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Sex Determination and Problems Arising from Indeterminate Sex

by

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Nowadays, although the meaning of sex, *i.e.* the male or female sex, is generally agreed to in a superficial sort of way by most doctors, yet when the term is considered in greater detail, it will be seen that the word 'sex' is insufficient to convey an exact meaning without qualifying it with certain adjectives, *e.g. chromosomal* sex, which means the sex chromosome complement in any particular individual; *chromatin* sex, *i.e.* whether there are chromatin dots in an individual's cells or not; the *gonadal* sex which describes the type of gonads, *i.e.* testis or ovary possessed by an individual; the *genital* sex which describes whether the internal or external genitalia are those of a normal male or a normal female; the *psychological* sex or the sex which is imposed on a child by the parents in their upbringing; the *social* sex or the behaviour of an individual in an accepted male or female manner. (Table I) In most instances, an individual who is male will have characteristics of the male sex in whatever context the word is used, *i.e.* he will have an XY chromosomal sex complement, testes, vas deferens, penis with scrotum, be brought up as a boy and behave like a man in society; and similarly if she is female. However, it is because it is possible for a single individual to have "cross sex characteristics" that such aberrations pose an acute problem not only to doctors but also to the individuals concerned, the parents and society in general. The sex of such individuals is described as *indeterminate.*

"SEX"

Type	Male	Female
Chromosomal	Xy	XX
Chromatin	O−ve	O+ve
Gonadal	Testes	Ovary
Genital (Int)	Vas, etc.	Uterus, etc
Genital (Ext)	Penis, etc	Vulva, etc
Psychological	Boy	Girl
Social	Manly	Womanly

TABLE I. Different kinds of Sex.

Embryology of Sex:

a) **Chromosome:**

It has been demonstrated in 1956 (1) that the human cell consists of 46 chromosomes in 23 pairs, one pair of which is the sex chromosomes. In the female the members of the pair are identical and hence can be denoted by the notation XX, while one of the members of the pair in the male is identical with female sex chromosomes, the other member is dissimilar and much smaller and has been

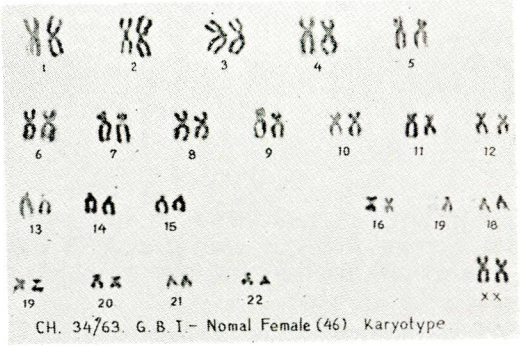


FIG. 1. Karyotype of normal female chromosomes. Note the sex chromosomes XX.

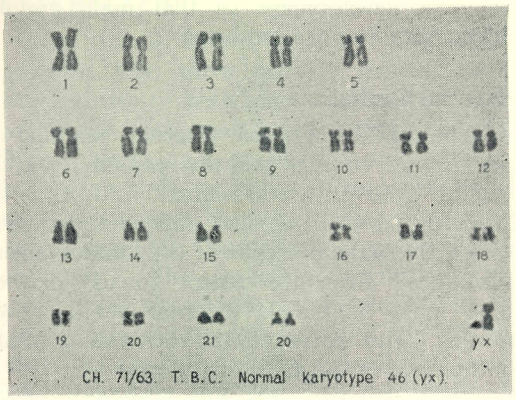


FIG. 2. Karyotype of normal male chromosomes. Note the sex chromsomes XY.

notationally designated XY. Figs. 1 and 2 show the chromosome constitution of a normal female and a normal male respectively. However, in the formation of the gamete, *i.e.* the male sperm and the ovum in the female, there must be a halving division of the total chromosome complement, so that at fertilisation when a sperm joins an ovum, the total normal number of 46 is made up again. Therefore there are 23 chromosomes only in a sperm and 23 in an ovum, and with reference to the sex chromosomes, in each gamete there will be only ONE sex chromosome instead of 2, so that the sperms may be X-bearing or Y-bearing while the ova will all be X-bearing. Depending on whether it is the X-bearing sperm that fertilises the X-bearing ovum, or whether it is the Y-bearing sperm that does so, a female or male zygote will result (Fig. 3). This is

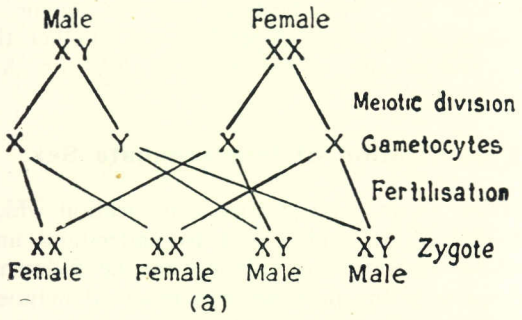


FIG. 3. Showing normal meiosis and fertilisation.

therefore the way in which chromosomally males or females are produced.

b) **Chromatin sex:**

It has been found that in the normal female, the cells of her tissues show a chromatin dot (2) which is situated at the periphery of the cells, while the male cells are devoid of this chromatin dot. Thus the normal female is chromatin +ve while the normal male is

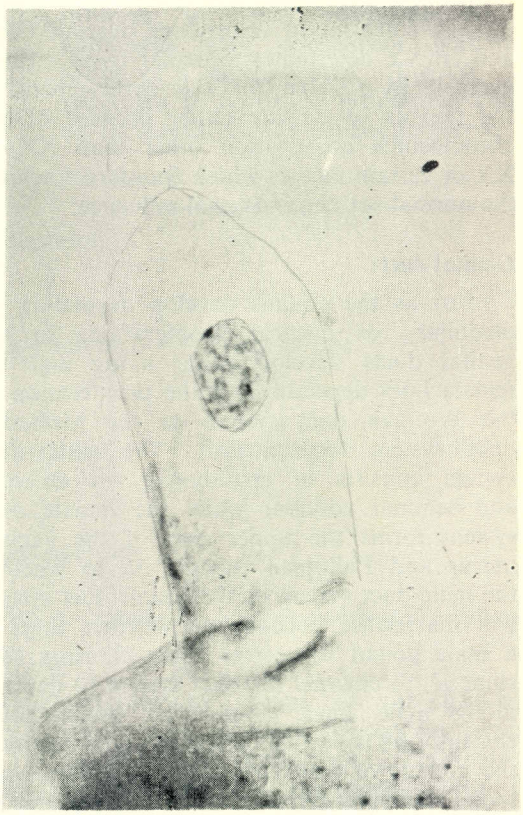


FIG. 4. Chromatin "dot" or Barr body in nucleus of buccal oral mucosal cell of female. Aceto-orcein stain.

chromatin -ve. It was Ohno (3, 4, 5) who showed that the chromatin dot in the female was derived from one of the 2 X's, and this led to the brilliant hypothesis of Lyon (6) regarding the genetic loss in this chromosome when it "assumes" the dot, and that it is a matter of chance which of the 2 X's would assume this dot and that such a change was determined very early in embryological life.

c) **Gonads:**

The testis or ovary develops from the genital ridge and the mesonephric body in the

embryo and at an early stage differentiates into an outer structure called the cortex and an inner structure the medulla. If the cortex develops subsequently with regression of the medulla, then an ovary emerges; while a testis is formed if the medulla develops with cortical regression. Obviously, it is the sex chromosomes which determine whether a testis or an ovary should develop and normally an XX constitution determines ovarian tissue and an XY determines testicular tissue. However, it is possible for the development of the gonads to deviate either because of sex chromosome constitution other than XX or XY or certain factors which counteract against the normal sex chromosomal influence.

d) **Genital duct:**

Just as the gonads develop depending on medullary or cortical proliferation, so the genital ducts develop either along male or female lines depending on the proliferation of the Wolffian duct system or the Mullerian duct system respectively. The male duct system consists of epididymis, vas deferens and seminal vesicles; while the female duct system forms the upper part of the vagina, uterus and Fallopian tubes. As to whether the male duct system or the female duct system will materialise depends on whether there is a male gonad to secrete "masculinising substances" to channel the duct system to develop along male lines. This has been shown elegantly by the work of Jost (7) who removed the gonads of embryo rabbits at a very early foetal stage and returned them in the womb of the mother rabbits. He found that if he removed foetal testes, the genital ducts and the external genitalia, will develop along female lines. If he removed foetal ovaries, again the internal and external genitalia will be female. Thus, if it happens that in the human male foetus, either because of contact with female sex hormones or because of the presence of substances which neutralise the "masculinising substance" or because of the inadequacy of these "masculinising substance", then the external and internal genitalia will develop along female lines, and depending on the degree of influence of these interfering substances there may be part male and part female preponderance in the genital duct or external genitalia development so that male pseudohermaphrodites are produced.

e) **External Genitalia:**

The undifferentiated external genitalia of the foetus consists of the genital tubercle, genital groove, urethro-labial fold and the labio-scrotal swelling, and depending on whether there is the presence of the "masculinising substance" from the foetal gonads or not, then male or female external genitalia will result. The parts of the external genitalia in the male or female is as follows:-

Primitive part	Male differentiation	Female differentiation
Genital tubercle	Penis	Clitoris
Genital groove	Total fusion	Vestibule
Labio-scrotal swelling	Scrotum	Labia Majora

Just as in the case of the genital duct maldevelopment due to extraneous factors, so can the external genitalia have part features of the male or female even if the gonads are "purely" of one sex. Of course, this ambiguity in the external genitalia can occur if in an individual there are 2 types of gonads, *i.e.* both testicular and ovarian tissue and such a person is called a *true hermaphrodite*. All other persons with ambiguous external genitalia are called false or *pseudohermaphrodites* and the adjective male or female is attached to the term depending on whether the sex chromosome constitution is XY or XX respectively.

Classification of Indeterminate Sex:

There is no cut-and-dried classification which is simple to remember and to understand and yet fully all-embracive. However, the following attempt suits the purpose in a practical manner, though overlap is inevitable. This classification recoginises 2 types of indeterminate sex, *i.e.* the intersexes on the one hand and the chromosomal sex abnormalities on the other.

a) **INTERSEXES:**

By this is meant, patients with external genitalia who show features not of one sex but features of both sexes and in this manner, are those with ambiguous external genitalia. They present as a problem in the assignation of sex at birth or later on, and can be subdivided into true hermaphrodites, male pseudo hermaphrodites and female pseudohermaphrodites. The pseudohermaphrodites are male if the sex chromosomes are XY and the gonads are testes; and female if the sex chromosomes are XX and the gonads are ovaries. The exception are the true hermaphrodites who have both testes and ovaries and whose sex chromosomes may be XX, XY or a mixture of XX and XY, *i.e.* sex chromosome mosaics.

1. **Male Pseudohermaphrodites:**

These are chromosomal and gonadal males but because of failure of the "masculinising substance" or presence of female hormones or failure of end organ response to the "masculinising substance", the genital duct system and the external genitalia take on some of the features of the female. From the appearance of the external genitalia, this category can be divided into 2 groups:-

a) Large phallus but with a hypospadias into which the vagina opens or the vagina opens into a separate introitus;

b) Small phallus but larger than a clitoris with other features almost similar to a normal female.

Group *b*) almost always feminise at puberty, *i.e.* develop breast tissue with oestrinisation of the vaginal mucosa but, of course, not menstruate. Group *a*), however, have no other female characteristics. In both groups, the testes may be in the labio-scrotal sac or in the inguinal region or in the abdomen. The genital duct development may show various degrees of feminisation such as uterus, tubes, vagina.

They are diagnosed by the fact that the sex chromosomes are XY, the gonads are testes and the cells are chromatin negative.

1. **Female pseudohermaphrodites:**

These are chromosomal and gonadal females but the external genitalia show some degree of masculinisation. According to the aetiology, this group can be divided thus:-

a) Those due to *adrenal hyperplasia; i.e.* the adreno-genital syndrome. This is a condition where a particular enzyme necessary for the elaboration of cortisone is missing in the adrenal as a result of which the precursors of cortisone are diverted to the overproduction of androgens with consequent masculinisation of a female foetus. (Fig. 5). The reduction in cortisone production causes an increased ACTH production from the

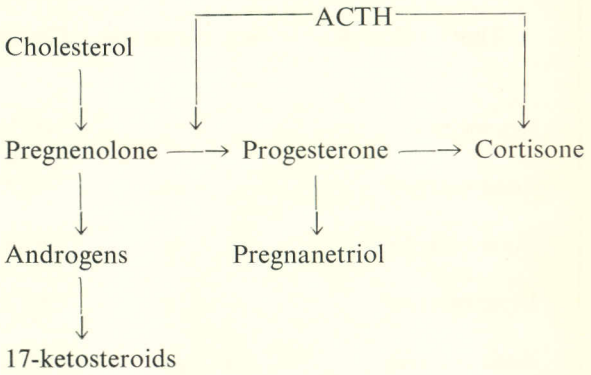


FIG. 5. Scheme for congenital adrenal hyperplasia.

pituitary by means of the feed-back mechanism resulting in further increase in androgen production. Therefore, it will be seen that the diagnosis of this condition is the measurement of the 24 hr. 17-ketosteroid level in the urine which will be far in excess of any normal infant.

b) Those not due to *adrenal* hyperplasia; such as:-

1. Use of progestogens during early foetal life to prevent habitual abortion. The progestogens have androgenic activity.
2. Arrhenoblastoma in the mother.
3. Idiopathic. In this group, the masculinisation of a female foetus is due to unknown factors. In this subgroup of female pseudohermaphrodites, the 17-ketosteroid level is normal.

3. **True hermaphrodites:**

Here there is a testis and an ovary or an ovotestis in a single individual and as a result there may be bisexual development of the genital ducts and the external genitalia. The chromosomal sex may be male, female or mixtures of both. It is not possible to diagnose this condition till biopsy of the gonads reveal both testicular and ovarian tissue. However, true hermaphrodites are extremely rare. As the following table will show true hermaphrodites where chromosome study has been done show the majority to be female, *i.e.* XX.

Tissue cultured	Sex chromatin	Chromosome karyotype	Author
Leucocytes	+ve	46(XX)	Harnden, Armstrong (1959) (8)
Bone marrow	+ve	46(XX)	Ferguson-Smith (1960) (9)
Bone marrow	+ve	46(XX)	,, ,,
Bone marrow	+ve	46(XX)	De Assis et. al (1960) (10)
Bone marrow	+ve	46(XX)	Gordon et. al (1960) (11)
Skin	+ve	46(XX)	Grumbach et. al (1960) (12)
Leucocytes	+ve	46(XX)	Wong (1964) (13)
Marrow and skin	−ve	46(XY)	Hirschhorn et. al. (1960) (14)
Marrow	−ve	46(XY)	Sandberg et. al. (1960) (15)
Marrow and testis	+ve	46(XX) & 47(XXY)	Sasaki et. al. (1960) (16)

Diagnosis of intersexes: In spite of the apparently complex situation, it is possible to rationalise the step-by-step procedures whereby a particular case of intersex may be assigned its category according to the classification above, and much of this classification has been due to the work of Wilkins (1960), (17). The following may be taken as a practical approach to the problem:-

1. A buccal smear is done with aceto-orcein stain and the result obtained in 5 minutes (Wong, 1962 (18)). *If it is chromatin positive, i.e.* if a chromatin dot is present, then the case is either a *female pseudohermaphrodite* (2 above) or a *true hermaphrodite* (3 above).
2. The chromosomal constitution can be established by leucocyte culture but in nearly all instances this is unnecessary.
3. A true hermaphrodite cannot be established till after biopsy of the gonads are carried out. Anyway, they are extremely rare.
4. The next step to narrow the field for the female pseudohermaphrodite is to collect the 24 hr. urine for 17-ketosteroids and if it is more than 2 mg. then the case is one of *adrenal hyperplasia* and treatment with cortisone will reduce the 17-ketosteroid level and halt the masculinisation process. If the 17-ketosteroid level is normal, then the case is one of female pseudohermaphrodites *not* due to adrenal hyperplasia.
5. If the buccal smear is *chromatin-negative*, then the case is one of *male pseudoher-*

maphroditism or *true hermaphroditism*, and as mentioned before the latter can only be definitely established by gonadal biopsies.

Management of intersexes: There are 3 specific problems and they include:-

a) Selection of the sex of rearing and this should be made as early as possible, before 2 years of age so that psychological maladjustments may not occur if the doctor's decision is contrary to that of the parents;

b) Surgery;

c) Specific treatment.

Since, there is specific treatment for only one of the intersexes, *viz.* female pseudohermaphroditism due to adrenal hyperplasia, it will be considered first.

1. **Female pseudohermaphroditism due to adrenal hyperplasia:** Hydrocortisone or prednisolone is given. This would depress ACTH production and would result in reduction of androgen production. DOCA supple nents may be needed if electrolyte disturbances are present. All the patients should be brought up as females and surgical amputation of the enlarged phallus may be carried out if necessary. These patients can marry and even give birth to children with adequate treatment.

2. **Female pseudohermaphrodites not due to adrenal hyperplasia:** All these patients should be brought up as females and surgical plastic operations on the external genitalia may have to be done to allow them to conform to normal female genitalia. All female pseudohermaphrodites have ovaries and female genital duct system. Again, they all function as normal females. The greatest danger in the case of female pseudohermaphrodites is the tendency to raise them as males in this country especially among the Chinese, with disastrous results later on in life.

3. **Male pseudohermaphrodites:** Here, the assignation of sex is beset with difficulties because in group (b) above, they feminise at puberty and should therefore be brought up as females. In this group, therefore, at laparotomy, the testes should be removed and oestrogens given at puberty. They will, of course be, sterile. In group (a) the decision to rear the infant as a male or female will depend on the external genitalia. If the phallus is developed enough to serve as a biological organ at puberty, then they should be brought up as males after surgical repair.

4. **True hermaphrodites:** The sex of rearing will depend on the predominance of male or female internal and external genitalia. If the genitalia is more male then the ovarian tissue should be removed and if the genitalia are more female, the testicular tissue should be removed. Requisite plastic operations on the external genitalia may have to be carried out as necessary.

(For case histories of intersexes in this country. the reader is referred to Wong (1961) (18))

(b) Sex Chromosome Defects:

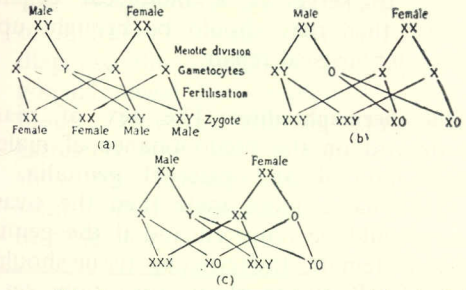
This other group really does not give rise to sex ambiguity at birth or before puberty and in fact even at and after puberty the external genitalia is of one sex, *i.e.* male or female. However, fertility is either reduced or totally absent. Biopsies of the gonads may show different degrees of absence, aplasia or hypoplasia and chromosome studies will reveal abnormalities of the sex chromosomes in number or structure.

1. **Turner's Syndrome:**

This condition is associated with a female phenotype but with associated congenital deformities such as stunting, cubitus valgus, shield-shaped chest, webbed neck, skeletal deformities, coarctation of aorta and lymphoedema of skin. The gonads are rudimentary and not ovaries at all. The chromatin sex is negative in spite of the female external appearance, and the chromosome constitution consists of only 45 chromosomes instead of 46, the absent chromosome being one of the X's of the XX female chromosome, *i.e.* 45 (XO). Such patients are, of course, sterile.

Such a chromosome constitution can arise from non-disjunction of meiosis or formation of the gamete as illustrated in

Fig. 6. Fig. 7 shows such a patient and Fig. 8 depicts her chromosome karyotype. Recently, it has been shown that chromatin-positive Turner's syndrome can occur, *i.e.* in patients with these clinical features but



Behaviour of sex chromosomes of males and females during meiotic formation of gametes and resulting zygotes
(a) Normal disjunction; (b) Non-disjunction in male;
(c) Non-disjunction in female.

FIG. 6. Results of non-disjunction at meiosis, and chromosomal sex deffers as a result of fertilisation.

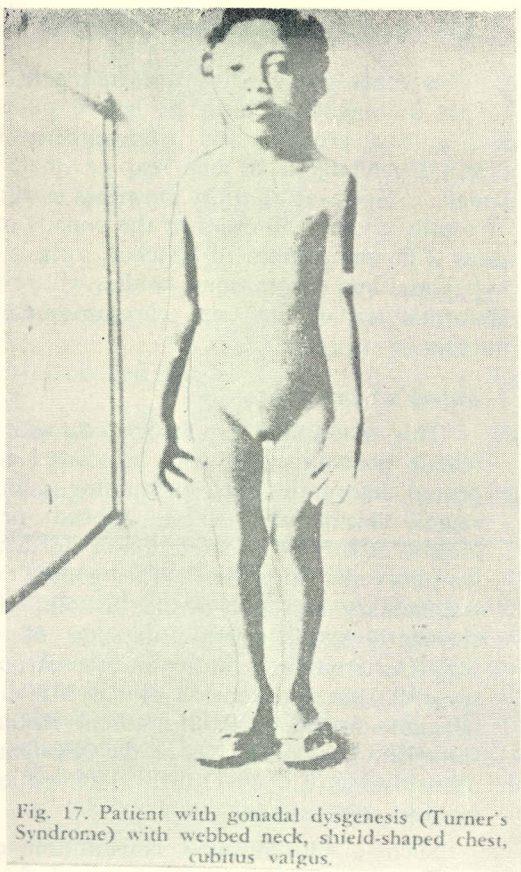


Fig. 17. Patient with gonadal dysgenesis (Turner's Syndrome) with webbed neck, shield-shaped chest, cubitus valgus.

FIG. 7. Turner's Syndrome. Note the webbing of neck, cubitus valgus and female phenotype.

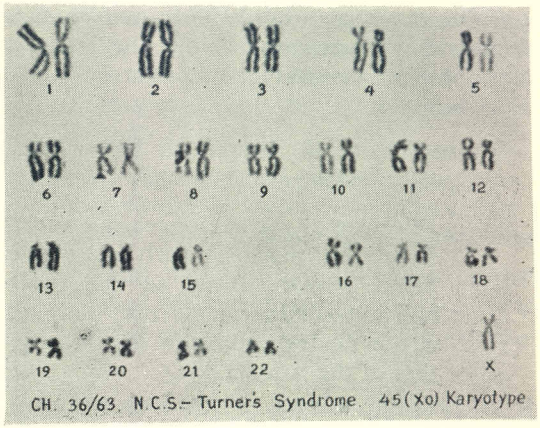


FIG. 8. Karyotype of Turner's Syndrome. Note the 45 chromosomes and only sex chromosome, i.e. the X-sex chromosome.

with a mixture of chromosome cell lines, *e.g.* XX/XO, *i.e.* mosaics.

2. **Klinefelter's Syndrome:**

This type of patient is phenotypically male with penis and small testes but with a female distribution of fat. They are sterile and the chromosome constitution is 47 (XXY). They are also mentally backward.

3. **Polysomal X-chromosomal conditions:**

This is a group of individuals who possess more than 2 X sex chromosomes, and they can be divided into 2 categories—those who are phenotypically female and those who are phenotypically male, the latter possessing in addition also the Y sex chromosomes. Two characteristics may be associated with this state, *i.e.* depression of fertility and mental deficiency. Because of the extra X sex chromosome, all these conditions will possess more than one chromatin dot in their cells, the total number of such dots being one less than the total number of X sex chromosomes.

4. **Abnormal X chromosomes:**

The above 3 examples of chromosome sex abnormalities concern abnormalities in number. However, in some patients with female phenotype, there are structural changes in the X sex chromosome which may be abnormally large or may be smaller as a

result of deletion. The types of cases described is shown in Table II.

	Female Phenotype			
Chromosome constitution	Sex chromatin	Fertility	I.Q	Reference
44 + XX (large X) 3 cases	+ve.	Primary amenorrhea	?	(66)
44 + X ∧ (½ X)	+ve (small)	Primary amenorrhea	?	(28 & 29)
44 + XX (large X)	+ve (large)	Primary amenorrhea	?	(28 & 29)
44 + Xx (small x)	+ve (small)	Primary amenorrhea	?	(28 & 29)

Table II. Sex chromosome defects affecting structural change in X-sex chromosome.

(For a more detailed explanation of sex chromosome abnormalities seen in this country together with references the reader is referred to Wong (1963) (19)).

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