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SEX CHROMATIN, SEX CHROMOSOMES AND SEX ANOMALIES*

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DEVELOPMENTAL anomalies of the reproductive system are encountered infrequently, in comparison with many conditions that confront physicians. Yet genital anomalies have to be studied carefully. They are a source of particular concern to the parents of an affected child, and the patient may bear a heavy burden on reaching maturity because the defect usually interferes with satisfaction of the basic instinct of reproducing one's kind. These problems should be handled with exceptional discretion and understanding.

On the principle that a sound basis for good management of a medical problem is provided by knowledge of its biological background, we propose to examine the *etiology* and *pathogenesis* of sex anomalies. This field has expanded so rapidly in recent years that the account will deal mainly with those anomalies having as their basis an abnormal sex chromosome complex, i.e. a complex other than the XX pair of females or the XY pair of males. These anomalies are of particular interest because the knowledge of genetic errors at the visible chromosome level, as distinct from effects of the submicroscopic genes, is a recent acquisition. In spite of excellent accounts of chromosomal abnormalities in Drosophila and other insects, most physicians might have guessed that an abnormality of the genetic material so gross as to be visible in the chromosome complement under the microscope would be lethal to the human embryo.

SEX CHROMATIN

The chromatin pattern of interphase nuclei is distinctive in the normal female and normal male, the presence of sex chromatin being a characteristic of the female. As has been described in detail elsewhere,[1-3] the sex chromatin is about 1μ in diameter, often planoconvex in shape, and usually lies against the inner surface of the nuclear membrane. In normal persons, the presence of sex chromatin is related to the XX complex and the absence of sex chromatin to the XY complex. There is the same relationship in patients with various forms of hermaphroditism. But, as will be described presently, in other sex anomalies abnormal sex chromosome complexes produce chromatin patterns that are indistinguishable from those of the normal male or female. Further, patients are encountered occasionally in whom the sex chromatin pattern is unlike that of normal individuals because of a variety of unusual sex chromosome complexes. Two sex chromatin masses in many nuclei, a chromatin pattern intermediate between that of the female and male, and differing chromatin patterns in nuclei of different tissues have been described; other variants may yet be found.

The sex chromatin pattern of interphase nuclei may be studied in buccal smear preparations,[4,5] in skin biopsy specimens[6] or in neutrophils of peripheral blood.[7] Neutrophils are unusual in that the sex chromatin is contained in an accessory nuclear lobule that occurs in 1-3% of these cells in females. The buccal smear test is preferred, because of its simplicity, for routine use as a diagnostic aid or when conducting mass surveys for research purposes.

SEX CHROMOSOMES

Recent technical advances have made it possible to obtain good metaphase figures of human chromosomes. The preparations are derived from fibroblasts in a fragment of skin that are grown *in vitro* for two weeks or so,[8-10] from bone marrow cells that are incubated for several hours at 37° C.,[11] or from leukocytes (probably monocytes) of peripheral blood after incubation at 37° C. for about three days.[12-14] The last method has several distinct advantages, but the chromosomes of both leukocytes and cutaneous fibroblasts should be examined if there is substantial reason to suspect that the chromosome complement may differ from one tissue to another. The chromosome complements shown in Figs. 1, 2, 4, 7, 9 and 10 were obtained from leukocytes of peripheral blood.

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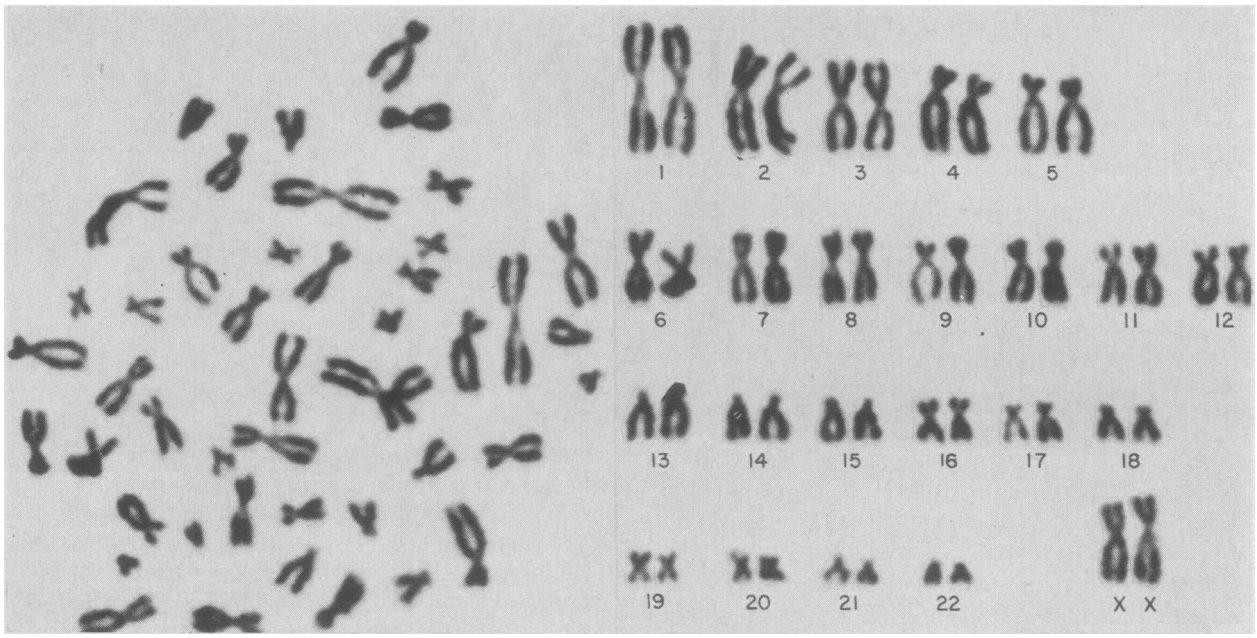

Fig. 1.—The chromosome complement of a normal female (44 autosomes and an XX sex chromosome complex).

The chromosomes of a dividing cell at metaphase of mitosis are shown in Fig. 1 (left). Each chromosome has divided into two chromatids or potential daughter chromosomes, but the chromatids are still held together at the centromere or attachment point for the spindle fibres. Further analysis involves arranging the chromosomes in pairs, one member of each pair being of paternal, the other of maternal, origin (Fig. 1, right).[15] The pairs are arranged in order of decreasing length, the sex chromosomes being set to one side because this pair is clearly different in the two sexes. Chromosome pairs 6 to 12 are much alike and identification of individual members of this group is difficult. The other chromosomes can usually be identified, mainly on the basis of differences in their lengths and the positions of their centromeres. Having arranged the chromosomes in this manner, one obtains the karyotype of the individual. Fig. 1 illustrates the karyotype of a normal female; there are 22 pairs of non-sex chromosomes or autosomes and an XX set chromosome complex, resulting in the normal chromosome number for man of 46. The X chromosomes are similar morphologically to the 6th pair of autosomes.

A metaphase figure from a normal male, and the resulting karyotype, are illustrated in Fig. 2. The autosomes are like those of a female but the sex

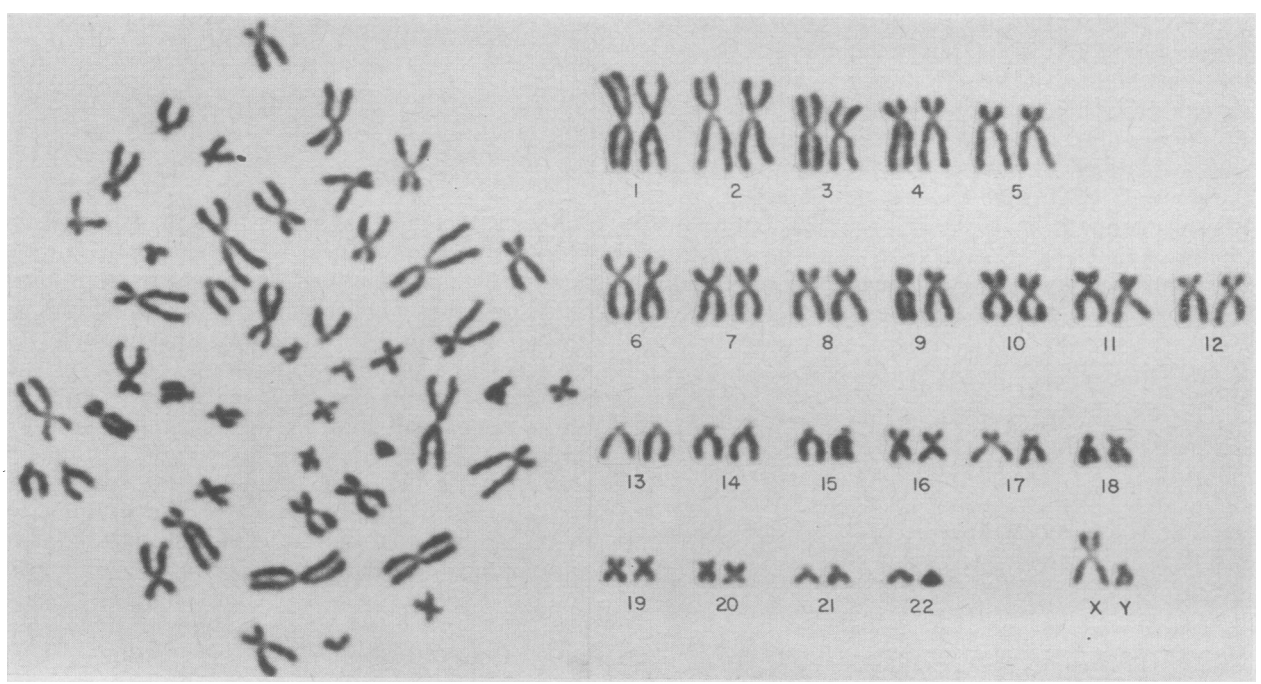

Fig. 2.—The chromosome complement of a normal male (44 autosomes and an XY sex chromosome complex).

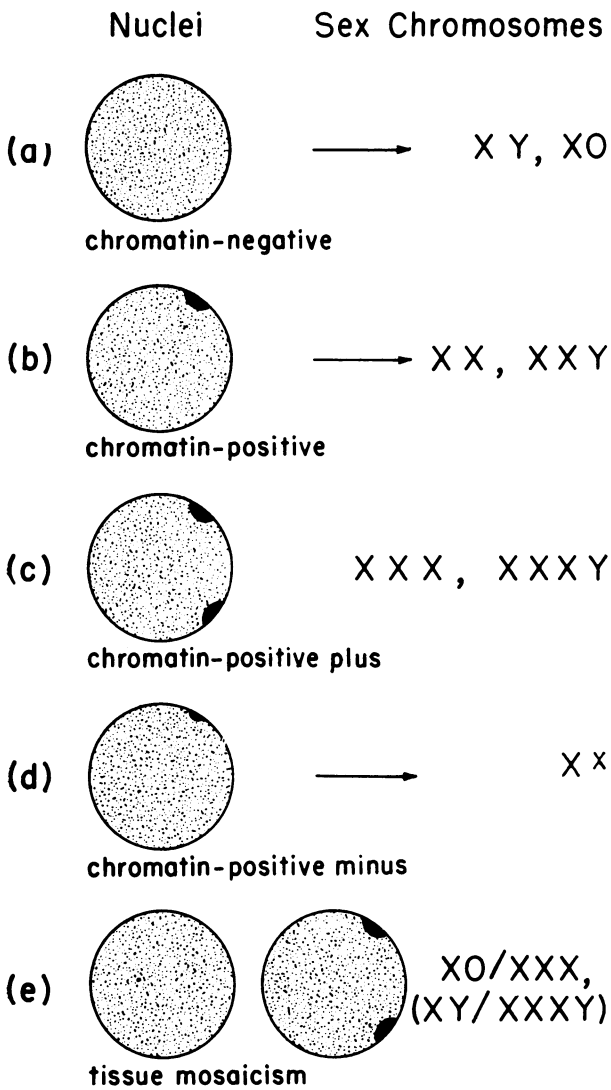

Fig. 3.—Correlations that have been demonstrated between the sex chromatin pattern and the sex chromosome complex.*

chromosome complex is XY. The Y chromosome is similar in size to autosomes 21 and 22.

A number of congenital defects have been described that are associated with autosomal abnormalities. Mongolism is of special importance in this connection, being characterized by the presence of three chromosomes at site 21, to give a chromosome number of 47.[16, 17] Fortunately, chromosomes 21 have a small pale region (the SAT-zone) at the end of the short arm, making it possible, in favourable preparations, to distinguish these autosomes from the other small chromosomes.

Relationship Between Sex Chromatin And Sex Chromosomes

The precise relationship between the sex chromatin and the sex chromosomes is not yet understood, but the correlations illustrated in Fig. 3 have been demonstrated. Chromatin-negative nuclei indicate that the sex chromosome complex is XY or XO (X nothing). Chromatin-positive nuclei indicate that the sex chromosome complex is XX or XXY. Doubly chromatin-positive nuclei (chromatin-positive plus) have been correlated with the presence of XXX or XXXY sex chromosome complexes. A chromatin pattern that lies between that of the male and female (chromatin-positive minus) has been described in a patient with a normal X chromosome paired with an X chromosome that has suffered partial deletion. A case of mosaicism is on record, in which the nuclei of one tissue were chromatin-negative, while in another tissue the nuclei had duplicated sex chromatin. The mosaicism in this particular patient was XO/XXX; an XY/XXXY mosaicism would produce a similar sex chromatin pattern.

The earlier hypothesis that the sex chromatin is formed by two X chromosomes that lie side by side and remain condensed (positively heteropyknotic) in the interphase nucleus appears to be untenable in view of the finding that two masses of sex chromatin are correlated with three X chromosomes (Fig. 3c). But the correlations shown in Fig. 3 are compatible with the view of Ohno *et al.*[18, 19] that the sex chromatin is formed by one X chromosome that behaves in a positively heteropyknotic manner. The factors that determine whether an X chromosome will or will not remain condensed in the interphase nucleus are unknown. However, it seems that as long as one X chromosome is in an extended state (and thus invisible in the interphase nucleus), the genetic requirements of the cell are satisfied and such additional X chromosomes as may be present can assume the compact or contracted state to form one or more masses of sex chromatin. But an unexplained exception to the foregoing generalization has been demonstrated in the form of two chromatin-positive subjects with gonadal dysgenesis and XO sex chromosome complexes.[20] The small Y chromosome does not appear to make a direct contribution to the sex chromatin.

The suggestion that one member of a pair of homologous chromosomes may differ from its partner in morphological, and perhaps physiological, characteristics is a novel one, but several cherished beliefs have been modified as a result of recent cytological investigations.

Sex Anomalies With Normal Sex Chromosome Complexes[21-25]

Female pseudohermaphrodites, most of whom are caused by fetal adrenal virilism, are chromatin-positive and have an XX sex chromosome complex. Male pseudohermaphrodites, including patients with the syndrome of testicular feminization, are chromatin-negative and have an XY sex chromosome complex. True hermaphrodites are either chromatin-positive or chromatin-negative; two chromatin-positive subjects were described as hav-

*Two new correlations may now be added to those noted in Fig. 3. We have found an XXYY sex chromosome complex in a mentally defective Klinefelter subject whose interphase nuclei were typically chromatin-positive ((b) above). Further, an XXXX sex chromosome complex was demonstrated in two mentally defective females, many of whose nuclei contained three masses of sex chromatin.—*The authors.*

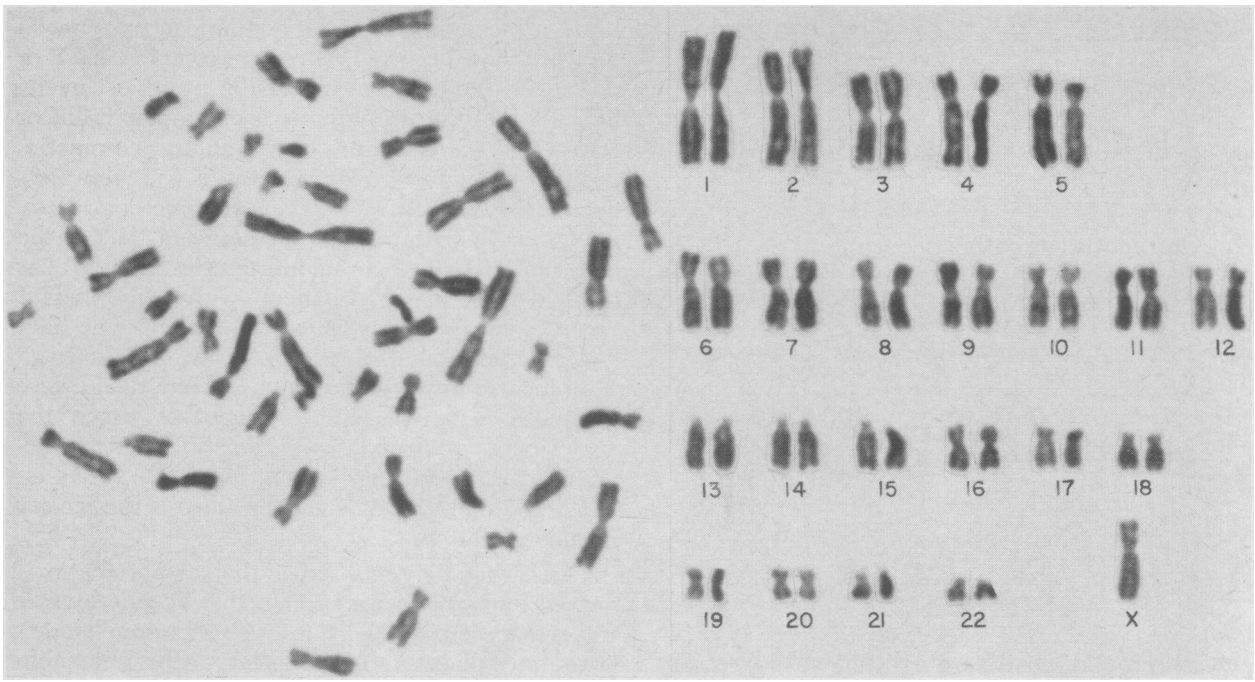

Fig. 4.—Chromosome complement of a female patient with gonadal dysgenesis (Turner's syndrome) and chromatin-negative nuclei (44 autosomes and a single X chromosome). Patient referred by Dr. E. R. Plunkett.

ing XX sex chromosome complexes. Patients with Turner's syndrome (gonadal dysgenesis) and chromatin-positive nuclei usually have XX sex chromosomes but, as mentioned above, the presence of XO complexes was reported in two such patients.[20] An XY sex chromosome complex was described in a rare case of "pure" gonadal dysgenesis with chromatin-negative nuclei, in which there were none of the congenital defects that usually accompany gonadal dysgenesis. Subjects with Klinefelter's syndrome (seminiferous tubule dysgenesis) and chromatin-negative nuclei were reported to have XY sex chromosomes. Only a few patients have been subjected to chromosome analysis in some of the foregoing categories and exceptions may come to light. In any event, the conditions now to be described are of special interest in connection with abnormal sex chromosome complements.

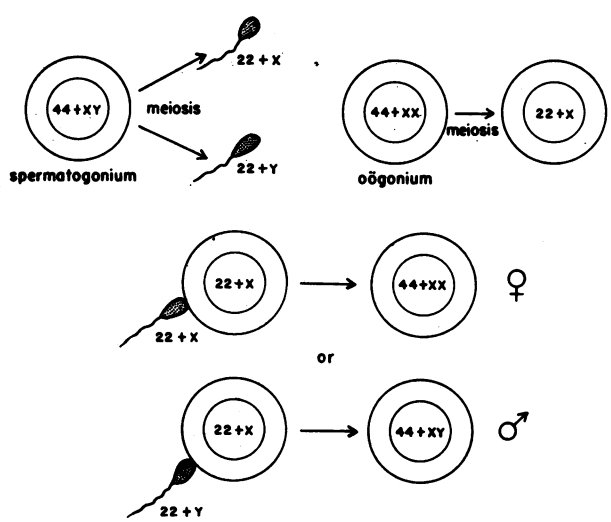

Fig. 5.—Diagrammatic representation of certain events in normal gametogenesis and fertilization.

CHROMATIN - NEGATIVE TURNER'S SYNDROME (GONADAL DYSGENESIS)

The patient with an XO sex chromosome complex is a female with dysgenesis of the gonads and shortness of stature. The name "Turner's syndrome" is applied when webbing of the neck and cubitus valgus are also present, but for convenience the terms "gonadal dysgenesis" and "Turner's syndrome" will be used interchangeably. Other associated defects occur less frequently. The nuclei are chromatin-negative and the buccal smear test is a useful diagnostic aid.[26] The presence of an incomplete XO sex chromosome complex in a chromosome complement of 45 was first demonstrated by Ford and his collaborators in 1959 and has been confirmed by several authors.[27-29]

Fig. 4 illustrates the chromosome complement of a 22-year-old girl with chromatin-negative nuclei and the clinical manifestations of Turner's syn-

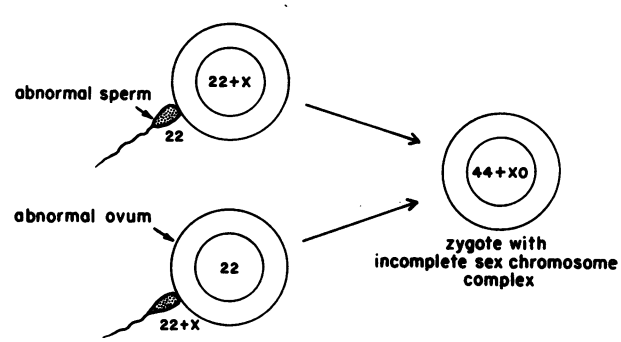

Fig. 6.—Diagrammatic representation of a possible explanation for the XO sex chromosome complex found in patients with Turner's syndrome and chromatin-negative nuclei.

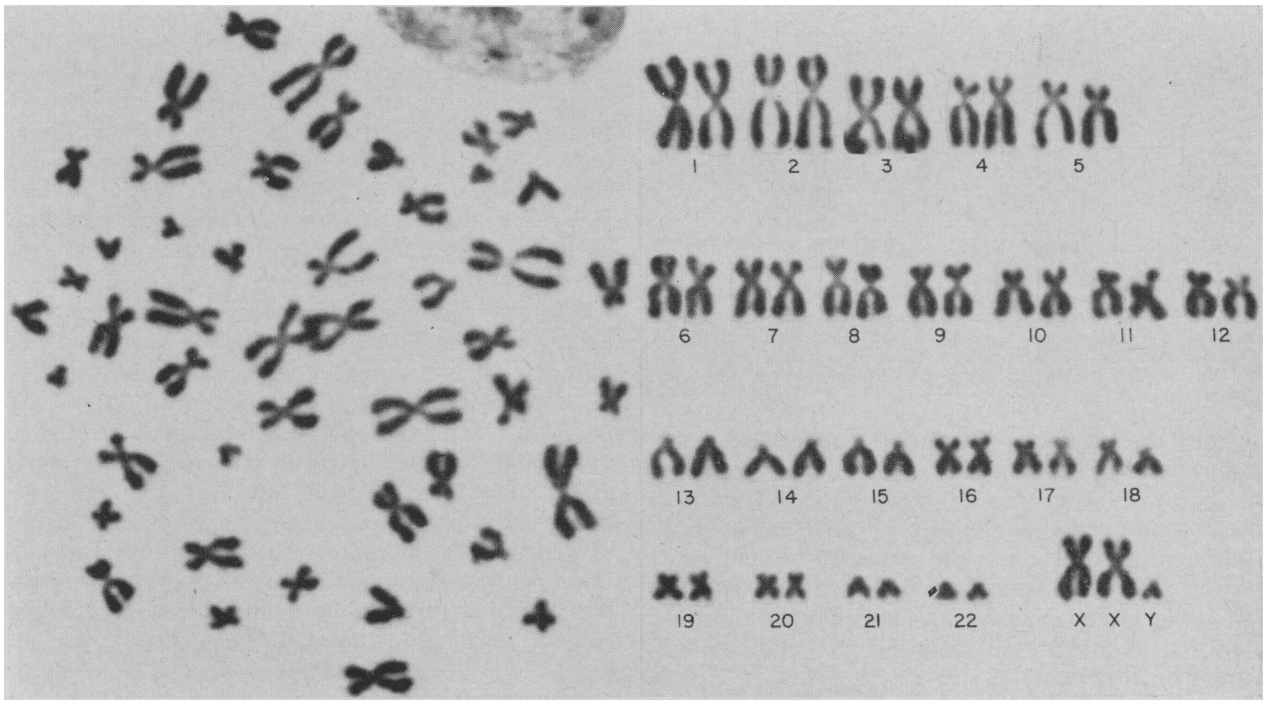

Fig. 7.—Chromosome complement of a male patient with seminiferous tubule dysgenesis (Klinefelter's syndrome) and chromatin-positive nuclei (44 autosomes and an XXY sex chromosome complex). Patient referred by Dr. E. R. Plunkett.

drome. Although the autosomes are normal, the sex chromosome complex is represented by a single X chromosome. The sex chromosome complex is neither male nor female, but incomplete, and the natural history of the condition must begin with events that are associated with conception. The chromosomal abnormality could result from an error in the segregation of a sex chromosome either in an early division of a normal fertilized ovum or by participation of an abnormal germ cell in fertilization. The latter possibility is assumed at this time, although the first merits consideration.

The main events in normal gametogenesis and fertilization are illustrated diagrammatically in Fig. 5. Spermatogonia undergo several divisions, including one that is reductional, giving rise to two classes of sperms, one bearing 22 autosomes and an X chromosome, the other 22 autosomes and a Y chromosome. Starting from oögonia, germ cell maturation in the female produces only one type of ovum, having 22 autosomes and an X chromosome. Fertilization of an ovum by an X-bearing spermatozoon produces a zygote with female potentialities, while if the sperm has a Y chromosome the zygote has male potentialities.

The consequences of the genetic differences imposed by the XX or XY complexes are seen primarily in diverging paths of gonadal development. Until the end of the second month of gestation, the gonads of male and female embryos are identical and consist of a central medullary portion and a peripheral cortical portion. When the sex chromosome complex is XY the medulla differentiates into a testis and when the sex chromosome complex is XX the cortex develops into an ovary.

These basic biological mechanisms break down in the sequence of events that lead to Turner's syndrome. Assuming that the fault usually lies in an abnormal gamete, it appears that the two components of the sex chromosome complex fail to segregate at the reductional meiotic division of gametogenesis. There are thus produced germ cells with two sex chromosomes or none, the latter being responsible for Turner's syndrome. The 44 + XO zygote would result from fertilization of a normal ovum by a sperm with no sex chromosome or from fertilization of an ovum with no sex chromosome by a normal X-bearing sperm (Fig. 6).

The incomplete sex chromosome complex is incompetent to produce normal gonads. They are small, the so-called "streak ovaries", and are, in effect, aplastic ovaries that lack follicles and are consequently sterile. The term "congenitally rudimentary ovary syndrome", suggested by Hamblen *et al.*,[30] is an accurate description of the main developmental defect.

The remainder of the reproductive tract develops along female lines in accordance with the principle established by Jost,[31] who showed that a chemical inductor or evocator substance of testicular origin is required to effect male differentiation of the internal and external genitalia. The syndrome should be suspected in infancy if there is lymphedema of the lower extremities and excessive looseness of the skin at the back of the neck. Shortness of stature, webbing of the neck, cubitus valgus or other associated defects draw attention to the possibility of the syndrome in childhood. From puberty onward there is primary amenorrhea, the breasts continue in their prepuberal state and

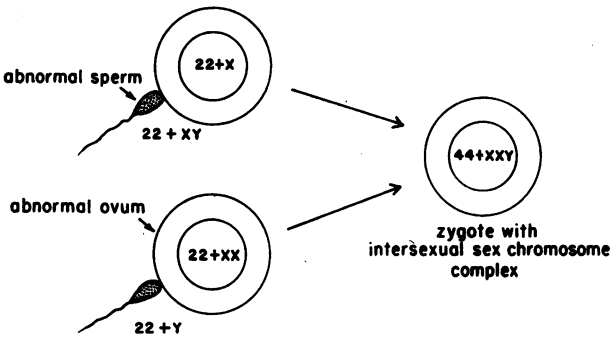

Fig. 8.—Diagrammatic representation of a possible explanation for the XXY sex chromosome complex found in patients with Klinefelter's syndrome and chromatin-positive nuclei.

the urinary excretion of pituitary gonadotrophins is elevated.[26] The diagnosis of gonadal dysgenesis or Turner's syndrome is confirmed by finding chromatin-negative nuclei in the buccal smear, but a chromatin-positive nuclear pattern does not rule out the syndrome. Administration of stilbestrol from the age of puberty is a logical and effective form of therapy, although the primary gonadal defect cannot be altered.

CHROMATIN-POSITIVE KLINEFELTER'S SYNDROME (SEMINIFEROUS TUBULE DYSGENESIS)

The infertile male with seminiferous tubule dysgenesis, whose condition is best known by the name Klinefelter's syndrome, is in several respects the opposite of the Turner's syndrome patient. The nuclei are chromatin-positive in the type of Klinefelter subject under consideration and, again, the buccal smear test is a useful diagnostic aid.[32] The presence of an intersexual XXY sex chromosome complex and a chromosome number of 47 was first demonstrated by Jacobs and Strong[33] and by Ford and his colleagues[34] in 1959. Fig. 7 illustrates a metaphase plate and the karyotype of a 25-year-old male with Klinefelter's syndrome and chromatin-positive nuclei.

The chromosomal error may originate during gametogenesis or in an early division of an XY-bearing zygote. In the former instance (Fig. 8), it is the gamete with an extra sex chromosome that is involved. The error may occur during spermatogenesis, so that the sperm contains both X and Y chromosomes, or it may occur during oögenesis, an ovum with two X chromosomes being fertilized by a Y-bearing sperm. In either event, the zygote contains 44 autosomes and an XXY complex and from this cell there develops the infertile male with dysgenesis of the seminiferous tubules.

The testes of prepuberal chromatin-positive males are normal except for a reduction in the number of spermatogonia in the seminiferous epithelium.[35-37] It is assumed, therefore, that the indifferent gonads of the early embryo develop into testes, when the sex chromosome complex is XXY, much as they do when the complex is XY. This inference, combined with the wholly inadequate gonadal development in the XO Turner's syndrome, indicates that the Y chromosome contains testis-promoting genes that can override the genetic influence of two X chromosomes on gonadal differentiation. Development of the XXY embryo proceeds along male lines under the influence of the evocator substance produced by the embryonal testes. Normal male development continues until early puberty and these individuals are, as a rule, recognized during childhood by finding chromatin-positive nuclei in the course of a mass survey with the buccal smear technique.

The first pathological change in the chromatin-positive XXY male occurs in early puberty and the pathology is found in the testes. The tubules undergo a process of fibrosis and hyalinization so that many of them are converted into small, irregular, hyaline masses that have no trace of seminiferous epithelium. Other tubules are less abnormal, although they are small and their epithelium consists of Sertoli cells only. In an occasional specimen, a few tubules show evidence of spermatogenesis, but there is no case on record of a Klinefelter subject producing enough spermatozoa to be fertile. The Leydig cells are arranged in large aggregates.

It can hardly be a coincidence that the collapse of testicular development takes place during early puberty when the pituitary begins to elaborate gonadotrophins in significant amounts. Under normal conditions, the tubules enlarge at puberty and the seminiferous epithelium acquires its full capacity for spermatogenesis in response to the stimulating effect of gonadotrophins. It is possible that, in the Klinefelter subject, there is an abnormal end-organ response to gonadotrophins because the cells of the seminiferous tubules contain an intersexual XXY sex chromosome complex.

Aside from consistently small testes, which are one inch or less in their longest dimension, the physical findings are highly variable. The limbs, especially the lower limbs, tend to be long in proportion to the trunk and there may be poor growth of facial hair and a gynecoid distribution of pubic hair. An occasional subject has gynecomastia. Aside from the testes, the genitalia are almost always well developed and a proportion of Klinefelter subjects have normally masculine bodily characteristics. Hormone studies show an elevated urinary excretion of gonadotrophins, while the 17-ketosteroid levels are usually in the low normal or subnormal range. Mental retardation is another variable component of the syndrome.[38, 39] It is evident from the foregoing clinical summary that the XXY Klinefelter subject will rarely be detected before puberty and could easily be missed after puberty unless specific attention is paid to a history of infertility or the finding of small testes during the course of a physical examination. The simple buccal smear test should be done when there is the slightest reason to suspect the syndrome.

The XXY infertile male occurs much more frequently than the XO infertile female, an observation that has no satisfactory explanation. From the

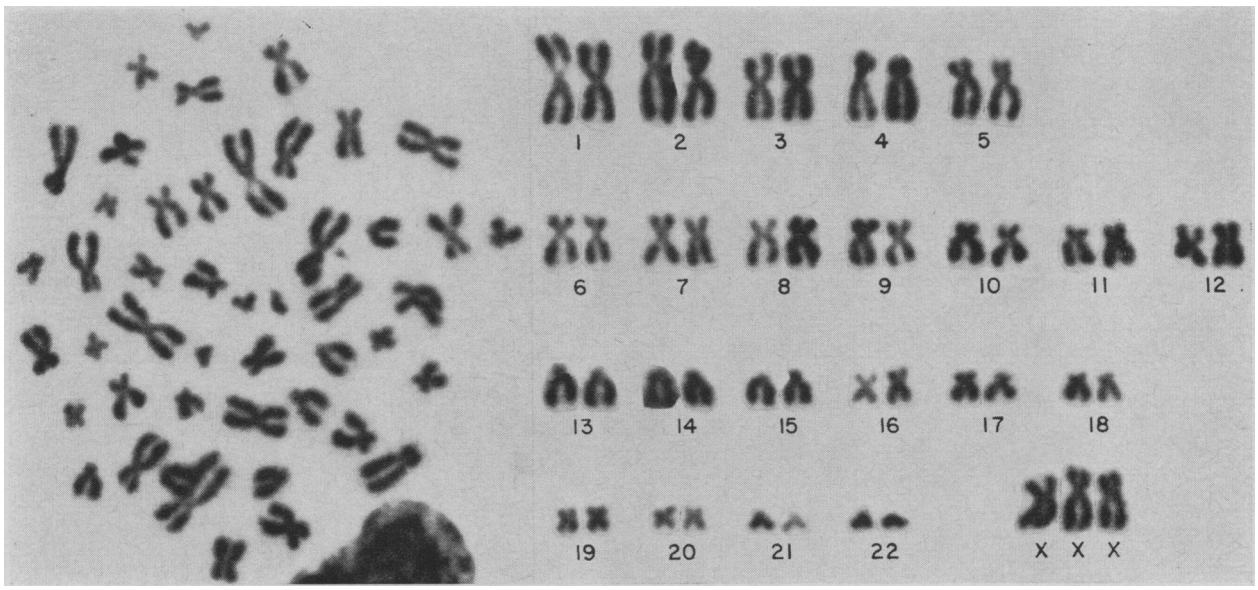

Fig. 9.—Chromosome complement of a mentally defective female patient with duplicated sex chromatin (44 autosomes and an XXX sex chromosome complex).

survey of newborns conducted by Moore[40] and on the basis of clinical experience, the frequency of the chromatin-positive Klinefelter syndrome appears to be of the order of 1:500 males in the general population. However, the frequency of the syndrome is about 1:100 among mentally retarded males.[41-46] It is of interest to note that the incidence 1:500 is similar to the frequency of mongolism in male newborns, a condition that is also related to the presence of an extra chromosome, although it is not a sex chromosome in mongolism.

UNUSUAL SEX CHROMATIN PATTERNS

Patients with unusual sex chromatin patterns, i.e. other than typical chromatin-positive or chromatin-negative nuclei, are less frequent among the sex anomalies than those described above. Jacobs *et al.*[47, 48] described two female subjects, a proportion of whose nuclei contained two masses of sex chromatin in the buccal smear preparation and whose karyotypes consisted of 44 autosomes and an XXX sex chromosome complex. Fig. 9 illustrates the chromosomes of a 60-year-old female with mental deficiency and schizophrenia, who has duplicated sex chromatin in interphase nuclei. This patient, who will be described in detail in a later publication together with several other mentally retarded females with duplicated sex chromatin, demonstrates that a triple X sex chromosome complex is compatible with fertility, for her hospital record states that she had nine pregnancies.

Duplication of the sex chromatin also occurs in phenotypic males, and then the sex chromosome complex is XXXY, giving a somatic cell chromosome number of 48.[49] Such patients are likely to be mentally retarded and indistinguishable from subjects with the Klinefelter syndrome clinically. Fig. 10 illustrates the chromosomes of a 15-year-old mentally defective male with the Klinefelter syndrome and duplication of sex chromatin in interphase nuclei. Details of this and of a similar patient will be published separately.[50]

There are several theoretical explanations for the occurrence of XXX or XXXY sex chromosome complexes in somatic cells. They are complicated and involve failure of normal X chromosome segregation either during gametogenesis or an early cleavage division of the fertilized ovum, or both. Further observations are required in order to select the most probable explanation for such unusual complexes as XXX and XXXY.

Jacobs *et al.*[48] described a phenotypic female with clinical manifestations of the gonadal dysgenesis (Turner's) syndrome, who had a small sex chromatin mass in about 10% of nuclei in a buccal smear preparation. The sex chromatin pattern was, therefore, intermediate between that of a normal male and normal female. The karyotype contained 46 chromosomes and the sex chromosome complex was interpreted as consisting of a normal X chromosome together with a portion of an X chromosome, the remainder having apparently been lost through chromosome breakage and partial deletion.

Finally, Jacobs *et al.*[48] have placed on record a case of mosaicism with respect to the sex chromatin pattern and the sex chromosome complex. Nuclei from the buccal mucosa contained two masses of sex chromatin but there were no $\frac{\varphi}{+}$-type neutrophils in the peripheral blood. Analysis of the chromosomes showed that the majority of cells cultured from a skin biopsy specimen contained 44 autosomes and an XXX sex chromosome complex, while cells of bone marrow and blood contained mainly cells with 44 autosomes and an XO sex chromosome complex. Blood cells with an XY complex and skin cells with an XXXY complex would produce the same kind of sex chromatin pattern and such a patient will probably be found. Still other forms of mosaicism have been described.[34, 51] Sex chromo-

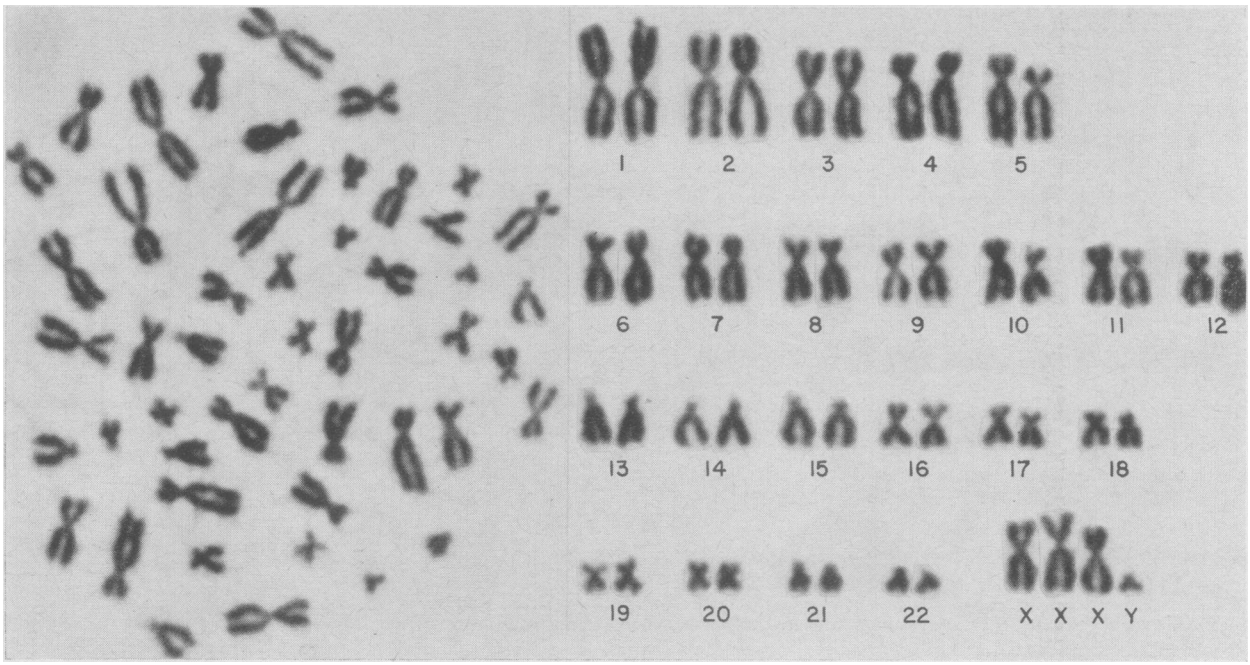

Fig. 10.—Chromosome complement of a mentally defective male patient with Klinefelter's syndrome and duplicated sex chromatin (44 autosomes and an XXXY sex chromosome complex).

some mosaicisms may orginate in non-disjunction of an X chromosome during early division of a normal zygote, or of a zygote that already has a chromosomal anomaly (e.g. to produce a hypothetical XY/XXXY mosaicism from an XXY zygote).

SUMMARY

Recent studies have demonstrated several abnormalities of the sex chromosome complex. The best known are the XO complex of chromatin-negative patients with Turner's syndrome (gonadal dysgenesis) and the XXY complex of chromatin-positive patients with Klinefelter's syndrome (seminiferous tubule dysgenesis). Females with duplication of the sex chromatin have an XXX sex chromosome complex and are likely to be normal except for some degree of mental retardation. Males with duplicated sex chromatin have an XXXY sex chromosome complex and are likely to belong to the Klinefelter syndrome and to be mentally retarded. One female patient is described in the literature whose sex chromatin pattern is intersexual and who has a normal X chromosome together with an X chromosome that has suffered partial deletion. Finally, there are instances of mosaicism, in which the sex chromosome complex differs in different body cells.

Although the new science of human cytogenetics has become complicated even in its very early stages of development, there is now offered the possibility of visualizing the natural history of several developmental errors from conception to maturity. The full realization of this goal will require a great deal of work in the general field of developmental biology.

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