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Bio-science Seminar - Chromosomal Disorders In Man

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BIO-SCIENCE SEMINAR—CHROMOSOMAL DISORDERS IN MAN*

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Subject: Chromosomal Disorders in Man

Moderator: Joel I. Hamburger, M.D.

Discussants: Martin Z. Fruchtman, M.D.

Raymond Guevin, M.D.

DR. HAMBURGER: The study of chromosomal disorders in man has been given great impetus by the recent development of two techniques: the buccal smear for sex chromatin, and direct study of human chromosomes. As a result of these developments, a multitude of reports have been submitted defining the chromosomal complements of numerous disorders of sexual and/or somatic differentiation. Understanding these disorders and the underlying hereditary mechanisms requires familiarity with some of the basic principles and terminology of modern cytogenetics. It is our purpose this evening to review this material. Dr. Fruchtman would you please define and compare the phenomena of meiosis and mitosis, and review for us the mechanism of sex differentiation?

DR. FRUCHTMAN: Meiosis is the process by which mature reproductive cells or gametes are produced (Fig. 1). In this process two major divisions take place, the first of which is reductional and the second, the equation division.
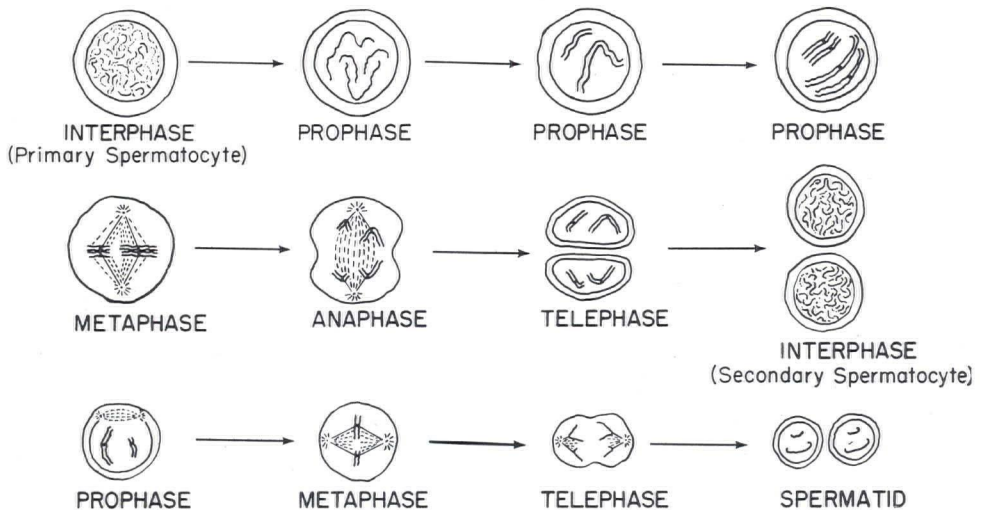

Figure 1
Meiosis.

In the initial phases of meiosis the immature reproductive cell, spermatogonium or oögonium, enlarges to become a primary spermatocyte or oöcyte. Next, the diffuse chromatin material within the resting nucleus condenses to form distinct chromo-

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somes, which in turn pair off and line up on the spindle fibers prior to separating into the two daughter cells, called secondary spermatocytes or oöcytes. In the case of the oöcyte one daughter cell preempts most of the cytoplasm, and its tiny sister cell is left as a polar body. Note that in late prophase each single chromosome consists of paired identical chromatids joined by a centromere or kinetochore. This first meiotic division is referred to as the reduction division because the resultant cells have one-half the original number of chromosomes. In the example of Figure 1 the full chromosomal complement is four. This is called the diploid number of chromosomes; the haploid number is two.

The second meiotic division is simply an example of mitosis, or the sequence of nuclear and cytoplasmic events by which a cell divides into two identical off-springs, each of which, in contrast to the products of the first meiotic division, is an exact replica of the mother cell. In the mitotic or equation division the diffuse chromatin of the interphase nucleus again forms distinct chromosomes in prophase. The chromosomes are split longitudinally into paired chromatids which line up on the spindle fibers. In anaphase a chromatid from each pair separates to opposite poles of the dividing cell to become a chromosome in its own right. In telephase the nuclear membrane envelopes the chromosomes and the cytoplasm divides producing two daughter cells each of which contain the same number of chromosomes as the mother cell. These cells are called spermatids or, in the female, ovum with its polar body. In the further maturation of the spermatids into spermatozoa the cytoplasm is drawn out into a tail leaving the head consisting primarily of the nucleus.

The union of sperm and ovum, forming a zygote, restores the diploid number of chromosomes. At the moment of fertilization genetic sex is established as seen in Figure 2. Females have two X chromosomes, whereas males have an X and a Y. As a consequence of meiosis all ova normally have a single X chromosome, while spermatozoa have either an X or a Y. Fertilization of an ovum by an X sperm produces a genetic female, whereas fertilization by a Y sperm, of course, produces a male.

In man the full chromosomal complement or diploid number is 46, 22 pairs or 44 non-sex chromosomes (autosomes) and two sex chromosomes.[2] The latter are paired X's in the female and unpaired X and Y in the male. Under unusual circumstances some individuals have chromosomal counts which differ from the normal or euploid state. Such a condition is called aneuploidy or heteroploidy, and if a simple multiple of the haploid chromosomal count exists it is termed polyploidy, examples of which are triploidy with 69, or tetraploidy with 92 chromosomes.

DR. HAMBURGER: Dr. Guevin, would you please explain for us the process by which the indifferent fetus, equipped with primordia of both sexes, develops into either a male or female individual?

DR. GUEVIN: Sex differentiation can be divided into three distinct phases, the first of which is gonadal differentiation beginning at the sixth week of intrauterine life. At this time the primitive gonad with both cortical (ovarian) and medullary (testicular) primordia, starts along the path to specific sexual orientation,
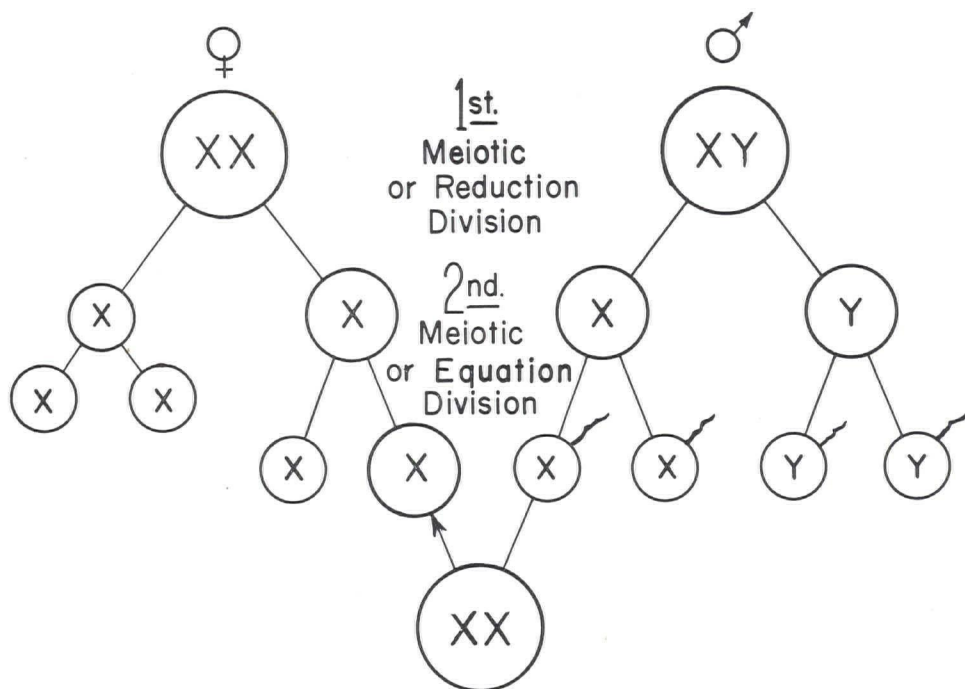
SEX DETERMINATION

Figure 2

Sex determination.

influenced by the balance of genetic factors established at the moment of fertilization (Fig. 3). In the case of XY genetic sex, medullary structures predominate and a testis will form, whereas in the XX individual, cortical structures develop into an ovary. The next phase, that of differentiation of the internal genitalia or genital ducts (Fig. 4), is postulated to be under the control of "evocators" produced by the developing gonad. These evocators are not hormones in the usual sense since their action is local and unilateral, as evidenced by the situation in the hermaphrodite with an ovary on one side, a contralateral testis, and genital ducts appropriate to the respective gonads. Similarly, Jost[6] has shown that in rabbits castrated before there has been any gonadal differentiation, a foetal testis grafted unilaterally will lead to the development of male internal genital ducts on that side. The third phase in sexual differentiation is the development of the external genitalia, and it is important to recognize that this step is entirely hormonally controlled. In contrast to the development of internal genital ducts, the final configuration of the external genitalia can be influenced by sex hormones, whether from gonadal, adrenal or exogenous sources (Figs. 5, 6).

The full significance of the foetal gonad influence on sex differentiation has greatly been clarified by Jost[6] who showed that foetal rabbits castrated prior to gonadal differentiation always develop as females. However, internal and external genitalia remain rudimentary (Fig. 4). The development of the male phenotype
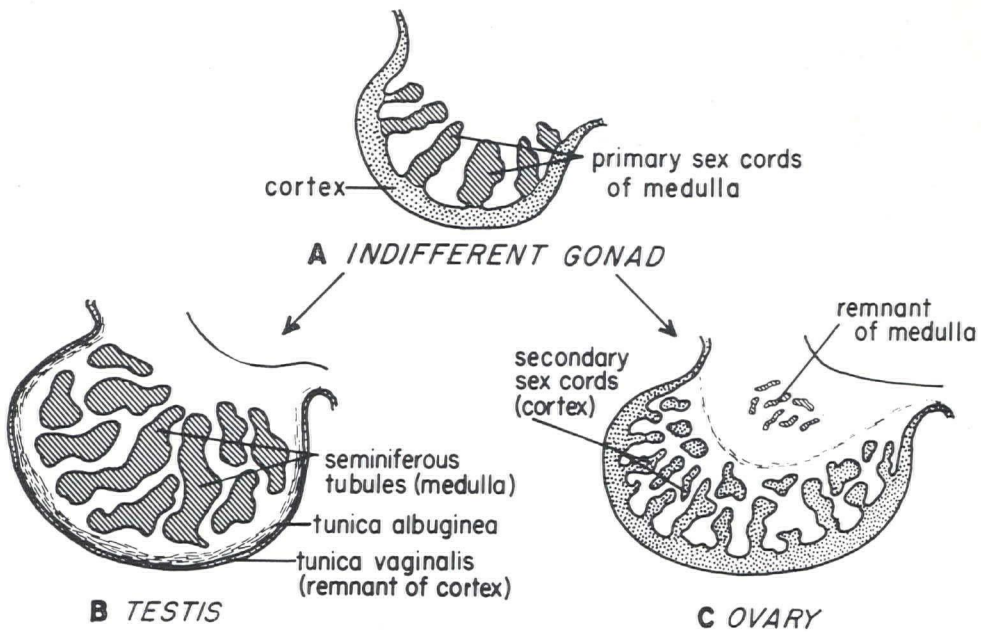
cortex — primary sex cords of medulla

A *INDIFFERENT GONAD*

secondary sex cords (cortex) — remnant of medulla

seminiferous tubules (medulla)
tunica albuginea
tunica vaginalis (remnant of cortex)

B *TESTIS*

C *OVARY*

Figure 3
Differentiation of the indifferent gonad (from Barr[44], by permission of the author).

indifferent gonad
Wolffian duct
Müllerian duct
urogenital sinus

A

normal ♀ — gonadectomized ♀ and ♂ — normal ♂

ovary

uterine horn

Müllerian vagina

testis

seminal vesicle

prostate

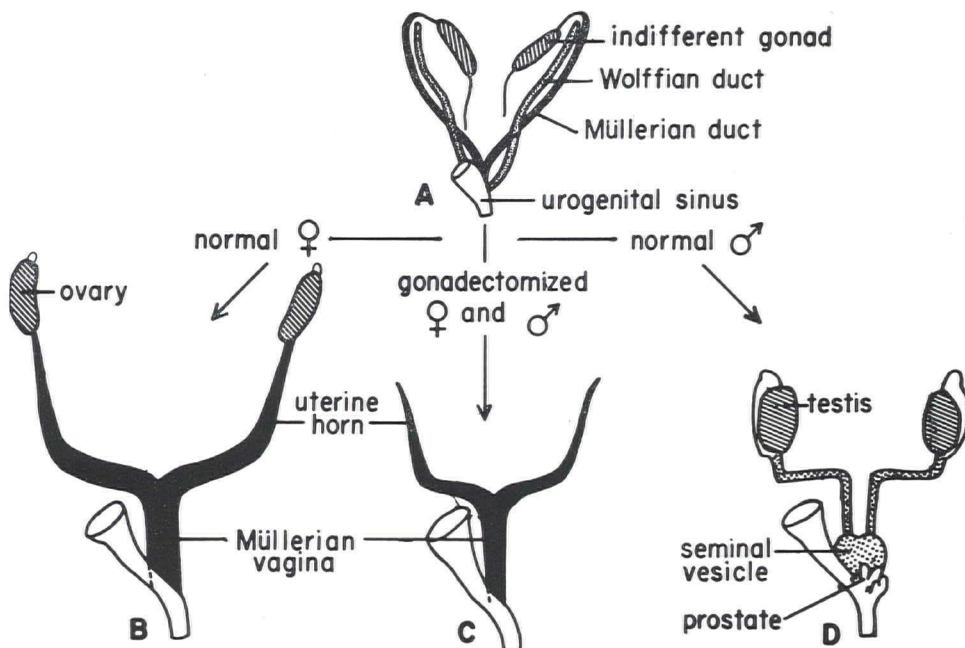**B**   **C**   **D**

Figure 4
Differentiation of genital ducts (from Jost,[45] by courtesy Cambridge University Press).
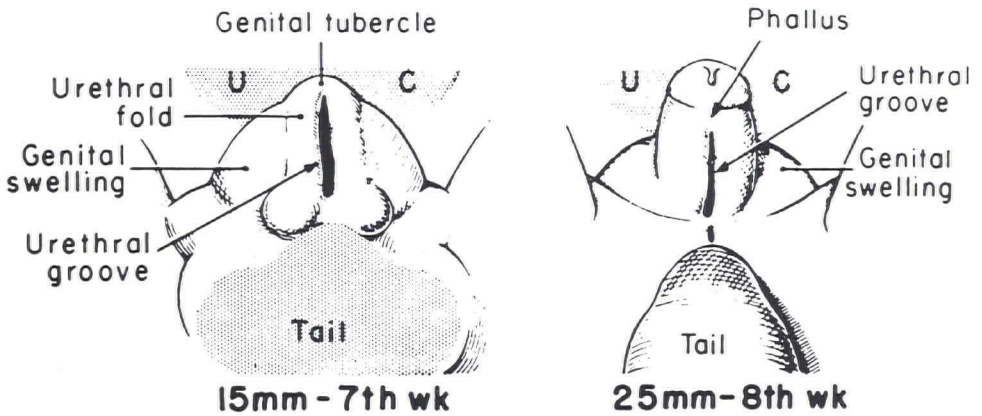
INDIFFERENT

Figure 5
Differentiation of external genitalia (as depicted in Astwood[3]).
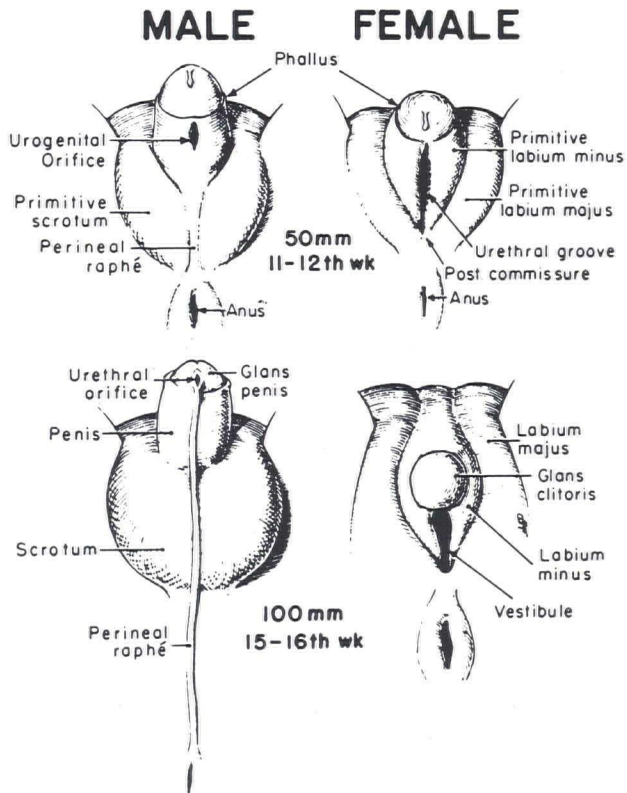
MALE   FEMALE

Figure 6
Differentiation of external genitalia cont. (as depicted in Astwood[3]).

thus requires the presence of specific masculinizing substances of gonadal origin, whereas the female phenotype can develop without such mediators, although sex structures will be somewhat immature. In summary, gonadal differentiation is genetically determined; differentiation of the internal genital ducts is determined by specific local evocator substances of gonadal origin; and differentiation of external genitalia is hormonally determined.

DR. HAMBURGER: Departures from the usual sequence of events in meiosis can lead to defective transmission of the genetic complement to the zygote, resulting in a mutation. Dr. Fruchtman, how might this happen?

DR. FRUCHTMAN: The important mechanisms include crossing over, translocation, deletion, inversion and non-disjunction (Fig. 7). We might briefly define these:
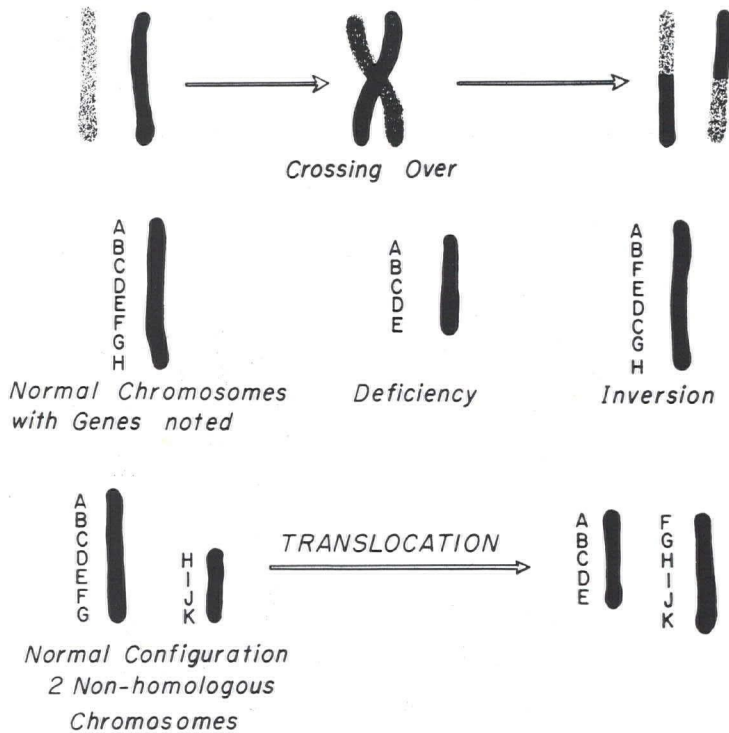
Figure 7
Mechanisms of aberrant genetic transmission.

1. Crossing over refers to the exchange of chromatin material between adjacent homologous chromosomes.

2. Translocation is a similar process but involves an exchange of chromatin material between chromosomes which are *not* paired or homologous.

3. Deletion is simply the loss of a segment of chromatin material from a chromosome, leaving the cell deficient in the genetic influence carried on that chromatin segment.

4. Inversion involves only a change in the order of genetic determinants on a given chromosome — none having been added or substracted.

5. Non-disjunction differs from the above processes in that entire chromosomes may be gained or lost as cells divide. As shown in Figure 8, the X chromosomes fail to separate at the second meiotic division so that the mature ovum contains both X chromosomes, while its polar body has neither. If such an ovum is fertilized by a Y sperm (as in this example) an XXY individual results. This, of course, is the sequence of events which lead to the anomalies of Klinefelter's syndrome.

The environmental factors which favor the occurrence of these mechanisms include increased temperature, radiation, drugs (e.g. colchicine) and advanced maternal age.[1]

DR. HAMBURGER: Since many chromosomal disorders in man result in abnormal sexual differentiation, the buccal smear technique for determining sex chromatin, a rapid method of screening for genetic sex, was hailed as a significant contribution to a better understanding of these disorders. Dr. Fruchtman, how are such smears prepared, and can you show us an example?
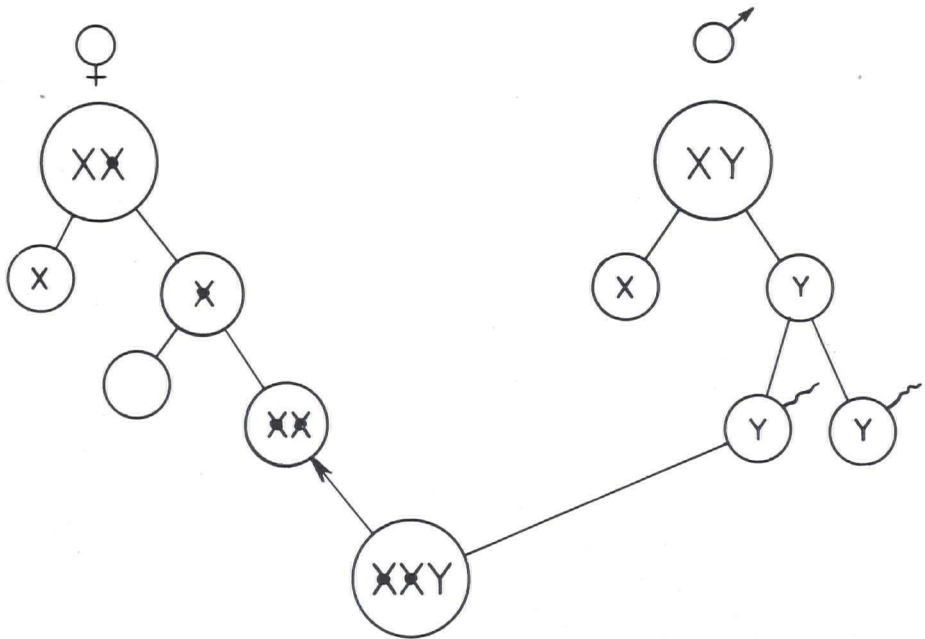
Transmission of color blindness in Klinefelter's Syndrome when neither parent is color blind

Figure 8

Transmission of color blindness in Klinefelter's syndrome when neither parent is color blind. (the X with the central dot carries the gene for color blindness).

DR. FRUCHTMAN: Buccal smears are prepared by gently scraping the buccal mucosa with a tongue blade and transferring the epithelial cells to a glass slide. The smear is fixed immediately in 95% alcohol, or in equal parts 95% alcohol and ether, and later stained with a basic dye. Some laboratories employ mild acid hydrolysis to accentuate the sex chromatin. Males are considered chromatin negative since less than 6 per cent of their nuclei contain sex chromatin. Females are normally chromatin positive since 40 to 80 per cent of their nuclei contain sex chromatin.

DR. HAMBURGER: Dr. Guevin, what does the sex chromatin mass represent?

DR. GUEVIN: In 1949 Barr first called attention to the significance of the clump of chromatin material found at the inner border of the nuclear membrane of the resting cell (Fig 9). This structure was regularly observed in cells of female origin but uncommonly, if at all, in those of male. This is the so-called sex chromatin mass, or chromatin bar. It measures about one micron in diameter and is composed of deoxyribonucleic acid. This mass of nuclear chromatin, clumped and heterochromatic (or heteropycnotic), is stainable and visible in the intermitotic nucleus, whereas the rest of the nuclear chromatin material becomes dispersed.
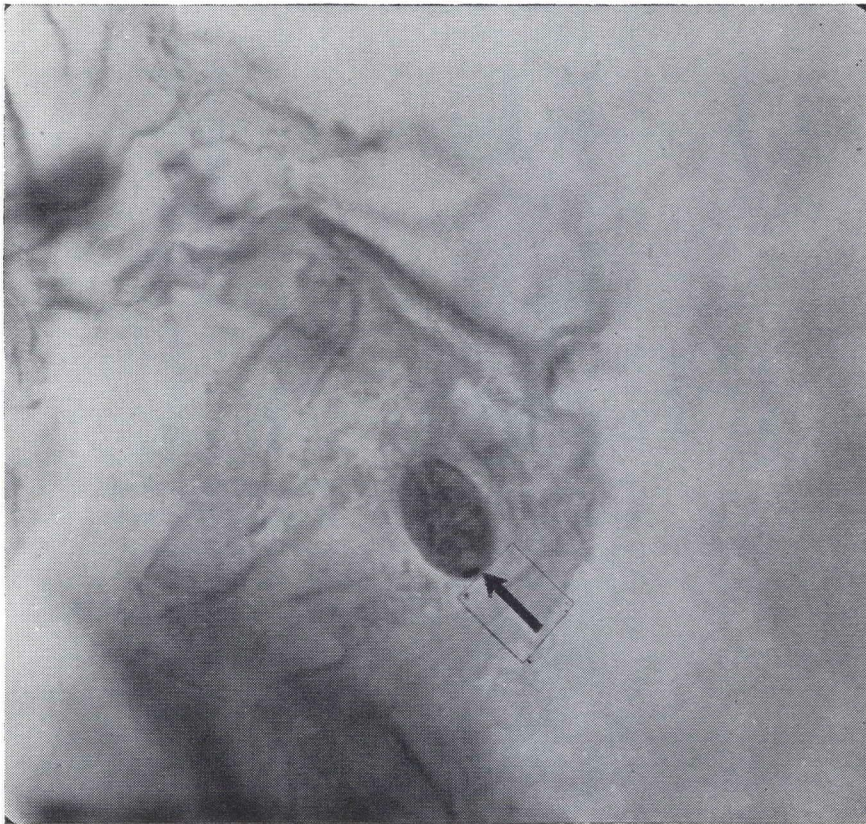

Figure 9
The sex chromatin mass.

In spite of Barr's recommendations of caution regarding the significance of his finding, the impression that nuclear sex is equivalent to genetic sex gained widespread acceptance.

In addition, it was accepted that in the female phenotype the chromatin bar represented fused portions of two X chromosomes, and that its absence in the male was due to the fact that only a single X chromosome is present. This reasoning is compatible with the observations both in chromatin-negative Turner's syndrome where only a single X chromosome is found, and in chromatin-positive Klinefelter's individuals with two X chromosomes. However, earlier studies in chickens, silkworms and some insects revealed that females with only one sex chromosome were chromatin positive.[8,36] Furthermore, in human studies two chromatin bars have been found in the XXX[9] and three in the XXXX females.[10] These observations seem to indicate that a single X chromosome can produce a sex chromatin mass, and that the number of such masses found is one less than the number of X chromosomes in the nucleus. Ohno[11] suggested that the heterochromatic X chromosomes, i.e. those which produce chromatin bars, are of paternal origin and that the maternal X remains dispersed (euchromatic or isopycnotic). However, in chromatin-positive Klinefelter's syndrome there is evidence that both X chromosomes are derived from the mother, but one chromatin bar is seen. If the X chromosome of paternal origin were always heterochromatic one would expect one-half of females to have two chromatin bars — one representing the X chromosome from the father and the other to have been derived from the materal grandfather.

An alternate viewpoint is that all X chromosomes are heterochromatic but that one chromosome has become dispersed to satisfy the metabolic and genetic requirements of the resting cell.[12] All additional X chromosomes would then remain in the heterochromatic state. Although having the virtue of simplicity, this exposition fails to account for the tetraploid state in chromatin-negative individuals with 92 chromosomes, including two X's and two Y's.[13]

An explanation which would cover all of the above situations assumes that each set of 44 autosomes can suppress heterochromaticity of one X chromosome.[13] There still remains, however, the recently described patient with Turner's syndrome with an XO chromosomal complement and a chromatin positive buccal smear. Perhaps the XO classification was an error of interpretation since it is well known that the X chromosome cannot positively be distinguished from autosomes of pairs six to twelve.[10,14] Hence, possibly two X chromosomes were present and one autosome was missing. An alternate view would involve translocation of the heterochromatic portion of the missing X chromosome to one of the autosomes, with deletion of the remainder of the X chromosome. In this particular research area it seems that exceptions have managed to remain one step ahead of explanations.

DR. HAMBURGER: Although the buccal smear is a valuable screening tool, definitive evaluation of chromosomal disorders, whether of sex chromosomes or of autosomes, necessitates actual chromosomal counting. Dr. Fruchtman, in general how is this done?

DR. FRUCHTMAN: In the preparation of cells for chromosomal counts, bone marrow is usually used, but skin and gonadal tissue, and leucocytes from peripheral blood have also been employed. Cells are cultured for five hours, and during the last two hours colchicine is introduced into the medium to arrest mitosis at metaphase. The cell culture is then treated for 10 minutes with a hypotonic solution causing the cells to swell and the chromosomes to disperse. The preparation is fixed, stained and squashed between a glass slide and coverslip to spread the chromosomes which may then be observed in one optical plane under the oil immersion objective. Photomicrographs are taken; the chromosomes are cut out and paired according to centromere position, arm lengths and ratios of their parts. The result is the karyogram or idiogram shown in Figure 10. One employs the term karyotype to refer to a specific chromosomal makeup, e.g., one with Klinefelter's syndrome might have an XXY karyotype. This should be differentiated from genotype which indicates the specific gene complement, and phenotype which refers to physical configuration.
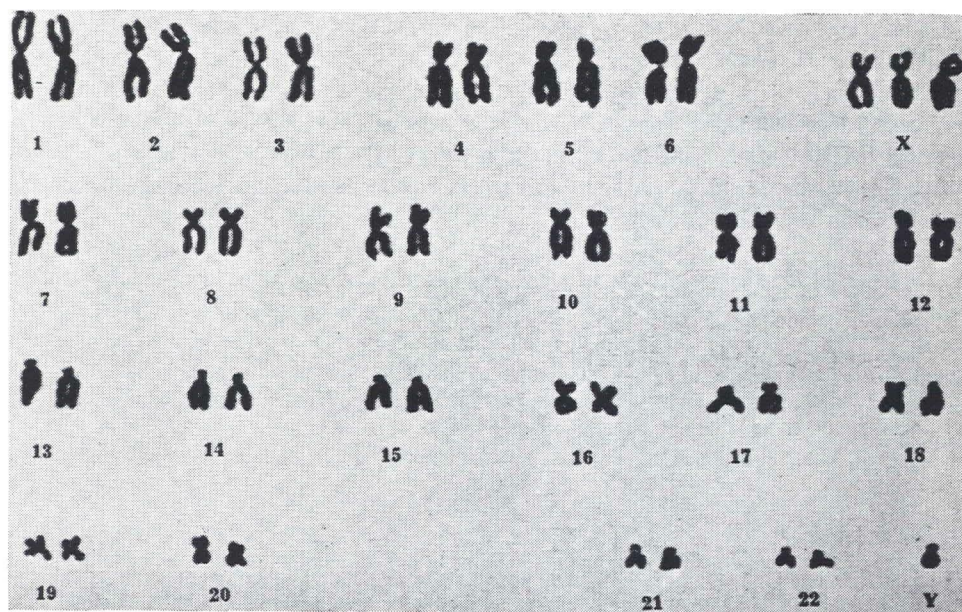

Figure 10

Karyogram of XXXY Klinefelter's individual (from Ferguson-Smith[36]).

DR. HAMBURGER: Dr. Fruchtman, what are some of the criticisms of this technique?

DR. FRUCHTMAN: The assumption that chromosomes of similar morphology are in fact homologus is fraught with danger. It is a fundamental principle of genetics that proof of the homologous nature of two chromosomes depends upon the demonstration of pairing during mitosis.[1]

Because of defects in the technical aspects of the method, it is unusual even in normal individuals for every metaphase cell to contain the same number of

chromosomes. Hence, in actual practice the range of chromosomal numbers is determined. The majority of atypical cells usually have less than the modal (or most common) number, having lost one or more chromosomes in the process of preparation. At times, however, cells are found with an extra chromosome which has been spuriously introduced. Nevertheless, the modal number usually predominates when enough properly prepared cells are counted. It is important not to include cells in the count when: (1), the chromosomes are poorly spread; (2), the cells are ruptured; or (3), one or more chromosomes are split at their centromeres into sister chromatids. It is generally assumed that counts differing from the modal count are artefacts, but they could represent either abnormalities during mitosis or mosaics. In a recent report Berman[15] has suggested the direct examination of marrow cells, avoiding incubation, as a means of reducing the artefact inherent in the method.

DR. HAMBURGER: Dr. Fruchtman, what is mosaicism and what criteria must be established to confirm the existence of a mosaic?

DR. FRUCHTMAN: Mosaic is a term applied to an individual in whom cells exist with more than one chromosomal number. For a mosaic to result it is apparent that the anomalous events must occur after fertilization of the ovum and formation of the single cell zygote. Any defect in maturation of germ cells would be reflected in the zygote itself and, hence, in all cells of the developing embryo. Furthermore, such an event must occur soon after the zygote is formed — probably in the first or second division — if the final individual is to have any significant number of atypical cells. As noted by Ford[16] the proportion of cells deviating from the species number may not be the exact fraction predicted on the basis of the time at which

Figure 11

Mosaicism.

MOSAICISM

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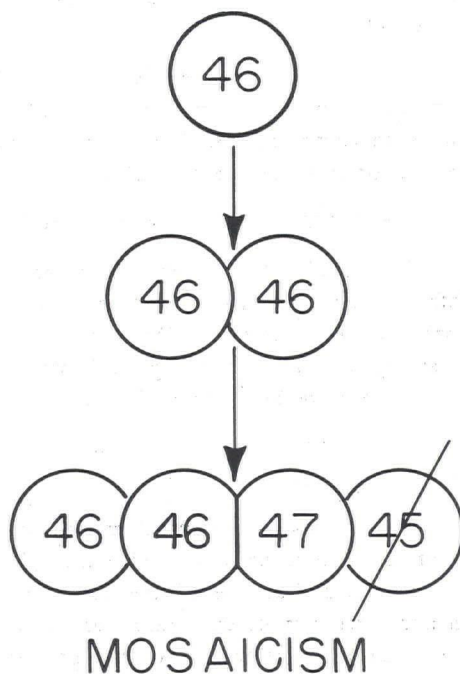
the anomalous mitotic division took place, since selective forces appear to operate which favor the survival and multiplication of cells with the more nearly normal chromosomal complement. For example, (Fig. 11) if the defect occurred at the second mitotic division of the zygote the result would be two normal and two abnormal cells. Neither abnormal cell might be viable, but if one survived and continued to divide, one might expect that the final individual would be a mosaic of two-third normal and one-third abnormal cells. However, as a result of unfavorable selective influences acting upon the abnormal cell the final mosaic ratio might actually be three to one. The degree of selectivity also could vary from organ to organ such that in marrow the percentage of abnormal cells might be 30, while in the skin 5 or less.

Since it is unusual for all metaphase cells to contain the same number of chromosomes even in normal individuals, Court-Brown[17] has suggested criteria which must be satisfied in the establishment of a mosaic:

(1) The number of cells with counts differing from the modal number must be greater than reasonably may be attributed to chance, usually about 30 per cent.

(2) This excess of atypical cells must be found in two or more cell cultures, preferably in two or more tissues.

(3) A high proportion of atypical cells should have the same karyotype, that is, the same chromosomes are deficient or in excess.

DR. HAMBURGER: Of the chromosomal disorders, the first to be described was the trisomy of chromosome No. 21 in mongolism. Dr. Guevin, how is this explained?

DR. GUEVIN: The trisomy of mongolism is presumed to result from maternal nondisjunction of chromosome No. 21 during oögenesis, producing an ovum with two such chromosomes. When such an ovum is fertilized by a normal sperm, trisomy results, or three of the No. 21 chromosome.[18]

DR. HAMBURGER: What is the karyotype of parents and offsprings of mongols?

DR. GUEVIN: Parents of mongols usually have a normal karyotype, but one father (with an apparently normal phenotype) has been reported with trisomy of chromosome nineteen.[20] This will be discussed later. When mongols are fertile, offspring are usually mongols; however, in some cases a normal karyotype and phenotype have been found.[19]

DR. HAMBURGER: How does familial mongolism differ from the more frequent sporadic cases?

DR. GUEVIN: Familial mongolism with a chromosomal count of 46 has been reported without an advanced maternal age factor. However, close study of some cells revealed that one of the autosomes of pair 22 was larger than its mate and an additional minute chromatin particle was present (Figure 12). This was apparently the result of translocation of the greater part of a third No. 21 chromosome to a

No. 22, with the extra chromatin particle representing the residual centric fragment of that third No. 21 chromosome. Hence, the result is essentially trisomy of the 21st chromosome.[21]

SPORADIC MONGOLISM

21	22

FAMILIAL MONGOLISM

21	22

Figure 12
Familial mongolism (numbers below chromosomes indicate their idiogram position — see Figure 10).

	PATIENT	MOTHER MAT. AUNT MAT. G. M.	FATHER BROTHER
15	∧	∧	∧ ∧
21	∧∧	∧	∧ ∧
15/21	X	X	
No. Chromosomes	46	45	46

Figure 13
Familial mongolism, alternate type (figures in column to the left
indicate pair number of chromosomes depicted).

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In another study a mongol boy was found to have 46 chromosomes while his mother, maternal aunt and maternal grandmother each had 45. Analyses of the idiograms revealed that the father and brother had normal chromosomal complements of 46. The boy, although having 46 chromosomes, had only one of pair 15 and an extra chromosome with the configuration of those in pairs six to twelve. The mother, maternal aunt and maternal grandmother had only one chromosome of pairs 15 and 21, but they also had the extra chromosome described above (Fig. 13). The interpretation is that the extra chromosome represents a fusion of one chromosome of pair 15 with one of pair 21. This results in a normal genetic complement in the female relatives, and provides for the equivalent of 47 chromosomes with trisomy of No. 21 for the mongol son.[43]

DR. HAMBURGER: Another example of trisomy is the XXY karyotype in Klinefelter's syndrome. Dr. Fruchtman, what is the evidence for maternal non-disjunction in this disorder?

DR. FRUCHTMAN: Non-disjunction during oögenesis was suggested by two observations. The first was that a mean maternal age of 33 years was observed for patients with this disorder in a population with an overall mean maternal age of 27.5 years.[22] Mental retardation is more frequent in the progeny of mothers with relatively advanced age, and mental retardation occurs with increased frequency in Klinefelter's syndrome. Since it is known that advanced maternal age predisposes to non-disjunction, the above has been considered suggestive evidence. The second observation, of greater genetic value, related to the occurrence of color-blindness in patients with Klinefelter's syndrome where both parents had normal color vision. Color vision is a sex-linked recessive trait carried on the X chromosome, and had the father been responsible for the patient's color-blindness he too should have been color-blind, since there is no corresponding gene on the Y chromosome to oppose the expression of this trait. Since neither the father nor the mother were color-blind, it must be presumed that the mother was a heterozygous carrier of the trait. Further-more, as shown in Figure 8, non-disjunction can be pinpointed to the second division of meiosis, since this is the only time at which a gamete could be produced[22] with two chromosomes carrying genes for color-blindness.

DR. HAMBURGER: Dr. Fruchtman, what other karyotypes have been observed with the picture of Klinefelter's syndrome?

DR. FRUCHTMAN: Examples of Klinefelter's syndrome have now been reported with karyotypes of XX, XXY, XY,[22] XXYY,[23] XXXY,[24] and a mosaic of XXY/XX.[25] These are explained on the basis of various combinations of non-disjunction and translocation.

DR. HAMBURGER: Klinefelter's syndrome and mongolism would appear to have much in common from the genetic standpoint. Dr. Fruchtman, would you summarize these similarities for us?

DR. FRUCHTMAN: Mongolism and Klinefelter's syndrome have the following in common: (1), they result from non-disjunction during oogenesis; (2), there is a relatively advanced maternal age; and (3), mental deficiency, the rule in mongolism,

occurs with increased frequency in Klinefelter's syndrome. As might be expected in disorders with common etiologic factors, it was not long before individuals were reported with both disorders and 48 chromosomes.[26,27] Also reported was a case with 49 chromosomes with trisomy at two autosomal positions;[26] and instances of triploidy[28] and even tetraploidy[13] have been seen. These observations indicate that non-disjunction can involve any or all chromosomal pairs in single or multiple fashion.

DR. HAMBURGER: A more recently described example of trisomy is the so-called "super-female". Dr. Guevin, could you describe these women?

DR. GUEVIN: These inappropriately named women with an XXX karyotype are usually underdeveloped sexually, cease menstruating at an early age, and are infertile. However, two fertile cases have been described and in both the offsprings were apparently normal males.[9,29,30]

DR. HAMBURGER: Dr. Guevin, what other instances of trisomy have been described and what is their significance?

DR. GUEVIN: Trisomy has been reported with chromosomes 15, 17, 19 and 22. In most cases there were associated congenital anomalies, but the trisomy of No. 19 chromosome was found in an apparently normal male who was studied only because he fathered a mongol. These reports suggest that trisomy may be found more frequently than might be expected, and since it can occur without overt evidence of abnormality, the mere association of trisomy and congenital anomalies does not prove a causal relationship. It is of interest that trisomy of No. 22 was found in an individual with Sturge-Weber syndrome.[20]

DR. HAMBURGER: Up to now we have been discussing instances in which extra chromosomes are present. Of equal interest are the chromosomal deficiencies. Gonadal dysgenesis or Turner's syndrome, although originally predicated on the basis of the triad of short stature, multiple congenital anomalies and gonadal dysgenesis, has achieved more solid footing since an XO karyotype was observed in a high proportion of cases. Dr. Guevin, what is the explanation for this XO chromosomal complement?

DR. GUEVIN: The XO chromosomal complement in Turner's syndrome results from non-disjunction during spermatogenesis with subsequent fertilization of an X ovum by a sperm without any sex chromosome.

DR. HAMBURGER: For a brief interval in the development of our understanding of chromosomal disorders, it seemed that the XO karyotype might be the most appropriate ground on which to define the limits of Turner's syndrome. The observation that chromatin-negative patients with Turner's syndrome had an XO karyotype was compatible with the prevailing concepts of sex differentiation that two X chromosomes are necessary for ovarian development, and that agonadal individuals develop in an immature female fashion. However, frequent reports of so-called varients of Turner's syndrome are a source of considerable confusion. Dr. Guevin, would you please describe some of these perplexing patients?

DR. GUEVIN: Twenty to 30 per cent of patients with typical features of

Turner's syndrome are chromatin-positive, and they presumably have an XX karyotype.[3] Also chromatin-negative individuals with an XO karyotype have menstruated regularly[31] and, in a carefully documented study, one such subject has given birth to a normal chromatin-negative male.[32] Males with undescended or dysgenetic testicles and the anomalies and stature of Turner's syndrome have been reported with an XY karyotype,[4,46] and in one instance with an XO karyotype.[34] More recently Grumbach[10] has reported a patient with Turner's syndrome and an XO chromosomal complement, but the buccal smear was chromatin-positive.

DR. HAMBURGER: Upon what basis might these observations be integrated into a unifying concept for Turner's syndrome?

DR. GUEVIN: The development of a unifying concept to encompass these seemingly incongruous observations, and perhaps others still to be described, necessitates thinking in terms of genes rather than of buccal smears or chromosomes. The fundamental factors controlling gonadal and somatic development clearly reside within genes. With this principle in mind, and recalling the various mechanisms by which mutations may be produced, possible explanations for the Turner's varients may be considered.

The chromatin-positive XX individuals at times have one X which is smaller than its mate.[35] Perhaps this represents the deletion of genes from the smaller X, leading to gonadal and somatic anomalies and a retention of the heterochromatin and chromatin positivity. Or both X chromosomes with the full number of genes could have functional impairment of the latter, perhaps on a biochemical or enzymatic basis. Some chromatin-positive patients with Turner's syndrome are XX/XO mosaics.[22] The menstruating and fertile XO subjects might be examples of translocation of genes from the missing X to an autosome, thus retaining relatively normal genetic capacity for gonadal and sexual development. The loss of the remaining genes from the missing X could be the basis for the somatic anomalies. Another possible explanation in these XO patients is an erroneous karyotype. Since the X chromosome cannot be separated with absolute certainty from autosomes of pairs 6 through 12, both X's might be present and one autosome absent, the latter resulting in somatic anomalies. The chromatin-positive, XO patients might have retained the heterochromatic portion of the missing X chromosome by means of translocation to an autosome. Similar explanations could be applied to each of the variants of Turner's syndrome, employing combinations of known mechanisms to account for the observed karyotype and phenotype.

Hence, it becomes apparent that the possession of an incomplete complement of functioning genes on the sex chromosomes is the fundamental factor common to all of these disorders. The involved genes might be those for sexual or somatic differentiation, or both. The karyotype reflects the qualitative and quantitative aspects of this deficiency, and the phenotype is the expression of these differences. Thus, Turner's syndrome should be considered a spectrum of disorders (Table 1) at one end of which are females with classical Turner's stigmata, an XO karyotype, but remaining fertile. At the other extreme are males with similar anomalies, an XY karyotype and, possibly, fertility. Between these extremes would fall the remaining variants.

Chromosomal Disorders

Table 1

Classification	Sex Chromatin	Karyotype	Phenotype	Anomalies
Turner's	—	XO	Fertile	Yes
Turner's	––	XO	Immature Menstruating	Yes
Turner's (Classical)	—	XO	Immature	Yes
Turner's	+	XX,Xx XX,XO XO	Immature	Yes
Pure Gonadal Dysgenesis	—	XY	Immature	No
Turner's—Male	—	XO	Cryptorchid Hypospadiac	Yes
Turner's—Male	—	XY	Cryptorchid Dysgenetic	Yes

DR. HAMBURGER: What is the karyotype of the true hermaphrodite? Dr. Fruchtman, can you help us?

DR. FRUCHTMAN: True hermaphrodites of course, have both ovarian and testicular tissue. Several karyotypes have been reported, including XX, XX/XO, XXX/XO and XXY/XO.[37,40] However, most reported cases have had 46 chromosomes and XX sex chromosomal complement.

DR. HAMBURGER: What is the genetic significance of the XX karyotype in the true hermaphrodite?

DR. FRUCHTMAN: It is of interest that hermaphrodites lacking the Y chromosome have been able to develop testes. This is in contrast to Harnden's case of pure gonadal dysgenesis in which the karyotype was XY and no gonadal tissue of any type was found.[33] Such cases challenge current concepts of sex differentiation which propose that testes do not develop without the Y chromosome and, as a corollary, testes do develop when the Y chromosome is present. Again, one must think in terms of gene dysfunction, translocation, deletion or combinations of these or perhaps other mechanism to explain such occurrences.

DR. HAMBURGER: Dr. Guevin, are there instances of autosomal deletion?

DR. GUEVIN: Turpin[22] has reported a 4 year old boy with multiple vertebral anomalies and 45 chromosomes. Missing was one of the group of small subterminal chromosomes. In addition, one of the larger subterminal chromosomes was remarkable in that it had much longer "arms" than its homologue. Perhaps this was the result of partial translocation with the deletion of remaining fragments.

DR. HAMBURGER: What observations have been made on the chromosomal complements in leukemia? Dr. Guevin, would you briefly answer this last question?

DR. GUEVIN: The increased incidence of leukemia in mongolism has resulted in considerable interest in the study of the chromosomal complements. In the

chronic leukemias they have been normal, but in acute leukemia abnormal chromosomal counts have been reported in two patients, and evidence for translocation existed in two others.[41,42] An additional patient with presumed radiation-induced acute leukemia had a modal number of 42 with many polyploid cells containing predominantly multiples of 21 chromosomes.[41]

DR. HAMBURGER: Dr. Smith, what would appear to be the future for studies of the chromosomes?

DR. SMITH: Your associates, Dr. Hamburger, have been quite effective in answering questions, and I judge that the three of you are better qualified to discuss this aspect. One must have solid footing in the knowledge of the past and present before attempting any projection into the future.

We have referred today to the gene as the final locus of intrachromosomal determinants for cellular development and function. It is tempting, therefore, to propose that inquiry into the nature of this genetic unit would be potentially fruitful. There is little question but that the assumption of the existence of such a genetic unit has served well to keep the peace between morphology and function. However, the evidence is fast accumulating, if not already at hand, that genes in the traditional sense do not exist. The brilliant contributions of the past few years in respect to the chemistry and molecular configuration of deoxyribonucleic acid (DNA), the substance which carries the master code of genetic information, permit us now to visualize for the first time the elemental structure of the system for transmission of cellular information. Instead of the mysterious gene, we now have a well-defined chemical template, a long spiral ladder of but three chemical moieties — sugar, phosphate and purine-pyrimidines.

The enthusiast might say that much of the future effort will be concerned with correlating the orderly structural variations of DNA with specific chromosomes. He might ask: what are the forces which control the pre-mitotic segregation of DNA material into the exact numbers of chromosomes characteristic of each species? However, these are tremendous challenges for ultra-micro physical chemistry, and the answers would seem to lie at the far end of such a long projection into the future.

As clinicians, we are faced with a great number of disorders, many poorly understood as to etiology, with such general designations as inherited, acquired, congenital, idiopathic, familial, constitutional, inborn error of metabolism, etc. The opportunity is unlimited to attempt a genetic deciphering of these in terms of a demonstrable defect in one or another of the autosomes. This, of course, means a painstaking effort at correlating specific autosomes with specific cellular and organ systems. It would seem to be a large area for future research. Progress in unravelling some of the mysteries surrounding sex differentiation has been possible by virtue of the geneticist's ability to recognize the sex chromosomes. No ready tag of identification is apparent for the autosomes but, judged by the resourcefulness shown to date, we can be confident that new contributions of significance will be added to this rapidly advancing field.

REFERENCES

1. Wagner, R. P., and Mitchell, H. K.: Genetics in Metabolism, New York, Wiley, 1955.

2. Tjio, J. H., and Levan, A.: The chromosome numbers of man. Hereditas 42:1, 1956.

3. Astwood, E. B., ed.: Clinical Endocrinology I. New York, Grune & Stratton, 1960.

4. Wilkins, L.: Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence, ed. 2, Springfield, Ill., C. C. Thomas, 1957.

5. Grumbach, M. M., VanWyk, J. J., and Wilkins, L.: Chromosomal sex in gonadal dysgenesis (ovarian agenesis): Relationship to male pseudohermaphrodism and theories of human sex differentiation, J. Clin. Endocrin. 15:1161, 1955.

6. Jost, A.: Problems of fetal endocrinology: The gonadal and hypophyseal hormones, Recent Prog. Hormone Res. 8:379, 1953.

7. Barr, M. L., and Bertran, E. G.: Morphological distinction between neurones of male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis, Nature 163:676, 1949.

8. Barr, M. L. and Bertram, E. G.: Sexual dimorphism in interphase nuclei, In, Symposium on Cytology in Human Genetics, Sept. 2, 1959, Pennsylvania State University.

9. Jacobs, P. A., Baikie, A. G., Brown, W. M., MacGregor, T. N., MacLean, N., and Harnden, D. G.: Evidence for the existence of the human "super female", Lancet, 2:423, 1959.

10. Barr, N. L., and Carr, D. H.: Sex chromatin, sex chromosome and sex anomalies, Proc., Northcentral Sect., Am. Urological Assoc., Nov. 30, 1960, Detroit, Mich.

11. Ohno, S., Kaplan, W. D., and Kinosita, R.: Formation of the sex chromatin by a single X-chromosome in liver cells *rattus norvegicus,* Exper. Cell Res. 18:415, 1959.

12. Smith, A.: Sex-chromatin body, Lancet 2:319, 1960.

13. More chromosome anomalies, Lancet 2:191, 1960.

14. Fraccaro, M., and Lindsten, J.: A child with 49 chromosomes, Lancet 2:1303, 1960.

15. Prowsner, E. R., and Berman, L.: The chromosomes of human bone marrow cells — morphologic study of material prepared promptly after aspiration, Proc., Third Meeting, Am. Soc. Hematology, Nov. 29, 1960, Montreal, Canada.

16. Ford, C. E.: Human cytogenetics: Its present place and future possibilities, Am. J. Human Genet. 12:104, 1960.

17. Court-Brown, W. M., Jacobs, P. A., and Doll, R.: Interpretation of chromosome counts made on bone-marrow cells, Lancet 1:160, 1960.

18. Lejeune, J., Gautier, M., and Turpin, R.: Etude des chromosomes somatiques de neuf enfants mongoliens, C. rend. Acad. Sc. 248:1721, 1959.

19. Lehmann, O., and Forseman, H.: Chromosome complements in a monogoloid mother, her child and the child's father, Lancet 1:498, 1960.

20. Hayward, M. D., and Bower, B. D.: Chromosomal trisomy associated with the Sturge-Weber syndrome, Lancet 2:844, 1960.

21. Penrose, L. S., Ellis, J. R., and Joy, D. A.: Chromosomal translocation in mongolism and in normal relatives, Lancet 2:409, 1960.

22. Ferguson-Smith, M. A.: Cytogenetics in man, A.M.A. Arch. Int. Med. 105:627, 1960.

23. Muldal, S., and Ochey, C. H.: The "double male": A new chromosome constitution in Klinefelter's syndrome, Lancet 2:492, 1960.

24. Ferguson-Smith, M. A., Johnston, A. W., and Handmaker, S. D.: Primary amentia and micro-orchidism associated with an XXXY sex-chromosome constitution, Lancet 2:184, 1960.

25. Crooke, A. A., and Hayward, M. D.: Mosaicism in Klinefelter's syndrome, Lancet 1.1198, 1960.

27. Lanman, J. T., Sklarin, B. S., Cooper, H. L., and Hirschhorn, K:. Klinefelter's syndrome in a ten-month-old mongolian idiot: Report of a case with chromosome analysis, New England J. Med. 263:887, 1960.

28. Book, J. A., and Santesson, B.: Malformation syndrome in man associated with triploidy (69) chromosomes), Lancet 1:858, 1960.

29. Stewart, J. S. S., and Sanderson, A. R.: Fertility and oligophrenia in apparent triplo-X female, Lancet 2:21, 1960.

30. Fraser, J. H., Campbell, J., MacGillivray, R. C., Boyd, E. ,and Lennox, B.: The XXX syndrome frequency among mental defectives and fetrility, Lancet 2:626, 1960.

31. Stewart, J. S. S.: Gonadal dysgenesis: The genetic significance of unusual variants, Acta Endocrin. 33:89, 1960.

32. Bahner, F., Schwarz, G., Harnden, D. G., Jacobs, P. A., Hienz, H. A., and Walters, K.: A fertile female with XO sex chromosome constitution, Lancet 2:100, 1960.

33. Harnden, D. G., and Stewart, J. S.: The chromosomes in a case of pure gonadal dysgenesis, Brit. M. J. 2:1285, 1959.

34. Bloise, W., DeAssis, L. M., Bottura, C., and Ferrari, I.: Gonadal dyspenesis (Turner's syndrome) with male phenotype and XO chromosomal constitution, Lancet 2:1059, 1960.

35. Griboff, S. I., and Lawrence, R.: A proposed genetic theory for the pathogenesis of certain congenital gonadal defects, Lancet 1:602, 1960.

36. Ferguson-Smith, M. A., and Johnston, A. W.: Chromosome abnormalities in certain diseases of man, Ann. Int. Med. 53:359, 1960.

37. Harden, D. G., and Armstrong, C. N.: The chromosomes of a true hermaphrodite, Brit. M. J. 2:1287, 1959.

38. Ferguson-Smith, M. A., Johnston, A. W., and Weinberg, A. N.: The chromosome complement in true hermaphroditism, Lancet 2:126, 1960.

39. DeAssis, L. M., and Bottura, C.: Chromosomal constitution in nuclear sex of a true hermaphrodite, Lancet 2:129, 1960.

40. Gordon, R. R., O'Gorman, F. J. P., Dewhurst, C. J., and Blank, C. E.: Chromosome count in a hermaphrodite with some features of Klinefelter's syndrome, Lancet 2:736, 1960.

41. Hirschhorn, K., Decker, W., and Cooper, H. L.: True hermaphroditism wtih XY/XO mosaicism, Lancet 2:319, 1960.

42. Baikie, A. G., Court-Brown, W. M., Jacobs, P. A., and Milne, J. S.: Chromosome studies in human leukemia, Lancet 2:425, 1959.

43. Carter, C. O., Hamerton, J. L., Polani, P. E., Gunalp, A., and Weller, S. D. V.: Chromosome translocation as a cause of familial mongolism, Lancet 2:678, 1960.

44. Barr, M. L.: Sex chromatin and phenotype in man, Science 130:679, 1959.

45. Jost, A.: In, Society for Endocrinology: Symposium on Sex Differentiation and Development, 1958. Ed. by C. R. Austin, London, Cambridge University Press, 1959, p.

46. Steiker, D. D., Mellman, W. J., Bongiovanni, A. M., Eberlein, W. R. and Leboeuf, G.: Turner's syndrome in the male, J. Pediat. 58:321, 1961.