SEX CHROMOSOME MOSAICISM IN MAN

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1. INTRODUCTION

THE basic chromosome complement of man has been known with certainty to be 46 for only four years (Tjio and Levan, 1956; Ford and Hammerton, 1956). During this time rapid advances have been made in the study of the somatic chromosomes of sexually

TABLE

Summary of known cytological details of human sexual abnormalities

Clinical conditions		Sex chromatin	Sex chromosome constitution	Reference
Description	Phenotype			
Klinefelter . .	Male	Positive	XXY	Ford *et al.* (1959*a*) Jacobs *et al.* (1959*a*)
Turner (Bonnevie Ulrich)	Female	Negative	XO	Ford *et al.* (1959*b*) Court Brown *et al.* (1960)
Super-female . .	Female	Positive	XXX	Jacobs *et al.* (1959*b*)
Testicular feminisation	Female	Negative	XY	Jacobs *et al.* (1959*c*)
Pseudo-hermaphrodite	Male	Positive	XX	Hungerford *et al.* (1959)

abnormal humans. In all the 23 reported cases the twenty-two pairs of autosomes appear to be normal but the disorders can be correlated with abnormal sex chromosome conditions (table).

Of particular interest is the suggestion by Ford (1959*a*, *c*) that possible mosaicism for the sex chromosomes may occur in both Turner's and Klinefelter's syndromes. In the former condition three cases of presumptive XO/XX mosaics were described and in the latter, one case of a presumptive XXY/XX mosaic.

In this paper confirmatory evidence is provided for the occurrence of XXY/XX mosaicism in man.

2. CASE NOTE

The patient was a 37-year-old male with a psychopathic personality. He was under-nourished but had normal skeletal proportions. Weight 73 kilo, height 175 cm., span 175 cm., sitting height 90 cm.,

interachromial width 38 cm., interiliac width 30 cm. His skin was pale and waxlike and he had numerous fine wrinkles on his face. There was complete absence of facial hair and his axillary and pubic hair was scanty, and the latter was of a feminine distribution. He had normal adult penis and scrotum but his testicles were very small and soft. His breasts were not enlarged. His urinary output of 17-ketosteroids was 2 mg./24 hours, of 17-OH steroids 13 mg./24 hours, and of gonadotrophins approximately 200 mg. (as national reference substance HMG24) which is the normal range for post-menopausal women.

3. MATERIAL AND METHODS

The material consisted of buccal smears and a bone marrow sample removed by sternal puncture, but a biopsy of the testicles was refused.

(i) Buccal smears. These were stained by a modification of the " Rapid Method " of Battaglia (1959). Albuminised smears were fixed in 1 : 3 acetic alcohol for 30 minutes and then hydrolysed in 1N HCl for 10 minutes at 60° C. This was followed by staining in Feulgen Reagent for one hour. Slides were then rinsed with two changes of 45 per cent. acetic acid and mounted temporarily, with gentle heating, in aceto-carmine.

(ii) Bone marrow. This was treated by a modification of the method of Ford *et al.* (1958). I am indebted to Dr C. E. Ford for details of this method which involved : (i) incubation for 7 hours with the addition after 6 hours of 0·04 per cent. Colcemid solution, 0·1 ml. of this solution being added for every 1 ml. of marrow suspension. (ii) Replacement of the supernatant after incubation by 8-10 ml. of 1·1 per cent. sodium citrate, the culture being maintained in this medium for 15 minutes at 37° C.

4. OBSERVATIONS

(i) Sex chromatin. A majority of cells of the buccal epithelium were chromatin positive. Out of 250 such positive cells 83 per cent. had a single chromatin particle (plate, fig. 1) but the remainder showed two such particles (plate, fig. 2; cf. Danon and Sachs, 1956).

(ii) Chromosomes. One hundred well-spread and undamaged cells were counted and the results were as follows:

Chromosome no.	46	47
Number of cells	45	55

Thirty-three of these cells were analysed in detail according to the method of Ford *el al.* (1958). Of these thirty-three cells thirteen had 46 chromosomes all of which were found to be of normal female karyotype (plate, fig. 3). The remaining twenty cells had 47 chromosomes and were XXY in constitution, the autosomes being of standard type (plate, fig. 4).

5. DISCUSSION

(1) *The occurrence of mosaicism*

There are three possible explanations for the results presented here:

(i) The observed variation has arisen through errors in technique. Ford *et al.* (1958) have summarised the preparative errors which may

lead to the spurious appearance of variation in the chromosome number. Provided care is exercised in determining whether any possible error of this kind is involved, as was done in the present case, there is no reason why such spurious variation should ever be reported (contra Court Brown *et al.*, 1960).

(ii) The aneuploidy observed may be associated with the normal processes of differentiation in the tissue examined.

This is a point which Court Brown *et al.* (1960) did not consider in formulating criteria for the diagnosis of a chromosomal mosaic, though clearly it is of primary importance.

The abnormal chromosome pattern recovered in this case is undoubtedly secondary since no such consistent variation occurs either in a majority of Klinefelter cases or in normal individuals. In bone marrow tissue the secondary diploidy which occurs in association with the typical Klinefelter trisomy is not a consequence of normal differentiation.

(iii) The tissue examined is a mosaic.

This last explanation is the only one tenable for the results obtained. It must be noted however, that this mosaicism, which occurs in the bone marrow, may not be uniformly distributed throughout the individual. This is a point which it is hoped to clarify by further study.

Court Brown *et al.* (1960) suggested that to establish mosaicism the variation should be demonstrable in two or more separate bone marrow preparations from the same individual. While this may be desirable it can barely be held necessary in all cases. In this instance the number of satisfactory cells obtained was considerably higher than is normally the case. For example, Court Brown *et al.* (1960) counted between 5 and 77 cells in the 84 individuals they studied, but in only six of these did the count exceed 50 cells; moreover, these counts included cells which were undoubtedly damaged. Is it to be concluded that two or more such samples are to be preferred to one sample of 100 technically satisfactory cells?

(ii) *The mode of origin of mosaicism*

Sex chromosome mosaicism is not unknown in lower organisms and in certain cases such as the Iceryine scale insects regular hermaphroditism is known to depend on a haplo-diplo form of mosaicism (Hughes Schrader, 1948).

A quite distinct condition exists in the classical and irregular female sex chromosome mosaics or gynandromorphs found in *Drosophila* spp.

This mosaic condition arises by irregularities in the early cleavage divisions of potentially female eggs which lead to the elimination of an X chromosome. Male characteristics then develop in tissues arising from this deficient cleavage cell (Morgan and Bridges, 1919).

This is because in *Drosophila* spp. the XO condition is male determining, sex depending on a balance between the relative number of X's and autosomes. In man on the other hand the Y chromosome is strongly male determining (Ford *et al.*, 1959*a*).

In man the origin of sex monosomics (XO) and trisomics (XXY and XXX) must be due to primary non-disjunction during gametogenesis, resulting in the union of one normal and one abnormal gamete. If this is followed by mitotic non-disjunction, sex chromosome mosaics will arise and Ford (1959*a*, *c*) has in fact explained the origin of Klinefelter and Turner mosaics in this way.

The stage in development in man at which mitotic non-disjunction occurs is not known. Evidently in Klinefelter mosaics it is not during the early cleavage divisions since this would lead to the production of gynandromorphism. As yet there are no established instances of gynandromorphs in man, though Hungerford *et al.* (1959) have described a case in which gynandromorphism cannot as yet be confidently ruled out.

Precisely where non-disjunction has taken place in the present mosaic cannot be determined. In this case, as in that of Ford's (1959*a*, *c*), observation has been confined to the bone marrow where ideal conditions exist for the identification of mosaicism. The occurrence of mosaicism in this tissue implies that non-disjunction must have occurred before the functional marrow tissue was produced.

Another unanswered question is the cause of mitotic non-disjunction. There are two possible explanations. Firstly, errors in the spindle mechanism which then lead to accompanying errors in chromosome behaviour; and secondly, errors in chromosome behaviour which arise from changed mechanical relationships of the chromosomes on a normal spindle. Both possibilities may depend either upon a change in the genotype or on the inherent mechanical properties of the chromosomes themselves. When non-disjunction is genotypically controlled this control may affect spindle formation directly or alternatively may produce disturbed chromosome behaviour on a normal spindle. Little attention has been paid to the relationship between spindle formation and the genotype. That the function and behaviour of this cell organelle can be influenced by the genotype is clear from the work of Wald (1936) who showed that the recessive gene "claret" in *Drosophila simulans* is, when homozygous, associated with a distortion of the first female maturation spindle leading to non-disjunction. This in turn leads to the production of female gynandromorphs and male intersexes (Sturtevant, 1929). As yet no instance of non-disjunction resulting from abnormal gene controlled chromosome behaviour is known. However, chromosome behaviour is subject to genotypic control and an abnormal genotype is known to produce abnormal behaviour (Rees, 1960).

In the same way when non-disjunction depends upon the inherent mechanical properties of chromosomes this may be because these

structures lead to the formation of an abnormal spindle or because of an inter-chromosomal effect on an otherwise normal spindle. No clear example is known of the first possibility but the second is well established. For example, when supernumerary chromosomes are present in rye mechanical difficulties on the spindle lead to non-disjunction of the extra chromosome (Håkannson, 1959).

As far as the present mosaic is concerned non-disjunction is probably best interpreted as a consequence of genetic unbalance. The phenotype of Klinefelter's syndrome must reflect an unbalanced genotype and this could, in particular environmental circumstances, produce a disturbance of the spindle, with consequent mitotic non-disjunction and mosaicism. The relationship between unbalance and non-disjunction is not however a necessary one since not all Klinefelters have been found to be mosaics; neither may it apply to all tissues in the individual.

One final point may be made concerning the production of the new cell type which determines mosaicism. In both Ford's case and the present one, the pattern of mosaicism would seem to be identical, a primary XXY condition leading by mitotic non-disjunction to an XX. Similarly in the three cases of Turner's syndrome described by Ford (1959*c*) the pattern was always consistent; an XO condition producing a secondary XX condition. Caution should be exercised in generalising from these few cases but there is a suggestive consistency in the pattern of non-disjunction in both mosaics. Ford (1959*a*) has proposed that in the Klinefelter mosaic he analysed, an XX cell arising from an XXY lineage must be regarded as having a selective advantage since it is a step towards normality. This suggestion is difficult to reconcile with the proportions of the two cell types recovered, both in his own case and in the present study. Indeed it would be easier to assume that both cell types are represented because neither is at a selective disadvantage whereas other non-disjunction products may be. Alternatively it may well be that the preferential loss of the Y chromosome can be explained at least in part on account of its smaller size.

6. SUMMARY

1. A case of XXY/XX sex chromosome mosaicism is described in a bone marrow sample from a man diagnosed as a Klinefelter.

2. The origin of the secondary XX condition in 45 per cent. of the cells is, in this instance, considered to be a reflection of an unbalanced genotype. This results in loss of the Y chromosome by mitotic non-disjunction.

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Note added in proof

The table of cytological details of human sexual abnormalities was complete at the time of submitting this paper to the press.