SEX CHROMOSOME ABNORMALITIES IN A POPULATION OF 1,662 MENTAL DEFECTIVES

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Following on the discovery of sexual dimorphism in interphase nuclei,[1] the examination for sex chromatin in buccal mucosal smears has become a recognized and routine means of detecting the majority of subjects with sex chromosome abnormalities. Several surveys have been carried out to determine the nuclear sex of mentally defective individuals. The data from some representative surveys are presented in Table I.

TABLE I. NUCLEAR-SEX ABNORMALITIES IN SURVEYS OF MENTAL DEFECTIVES*

Survey	Number studied	Number abnormal	Number of chromatin bodies
Males: Mild defectives			
Prader *et al.*[14] (1958) ..	336	8	1
Ferguson-Smith[5] (1959) ..	663	8	1
De la Chapelle and Hortling[4] (1960)	342	3	1
Israelsohn and Taylor[9] (1961)	1,556	7	1
Males: Major defectives			
Mosier *et al.*[13] (1960) ..	1,252	10	1
Barr *et al.*[2] (1960)	1,506	11	1
		3	2
Ferguson-Smith[6] (1960) ..	916	7	1
		2	2
		23	1
Maclean *et al.*[11] (1962) ..	2,607	4	2
		1	3
This survey (1963)	763	5	1
Females: Major defectives			
Fraser *et al.*[7] (1960)	595	4	2
Johnston *et al.*[10] (1961) ..	827	3	2
Maclean *et al.*[11] (1962) ..	1,907	8	2
		1	0
This survey (1963)	899	4	2
		1	0

*(after Israelsohn and Taylor, 1961)

The present paper gives the results of a survey on the entire population of 1,662 mental defectives at the Witrand Mental Institution at Potchefstroom. Ten subjects were found to have a discrepancy between their nuclear and phenotypic sex, and chromosome studies were made on all of these.

TECHNIQUES

Buccal Smears

The lacto-aceto-orcein squash technique for oral mucosal smears, as described by Sanderson and Stewart (1961),[15] was employed in this survey.

A standard stock solution is prepared by dissolving 1 gram of synthetic orcein in 45 ml. of glacial acetic acid, which is then boiled, cooled and filtered. Equal parts of the stock solution and 70% lactic acid are then mixed and filtered.

Ideally, the patient's mouth is cleaned before taking the specimen and this precaution is especially necessary in low-grade defectives, where oral hygiene is poor. The inside of the cheek is scraped firmly with a wooden spatula. The initial scrapings are best discarded since these often represent superficial, degenerating cells. Desquamated cells and saliva are then deposited on a clean microscope slide, intimately mixed with a drop of stain and covered by a thin coverglass. Firm pressure is applied by placing a few layers of filter paper over the coverslip and stroking in one direction using a spatula and maintaining pressure all the time. In this way excess stain is expressed from below the coverslip and absorbed by the filter paper, and the stained nuclei are evenly dispersed, enlarged and flattened. Because of the hygroscopic property of lactic acid, such smears were shown[15] not to dry out for as long as 6 weeks at room temperature. This, however, has not been our experience (in the dry climate of the Witwatersrand) and to keep preparations for longer than a few days, it was necessary to seal the edges of the coverglass with a quick-drying nail varnish. These preparations remained suitable for about 4 weeks.

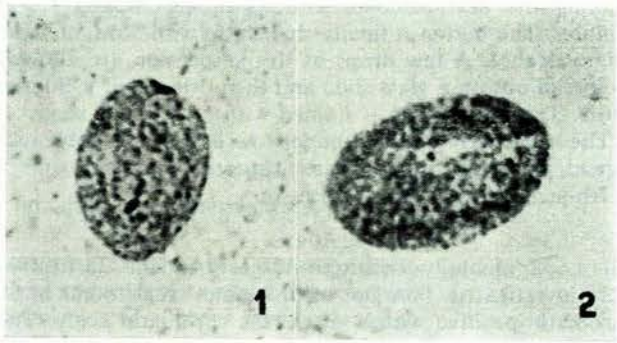

Fig. 1. Typical chromatin-positive nucleus from a normal female.
Fig. 2. Typical chromatin-negative nucleus from a normal male.

The smears were scanned with a 1/7th-inch oil-immersion lens, using a peacock-blue filter. Unsatisfactory smears, resulting from contamination with bacteria or food particles, necessitated repeat preparations in about 25% of cases. Nuclei were considered to be chromatin positive when a distinct nuclear body was seen lying adjacent to the nuclear membrane (Fig. 1). 50 *suitable* nuclei were counted in each case. The nuclear sex was recorded as chromatin negative if no Barr bodies were observed (Fig. 2). In preparations with sex chromatin present, at least 30 consecutive nuclei were examined, and if there was no suggestion of multiple sex chromatin bodies being present, the nuclear sex was recorded as chromatin positive. The mean frequency of chromatin positive status in normal females was found to be 33·4% with a range of 14 - 48%. In those smears where the nuclei contained more than one chromatin body or in ambiguous cases, further specimens were obtained, and in these at least 100 suitable nuclei were counted.

The above method has several advantages: Preparation is simple and a minimum of equipment is required; sexing is rapid (800 patients were screened by a two-man team within 2 days) and fine details of nuclear structure are well demonstrated.

Chromosome Studies

Peripheral blood cultures were performed by one of us (C.W.), using a modification of the technique described by Moorhead *et al.*[12] 10 ml. of venous blood are collected in a heparinized syringe, a sterile cork affixed to the needle and the blood allowed to stand and settle for 1 - 2 hours. The top half of the leukocyte-rich plasma is then injected directly into the culture bottle containing medium 199, A B serum and phytohaemagglutinin. The mixture is incubated at 37°C for 72 hours. Colchicine, 10^{-6} M. is added during the last 2 hours of incubation. One volume of cell suspension is then treated with 3 volumes of hypotonic sodium citrate or distilled water and allowed to stand for 7 minutes. After centrifuging for 3 minutes, the supernatant fluid is drawn off and about 5 ml. of acetic-alcohol 1:3 is carefully added so as not to disturb the 'button'. After 30 minutes the cells are resuspended by

shaking the acetic-alcohol and again centrifuged for 3 minutes. The button is finally shaken up with 5 ml. of fresh acetic-alcohol. A few drops of this suspension are allowed to spread out on a glass slide and then dried over a Bunsen flame. The preparation is stained with Leishman's stain.

The slides were scanned under low power and only well-spread metaphase plates were inspected in detail with a 1/7th-inch oil-immersion objective.

RESULTS

All 1,662 mentally defective subjects in the Institution were investigated. Five out of 763 males were found to be chromatin-positive with a single sex chromatin body. The frequency of chromatin-positive males was thus 6·6 per 1,000. Of 899 females, 5 had sex chromatin aberrations, an overall incidence of 5·6 per 1,000. Four of the 5 were found to have 2 sex chromatin bodies in a proportion of their nuclei—a frequency of 4·4 per 1,000—and 1 was chromatin negative.

The chromosome studies are summarized in Table II. Four patients exhibited the triplo-X constitution of super-females, 4 patients were phenotypically and genotypically examples of the Klinefelter syndrome with an XXY complement, and 2 patients (5, 9) had less clear-cut anomalies.

Case 5 showed two modal counts, one cell-line consisting of 45 chromosomes with an XO constitution, the other cell-line of 45 chromosomes plus a small fragment. This fragment is either the result of partial deletion of the long arms of a Y chromosome, or of both arms on either side of the centromere of an X chromosome. The occurrence of a single deletion is easier to conceive than a double one and the former explanation, therefore, seems to us the more likely. This patient, then, is a mosaic with an XO/X fragment-Y, constitution. The patient is aged 3 years, and there are no physical abnormalities present.

Case 9 showed a modal number of 48. On analysis, 16 group C and 6 small acrocentric chromosomes are present. The genotype is XXY with the addition of a small acrocentric chromosome. The latter may belong to the G group; it may be an extra Y chromosome or it may represent an abnormal trisomic chromosome with deletion of one of its arms. Fairly large satellites are present and the long arms are far apart, both features of chromosome 21. However, there are no clinical evidences of Down's syndrome at all. The chromosome complement may therefore be either XXYY or XXY trisomy 21.

Full clinical and chromosomal data on all the abnormal patients will not be presented here. A summary of the findings, however, is tabulated in Table II, and the two unusual cases have been considered in somewhat greater detail above. The 4 super-females are the first to be described in this country and will be reported in full elsewhere. Four representative karyotypes of our cases are demonstrated in Figs. 3 - 6.

DISCUSSION

The value of the lacto-aceto-orcein squash technique is demonstrated by the rapidity and ease with which this large survey was carried out and by our results which are consistent with comparable surveys overseas. The method should become part of the equipment in the physician's or endocrinologist's consulting room or clinic.

As in the survey of Maclean *et al.*,[11] reliable I.Q. estimates on our cases were not available. Again, because the subjects were inmates of a mental institution, we have classed them as 'major defectives'. In fact, the difference in occurrence of aneuploidy between high and low grade cases was insignificant, 4 patients being feeble-minded, 2 imbeciles and 4 idiots. Our results provide figures of incidence for this country (since this is the first survey of its kind undertaken here) which need to be compared with those from other surveys (Table I).

With regard to chromatin positive males, our finding of an incidence of 6·6 per 1,000 is compatible with that of Mosier *et al.*[13] (7·9 per 1,000), Barr *et al.*[2] (7·3 per 1,000), Ferguson-Smith[6] (7·6 per 1,000) and Maclean *et al.*[11] (8·8 per 1,000). On pooling the data, an overall frequency for all males with abnormal nuclear sex, of 65/7,044, or 9·2 per 1,000 is obtained.

Out of our 899 female cases 4 (4·4 per 1,000) had an XXX constitution, which compares favourably with the findings of Fraser *et al.*[7] (4 in 595 or 6·7 per 1,000), Johnston *et al.*[10] (3 in 827 or 3·6 per 1,000) and Maclean *et al.*[11] (8 in 1,907 or 4·2 per 1,000). These sets of data do not differ significantly, and when pooled give a frequency of 19 out of 4,228, or 4·5 per 1,000.

The frequency of the XO status in female defectives is given as 0·4 per 1,000.[11] Our case is presumably an XO/X(Y) or (x) mosaic. The incidence of chromatin negative females in our series is thus 1/899, or 1·1 per thousand.

The figures make it apparent that sex chromosome aberrations are not uncommon in oligophrenic populations. There is, in fact, a significant association between oligophrenia and an increased complement of X chromosomes in both phenotypic males and females.[8, 11] Absence

TABLE II. SUMMARY OF CHROMOSOME FINDINGS

Case number	*Phenotypic sex*	*Percentage sex chromatin*			*Chromosomes counted*									*Sex chromosomes*
		—	+	++	<44	44	45	46	47	48	48>	*Modal number*	*Total*	
1	F	20	36	44	1	—	—	—	19	—	—	47	20	XXX
2	F	16	48	36	—	1	2	1	26	—	—	47	30	XXX
3	F	25	45	30	—	—	—	1	24	—	—	47	25	XXX
4	F	19	41	40	—	1	—	1	26	2	—	47	30	XXX
5	F	100	—	—	—	1	11	48	—	—	—	45/46	60	XO/XY fragment
6	M		39	—	1	—	—	2	17	—	1	47	21	XXY
7	M		34	—	1	—	—	—	15	—	—	47	16	XXY
8	M		20	—	—	1	1	1	16	—	—	47	19	XXY
9	M		27	—	—	2	—	1	1	21	—	48	25	XXYY or XXY trisomy 21
10	M		32	—	1	2	1	3	18	—	—	47	25	XXY

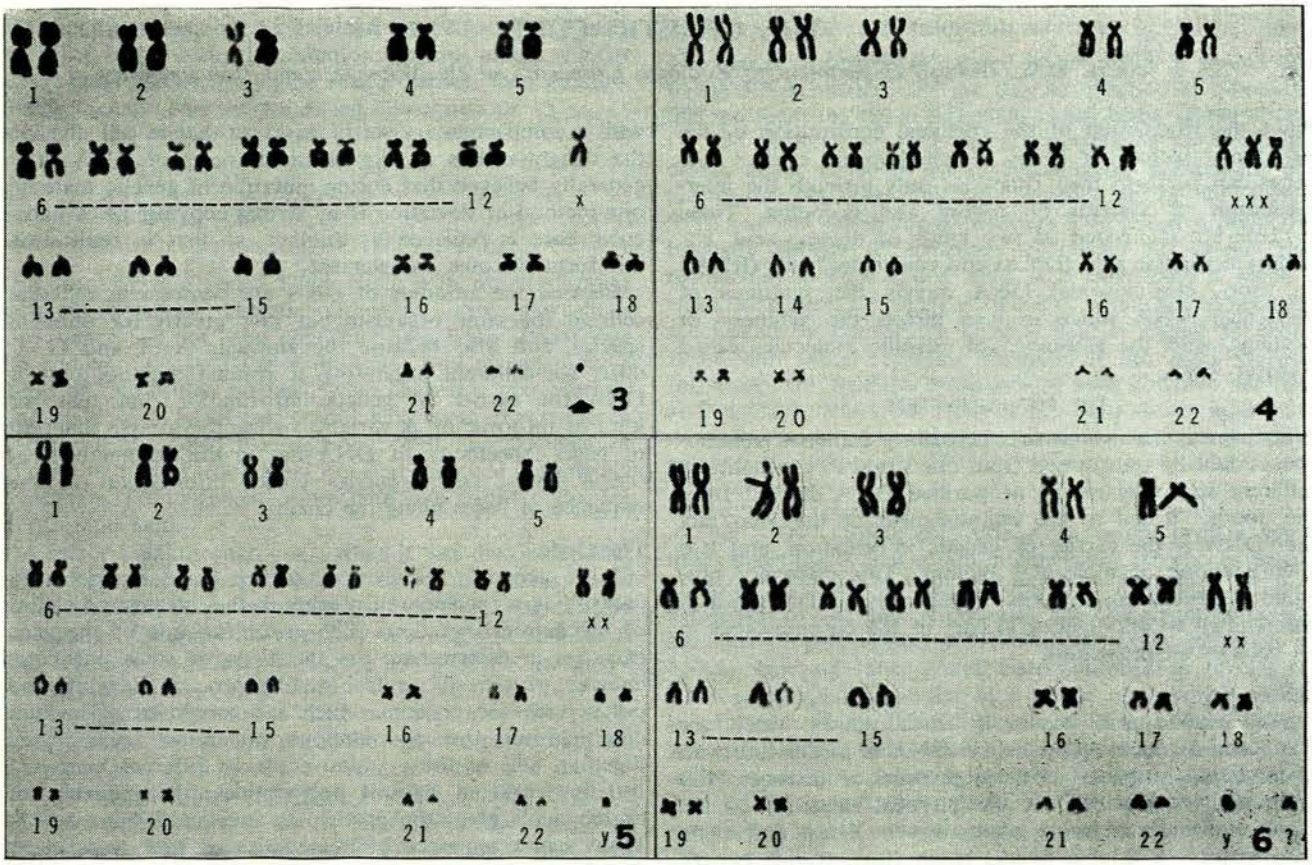

Fig. 3. Case 5. Karyotype of chromosomes at metaphase. This patient shows a mosaic pattern with two distinct cell lines. The particular cell pictured here had a modal count of 46 and a presumptive XY-fragment constitution. An arrow indicates the small chromosome fragment.
Fig. 4. Case 1. Super-female with a modal count of 47 and a triplo-X genotype.
Fig. 5. Klinefelter syndrome. There are 47 chromosomes present in each cell and the sex complement is XXY.
Fig. 6. Case 9. The modal number is 48 and the presumptive constitution is XXYY or XXY trisomy 21.

of an X chromosome, however, as in the XO constitution of gonadal dysgenesis, is not believed to be associated with significant mental subnormality[11] and the fact that we found no true case in our study, adds weight to this suggestion.

The intellectual impairment, in poly-X cases, seems to be due to the chromosome imbalance as such, rather than to specific genes on the sex chromosomes.[8] Relatively mild malformations result from sex chromosome anomalies as compared with those seen with autosomal aneuploidy. This may be accounted for on the basis of the Lyon hypothesis, where suppression of X chromosomes in excess of 1 explains their less damaging effect, as compared with extra autosomes on the developing embryo.[3]

SUMMARY

The nuclear sex status of 1,662 mental defectives at the Witrand Mental Institution was studied.

Ten cases with sex chromatin abnormalities were detected on buccal smear examination and chromosome cultures were carried out on them. The incidence of chromatin positive males was 6·6 per 1,000, of XXX females 4·4 per 1,000 and of chromatin negative females 1·1 per 1,000. These figures are in accord with those of comparable surveys done in other countries.

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