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CURRENT PROBLEMS IN RESEARCH

Sex Chromatin and Phenotype in Man

Disagreement between nuclear sex and phenotype raises questions about the cause of sex anomalies.

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It is now firmly established that there is a sexual dimorphism in the structure of intermitotic nuclei of man and certain other mammals. The difference between the sexes is that a special mass of chromatin or chromocenter, the sex chromatin, is clearly visible in nuclei of normal females but not in those of normal males. In normal individuals, at any rate, the presence or absence of sex chromatin is probably related to the XX sex chromosome complex of females and the XY sex chromosome complex of males.

A discrepancy between nuclear structure and the more obvious sexual features of the phenotype is found in certain developmental sex anomalies in man. For example, the phenotype is predominantly female in the Ullrich-Turner syndrome (gonadal dysgenesis), although the nuclei are usually indistinguishable from those of normal males. Similarly, the nuclei have a male structure in the syndrome of testicular feminization, but the external anatomy is strikingly feminine. Conversely, many phenotypical males with the Klinefelter syndrome (seminiferous tubule dysgenesis) have nuclei that are indistinguishable from those of normal females.

The sexual dimorphism of intermitotic nuclei has become a useful diagnostic aid, even when used empirically, in dealing with the sex anomalies (*1*). But the ultimate aim is an understanding of the biology of the sex anomalies as a prelude to preventive measures. This requires information from the study of abnormal sex development in subhuman forms (*2*) and from the study of human chromosomes in dividing cells, through recently developed techniques (*3, 4*). This article represents an attempt to summarize current concepts of the pathogenesis of some syndromes encountered in clinical medicine, and to point out the many gaps in our knowledge that have to be filled before etiological factors can be fully understood.

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Normal Gonadal Differentiation

Gonadal ridges appear in the human embryo at about the fourth week of gestation. Their structure is identical in male and female embryos until the seventh week (Fig. 1, *A*). The cellular cortex of the indifferent gonad has the potentiality of developing into an ovary. The medulla, consisting of primary sex cords in a mesenchymal stroma, has the potentiality of developing into a testis (*5*). Primordial germ cells can be identified in cortex and primary sex cords from the sixth week onward, having migrated into the gonad from the region of the extra-embryonal entoderm.

The fate of the indifferent gonad is established by the balance between male-determiners and female-determiners in the genotype (*6*). When the sex chromosome complex is XY, male-determining genes on autosomes predominate over female-determining genes on the single X chromosome. The medulla begins to develop, and the cortex to regress, at the seventh week. The primary sex cords become seminiferous tubules, and interstitial cells appear between them, while the cortex becomes the thin visceral layer of the *tunica vaginalis* that adheres to the *tunica albuginea* (Fig. 1, *B*). It is noteworthy that the endocrine component of the testis, consisting of interstitial or Leydig cells, is well developed in the embryonal testis and again after puberty but is inconspicuous in the intervening period.

When the sex chromosome complex is XX, female-determiners on the two X chromosomes outweigh male-determiners on autosomes (*7*). Beginning at the ninth week, the cortex develops into an ovary through the ingrowth of secondary sex cords, and the medulla regresses (Fig. 1, *C*). Interference with this crucial step of differentiation of bipotential gonads into testes or ovaries, at about the end of the second month of embryonic development, appears to be the point of departure for most sex anomalies in man. The genetic balance between male-determiners and female-determiners may be altered by a mutant gene or by an abnormality of one or more of the chromosomes that bear these determiners. But experimental evidence testifies to the frequent adverse effects of various nongenetic factors on gonadal differentiation. Evidence for possible genetic or nongenetic factors that might interfere with normal gonadal differentiation has to be sought for each type of sex anomaly.

Normal Differentiation of Internal and External Genitalia

Wolffian ducts (primordia of epididymides, *vasa deferentia*, and seminal vesicles) and Müllerian ducts (primordia of Fallopian tubes, uterus, and vagina) are both present when gonadal differentiation begins, and the external genitalia are also in a bipotential state. Much experimental work bears on the factors responsible for development of internal and

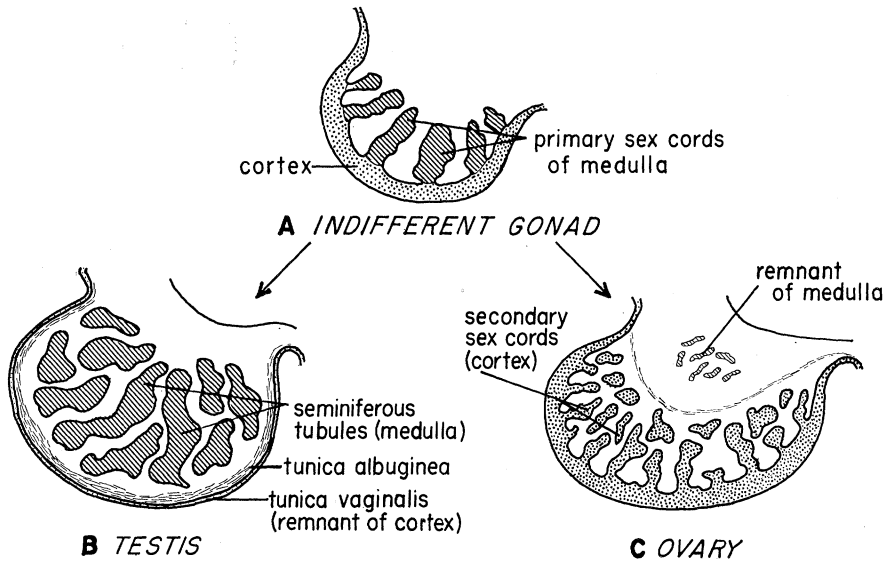

Fig. 1. Diagrammatic representation of development of a testis from the medullary component, and an ovary from the cortical component, of the indifferent gonad of an early embryo. [Modified from Grumbach and Barr, *Recent Progr. in Hormone Research* (*26*), courtesy Academic Press, New York]

external genitalia in a direction which is consistent with the male or female character of the gonads. Treating embryos with androgens or estrogens, transplanting an embryonal gonad into an embryo of the opposite sex, and depriving an embryo of the influence of embryonal gonads have all yielded pertinent results. The consequences of surgical removal of gonads in rabbit and rat embryos and the destruction of gonads by x-rays in mouse embryos form the basis of current hypotheses concerning the pathogenesis of sex anomalies in man (*8–10*). The outstanding work of Jost on rabbit embryos illustrates the results obtained in such experiments (Fig. 2).

Gonadal differentiation into testes or ovaries begins on the 15th day in the rabbit, while differentiation of the duct system begins on the 20th day and is virtually complete by the 28th day, which is about two days before birth (Fig. 2, *A-B* and *A-D*). Gonadectomy of female embryos at any stage or of male embryos at about the 20th day is followed by maturation of the duct system and the external genitalia in a female direction, although the uterus is rather smaller than normal (Fig. 2, *A-C*). There is normal maturation of the male genitalia if removal of the testes is delayed beyond the 24th day. These observations indicate that ovaries are not essential for female development, but that the action of an inductor or evocator from the interstitial cells of the embryonal testes is necessary during a critical period for male development. Although much remains to be learned of the factors controlling embryogenesis of the reproductive system, the requirement of a masculinizing evocator of testicular origin to counteract a tendency of all embryos to feminize is a keystone in current concepts of the pathogenesis of congenital errors of sex development.

The masculinizing evocator appears to have a local action on adjacent tissues, since a unilateral graft of embryonal testis into a female embryo stimulates the Wolffian duct and suppresses the Müllerian duct on the side of the transplant preferentially (*9, 10*) (Fig. 3). A similar asymmetry follows unilateral gonadectomy of male embryos between the 20th and 24th day and occurs in human true hermaphrodites when there is testicular tissue on one side only. The substance seems to act in the manner of embryonal evocators generally, which is consistent with experimental evidence that the evocator substance differs in its physiological effects, and probably in chemical composition, from testosterone and other androgenic hormones.

The Müllerian ducts of rat embryos in the early undifferentiated stage persist and grow in vitro, and the Wolffian ducts regress, regardless of the sex of the donor (*9, 10*). This suggests that the inherent tendency of embryos to feminize is genetically controlled, rather than the result of an exogenous factor such as maternal estrogens. There is little information relating to genetic mechanisms that may operate in this connection. The genes involved must be other than the male-determiners and female-determiners whose balance controls gonadal differentiation, since female differentiation can occur whether the sex-chromosome complex is XX or XY.

Sexual Dimorphism of Intermitotic Nuclei

Chromosomal, gonadal, and phenotypical sex are normally in agreement. But the sex-chromosome complement may be inconsistent with the main fea-

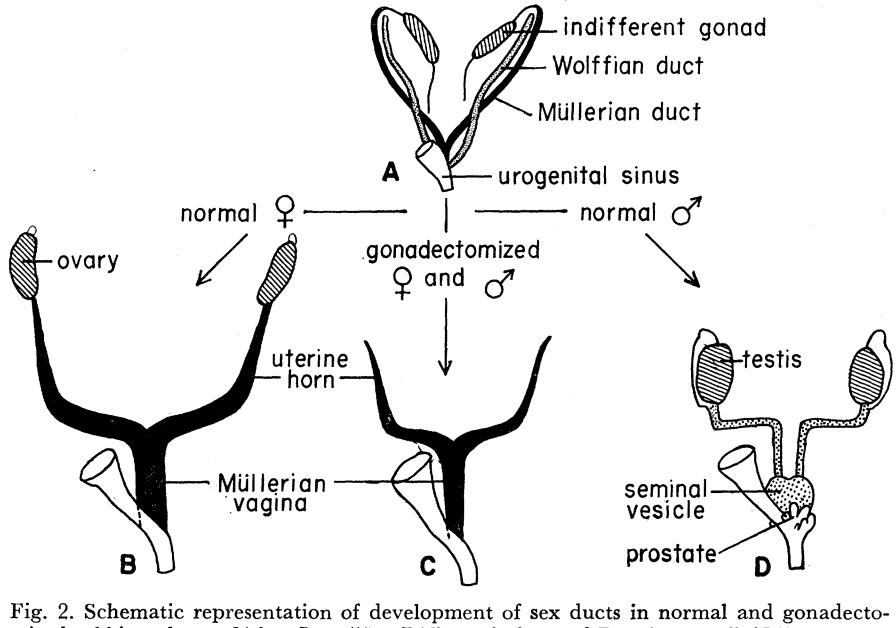

Fig. 2. Schematic representation of development of sex ducts in normal and gonadectomized rabbit embryos. [After Jost, "Sex Differentiation and Development" (*51*), courtesy Cambridge Univ. Press, London]

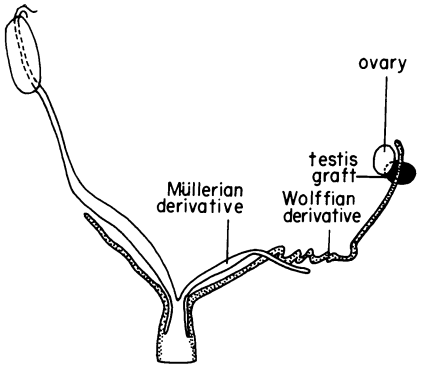

Fig. 3. Genital tract of a 28-day-old female rabbit embryo in which a testicular graft from a 21-day-old embryo had been implanted adjacent to an ovary on the 20th day of development. [After Jost, *Arch. anat. microscop. morphol. exptl.* (*9*), courtesy Masson, Paris]

tures of the phenotype when there has been an error in gonadal differentiation. Consequently, the "tests of chromosomal sex," having as their basis a sexual dimorphism in the structure of intermitotic nuclei, are useful diagnostic aids in clinical practice and raise new problems in connection with the etiology of sex anomalies.

The sex chromatin that characterizes nuclei of females is usually adherent to the inner surface of the nuclear membrane and is often so closely related to the membrane as to have a planoconvex outline (Fig. 4, *a* and *b*) (*11*). It is about 1 micron in diameter and can be resolved frequently into two components of equal size. The sex chromatin shares with the rest of the chromatin an affinity for basic dyes and, like the rest of the chromatin, reacts positively to tests for deoxyribonucleic acid, staining readily with the Feulgen technique and with methyl green. In particularly favorable circumstances, as in the study of whole mounts of thin membranes, the sex chromatin can be identified in virtually every nucleus. In sections of tissues 5 microns in thickness, sex chromatin can be identified in 60 to 80 percent of the nuclei, depending on the technical quality of the preparations and such factors as the size of the nuclei and the coarseness of the general chomatin particles. A chromatin mass larger than other chromatin particles of the nucleus is encountered in up to 10 percent of the cells in sections of tissues from males. This particular mass of chromatin is seldom as large as the sex chromatin of females, and its significance is uncertain.

Neutrophils have a different kind of sexual dimorphism (Fig. 4, *c*) (*12*). In a small proportion of neutrophils of females (1 to 10 percent, average about 3 percent), there is an accessory nuclear lobule; such a lobule is encountered with the greatest rarity, if at all, in neutrophils of males. The relation of the accessory nuclear lobule to the sex chromatin is not known.

Nuclei have been examined for sex chromatin in 24 mammalian representatives, more extensively in some than in others (Table 1) (*13*). In man and monkey, the imprint of sex on nuclear structure is present in the various tissues and organs, cells with small pyknotic nuclei excepted. This is also true of those carnivores that have been studied extensively and probably holds for carnivores generally. Nerve-cell nuclei bear a clear imprint of sex in the few representatives of the order Artiodactyla that have been studied, but the nuclear chromatin is too coarse in nonnervous tissues to allow identification of the sex. In the Virginia opossum, the only marsupial that has been examined, sex chromatin is present in nuclei of both sexes, but the size is significantly larger in females. There are multiple large particles of chromatin in nuclei of the rabbit and of rodents, so these animals are unsuitable for work that depends on the sex characteristics of intermitotic nuclei. But there are exceptions, for the sex chromatin can be identified in motor neurons of female rats and hamsters and in ameloblasts of newborn rats, and the sex-identifying variant that occurs in neutrophils of man is also present in those of the rabbit. Representatives of an order appear to have similar nuclear characteristics with respect to the coarseness of the chromatin particles and the clarity of the sex chromatin of females.

Nuclear dimorphism according to sex is lacking in the very early stages of embryonic development. In the cat, for example, this feature could not be detected in the morula stage and was seldom seen in blastocysts. Neither has sex chromatin been described in ova of primary follicles. But nuclear dimorphism is clearly established in embryos of the cat well before gonadal differentiation, and the imprint of sex is visible in resting nuclei of human and macaque embryos from the 12th to the 19th day onward (*14*).

Since the sex chromatin is a Feulgen-positive chromocenter, it presumably represents positively heterochromatic regions of chromosomes—that is, regions that are dense and prominent when the euchromatic regions are indistinct. The fine details of the sex chromatin, especially its bipartite structure and its connection with a delicate thread that may also be double, as well as the multiple masses of sex chromatin that are present

Table 1. Sexual dimorphism in cell nuclei of mammals.

Representatives	Dimorphism present?
Primates	
Man	Yes
Monkey	Yes
Carnivora	
Cat	Yes
Dog	Yes
Mink	Yes
Marten	Yes
Ferret	Yes
Racoon	Yes
Skunk	Yes
Coyote	Yes
Wolf	Yes
Bear	Yes
Fox	Yes
Artiodactyla	
Goat	Yes
Deer	Yes
Swine	Yes
Cattle	Yes
Marsupialia	
Opossum	Yes
Lagomorpha	
Rabbit	No
Rodentia	
Rat	No
Hamster	No
Mouse	No
Guinea pig	No
Ground hog	No

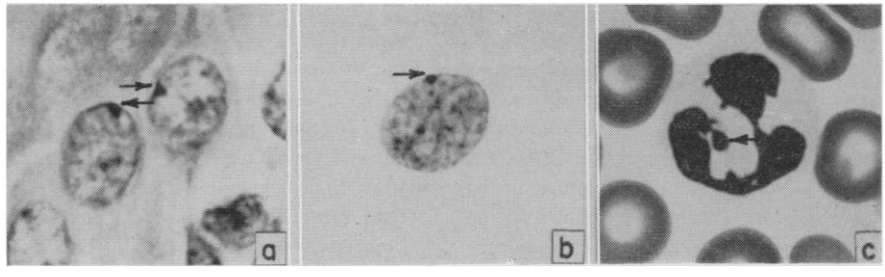

Fig. 4. Nuclei of human females. (*a*) Nuclei in epidermis of a skin biopsy specimen (hematoxylin-eosin); (*b*) nucleus in an oral mucosal smear (cresylecht violet); (*c*) neutrophil in a blood film (Giemsa). (× about 1680.) [After Barr, *Brit. J. Urol.* (*52*), courtesy Livingstone, Edinburgh]

in polyploid nuclei, suggest that it is formed from heterochromatic regions of a pair of homologous chromosomes (*15*). Although an alternative interpretation has been suggested (*16*), the weight of evidence favors the view that the bipartite sex chromatin of females is formed by heterochromatic regions of the two X chromosomes, and that a definite chromocenter is not formed by the nonhomologous sex chromosomes of the heterogametic sex (*17*). This interpretation implies somatic pairing, for the X chromosomes at any rate (*18*). Somatic association of heterochromatic X chromosomes has, indeed, been described in ovarian follicular cells of the mouse (*19*).

The sex chromosomes vary in their heterochromaticity in different cell types, depending, possibly, on differences in the immediate environment of the chromosomes. As mentioned above, sex chromatin has not been described in ova and is lacking in the very early stages of embryonic development, while the XX complex forms a definite chromocenter throughout the rest of the life span of females. Conversely, the XY complex is strongly heterochromatic in prophase of meiosis but seldom produces a recognizable chromocenter in somatic cells. Other variants are on record. For example, the X and Y chromosomes form separate chromocenters of the same size in somatic cells of the ground vole, so that nuclei of males and females cannot be distinguished from each other; the multiple X chromosomes of certain insects form individual chromocenters, the sexes being divergent with respect to the number of these chromocenters that are formed; and the XY, rather than the XX, complex forms a distinctive chromocenter in somatic cells of the silkworm and the spruce budworm, in which the female is the heterogametic sex (*20, 21*).

Tests of Chromosomal Sex in Clinical Medicine

Application of the principle of nuclear sexual dimorphism to the study of patients with sex anomalies requires only an easily obtainable source of cells (*12, 22*). A skin biopsy specimen may be studied, since the sex characteristics of the nuclei are well defined in the mature spinous cells of the epidermis and in the large spherical nuclei of hair follicles (Fig. 4, *a*). A smear preparation of oral epithelium is particularly easy to obtain and is the favored procedure on that account (Fig. 4, *b*). Although whole nuclei are present in oral smears, the incidence of nuclei with unequivocal sex chromatin is low (30 to 60 percent) in chromosomal females, and in an occasional preparation the sex chromatin is less conspicuous than usual because of being much flattened against the nuclear membrane. However, these factors do not seriously lessen the usefulness of the oral-smear method, because chromocenters at the nuclear membrane that could be interpreted as sex chromatin occur with the greatest rarity in smears from chromosomal males. The neutrophil method (Fig. 4, *c*) gives the same information as is derived from the more conventional skin-biopsy and oral-smear techniques. Preparations of high technical quality are required for each of the tests. The preferred technical procedures are given elsewhere (*23*).

A correlation between the presence of sex chromatin and XX sex chromosomes, or the absence of sex chromatin and XY sex chromosomes, can logically be assumed for normal individuals. But the interpretation is not necessarily so straightforward in the sex anomalies (*24*). The possibility of a chromosomal abnormality need not be considered when the congenital error clearly does not involve the genetic sex-determiners. For example, the sex chromatin indicates the XX complex in the adrenogenital syndrome, where the fetal adrenal cortex is at fault, and when there is partial masculinization of the external genitalia in a female newborn whose mother received progestins during pregnancy (*25*). In addition, a chromosomal abnormality, other than sex chromosomes that are inconsistent with the phenotype, need not be suspected if the congenital error can be clearly attributed to a mutant gene or genes among the sex-determiners.

If the foregoing conditions are not fulfilled, the presence of sex chromatin in the "tests of chromosomal sex" means only that the nuclei contain heterochromatic regions of two X chromosomes. One of these chromosomes may be defective in its euchromatic region, there may be an unusual sex chromosome complex (such as XXY), or the autosomes that bear male-determiners may be in some way abnormal. Conversely, absence of sex chromatin in the "tests of chromosomal sex" indicates that two normal X chromosomes are not present. The sex chromosome constitution could, in theory, be XO, or there could be an abnormality of the autosomes carrying male-determiners. Awareness of the possibility of chromosomal abnormalities as a basis for some genetic sex anomalies should stimulate study of whole-chromosome complements by techniques that are now available.

Congenital Errors of Sex Development in Man

The hermaphrodite group was the main center of interest until recently. Hermaphrodites were known in ancient times and have always attracted attention because of the bizarre intersexual morphology of the external genitalia. There are three main varieties. Both testicular and ovarian tissues are present in *true hermaphrodites*. The nuclei have a female chromatin pattern in some patients and a male chromatin pattern in others. *Male pseudohermaphrodites* have testes, and the nuclei are always male. The internal and external genitalia have an intersexual morphology in true hermaphrodites and male pseudohermaphrodites, but the details vary widely from one subject to another. Through some physiological deficiency, the evocator produced by the embryonal testes has failed to bring about full masculinization of the reproductive system in the male pseudohermaphrodite.

Female pseudohermaphrodites have ovaries and essentially normal female internal genitalia. But there is persistence of the fetal urogenital sinus, clitoral hypertrophy, and at times partial fusion of the labioscrotal folds to produce intersexual external genitalia. With few exceptions, the condition is the result of hyperplasia of the fetal adrenal cortex and elaboration of androgenic steroids in excessive amounts. The sex chromosome complement is always XX. The hermaphrodite group is described in detail in the classical book by Young, and the discussion is bought up to date in recent publications (*16, 26, 27*). The following account is limited to errors of sex development in which there is an extreme divergence between the phenotype and the nuclear chromatin pattern.

Gonadal Dysgenesis

Gonadal dysgenesis (or virtual agenesis) is encountered as a component of the Ullrich-Turner syndrome. The individual has a female phenotype, with essentially normal external genitalia,

vagina, uterus, and tubes. The principal defect is in the gonads, which are represented by slender streaks of connective tissue, simulating ovarian stroma, attached to the broad ligaments (Fig. 5). Derivatives of mesonephric ducts may be present, but there is rarely any evidence of ovarian follicles or seminiferous tubules in the typical syndrome, except possibly during the neonatal period (*28*). Various congenital abnormalities are associated with gonadal dysgenesis. Shortness of stature is almost the rule, and there are often cutaneous folds at the sides of the neck. Less frequently, there may be a variety of skeletal or vascular anomalies and a number of other defects. Urinary excretion of pituitary gonadatropins is elevated after the age of 10 years, and secondary sex characteristics fail to develop naturally at puberty.

Both Jost and Wilkins suggested that a proportion of individuals with gonadal dysgenesis might be chromosomal males, the embryos having feminized in the absence of the masculinizing evocator of testicular origin (*9, 10, 29*). This prediction was verified promptly (*30*) when tests of chromosomal sex became available, at least to the extent that 80 percent of subjects with gonadal dysgenesis are now known to have a male chromatin pattern, while the remainder have a female chromatin pattern (*31, 32*).

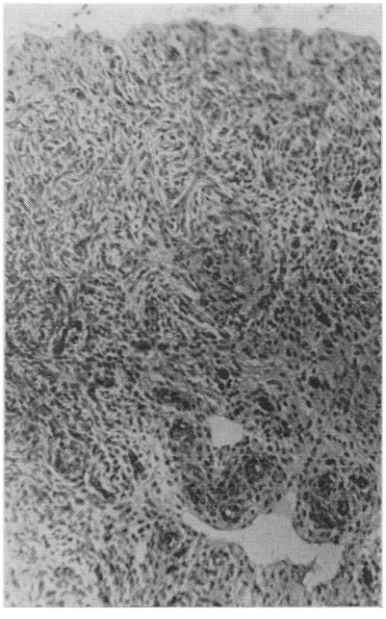

Fig. 5. Gonad consisting entirely of connective tissue that simulates ovarian stroma, in a 4-year-old girl with gonadal dysgenesis and a male chromatin pattern. (Hematoxylin-eosin.) (×125) [Courtesy Melvin M. Grumbach]

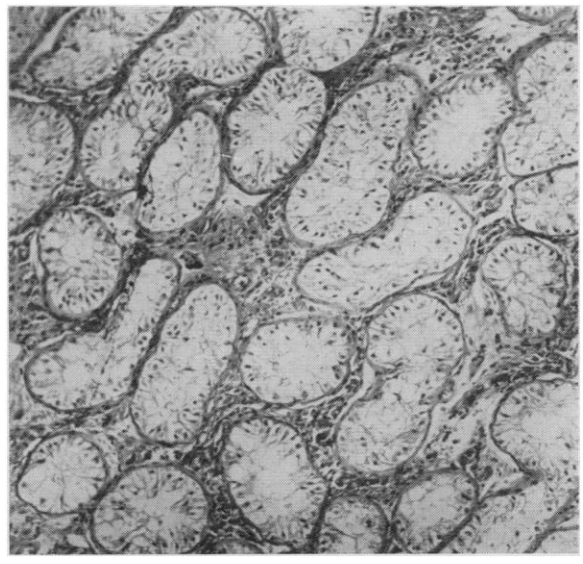

Fig. 6. Testis from a subject with the syndrome of testicular feminization. There are adequate Leydig cells, but the seminiferous tubules show the inhibition of spermatogenesis that occurs in undescended testes. (Hematoxylin-eosin.) (×85)

But the etiological factor responsible for failure of gonadal development and for the associated anomalies remains obscure. A maternal factor is a possibility, although none has yet been demonstrated (*28, 31*). Or an adverse nongenetic factor may originate in the zygote, following, for example, the fertilization of an overripe ovum (*33*). If the etiology of the Ullrich-Turner syndrome proves to be nongenetic or if a mutant gene with pleiotropic manifestations is responsible, the sex chromosome complement would be XY or XX, in accordance with the structure of the intermitotic nuclei. Tests of color vision indicate that those with sex chromatin bear the XX complex and that those without sex chromatin bear the XY (or XO) sex chromosome complex (*34*). Other methods of study, especially the examination of entire chromosome complements at metaphase, are needed to investigate the possibility of chromosomal anomalies that would not be visible in intermitotic nuclei (*21, 35*).

Whatever the etiology of the virtual agenesis of the gonads proves to be, embryological development proceeds along female lines in accordance with the principle that an evocator of testicular origin is required for masculinization.

Testicular Feminization

The syndrome of feminizing testes is another condition in which the phenotype is predominantly female, although the intermitotic nuclei have a male chromatin pattern and probably contain the XY sex chromosome complex (*26, 36*). The syndrome is in some respects an extreme form of male pseudohermaphroditism, but there are unique features that justify its consideration as a distinct entity.

The external genitalia are normally female in the typical syndrome, but pubic hair is often lacking. The vagina is a blind pouch, uterus and tubes being usually absent. Testes are present bilaterally in the pelvis or inguinal regions, accompanied by epididymides and proximal portions of *vasa deferentia*. The seminiferous tubules are immature (Fig. 6) because of the undescended position of the testes. Leydig cells are present in normal numbers. There is a normal female habitus, and the breasts develop well at puberty. The secondary sex characteristics are, in fact, strikingly feminine, and primary amenorrhoea may be the only overt indication of an abnormality of the reproductive system.

The syndrome is a hereditary anomaly that is transmitted by normal mothers. In families that include these subjects, there is a normal sex ratio if those with testicular feminization are added to normal males. The anomaly is probably caused by a mutant gene, but examination of the chromosome complement at metaphase is required to rule out a chromosomal abnormality consistent with male-type intermitotic nuclei. A quantitative or qualitative defect in the production of the evocator by interstitial cells is probably responsible for failure of male development in those parts of the reproductive system that are farthest from the testes. The interstitial cells clearly have a perverse metabolism, as shown by the development of feminine secondary sex characteristics at puberty and by the onset of menopausal symptoms if the testes are removed.

Seminiferous Tubule Dysgenesis (Klinefelter's Syndrome)

The Klinefelter syndrome includes subjects in whom there is a discrepancy between the phenotype and nuclear structure which is the reverse of that found in most individuals with gonadal agenesis and in those with the syndrome of feminizing testes.

The reproductive system has undergone normal male development, except that the testes are small and sperms are lacking from the semen. Eunuchoid traits may be present, and there is gynecomastia occasionally. Increased urinary excretion of pituitary gonadotropins is almost the rule, and the level of urinary 17-ketosteroids may be decreased (*37*). There was no reason to suspect a discrepancy between nuclear structure and the phenotype in any of these subjects, and the discrepancy was noted in the routine application of tests of chromosomal sex to various types of disorder of the reproductive system (*38*). The proportion of subjects who satisfy the clinical requirements for inclusion in the syndrome and who have a female chromatin pattern is not known exactly; three out of four may be a reasonable assumption.

The unusual histological structure of the testes is the most significant finding, and it differs to some extent, depending on whether the nuclei are female or male. The seminiferous tubules are highly abnormal when the nuclei are female. They are commonly represented by hyaline masses (Fig. 7, *a*), or there may be small tubules, with a thickened *lamina propria,* that contain Sertoli cells or epithelial-like cells of a type difficult to identify (Fig. 7, *b*). Spermatogonia or even more mature germ cells are present in a few tubules of some individuals. Spermatogenesis to the stage of mature sperms is encountered only rarely but is compatible with a female chromatin pattern in the somatic cells. The appearance of Leydig cells in large clumps contrasts with their scattered arrangement in normal testes. When the nuclei are male, the tubular defects are less severe and the Leydig cells are in smaller aggregates.

The results of family studies suggest a genetic etiology of the Klinefelter syndrome (*39*). If a mutant gene among the sex-determiners is responsible, the sex-chromosome complex would be XX or XY according to the chromatin pattern. Tests of color vision have yielded conflicting results (*40*). However, there may be an XXY complex or a deficiency in the euchromatic portion of an X chromosome when the nuclei are female, or there may be an abnormality of the autosomes that bear male-determiners whether the nuclei are female or male. In this condition, probably even more than in the other sex anomalies, it will be necessary to study the entire chromosome complement in metaphase. A beginning in this direction has been made (*4, 41*).

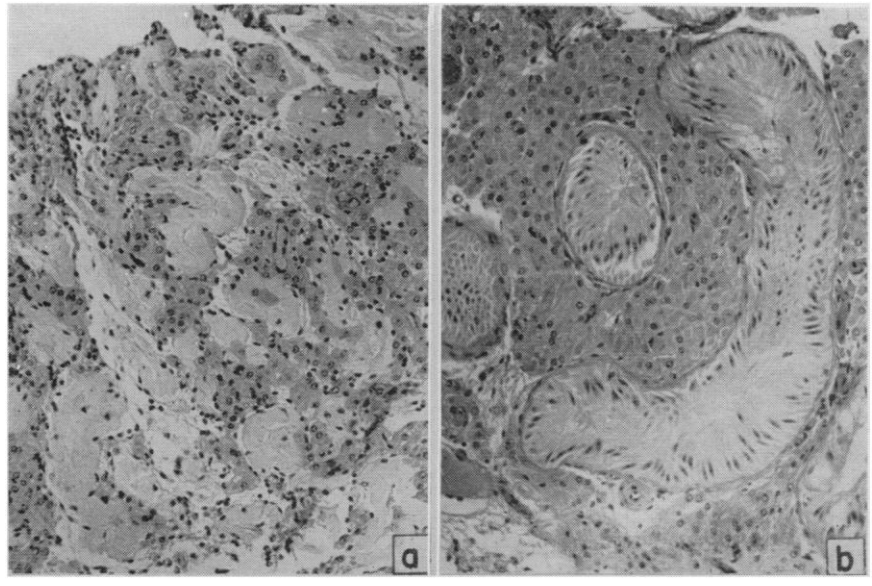

Fig. 7. Photomicrographs illustrating gonadal structure in seminiferous tubule dysgenesis (Klinefelter's syndrome) in a subject with a female chromatin pattern. The tubules may be reduced to hyalinized masses (*a*), or they may have thickened fibrous tunics and grossly defective epithelia (*b*). The Leydig cells are in large aggregates. Hematoxylin-eosin.) (× 125)

Whatever the precise etiological factor may be, testis-like gonads develop from the indifferent gonads of the early embryo regardless of whether the nuclei have female or male characteristics. Although there is severe dysgenesis of the seminiferous tubules, the abundant interstitial cells produce the evocator that masculinizes the embryo, resulting in an individual who has a male phenotype. A full understanding of the syndrome of seminiferous tubule dysgenesis is particularly desirable because of its association, in many instances, with some degree of mental retardation (*42*).

Concluding Remarks

The principle of sexual dimorphism in the structure of intermitotic nuclei is well established and forms the basis of the "tests of chromosomal sex" that are valuable adjuncts to diagnosis in clinical medicine. An attempt has been made to point out deficiencies in our knowledge that may attract the attention and interest of biologists. The etiology of the sex anomalies is in need of clarification, and a study of entire chromosome complements would be especially helpful. Other lines of investigation are suggested by the problems discussed during the Symposium on Nuclear Sex that was held in London, England, in September 1957 (*43*).

Addendum

Several reports that have an intimate bearing on this subject appeared after submission of the manuscript.

At the time of preparation of the paper, there were two reports that dealt with the sex chromosome complex in a sex anomaly, as determined by the new cytological techniques, but they were in disagreement (*4, 41*). Ford *et al.* (*44*) have now described an XX/XXY mosaicism in a patient with the Klinefelter syndrome and a female chromatin pattern, which approaches the description of an XXY complex in a similar patient by Jacobs and Strong (*41*). I understand that the presence of an XXY complex in such subjects has been confirmed by unpublished work in several laboratories. On the basis of this finding, Stern (*45*) was able to resolve the seemingly divergent results that had been recorded in connection with tests of color vision in patients with the Klinefelter syndrome (*40*).

The presence of an extra chromosome, which is one of the smallest autosomes, has been demonstrated in mongolism

(*46*). A somatic cell chromosome number of 48, rather than the normal 46, has been found in a unique individual with both mongolism and the Klinefelter syndrome (*47*). One of the extra chromosomes was the small autosome that occurs in mongolism; the other was contributed by the unusual XXY sex chromosome complex of the Klinefelter syndrome. Important observations are also being made on the sex chromosome complement in cases of gonadal dysgenesis or Turner's syndrome, in which a single X chromosome, unpaired with either another X chromosome or a Y chromosome, has been described (*48*).

These observations necessitate a revision of the currently accepted hypothesis of genetic sex-determining mechanisms in man, which are based on cytogenetic studies in *Drosophila*. It now appears that the Y chromosome, far from having a passive role in sex determination, contains potent male-determining genes. In fact, the gonads have a nearly normal testicular structure in an XXY chromosome-bearing individual until the age of puberty, when the testicular pathology that is characteristic of the Klinefelter syndrome develops rapidly (*49*). The Y chromosome of the mouse has also been shown to bear male-determining factors (*50*). But the details differ in the two species, for the XO sex chromosome arrangement results in a fertile female in the mouse and an infertile female in man.

The view that the sex chromatin is an XX chromosome marker is consistent with an XXY- complex for patients with Klinefelter's syndrome and a female chromatin pattern, and with an XO arrangement for patients with Turner's syndrome and a male chromatin pattern. But the important significance of the recent observations on chromosomal abnormalities is the clear demonstration of aneuploidy as a cause of some developmental errors in man. The next few years are certain to bring developments of the first importance in the field of human cytogenetics and in the application of cytogenetics to certain aspects of clinical medicine.

References and Notes

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