Presence of Brightly Fluorescent Material in Testes of XX Males*

Summary. The case of a phenotypic male with a predominantly 46,XX karyotype is presented. Examination of quinacrine mustard stained frozen sections of testicular tissue revealed that 29% of Sertoli cells contained a brightly fluorescent spot, presumably representing the Y chromosome. Interestingly, these cells were the only X chromatin negative cells observed in the testis. It is concluded that it is hazardous to regard any case as a pure 'XX male' without extensive study of all available tissues, including fluorescence of testicular tissue.

In spite of increased recognition and reporting of cases of 'XX males' (46,XX) in recent years (de la Chapelle, 1972; Nicolis et al, 1972), the precise cause or causes and pathogenesis have not been clearly elucidated.

In a case recently studied in our laboratory frozen sections of testicular biopsy specimens, stained with quinacrine mustard exhibited intensely staining bodies in sex chromatin negative Sertoli cells of seminiferous tubules.

In addition, this is the first report in the literature, to our knowledge, of this disorder in a black person, although we are aware of two other cases (Wertelecki et al, 1972; L. Weiss, personal communication).

Case Report

A 19-year-old black man was referred to the endocrinology clinic for obesity and hypogonadism. He was born at full term to a 28-year-old mother and a 31-year-old father. Pregnancy and delivery were unremarkable. Birth weight was 2724 g. There was no known consanguinity. His early childhood was described as normal except for his being 'slow in school work' necessitating transfer to a special school.

His weight was normal until 12 years of age when it began to increase rapidly. He weighed 55 kg at age 12, 78.5 kg at age 14, and 88.5 kg at age 15. The parents

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weights and heights are: father, 74·9 kg and 170 cm and mother, 67·2 kg and 155 cm. The patient's sister, his only sib, is of normal weight and height.

Axillary, pubic, and facial hair began to grow at approximately 17 years of age and acne developed. The facial hair has remained sparse and has not required shaving. Libido has been good. He denies sexual impotence. There was no history of testicular trauma or inflammatory disease. The patient denied alcohol indulgence or narcotic abuse.

Past medical history includes measles, chicken pox, tonsillectomy, circumcision, and one episode of acute cystitis. There was also bilateral slipping of femoral capital epiphyses at ages 13 and 14 presumably due to obesity.

The family history is unremarkable except for a maternal uncle who is institutionalized for schizophrenia. He exhibits signs of hypogonadism and has a 46,XY karyotype.

Physical examination of the patient reveals an obese, slightly retarded young man (Fig. 1). Weight is 115 kg; height, 170 cm; arm span, 175 cm; head to pubis, 78·6 cm; pubis to heel, 91·4 cm. The voice is normally deep and facial hair is sparse. Axillary and pubic hair are normal in amount and of male distribution. Some acne is present. The breasts are enlarged but on palpation seem to consist primarily of adipose tissue. The testes are descended, of normal consistency, and are small, measuring 2·5×2·0×1·4 cm and 2·2×2·0×1·2 cm left and right, respectively. The phallus and prostate are normal. No other abnormalities were noted on physical examination. Colour vision of the patient and his father are normal as measured by pseudo-isochromatic plates (American Optical Company). Neuropsychological studies show no pathological trends but indicate retarded psychosexual development. His full scale IQ is 83, verbal IQ 77, and performance IQ 94.

The significant hormone determinations are shown in Table I. Other pertinent laboratory studies include: erythrocyte glucose-6-phosphate dehydrogenase, 10·6 IU/g haemoglobin (normal, 8·9 ± 2). Determination of Xg\(^a\) blood group showed the patient to be positive, his mother was positive, and his father negative.

Dermatoglyphics show an ulnar loop in all fingers of both hands, an ad angle of 44\(^v\) in the left and 42·5\(^v\) in the right hand; there were no special patterns in the interdigital areas and no simian line. Cystourethrogram was normal. The patient was unable to provide an ejaculate for study.

Bilateral testicular biopsy was performed. A portion of testis was immediately frozen for later sectioning. Skin and testicular tissue were cultured for cytogenetic studies by the method of Basrur, Basrur, and Gilman (1963). Histological examination of the testicular specimens showed total absence of spermatogonial elements, the seminiferous tubules being lined entirely by Sertoli cells. There was minimal interstitial fibrosis of tubular walls, and the Leydig cells appeared to be slightly increased in number.

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Normal value (male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-hydroxysteroids (urine)</td>
<td>6·4 mg/24 hours</td>
<td>3-10 mg/24 hours</td>
</tr>
<tr>
<td>17-ketosteroids (urine)</td>
<td>9·6 mg/24 hours</td>
<td>8-16 mg/24 hours</td>
</tr>
<tr>
<td>Human pituitary gonadotropin (urine)</td>
<td>48 mouse units</td>
<td>6-48 mouse units</td>
</tr>
<tr>
<td>Serum testosterone</td>
<td>0·2 μg/100 ml</td>
<td>0·2-1·1 μg/100 ml</td>
</tr>
<tr>
<td>Serum follicle stimulating hormone</td>
<td>15 mIU/ml</td>
<td>11-23 mIU/ml</td>
</tr>
<tr>
<td>Serum luteinizing hormone</td>
<td>40 mIU/ml</td>
<td>2-24 mIU/ml</td>
</tr>
</tbody>
</table>

TABLE II

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Test</th>
<th>No. of Cells Counted</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal mucosa</td>
<td>X chromatin Fluorescence (quinacrine mustard)</td>
<td>600</td>
<td>150 positive (25%)  Brightly fluorescent body in 3 cells (1·4%)</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>Chromosome analysis Fluorescence (quinacrine mustard)</td>
<td>100</td>
<td>99-46,XX;1-47,XXY Brightly fluorescent body in 5 cells (1%)</td>
</tr>
<tr>
<td>Testes</td>
<td>X chromatin Culture and chromosome analysis Fluorescence (quinacrine mustard) on frozen sections</td>
<td>90 Leydig cells 90 Sertoli cells 48 260 Sertoli cells 100 Leydig cells</td>
<td>20 positive (23%) 0 positive 46,XX Brightly fluorescent body in 76 cells (29%) No fluorescent body observed</td>
</tr>
</tbody>
</table>
FIG. 2. 47,XXY karyotype. The only metaphase in which a Y chromosome could be identified.

FIG. 3. Fluorescence photomicrograph of frozen sections from testicular tissue illustrating the Y body in X chromatin negative Sertoli cells. (×800.)
**Cytogenetic Studies.** Methods for studying peripheral blood leucocytes and fluorescence techniques were based on those of Moorehead *et al* (1960) and Caspersson *et al* (1970a), respectively. Frozen sections, approximately 5 μm thick were cut in a cryostat, fixed at 95% ethanol for 15 min and then air dried before quinacrine mustard staining. The results are listed in Table II. Of special interest was the finding of only one cell (peripheral blood leucocyte) in which a Y chromosome could be identified (Fig. 2). The very low incidence of brightly fluorescent bodies in buccal mucosal cells and peripheral blood leucocytes is consistent with the findings in normal female buccal smears (Robinson, 1971) and peripheral blood leucocytes (Conen, Lewin, and Vakil, 1971), and must be discounted as representing a Y chromosome. A striking finding was the presence of an intensely fluorescent body in 76 of 260 (29%) Sertoli cells examined (Fig. 3). It is unlikely that these spots represent the inactive X chromosomes as reported by Mukherjee, Moser, and Mitrowski (1972) because of their smaller size, location, and occurrence in other than cultured cells.

**Discussion**

It has been pointed out (Caspersson *et al*, 1970b) that caution must be used in interpreting the presence of brightly fluorescent bodies in interphase cells since such bodies occasionally represent structures other than the Y chromosome. Conversely, the Y chromosome may not always be manifested by a brightly fluorescing body (Borgaonkar and Hollander, 1971). It is indeed possible that the Sertoli cells observed in the testicular biopsy specimens in the present case may not be displaying a true fluorescent Y body. However, the lack of such brightly fluorescent bodies in other tissues examined with the quinacrine fluorescent technique and the fact that only the cells displaying such brightly fluorescent bodies (Sertoli cells) did not contain an X chromatin body, strongly suggests that we are dealing with a Y chromosome rather than one of the other structures known to simulate the Y body.

Our interpretation of the studies of testicular tissue is that the Leydig cells have a 46,XX chromosomal constitution, and that the Sertoli cells have either a 47,XXY or 46,XY chromosomal constitution, the latter being more likely because of the lack of recognizable X chromatin in those cells.

Three main classes of theories have been proposed to explain the occurrence of male differentiation with testicular development in individuals with 46,XX chromosomal constitution (de la Chapelle, 1972).

These are the gene theory, the interchange theory, and the mosaicism or eliminated Y theory. According to the mosaicism theory, there is undetected or very limited mosaicism of a Y-containing cell line. A variation of this theory, explaining the inability to find a Y chromosome after extensive searching, suggests that the Y-containing cell line is lost after triggering male differentiation at a very early stage of development of the zygote.

The present case brings the number to at least seven (de la Chapelle, 1972) of cases in which a small number of Y chromosomes (or possible Y chromosomes) have been detected in otherwise 46,XX individuals, providing strong suggestive evidence that a substantial number of 'XX males' are indeed examples of the third mentioned theory, ie, mosaicism. Hence, it seems that the weight of evidence dictates consideration of the possibility that many, if not most cases of 'XX males' represent mosaicism, such as XX/XXY or XX/XY, the Y being occasionally, but more often not, detected. Several investigators (Therkelsen, 1964; Boczkowski et al, 1969; de la Chapelle, 1972) have called attention to the sex chromatin disparity in testicular tissue. In those instances sex chromatin was observed in cells of tubular walls and in Leydig cells, but was not detected in Sertoli cells. Our identical observations with the additional knowledge that only Sertoli cells contained the brightly fluorescent body on quinacrine mustard staining suggest that the Sertoli cells carry the XY line. This does not account for the failure to detect the Y chromosome in such cases where testicular tissue was cultured, but it is possible that the XY Sertoli cell is at some relative disadvantage in growing and dividing under the usual conditions of cell culture for cytogenetic analysis. Indeed, de la Chapelle *et al* (1964), by histo-chemical studies, showed that the majority of the cultured cells and all the karyotyped cells from the testis of his first case were probably of Leydig cell origin.

On the basis of the experience in the present case, ie, failure to detect Y chromosomes in blood cultures except on extensive repeat studies, absence of Y chromosomes in testicular culture, and presence of intensely fluorescent bodies in many interphase testicular cells, it becomes increasingly difficult to regard as pure 'XX males' any of the cases in which all of these studies (including quinacrine staining of fresh frozen sections of testicular material) have not been carried out.

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dehydrogenase determination and Drs Kenneth Adams, Steven Myers, and Phillip Rennick (Lafayette Clinic, Detroit, Michigan) for the neuropsychological studies. Hyland Laboratory, California, kindly provided the Xg* anti-serum.

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REFERENCES


Three Translocations Involving C- or G-group Chromosomes

Summary. Three translocations each involving C or G chromosomes are reported. A familial translocation t(Cq+ ; Eq-) was identified to be rcp(6;18) (q2;q1) and two malformed children were then found to have a 46,XY(or XX),-6, +der(6) constitution. One of the carrier’s pregnancy in this family was monitored by amniocentesis and a fetus was identified as being a male translocation carrier (balanced). Two other translocations were identified as rcp(11;14) (q12 or 13;q32?) and t(17;22) (p12 or 13?;q11?), respectively.

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Individual chromosomes in the C- or G-group cannot be distinguished with certainty either by conventional karyotype analysis or autoradiography. However, various Giemsa-banding and quinacrine fluorescence techniques have made it possible to recognize every individual chromosome pair by their characteristic patterns of bands (Caspersson, Lomakka, and Zech, 1971; Evans, Buckton, and Summer, 1971).

In the present report the chromosomes involved in three different structural rearrangements, including two Cs and one G, were identified by the techniques of both Giemsa-banding and quinacrine fluorescence. Further, the points of exchanges were determined within narrow limits.

Materials and Methods

Metaphase spreads were obtained by conventional leucocyte culture technique. Slides were flooded with ice chilled trypsin solution (freshly prepared, Difco 1:250 trypsin, 0-05% in Ca ++ and Mg ++ free Hanks’ solution) for 10 to 30 seconds, rinsed in physiologic...