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Hormonal and Clinical Aspects of Hermaphroditism and the Testicular Feminizing Syndrome in Man [and Discussion]

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Hormonal and clinical aspects of hermaphroditism and the testicular feminizing syndrome in man

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Contents

Intersexual conditions in man may be classified, on the grounds of the gonadal make up, into two groups: true and pseudohermaphrodites. The former have ovarian and testicular tissue, while in the latter only one type is found (female and male varieties). In one quarter or more of true hermaphrodites there is chromosome mosaicism and the presence of a Y chromosome in at least one of the cell lines, in most cases, explains the error of sex determination. However, in the many 46, XX and in the fewer 46, XY cases, the origin of the gonadal intersexuality is not clear, though both genetic and epigenetic influences may be at work. It would seem that, as a result of abnormal development, the right gonad would more easily be transformed into a testis and the left into an ovary.

In many pseudohermaphrodites, the anomaly of sex differentiation results from an inherited abnormality of adrenal steroidogenesis acting on the sex structures during embryonic development and persisting during postnatal life.

A relatively common form of male pseudohermaphroditism is the syndrome of testicular feminization. This is characterized by a perfectly feminine body habitus but absence of sex hair and of uterus, and by extreme hypoplasia, or absence, of Müllerian or Wolffian derivatives. The gonads, often intra-abdominal, are testes, usually sterile. The overall evidence is that these testes produce testosterone, probably at normal male levels, and possibly oestrogens in a similar fashion, though the intra-abdominal situation of the gonad and some variables of its structure, of the clinical condition and of the techniques used may underly the variability of the findings. Evidence supports the idea that the condition is caused by target-organ resistance, which seems to rest on the inability of the target organs to convert testosterone into dihydrotestosterone, which appears to be concerned with the androgen response of the target organs. This same lack of responsiveness during embryonic development would account for failure of male differentiation, and such a mechanism would support the idea that the normal foetal male hormone is testosterone. The conversion normally appears to be controlled by a specific 5α-reductase and, in view of the fact that testicular feminization is an inherited condition seemingly caused by point mutation, it is possible that the enzyme itself may be abnormal or absent. The exact mode of inheritance of testicular feminization is unknown. Linkage studies so far have not resolved between sex-linked and autosomal sex-limited transmission, though the presence of a demonstrable biochemical defect may now help in resolving the point at issue.

Introduction

In medicine and human biology, the term hermaphroditism or intersexuality is used to describe any type of discrepancy of the sex structures, of congenital origin, that results in a mixture of female and male characteristics. The word hermaphroditism, though generally used

synonymously with intersexuality, is sometimes more restricted to mean true hermaphroditism as opposed to pseudohermaphroditism. These two terms, true and pseudo, are used in strict and exclusive dependence on the make up of the gonads of an intersexual individual; if both ovarian and testicular tissues are present in the same subject, the individual is called a true hermaphrodite. On the other hand, a pseudohermaphrodite is a subject in whom ambiguity exists only in the external or internal genitalia but not in the gonads: the subject is classified as a female pseudohermaphrodite if only ovaries are present, and as a male pseudohermaphrodite when there are only testes.

The distinction between true and pseudohermaphrodites is important also from a causative viewpoint: in the true the error involves the sex-determining mechanism, whereas in the pseudo the error is of sex differentiation.

There are numerous classifications of intersexual states, based on a simple anatomical description, on aetiological considerations (more or less hypothetical), pathogenesis, or on various hybrid mixtures (see Ashley 1962; Lennox 1966). However, a simple anatomo-clinical classification is useful. We realize, of course, that, as in all types of clinical classifications, aetiological knowledge may cut across classification boundaries and that aetiologically homogeneous conditions may be dissected from the group of clinically classified disorders if one so wishes.

In the space available I shall deal with only two main topics: the true hermaphrodites, about whom there is little to say from the hormonal standpoint, and the syndrome of feminizing testes.

Table 1. Classification of true hermaphrodites

lateral	
unilateral	
bilateral	
variant of lateral: mixed gonadal dysgenesis	

Table 2. True hermaphrodites (1899–1969)

lateral	97	
unilateral	133	
bilateral	57	
?unilateral/?bilateral	23	310
variant of lateral hermaphrodites (streak and testis)		29
	total	339

True hermaphroditism, biological considerations

Classification

True hermaphrodites have either testis and ovary or a combined gonad, an ovotestis in which the two elements may be very intermingled or more or less discrete, sometimes so discrete as to constitute two separate but adjoining gonads on the same side of the body. They are classified according to the distribution of gonads on the two sides (table 1), but it should be stressed that it is not unusual in human true hermaphrodites to have reliable data on the mixed structure of the gonad on one side only and no information for the other side.

The testis may be devoid of germ cells. There may be a uterus, and other Müllerian or Wolffian derivatives may be found, or both may be present. The external genitalia are more or less ambiguous, sometimes more masculine, at other times almost frankly feminine.

In the last 70 years some 310 cases of true hermaphroditism have been described, mostly as isolated case reports. Two general reviews (Overzier 1963; Neimann & Fonder 1967) have listed 250 cases. The remainder have been published since (see special references on true hermaphroditism, p. 204), and I have included in the figures our own five cases (table 2).

A word should be said about a variant of intersexuality that is not usually classified with the true hermaphrodites. It has recently been customary to consider this a distinct and specific syndrome (mixed gonadal dysgenesis—see Sohval 1963) in which one gonad is a more or less well recognizable testis and the other a so-called 'streak', a connective tissue structure with some resemblance to ovarian stroma but devoid of germinal elements. Most subjects with this gonadal make up have female external genitals, though sometimes somewhat masculinized, and usually chromosome mosaicism of the 45,X/46,XY type. The definition of true hermaphroditism does not exclude sterility of the gonad. Thus in this respect the cases could be acceptable. However, the main difficulty in relating these cases to true hermaphrodites is the fact that there is little specific in a streak to suggest *per se* its ovarian origin. Thus, in the ovary, unlike the testis, absence of germinal cells makes organ recognition, and hence classification of hermaphroditism by the gonadal make up, difficult if not impossible. Nevertheless, I think these cases could be classified with the true hermaphrodites for two reasons: firstly, the streak in these cases is like the streak seen in older females with the 45,X condition. In this condition, in foetal life the gonad is an ovary with normal primordial germ cells, and even in the newborn primordial follicles may be found, though their numbers may be small. Extrapolating to mixed gonadal dysgenesis we might consider the streak as a remnant of a dysgenetic ovary. An additional point is that the presence of a few primordial follicles in these cases has seldom been excluded by complete and careful histological examination of a whole streak. Secondly, among the mosaics with true hermaphroditism, the 45,X/46,XY condition is relatively common.

I have listed 29 cases with mixed gonadal dysgenesis with 45,X/46,XY mosaicism (see review by Bitan, de Grouchy, Jolly & Bach 1968; special references on mixed gonadal dysgenesis, p. 204), including two cases studied at Guy's Hospital, but I would not like to convey a wrong impression and therefore two points must be made; not all cases of the mixed gonadal dysgenesis variant have this type of chromosome make up, though most have, and not all 45,X/46,XY mosaics have this type of gonadal make up.

TABLE 3. DISTRIBUTION OF TRUE HERMAPHRODITES BY NUCLEAR SEXING, CHROMOSOMES, ETC.

neither chromosomes nor nuclear sexing	111	
nuclear sexing only	91	
chromosomes	108	310
chromosomes of variant (45,X/46,XY)		29
	total	339

Chromosome findings

In about one third of the true hermaphrodites in the narrow sense of the word, chromosome studies have been done and in almost another third nuclear-sexing results are available (table 3).

The sex chromosome findings are of interest in relation to the causes of the upset of the sex-determining mechanism. Of the 108 chromosomally classified true hermaphrodites (table 4), and excluding the 29 special cases of 45,X/46,XY mosaicism, one quarter are mosaics, and of these the great majority carries a Y chromosome in one cell line. The 45,X/46,XY mosaics with clear cut ovarian as well as testicular tissue have already been commented on. The 46,XX/46,XY cases are interesting, as many or all of them reflect a double fertilization event, for example fertilization of two special ova by two spermatozoa, an X and a Y, or fertilization of ovum and polar body chromosome set (rather than polar body as such), or fertilization of

a single ovum by two spermatozoa, the ovum undergoing division before fusion of its two chromosome sets with the two male representatives. We should also consider the fusion into a single blastomere of two normal, normally fertilized eggs, a sort of dizygotic twinning in reverse, though there is no evidence that this might occur as a natural phenomenon. The presence of specific genetic parental contributions from each half set of chromosomes has been demonstrated in some cases, thus pinpointing some of the above possibilities (see Race & Sanger 1968).

One need hardly say that not all 46,XX/46,XY's will be externally or, indeed, internally hermaphroditic and, if the experience from fused mouse blastocysts can be considered relevant, at least a proportion may turn out to be straightforward males.

TABLE 4. CHROMOSOMAL FINDINGS IN 108 TRUE HERMAPHRODITES (EXCLUDING 29 CASES WITH 45,X/46,XY MIXED GONADAL DYSGENESIS)

46,XX 59		
46,XY 21		
mosaics and chimaeras 28	with Y	11 45,X/46,XY 6 46,XX/46,XY 5 46,XX/47,XXY 4 others
	without Y	2

The presence of a Y chromosome in the true hermaphrodites explains the presence of testicular tissue. As for the rest it is pertinent to ask oneself how testicular tissue is produced in the face of an XX complement indistinguishable from that of normal females—and conversely how ovarian tissue arises in the XY individuals. Of course, it could always be argued that some of these true hermaphrodites may be undetected or hidden mosaics, and, needless to say, the 46,XX and the 46,XY groups need not be aetiologically homogeneous. There are two sets of possibilities: either an error at the epigenetic level of the normal and very efficient system of gonadal differentiation, or a genetic effect. For the first we might consider that the timing of gonadal differentiation may vary somewhat and that, when the normal variation is exceeded, development of the wrong gonad ensues. In a sense we may be dealing with a developmental threshold effect. The second, or genetic mechanism, may be explained by the operation of a mutant gene like the situation of polled and intersexuality in the XX goat. Alternatively, the genetic error may be chromosomal and result either from hidden or undetected translocation of Y chromosome material, containing the male determining part, on to the X. It has been suggested that in this case the extent of testis formation may be related to unevenness of activation of the Y-carrying X chromosome (Ferguson-Smith 1966). Rather circuitous evidence in support of the hypothetical translocation comes from 46,XX males with testes in whom a male and not a female frequency of the Xga antigen was found by Race & Sanger (1968). Although there are two X chromosomes, this finding suggests that a single *Xg* locus is present. But there may be other perhaps less plausible explanations. As far as I know, sufficient Xg data are not available for the 46, XX true hermaphrodites.

Gonadal findings

Turning now to the gonadal findings in the 310 true hermaphrodites (table 2), almost half the cases are unilateral, one third lateral and one fifth bilateral. In about 7% of the reported cases a gonadal classification is impossible because only one intersexual gonad was reported and data for the other side are either not available or are inconclusive. On the whole about half

the left gonads are ovaries, as compared with a fifth or so on the right side, whereas for the testes the situation is reversed but with a smaller difference between the two sides (figure 1).

However, a comparison of gonads by sex-chromosome complement is more probing. The ovotestis in a 46, XX subject can be considered an incomplete attempt at producing a testis and in a 46, XY subject at making an ovary. The frequencies of the types of gonads in relation to sex chromosomes and side are set out in table 5. The two nuclear-sexing groups have been pooled

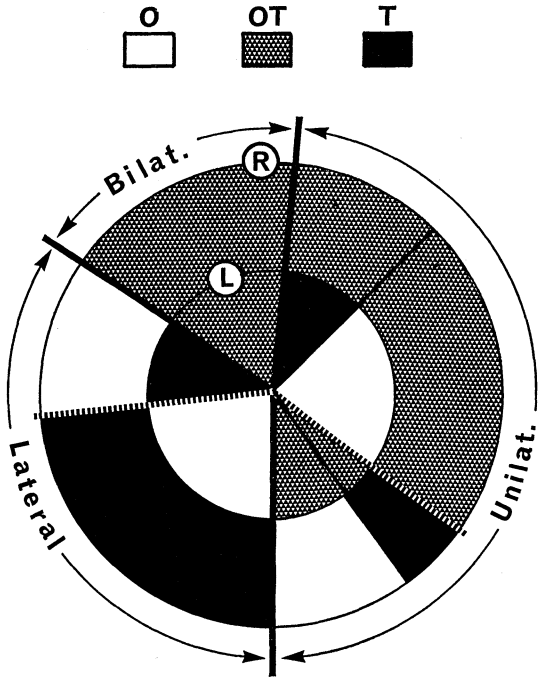

FIGURE 1. Diagram of the types of gonads found on the right (outer circle) and on the left (inner circle) sides in 287 true hermaphrodites whose gonadal structures on both sides have been described.

TABLE 5. GONADS IN RELATION TO SIDE AND SEX-CHROMOSOME COMPLEMENTS IN 132 LATERAL OR UNILATERAL TRUE HERMAPHRODITES

	type of gonad	right	left
46, XX and chromatin positive	ovary	28	43
	testis or ovotestis	56	41
	testis	25	14
46, XY and chromatin negative	ovary or ovotestis	23	34

with the appropriate sex-chromosome complements, though there may be a number of mosaic subjects among them. In the 46, XX taken together with the chromatin-positive true hermaphrodites, the appropriate gonad, the ovary, is significantly more often on the left side and the inappropriate one, testis or ovotestis, on the right, whereas for the male complements the reverse is true. We might thus infer that during gonadal differentiation, at least abnormal differentiation, the right gonad may more easily become a testis and the left an ovary.

The interest of this observation lies in the fact that asymmetry of gonadal development has been commonly observed among vertebrates and that its origin and consequences have attracted a great deal of attention. In some classes of vertebrates, it is the left gonad that tends to be

vestigial. In birds, in whom this phenomenon has been extensively studied, the right gonad of the female generally (but not necessarily in all groups or in all individuals to the same extent) undergoes involution and becomes non-functional as an ovary. However, if in pullets, for example, the left ovary is removed, the right gonad evolves into a testis, masculinizes the bird and may produce sperm. In birds it is the right gonad that, predominantly medullary in origin, exercises a strong feminizing influence on the embryo (for a discussion see Domm 1939; Franchi, Mandl & Zuckerman 1962; Jost 1965). It could be that also in man the right gonad has the same medullary predominance and testis potentiality and, if this is so, the hypothesis of an epigenetic origin of the gonadal intersexuality in many cases would be favoured.

Pseudohermaphroditism

Classification

We turn now to pseudohermaphrodites and to their overall classification which is rather complex.

First the female pseudohermaphrodites with ovaries, in a proportion of whom the developmental genital anomaly may be caused by virilizing hormones: the androgenic group (table 6). The more important category of this group is the adrenogenital syndrome and either the

Table 6. Classification of female pseudohermaphrodites

androgenic	foetal: adrenogenital syndrome (21 and 11 hydroxylase deficiencies)
	external (iatrogenic)
	maternal
non-androgenic	with urinary tract anomalies
	without urinary tract anomalies

Table 7. Classification of male pseudohermaphrodites

testicular feminization syndrome	complete
	incomplete
adrenogenital syndrome (3β-hydroxysteroid dehydrogenase deficiency)	
lipoid hyperplasia of adrenal cortex (desmolase deficiency)	
chromosomal	
others	

commoner 21-hydroxylation block or one of 11-hydroxylation during adrenal hormonal biosynthesis *in utero* may be responsible for virilizing the female foetus more or less extensively (see review by Bongiovanni, Eberlein, Goldman & New 1967) (figure 2).

Among the male pseudohermaphrodites (table 7), the testicular feminization syndrome is an important clinical entity which will be discussed here. Other causes of male pseudohermaphroditism are a rare form of the adrenogenital syndrome which affects males and is the result of a 3β-hydroxysteroid dehydrogenase deficiency (Bongiovanni *et al.* 1967), and the lipoid hyperplasia of the adrenal cortex that results from an even earlier block in the conversion of precursors of adrenal hormones (Prader & Siebenmann 1957; O'Doherty 1964). The block may be caused by a deficiency of the side-chain splitting enzyme, desmolase (Prader & Anders 1962) (figure 2).

All these adrenal disorders, most of which are exceptionally rare, are genetically determined and apparently inherited as recessives.

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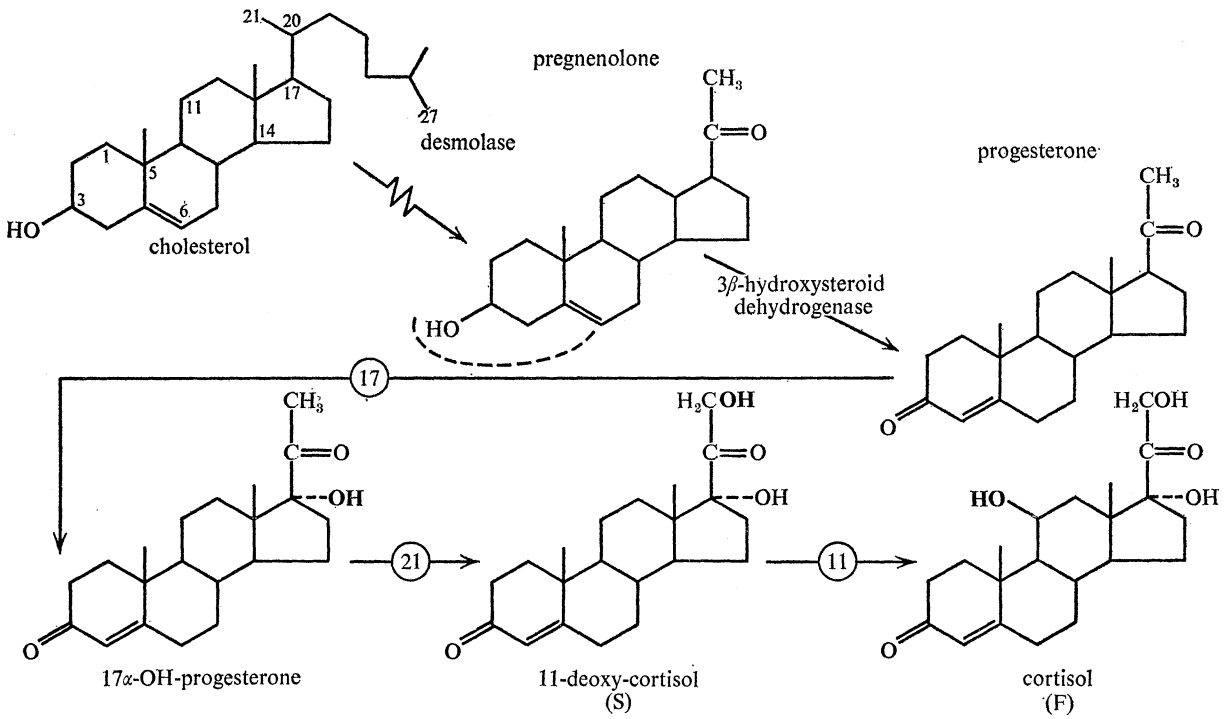

FIGURE 2. Some steps in the pathway of adrenal steroidogenesis whose block may lead to pseudohermaphroditism (modified from Bongiovanni *et al.* 1967) (see text).

Other types of pseudohermaphrodites, both females and males, will not be discussed here though they are mentioned in the tables.

Testicular feminization

Clinical aspects

The syndrome of testicular feminization belongs to the group of male pseudohermaphrodites, namely subjects with testes and therefore normal sex determination but abnormal sex differentiation. Perhaps one in five male pseudohermaphrodites may have this syndrome (Netter, Lumbroso, Yaneva & Bellaisch 1958). The syndrome has been known for a number of years, but it was J. McLean Morris who really drew attention to it in 1953, by abstracting from the literature 80 cases and adding two personal observations, and gave it a name. The clinical features of the complete syndrome are:

(1) Female habitus with normal breast development and female external genitals without clitoral enlargement.

(2) Absence of sex hair.

(3) Absence, or the presence of only very rudimentary Müllerian or Wolffian derivatives.

(4) The presence of testes, usually without spermatogenesis, intra-abdominal or along the paths of the inguinal canals.

Morris thought that the testis may secrete androgens and oestrogens, though that the latter may be abnormal. He also drew attention to the risk of gonadal tumours, sometimes malignant, and to the strong familial tendency.

Since Morris's description, the syndrome has been the object of much speculation, particularly aetiologically and pathogenetically, and numerous cases have been reported, some more,

others less closely conforming to Morris's criteria. Altogether some 300 cases of the syndrome have been published (Hauser 1963; Morris & Mahesh 1963; Zurli, Borghi & Giusti 1965). Difficulties arise from the many sporadic cases and particularly those with clitoral enlargement; Morris & Mahesh (1963), though seemingly not ruling out the fact that cases with and without clitoral enlargement may occur in the same family, set the latter cases apart in an incomplete group which may or may not be a closely related entity.

Patients with this syndrome may first present after the age of expected puberty with primary amenorrhoea and have been described as 'often voluptuously feminine' (McKusick 1968). The clinical impression of them, as a group, is that they show remarkably good adjustment and social adaptation and excellent drive and purpose. Their sexual orientation and other attributes, also in respect to rearing children, are absolutely feminine (Money, Ehrhardt & Masica 1968). Usually the breast is well developed, sometimes excessively, but with under-pigmented areolae. Although some of these patients may be good at school games, they do not seem to be top athletic performers. The average height, as adults, in 16 cases at Guy's Hospital (propositi only) was 170 cm, which is taller than the average female (162 cm) and shorter than the average male (175 cm). Pubic and axillary hair is generally absent, but in some 10 % of cases it may be normal for a female. The question of sex hair is of some importance, as its presence is, for some authors, an indication of the incomplete form. However, there have been reported a very few families in which affected sibs with and without sex hair were present. In the 33 postpubertal cases seen at Guy's, sex hair was either completely absent or nearly so.

The external genitalia are feminine but the labia may be under developed. The vagina is usually about 4 to 6 cm deep, though exceptionally it may be a mere dimple. It ends blindly and specifically the cervix is absent. Definite clitoral enlargement is considered the hallmark of the incomplete syndrome.

Inguinal herniae, otherwise rare in females, are present in about two thirds of the patients. They almost always contain the testis and are usually bilateral. However, labial gonads are rare. The testes are of modest size and brown on section, often septated by bands of connective tissue and sometimes with yellowish nodules which represent so-called Sertoli cell adenomas. Histological studies show that the testicular tubules are rather polymorphic and usually there is no spermatogenesis, so that the tubules are generally lined only with Sertoli cells. These often are very plump and have an appearance of activity. However, undifferentiated germ cells, particularly in the extra-abdominal testes, are reported with some frequency and some evidence of spermatogenesis has been reported in a few cases. Hyperplasia of Leydig cells is extremely common. They never contain Reinke's crystalloids (for a discussion on their significance see Wolstenholme & O'Connor 1967) but may, as may the Sertoli cells, appear normal at electron microscopy (Gordon, Miller & Bensch 1964; Dadoune, Abelanet & Delarue 1966; Smith, Leeson, Bunge & Anderson 1967). The large and more cristated mitochondria in the Leydig cells resemble those in the foetal testis. The smooth endoplasmic reticulum is typical of those mature cells that, in this or other organs, are associated with steroid hormone production.

One may find attached to the testis a variable sized body of connective and smooth muscle tissue which can be interpreted as similar to myometrium or at any rate of Müllerian origin. In a proportion of cases a fallopian tube may be detected histologically, or epididymal structures may be seen on sectioning the connective tissue near the gonad. A narrow band or rod of connective tissue may be found coursing medially from the gonads, without a lumen but containing sparse muscle fibres. Sometimes these bands unite on the midline and form a short narrow band

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which, however, on sectioning has never revealed a lumen. Thus a true uterus and cervix are never found. A vas rudiment may be thought to be present in some cases. It might be estimated that some vestige of Müllerian or Wolffian structures is found in perhaps three quarters of cases.

A feature of practical as well as theoretical interest is the occurrence of malignancy in these testes. Hence prophylactic gonadectomy is generally advised. Morris (1953) originally estimated this at 8 % but others have given lower prevalence estimates. Among the 39 cases, mostly young adults, seen at Guy's there were two fatal cases with malignancy, both familial. The usual diagnosis is seminoma and dysgerminoma but, particularly in the older literature, other diagnoses were made. In general tumours have been found in subjects over 20, more commonly over 30 when the frequency of malignancy has been estimated at about 20 % (Morris & Mahesh 1963). However, one case has been seen in a younger girl of just under 20 and another, in one of our families, in an infant of 18 months (see pedigree, figure 3). Tumours seem more likely in intra-abdominal testes and in patients who have obvious pubic hair, in whom the incidence may be four times higher than in the rest.

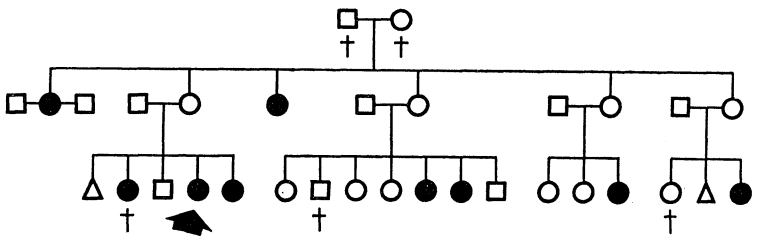

FIGURE 3. A partial pedigree of a family with testicular feminization (kindred PRU 985) (see text); ●, affected; †, dead.

Frequency and inheritance

Various estimates have been given of the frequency of the condition, and the lowest estimate is around one in 120000 (Jagiello & Atwell 1962). The condition is clearly inherited (Taillard & Prader 1957). Before 1959, studies with X-linked colour blindness had given a hint that a single X chromosome was present in these patients, contrary to some suggestions that were being discussed at the time about a chromosomal origin of the condition. Since then the presence of an XY sex chromosome complement, like in normal males, has been repeatedly confirmed and Xg studies (Sanger, Tippett, Gavin, Gooch & Race 1969) find a male frequency of this sex-linked blood group antigen. The evidence suggests that the disorder arises through a point mutation (*tf*) and it has been possible to arrive at an indirect estimate of the gene-mutation frequency on the grounds of the usual assumptions about the effect of the gene in carriers (Jagiello & Atwell 1962). A number of good pedigrees have been reported and there is an overall excess of females, true and apparent (figure 3). By correcting for ascertainment bias, the ratio of females to affected and to normal males is close to 2:1:1 (Morris & Mahesh 1963). Two pairs of monozygotic concordant and one of dizygotic discordant twins have been reported (see Zurli *et al.* 1965). The family transmission pattern is through unaffected mothers to half the sons, on an average, who are affected. Evidence for maternal transmission comes also from women who have had affected children by different husbands. This type of evidence is more easily obtained in animals; testicular feminization has been reported in three out of eight offspring of a cow of the NRF breed, mated to different bulls (Nes 1966).

The question is whether *tf* is on an autosome or on the X, the alternatives for inheritance being: autosomal sex-limited transmission—the gene behaving as a dominant in males and as a

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recessive in females—or sex-linked transmission. In sex-limited transmission, affected males may produce affected sons, but not so with sex-linked transmission. However, this criterion fails in testicular feminization because of sterility of those affected. Linkage with known X chromosomal or autosomal loci is another approach. Sanger and her colleagues (1969) (see also Boczkowski 1968) tested a number of opportunely segregating families for measurable linkage between the *tf* and *Xg* loci and between the clinical syndrome and autosomal blood group antigens, with negative results so far. We have data on very few families with other autosomal markers which again do not reveal linkage between *tf* and the specific autosomal loci, nor has the search for possible autosomal marker chromosomes been successful in the patients. In one of our families, in which deutan colour blindness segregates, several recombinants have occurred, and Stewart (1959), Aubert, Arroyo, Mercier & Clair (1967), Boczkowski (1968) have also observed recombinants in their families. Families are known in which no recombinants between testicular feminization and colour blindness have been found (see, for example, Stewart 1959; Southren & Saito 1961). Thus, if *tf* is on the X chromosome, it may be beyond the surveying power of the *Xg* or of the *deutan* and *protan* and their closely linked loci (Nilsson, Bergman, Reitalu & Waldenström 1959), or at least at some distance from the two gene clusters on the human X.

Another suggestion to help with the location of *tf* is to study carriers of the gene for patchiness of distribution of the phenotypic changes of the syndrome as in one case mentioned by McKusick (1968). The idea is that the X inactivation phenomenon may allow one to detect a patchy phenotype in carriers, and thus from patchiness deduce X linkage. Indeed there is some evidence that carriers may partially manifest the testicular feminization phenotype in respect to sex hair and menses. However, it would be difficult to secure convincing evidence from the clinical phenotype. It would seem that the biochemical phenotype, which will be discussed later, could well be used for detecting patchiness in carriers which, if proven, would support the idea that *tf* may be on the X chromosome.

Pathogenesis: hormonal mechanisms

The most intriguing aspect of the syndrome of feminizing testes concerns its pathogenesis. For example it was suggested that it may be caused by an androgen inhibitor, an idea discussed recently also by Dorfmann (1964, quoted by Neher, Kahnt, Roversi & Bompiani 1965) and, on the grounds of animal experiments, by Neumann & Elger (1966). Wilkins (1950) conducted an important clinical experiment when he administered androgens to a 30-year-old affected person. First he used injections of testosterone into the pubic skin and, having obtained no hair growth in a month, he used methyltestosterone in increasing doses for three months without evidence of masculinization, though the skin biopsy revealed the presence of hair follicles. He thought therefore that this constituted support for the hypothesis that the underlying mechanism of this condition might be an absent or very poor response of target organs to androgens, an idea not without precedent in endocrinology and human genetics. This would account for the absent masculinization *in utero* and for the later failure of virilization at puberty. Subsequently the skin findings were confirmed and the androgen experiment repeated. It might be thought, in criticism of some of these experiments, that neither the type nor the total quantity of androgenic compounds used was adequate and that the presence of endogenous oestrogens, if any, or other testicular secretion, might have counteracted their effect. There is evidence, however, that, even after gonadectomy, no response can be obtained (Southren 1965); also, large doses of

androgens were sometimes used without effect and, conversely, in a case of the incomplete form of testicular feminization, a marked response was elicited (Morris & Mahesh 1963). At any rate, other facts had to be accounted for. For example, the cornification of the vagina, admittedly modest, and the breast development, though abnormal in detail, suggested the presence of oestrogens, while the effect of gonadectomy on these two organs, as well as the production of menopausal symptoms in some of the patients, suggested a testicular source of hormones.

As alternatives to the hypothesis of androgen insensitivity, ideas were put forward concerning abnormalities of type *or* quantity of androgens *and* oestrogens secreted by the patients, and a number of hormonal investigations were aimed at testing some of these suggestions. It should be said that in general all results have been variable, sometimes frankly contradictory, and one could point to a number of possible contributory factors. The syndrome itself may be non-homogeneous and, in particular, sporadic or incomplete cases may be different entities from familial complete examples which appear to be the more uniform. Age, too, can be important. Position of gonads, their structure and possibly the phase when the investigation is carried out, could make a difference. Although there is no evidence that in these subjects there may be cyclical hormonal fluctuations as in normal women, some vague pointers suggest this possibility which is not too remote if one considers the interrelationship between hormones and brain priming. The suitability of controls, when used, is another problem and technical factors, especially in *in vitro* tests, are most important variables. Results and findings are well summarized by Morris & Mahesh (1963); Simmer, Pion & Dignam (1965) and Southren (1965).

Few deductions about hormone production can be made from the described urinary assays. In general in most cases the level of urinary gonadotropins has been found to be normal or somewhat elevated, that of the 17-ketosteroids increased in at least half the cases, compared with normal women, and in a similar proportion of cases the levels of urinary oestrogens have been found higher than in normal males, though generally at the lower limits for normal women, while the vaginal cornification index has suggested a rather inadequate but frank oestrogen effect. Often, but not invariably, the administration of ACTH has increased the output of neutral 17-ketosteroids, while different fractions have not always behaved consistently. On the whole administration of human chorionic gonadotropins (HCG) has resulted in an increase of the urinary 17-ketosteroid output, whether dexamethasone suppression was used at the same time or not. What is more to the point, orchidectomy has generally depressed the ketosteroid and oestrogen output in the urine, and the cornification of the vagina, while the gonadotropins excretion has risen even when their level was high pre-operatively.

Conversely, studies of testosterone in the blood have shown levels within the normal range for males (Southren 1965; Hudson & Coghlan 1968) and the measurement of the testosterone production rate in the blood (Simmer *et al.* 1965) has shown normal values for males (Southren 1965; Jeffcoate, Brooks & Prunty 1968). The urinary production rate in some cases has shown male values, without the discrepancy between blood and urine values that is characteristic of normal women (Hudson & Coghlan 1968). Other androgenic metabolites have also been studied (Pion, Dignam, Lamb, Moore, Frankland & Simmer 1965) and the daily production rates of dehydroepiandrosterone and its sulphate were normal, and their ratio was similar to that in males (Deshpande, Wang, Bulbrook & McMillan 1965).

Attempts have been made to investigate the source of testosterone found in the blood of subjects with the feminizing syndrome. The testosterone levels have shown significant increases

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in response to both ACTH and HCG (Pion *et al.* 1965; Southren 1965; Hudson & Coghlan 1968) and in some cases important testosterone metabolite levels in the plasma have been lowered by dexamethasone block. Castration has produced, as indicated, a fall of plasma testosterone which, however, could still be made to rise moderately with ACTH but not with HCG (Pion *et al.* 1965; Southren 1965). These tests suggest that the major source of plasma testosterone in this syndrome is the testis but it is possible that the adrenal cortex contributes a proportion of the hormone, unlike normal males. All these results, it must be noted, are from few cases.

More direct information on the source of testosterone and other steroids comes from the study of their plasma concentrations in the spermatic vein (Morris & Mahesh 1963; Southren 1965; French, Van Wyk, Baggett, Easterling, Talbert, Johnston, Forchielli & Dey 1966), or better from comparisons of concentrations in artery (or in the systemic circulation) and vein (see Simmer *et al.* 1965, table VIII; Deshpande *et al.* 1965; Rivarola, Saez, Meyer, Kenny & Migeon 1967). In the cases investigated the concentration of testosterone and of two other principal metabolites of the intra-testicular steroid pathway, dehydroepiandrosterone and androstenedione and its sulphate were usually measured. Several points emerged: (1) the venous concentration was very high compared with the arterial level; (2) the overall highest concentration of testosterone in the vein was probably at the lower limits of normality for males; (3) surprisingly high amounts of dehydroepiandrosterone, and particularly its sulphate, were found.

Details of the pathway of steroid synthesis in the testis of the feminizing syndrome have been studied *in vitro* by several workers. The techniques of preparation of the tissues, the cofactors added, the duration of the incubation, the methods of isolation and identification of the compounds have varied between different laboratories so that, even on this count, strict comparisons are impossible. Naturally, different isotopically labelled precursors were employed, depending on the specific conversion under scrutiny. On the whole it was found that the major precursors, pregnenolone, progesterone, dehydroepiandrosterone and androstenedione, can all be converted to testosterone, and thus that a number of enzyme systems relevant to these conversions are present and functional. Extrapolation from these *in vitro* studies to the *in vivo* situation is fraught with difficulty. However, two main points of interest in relation to androgens emerged:

(1) The preferential route to testosterone seems to be via pregnenolone rather than progesterone (David, Wiener, Ross & Landau 1965; Charreau & Vilee 1968), the opposite of what seems to be the case in the adult testis. The possibility that this may result from a relative deficiency of 3β-hydroxysteroid dehydrogenase has been put forward, and David *et al.* (1965) have drawn an analogy between this and adrenal male pseudohermaphroditism due to a block of this enzyme. Also Neher *et al.* (1965) found that testosterone formation *in vitro* could occur, but mostly starting from androstenedione, a fact that suggested deficient 3β-hydroxylation. Interestingly, *in vitro* work in the experimentally cryptorchid rat has suggested a similar preferential pathway due to decreased activity of a 3β-hydroxysteroid dehydrogenase which seems to be (or whose synthetic pathway seems to be) heat sensitive (Inano & Tamaoki 1968). Similarly in the bull the production of testosterone by the intra-abdominal testis is diminished, a process that has been postulated to be related to temperature (Mann, Rowson, Short & Skinner 1967), while in both ram and bull *in vivo*, the cryptorchid production of androstenedione is unimpaired and the activity of 3β-hydroxysteroid dehydrogenase is impaired in spite of the appearances of a hypertrophic interstitium (Skinner & Rowson 1968).

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(2) There is some doubt (compare Charreau & Villee 1968 with Forleo & Ingiulla 1967) about the efficiency of testosterone production *in vitro*.

As for oestrogens, only a proportion of workers (Morris & Mahesh 1963; Sharma, Dorfman & Southren 1965; Southren, Ross, Sharma, Gordon, Weingold & Dorfman 1965) claim that oestrone and 17β-oestradiol were produced (Griffiths, Grant & Whyte 1963; David *et al.* 1965; Pion *et al.* 1965; Forleo & Ingiulla 1967). However, the production in these experiments of a steroid similar to equilenine is claimed (Southren 1965), though others have not been able to confirm this (Grant, Griffiths & Pierrepoint 1967). The limitations of *in vitro* studies are particularly clear because comparisons of blood levels in spermatic vein and artery (or peripheral blood) revealed the presence in the former of concentrations of oestrogens between 2 and 30 times higher than in the artery (French, Baggett, Van Wyk, Talbert, Hubbard, Johnston, Weaver, Forchielli, Rao & Sarda 1965; Pion *et al.* 1965; Simmer *et al.* 1965). Higher gonadal vein than peripheral circulation plasma levels of oestradiol were found by French *et al.* (1965) who also noticed *in vitro* synthesis of this steroid, and the findings of Ikkos, Tillinger & Westman (1959) give some measure of support to a testicular origin of some of the urinary oestrogens, while there was no evidence of peripheral conversion of testosterone to oestrogens (French *et al.* 1965, 1966). Urinary secretion of oestrogens was enhanced by ACTH and HCG but particularly by the latter, while orchidectomy depressed their output by about one third (Deshpande *et al.* 1965). On the whole these steroids in the urine have, as already stated, been found to be within the normal male range or elevated in about one third of the cases (Deshpande *et al.* 1965; Simmer *et al.* 1965).

From all this work the idea of a target organ defect in respect to testosterone, in the face of a relatively normal steroid biosynthesis (French, Spooner & Baggett 1967; Kase & Morris 1965), gains indirect support. Indeed, even the defective spermatogenesis in testicular feminization has been considered a possible lack of response to testosterone (Southren 1965) and certainly the lack (French *et al.* 1966) or inadequacy (David *et al.* 1965) of metabolic response to this hormone points in the same direction.

Recent work on the metabolism of testosterone at tissue level by Bruchovsky & Wilson (1968*a*) in pursuance of the observations of Farnsworth & Brown (1963) has, however, offered a new approach to the problem of testicular feminization at the very level at which, in the adult, some of its defects are manifest.

The background work carried out in the rat demonstrates the speedy transformation by accessory sex tissues, for instance the prostate, of testosterone into dihydrotestosterone (5α-androstan-17β-ol-3-one), androstanediol and androsterone (figure 4). The evidence is that testosterone concentrates rapidly in the nuclei of the cells of the target organ and that between 50 and 75 % of it is transformed enzymically by a 5α-reductase ($NADPH_2$: Δ^4-5α-3-ketosteroid oxido-reductase) into dihydrotestosterone. Not only is this specific enzyme inside the nuclei of the target organ cells, but it appears to be associated with the nuclear chromatin (Bruchovsky & Wilson 1968*b*). It is suggested that only the dihydro-product, which is apparently a potent androgen, acts on the target organ in whose cells it enhances RNA polymerase activity and RNA production, and increases protein synthesis. The cytoplasm of these cells has an additional enzyme for the conversion of dihydrotestosterone to the relatively less active androgen, androstandiol. Furthermore, though the specific enzyme may be present in some non-target organs like the liver, measurable quantities of dihydrotestosterone are not found in either nuclei or cytoplasm, either because of the competitive and rapid formation of other metabolites or

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because of the fast transformation of the dihydro hormone into these metabolites. These facts, namely the production of dihydrotestosterone within the nucleus and its efficient transformation in the cytoplasm, suggest that assessment of androgenic potency of exogenous dihydrotestosterone may be difficult because both site of formation and localization of the dihydro-product seem relevant to its specific activity. Concerning such action, it would appear premature to think of it in terms of molecular effects on genes or chromosomes. The general features of the activity of 'growth and development' hormones in respect to multiplicity of action, specificity of receptors and similarity of response of the same tissue to different hormones have been succinctly stressed by Tata (1969).

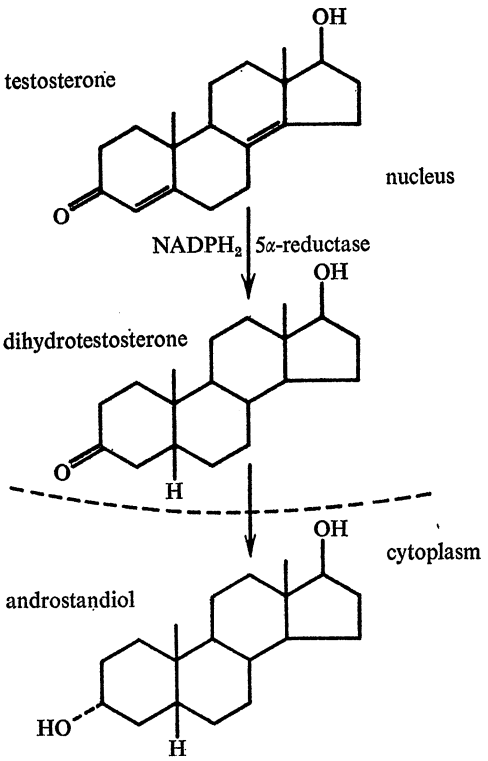

FIGURE 4. Dihydrotestosterone production in target organ cells (see text).

Taking a lead from the results of the experimental work, Northcutt, Island & Liddle (1969) have compared the efficiency of dihydrotestosterone production by suprapubic skin, pubic hair follicles, epididymis and vas deferens of normal males and of subjects with the syndrome of feminizing testes. They have studied also suprapubic skin of normal females. The results have shown at least a 20-fold greater efficiency of the normal male and female target organs. These results, confirmed by Heinrichs, Karsznia, Wyss & Herrmann (1969), suggest that there may be evidence for a specific target organ defect in testicular feminization. One should stress that the investigations have not been extensive and much remains to be done, not only in respect to the specific derangement in the syndrome of feminizing testes, but in respect to a number of fundamental problems like that of the potency and activity of dihydrotestosterone, the confirmation of its target organ specificity and the study of its mode of action. The finding has obviously implications that transcend those of sex activity of the hormone.

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Meanwhile it is interesting that a technically and also otherwise independent study, by means of intravenously administered tracer substances, should have pinpointed an important metabolic difference between males on the one hand and females, hypogonadal males and females, and other subjects, for instance with testicular feminization, on the other (Mauvais-Jarvis, Floch & Bercovici 1968). The difference is in the conversion of testosterone to dihydrotestosterone which is a characteristic of the normal adult male. Patients with testicular feminization, like normal women, children and hypogonadal patients, seem to have a low capacity to carry out this transformation and therefore to excrete urinary androstandiol or its conjugation product, while they secrete instead androsterone sulphate in their urine. In patients with testicular feminization this lack of conversion is remarkable because of the relatively good secretion of testosterone by their gonads. In the light of the target organ findings, it is possible that these observations reflect, in essence, the absence of the major male-sex accessory target organs in testicular feminization patients, but the inertness of those target organs that are present can be expected to contribute to the observed overall conversion defect. In fact a 5α-reductase deficiency was hypothesized by Mauvais-Jarvis *et al.* (1968). Recent work by this group, based on a comparison of the metabolism of distinctively marked testosterone, given both intravenously and by injection into the abdominal and thoracic skin, gave support to an *in vivo* specific 5α-reduction by the (target) skin of normal males and a deficiency of this in testicular feminization (Mauvais-Jarvis, Bercovici & Gauthier 1969).

Summary on origin

We can arrive at these summary conclusions, which must be considered provisional, about the hormonal mechanism of origin of the complete syndrome of feminizing testes:

(1) In postnatal life there is a target organ defect, shown to exist in the supra-pubic skin—which is considered to be a target organ—of the conversion of testosterone into its active derivative, dihydrotestosterone. This conversion is normally mediated by a specific 5α-reductase. Because of the point mutational origin of testicular feminization, it is possible that the primary defect may be an abnormality of structure and formation or an absence of this enzyme.

(2) The intra-testis pathway of testosterone production—which in any case appears lower than normal—might be unusual for a mature testis, as it appears to be essentially through pregnenolone presumably because of a deficiency of a 3β-hydroxysteroid dehydrogenase. *In vitro* animal experiments, which however may not apply to the *in vivo* situation in man, show that in the undescended testis the activity of this enzyme is reduced, with consequent preferential utilization of the pregnenolone pathway and curtailment of testosterone production.

(3) A feature of the testis in the syndrome is the high production of dehydroepiandrosterone and its sulphate, and this might be ascribed to the same enzyme block.

(4) Evidence suggests that oestrogens may be produced in perhaps normal amounts by the testis in this syndrome and as part of the usual metabolic processes of normal males.

(5) All this should provide a complete explanation of the features of testicular feminization. However, in the light of present uncertainties a perfect fit between hormonal findings and this syndrome cannot be expected. Nevertheless, these findings would seem to explain satisfactorily the essential clinical features of the disorder in the adult, namely the failure of pubertal virilization, the usual absence of sex hair and the female breast development. The presence of hair, however, in some patients is unaccounted for by the target organ theory. It is not excluded that other hormonal mechanisms may operate or that there may exist alternative pathways for the

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specific conversion of testosterone into dihydrotestosterone. These may be much less efficient in general but in some few affected subjects they may be more successfully used in the conversion of testosterone than in the majority of patients. Other features, like failure of testis descent or absence of spermatogenesis, may not be easily comprehended without further *ad hoc* postulates.

(6) The abnormal embryonic development of internal and external genitals may be explained by extending the evidence on the biochemical defect in a target organ of adults, to the Wolffian elements in the embryo which would be unresponsive to embryonic testicular androgen (testosterone, let us assume) and consequently would almost completely fail to develop. If the alleged biochemical defect resulted from a genetically determined enzyme defect, the idea that testosterone itself is the hormone concerned in normal male differentiation would gain support.

(7) The grossly deficient or absent Müllerian derivatives might be explained as involution caused by the action of another special hormone from the embryonic testis, different from testosterone, and main androgen, to which the ducts would be sensitive, or there may be other explanations for what happens in testicular feminization. For example, one might visualize, in normal embryos, a system of control of the Müllerian ducts based on the one single hormone but operating through a double mechanism: concentration of hormone and time in development. In testicular feminization these mechanisms would be deranged by target organ failure. But this must remain hypothetical at the present time.

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(*b*) *Mixed gonadal dysgenesis*

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Discussion on paper by P. E. Polani, p. 187

G. W. Harris: What is known about the psychology of patients with testicular feminizing syndrome? When they adopt children, they showed well-marked maternal behaviour, although this could obviously be for a variety of reasons.

P. E. Polani: There is little information available and most work is based on clinical impressions. The patients appeared to be very feminine, intelligent, good mothers and good wives.

R. V. Short: In my laboratory Jeffcote has made extracts of testicular tissue from the stallion, bull and boar, in an attempt to find dihydrotestosterone, but could not find any. Testosterone must be regarded as having different modes of actions in different organs and perhaps it would be too much to expect to find dihydrotestosterone in all target organs, including the brain. What are the reasons for the disappearance of germ cells from the testis in testicular feminization patients?

P. E. Polani: Variations in hormone action could also occur with different developmental ages. The fate of germ cells in patients with testicular feminization is not known. Occasionally a few germinal elements are found in their testes. Stages of spermatogenesis up to spermatids have been reported in these patients too, although presumably only in those cases where the testes are in the inguinal canal or labium. A convincing case report of spermatogonia in an intra-abdominal testis has not been found.

C. E. Ford: What is the variation in the proportion of testicular and ovarian tissue in true hermaphrodities? If there was a continuous range in distribution, the two ultimates would be the normal female on the one hand and the XX male on the other. Would Polani consider the XX male as possibly one extreme of the true hermaphrodite or as a completely independent syndrome?

P. E. Polani: The distribution of cell types is not known and will be difficult to discover because much of the human material is so selective. Such a variation probably does exist and the XX male may be an extreme of this distribution. I tried to calculate the relative proportions of XX males and XX true hermaphrodites, and XX males appeared to be several times more common. But this calculation is based on estimates of the incidence of XX males in relation to the known proportion of Klinefelters, and of XX true hermaphrodites in relation to the proportion of true hermaphrodites and of total hermaphrodites. It is therefore a most devious calculation.

J. L. Hamerton: In the genetically determined intersexual condition in the goat there was a continuous range of variation from the XX male pseudohermaphrodite through the true hermaphrodite with ovarian and testicular tissue, to what could only be called the XX male. XX males are rare and have normal male external genitalia and testes but XX sex chromosomes. A similar gene exists in the pig, but there are more true hermaphrodites than male pseudo-hermaphrodites in this species. Similar genes may also exist in the cow, sheep and horse. There is now evidence in man of familial cases of male pseudohermaphroditism, and of male pseudo-hermaphroditism with XY pure gonadal dysgenesis in the same family. There seems to be a strong evidence of a genetic mechanism with a segregating gene varying in its expression and penetrance, and possibly giving rise to the whole range of sexual abnormalities. If there are any cases in man of XX male pseudohermaphroditism it would strengthen the genetic hypothesis.

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P. E. Polani: I do not know of examples of externally quite feminized XX male pseudohermaphrodites. The XX male of Klinefelter type does not have active spermatogenesis and is infertile. True hermaphroditism may occur in families: two of the cases came as pairs of sibs, one was a chromatin-negative true hermaphrodite with a similar sib, and the other was an XX true hermaphrodite with a similarly affected sib. Only one of these XX true hermaphrodites had both ova and spermatogenesis, a case reported by McGovern and studied chromosomally by German.

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