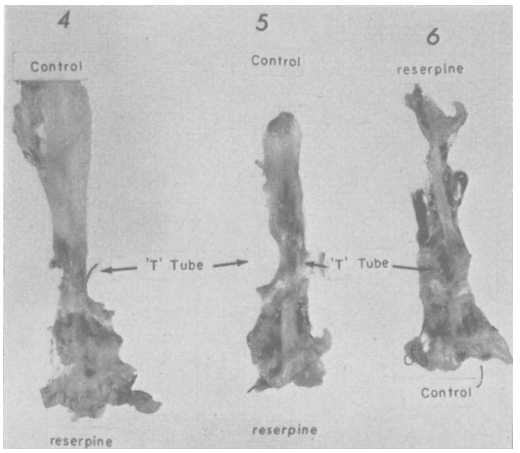

FIG. 2. Perfused preparations representing Group I or divided cat esophagus. Specimen 4: control side grade 0 digestion; reserpine treated side grade 1+. Specimen 5: control side grade 1+ digestion; reserpine treated side grade 3+. Specimen 6: control side grade 2+ digestion; reserpine treated side grade 5+.

This is revealed in increased digestion observed in the divided esophageal perfusion as well as in experiments using a separate cat's esophagus.

The mechanism this decreased tissue resistance produced by reserpine remains obscure. Increased ACTH release through hypothalamic stimulation(6) with resultant corticosteroid secretion may play a role in this increased tissue susceptibility. Serotonin release(7) and local factors such as vascular spasm or decreased blood flow because of hypotension may be important. These various possibilities are being investigated by use of this preparation.

Summary and conclusion. The I.V. administration of reserpine lowers significantly the susceptibility of the intact cat's esophagus to injury by digestive action of human gastric juice.

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Familial Primary Amenorrhea Due to Testicular Feminization: A Human Gene Affecting Sex Differentiation.*† (25456)

THEODORE T. PUCK, ARTHUR ROBINSON AND J. H. TJIO
Dept. of Biophysics, Florence R. Sabin Labs., University of Colorado Medical Center, Denver

The chromosomal constitution of individuals suffering from gonadal dysgenesis (Turner's syndrome) has been demonstrated to consist of only 45 chromosomes, the autosomes of which were normal in number and morphology, but the sex chromosome condition being XO(1,2). Such patients do not undergo ordinary developmental changes of puberty, but remain essentially sexually infantile females. The Barr Test for nuclear chromatin, which when positive indicates existence of at least 2 X chromosomes, is negative. Chromosomal delineation of the somatic cells of these patients demonstrated that in man, the Y chromosome contains genes necessary for development of male characteristics, a situation unlike that of *Drosophila,* but similar to that in the mouse(3). Another patient with clinical diagnosis of female pseudohermaphroditism was studied(2) and reported to display cytologically normal female (XX) chromosomes. Hungerford, *et al.* reported a case of true hermaphroditism(4) to have displayed an XX chromosomal condition. The present report deals with another female patient, exhibiting primary amenorrhea asso-

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ciated with testicular feminization (male pseudohermaphroditism). This condition is of especial interest because, while it resembles gonadal dysgenesis in some features, it differs markedly in others, among these being the presence of a strong hereditary component. The genetic basis of a condition clinically like this has been discussed but not resolved(5a, 6a,6b). A detailed clinical report of this 25-year-old woman will be presented elsewhere. The diagnosis appeared unequivocal and was based, among other findings, on female external genitalia, well developed breasts and feminine habitus, primary amenorrhea, negative Barr Test for nuclear chromatin, a normal urinary excretion of follicle stimulating hormone and 17-keto steroids, and a pedigree indicating transmission of the defect only through the maternal line.

Methods. Samples of skin were obtained, and the cells cultivated and examined for chromosomal morphology, by standard methods previously described(7,2). The one modification over previous procedures consisted in sampling an area of healing superficial skin lesion instead of normal skin, a substitution which appears to shorten the time needed to establish a stably growing culture. A piece of skin from the back of the neck or behind the ear is anesthetized with dichloro-tetrafluoro-ethane (Frigiderm, W. T. Brachvogel, Los Angeles, Calif.), then abraded over an area 1 x 0.1 cm, to depth just sufficient to reach the dermis by means of a Dermabrasion instrument (Mill-Bilt Equipment Co., N. Y.). The abraded area is covered with a Bandaid, and allowed to develop a scab. Seventy-two hours after the abrasion, the scab is washed in 70% alcohol, dried, lifted off aseptically with forceps and deposited in Petri dish containing nutrient solution, fortified with tested human cord serum, as previously described (7a). The scab, which may weigh about 5 mg, is cut in about 10 pieces with sterile scissors, the medium becomes cloudy with detached cells. The dish is incubated, with regular medium changes as previously described, in incubator at 37°C, containing 5% CO_2 in air almost saturated with water, with temperature and gas composition carefully regulated. In about 7 days the cells multiplied when they may be trypsinized, and re-seeded into new vessels. The cultures so obtained, like others described by us(7a), grow with a generation time of approximately 20 hours, and maintain stable chromosomal constitution throughout production of virtually unlimited numbers of progeny, so long as the specified conditions are maintained. The healing wound forms a new scab after the first one is removed. This may be re-sampled if necessary. No scar results from this procedure.

Results. Typical chromosomal complements from cells of our patient are shown in Fig. 1. There are 46 members. Ideograms from 10 different mitoses have been prepared by arranging chromosomes in accordance with the standard classification scheme of Tjio and Puck(8). A typical example is shown in Fig. 2. It is evident that the autosomes are normal but the sex chromosomal constitution is XY, rather than female, XX. In examination of more than 100 mitoses, the chromosome number of 46 was uniformly obtained except in 4 cells, 2 of which had 44 and 2 of which had 45 chromosomes, the supposition being that these represented chromosome losses occurring during fixation. The Y chromosome was always clearly identifiable.

In our patient, the only pedigree data so far available consist in written reports supplied by the patient and her mother. Efforts are being made to study other members of the family, who are widely distributed. Against the time when these additional data can be obtained directly, a preliminary summary is furnished here of the familial history, which seems justified, in view of the unusual intelligence and cooperativeness of the patient and her mother, and the ease with which the condition of primary amenorrhea can be recognized. The data indicate that primary amenorrhea has occurred in some females of every generation in 4 consecutive generations. The number of apparent females outnumbers the males by approximately 3 to 1. The condition has been transmitted exclusively in the maternal line, typical of this form of primary amenorrhea(5a). Hence, a defect on the Y chromosome as the causative factor of this condition is ruled out. No known consanguinity has occurred in the family through-

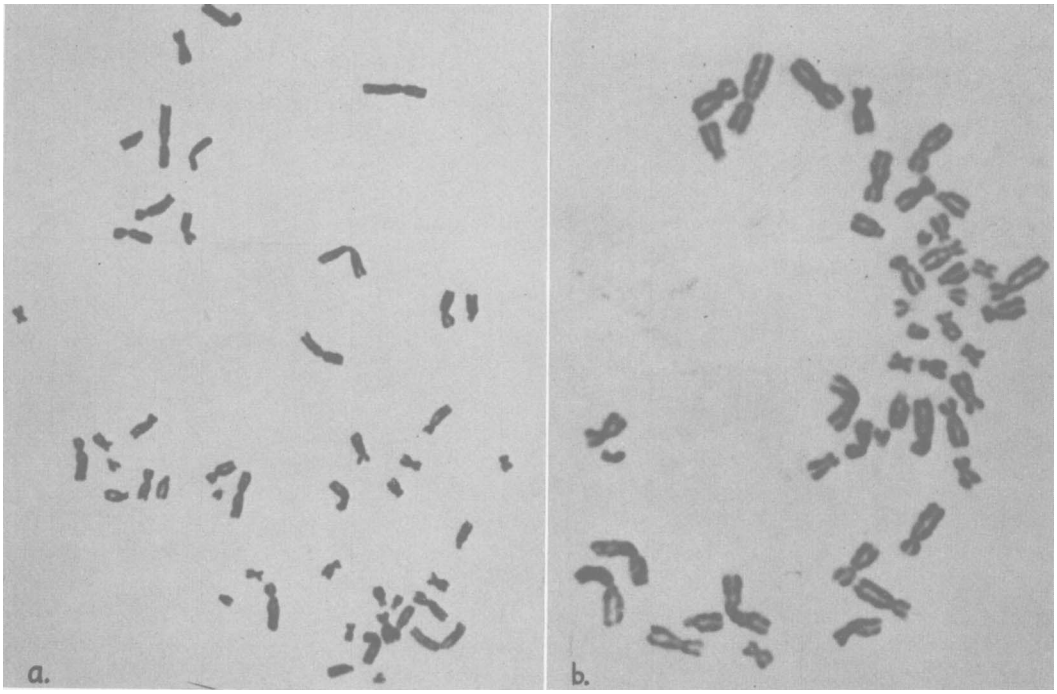

FIG. 1. Typical chromosomal complements of this patient's cells grown in tissue culture as described.

out these 4 generations. Of particular importance is the fact that the patient's mother, maternal grandmother, and two maternal cousins, have all borne children, but did not initiate menses until ages of 19 to 21. This delayed menarche in families of patients like that described here has been rarely recorded, although it is mentioned in one member of a family studied by Pettersson and Bonnier(6).

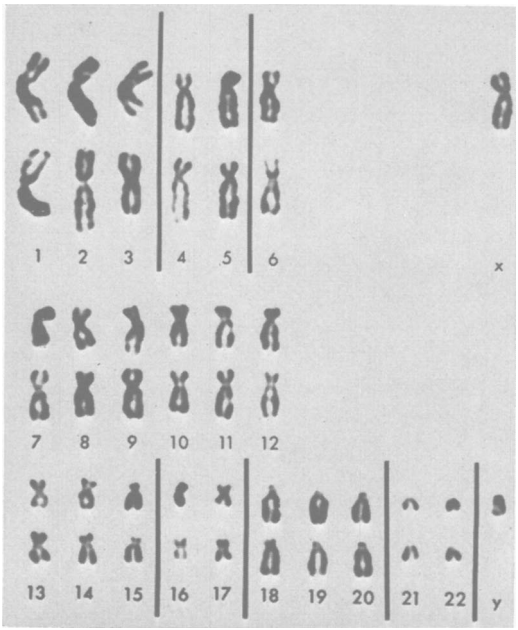

FIG. 2. Ideogram of chromosomes of the patient here described, showing the typical male chromosomal pattern.

The nature of the primary genetic lesion in cases exhibiting a condition clinically like the present one has been reviewed(5a). The genetic evidence has been carefully weighed and the conclusion reached that these individuals have an XY chromosomal condition with a defective gene, which could be either a sex-linked recessive or a sex-limited autosomal dominant; a smaller alternative probability was judged to exist that the XXY condition or some other chromosomal anomaly is the causative factor(5a).

The chromosomal constitution of this patient rules out the XXY possibility. An XY/46 chromosomal constitution has also been ascribed to a patient of this type by Chu and Giles(5b), and to 4 other patients with presumably the same condition, reported by Lennox(5c). It has also been described by Severinghaus(5d) in a male pseudoherma-

phrodite of somewhat more male type than the present patient. While the detailed chromosomal constitutions and clinical findings in the cases of Lennox and of Chu and Giles have not yet been presented, the concordance of these results as well as the genetic data previously available makes it at least a reasonable presumption that the disease condition here considered is fairly regularly associated with the given chromosomal constitution. Moreover, similarities in findings in all these cases also make it very unlikely that the chromosome here described as a Y is in reality a portion of a fragmented X.

To decide whether the genetic defect is carried on the X chromosome, or whether it is autosomal but is never transmitted through the male line because of its sterilizing influence on male carriers, data are needed from families containing this defect along with known markers, especially sex-linked ones like color blindness or hemophilia as Grumbach and Barr have noted(5a). Evidence with some bearing on this question is available from a recently reported case of hemophilia in a "girl" baby with a 46/XY chromosomal constitution(9). While that child was too young for the decision to be made as to whether her condition was unequivocally classifiable as pseudohermaphroditism with feminizing testes, it seems highly likely, in view of the correspondence obtained between clinical diagnosis and chromosomal constitution in all other studies so far available in such patients. Moreover, the maternal pedigree presented for that child indicates a very high preponderance of females, as would be expected in this condition.

On this assumption, and assuming further the absence of other complicating factors, one can examine the distribution of both these defects in that family: If the sex-differentiation defect were on the X chromosome and closely linked to the hemophilia gene, it could be present either on the same X chromosome as the latter, or on the other X chromosome. In the former case, the sex differentiation defect would virtually always accompany the sex-linked defect in the chromosomal males. In the latter, the 2 defects would not appear in the same XY individual. Thus, it would not be possible to find 2 siblings, both with the XY constitution, both exhibiting the hemophilia defect, but only one of which possessed the sex differentiation defect here considered. But in the pedigree of the patient reported in data of Nillson *et al.*(9), a sexually normal male and the pseudohermaphroditic patient appear in the same set of siblings, both exhibiting the hemophilia condition. Therefore, the conclusion may be drawn, subject to the uncertainties noted, that the sex differentiation gene in this case is autosomal, or else, if sex-linked, is so far distant from the hemophilia gene as to permit crossing over with high probability. It cannot be assumed, however, from the clinical resemblance between 2 independently arisen hereditary conditions, that they have been caused by a mutation at the same locus, no matter in how many details their syndromes agree.

Accumulation of additional pedigrees of cases clinically like our present one, involving at the same time differences in other marker genes, should eventually permit a determination of whether the genetic basis of the condition in question is uniform, and if so, whether it is present on the X chromosome or on a given autosome; or whether the condition represents a genetically heterogeneous class of cases, the mutant genes for which may be at any one of a number of sites, like those causing intersexuality in *Drosophila* and those causing the waltzer or shaker syndrome in mice(10).

In Turner's syndrome the XO condition is present and menses, plus other aspects of normal female development, fail to occur. While the condition of such an XO patient indicated that genes on the Y chromosome are necessary for development of maleness, data from the kind of patients here considered indicate that these are not sufficient for full expression of male character. The gene responsible for the condition of pseudohermaphroditism with feminizing testes in XY individuals like our patient appears to exercise an important influence on development in both sexes: in the heterozygous condition in an XX individual, it appears capable of delaying menarche for about 8 years. When present with an XY chromosomal constitu-

tion, however, it prevents expression of maleness, producing an essentially female condition, but without reproductive function.

After this paper was submitted, Jacobs *et al.*(11) described bone marrow cell analysis from 4 patients with testicular feminization, all of whom had a 46/XY chromosomal constitution. Thus the correlation between XY sex chromosomes in females and the condition of testicular feminization is strengthened. Moreover, in one of these cases, as in the case of hemophilia discussed by us, a color blind male pseudohermaphrodite and a color blind normal male occurred in the same set of siblings. Therefore, since the gene responsible for testicular feminization is not closely linked to *either* of 2 different sex-linked genes, its autosomal location becomes highly probable.

Summary. A female patient with "male pseudohermaphroditism with feminizing testes" had a 46/XY chromosomal constitution, a fact confirming clinical and genetic deductions drawn by previous investigators largely on the basis of pedigree data, and in accord with recent brief reports on the chromosome condition in other such patients. A detailed chromosomal analysis is provided. Some previous cases having similar clinical manifestations had been recognized by previous workers as being caused either by an autosomal dominant or a sex-linked recessive genetic defect. Analysis of pedigrees from similar cases demonstrates the causative gene defect to be not strongly linked to either of 2 sex-linked genes, hemophilia and color blindness. Hence it is probably autosomal. Of particular importance is the finding that in the family of our patient, all female transmitters of the defect for whom data were available exhibited a delayed menarche of approximately 8 years. This situation has been rarely noted previously, and requires study as to its universal accompaniment of this condition. It is evident that in the present case the presence of this gene in heterozygous condition in an XX individual causes a delay of menarche, while in an XY individual it results in suppression of the male characteristics and a partial development of female traits. It is pointed out that more than one gene locus might be able to produce clinical effects like those here described. Study of the action of such genes appears highly important to problems of human development.

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Summation of Protein Anabolic Effects of Testosterone Propionate and Growth Hormone.* (25457)

CHARLES D. KOCHAKIAN†
University of Alabama Medical Center, Birmingham

Simultaneous administration of testosterone propionate and a growth hormone preparation at their respective maximum effective

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