$ mother and (**B**) normal XXp$^+$ sib of (**C**) affected Xp$^+$Y female proband and (**D**) affected Xp$^+$Y female fetal sibling. Extra band is associated with multiple abnormalities in proband and fetal sibling, including H-Y$^-$ cellular phenotype. Evidently, Xp$^+$ is nonrandomly inactivated in XXp$^+$ females of this family. From giemsa-banded karyotypes prepared by Dr Renée Bernstein (see reference 55).

Consider the following case study (55): A 46,XY karyotype was discovered in a severely retarded female with multiple phenotypic abnormalities. The Y chromosome was intact, but there was an additional band on the short arm of the X, which the child had inherited from her mother (Figure 6). (The abnormal chromosome was also present in a normal 46,XX younger sib). The affected child died at 5 years of age. Autopsy revealed female genitalia internally, including microscopic ovarian remnants containing ovarian stroma and degenerating follicles. There was no evidence of testicular differentiation. The mother became pregnant again. Study of cells from the amniotic fluid revealed a 46,XY karyotype. There was an additional band on the short arm of the X and on this basis the pregnancy was terminated. The 20-week-old fetus was a female with multiple abnormalities similar to those of the proband.

Ovaries were present bilaterally. Fetus and proband were typed H-Y antigen negative.

Videodensitometric analysis of the abnormal chromosome indicated that the extra band may have arisen as a duplication of part of Xp. If there were a regulatory gene on Xp whose function it was to restrict excessive synthesis of H-Y, duplication of this gene could be expected to reduce production of H-Y below a threshold required for testicular differentiation. The gonad would now differentiate as an ovary (as in the fetus); in the absence of the second X chromosome, the ovary would later degenerate (as in the proband). Alternatively, if the function of the regulatory gene were to initiate synthesis of H-Y, duplication of Xp might block that function via a "position effect" for example, thereby thwarting production of H-Y altogether.

XY gonadal dysgenesis: evidence for a gonad-specific H-Y receptor. Of eleven human females with XY gonadal dysgenesis that have been typed in our laboratory, it is noteworthy that seven were unambiguously H-Y$^+$. At first sight this might seem paradoxical, for if H-Y is the inducer of the mammalian testis, how can we account for gonadal dysgenesis in females with both H-Y$^-$ and H-Y$^+$ cellular phenotypes?

Yet presence of H-Y antigen and absence of H-Y antigen in different cases of gonadal dysgenesis representing failures of primary sex determination are no more paradoxical than presence of testosterone and absence of testosterone in different cases of male pseudohermaphroditism representing failures of secondary sex determination. Indeed a clue to the nature of H-Y$^+$, 46,XY gonadal dysgenesis is provided by the presence of testosterone in phenotypic females with testicular feminization syndrome (TFS). We have seen that TFS is caused by androgen insensitivity; testosterone is produced, but target organs are unresponsive because the androgen receptor protein is defective or absent.

Suppose that H-Y antigen were disseminated like a hormone, and that testicular organogenesis required binding of disseminated H-Y to its specific receptor in the gonadal primordium. Then in cases of specific receptor failure, H-Y would be present in somatic tissues as a stable portion of the plasma membrane, but it would be *functionally* absent in the gonad. The result would be failure of testicular differentiation despite "presence" of serologically detectable H-Y antigen. In fact, there are now reasons for believing not only that H-Y is released in "free" form and that gonadal cells bear H-Y receptors, but also that uptake of disseminated H-Y is a prerequisite of normal testicular differentiation. To cite a few examples: 1) H-Y is detected in masculinized gonads of the bovine freemartin fetus (56); 2) H-Y occurs as a free molecule in rat epididymal fluid (57), in supernatant fluid of mouse testicular cell preparations (58), and in the supernatant medium of cultured Daudi cells (59 and see below); 3) H-Y binds specifically to cells of the ovary, and to lesser degree to cells of the testis (57) (presumably coated with molecules of indigenous H-Y); 4) the binding reaction triggers testicular differentiation in XX gonadal cells of the fetal calf (60), and in dispersed XX cells of the neonatal rat (61); 5) an early consequence of this reaction is the appearance of HCG receptors in the newly converted XX testicular cells (62).

In vitro considerations

β_2m-HLA anchorage sites for H-Y antigen. If H-Y antigen is the inducer of the mammalian testis, why should it be found in all tissues of the normal male? In order to induce a particular organogenetic event, the relevant inducer must be present before initiation of that event; e.g. H-Y is detected in the eight cell preimplantation mouse embryo several days before the initiation of testicular organogenesis in the mouse (63). Thus, ubiquitous expression of H-Y may simply reflect its need for early expression, and its corresponding escape from the regulatory influences present in the lower vertebrates, in which gonadal differentiation may be affected by steroid hormones. Alternatively, H-Y may have other functions; but function notwithstanding, ubiquitous expression of an antigenic cell surface molecule that is disseminated imposes the requirement of a ubiquitous stable membrane anchorage site for that molecule. In view of the profound influence of major histocompatibility complex (MHC) cell surface antigens on the expression of H-Y transplantation antigen, it has been proposed that in association with beta-2-microglobulin (β_2m), cell surface components of the MHC (HLA in humans) act as the non-specific membrane anchorage sites for all organogenesis inducing proteins, including H-Y (64).

If this notion is correct, male cells that have lost β_2m-HLA should be unable to accommodate H-Y on their membranes. Indeed β_2m(−), HLA(−) cultured "Daudi" cells (from a human 46,XY Burkitt lymphoma) absorb considerably less H-Y antibody than is absorbed by cells cultured from 46,XY Burkitt lymphomas that have retained β_2m-HLA. When Daudi cells are cocultured with female cells of the HeLa D98

line, β_2m supplied by the latter restores Daudi HLA and H-Y to the surface of (Daudi × HeLa) somatic cell hybrids (59 but see 11). Cultured Daudi cells are an excellent source of soluble H-Y antigen, apparently secreted in the absence of the β_2m–HLA carrier, and as noted above, this H-Y has been used to induce testicular transformation of bovine fetal ovarian cells.

From this perspective, H-Y antigen utilizes two "receptors": the specific receptor of the gonad and the non-specific β_2m–MHC stable membrane anchorage site of all cells, gonadal and somatic. It follows that testicular organogenesis requires saturation of both non-specific anchorage sites and specific receptors with molecules of disseminated H-Y antigen. According to this view, abnormal gonadal differentiation would result from *reduced synthesis* of H-Y antigen due to paucity of Y-bearing cells (in an XX/XY chimeric primordium) or paucity of H-Y genes (in an XX primordium containing sub-threshold numbers of H-Y genes), or *abnormal display* of H-Y antigen due, for example, to anomalous binding characteristics of either non-specific anchorage site or specific receptor.

Binding of H-Y antigen to its gonad-specific receptors is inhibited by a supernatant of the fetal ovary. The question arises whether ovarian differentiation is a passive or active process. Is the ovary determined by absence of a testis inducer or is the ovary actively induced by a "female" molecule corresponding to H-Y of the male? If it were mere absence of H-Y that caused organogenesis of the ovary, we should expect hermaphroditic differentiation whenever XX/XY gonads fail to become testes. But the fact is that when XX/XY chimeric gonads fail to organize testes, they organize ovaries, not ovotestes. It appears that XX cells can transmit a signal to XY cells, inducing the latter to engage in ovarian differentiation (reviewed in reference 65). Indeed XY cells can become functional oocytes (66).

Suppose there were an ovary inducer molecule. According to what has already been said, this molecule might be expected to bind β_2m–MHC nonspecific carriers and also to engage its own gonad-specific receptor. If testis-inducing H-Y and a putative ovary-inducing molecule did use the same β_2m–MHC carrier, or if engagement of one inducer and its specific receptor precluded engagement of the other inducer by an adjacent receptor (via changes in membrane topography or steric interference for example), then the two inducers would compete for receptors when both inducers were present. The outcome might be determined by timing of dissemination, or alternatively, by affinity of a particular inducer for a specific receptor or non-specific membrane anchorage site.

The idea of competition has been tested: since ovarian cells are H-Y negative, they do not absorb H-Y antibody. If these cells possessed H-Y antigen receptors they might acquire H-Y, provided that the molecule were available, as in the supernatant fluid of testicular cell preparations. Having thus acquired H-Y antigen, ovarian cells should absorb H-Y antibody. In our laboratory, adult dog ovary cells absorbed H-Y antibody after their exposure to supernatant fluid of the dissociated mouse testis. But they did not absorb H-Y antibody, i.e. they did not take up H-Y antigen, when they were first exposed to a supernatant of the newly differentiated *fetal* ovary. There must be a molecule elaborated by the fetal ovary that can block reaction of H-Y antigen with its gonad-specific receptor. However, inhibition of H-Y binding in this system could be due to a competitive inhibitor (able to promote ovarian differentiation in an XX/XY chimeric gonad for instance) or even to non-specific competition from any of several "junk" proteins secreted by the fetal gonad. So it remains to be determined whether ovarian differentiation could be induced in the indifferent XY gonad with a supernatant of the fetal ovary (58).

REFERENCES

1. Gordon H: Ancient ideas about sex determination, Genetic Mechanisms of Sexual Development. Edited by HL Vallet, IH Porter. New York, Academic Press, 1979, pp 1–32
2. Iwata H, Nagai Y, Stapleton DD, Smith RC, Ohno S: Identification of human H-Y antigen and its testis-organizing function. Arthritis Rheum 22:1211–1216, 1979
3. Eichwald EJ, Silmser CR: Untitled communication. Transplant Bull 2:148–149, 1955
4. Goldberg EH, Boyse EA, Bennett D, Scheid M, Carswell EA: Serological demonstration of H-Y (male) antigen on mouse sperm. Nature 232:478–480, 1971
5. Scheid M, Boyse EA, Carswell EA, Old LJ: Serologically demonstrable alloantigens of mouse epidermal cells. J Exp Med 135:938–955, 1972
6. Koo GC, Stackpole CW, Boyse EA, Hammerling U, Lardis M: Topographical location of H-Y antigen on mouse spermatozoa by immunoelectronmicroscopy. Proc Nat Acad Sci USA 70:1502–1505, 1973
7. Koo GC, Boyse EA, Wachtel SS: Immunogenetic techniques and approaches in the study of sperm and testicular cell surface antigens, Immunobiology of Gametes. Edited by M Edidin, MH Johnson. Oxford, Alden Press, 1977, pp 73–84
8. Tokuda S, Arrington T, Goldberg EH, Richey J: Simpler

technique for serological detection of H-Y antigen on mouse lymphocytes. Nature 267:433–434, 1977
9. Koo GC, Goldberg CL: A simplified technique for H-Y typing. J Immunol Meth 23:197–201, 1978
10. Galbraith GMP, Galbraith RM, Faulk WP, Wachtel SS: Detection of H-Y antigen by fluorescence microscopy. Transplantation 26:25–27, 1978
11. Fellous M, Gunther E, Kemler R, Wiels J, Berger R, Guenet JL, Jakob H, Jacob F: Association of the H-Y male antigen with β_2-microglobulin on human lymphoid and differentiated mouse teratocarcinoma cell lines. J Exp Med 147:58–70, 1978
12. Shalev A, Berczi I, Hamerton JL: Detection and cross-reaction of H-Y antigens by haemagglutination. J Immunogenetics 5:303–312, 1978
13. Billingham RE, Silvers WK: Inbred animals and tissue transplantation immunity. Transplant Bull 2:399–406, 1959
14. Miller L: The Eichwald-Silmser phenomenon in an inbred strain of platyfish. Transplant Bull 30:147–149, 1962
15. Chai CK: The effect of inbreeding in rabbits. Transplantation 6:689–693, 1968
16. Gilmour DG: Histocompatibility antigen in the heterogametic sex in the chicken. Transplantation 5:699–706, 1967
17. Silvers WK, Yang SL: Male-specific antigen: its homology in mice and rats. Science 181:570–572, 1973
18. Wachtel SS, Koo GC, Zuckerman EE, Hammerling U, Scheid M, Boyse EA: Serological crossreactivity between H-Y (male) antigens of mouse and man. Proc Natl Acad Sci USA 71:1215–1218, 1974
19. Wachtel SS, Koo GC, Boyse EA: Evolutionary conservation of H-Y ("male") antigen. Nature 254:270–272, 1975
20. Pechan P, Wachtel SS, Reinboth R: H-Y antigen in the teleost. Differentiation, in press
21. Shalev A: Unpublished observations
22. Wachtel SS, Ohno S, Koo GC, Boyse EA: Possible role for H-Y antigen in the primary determination of sex. Nature 257:235–236, 1975
23. Jost A: Hormonal factors in the sex differentiation of the mammalian foetus. Philos Trans R Soc Lond (Biol) 259:119–130, 1970
24. Wilson JD, MacDonald PC: Male pseudohermaphroditism due to androgen resistance: testicular feminization and related syndromes. The Metabolic Basis of Inherited Disease. Fourth edition. Edited by JB Stanbury, JB Wyngaarden, DS Frederickson. New York, McGraw-Hill, 1978, pp 894–913
25. Morris JM: The syndrome of testicular feminization in male pseudohermaphrodites. Am J Obstet Gynecol 65:1192–1211, 1953
26. Attardi B, Ohno S: Cytosol androgen receptor from kidney of normal and testicular feminized (Tfm) mice. Cell 2:205–212, 1974
27. Keenan BS, Meyer WJ III, Hadjian AJ, Jones HW, Migeon CJ: Syndrome of androgen insensitivity in man: absence of 5α-dihydrotestosterone binding protein in skin fibroblasts. J Clin Endocrinol Metab 38:1143–1146, 1974
28. Wilson JD, Griffin JE, George FW: The mechanism of phenotypic sex differentiation. Arthritis Rheum 22:0000–0000, 1979
29. Koo GC, Wachtel SS, Saenger PS, New MI, Dosik H, Amarose AP, Dorus E, Ventruto V: H-Y antigen: expression in human subjects with the testicular feminization syndrome. Science 196:655–656, 1977
30. Bennett D, Boyse EA, Lyon MF, Mathieson BJ, Scheid M, Yanagisawa K: Expression of H-Y (male) antigen in phenotypically female Tfm/Y mice. Nature 257:236–238, 1975
31. de la Chapelle A: Analytic review: nature and origin of males with XX sex chromosomes. Am J Hum Genet 24:71–105, 1972
32. Bennett D, Mathieson BJ, Scheid M, Yanagisawa K, Boyse EA, Wachtel SS, Cattanach BM: Serological evidence for H-Y antigen in *Sxr*, XX sex-reversed phenotypic males. Nature 265:255–257, 1977
33. Selden JR, Wachtel SS, Koo GC, Haskins ME, Patterson DF: Genetic basis of XX male syndrome and XX true hermaphroditism: evidence in the dog. Science 201:644–646, 1978
34. Wachtel SS, Basrur P, Koo GC: Recessive male-determining genes. Cell 15:279–281, 1978
35. Ohno S: Major Sex-Determining Genes. Berlin-Heidelberg-New York, Springer-Verlag, 1979, pp 81–84
36. Wachtel SS, Koo GC, Breg WR, Thaler TH, Dillard GM, Rosenthal IM, Dosik H, Gerald PS, Saenger P, New M, Lieber E, Miller OJ: Serological detection of a Y-linked gene in XX males and XX true hermaphrodites. N Engl J Med 295:750–754, 1976
37. Saenger P, Levine LS, Wachtel SS, Korth-Schutz S, Doberne Y, Koo GC, Lavengood RW, German JL, New MI: Presence of H-Y antigen and testis in 46,XX true hermaphroditism: evidence for Y-chromosomal function. J Clin Endocrinol Metab 43:1234–1239, 1976
38. Winters SJ, Wachtel SS, White BJ, Koo GC, Javadpour N, Loriaux L, Sherins RJ: H-Y antigen mosaicism in the gonad of a 46,XX true hermaphrodite. N Engl J Med 300:745–749, 1979
39. Fredga K. Gropp A, Winking H, Frank F: Fertile XX- and XY-type females in the wood lemming *Myopus schisticolor*. Nature 261:225–227, 1976
40. Fredga K, Gropp A, Winking H, Frank F: A hypothesis explaining the exceptional sex ratio in the wood lemming (*Myopus schisticolor*). Hereditas 85:101–104, 1977
41. Wachtel SS, Koo GC, Ohno S, Gropp A, Dev VG, Tantravahi R, Miller DA, Miller OJ: H-Y antigen and the origin of XY female wood lemmings (*Myopus schisticolor*). Nature 264:638–639, 1976
42. Wachtel SS, Koo GC, Breg WR, Elias S, Boyse EA,

Miller OJ: Expression of H-Y antigen in human males with two Y-chromosomes. N Engl J Med 293:1070–1072, 1975
43. Koo GC, Wachtel SS, Krupen-Brown K, Mittl LR, Breg WR, Genel M, Rosenthal IM, Borgaonkar DS, Miller DA, Tantravahi R, Schreck RR, Erlanger BF, Miller OJ: Mapping the locus of the H-Y gene on the human Y-chromosome. Science 198:940–942, 1977
44. Simpson JL: Gonadal dysgenesis and abnormalities of the human sex chromosomes: current status of phenotypic-karyotypic correlations. Birth Defects 11:23–59, 1975
45. Narita O, Manba S, Nakanishi T, Ishizuka N: Pregnancy and childbirth in a true hermaphrodite. Obstet Gynecol 45:593–595, 1975
46. Mayou BJ, Armon P, Lindenbaum RH: Pregnancy and childbirth in a true hermaphrodite following reconstructive surgery. Br J Obstet Gynaecol 85:314–316, 1978
47. Cattanach BM, Pollard CE, Hawkes SG: Sex-reversed mice: XX and XO males. Cytogenetics 10:318–337, 1971
48. Basrur P-K, Kanagawa H: Anatomic and cytogenetic studies on 19 hornless goats with sexual disorders. Ann Genet Selection Animale 1:349–378, 1969
49. Hamerton JL, Dickson JM, Pollard CE, Grieves SA, Short RV: Genetic intersexuality in goats. J Reprod Fertil 7(suppl):25–51, 1969
50. Soller M, Padeh B, Wysoki M, Ayalon N: Cytogenetics of the Saanen goats showing abnormal development of the reproductive tract associated with the dominant gene for polledness. Cytogenetics 8:51–67, 1969
51. de la Chapelle A, Koo GC, Wachtel SS: Recessive sex-determining genes in human XX male syndrome. Cell 15:837–842, 1978
52. Kasdan R, Nankin HR, Troen P, Wald N, Pan S, Yanaihara T: Paternal transmission of maleness in XX human beings. N Engl J Med 288:539–545, 1973
53. Ohno S: Sex Chromosomes and Sex-linked Genes. Berlin-Heidelberg-New York, Springer, Verlag, 1967
54. Short RV: Sex determination and differentiation of the mammalian gonad. Int J Androl 2(suppl):21–28, 1978
55. Bernstein R, Jenkins T, Dawson B, Wagner J, Dewald G, Koo GC, Wachtel SS: Female phenotype and multiple abnormalities in siblings with a Y-chromosome and partial x-chromosome duplication: H-Y antigen and Xg blood group findings. J Med Genet In press
56. Ohno S, Christian LC, Wachtel SS, Koo GC: Hormone-like role of H-Y antigen in bovine freemartin gonad. Nature 261:597–599, 1976
57. Muller U, Aschmoneit I, Zenzes MT, Wolf U: Binding studies of H-Y antigen in rat tissues: indications for a gonad-specific receptor. Hum Genet 43:151–157, 1978
58. Wachtel SS, Hall JL: H-Y binding in the gonad: inhibition by a supernatant of the fetal ovary. Cell 17:327–329, 1979
59. Beutler B, Nagai Y, Ohno S, Klein G, Shapiro IM: The HLA-dependent expression of testis-organizing H-Y antigen by human male cells. Cell 13:509–513, 1978
60. Nagai Y, Ciccarese S, Ohno S: The identification of human H-Y antigen and testicular transformation induced by its interaction with the receptor site of bovine fetal ovarian cells. Differentiation 13:155–164, 1979
61. Zenzes MT, Wolf U, Engel W: Organization in vitro of ovarian cells into testicular structures. Hum Genet, 44:333–338, 1978
62. Muller U, Zenzes MT, Bauknecht T, Wolf U, Siebers JW, Engel W: Appearance of hCG-receptor after conversion of newborn ovarian cells into testicular structures by H-Y antigen in vitro. Hum Genet 45:203–207, 1978
63. Krco CJ, Goldberg EH: Detection of H-Y (male) antigen on 8-cell mouse embryos. Science 193:1134–1135, 1976
64. Ohno S: The original function of MHC antigens as the general plasma membrane anchorage site of organogenesis-directing proteins. Immunol Rev 33:59–69, 1977
65. Wachtel SS: Immunogenetic aspects of abnormal sexual differentiation. Cell 16:691–695, 1979
66. Ford CE, Evans EP, Burtenshaw MD, Clegg HM, Tuffrey M, Barnes RD: A functional "sex-reversed" oocyte in the mouse. Proc R Soc Lond (Biol) B 190:187–197, 1975
67. Wachtel SS: The genetics of intersexuality: clinical and theoretical perspectives. Obstetrics and Gynecology. In press 1979