C. E. BLANK *ET AL.*: CHROMOSOME MOSAICISM
IN A HERMAPHRODITE

J. FORSHAW AND E. H. MOORHOUSE: AMYLOIDOSIS
AND CHRONIC ULCERATIVE COLITIS

FIG. 1.—Biopsy of right gonad taken at 10 weeks, showing essentially normal testicular tissue. ($\times 205$.)

FIG. 2.—Right gonad removed at 14 weeks, showing essentially normal testicular tissue. ($\times 205$.)

FIG. 3.—Section from left gonad. ($\times 205$.)

FIG. 1.—Case 1. Section of cortex of kidney showing amyloidosis of glomeruli and interstitial fibrosis with round-cell infiltration. (Congo red. $\times 115$.)

FIG. 2.—Case 1. Section of medulla of kidney, showing tubular atrophy and tubular and interstitial amyloidosis. (Congo red. $\times 115$.)

Chromosome Mosaicism in a Hermaphrodite

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[WITH SPECIAL PLATE]

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The chromosome complement in 16 patients with convincing evidence of hermaphroditism has now been investigated. A normal female karyotype (44 autosomes+XX) was reported in 12 cases¹⁻¹⁰ and a normal male karyotype (44+XY) in one.^{11 12} Ferguson-Smith *et al.*¹³ reported a patient who appeared to have a mixture of cell types (mosaicism); one cell line appeared to have a 44+XX complement and a second was thought to have a 44+XXX complement. Gartler and his colleagues¹⁴⁻¹⁶ described a 44+XX/44+XY mosaic. Three cell lines, 44+XX, 44+XXY, and 44+XXYYY, were observed in a hermaphrodite described by Fraccaro *et al.*¹⁷

44+XY/44+XO mosaics in whom there is some histological support for the diagnosis of hermaphroditism have also been described.^{18 19}

We describe here a case of a hermaphrodite with presumptive 44+XX/44+XXYY chromosome mosaicism.

Case Record

An apparent male child with hypospadias and an undescended testis (Fig. I) was born on 18 April 1963. The mother was aged 20 and the father 23; two earlier pregnancies had resulted in the birth of normal boys and a third had ended in a miscarriage at

FIG. I.—External genitalia when first seen.

three months. The mother had been well throughout the pertinent pregnancy, and cannot recall having tablets or injections in the early months. The parents are not blood relatives.

The child was admitted for further investigation on 16 June. An examination of blood and buccal mucosa for the presence of sex chromatin was requested and a careful examination under anaesthesia planned. Nuclei were shown to be sex-chromatin-positive (see below). Under anaesthesia a small amount of radio-opaque material was introduced into an apparent external urethral opening. X-ray appearances strongly suggested a distended vagina, a small

uterus, and one fallopian tube. An intravenous pyelogram showed normal urinary tracts and a normal bladder.

The abdomen was opened on 25 June, when the child was 10 weeks old. On the left side of the pelvis was a unicornuate uterus and a normal-looking fallopian tube and ovary, the latter

FIG. II.—External genitalia three months after reconstructive surgery.

of uniform appearance throughout. A biopsy was taken from the length of the ovary. On the right side of the pelvis was a vas deferens passing from the internal ring to the region of the bladder base. The scrotum on the right side was then opened and a normal-looking testis and epididymis exposed; a biopsy was taken from the testis. The abdomen was closed. The case was carefully reviewed and a decision was taken that the child should be brought up as a girl. Further surgery was then necessary. On 29 July the phallus was removed by excision of the corpora cavernosa and some three-quarters of the glans; the remainder of the glans was left attached to skin on the ventral aspect of the organ and was sutured back into the position of the normal clitoris. The testis was removed and the scrotum reconstituted to conform in appearance with the left side of the vulva (Fig. II). Nothing more was done at that time, the question of any further surgical procedure

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being postponed until later. The sex was legally changed to female.

Histology

Right Gonad (Testis).—Two separate examinations were made of the right gonad. The first was of a small biopsy specimen which consisted of seminiferous tubules lying among loose connective tissue in which were more than the usual number of Leydig cells. The second examination was made on the removed testis, which measured 30 by 15 by 9 mm. and consisted chiefly of oedematous tissue in which were embedded masses of seminiferous tubules. In both specimens the tubules were of approximately normal size and were without a lumen (Special Plate, Figs. 1 and 2). Two quite distinct types of tubule epithelial cells were observed—a small cell

rounded by a fibrous capsule. No urethra or embryological remnants were seen.

The patient is then a hermaphrodite.

Sex-chromatin and Chromosome Analysis

The Patient.—One hundred suitable nuclei in buccal-smear preparations derived from the patient were examined for sex chromatin. Twenty-five nuclei had a single Barr body of normal size. No cell had a nucleus with more than one Barr body. A count of 25% is rather low, but may be regarded as within the normal range for an individual with two X chromosomes. Seven drumsticks were identified in 500 polymorphonuclear leucocytes examined. The connective tissue and interstitial cells of both gonads contained nuclei with a single Barr body. About 50% of the small seminiferous tubule epithelial cells contained a single Barr body.

Chromosome preparations were derived from peripheral blood by a modification of the method described by Moorhead *et al.*,²⁰ but were unsuitable for chromosome analysis. Preparations were derived from dermis by a modification of the method described by Harnden.²¹ Specimens of both gonads were sent to Dr. D. G. Harnden, Clinical Effects of Radiation Research Unit, Western General Hospital, Edinburgh, for culture (method described by Harnden²¹) and chromosome analysis. Chromosome counts are summarized in the Table.

Sixty-five of those cells with a count of 46 chromosomes were analysed in detail; each had a chromosome complement (Fig. III) indistinguishable from that of a normal female. Each of the 19 cells with a count of 48 chromosomes was shown to have 16 chromosomes in the X-6-12 group, two of which were presumed to be X chromosomes, and six chromosomes in the Y-21-22 group (Fig. IV). One of the small acrocentric chromosomes clearly had the size and shape of a Y chromo-

some. A second small acrocentric chromosome was best interpreted on morphological evidence as a Y chromosome. The karyotype of the cells with 48 chromosomes was therefore thought to be 44+XXYY. Four cells had a count of 47 chromosomes. Three of these were observed in the skin culture; two were shown to have a presumptive 44+XXY karyotype and the third a presumptive 43+XXYY karyotype with a member of the 13-15 group missing. In the one cell with a count of 47 chromosomes found in the culture

FIG. III.—Karyotype of cells with a count of 46 chromosomes. Presumptive 44+XX.

with an ovoid nucleus (presumably resting spermatocytes or Sertoli cells) and a less frequent larger cell containing many large vacuoles (presumably spermatogonia). The interstitial cells which lay outside an apparently normal basement membrane, and the laminated connective-tissue envelope of the spermatoc cords, were unusually prominent and well formed. Cells in mitosis were not seen and there was no evidence of spermatogenesis.

Left Gonad (Ovary).—The tissue examined consisted of two small fragments, each measuring less than 1 mm. in diameter. The structure of these fragments appeared, in section, to be identical and consisted essentially of a dense mass of primary follicles (Special Plate, Fig. 3). Most of the cells within the follicles were uninuclear but a few contained two nuclei. The cells in the walls of these follicles varied considerably in appearance. Only one of the thousands of primary follicles examined in serial sections showed any evidence of maturation, and that consisted only of cubical formation of the follicular cells. The interstitial cells were densely packed and not obviously abnormal.

Phallus.—A cylindrical mass of tissue removed from the phallus consisted of two masses of apparently normal erectile tissue sur-

Chromosome Counts in Preparation Derived from Patient

Tissue	Chromosome Counts									
	< 45		45		46		47		48	
	C.	A.	C.	A.	C.	A.	C.	A.	C.	A.
Skin	0	—	1	1*	84	20†	3	3‡	12	12§
Right gonad (testis)	0	—	2	2*	25	25†	0	—	3	3§
Left gonad (ovary)	1	1	4	4*	20	20†	1	1‡	4	4§

C. = Counted. A. = Analysed.

* The karyotype was variable in these seven cells. † Presumptive 44A+XX.

‡ The karyotype was variable in these four cells. § Presumptive 44A+XXYY.

derived from the left gonad the chromosome complement could not be determined. In those cells with a count of 45 the missing chromosome was variable.

It is concluded that two cell lines were present in each of three separate cultures and the karyotypes were interpreted as 44+XX and 44+XXYY. Although it is possible that a third cell line, 44+XXY, is present, this cannot be regarded as established because cells with this complement were few and observed in only one culture.

The patient is then a presumptive 44+XX/44+XXYY mosaic.

Parents of Patient.—The sex-chromatin (buccal-smear) findings with regard to the parents of the patient were normal. The chromosome complement of the father (peripheral-blood culture) was also normal. Suitable preparations for chromosome analysis were not obtained from culture of the mother's blood.

Blood-group Findings

Blood-group studies were made on the patient, her father, and her mother. All tests were read microscopically and there was no

smear and buccal-mucosa preparations were examined and shown to be sex-chromatin-positive. Full investigation to determine the precise condition of the genitalia, the nature of the gonads, and the chromosome complement followed. A diagnosis of hermaphroditism was then established.

It has been recommended²²⁻²⁴ that in cases of doubtful sex the more appropriate sex should be assigned in the first few months of life. In this way psychological difficulties are avoided. This practice was adopted here. We considered that the external genitalia could be made to conform to either sex but that the plastic surgery necessary was simpler if the female sex were chosen. Preliminary plastic procedures were carried out on the external genitalia in order that nothing obvious should interfere with the concept of the child as a normal girl, and the testis was removed for this reason and also to eliminate the possibility, however slight, of masculine changes near to puberty. At a later date exploration of the vagina and enlargement of the introitus will be undertaken. The parents have been emphatically told that they have a girl

who some years hence will require some surgery of probably minor extent.

Histology of Gonads

Examination of biopsy specimens from the right gonad suggested the presence of testicular tissue only. Two samples of the testis were available for study, one taken at 10 weeks and the other at 14 weeks. While no difference was apparent in the seminiferous tubules the amount of interstitial tissue was much less in the second specimen. The Leydig cells in both specimens were more prominent than usual and they were relatively more prominent in the 10-week tissue than in the later specimen. The normal testis at birth contains a very large amount of Leydig-cell tissue, and this largely involutes during the first six weeks after birth. The appearance of the testis in this child suggests either the presence of

more than normal tissue at birth or more delay in involution. Spermatogenesis was absent, but this is normal.

The left gonad appeared to consist entirely of ovarian tissue. Although this child was 10 weeks old at the time of biopsy the primordial follicles were still in a state of proliferation and maturing follicles were not apparent. While it is common to find binuclear primordial follicles in infants up to 40 weeks' gestation it is rare to find them after. Again, maturing follicles are usually prominent in the ovary of the newborn infant. It would then appear that this ovary is immature for the child's age.

The phallus removed at operation contained no urethra or other vestigial remnants, and probably represents an overgrown genital tubercle rather than a true penis.

FIG. IV.—Karyotype of cells with a count of 48 chromosomes. Presumptive 44+XXYY.

evidence of mixed field agglutination results, indicating that there was only one red-cell population.

Patient: A₁, NsNs, P₁+, R₁R₂, Lu(a-), Kell(a-), Le(a+b-), Fy(a-), Jk(a+b-), Wr(a-), C^w-.

Mother: O, MSNs, P₁+, R₁R₂, Lu(a-), Kell(a-), Le(a+b+), Fy(a+), Jk(a+b+), Wr(a-), C^w-.

Father: A₁, MsNs, P₁+, R₁R₂, Lu(a-), Kell(a-), Le(a-b-), Fy(a-), Jk(a+b-), Wr(a-), C^w-.

Discussion

Clinical Features

When first seen this child appeared to be a boy with one incompletely descended testis and severe hypospadias. Blood-

Chromosomal Aspects

A consideration of the significance of the chromosomal findings requires some clarification of the term "hermaphrodite." The term is used here to indicate an individual who possesses both ovarian and testicular tissue, identified as such histologically. There are some reported cases of hermaphroditism where chromosome studies have been performed in which it seems to us that the diagnosis has not been established beyond doubt. Miller *et al.*¹⁹ have reported two cases with dysgenetic gonadoma and "ovarian stroma." In neither case were ovarian follicles observed (O. J. Miller, personal communication, 1963) and seminiferous tubules were observed in one patient only. A second case reported by Ferguson-Smith *et al.*¹³ with a chromosomal fragment in some cells, had seminiferous tubules and "ovarian stroma." In our view stroma in the absence of follicles is scarcely a sufficiently definitive structure to permit the certain appellation "ovarian." The case described by Hirschhorn *et al.*¹⁸ appears to us, and also to Hirschhorn and his colleagues, not proved beyond doubt, for although two ovarian follicles were identified some reservation with respect to the presence of seminiferous tubules was expressed. It is perhaps wise, for the present, not to include the cases reported by these authors as established cases of hermaphroditism.

Three of 16¹⁻¹⁷ previously published cases of hermaphroditism with convincing evidence of ovarian and testicular tissue, where the chromosome complement has been investigated, have abnormal karyotypes. One of the two patients described by Ferguson-Smith *et al.*¹³ appeared to have two cell lines with different chromosome complements; one cell line had a chromosome complement indistinguishable from that of a normal female and the other had 47 chromosomes, the extra chromosome being a member of the X-6-12 group. Gartler and his colleagues¹⁴⁻¹⁶ described a mosaic where one cell line had a chromosome complement indistinguishable from that of a normal female and the other a complement indistinguishable from that of a normal male. The third of these patients, described by Fraccaro *et al.*¹⁷ was also a chromosome mosaic; three cell lines, 44 + XX, 44 + XXY, and 44 + XXXYY were observed.

The chromosome findings in the patient described by us may perhaps be usefully compared with those in the patients described by Gartler *et al.* and Fraccaro *et al.* In each case there is a cell line with 44 autosomes and a presumptive XX sex-chromosome complement and a cell line with 44 autosomes and one or more presumptive Y chromosomes. It might be suggested that a cell line without a Y chromosome, but with two X chromosomes, would favour the local development of ovarian tissue, and a cell line with one or more Y chromosomes the local development of testicular tissue. It must, however, be noted that a Y-bearing cell line was not observed in the mosaic described by Ferguson-Smith *et al.*

The distribution of cell types in the patient described by Gartler *et al.* is interesting. The culture grown from tissue derived from the ovarian part of the ovotestis (right gonad) had a preponderance of XX-bearing cells and that from the testicular part of the ovotestis a preponderance of XY-bearing cells. In the culture derived from tissue from the ovary only XX cells were observed. It might then be suggested that the frequency of chromosomal types noted in cells grown from the differentiated gonad reflects the frequency of chromosomal types in the cells important to the development of the gonad. The

distribution of cell types in our patient, however, does not support this hypothesis. Although ovarian tissue only was observed in one gonad and testicular tissue only in the other, the cells grown from each gonad had about the same frequency of Y-bearing cells.

The blood-group findings¹⁶ in the XX/XY mosaic described by Giblett *et al.* suggest that this mosaic resulted from the inclusion in the same embryo of cells derived from the fertilization of two egg nuclei by two spermatozoa. The blood-group findings in our patient do not suggest that her mosaicism has a similar origin. Perhaps the simplest explanation is that of non-disjunction in a zygote, or in a cell line, with 47 chromosomes and an XXY sex-chromosome complement. The inclusion of both Y chromatids in a single nucleus would result in two new cell lines, one with 44 autosomes and an XXXY sex-chromosome complement and the other with 44 autosomes and an XX sex-chromosome complement.

Summary

The clinical, pathological, and chromosomal findings in a case of hermaphroditism are described. The patient is a chromosome mosaic; one cell line has 44 autosomes and an XX sex-chromosome complement and a second cell line has 44 autosomes and a presumptive XXXY sex-chromosome complement. Some aspects of chromosome mosaicism relating to gonadal pathology are considered.

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