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Monozygotic Twins of Different Sex

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A pair of monozygotic twins of different apparent sex are described. We consider the discordance as to sex to be due to the loss of a Y chromosome at one of the cell divisions preceding the formation of the germinal discs derived from a single zygote.

Case Reports

Case 1. J. P. (female) presented with amenorrhoea at the age of 15 and was diagnosed as a case of Turner's syndrome. She was included in a sex chromatin survey of endocrinological cases, and noted to be a twin at this investigation.

On examination her height was 4 ft. 7 in. (140 cm.); span 4 ft. 9½ in. (146 cm.); height to symphysis 2 ft. 3½ in. (70 cm.); and weight 6 st. 6 lb. (29 kg.). There was no webbing, no breast development, and no axillary hair, but there was scanty pubic hair. There were no other abnormalities apart from shortness and immaturity.

She was treated by cyclic medication with oestrogen and progesterone (stilboestrol 3 mg./24 hr. for 20 days; ethisterone 180 mg./24 hr. for 10 days. Menstruation became established and the breasts developed. During the next five years she grew 4·5 in. (11 cm.) and put on 28 lb. (12·7 kg.). On examination at the age of 21 she showed no abnormality apart from shortness and numerous moles, particularly on her arms. She was engaged to be married.

Buccal smear: no Barr bodies seen; blood film: no drumsticks seen. Chromosome analysis (Table I): lymphocytes showed XO/XY mosaicism; fibroblasts, XO cells only seen.

Case 2. W. H. P. was the twin brother of J. P. There was no evident abnormality apart from shortness (5 ft. 1½ in.), heterochromia of one iris, and a curious ability to approximate his elbows behind his back. He was of muscular build, with a deep voice and good beard growth, and was married. His genitalia appeared completely normal: the testes were of normal size and consistency and both vasa were present. Conception had not occurred 12 months after marriage: no contraception was practised. The left iris was almost entirely green-brown in colour, while the right was blue and identical to his sister's irides.

Chromosome analysis: lymphocyte culture XO; fibroblast culture XO (Table I). There was also evidence of autosomal mosaicism for a structural abnormality in the fibroblast cultures. These findings will be discussed elsewhere by Dr. Kahn.

Blood and serum groups (Dr. R. R. Race and Dr. Ruth Sanger; Professor H. Harris and Dr. Elizabeth Robson) showed no discrepancy (Table II).

Investigations

Radiographs (Dr. R. Astley). There were distinct *similarities* in the rib shapes of both twins, and *appreciable differences* in the pelvis. The boy's was undoubtedly male and the girl's was female in type. Hers had a more transverse and a less conical shape, a wider inlet, a wider pubic arch, and generally lighter structure. Her ossification was less advanced, the secondary centres of the iliac crest and ischio-pubic ramus being present but not fused, whereas both were fused in her brother.

All epiphysial centres at the elbows were fused. The girl showed some buttressing of the external supracondylar ridge.

The knees of both twins showed possible slight overgrowth of the medial epiphysial condyle. The trabecular pattern was coarser in the girl.

The hands of the boy, but not the girl, had a short left fourth metacarpal and a slightly short right fourth metacarpal. The girl's bones were more slender and ossification was slightly less advanced, the radial epiphysial lines not being completely closed.

The girl's metatarsals were more slender. In both, the metatarsals were of normal length.

TABLE I

ANALYSIS OF LYMPHOCYTE CULTURES IN XO/XY TWINS

		XO	XY	Total	% XY
Female twin (J. P.)	Lymphocyte 1	45	7	52	13
	Lymphocyte 2	36	2	38	5
	Fibroblast 1	59	?1	60	0
Male twin (W. H. P.)	Lymphocyte 1	10	?1	11	?0
	Lymphocyte 2	27	0	27	0
	Fibroblast 1	62	0	62	0
	Fibroblast 2	81	0	81	0

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TABLE II

BLOOD GROUPING AND SERUM PROTEINS

	ABO	Rhesus	P_1	MNSs	Lu^a	Kell	Lewis a b	Duffy a b	Kidd a b	Xg^a	Haptoglobins	Transferrins
Father	A_1	R_2R_2	+	MsNs	−	−	−	+ +	+ −	+	2·1	C
Female twin (J. P.)	O	$R_1^W R_2$	−	MSMs	−	−	− +	+ −	+ −	+	1·1	C
Male twin (W. H. P.)	O	$R_1^W R_2$	−	MSMs	−	−	− +	+ −	+ −	+	1·1	C

Hand and Foot Prints (Professor L. S. Penrose). As is usual in identical twin pairs, the general dermatoglyphic patterns were closely similar (Fig. 1). The only marked difference concerned the left index finger: on this digit the girl showed a large ulnar loop and the man a small radial loop.

The total ridge count, or index of finger pattern intensity, is normally greater in males than in females by about 20 ridges, but in females with Turner's syndrome it tends to be raised, on the average, to 20 ridges above the male mean. In the present instance (Table III), to correspond with the brother's reading of 115, the sister should have a reading of 97, but, as a case of Turner's syndrome, the value of 132 was just at the expected level. There were no other features on the hands characteristic of Turner's syndrome.

Electrocardiograms (Dr. Clifford Parsons). The tracings were strikingly similar, as is usual in identical twins (Fig. 2). No abnormality was evident.

Discussion

The obvious difference in the sexuality of these twins must presumably be the expression of some chromosomal difference and, in view of the Y chromosome probably being necessary for male determination in man, a substantial proportion of XY cells in some tissues must be assumed. The fact that none could be demonstrated is probably mainly of interest in showing that the tissues available for culture are not necessarily represen-

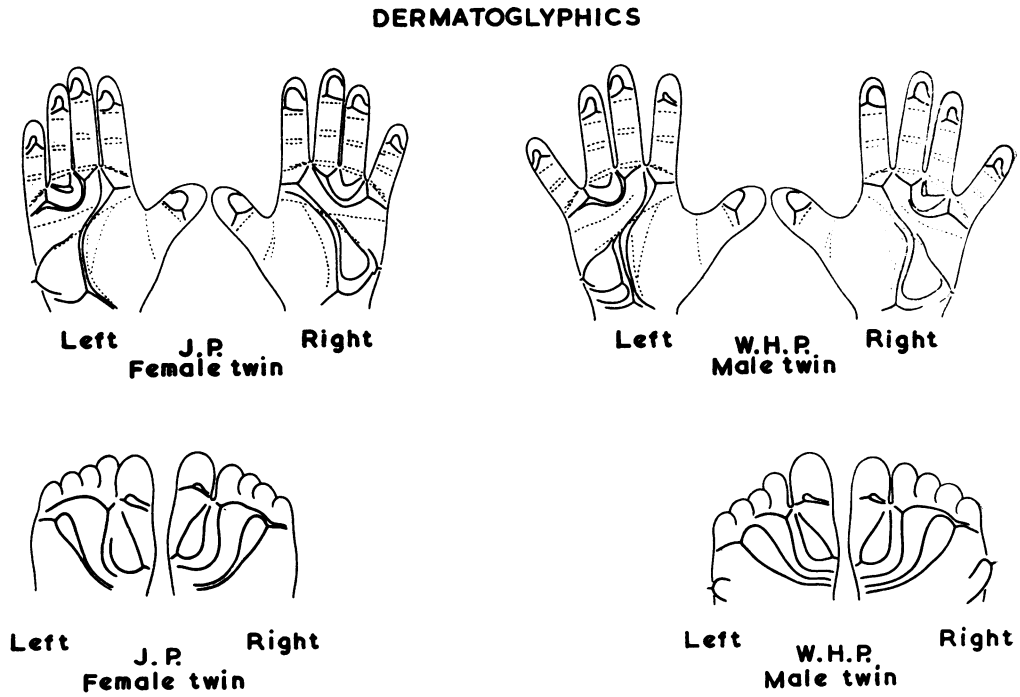

FIG. 1. Hand and foot prints of male and female twins, J. P. and W. H. P.

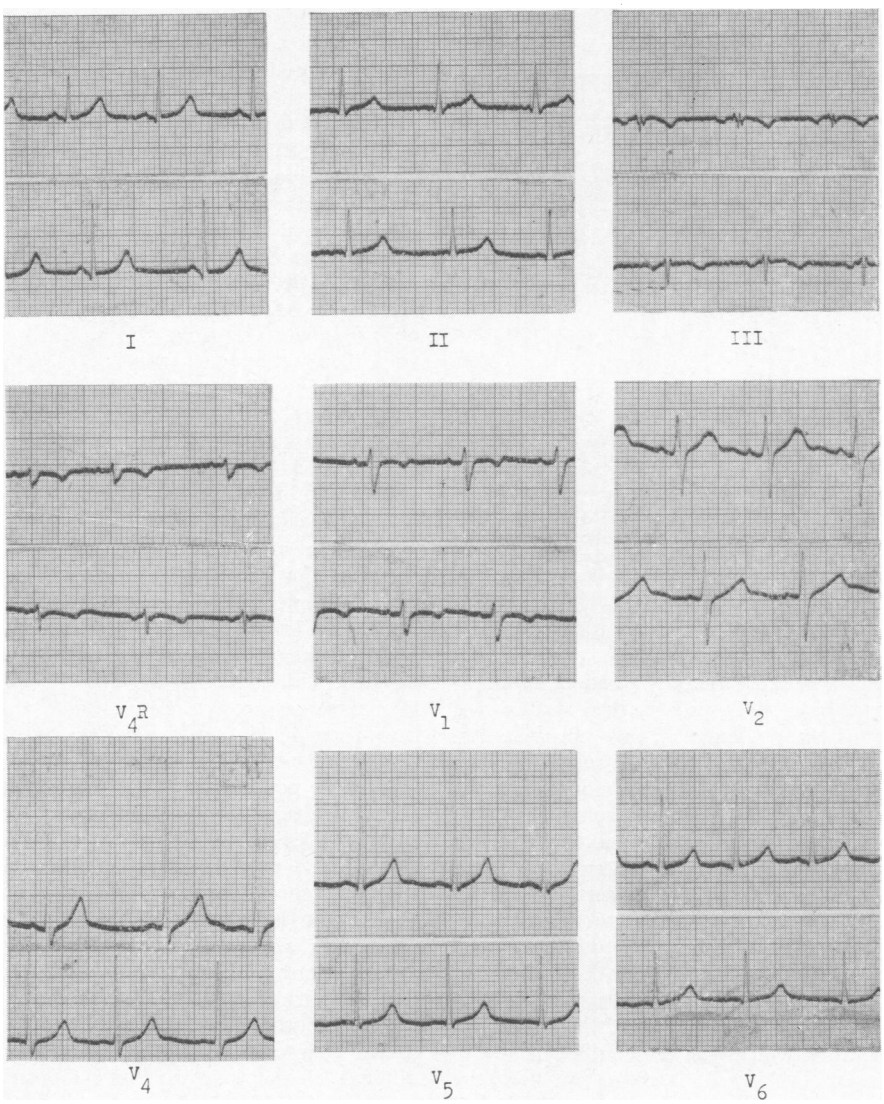

FIG. 2. Comparison of electrocardiograms of the twins. Male (top of each row), and female (bottom of each row).

tative of those determining the differences in phenotype.

Both the probability of a chromosomal abnormality arising synchronously in two independent zygotes and the probability that two independent zygotes should lead to embryos which, so far as can be tested, are of identical genotype, are very low. The chance that both these events, which should be unrelated, could occur successively is so remote that a monozygotic origin of these twins must be assumed. Both the very mild manifestation of the XO phenotype in the girl and the absence of any signs of abnormality in the boy are very remarkable. It might be expected that the boy would show stigmata of Turner's syndrome and, in fact, resemble the so-called male Turner. No such case has yet been described: in two other cases of XO/XY mosaicism, which we have investigated because of intersexuality, no sign of Turner's syndrome was present. On the other hand, both

TABLE III

DERMATOGLYPHIC PATTERNS OF XO/XY TWINS

	Site					Total
	Finger tips: dermal ridge counts	V	IV	III	II	I	
W. H. P.	L	7/0*	12/0	13/0	0/3	20/0	115
	R	5/0	11/3	12/0	15/0	17/0	
J. P.	L	6/0	10/0	12/0	17/0	20/0	132
	R	6/0	12/0	14/0	18/0	17/0	
	Palms a-b ridge count		Left		Right		
W. H. P.			43		40		83
J. P.			44		44		88
	Maximal *atd* angle		Left		Right		
W. H. P.			40°		36°		76
J. P.			40°		39°		79
	Soles Hallucal pattern ridge count		Left		Right		
W. H. P.			22		21		43
J. P.			22		17		39

* 7/0 indicates a radial count of 7 and an ulnar count of 0 ridges, 11/3 indicates a radial count of 11 and an ulnar count of 3 ridges etc. The total count is the sum of the figures in heavy type. When one count is zero the pattern is a loop: 7/0 shows an ulnar loop, 0/3 shows a radial loop. When neither count is zero—e.g. 11/3 the pattern is a whorl. The larger number for each finger is used for the total count.

boys and girls may show many stigmata common in Turner's syndrome, including marked webbing of the neck, without evidence of any chromosomal abnormality. The so-called male Turner syndrome cannot be regarded as a clinically homogeneous group, and now that the chromosomal basis of Turner's syndrome is well established, the eponym is usefully restricted to girls in whom there is an XO complement, or a deficiency of part of a second X chromosome in a substantial proportion of cells.

The hypothesis of Gartler and Sparkes (1963) that Turner's syndrome is due to the death of approximately half the cells at one stage of embryogenesis, as a result of inactivation of the solitary X in those cells in which it was arbitrarily selected to form the late-replicating X, is consistent with the mild expression found in association with XO/XY mosaicism, the XY component not being exposed to any such risk of embryonic death. Unfortunately, due to biased ascertainment in most series of Turner's syndrome, including our own, evidence of the relative effects of an XO constitution, as opposed to an XO/XX mosaic constitution, is not available. Although no Y chromosome could be demonstrated in the male twin, the most likely explanation of his apparently normal sexual development is that he has a Y chromosome in a substantial proportion of cells. His male development, in spite of substantial proportions of XO cells, is most simply explained as the result of certain key organs, such as endocrine cells of the testes, being predominantly XY, and to such expressions of maleness as normal male genitalia, facial hair, size of larynx, etc. being due to XO cells being fully competent to respond to male-determining stimuli. We have evidence from other cases of mosaicism that some tissues are consistently of one cell type. We have studied both an XO/XX mosaic and an XO/XR/XRR mosaic (where R denotes a ring chromosome), in whom no Xg^a activity could be demonstrated in spite of both having an Xg(a+) father. In another XO/XY mosaic which we have studied, the majority of fibroblasts cultured from both the gonadal streak and the testis were XO (90% and 70%, respectively), while marrow and lymphocyte cultures were almost entirely XY (80% and 95%, respectively). In one mongol mosaic, studied in collaboration with Dr. J. D. A. Delhanty, no normal cell was found in 100 fibroblasts, though 34% of lymphocytes were normal.

These results are of considerable interest in showing that, at the time of study, certain cell types do not have common stem cells. The hypothesis that lymphocytes are the universal replacement cell for mesoderm can now be rejected on direct evidence in relation to fibroblasts. In the two cases referred to above no Xg^a activity was demonstrable, though drumsticks were present and the father was Xg^a positive: paternity was as assured as possible from extensive blood grouping (Dr. Race and Dr. Sanger). This provides very suggestive evidence that the erythroid and myeloid elements do not form from a common stem cell in health: if this is so in chronic myeloid leukaemia, a condition in which both erythroid and myeloid precursors show the same chromosomal abnormality, these cells may be derived from sources that are normally dormant.

The hypothesis that differentiation of the primary sexual characters, which is brought about by the differential growth of structures originally identical in both sexes, is entirely a result of differential hormonal stimulation originating from the gonads, and is not due to the differential responses of XY, XX, or XO cells in the tissues, is entirely consistent with these observations. The probability that the twins were connected by arterial anastomoses (most monozygotic twins are monochorionic, and arterial anastomoses are almost invariably present between monochorionic placentae) is not excluded by the absence of any freemartin effect, if it is assumed that the proportion of the hormonal pool utilized by the tissues per heart beat greatly exceeds the proportion of the blood volume exchanged: this is a reasonable assumption since the half-life of some

steroids is known to be less than two hours (Mills, 1962) and no sustained twin-to-twin flow is possible.

In the rabbit it has been shown that a basically female phenotype develops if the testes are removed sufficiently early in foetal life (Jost, 1946). In the cow, a species in which twin placentae are usually connected by substantial blood vessels, the freemartin condition is well known and even features in Roman literature (taura): however, its development may be due in part, or even entirely, to cellular migration rather than to hormonal exchange (Ohno, Trujillo, Stenius, Christian, and Teplitz, 1962). The demonstration that the cells established from tissue culture of the gonad, which are probably not germinal cells, may be uniformly representative of the host cell line is not inconsistent with germinal mosaicism (Goodfellow, Strong, and Stewart, 1965). As twins of different sex and normal sexuality can develop in the marmoset in spite of placental communications sufficient to lead to chimaerism both of the lymphocytes and of the germ cells (Benirschke and Brownhill, 1963), the question of the relative contribution of hormonal environment and tissue responsiveness in sex differentiation in man cannot be regarded as capable of resolution by purely anatomical observations on other species. Since periodical reciprocal interchanges of stem cells may lead to a rapid approach to equilibrium in the stem cell population, chimaerism need not imply a shared hormonal background. Until dynamic studies on placental mixing are undertaken, it cannot be concluded that the mechanisms of sexual differentiation in the cow, the marmoset, and man differ.

The absence of masculinization in the girl, whose feminizing potentiality was impaired by a solitary X chromosome and who also probably shared a placental circulation with her brother, suggests that the freemartin condition may be unlikely to arise in man. Chimaerism, by which we imply an individual whose cells are derived from more than one zygote, can lead to fertile women with substantial proportions of XY red cell precursors (Dunsford, Bowley, Hutchinson, Thompson, Sanger, and Race, 1953). Placental connexions between dizygotic twins must be very rare in man. In a series of 500 dichorionic twin placentae anastomoses were present in only one, and this pair showed no discrepancy on extensive blood group, red cell, and serum enzyme typing (A. H. Cameron, J. H. Edwards, and J. Wingham, 1966, unpublished data). Uchida, Wang, and Ray (1964) recorded no anastomoses in 409 dichorionic placentae. Chimaerism is known to be very rare in man, since about one adult in 60 is a dizygotic twin, and in about 34% of such twins chimaerism would lead to an inconsistency in ABO blood grouping, which would be detected in testing donor blood and in blood referred from most antenatal clinics (see Race and Sanger, 1962, p. 360). Nevertheless, it is of interest to note that if the female twin had been studied before the techniques of nuclear sexing and chromosome analysis were available she might have been described as a freemartin.

We have also observed another pair of apparently female monozygotic twins showing a mosaic condition of the sex chromosomes (XO/XX) who were, in consequence, of different phenotype. These twins were born at home after 36 weeks' gestation, twinning not having been diagnosed, and were referred to hospital for treatment of prematurity. The father was a Pakistani aged 43, the mother a German aged 25. There was one previous daughter. The blood and serum grouping, the skin colour of the twins, and interview with the parents were all strongly against any suggestion of illegitimacy. The placenta was examined only by the midwife, who reported it to be single, with a constriction dividing it into two distinct zones, suggesting it was probably dichorionic. The twins were of different size (Twin I, Marion, 3 lb. 12 oz. (1·7 kg.), Twin II, Claire, 4 lb. 10 oz. (2·1 kg.)), and the smaller showed oedema of the feet and slight webbing of the neck. Nuclear sexing showed no abnormality in the larger twin, but the smaller showed no Barr bodies in the cells of the buccal mucosa, though drumsticks were present in both twins. Extensive blood and serum grouping performed by Dr. Race and Dr. Sanger and by Professor Harris and Dr. Robson was entirely consistent with monozygosity. The Xg^a findings, apparently confirmed on a second test conducted on a very small volume of blood, were themselves almost diagnostic of Turner's syndrome, since both twins were Xg (a−), as was the mother, while the father was Xg (a+). A third test showed weak activity in both twins. The palm and foot prints were strikingly similar (Table IV). Chromosome analysis of lymphocyte cultures gave results shown in Table V. The original skin cultures failed and further biopsies have been refused.

Cases of males ascertained because of defective sexual development, in which testicular tissue was present but in which only XO or XX cell lines were demonstrated, have been reported. In view of the rarity of such cases, and the fact that cases with similar phenotypes have been found to show either XO/XY or XX/XXY mosaicism or XX/XY chimaerism, the findings are not inconsistent with the likely, and at present indestructible, hypothesis that a Y chromosome is necessary for testicular development and that such a Y chromosome may resist detection by conventional cytological techniques.

TABLE IV

DERMATOGLYPHIC PATTERN OF XO/XX TWINS

Twin	Side	Site					Total
		Finger tips: dermal ridge counts					
		V	IV	III	II	I	
	L	7/o	9/o	o/o	5/o	o/o	
I	R	13/o	o/o	o/o	o/o	o/o	34
	L	3/o	10/o	o/o	o/o	o/o	
II	R	8/o	o/o	o/o	o/o	o/o	21
		Palms					
		a-b ridge count					
		Left			Right		
I		27			27		54
II		37			32		69
		Maximal *atd* angle					
		Left			Right		
I		70°			57°		127°
II		67°			69°		136°
		Soles					
		Hallucal ridge pattern count					
		Left			Right		
		21			No pattern e triradius		21
II		No pattern (e)/f triradius			17		17

Notation as in Table III.

TABLE V

ANALYSIS OF LYMPHOCYTE CULTURES AND INCIDENCE OF POLYMORPHS WITH DRUMSTICKS IN XO/XX TWINS

Age	Lymphocytes			Polymorphs			Xg[a]
	XO	XX	%XO	No.	Drumsticks	%Drumsticks	
			Twin I: Marion				
10 dy.	16	28	36	500	5	1·0	—
8 mth.	6	24	20	1000	6	0·6	—
20 mth.	6	13	30	2134	13	0·6	W
			Twin II: Claire				
10 dy.	15	28	35	580	9	1·6	—
8 mth.	2	47	4	1000	16	1·6	—
20 mth.	4	20	17	1016	14	1·4	W

As both Turner's syndrome and monozygotic twins are fairly rare events (about 1 in 5,000 and 1 in 240 in pregnancies terminating after the seventh month, respectively), the concurrence of Turner's syndrome and twinning is remarkable. A very similar pair of XO/XY twins, in whom the monozygotic state was confirmed by reciprocal skin grafts and subsequent chromosome analysis, have been described (Turpin, Lejeune, Lafourcade, Chigot, and Salmon, 1961; Lejeune and Turpin, 1961). In addition, an XO/XX monozygotic pair have been described in Denmark (Mikkelsen, Frøland, and Ellebjerg, 1963). It can be assumed that the events of twinning and non-disjunction are not unrelated, though it is difficult to relate them. A systematic study of the cells adhering to the amnion stripped from the placenta of almost all twins born in the City of Birmingham since January 1, 1964 has revealed no anomaly in over 500 pairs.

One possible reason for this high frequency of mosaic Turner's in twins is that the more normal twin assisted the pair to survive the hazards of early pregnancy, a period in which it is now clear that the great majority of aneuploid zygotes perish. The series of Carr (1963, 1965) suggests that about 1 abortion in 20 is due to an XO anomaly. Assuming 20% of pregnancies abort, this would place the incidence of loss of an X or a Y chromosome at about 1% of conceptions, and the proportion surviving early pregnancy at about 2%. Similar incidences at conception are suggested for autosomal aneuploidy. Another possible explanation is that the XO blastocyst tends to be long and slender and predisposed to the instability leading to duplication: this presumably underlies the monozygotic dichorionic twinning which appears to occur in about one pregnancy in a thousand.

Further evidence of an extraordinary association between twinning and non-disjunction is provided by the slight excess of twins in the sibs of Lindsten's cases (Lindsten, 1963), by the combination of Klinefelter's syndrome and mongolism in monozygotic twins in both Holland (Hustinx, Eberle, Geerts, Ten Brink, and Woltring, 1961) and France (Turpin, Thoyer-Rozat, Lafourcade, Lejeune, Caille, and Kesseler, 1964), by a triploidy/trisomy twin pair (Carr, 1963), and by one case of trisomy 13–15 in both of a pair of monozygotic twins (P. A. Davies, W. Aherne, and J. H. Edwards, 1963, unpublished data).

The different eye colours of the male twin are interesting, and easily explained in terms of the presumed XO/XY mosaicism, on the assumption that genes for eye colour are present on the Y chromosome. Although suggestive, there is no other evidence suggesting Y linkage for eye colour; as many other characters have been related to Y-borne inheritance on inadequate evidence the suggestion must be considered cautiously.

Summary

The twin brother of a girl presenting with Turner's syndrome was investigated and found to be consistently XO on both lymphocyte and fibroblast culture. The girl showed a small proportion of XY lymphocytes.

Data are presented implying that both cases are XO/XY mosaics and are derived from the same zygote.

A pair of twins with XO/XX mosaicism are also described.

We are deeply indebted to Dr. A. C. Crooke for introducing us to the female twin of the XO/XY pair and to Dr. B. D. Bower for referring the XO/XX pair for investigation; to Professor Douglas Hubble and

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