Original article and commentary
by Edgar J Schoen, MD

Kaiser Permanente Medicine 50 Years Ago:

The Development of Sex

This Perspective article, as the author notes, is the first one written by the original author. Dr Edgar J Schoen was born in 1925 and has served as a full-time Kaiser Permanente Staff physician since 1954, a 48-year period believed to be a record of longevity. But his service is highly distinguished in many ways additional to sheer length. His education and training were at the University of Illinois (BS), New York University (MD), and the Massachusetts General Hospital. He was a Staff Pediatrician and Pediatric Endocrinologist at the Oakland Medical Center, where he was Chief of Pediatrics for 24 years. His curriculum vitae details numerous societies and areas of public service plus 124 publications. Major current special medical interests include perinatal screening and health care delivery to children.

Dr Schoen's avocations include running and a vintage car with a Kaiser Permanente connection. Many younger Kaiser Permanente physicians may not know that Henry Kaiser made a foray into the automobile business after WWII. This ended in 1954, the year that the very sporty Darrin (named for the designer) model (see photo) was introduced. Fewer than 500 Darrins were made. Dr Schoen acquired one in 1956 and has maintained it in superb condition. A recent newspaper story about Dr Schoen and his car was headlined: "Still Running After All These Years: Rare Auto, Owner Have Much in Common." He says: "the car is better-looking than I am, and has fewer mechanical problems." Perhaps, but it is apparent that his intellect and wit are fully intact.

—Arthur Klatsky, Editor

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Normal Sex Development

The processes that result in a male or a female individual are: 1) sex determination, the genetic phenomenon that results in the sex genotype (the "constitutional" or "genetic" sex), and 2) sex differentiation, the process, governed by hormonal factors, that results in the phenotype sex (visible sex characteristics).

1. Sex Determination

The genetic sex of the embryo is determined by chromosomal pairing, which occurs upon meeting of sperm and ovum. The normal female inherits the chromosome pair XX; the normal male, the chromosome pair XY. All tissue cells of the female, at subsequent stages of development, are normally characterized by the presence of the XX chromosome pair; all tissue cells of the male contain the XY chromosome pair.

2. Sex Differentiation

Sex differentiation involves two groups of factors: a) gonadal and b) hormonal.

a. *Fetal gonadal factors.* The primordial gonad, according to Witschi,[1] is sexually undifferentiated. It consists of a cortex and a medulla. In the female, the cortex develops into the ovaries, while the medulla degenerates. In the male, the cortex degenerates and the medulla develops into the testes.

b. *Hormonal factors (fetal and maternal).* The testis, after it has developed from the medulla of the primordial gonad, produces hormones that contribute to the differentiation of the male sex organs; the hormonal function of the fetal ovary is less well defined. The influence of the maternal hormones has been demonstrated by removing the gonads early in the fetal life of rabbits: all fetuses from which the gonads have been removed develop as females, with female secondary sex characteristics, presumably in response to the activity of the placental hormones.[2] The testis of the male fetus is believed to produce hormones that counteract the maternal female hormones, to permit male sex differentiation.[3]

Aberrations from the normal path of sex development may occur at any of the three major periods implied by the above: the chromosomal pairing stage, the phase of

EDGAR J SCHOEN, MD, is senior consultant in pediatrics and director of regional perinatal screening in the genetics department for TPMG. He joined TPMG in 1954. At the time this original article was written, Dr Schoen was in the Department of Pediatrics and the Section of Endocrinology, Kaiser Foundation Hospital, Oakland, CA. E-mail: edgar.schoen@ncal.kiperm.org.

gonadal differentiation, or as a result of hormonal changes at any period of life.

Abnormal Sex Determination

The clinical test for determining chromosomal sex, developed in 1953 by Moore and Barr,[4] made possible a new phase in the investigation of states characterized by abnormal sexual development. These workers observed that in normal female subjects, the cells of many tissues (not of gonadal tissue alone) contained a chromatin body which they believed to represent an XX chromosome. The tissue cells of normal male subjects which contain the XY chromosome, do not contain the chromatin body. Buccal mucosal cells are used most frequently for the clinical test of "chromosomal sex" based on this observation.

Chromosomal sexing has been used in the investigation of states characterized by intersexuality. When chromatin bodies were found, the individual was said to be of the "female chromosomal sex"; when none were present, of "male chromosomal sex." Wilkins[5] noted in 1954 that chromatin bodies were absent from the nuclei of a majority of a series of apparent females with ovarian agenesis (Turner's syndrome), and considered these persons to be chromosomal males. In a majority of a group of patients with Klinefelter's syndrome[6] (hypogonadism, gynecomastia, azoospermia, eunuchoid body build, hypergonadotropinism), chromatin bodies were found in the tissue cell nuclei, and the subjects were judged chromosomal females.

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These observations led to new hypotheses of sex determination and sex differentiation. Witschi and his group[1] expressed the opinion that this apparent "sex reversal" in persons with Klinefelter's syndrome, in those with Turner's syndrome, and in persons with

Dr Schoen in his 1954 Darrin.

Commentary
by Edgar J Schoen, MD

As someone who has "been there, done that," I have been following with interest the comments of today's PMG physicians on the publications of their predecessors in the pioneering, though long defunct, Kaiser Foundation Medical Bulletin. I was particularly intrigued by a surgical article written by one of our founders, Cecil Cutting, 50 years ago and analyzed by my current Physician-in-Chief, Tom McDonald. Tom pointed out that he was born the month the article was written. Similar commentaries on other articles were written by current physicians who were anywhere from toilet training to kindergarten when their PMG ancestors were publishing in the Kaiser Foundation Hospitals journal. Art Klatsky, a *Permanente Journal* Editor, sensed a unique variation on this theme. How about a current PMG physician commenting on his own article written more than four decades ago? Although this approach is unusual from the standpoint of history, individual endurance, and longevity, it is highly unlikely that the commentator will find great fault with the original author. But here goes.

In 1959, when I wrote an article on sexual differentiation in the *Kaiser Foundation Medical Bulletin,* I was a 30-something Brooklyn wiseguy trained in Boston. Now, as a 70-something California wiseguy with a 48-year TPMG career, my viewpoint on the topic is not radically different than it was four decades earlier. The principles of sexual differentiation, just as with personal sexual function, remain the same—it's just the details that have changed.

As I looked back at my old article, I was surprised at how much we knew about sexual differentiation in the late 1950s. There had been great advances in both genetics and endocrinology in the previous decade. We had a rough clinical test for chromosomal sex—the buccal smear for "sex chromatin." A chromatin body in the buccal cells on the smear indicated more than one X sex chromosome. This was considered to be a "female sex chromatin" pattern—later amended to "chromatin-positive" when it was realized that men with Klinefelter's syndrome had more than one X chromosome in addition to the Y. And women with XO Turner's syndrome were chromatin-negative though obviously not males. This chromosomal confusion became a lot clearer early in 1959, when a practical technique was developed for viewing chromosomes directly. Up until that time, whether humans had 46 or 48 chromosomes

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was not known for sure. Not only did it become apparent that 46 chromosomes was normal but that there was a logical explanation for the discrepancies in chromatin bodies. This realization was best illustrated by a reported case of a patient with both Down syndrome and Klinefelter's syndrome and with 48 chromosomes—an extra chromosome 21 for Down syndrome and an extra X chromosome courtesy of Klinefelter's syndrome.

Some exciting things happened in the 1950s with hormones as well. It was known that for the first six weeks or so of fetal life, the gonads were undifferentiated, after which they developed into a testis or ovary with the testes secreting testosterone. This fetal testosterone was found to be essential for further male sexual development. Studies from France showed that if fetal gonads were removed before differentiating, all the fetuses developed as females. On the other hand, presence of fetal androgens in the first two months of gestation, as occurs in the adrenogenital syndrome as a result of an enzymatic block in steroid formation, leads to virilization of the female fetus and development of ambiguous genitalia (clitoral hypertrophy and formation of a urogenital sinus).

Well, that was then. How about now? What we've done is filled in some of the blanks in the sexual differentiation process. We know, for instance, that the change from the undifferentiated gonad to a testis requires a gene called SRY, which is located on the short arm of the Y chromosome. Fetal testosterone stimulates the wolffian structures (epididymis, vas deferens, and seminal vesicles), whereas a substance called antimüllerian hormone suppresses development of the fallopian tubes, uterus, and vagina. We now know that the active metabolite of testosterone is dihydrotestosterone and that, in a condition known as "testicular feminization," an XY male with plenty of testosterone being formed by a fetal testis develops as a female because of an enzyme defect that prevents testosterone being converted to dihydrotestosterone. We know the gene locus for many enzymes and syndromes owing to new sophisticated techniques, and, with characterization of the human genome, there is more to come.

The scientific advances continue at an accelerating rate in both genetics and endocrinology, but, from the clinical standpoint, management of disorders of sexual differentiation in 2002 would still be recognizable by a 1959 pediatric endocrinologist. *Plus ça change, plus c'est la même chose.* Except when I look in the mirror, of course. ❖

other anomalies of sex development, resulted from abnormal evolvement of the germ cells of the gonads. A defect in the cortex of the primordial gonad of a chromosomal female might result in the formation of a testis in the female fetus, producing a "pseudomale" (Klinefelter's syndrome). A defect in both cortex and medulla of the primordial gonad might result in an agonadal state, whereupon the individual, whether a chromosomal male or a chromosomal female, would evolve as a female under the influence of maternal hormones. These hypothetical considerations were supported by experimental work cited by Witschi.[1]

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Moore,[7] in 1959, reported his study of buccal mucosal smears from 3715 infants, 1804 of whom were female. Although the buccal smears of all of the female infants indicated chromosomal female sex, the tissue cells of five of the 1911 male infants contained chromatin bodies characteristic of the female. These five cases he considered to be instances of "sex reversal", but recent work indicates that the chromatin bodies may not represent the normal female sex chromosome pair (XX), and that lack of chromatin bodies does not necessarily represent the normal male chromosome pair (XY). Ford, Jacobs, and their co-workers,[8-12] using techniques permitting direct visualization of chromosomes in human cells, demonstrated that the tissue cells of a patient with Klinefelter's syn-

drome contained abnormal chromosomes in the grouping XXY, and that the tissue cells of a patient with Turner's syndrome contained abnormal chromosomes in the distribution XO. In the light of these studies, the terms "chromosomal female" and "chromosomal male" were discarded by these and other investigators, and were replaced by the descriptive terms, *chromatin positive* and *chromatin negative.*

In addition, these workers found an important abnormality in the total number of chromosomes present in the tissue cells of the intersexes studied, and in patients with certain diseases, which appear to be connected with chromosomal abnormalities that were hitherto unsuspected. The observations were made possible by the technique developed by Ford, Jacobs, and Lajtha[8]: marrow cells are grown in tissue cultures: hypotonic saline or citrate is added to expand the cell and to separate the chromosomes, and mitosis is stopped in the metaphase by means of colchicine. A "squash preparation" is made from the cell suspension, which has been stained by Feulgen's method, and the chromosomes can then be counted and paired. Prior to the application of this technique, the normal human tissue cell was believed to contain 48 chromosomes. It was now learned that such cells normally contain 46 chromosomes, in 23 pairs; one of the pairs consists of sex chromosomes, an XX pair in the female and an XY pair in the male. The cells of persons with Klinefelter's syndrome (which are chromatin positive) contained 47 chromosomes instead of the normal 46; they had three sex chromosomes (XXY) instead of the normal "chro-

mosomal female" pair.[11] The cells of persons with Turner's syndrome (which are chromatin negative) contained 45 chromosomes instead of the normal 46; the single sex chromosome was half the female component, designated as the XO sex chromosomal pattern.[9] The cells of both male and female mongoloid subjects were found to contain 47 chromosomes;[12] the extra chromosome appears not to be a sex chromosome, but an unusual type in the smallest size range. The cells of a patient with both mongolism and Klinefelter's syndrome were observed to contain 48 chromosomes.[10] One of the extra chromosomes was present in the configuration XXY; the other was an additional small chromosome which, in the author's opinion, appeared likely to be characteristic of mongolism. An abnormal number of chromosomes has been found in several cases of acute leukemia—an observation which derives increased interest from the relatively high incidence of leukemia among patients with mongolism.

These discoveries of relationships between abnormal chromosomal structure and/or pairing at the microscopic level on the one hand, and grossly apparent anatomic and physiologic aberrations on the other have lent impetus to the investigation of sex determination.

Abnormal Sex Differentiation

The endocrine bases for abnormalities of sex differentiation have been somewhat more accurately understood for a number of years, but additional clarification has been gained regarding the point in the individual's growth at which these aberrations occur. Developmental errors or trauma to either the medulla or the cortex of the primordial gonadal folds may result in a somatic sex pattern opposite to the chromosomal pattern.

Other instances of sexual maldevelopment, intersex, or apparent sex reversal may be attributed to hyperplasia or tumor of the gonads or of the adrenal cortex in the fetus, at subsequent stages of growth, or in adult life. Advances in steroid hormone assay techniques have resulted in a clearer understanding of sexual development due to endocrine dysfunction. For example, the virilization that occurs in persons with congenital adrenal hyperplasia is known to result from a lack of the enzyme "21-hydroxylase."[13]

Recent findings have thus fundamentally altered concepts for sex determination and sex differentiation. A field for investigation has been opened, which ranges from sexual maldevelopment through a wide variety of inherited and congenital abnormalities, both mental and physical, to leukemia and other neoplastic diseases, which may ultimately prove to be associated with chromosomal deviations or defects. ❖

References

1. Witschi E, Nelson WO, Segal SJ. Genetic, developmental and hormonal aspects of gonadal dysgenesis and sex inversion in man. J Clin Endocrinol. 1957;17:737.
2. Jost A. Recherches sur le contrôle hormonal de l'organogenèse sexuelle du lapin et remarques sur certaine malformations de l'appareil genital humain. Gynec et obst 1950;49:44.
3. Lillie FR. The free-martin; a study of the action of sex hormones in the foetal life of cattle. J exp Zool 1917;23:371.
4. a. Moore KL, Barr ML. Morphology of the nerve cell nucleus in mammals with special reference to the sex chromatin. J Comp Neurol 1953;98:213.
   b. Moore KL, Barr ML. Nuclear morphology, according to sex, in human tissues. Acta anat 1954;21:197.
5. Wilkens L, Grumbach MM, Van Wyk JJ. Chromosomal sex in "ovarian agenesis." J Clin Endocrinol 1954;14:1270.
6. Grumbach MM, Blanc WA, Engle ET. Sex chromatin pattern in seminiferous tubule dysgenesis and other testicular disorders: relationship to true hermaphrodism and Klinefelter's syndrome. J Clin Endocrinol 1957;17:703.
7. Moore KL. Sex reversal in newborn babies. Lancet 1959;1:217.
8. Ford CE, Jacobs PA, Lajtha LG. Human somatic chromosomes. Nature (London) 1958;181:1565.
9. Ford CE, Jones KW, Polani PE, DeAlmeida JC, Briggs JH. A sex-chromosome anomaly in a case of gonadal dysgensis (Turner's syndrome). Lancet 1959;1:711.
10. Ford CE, Jones KW, Miller OJ, et al. The chromosomes in a patient showing both mongolism and the Klinefelter syndrome. Lancet 1959;1:709.
11. Jacobs PA, Strong JA. A case of human intersexuality having a possible XXY sex-determining mechanism. Nature (London) 1959;183:302.
12. Jacobs PA, Baikie AG, Court Brown WM, Strong JA. The somatic chromosomes in mongolism. Lancet 1959;1:710.
13. Bongiovanni AM, Eberlein WR. Defective steroidal biogenesis in congenital adrenal hyperplasia. Pediatrics 1958;21:661.

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