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Analytic Review:
Nature and Origin of Males with XX Sex Chromosomes

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I. INTRODUCTION

Since the first reports in 1964 of males with the karyotype 46,XX [1–3], over 40 such cases have been reported. A number of problems pertaining to these cases are as yet unresolved. First, there is the question of symptomatology. It is not clear whether 46,XX males form a clinical entity of their own or whether they should be classified merely as variants of Klinefelter's syndrome, as defined clinically. Second, the etiology of the condition has not been clarified. Third, it remains to be considered what pathogenetic mechanisms contribute to the clinical features seen in these individuals.

At the present time, a review of the available data may serve to throw some light on these questions. The data presented here will not solve the problems, but it is hoped that by summarizing the present knowledge, it will be easier to define more sharply the tasks of future research in the field. Moreover, a presentation of the solutions that appear to me most likely in the light of present data may provoke discussion which will contribute toward the solution of the questions that are as yet unsettled.

II. DEFINITION

Clinically, these individuals have a male phenotype, male psychosexual identification, testes or gonads of testicular type without macroscopic or microscopic evidence of ovarian tissue, and absence of female genital organs. In view of the wide variety of existing pseudohermaphroditic and intersexual states, there will be a critical overlap of some cases. Such cases will be dealt with separately. The definition of female chromosomes, that is, the karyotype 46,XX, will be discussed in depth in the section on cytogenetics.

By definition, true hermaphrodites have both testicular and ovarian tissue. They mostly have the karyotype 46,XX, but 46,XY and various mosaics are common. From the point of view of etiology and pathogenesis, it is equally important to try to explain how testicular tissue has developed in these intersex states in the absence of a Y chromosome.

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III. Incidence

Five surveys of the karyotypes of newborn babies are summarized in table 1.

TABLE 1

Incidence of Chromosomal Abnormalities in Newborn Males

Reference	No. Males Investigated	No. Babies with Gross Numerical or Structural Chromosome Abnormalities				
		47,XXY	47,XYY	47,XY,G+	Other	46,XX
[4]*,†	1,931	4	0	0†	8	0
[5]	1,066	1	4	0	1	0
[6]	2,222	4	3	2	3	0
[7]	517	2	2	1	7‡	0
[8]	3,496	3	5	5	6	1
Total	9,232	14	14	8	25	1

* Additional data quoted from [9].

† Sampling was performed in this study. Infants with malformations were excluded.

‡ The sex of a number of individuals with abnormal karyotypes (several mosaics) was not indicated. Therefore, 7 is a minimum figure.

From these data it emerges that one XX male was found among 9,232 newborn males tested. In the same series, 14 individuals were XXY and another 14 were XYY. Sex chromatin surveys have revealed two individuals with XX among 61,742 newborn males tested [10–12] and no XX males among 21,252 mentally retarded or mental hospital patients [10, 13].

The overall incidence of the 46,XX male condition at birth, on the one hand, and in mentally defective individuals or mental hospital patients of all ages, on the other, thus appears to be low (three in 70,974 and none in 21,252, respectively). By contrast, the incidence of the XXY and XYY conditions is of the order of one in 700 newborn males (table 1). If these figures are borne out by larger samples in future investigations, it would appear that the incidence of XX males is extremely low, and the XXY and XYY conditions may each turn out to be 10 to 100 times more frequent at birth. The incidence of the XX male condition is not frequent among the mentally retarded (see below).

IV. Clinical Features

General Appearance, Body Proportions, Malformations

By definition, the general appearance of these individuals is typically male. This is documented in three individuals whose pictures are shown in figure 1. Such features as muscularity, distribution of fat, and general body proportions are mostly reported to be of normal male type. A few cases are described as "gynoid and macroskèle" (case 23),* or "macroskèle" (case 29).

* Case numbers in parentheses refer to case designation numbers given to each individual described in table 2.

As shown by Frøland [16], XXY males are longer-legged than normal XY males. Measurements in XX males indicate normal proportions between legs and trunk, but the numbers are too small to permit conclusive statements to be made. The arm span data do not show any consistent trend.

Associated malformations are rarely found. Among those reported are: bilateral hydrocele (case 12), clinodactyly of fifth fingers (case 12), bilateral deafness (case 13), partial deafness (case 26), pseudotruncus arteriosus, microcephaly, and enophthalmy (case 35). Skeletal malformations have not been reported. Obesity occurs occasionally.

PSYCHOSEXUAL ORIENTATION, PSYCHOLOGIC FEATURES, INTELLIGENCE

Psychosexually, XX males by definition have a male orientation. Many of the cases described were married. Psychologic features are often reported as normal. As in Klinefelter's syndrome [32, 33], a variety of psychologic disturbances, notably psychasthenia, have been reported in XX males. Aggressiveness was not encountered in any significant degree. Psychosis was not reported.

It is well established that the prevalence of Klinefelter's syndrome is about four times as high among the mentally retarded as among newborn babies [10, 34, 35]. Also, mild mental retardation is a common feature in patients with Klinefelter's syndrome, although there is considerable variation among the different series, reflecting the largely different modes of ascertainment [16, 33, 34, 36, 37]. In XX males, the situation may be different. As indicated above, sex chromatin screening revealed no XX males among 21,252 males who were mentally retarded or in mental hospitals.

Of the 45 males described in table 2, the intelligence status was only indicated in 20. Of these, two were reported as mentally retarded (cases 23 and 30). In five cases, IQs between 75 and 96 were reported, and in the remaining 11, the intelligence was considered normal, often without IQ tests. One patient (case 19) had a Bachelor of Arts degree.

It therefore appears that, while a proportion of XX males is indeed to be found in the range of the feeble-minded, the incidence of XX males among the mentally retarded may not be higher than among newborn babies. These data, being derived from relatively small samples, have to be interpreted with caution.

HEIGHT

The mean heights of XX males and some other populations are seen in table 3. It is quite apparent that XX males (mean 168.2 cm) are shorter than XXY males (mean 177.4 cm). The XX males may even be significantly shorter than normal XY males, but they are taller than 46,XX females (table 3). To avoid bias, only groups of patients with the same ethnic and geographic background should be compared. When XX males and XXY males from the United Kingdom were studied in this respect, similar differences were observed. The limited number of cases does not permit other groups of individuals to be similarly compared. The difference in height distribution among various groups of individuals is depicted graphically in figure 2.

TABLE 2

CLINICAL AND GENETIC DATA ON 45 MALES WITH KARYOTYPE 46,XX REPORTED IN THE LITERATURE*

CASE DESIGNATION	CODE OF PATIENT IN ORIGINAL PAPER	AGE (YEARS)	HEIGHT (cm)	GYNECOMASTIA	IQ†	COLOR VISION‡	Xg(a) BLOOD GROUP§			PARENTAL AGES AT BIRTH OF PROPOSITUS		REFERENCE
							Propositus	Mother	Father	Mother	Father	
1	...	19	172	—	N	N	—	—	+	28	26	[1]
2	1	29	167	—	N	N	—	—	+	19	27	[14]
3	2	6	111	...	84	N	+	+	+	18	20	[14]
4	...	30	167	—	...	...	...	...	...	...	...	[2]
5	50/60	19	160	...	...	...	...	...	...	25	...	‖
6	5/61	25	167.5	+	...	N	+	...	...	28	33	
7	68/61	60	...	+	...	N	...	...	...	43	42	
8	13/62	33	156	...	...	...	...	...	...	27	...	
9	101/65	25	...	...	...	...	...	...	...	...	...	
10	73/66	34	182	—	...	N	+	+	+	35	33	
11	16/67	20	171	—	...	N	—	—	...	37	44	
12	167/67	<1	...	...	...	...	+	+	+	27	34	
13	276/67	55	168	±	76	...	...	...	...	...	...	
14	HMcG	16	167.5	+	...	N	+	...	...	32	29	M. A. Ferguson-Smith (personal communication, 1970)
15	TMcG	30	167.5	—	...	N	—	—	...	31	41	
16	A.H.	76	...	+	...	N	+	...	...	...	...	
17	A.P.	23	...	—	...	N	+	...	...	28	30	
18	1	17	154	—	N	N	+	+	+	26	31	[15]
19	2	35	180	—	N	N	+	+	+	35	37	[15]
20	49	15	162	+	...	...	+	+	...	32	...	[16]
21	50	20	172	—	...	...	+	+	+	30	33	[16]
22	1	15	157	—	91	N	+	+	...	23	...	[17]
23	2	36	170	—	<N	...	...	...	...	...	...	[17]
24	...	10	135	...	75	...	+	+	+	30	38	#
25	...	33	...	+	N	N	...	...	...	25	26	[18]
26	...	41	167	—	...	N	...	...	...	39	40	[19]
27	...	22	170	—	N	N	+	+	—	23	28	[20]
28	...	31	163	—	96	N	+	+	...	18	...	[21]
29	DEL.	32	160	—	N	N	+	+	+	28	30	[22]
30	OSM.	15	163	+	<75	N	...	...	...	24	25	[22]
31	1	25	175	+	N	...	+	+	+	23	26	J. R. H. Pinkerton and M. Seabright (personal communication, 1970)
32	2	6	96.5	...	N	...	+	+	+	25	30	
33	...	16	165	...	N	...	+	+	+	29	29	A. Prader (personal communication, 1970)
34	II, 4	1	...	N	...	...	...	...	...	33	35	[23]

TABLE 2 (*continued*)

Case Designation	Code of Patient in Original Paper	Age (Years)	Height (cm)	Gyneco-mastia	IQ†	Color Vision‡	Xg(a) Blood Group§			Parental Ages at Birth of Propositus		Reference
							Propositus	Mother	Father	Mother	Father	
35	...	<1	...	...	...	...	...	...	...	28	30	A. Berger and M. Gautier (personal communication, 1970)
36	S.B.	...	161	—	N	...	...	...	...	28	18	H.A. Lubs (personal communication, 1970)
37	PEP	...	161	...	...	...	+	+	+	...	...	J.B. Bijlsma, A.Haak, and L.N. Went (personal communication, 1970)
38	B.G.	...	...	...	...	...	—	+	+	29	31	P. E. Polani (personal communication, 1970)
39	...	...	...	...	...	...	...	...	...	24	25	
40	...	30	...	...	N	...	...	...	...	...	...	[24]
41	F.A.	<1	...	...	...	...	...	...	...	30	...	A. G. Bell and J. D. Gossage (personal communication, 1970)
42	D.K.	1	...	...	...	...	...	...	...	22	22	
43	...	25	154	—	...	...	+	—	+	...	...	[25]
44	D.C.F.	...	150	+	...	...	...	...	...	...	...	M. T. Matton (personal communication, 1970)
45	G.E.	41	162	...	...	N	...	...	...	49	34	J. Lindsten (personal communication, 1970)**

* Similar cases which did not appear to fulfill the criteria for XX males used here have been described [26–30].

† N denotes that IQ was not measured but intelligence was reported as normal.

‡ N denotes normal.

§ Some of the results have been reported directly to the author by R. Sanger and R. R. Race (personal communication, 1970).

‖ All cases studied by the Edinburgh group have been described in a personal communication (1970) by W. H. Price to the author. Some of the cases have been published elsewhere. For identification of each patient, readers are referred to the code number.

ƨ This case was reported in detail in 1963 [31]. The karyotype was interpreted as 46,XX plus translocation of a fragment of the Y chromosome onto the short arms of one chromosome of group D and one of group G. On renewed chromosome examination of the propositus, and after the karyotypes of the parents had been determined as well, this finding was reinterpreted (J. de Grouchy, personal communication, 1970). The karotype is now interpreted as 46,XX,Dp+,Gp+, both marker acrocentric chromosomes having been inherited from the parents. Hence, the patient does not have any detectable Y chromosome material and belongs to the group of XX males discussed here.

** Data from this patient were not included in tables 3 and 8.

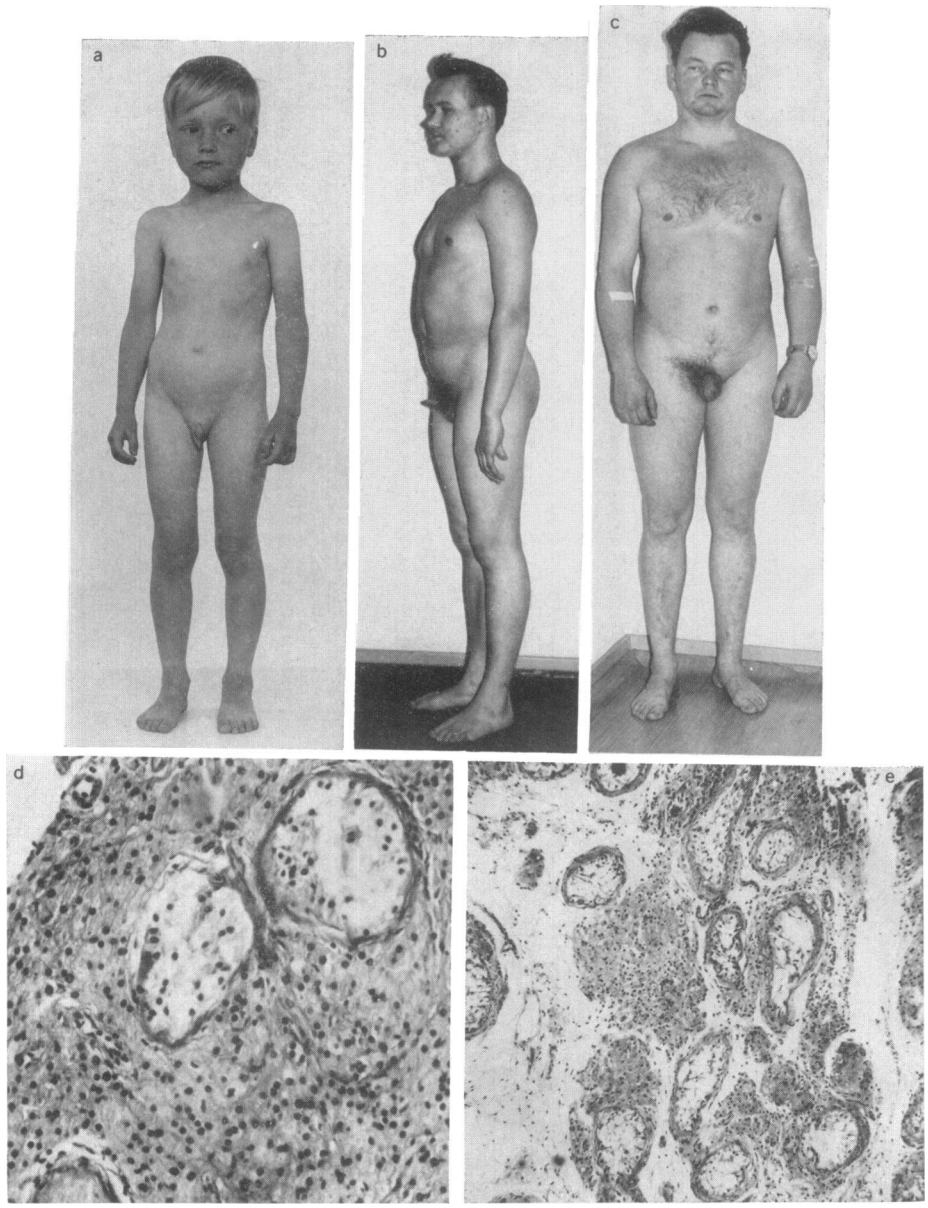

FIG. 1.—Three males with the karyotype 46,XX. *a*, Case 3. *b*, Case 1. *c*, Case 2. *d*, Testicular section from case 1. *e*, Testicular section from case 2. Note patent tubules with decreased diameter and without hyalinization but containing Sertoli cells only. Very numerous Leydig cells. Reprinted by permission from *Humangenetik* (*a*, *c*, and *e*) and *Acta Medica Scandinavica* (*b*).

TABLE 3

MEAN HEIGHT OF 46,XX MALES COMPARED WITH NORMAL MALES, NORMAL FEMALES, AND MALES WITH XXY KLINEFELTER'S SYNDROME*

Reference	Population	Country of Origin	No. Cases	Mean Height (cm)	SE
This paper	46,XX males	All countries	19	168.2	±1.52
	46,XX males	Subgroup United Kingdom	8	168.4	±2.87
	46,XX males	Subgroup other than United Kingdom	11	168.1	±1.72
[3,16]	47,XXY males	Denmark and United Kingdom	73	177.4	±0.77
[16]	47,XXY males	Subgroup Denmark	33	176.8	±1.13
[3]	47,XXY males	Subgroup United Kingdom	40	177.8	±1.06
[16]	Normal males	Denmark	...	173.5	...
P. Kajanoja (personal communication, 1970) ...	Normal males	Finland	...	173.5	...
P. Kajanoja (personal communication, 1970) ...	Normal females	Finland	...	~162	...

* The difference between the mean heights of XX males (168.2 cm) and XXY males (177.4 cm) is statistically significant ($t = 5.65$; $P < .001$).

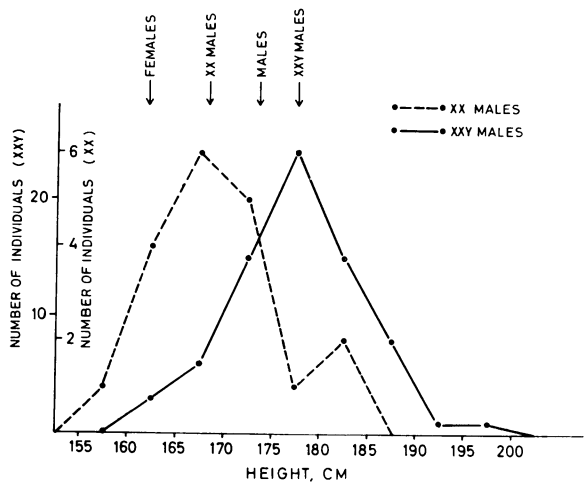

FIG. 2.—Distribution of heights in 19 males with 46,XX and 73 males with 47,XXY (table 3). Arrows indicate mean values.

SECONDARY SEX CHARACTERISTICS

Gynecomastia: The presence or absence of gynecomastia was recorded in 28 adult cases. Among these, nine individuals showed unambiguous gynecomastia (32%). In the seven series of patients with Klinefelter's syndrome reviewed by Frøland [16, table 5.5], the corresponding incidence was 110 in 272 cases (40%). Since gynecomastia itself is often the incentive to seek medical aid, the figures are maximum estimates. In any event, the incidence of gynecomastia in XX males is similar to that in XXY Klinefelter's syndrome.

Hair: Facial hair is almost always decreased, most patients having to shave less than once a day and often only once in 3–7 days. Similarly, pubic and body hair generally has a female-type distribution and is decreased in abundance. The same is true in most cases of Klinefelter's syndrome [16]. Among XX males, striking examples of normal hair growth, both facial and pubic, have been reported. One such individual (case 2) is depicted in figure 1.

GENITALIA

As in XXY Klinefelter's syndrome, the penis and scrotum are normal in the majority of XX males, although decreased in size in some cases. Figure 1 shows the situation in three males with XX. Testicular size is almost invariably decreased, the only exceptions being cases 2 and 4, in which the patients had normal or nearly normal-sized testes. The consistency of the testes varies from firm (mostly in younger patients) to soft (mostly in older patients). In the majority of the cases where testicular size was measured at operation, the size was given as 1–2 cm. In the few prepubertal cases, the size was noted to be normal.

Retention of one or both testes was reported in six of the 35 patients whose clinical descriptions were detailed. This incidence is similar to that found by others in XXY Klinefelter's syndrome [16].

Hypospadias was reported in four individuals (cases 3, 14, 24, and 34). It also occasionally occurs in Klinefelter's syndrome and is very frequent in various forms of pseudohermaphrodism or hermaphrodism. In X/XY mosaicism with male phenotype, hypospadias is nearly always present [38].

Taken together, the anatomy of the genitalia in XX males is apparently indistinguishable from that of subjects with XXY Klinefelter's syndrome.

TESTICULAR HISTOLOGY

Detailed description of the testicular histology is available for a number of XX males, whereas some cases have been very incompletely reported so far. In two infants, 2 and 8 months old, respectively (cases 35 and 12), normal or near-normal testicular histology was found. In two further children, 1 and 2 years old (cases 34 and 24), the histology was nearly normal but spermatogonia were lacking. In two individuals, testicular biopsies yielded no tubules at all. The tissue was extremely undifferentiated in one (case 3) and resembled Leydig cells in the other (case 26).

In many cases, the testicular histology resembled that usually found in Kline-

felter's syndrome (fig. 1). The most common features are: absence of spermatogonia, diminished diameter of the tubules, hyalinization and obliteration of tubules, peritubular fibrosis, and hyperplasia and polymorphism of Leydig cells. Immature spermatogonial cells are occasionally seen (here in cases 1, 22, and 33; these patients were all under 20 years of age at the time of examination).

However, notable exceptions to this general pattern are to be found among XX males. In a number of cases the testicular histology recalled the syndrome of germinal cell aplasia or del Castillo's syndrome. Tubules of normal or slightly diminished diameter, containing Sertoli cells only, are a characteristic feature of this syndrome. Slight thickening of the tubular membranes, slight peritubular fibrosis, and annular hyalinization of some tubules occur. Leydig cells are normal or moderately increased in number but do not show polymorphism or adenomatous growth. The most striking example of this type of testicular histology is found in case 27. Features similar to but intermediate between those mostly found in XXY Klinefelter's syndrome and those of germinal cell aplasia were encountered in cases 19, 4, 2, and 1.

To summarize, the histologic features of the testes in XX males closely resemble those of XXY Klinefelter's syndrome. In a minority of the cases, a picture recalling germinal cell aplasia or del Castillo's syndrome is seen. Cases with histology intermediate between these two syndromes are encountered more often. Considering the relatively wide limits of variation in XXY Klinefelter's syndrome, it is not yet clear whether there is a real qualitative difference in testicular histology between the syndromes.

HORMONES

Comparison of hormone measurements performed in different laboratories is of limited value. In a few published cases, hormone determinations in 46,XX males allow certain tentative conclusions and generalizations to be drawn.

Total 17-ketosteroids in the urine of postpubertal individuals reflect steroid secretion from the adrenals and testes and, to a lesser extent, from the ovaries. Of the fractions of 17-ketosteroids in males, dehydroisoandrosterone (DHA) is an adrenal androgen, and androsterone and etiocholanolone are metabolites of certain adrenal, testicular, and ovarian steroids. The steroids in the "residual fraction" (11-hydroxyketosteroids and 11-keto-17-ketosteroids) are mainly of adrenal origin. From table 4 it is quite apparent that no gross differences occur between the values found in XXY Klinefelter's syndrome and XX males.

Whenever stimulation by pituitary gonadotrophins has been attempted, the response, as measured by excretion of androgen metabolites, has been reported as poor. It thus appears that in the XX condition, as in Klinefelter's syndrome [39], the Leydig cells are deficient in hormone production.

The 17-hydroxycorticosteroids are mainly metabolites of the corticosteroids produced by the adrenal cortex. They are reported as normal or at the lower limit of normal in the majority of XX male cases, suggesting absence of involvement of the adrenal cortex in the pathology of the syndrome.

TABLE 4

MEAN 24-HOUR URINARY EXCRETION OF TOTAL AND FRACTIONATED 17-KETOSTEROIDS (mg) IN THREE MALES WITH KARYOTYPE 46,XX (CASES 20, 21, AND 30 IN TABLE 2), COMPARED WITH NORMAL MALES, NORMAL FEMALES, AND MALES WITH XXY KLINEFELTER'S SYNDROME [16]

Compound	XX Males ($N = 3$)	Normal Males ($N = 58$)	Normal Females ($N = 58$)	XXY Males ($N = 29$)
Total 17-ketosteroids	9.3*	14.5	8.38	9.94
Dehydroisoandrosterone	2.4	2.25	1.17	1.11
Androsterone	1.5	3.52	1.81	2.00
Etiocholanolone	2.0	3.53	2.28	2.38
Residual fraction	3.2	3.51	2.84	3.83

* The mean value of urinary total 17-ketosteroids in 10 XX males (cases 1, 2, 4, 18, 19, 20, 21, 23, 25, and 30) was 8.75 mg/24 hr.

Pituitary gonadotrophin excretion in urine is assessed in numerous ways, including biologic, immunologic, and chemical methods. Normal values vary widely from one laboratory to another. The majority of XX males reported proved to have increased excretion of pituitary gonadotrophins, while the minority had normal values. In the original description of Klinefelter's syndrome [40], increased gonadotrophin excretion was discovered in all the patients involved; however, it has since been established that a proportion of the XXY cases do have normal values [16, 37, 41]. Therefore, the finding of elevated gonadotrophin excretion in a majority of XX males is consistent with the hypothesis that the deficiency of the Leydig cells is directly responsible for the increase in the production of gonadotrophins by the pituitary [39], as in Klinefelter's syndrome.

Estrogen excretion, both as the total amount and when fractionated into estrone, estradiol, and estriol, was mostly decreased or within the lower range of normal for males. The same situation prevails in XXY Klinefelter's syndrome [42].

DERMATOGLYPHICS

In Turner's syndrome, the dermatoglyphics show characteristic traits, and in XXY Klinefelter's syndrome, too, there are significant deviations [43]. In the XX males in whom dermatoglyphics were studied (e.g., cases 18, 19, 22, 23, 26, 27), nothing strikingly abnormal was found. Abnormal but uncharacteristic features were reported in cases 28, 29, and 35. No consistent pattern emerges. No similarity with the features commonly found in XXY Klinefelter's syndrome has been reported.

V. ETIOLOGY

THEORIES

It is possible to devise several theories to explain the development of testes and a male phenotype without any apparent Y chromosome material. For practical purposes, it may be appropriate to condense several hypotheses into three main classes of theories which will be discussed here.

1. *The Gene Theory*

This hypothesis involves mutations in postulated sex-determining autosomal genes or sex-determining mechanisms other than the chromosomal one. Most mammalian species have an XX/XY sex-determining mechanism. In some species, a Y chromosome is not necessary for the formation of testes and a male phenotype. Such is the case in *Drosophila,* where the XX and XXY are females and the XY and X are males [44]. Thus, the Y chromosome does not determine whether a fly becomes a male or a female, but its presence is necessary to ensure the mobility of the sperm [45]. In mice, the Y chromosome is male-determining, since X mice are (fertile) females [46] and XXY individuals are (infertile) males [47]. In man, all available evidence suggests the unique male-determining nature of the Y chromosome; X, XX, XXX, and XXXX are females, while XY, XXY, XXXY, and XXXXY are males. The ratio of X chromosomes to autosomes or to Y chromosomes appears not to have any sex-determining function. No evidence has been put forward of any autosomal genes influencing male sex determination in man [48, 49].

The idea of a mutant autosomal gene causing human XX zygotes to develop into phenotypic males should not be too easily dismissed, however, because such genes do occur in other species. Striking phenotypic similarities exist between human XX males and XX males caused by the *tra* gene in *Drosophila melanogaster,* the *ise* gene in *Drosophila virilis,* the *polled* gene in Saanen goats, and the *Sxr* gene in mice. These conditions are described in greater detail below.

If genetic mechanisms of this type were the cause of human XX males, a number of corollaries would be expected. First, consanguinity among parents of XX males should occur but has not been observed in any of the cases described. Close consanguinity has been specifically ruled out in a number of cases, and detailed pedigree studies in cases 1, 2, 3, 18, and 19 failed to provide any positive evidence.

Second, a high incidence of XX males would be expected among sibs, cousins, or uncles of the propositi. Such familial occurrence has not been reported apart from one family [23], in which an XX male and an XX true hermaphrodite occurred in the same sibship (see below). While in many instances the karyotypes of male relatives have not been determined, detailed pedigree analyses have failed to reveal any increased incidence of childless male cousins or uncles (e.g., cases 1, 2, 3).

Third, as pointed out by Sturtevant [50], the sex ratios of the offspring change as a result of male-determining autosomal gene mutants. To test this hypothesis in human XX males, the number and sex of close relatives of XX males were tabulated (table 5). The sex ratios of sibs, uncles and aunts, and cousins do not clearly differ from those expected among the offspring of random matings. A tendency toward more females may be noticeable, especially among the cousins. But if a male-determining autosomal gene mutation were responsible for the XX male cases, there should be more males than females.

Thus, genetic findings fail to provide any evidence in favor of, but do not rule out, the gene theory.

TABLE 5

FAMILY DATA FROM FIVE FAMILIES

CASE DESIGNATION (TABLE 2)	NO. SIBS OF PROPOSITUS		NO. PARENTS' SIBS				NO. COUSINS			
			Father		Mother		Paternal		Maternal	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
1	1	1	...	3	...	3	1	3	...	2
2	...	...	5	1	4	1	4	7	7	4
3	1	...	6	2	4	4	5	11	6	9

	PATERNAL AND MATERNAL COUSINS	
	Males	Females
18	5	7
19	10	19

2. *The Interchange Theory*

If all or part of the Y chromosome were located within another chromosome, the genes involved might exert their normal male-determining effect.

According to measurements [51], the relative length of the entire Y chromosome is 8.9 and that of the longest available chromosome arm, the long arm of chromosome no. 2, is 49.0. If the Y chromosome were inserted into the long arm of chromosome no. 2, the increment in the length of that arm would comprise 18.2%, which would be readily detectable by eye or with the aid of measurements. If only the long arm of the Y were inserted, the increase in length would still amount to 15.5%. The interindividual variation in Y chromosome length in normal males [52, 53] does not provide an explanation either, since those fathers of XX males whose karyotypes have been studied have had Y chromosomes of average or even exceptional [1] length. Therefore, translocation or insertion of only part of the Y chromosome into another chromosome has to be considered.

Translocation between the Y and an autosome at paternal meiosis and subsequent transmission to the zygote of one of the elements involved in the interchange would produce the kind of translocation heterozygote postulated by Griboff and Lawrence [54]. Such a situation may prevail in *Herpestes auropunctatus,* as suggested from cytological evidence at meiosis [55]. A similar mechanism is possibly involved in *Ellobius lutescens* [56]. In the published reports of human XX males, no indication of any morphologic abnormality has been found, but if the segment of the Y involved were small, it would not necessarily be detectable cytologically at mitosis, and meiosis does not occur in XX males.

The X and Y chromosomes associate during the first meiotic division in the human male. Therefore, the hypothesis of an X-Y interchange by translocation or crossing-over put forward by Ferguson-Smith [57] seems more plausible than an

autosome-Y interchange. At prophase I, the X and Y are seen to be associated terminally [58], the short arm of the X adjoining the short arm of the Y at diakinesis-metaphase I [59], without cytological evidence of a chiasma. However, a synaptinemal complex has been found near or at the site of the end-to-end association of the human X and Y [60]. While the synaptinemal complex is neither responsible for nor a consequence of pairing or recombination, it may be required for the more regular and extensive exchange at meiosis [61]. A possible homology between the X and Y is therefore suggested.

The apparent lack of transmission of the Xg^a gene from three Xg(a+) fathers to their XX male sons could be caused by loss of the Xg locus due to the postulated reciprocal translocation. Further alleged support came from the hypothesis that the Xg locus was located on the short arm of the X [62]. However, the hypothesis was derived from data on Xg inheritance in individuals with a structurally abnormal X chromosome, and more recent evidence of the same type [63] now indicates that the Xg locus is always inactivated if located on a structurally abnormal X. Therefore, the assignment of the Xg locus to the short arm of the X chromosome no longer seems tenable.

Abnormal inheritance of the Xg locus in three families appeared to indicate that six normal XY, Xg(a+) males were sons of Xg(a—) mothers, thus breaking the rules of X-linked inheritance for Xg [64–67]. This could be explained more easily by invoking the interchange theory if the Y chromosome of the fathers carried a translocated Xg^a allele, which would be transmitted with the Y chromosome to their sons.

3. *The Mosaicism or Eliminated Y Chromosome Theory*

According to this theory, a stemline containing a Y chromosome may have existed and triggered testicular differentiation at an early embryonic stage. Subsequently, the stemline containing a Y chromosome could have become lost or extremely circumscribed. This could be brought about in two ways: (1) In a normal XY zygote, mitotic nondisjunction produces an XX and a YY line (or an XXY and a Y, or both). The YY or Y lines, being lethal, would be eliminated. Later, the XY or XXY line is postulated to be lost or extremely circumscribed. This theory requires only one nondisjunctional event. (2) An XX ovum produced, say, by nondisjunction is fertilized by a normal Y-containing sperm producing an XXY zygote. In a later stage, a mitotic error produces XX and XXYY, of which the latter becomes eliminated. This alternative requires two nondisjunctional or other divisional errors (one at meiosis, one at mitosis) and the subsequent elimination of two stemlines, both of which are known to be viable (XXY and XXYY).

When this theory was first proposed [1], the zygote's karyotype was believed to have been XXY. An original XY line may be another plausible hypothesis, since it involves fewer divisional errors than the XXY alternative. It will be argued in greater detail below that those XX males who are mosaics have an infrequent cell line with either XY or XXY sex chromosomes, which suggests that different causal mechanisms may have operated in different cases.

AVAILABLE EVIDENCE

The Blood Group Xg

The X-chromosome-borne blood group Xg is a useful marker in cases of sex chromosome aneuploidy. Gene frequencies were reported by Noades et al. [66]. Geographical differences do exist. In the following, XX males will be pooled without regard to their ethnic or geographic background and compared with gene frequency data derived from all the 3,418 samples [66]. While it would have been preferable to compare the gene frequency data from each geographical area with those of a corresponding group of XX males, this is not possible because of the small numbers of XX males. The procedure can be defended because the majority of XX males belong to precisely the same geographical groups as the control population.

Xg group phenotype frequencies. The frequency of Xg phenotypes in XX males may be compared with the known frequencies in normal males, in normal females, and in individuals with XXY Klinefelter's syndrome. Implications with regard to the principal etiologic theories are as follows:

1. A male-type distribution in XX males could result from either interchange or mitotic error in an original XY zygote, giving rise to the XX karyotype.

2. A female-type distribution would be expected if the XX males had arisen from XXY zygotes having one maternal and one paternal X (X^MX^PY), or, alternatively, a female-type distribution would also be expected if XX males were chromosomally normal.

3. A distribution like the one in XXY Klinefelter's syndrome would be expected (1) if the zygotes had originally been XXY (both X^MX^PY and X^MX^MY) or (2) if the XX male population were a mixture of individuals arising by mitotic nondisjunction from either XY or XXY zygotes.

The results of Xg grouping in XX males are seen in table 6. Using χ^2 tests, the

TABLE 6

Xg GROUP PHENOTYPE FREQUENCIES IN MALES WITH KARYOTYPE 46,XX AND COMPARISON BY χ^2 TESTS WITH EMPIRICALLY EXPECTED DISTRIBUTIONS IN NORMAL FEMALES, NORMAL MALES, AND MALES WITH XXY KLINEFELTER'S SYNDROME*

PHENOTYPE	OBSERVED XX MALES†	EXPECTED					
		XX Females	χ^2_1	XY Males	χ^2_1	XXY Males	χ^2_1
Xg(a+)	27	29.17	1.39	21.75	3.72	27.98	0.22
Xg(a—)	6	3.83		11.25		5.02	

* The frequencies in the control series were derived from individuals of North European and North American extraction only, while the Xg groups of XX and XXY males were from all cases available. All data given in this table were kindly provided by R. Sanger and R. R. Race (personal communication, 1970).

† These figures include most of the data shown in table 2 but also some that do not occur elsewhere in this paper.

data are compared with the known distributions in XX females, XY males, and XXY males. It is shown that the Xg distribution in XX males is not significantly different from any one of the three groups with which it is compared. At present, the distribution in XX males is farthest from expectation in XY males and nearest to expectation in XXY Klinefelter's syndrome.

A note of caution has to be sounded here because of the small numbers of individuals tested. By the time 17 XX males had been tested, the distribution best fitted the expectation for XY males [68], and this was taken to support the interchange hypothesis. Furthermore, only one of 17 fathers of 46,XX male sons is Xg(a—) (table 2). While this fact may have biological significance, it may also be due to chance alone and may distort the phenotype distribution figures.

The results appear to support the concept that XX males may arise through mitotic nondisjunction in XY or XXY zygotes, or both. Since there is no maternal age effect (see below), a significant number of X^MX^MY zygotes (maternal-age-dependent cases) appears unlikely. In view of this, it may not be possible to attempt a calculation of the derivation of the X chromosomes from the phenotype frequencies alone [69] in XX males. According to R. Sanger and R. R. Race (personal communication, 1970), the proportion of X^MX^MY was 72.5% and that of X^MX^PY was 27.5% in a series comprising over 200 XXY Klinefelter patients. Furthermore, when both X chromosomes are maternal, it appears that in 26.8% of the total cases they were identical (in terms of the Xg locus) and in 45.7% were different.

Source of the X chromosomes. If the X-Y interchange theory is disregarded, the distribution of Xg phenotypes has yielded information on the source of the X chromosomes in three families. In two of them (cases 1 and 2), the father's only X chromosome being Xg^a, the XX male son [Xg(a—)] must have inherited both his X chromosomes from his mother (X^MX^M). This could result from postzygotic mitotic nondisjunction or from meiotic nondisjunction at the first or second division. In a third family (case 38), both the X chromosomes (or Xg loci) of the XX male must have come from the mother (who must be Xg^aXg), and hence the propositus could be designated $X^{M1}X^{M1}$ or $X^{M2}X^{M2}$. This indicates that he has received the same maternal Xg locus or chromosome *in duplo*. Three mechanisms could be responsible for this: (1) mitotic nondisjunction of an XY zygote, (2) nondisjunction during the second meiotic division, or (3) nondisjunction during the first meiotic division accompanied by crossing-over between the Xg locus and the centromere. At the present time it is not possible to ascertain with certainty which of these three mechanisms has been active, but the absence of a maternal age effect (see below) suggests that postzygotic nondisjunction is the most likely explanation for the Xg data in the above families.

The Serum Group Xm

Another useful X chromosome marker, the serum group Xm, is related to antigenic variation residing in the α-2-macroglobulin of human serum [70]. The inheritance pattern is quite regular, obeying the same rules of X-linkage as those

described above for Xg. Limited data on linkage relations between the Xm group and other X-borne loci indicate that the Xm locus is located within measurable distance of the deutan locus [71]. At the present time it cannot be settled whether there is linkage between Xg and Xm, but close linkage is not indicated [72].

The Xm group has been tested in the families of cases 1 and 2 (table 7).

TABLE 7

Xm Serum Group Phenotype Distribution in Families of Cases 1 and 2 (Table 2)* Compared with Xg Blood Group Distributions

Case Designation	Xm(a)	Xg(a)
Case 1:		
XX male propositus	−	−
XX mother	−	−
XY father	+	+
XX sister	+	+
XY brother	−	−
Case 2:		
XX male propositus	+	−
XX mother	+	−
XY father	−	+

* K. Berg and A. de la Chapelle (unpublished observations, 1967).

Conclusions are as follows: In case 2, the Xm groups are uninformative, since the data are compatible with both an X^MX^P and an X^MX^M constitution in the XX male propositus. In case 1, Xg and Xm are identically inherited. This appears to confirm the previous assumption that both X chromosomes are of maternal origin in the XX male propositus (X^MX^M), and favors the theory that XX males arise from XY or XXY zygotes. However, if the X-Y interchange hypothesis is considered, the data suggest that both the Xg and Xm loci have been involved in the interchange. From the linkage data quoted above [72], it would seem that close linkage between Xg and Xm is improbable, since the most likely recombination fraction is given as 0.34; the 90% confidence limits are 0.18–0.48. Therefore, the interchange would have to comprise a long segment of the X, and there is no available genetic indication of this. Such a large interchange is indeed unlikely to occur in man, in view of the very short or nonexistent homologous segment common to both the X and the Y.

When further known or hitherto unknown markers borne on the X chromosomes are tested in the future, it should be possible to establish whether in any XX males the Xg locus has undergone interchange or recombination and another locus has not. This would support the interchange theory. However, such inheritance has so far not been observed, and positive evidence in favor of the interchange theory is therefore lacking.

Color Vision

Color vision data have been reported in 20 males with XX, and in all of them it was normal (table 2). This distribution is closer to that of females than to that of males, thus matching the Xg group distribution data and lending added support to the interpretations of the Xg results. Moreover, its bearing on the etiologic hypotheses is as follows:

Gene theory. The distribution is not at variance with expectation provided the gene theory is correct.

Interchange theory. If the color vision loci were involved in the presumed interchange, a male distribution would be expected. The fact that the distribution is closer to that expected for females does not, of course, rule out interchange, but the absence of linkage between the Xg and the color vision loci [67] would imply that a very large proportion of the X chromosome was involved, which is unlikely.

Mosaicism theory. If a notable proportion of XX males were products of mitotic errors in XY zygotes, then the two X chromosomes should be identical ($X^{M1}X^{M1}$ or $X^{M2}X^{M2}$) and the colorblindness incidence should be of the male type. The actual distribution appears to indicate that it is unlikely that all or even a majority of the cases have arisen in this way.

Since results of color vision tests have only been reported in 20 cases, the data are not statistically significant and are of suggestive value only. If by the time 38 cases have been tested, no colorblind XX male has yet been found, the probability that the distribution is of the female rather than the male type will be over 95%.

Parental Age

Maternal nondisjunction is associated with an elevated maternal age at the birth of the aneuploid propositus in G trisomy [73] and in XXY Klinefelter's syndrome [68, 74] but not in X Turner's syndrome [75]. When nondisjunction appears to be paternal in Klinefelter's syndrome [68] and in Down's syndrome [73], both paternal and maternal ages are probably normal.

Maternal ages at birth are given for 35 of the cases listed in table 2. A comparison of maternal and paternal ages at birth of XX males, XXY males, and all births are given in table 8. From the mean values, it is apparent that the ages of the mothers of the XX males (mean 27.8 years) do not differ from those of the total population (means 27.5 and 29.1 years). By contrast, mothers of XXY males are older (32.6 years). When the distribution of maternal ages is depicted graphically (fig. 3), the older age of the mothers of XXY individuals is also apparent and the curve is bimodal, as expected. It is hard to compare paternal ages at birth independently for different groups of aneuploid individuals, since paternal age is dependent on maternal age [78].

Judging from these data, a maternal age effect appears to be lacking in XX males. A possible interrelationship between paternal age and possible X-Y interchange is not indicated, and is entirely speculative in any case.

TABLE 8

MEAN PARENTAL AGES AT BIRTH OF 46,XX MALES COMPARED WITH AGES AT BIRTH OF XXY MALES AND ALL BIRTHS*

REFERENCE	POPULATION	COUNTRY OF ORIGIN	MOTHER			FATHER		
			No. Cases	Mean Age (Years)	SE	No. Cases	Mean Age (Years)	SE
This paper	46,XX males	All countries	35	27.8	±0.97	29	30.8	±1.19
	46,XX males	Subgroup United Kingdom	14	29.6	±1.50	12	33.2	±1.78
	46,XX males	Subgroup other than United Kingdom	21	26.7	±1.24	17	29.1	±1.50
[3,16]	47,XXY males	United Kingdom and Denmark	133	32.6	±0.65	127	35.5	±0.72
[3]	47,XXY males	Subgroup United Kingdom	90	32.8	±0.76	86	35.5	±0.80
[16]	47,XXY males	Subgroup Denmark	43	32.1	±1.26	41	35.7	±1.47
[76]	47,XXY males	Canada	35	31.1	±1.1	34	35.0	±1.5
[76]	All births	Ontario, Canada 1939	...	27.5	...	...	31.9	...
[16]	All births	Denmark	...	29.1	...	...	...	...

* The difference between the mean maternal age at birth of XX males (27.8 years) and XXY males (32.6 years) is statistically significant ($t = 3.08$, 166 df, $P < .01$).

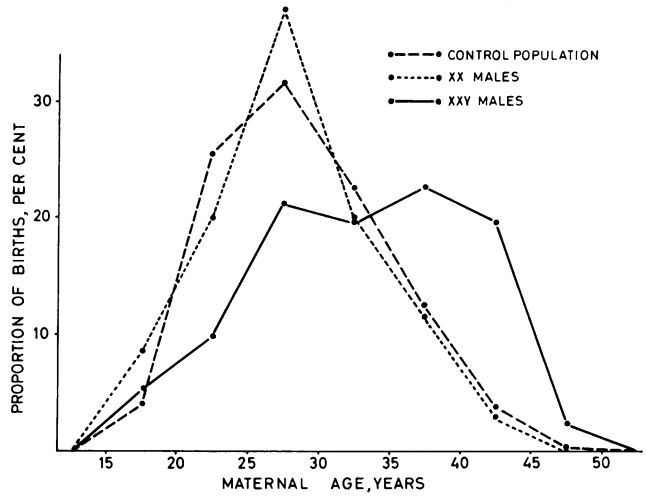

FIG. 3.—Distribution of maternal ages in a control population [77], in 35 XX males, and in 133 XXY males (table 8).

The absence of a maternal age effect argues against a significant proportion of XX males being the result of maternal meiotic nondisjunction. In fact, the odds are in favor of postzygotic mitotic nondisjunction in XY zygotes rather than meiotic nondisjunction producing an XXY zygote. Furthermore, the gene theory would imply absence of any maternal age effect. The evidence might also be considered to be in favor of the interchange hypothesis over the nondisjunction hypothesis. The apparent lack of any bimodality of the distribution of maternal ages (fig. 3) may be tentatively regarded as evidence against meiotic nondisjunction in a significant proportion of the cases.

The parental ages at the birth of XX males in whom the origin of the X chromosomes appears to be ascertained by the Xg group distribution are potentially informative [76]. The mean maternal age at birth of the three $X^{M}X^{M}$ males (cases 1, 2, and 38) is quite low (25.3 years), indicating support for the theory of postzygotic mitotic nondisjunction in an XY zygote (which would always produce the $X^{M1}X^{M1}$ or $X^{M2}X^{M2}$ situation). However, the very small numbers of informative families tested make it necessary to await further data before any definite conclusions can be drawn.

VI. CYTOGENETICS

By definition, 46,XX males possess no cells with a Y chromosome. There is no reason to doubt that the karyotypes have been correctly interpreted as 46,XX in the majority of the reports reviewed in this paper. This view is sustained by the finding of 16 chromosomes with normal morphology in group C and four chromosomes in group G. The presence of Barr bodies in numerous tissues and of drumsticks in polymorphonuclear leukocytes also indicates that there are two

X chromosomes. Furthermore, labeling with tritiated thymidine demonstrates the presence of two X chromosomes.

SEX CHROMATIN

In one paper [22], it was reported that Barr bodies were less frequent and smaller in two XX males than in XXY subjects. It was also stated that XXY individuals have larger and more frequent Barr bodies than normal XX males, but no quantitative data in support of this claim were presented. These findings have not been confirmed by others [15].

The sex chromatin pattern of Sertoli cells deserves comment. In an XX male, Therkelsen [2] found that the Sertoli cells were sex-chromatin-negative, whereas these cells from an XXY individual were sex-chromatin-positive. Subsequently, Frøland (quoted in [2]), examining 12 individuals with XXY, found Barr bodies in the Sertoli cells in eight, but not in the remaining four cases. In cases 1 and 2, the Leydig cells were sex-chromatin-positive and the Sertoli cells were sex-chromatin-negative (A. de la Chapelle, unpublished results, 1970), and the same pattern was found in case 28. Presumably, factors affecting the staining properties of various testicular cells and the expression of sex chromatin in them may account for these discrepancies, whose significance needs further clarification. Ideally, karyotypes should be prepared from clones cultured from single cells of known origin.

AUTORADIOGRAPHY

When 46,XX cells are labeled with tritiated thymidine at the end of the S period, one of the presumptive X chromosomes is clearly more heavily labeled at metaphase than any other chromosome [79–81]. Such a phenomenon only occurs in a proportion of all labeled cells (30%–60%), even when there is only end-of-S labeling [82, 83]. Why so many cells do not display a preferentially labeled X is poorly understood.

In previous autoradiographic work involving the X chromosome, two important limitations had to be recognized. First, the X chromosomes were not identifiable by morphologic criteria. Therefore, only cells clearly displaying a differentially labeled X were chosen for such purposes as grain distribution analysis or grain counting. If these cells comprise only 30%–60% of all labeled metaphases, approximately one-half of the presumably informative cells are omitted from the analysis. When the criterion for choosing a cell is the heterocyclic behavior itself, this may obscure significant differences in pattern.

Second, the X chromosome that does not display heterocyclic DNA synthesis cannot be distinguished from the autosomes of group C by conventional techniques. It is believed that in normal females the random inactivation of one or the other of the X chromosomes [84] is associated with heterocyclic behavior. However, there is preferential nonrandom inactivation of structurally abnormal X chromosomes (see below). Therefore, since this paper deals with X chromosomes which may be either structurally abnormal (X-Y interchange hypothesis) or normal

(gene hypothesis, mosaicism hypothesis), entirely different patterns of inactivation and heterocyclic behavior may be expected.

In over half of the 46,XX male cases reported here, autoradiographs have established late-synthesizing behavior of one C group chromosome, indicating beyond reasonable doubt that two X chromosomes are present. Only a few quantitative approaches have been tried. One possibility is to establish the grain counts over the late-synthesizing X relative to the grain counts over all other chromosomes. When this approach was used in three cases [15, 85], no difference between the heterocyclic X of XX males and normal XX females was indicated.

From previous work [86, 87], the patterns of grain distribution in the late-synthesizing X chromosome have become known. In an attempt to establish whether the pattern of grain distribution in XX males was similar to that of the heterocyclic X of normal females, linear grain densities were calculated for the late-synthesizing X of two XX males [85]. There was no clear difference in comparison with the X chromosomes of an XXXXY individual [88] or with the patterns seen in normal females by numerous authors (e.g., [89, 90]).

Structurally abnormal X chromosomes are often or always preferentially heterocyclic in man [83]. Whether this also applies to the postulated product of an interchange between "homologous" portions of the X and Y chromosomes is not known. Conversely, in an X/autosome translocation in mice, segments of the X chromosome attached to an autosome appear to remain active in all cells while the normal X is inactive [91]. Also, autosomal segments translocated into the X chromosome in mice are only partially inactivated [92]. In man, X/autosome translocations may be heterocyclic [82, 93].

The human Y chromosome is later labeling than the G group autosomes [83, 86] and in many XY cells is more heavily labeled than any other chromosome. In a bovine cell line, an unaltered labeling pattern in a segment of Y chromosome translocated into an autosome was found [94]; a similar observation was made in hamster cells [95]. Rarely have Y/autosome translocations been reported in man, and the evidence in these cases is not entirely convincing [83, case 48; 96]. No X/Y translocations have been reported in man.

The autoradiographic studies of chromosomal DNA synthesis in XX males reviewed here may be of limited significance in the solution of the problems under consideration. First, would an X-Y interchange alter the grain count or the linear grain density? From the data accumulated in the literature, no prediction can be safely made. It appears logical to assume that only if large chromosome segments were involved would the grain counts and distribution be affected. Even a small segment might conceivably have a dramatic effect if it affected the hypothetic inactivation center [92].

Second, since in a broad sense, XX males have a numerical abnormality of the X chromosome, it may be asked whether X chromosomes from individuals with numerical aberrations of the X chromosomes have abnormal autoradiographic patterns. The answer has already been provided: they do not [83, 89].

MOSAICISM

Table 9 lists six cases of 46,XX individuals in whom a very small number of

TABLE 9

PREDOMINANTLY 46,XX CASES WITH EXTREMELY INFREQUENT OR CIRCUMSCRIBED CELL LINES CONTAINING A Y CHROMOSOME

Reference	Case Description	Cytogenetic Features
[97]	True hermaphrodite with testis and ovary	46,XX in blood, bone marrow, skin, and first testis culture (altogether 214 cells); in a second testis culture, 30% of cells were 46,XY
[98]	True hermaphrodite with two ovotestes; younger brother is XX male (case 34, table 2)	46,XX in blood, skin, fascia, and right gonad; left gonad, two cells with 46, XY; total no. cells, 134
[15]	Male with two testes	46,XX only in two blood cultures (205 cells); in two to five cells, 47,XXY was probable (skin and testis cultures); total no. cells, 568
[99]	Male with two testes	In blood, two cells with 47,XXY of 210 cells; in skin and testis, possibly one cell with 47,XXY of 99 cells
L. S. Penrose (personal communication, 1970)	Male with two testes (IQ, 45; maternal age, 26; paternal age, 26; height, 173 cm).	Blood, one cell with 47,XXY of 261 cells; in first skin culture, only 46,XX in 96 cells; in second skin culture, one to three cells with 47,XXY of 96 cells
[3]	Male with hypospadias and gynecomastia (maternal age, 32; paternal age, 29; height, 167.5 cm) (case 1/61).	46,XX only in blood cells; in skin, 25 cells with 46,XX and two cells with 47,XXY

XY cells (two cases) or XXY cells (four cases) were found on examination of large numbers of cells or on repeated examinations. As is evident from the table, a remarkable feature was the extreme scarcity of Y-containing cells, so extreme that some authors have been reluctant to identify the Y chromosomes [15, 98]. In one case [97], the XY cells were numerous, about 30%, but only in a tissue culture established from a second testis biopsy after blood, bone marrow, skin, and the first testicular biopsy had revealed only XX.

It should be noted that the phenotype was that of true hermaphrodism in the two cases with an XY line of cells, while the phenotype was male in the four cases in which XXY cells were found. Among XX/XY cases with hermaphrodism, a few [100–102] appear to have been caused by double fertilization, as evidenced by blood groups. Such chimera-type composite zygotes may indeed account for an unknown proportion of all mosaics [103].

The existence of mosaics (or chimeras) of the type described in table 9 empha-

sizes the importance of examining as many cells as possible before mosaicism can be ruled out. Strictly speaking, mosaicism would only be ruled out after every cell in the organism had been studied, which is not feasible. An instructive case is the male cotwin in a monozygotic twin pair [104]. This individual had only 45,X cells, although the authors presented strong arguments that he must, in fact, be an X/XY mosaic.

Ford [103] has critically discussed what criteria should be used in the diagnosis of mosaicism. Table 10 (adapted and extended from his paper) shows the

TABLE 10

PROBABILITY THAT RANDOM SAMPLE OF CELLS FROM MOSAIC SUBJECT WITH TWO CELL LINES WILL NOT INCLUDE AT LEAST ONE CELL OF MINOR CELL LINE

FREQUENCY OF MINOR COMPONENT	NO. CELLS STUDIED IN SAMPLE				
	10	20	50	100	500
.05	.598	.358	.077	.0059	<.001
.01	.904	.818	.605	.366	.0066
.005	.951	.904	.778	.605	.0813

probability that the minor cell line will not be detected. It will be seen that, if the frequency of the minor cell line is 0.05 (i.e., 5%), there is a 36% probability that it will not be found among 20 cells studied and an 8% probability if the sample consists of 50 cells. When the minor cell line constitutes 0.5%, there is an 8% probability that it will not be found even among 500 cells.

While hundreds or thousands of cells have been studied and found to be 46,XX in many of the individual cases forming the basis of this review, in other cases only a limited number of cells have been analyzed or the number of cells has not been reported. It therefore appears logical to suppose that on closer scrutiny, additional mosaics might be detected in at least a proportion of these. This lends further support to the hypothesis that mosaicism (previously eliminated or persistent) may be a frequent etiologic factor in the XX male condition.

Selection of cell lines in vitro must be considered as an alternative explanation of the absence of the Y chromosome. Blood cultures [105, 106] entail 2–4 days of in vitro culture, and fibroblast cultures from various sources [107, 108] are usually allowed to grow for 2–4 weeks before being harvested. However, the bone marrow technique [109] reflects the in vivo situation reasonably well, since only 1–3 hours of incubation in vitro is necessary to obtain a colchicine effect. Therefore, total elimination of a Y-containing cell line due to factors active in vitro is unlikely. In an odd case, an XX cell line was believed to have arisen spontaneously in an XXY fibroblast culture in vitro, but contamination was thought to have been one possible explanation [110].

FLUORESCENCE MICROSCOPY AFTER STAINING WITH QUINACRINE MUSTARD

The alkylating agent quinacrine mustard binds to DNA, causing metaphase chromosomes to fluoresce in ultraviolet light [111, 112]. With this method, human chromosomes display a characteristic pattern of bands which is readily visible under the microscope and can be recorded with the aid of the reflection photometer developed by Caspersson's group [113]. With the aid of the criteria laid down [113], each human chromosome pair can now be accurately identified.

Apart from the typical banding, another feature of the human karyotype is especially conspicuous, namely, the very bright fluorescence in the distal half of the long arm of the Y chromosome. This fluorescence of the Y chromosome can be identified even in interphase nuclei [114, 115].

The fluorescence that occurs after staining with quinacrine mustard has provided a powerful new tool for use in the search for Y chromosome material in XX males. First, if an entire Y chromosome were present in all or some of the cells of such an individual, this should be readily distinguishable. Second, if there were very few cells containing a Y chromosome, even as low a proportion as 0.5% of all cells, such cells would probably be detectable [116]. Since the interphase method is rapid and simple, such screening can easily be done. Third, the precise identification of both X chromosomes allows a detailed study of their morphology and fluorescence pattern. If a portion of the brightly fluorescent part of the Y chromosome were translocated onto one of the X chromosomes (or an autosome), it would probably be readily detectable.

These points have been studied by two groups of workers. Blood and skin cells from two XX males failed to display any brightly fluorescent material derived from the Y chromosome [117]. In an extensive study of eight XX males and one XX true hermaphrodite, brightly fluorescent Y chromosome material was likewise shown to be absent in hundreds of metaphase cells and several thousand interphase cells [118]. Moreover, microscopy and reflection photometric analysis of the X chromosomes failed to indicate any abnormality of the fluorescence pattern in these chromosomes.

Conclusions to be drawn from these experiments are: (1) no brightly fluorescent part of the Y is present in the ten XX males so far investigated; (2) no evidence of low-grade mosaicism with a cell line having a Y chromosome emerges from a study of several thousand interphase cells from blood, skin, and testis cultures; and (3) since the fluorescence pattern of the X chromosomes was indistinguishable from that found in normal females, no evidence in favor of an X-Y interchange has been produced.

It must be emphatically stressed that failure to demonstrate low-grade mosaicism does not establish its absence, because its extent may be very circumscribed. Alternatively, the postulated Y-containing cell line may have been eliminated altogether. Likewise, the normal fluorescence of the X chromosomes does not disprove the interchange hypothesis, because translocation of a very small portion of the Y chromosome would not necessarily alter the fluorescence. Clinical evi-

dence suggests that the weakly fluorescent paracentric regions of the Y chromosome may contain the male determinants [118].

VII. True Hermaphrodism

There are extensive reviews on the subject of hermaphrodism [119–121]. Clinically, a variant of hermaphrodism has been termed "mixed gonadal dysgenesis" [122, 123]. It is questionable whether this syndrome is sufficiently different from true hermaphrodism to form a group of its own.

In 108 cases reviewed by Polani [121], 47 had a Y chromosome and 61 did not. The number of mosaics with a Y chromosome was 26. To this number one might add at least 40 cases with 45,X/46,XY mosaicism and "mixed" gonadal dysgenesis described in the recent literature. Then one-third to one-half of all cases are mosaics with a Y chromosome in at least a portion of the cells. Sex chromosome mosaicism might be accepted as a "natural" explanation of intersexual states and hermaphrodism. It then remains to be explained how true hermaphrodism has developed in the pure XX cases (nearly one-half of all hermaphrodites) and XY cases (nearly one-fourth of all hermaphrodites).

Tissue cultures derived directly from gonadal tissue in mosaics should settle the question of whether or not XY is predominantly found in testicular tissue and X or XX in ovarian tissue. Surprisingly few such studies have been reported. The expected distribution of stemlines was not regularly observed. In one instance of XX/XY chimerism, both stemlines were present in a blood culture but in a testis culture only XX was detected [124]. As a general rule, both stemlines are most often found in blood cultures. In several X/XY mosaics, only the 45,X stemline was found in testis cultures, whereas the 46,XY stemline was present in blood only [125]. Similar unexpected distributions of stemlines have been reported in natural chimeras in cattle [126] and horses [127] and in experimental chimeras in mice (C. E. Ford, personal communication, 1970).

An explanation often offered for failure to detect the "appropriate" stemline in the "appropriate" gonad is that whenever explants from solid organs or tissues are cultured in vitro, the cells that grow most actively are the fibroblasts. Therefore, karyotypes are prepared only or predominantly from fibroblasts which presumably lack organ specificity in the cytogenetic sense. However, in at least a number of instances, tissue cultures from testicular material have consisted of epithelial cells [126] or of a mixture of presumed Leydig cells and fibroblasts [1].

It should not, of course, be forgotten that the most obvious explanation of the failure to detect the "appropriate" karyotype is that it is not present. It therefore appears logical to extrapolate and claim that XX males also, at least in part, are the result of detected or undetected mosaicism, since a few mosaic cases have been found (table 9). However, such extrapolation is tentative, and the possibility of other mechanisms must be kept in mind. It remains to be seen why the postulated Y-containing stemline has been completely eliminated in most XX males but persists more often in hermaphrodites.

Familial hermaphrodism is of interest in this context. In one family [128],

three sibs were true hermaphrodites, with male phenotype and 46,XX only in several tissues. A common genetic or epigenetic factor would appear to be the most logical explanation, as may also be the case in another family [129]. However, in the sibship of four described by Berger et al. [23], there was one XX/XY true hermaphrodite and one XX male (tables 2 and 9). While the most plausible explanation appears to me to be that XX/XY mosaicism accounted for both cases of abnormal sex differentiation, this was not considered most likely by the authors. They concluded that a nonkaryotypic genetic mechanism was responsible. However, in none of the above three families has any direct evidence for such a genetic factor (e.g., familial occurrence outside the actual sibships) been found.

VIII. XX Male Animals

SWINE

In a male pig, Hard and Eisen [130] found a normal female karyotype (38,XX) in three peripheral blood cultures and in fibroblasts from a skin culture. The phenotype was normal except for hypospadias. Testicular histology showed hypoplasia of tubules but no hyalinization or thickening of the basement membrane. Absence of spermatogonia and abnormalities of the sustentacular cells were noted, and there was Leydig cell hyperplasia. This porcine case is remarkably similar to the human XX males. The description of the testicular histology strongly resembles cases 1, 2, 4, 19, and 27 of the present report.

Numerous pigs with varying degrees of intersexuality and an XX complement have been found [131]. Furthermore, at least five XX/XY mosaics or chimeras are on record [132, 133].

GOATS AND MICE

A significant contribution to our understanding of the etiology of intersexuality has been extracted from an intersex condition in Saanen goats [134, 135]. In brief, Saanen goats have a high incidence of characteristic abnormalities of the reproductive organs. All or nearly all of these can now be ascribed to a single autosomal mutation, *polled*, inherited in the Mendelian way.

In XX animals, the homozygous state of the gene for polledness leads with complete penetrance to pseudohermaphrodism. In some animals, the clinical condition is so extreme that these animals correspond to human XX males in the sense used in this paper. This was the situation in three of the 19 cases studied by Soller et al. [134]. In other cases, the external and internal sex organs are ambiguous, with or without testicular tissue. In XY animals, this genotype leads with partial penetrance to the development of male subfertility (epididymal sperm granuloma type).

An autosomal dominant mutation, *Sxr*, transforms XX mice into phenotypically normal males with small testes which, in the adult, are devoid of germ cells [136]. During fetal and early postnatal development, spermatogonia, but no meiotic divisions, are seen. The 39,X *Sxr* mice are phenotypically normal males.

Spermatozoa are produced, but none of seven males tested proved to be fertile. Cattanach et al. [136] found no evidence of a Y/autosome translocation and proposed that *Sxr* may be analogous to similar mutations in other mammals.

The lesson to be learned from the intersexuality seen in goats and mice is that a defect in an autosomal gene is sufficient to bring about an almost completely male phenotype in XX individuals. In certain cases, there even appears to be normal spermatogenesis [137]. As speculated by Soller et al. [134], it seems likely that a change in the specificity of a normal gene product is involved rather than the acquisition of a totally new biochemical activity. Thus, the normal allele of the *polled* gene may be associated with sex determination under normal conditions.

If these data are extrapolated to human pathology, XX males could arise through simple gene mutations. However, in the case of human XX males, the virtual absence of familial occurrence or consanguinity provides no evidence in support of such a hypothesis.

FREEMARTINISM IN CATTLE

Blood group and cytogenetic data show that an exchange of cells may occur between cotwins through anastomosed placental vessels. When the twins are of different sexes, the female cotwin may become a sterile freemartin with ambiguous genitalia. By contrast, the male cotwin generally has an entirely normal male phenotype and is fertile. Interestingly, XX/XY chimerism can apparently be found in both sexes. It is not clear to what extent the abnormal sex differentiation of the female cotwin is brought about by the transfused XY cells, by humoral agents, or by both.

Questions pertaining to the present problem involve to what extent the "non-self" stemline becomes intermingled in the various tissues of the recipient cotwin, and whether or not it tends to become eliminated, leaving behind only the "self" stemline. Histologically normal testicular tissue of two newborn bull calf cotwins of freemartins was found to contain approximately 20% and 70% of XX cells, respectively [138]. Similar results were obtained in an extensive study by Weiss and Hoffmann [126], who also showed that, with increasing age of the animal, the XX cells tended to persist only in the peripheral blood while there was a clear-cut process of elimination of the XX cells from the testes.

DROSOPHILA

A recessive autosomal gene, *tra,* lying between locus 44.0 and locus 45.3 in the third chromosome of *Drosophila melanogaster* transforms XX zygotes into sterile males [50]. The phenotype of these males has features remarkably similar to those of human XX males. The gene is without effect in XY individuals. It has been shown that the sterility of these XX *tra/tra* individuals is due to the gene and not to the absence of a Y chromosome, because XXY *tra/tra* individuals were also sterile.

The mode of inheritance of the *tra* gene was found to be regular. Thus, if both

parents are heterozygous, the sex ratio will be three females to five males. The mating between a heterozygous female and a homozygous male will give one female to three males. A very similar gene, *ise*, has been described in *Drosophila virilis* [139, 140].

OTHER ANIMALS

In the testes of three male chimera marmoset monkeys, XX and XY cells in meiosis as well as in spermatogonial mitoses were present [141]. That even meiotic cells were XX is remarkable and appeared to confirm the assumption [138] that even primordial germ cells circulate and may become exchanged between cotwins, as was later more convincingly shown [142]. Unusual sex chromosome inheritance in various mammals has recently been reviewed [143].

IX. Summary

Phenotypic males with the karyotype 46,XX are reviewed. The incidence of the condition at birth is low, one in over 9,000 newborn males. The XX male condition may turn out to be 10 to 100 times less frequent than the XXY or the XYY condition. Clinically, XX males resemble individuals with Klinefelter's syndrome. This is true, for instance, of their general appearance, psychosexual orientation, intelligence, secondary sex characteristics, testicular histology, and hormonal status. However, a striking difference in height is noticeable, XX males being shorter (mean 168.2 ± 1.52 cm) than XXY males (mean 177.4 ± 0.77 cm).

Three main classes of theories concerning the etiology of XX males (and XX true hermaphrodites) are presented. At the present time, it is not possible to decide conclusively whether any of these mechanisms accounts for all or most XX males.

The gene theory postulates that testes and a male phenotype occasionally develop in the absence of Y chromosome material. Genetic or epigenetic mechanisms hitherto undescribed in man must be invoked. Genes of the type postulated are well known in other species (e.g., the *tra* and *ise* genes in *Drosophila*, the *polled* gene in goats, and the *Sxr* gene in mice). Epigenetic mechanisms of the type leading to freemartinism in cattle have to be considered. One main argument against the gene theory is the virtual absence of familial occurrence of the condition. At present there is no indication of the occurrence of mechanisms of this kind in man.

The interchange theory postulates that homologous portions of the X and Y chromosomes have become interchanged, that is, that male-determining genes from the Y chromosome are located in one of the X chromosomes of XX males. Indirect evidence in favor of this hypothesis comes from the atypical inheritance of the blood group Xg in three males with XX and in a number of normal families. Alternative explanations of the Xg findings are possible, and appear equally likely. In these three XX males, both X chromosomes may be of maternal origin. Indirect evidence against the interchange theory is derived from the distribution

of Xg phenotypes among XX males, the inheritance of the serum group Xm in one family, quantitative autoradiographic studies of the DNA synthesis in the X chromosomes of XX males, and the absence of any alteration in the fluorescence pattern of the X chromosomes after staining with quinacrine mustard.

According to the mosaicism or eliminated Y theory, there may be undetected or extremely circumscribed mosaicism involving a cell line with a Y chromosome. Alternatively, an earlier existing line with a Y chromosome may have been completely eliminated. Direct evidence in favor of this hypothesis is the proven existence of at least six individuals with low-grade mosaicism involving a Y chromosome. However, the absence of a maternal age effect in XX males argues against a misdivision at maternal meiosis, while four cases of low-grade XX/XXY male mosaicism presumably originated from XXY zygotes. Therefore, XY may have been the original karyotype in many instances. Indirect evidence against mosaicism is the failure to detect any Y chromosomes in thousands of cells from 10 XX males investigated with the aid of the fluorescence that appears after staining with quinacrine mustard.

Of the three hypotheses, only the mosaicism theory is backed by direct evidence. It is therefore assumed that at least some of the XX male cases (and XX true hermaphrodites) have resulted from the triggering of male differentiation by a line of cells containing a Y chromosome (e.g., XY or XXY). Later, this cell line becomes very scarce, circumscribed, or is altogether eliminated. Other cases may be caused by other mechanisms, for example, by autosomal masculinizing genes, epigenetic or environmental factors, or chromosomal interchange.

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* Since completion of this paper, five further XX males have been described: H. O. Powers et al. (*J Clin Endocr* 31:576–579, 1970); J. Guyot (*Caryotype 46,XX et phenotype masculin,* thesis, Paris, R. Vezin, 1971, pp 1–77); C. Turc (personal communication, 1971); and D. Engelhardt and H. J. Karl (two cases; personal communication, 1971).

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