ence of larval and early postlarval bivalves in ancient sediments and have discussed methods of species-level identification (*3*, *14*). Original aragonitic structures have been found to be extremely well preserved in some deposits as old as the Late Cretaceous (*3*). Prodissoconch-dissoconch boundaries of specimens from such sediments are readily distinguished (Fig. 2) and, when coupled with size distribution data for larval specimens, can be used in defining maximum sizes at metamorphosis. This approach should permit the reconstruction of both relative and absolute temperatures.

1) *Relative temperatures*. Detailed examination of changes in prodissoconch dimensions in a species through time at a single locality, or in a series of localities along a single horizon, should provide an indication of temporal or spatial temperature gradients for any interval containing adequately preserved faunas. Such paleotemperature gradient reconstructions should serve as an independent test of data derived from other microorganisms or stable isotope analyses.

2) *Absolute temperatures*. The long geologic duration of most bivalve species (*15*) suggests that absolute paleotemperature estimates might be achieved for Holocene, Pleistocene, and Pliocene environments by using regressions of prodissoconch length on temperature for a variety of extant species. However, as with other quantitative paleoclimatic techniques (*16*), the assumption of evolutionary stasis within species with regard to physiological processes may not be entirely warranted, and should be used with caution.

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Genetically Determined Sex-Reversal in 46,XY Humans

Abstract. *Evidence is presented for the existence of a gene, probably on the X chromosome, which prevents testis differentiation when present in 46,XY human embryos. Affected 46,XY women are not completely normal because of premature ovarian involution, as a result of which they have "streak gonads" similar to those of 45,X women.*

In most mammals the XY and XX sex chromosome complements determine male and female sex, respectively. A genetic determinant on the Y is responsible for differentiation of the indifferent embryonic gonad into testis, which otherwise becomes ovary. The testis, in turn, produces substances that suppress Müllerian and promote Wolffian ductal development and masculinize the indifferent external genital structures. In the absence of a testis, Müllerian ducts differentiate, Wolffian ducts fail to differentiate, and the external genitalia are not masculinized.

It is well known that sex-chromosome imbalance can interfere with normal sexual development. In fact, the delineation of the various clinical syndromes which result from human sex-chromosome imbalance has played a pivotal role in clarifying normal mechanisms of sexual differentiation. Less widely recognized but no less interesting, and potentially more informative, are single genes that also are capable of disturbing these mechanisms (*1*, chaps. 5, 6, and 8).

By 1971 (*2*), indirect evidence had accumulated pointing to the presence of a human gene in this class, one that was capable of preventing differentiation of the testis in individuals with the chromosome complement 46,XY (and thus, secondarily, of male external genitalia, as was mentioned above). The phenotype predictable for the 46,XY individual carrying such a gene would have just been that of the female. This prediction has been correct, except that the gonad of 46,XY females thought to carry the gene has been not the functional ovary predicted for phenotypic females; instead, it has been the so-called streak gonad, a structure devoid of germ cells and indistinguishable from the structure found in adult 45,X females, who have Turner's syndrome (*1*, pp. 259–293). (Occasionally the streak in XY gonadal dysgenesis is replaced by a neoplasm such as gonadoblastoma or dysgerminoma.) The clinical syndrome—streak gonads in a 46,XY female—is best termed XY gonadal dysgenesis, replacing the earlier "pure gonadal dysgenesis" which refers to several entities [see (*2*) for the argument]. Normal female internal and external genitalia are found in both XY gonadal dysgenesis and 45,X gonadal dysgenesis (Turner's syndrome). The major clinical difference between these two conditions is a normal height in XY gonadal dysgenesis and shortness in Turner's syndrome. In addition, in XY gonadal dysgenesis certain other anatomical defects characteristic of Turner's syndrome besides short stature usually are absent.

The foregoing comparison makes it easy to understand why the presence of an undetected monosomic cellular component (45,X) has always loomed high as a possible explanation for the gonadal streaks found in 46,XY females. But we and our colleagues have been impressed by the occasional familial clustering of 46,XY individuals with gonadal streaks

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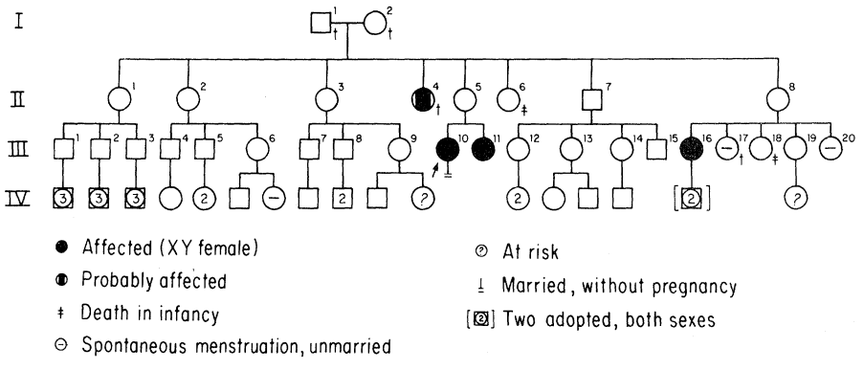

Fig. 1. Pedigree showing segregation of the "trait" of a female phenotype in individuals with the chromosome complement 46,XY (filled-in symbols). Individual II.4 was probably affected also. [For earlier reference to III.10 and III.11, see (*4*) and to III.16, see (*26*)]. Because those affected are sterile, this pedigree by itself fails to discriminate between an X-linked recessive mode of inheritance and an autosomal dominant affecting only XY individuals.

or gonadoblastomata (*1*, pp. 296–301; *2–4*). (Familial 45,X Turner's syndrome is extremely unusual.) The absence of parental consanguinity and a characteristic distribution of affected individuals in several reported pedigrees (*2–5*) led us to suggest X-linked recessive inheritance (*6*). We report here observations in one family which not only constitute strong evidence for the existence in humans of an X-borne gene that in mutant form results in sex-reversal (*7*) in 46,XY individuals but also provide further insight into the genetic control of normal human gonadal differentiation.

Affected in this family (Fig. 1) are two sisters (III.10 and III.11 in the pedigree), their mother's sister (II.4), and their mother's sister's daughter (III.16). All four were phenotypic females who by the time they were studied in adult life had gonadal streaks or gonadoblastomata (histologically unproved in II.4) in place of normal ovaries. At birth and throughout childhood each had appeared to be a normal girl. At the time expected for puberty, axillary and pubic hair developed; breast development and menstruation failed to occur but were inducible by the administration of the appropriate hormones. (In III.16, minimal breast development did occur, probably an indirect effect of her gonadoblastomata.) All four affected women in this family were of normal or increased height (176.5 cm, 168.0 cm, 171.5 cm, and "tallest woman in the family") and did not display the anatomical malformations and facies of Turner's syndrome. Their genitalia were unambiguously female. Each had a normally developed uterus and fallopian tubes the size expected in prepubertal girls. Endocrinological studies of individuals III.10 and III.11 showed greatly elevated concentrations of follicle stimulating hormone and luteinizing hormone; plasma testosterone in III.11 was 0.028 μg/100 ml. Pelvic surgery was performed between the ages of 19 and 29 in all four cases. Individuals III.10 and III.11 had bilateral streak gonads which both grossly and histologically resembled the streaks found in 45,X gonadal dysgenesis. In III.16 the gonads resembled the streaks grossly, one being somewhat larger than the other and more ovary-like in appearance; however, each proved to be gonadoblastoma. For II.4, who died postoperatively at age 19 in the 1920's, pathology records are unavailable; but it is known that a purplish, probably calcified abdominal tumor was removed. The chromosome complement of the three affected women living is 46,XY (Table 1). The large number of cells examined from multiple tissues, including one or both gonads in III.10, III.11, and III.16, constitute strong evidence against the existence of mosaicism. The X chromosome of III.11 and III.16 were shown by several banding techniques to be normal in morphology; their Y chromosomes also appeared normal. The distribution of affected individuals in this family is best explained on the basis of segregation of a single mutant gene located on the X [but see (*8*)], a gene without a known effect in 46,XX heterozygotes.

The gross and histological similarity between the gonadal structures found in individuals III.10 and III.11 when they were 29 and 28 years old and those found in adult 45,X females suggests that the early developmental history of the gonads in the two conditions also is similar. (Embryonic 46,XY female material has never been studied, as far as we know.) On the basis of available reports concerning the histology of gonads in 45,X embryos (*9*), the early 45,X gonad resembles that seen in normal 46,XX female embryos; germ cells normal in appearance are present. By the third intrauterine month, however, histological abnormalities develop, with an abnormal increase in connective tissue; germ cells persist, but formation of primordial follicles appears to be defective. By the neonatal period (*10*), only a streak may remain, or variable numbers of germ cells and follicles may persist, and by puberty, a streak is usually the only gonadal remnant. The essence of the situation, at least in 45,X, appears to be excessive attrition of germ cells before birth [as

Table 1. Summary of the study of 1300 metaphases from the three living affected women.

Individual and tissue*	Numbers of metaphases having the complement	
	46,XY†	Other than 46,XY‡
III.10		
Lymphocytes	103	4
Skin (HG 703)	50	
Round ligament (HG 704)	50	
Right gonad (HG 705)	200	
Left gonad (HG 706)	200	
Left fallopian tube (HG 707)	50	
III.11		
Lymphocytes	102	3
Skin (HG 748)	50	
Right gonad, medial portion (HG 747)	199	1
Right gonad, lateral portion (HG 744)	200	
III.16		
Lymphocytes, specimen 1	24	
Lymphocytes, specimen 2	40	
Right gonad	11	
Left gonad	12	1§

*Names of fibroblast cell lines given in parentheses. These lines are banked in liquid nitrogen at the New York Blood Center. †Including ruptured cells showing 44 or 45 chromosomes but in which a Y was identified. ‡Cells with fewer than 45 chromosomes in which a Y was not identified specifically. §A chromosome from group C(6-X-12) missing; unbanded chromosomes.

concluded also by Hamerton (*11*)], so that the supply of ova becomes exhausted not by middle age, as is normal, but earlier, certainly before puberty should begin. For the present, and to the extent that the strikingly similar histology permits, it can be assumed that the gonadal streak of adult XY gonadal dysgenesis is an ovary during embryonic life.

A first question raised by the observations we have made in this family is the following: How does the abnormal gene segregating here act to prevent testicular differentiation when present in the genome of 46,XY individuals? If it were not for consideration of this family [in conjunction with the few others known (*12*)], the Y chromosome might have been thought adequate to lead, by itself, to the differentiation of testis in the indifferent human embryonic gonadal ridge. However, in this family, three different Y chromosomes (that is, from three different fathers) were represented, and the phenotype was the same—streak gonad (or gonadoblastoma) in an otherwise normal female. Thus, the Y is not enough. Rather, an interaction must occur between products of genetic determinants on the Y and the X if embryonic testis rather than ovary is to develop [as first suggested in (*13*)]. The Y-borne locus essential for the testis to differentiate may require induction by the postulated non-Y-borne gene, in which case the gene on the Y would be classed as "structural." Alternatively, the Y-borne locus could itself have a controlling effect and induce a locus on the X, whose product would then bring about testicular differentiation. [The locus on the Y would be either the same as or closely linked to the histocompatibility locus called H-Y (*14*), which is positioned near the centromere of the Y as concluded earlier (*15*). A study of the H-Y antigen in our family is to be reported after the testing system has become more standardized than at present (*16*).]

In the absence of a Y chromosome in the genome of a mammalian embryo, interacting physiological mechanisms result in the female phenotype: the indifferent gonad becomes ovary, Müllerian and Wolffian ducts become uterus and tubes, and external genitalia become female. In this sense, male development would be viewed as a perturbation of this natural tendency, the result of the action of a special determinant borne on the Y chromosome. This special Y-borne determinant causes the indifferent gonad to differentiate into testis, whereupon the sequence of events leading to the development of complete maleness described at the outset of this report is brought into action. In the family studied, however, when an XY individual inherits the mutant gene for sex reversal the initial action expected of the Y fails to occur, and the female phenotype results. In relation to this variation from normal in the human, it is interesting that in *Myopus schistocolor* Lilljebord (wood lemming), a species of rodent in which a striking excess of females occurs, Fredga *et al.* (*17*) have shown that normal females may have either an XX or an XY sex-chromosome constitution. They suggest that femaleness in XY lemmings is due to an X-linked gene. Both XX and XY adult female lemmings have ovaries and are fertile. In contrast, in the XY females in our human family, the gonads, which, as discussed above, probably were ovaries during embryonic life, involute abnormally rapidly at some time before puberty, with amenorrhea and infertility being the clinical consequences, that is, follow the same course as in 45,X Turner's syndrome where, as a rule, the ovaries are essentially exhausted of follicles by the time of birth. It can be concluded, therefore, that in mammals, species differences exist in the manner in which ovaries will develop in individuals carrying a single X chromosome [see (*18*) for discussion of this variation]. [Incidentally, we wonder whether the same X-borne locus mutant in the lemmings is the one mutated in our family, in view of the evolutionary conservation of loci on the X, first recognized by Ohno (*19*)].

Thus, a second question can be posed: If ovarian differentiation is initiated in XY human females, why does oogenesis not proceed normally, as in females with two X chromosomes? Is degeneration into streak gonad perhaps the effect of the mutant gene; is it the effect of genes on the Y other than the one responsible for testicular differentiation; or, as seems more probable, is degeneration into streak the consequence of hemizygosity for some particular locus on the X, a locus which must be homozygous in the human if completely normal oogenesis is to occur? In the human XY female, this infertility very well may, as was suggested above, be on the same basis as in the human monosomy-X female—premature ovarian involution. In contrast, in some species a single X does suffice to sustain a functional ovary into adult life, for example, in the monosomy-X *Mus musculus* (mouse). The 39,X mouse is a fertile female (*20*), normal (*21*) except for having a shortage of oocytes, a reduced number of litters and live offspring, and an overall shortened reproductive life (*22*). The difference in ovarian development between the 45,X human and the 39,X mouse appears therefore to be quantitative rather than qualitative. Both 46,XY gonadal dysgenesis and 45,X gonadal dysgenesis probably do point to the existence of a locus on the X chromosome that is of major importance in oogenesis, and that locus must be active on both X chromosomes in human germ cells if normal ovarian function is to be sustained beyond intrauterine life. Lyon had reached the same conclusion and interpretation of the pertinent data as of 1974 (*18*), as had Hamerton even earlier (*11*).

Relating to this possibility, the argument has been presented (*15*) that the X and Y chromosomes do bear homologous segments, located very possibly near the ends of their short arms, segments that contain genes related to traits other than sexual development. Hemizygosity for this segment results in short stature and not uncommonly in major anatomical malformations—that is to say, the main somatic anomalies of 45,X Turner's syndrome. In XY gonadal dysgenesis, hemizygosity for this segment is not present, of course, nor are the short stature and malformations of Turner's syndrome. This could have been predicted. But, the fact that the gonads in XY gonadal dysgenesis reach an end stage essentially like that in X monosomy (that is, streaks) indicates that the Y chromosome does not carry the postulated ovary-sustaining locus, in its homologous segment or anywhere else. Actually, homozygosity for this locus on the X may be important in oogonial function and the normal maturation of eggs in all mammals in view of the fact that both the XY female lemmings mentioned above (*17*) and females of a certain vole, *Microtus oregoni*, the females of which normally have X-monosomic somatic cells (*23*), utilize an unusual cytological mechanism by which oocytes and possibly the entire germ line do acquire two X chromosomes, presumably through nondisjunction (*24*). Probably it is for these same reasons that both of the X chromosomes are genetically active in oocytes in the normally developed human female (*25*) whereas in somatic cells dosage compensation is accomplished through inactivation of one of the two X's.

Thus, our analysis of XY gonadal dysgenesis, a very rare disorder of human sexual development, permits us to conclude that the Y chromosome alone is insufficient to cause the indifferent embryonic gonad to become testis. Instead, an interaction must be postulated between a locus near the centromere of the Y and one somewhere on the X (*8*). Two plausible models for this interaction are (i) a

structural gene for testis differentiation located on the X is induced by a controlling element on the Y; or, (ii) the structural gene is on the Y, its controlling element on the X (*8*). In our family the X-borne locus (*8*) is mutant and fails in the interaction with the locus on the Y. The analysis of this rare genetic disorder indicates also that a single human X, although sufficient for differentiation of ovary, is insufficient for the sustenance into adult life of normal oogenesis.

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Manufactured Hexaparental Mice Show That Adults Are Derived from Three Embryonic Cells

Abstract. *Two female chimeric mice have been produced by aggregates of three genetically marked eight-cell embryos. All three embryonic genotypes are clearly expressed in the pigment pattern of the adults. These hexaparental mice together with their littermates demonstrate that, in the 64-celled blastocyst, at least three cells, and probably only three, are the source of all adult tissues.*

Chimeric mice produced by aggregating cleavage-stage embryos in vitro are being extensively used in studies of mammalian development (*1*). Genetic markers such as coat color mutants are usually incorporated in the embryos in order to make chimerism in the adult obvious. Thousands of such chimeric mice have now been produced in many laboratories. However, all adult chimeras so far manufactured have manifested only two genotypes—that is, have been derived from just two embryos. We report here the first adult chimeric mice derived from three aggregated embryos, and therefore having six genetic parents—hexaparental mice.

The coat color phenotypes used in this investigation were yellow, black, white, and dilute brown. In genotype, all were non-agouti (*aa*). Recessive yellow (*e/e*, *B/B*) mice are yellow because yellow is epistatic to black. These mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. An albino strain was segregated in our laboratory from CD-1 (ICR) mice (Charles River Breeding Laboratories, Wilmington, Mass.) to provide the albino embryos (*c/c,a/a, B/B, E/E*). Homozygous albino animals produce no pigment. Albino females mated to black males produced the black embryos used in these experiments. These embryos were heterozygous for albinism (*c/+*) but homozygous black (B/B). Dilute brown embryos (*a/a, b/b, d/d*) were obtained from a stock maintained in our facility.

Two series of experiments were conducted: one aggregating three embryos (black, white, and yellow) and another aggregating four embryos (black, white, yellow, and dilute brown). The results from the experiments aggregating three embryos are discussed first.

In manufacturing the chimeric mice, embryos composed of four or eight cells were first flushed from the oviducts of hormonally superovulated females. After the zonae pellucidae were removed with pronase, black, albino, and yellow embryos were placed in a triangular configuration in microdrops of phytohemagglutinin (PHA)-containing medium (*2*) under oil for 15 minutes and cultured in vitro without PHA for 24 hours. These trios were periodically observed with an inverted microscope in a 37°C constant temperature room. A gas phase of 5 percent CO_2 in air was maintained over the culture medium, except during the observation periods. The embryos were transferred to pseudopregnant females for gestation. Mosaicism was scored after formation of hair pigmentation—about 7 to 10 days after birth.

Preliminary experiments were conducted to ensure that the embryos of all three genotypes (black, white, and yellow) were equal in developmental ability. Pairwise combinations of the three genotypes were constructed. Three yellow ↔ white, six black ↔ white, and two black ↔ yellow chimeras were produced, indicating that the three geno-

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