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AN XXXX SEX CHROMOSOME COMPLEX IN TWO MENTALLY DEFECTIVE FEMALES*

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AMONG THE abnormalities of human chromosomes that are being discovered in rapid succession, some involve the sex chromatin complex in patients with a female, or predominantly female, phenotype. These chromosomal anomalies fall into two categories, the first being abnormal only in the sense that the sex chromosome complex is contrary to the predominating features of the phenotype. Under this heading, in which the sex chromosome complex is XY, are included the syndromes of testicular feminization[1-3] and "pure" gonadal dysgenesis.[4, 5] In the first of these syndromes, there appears to be a defect in the action of the masculinizing evocator substance that is elaborated under normal conditions by the fetal testes. In the second syndrome, the failure is primarily morphological rather than biochemical, for there is minimal and non-functional gonadal development.

The etiological factor in most cases of testicular feminization and "pure" gonadal dysgenesis probably lies at the gene, rather than the chromosome, level and there may be a connection between the genetic backgrounds of the two syndromes. This possibility receives some support from the recent study, by our group, of two sex chromatin-negative sisters, one with "pure" gonadal dysgenesis and the other with male pseudohermaphroditism and a predominantly female phenotype.

The second category of sex chromosome anomaly in females consists of instances in which the X chromosome material is either deficient or present in excess, the Y chromosome being absent. A deficiency of X chromosomes is best documented in connection with chromatin-negative gonadal dysgenesis (including Turner's syndrome), where the sex chromosome "complex" consists of a single X chromosome (XO) or, in some cases, an XO/XX mosaicism.[6-16] But gradations of X chromosome deficiency will perhaps be found, for there is on record a patient with gonadal dysgenesis who has a normal X chromosome associated with an X chromosome that is incomplete.[17] Sex chromatin is usually lacking in XO individuals, although there can be concurrence of chromatin-positive nuclei and an XO sex chromosome constitution.[18-19]

Excessive X chromosome material occurs in the triplo-X female who is likely to be normal (and fertile), aside from mental retardation.[11, 17, 20-22] There is a report of some cells having too few, and others too many, X chromosomes (XO/XXX mosaicism) in a patient with defective development of the reproductive system.[17] The latter finding was confirmed in an additional patient, in unpublished work in our laboratory.

Mental retardation appears to be more common among persons with sex chromosome anomalies than it is in the general population. The purpose of this paper is to describe two patients, found during a buccal smear survey of institutionalized defectives, who have what we interpret as a hitherto unreported sex chromosome complex, namely, XXXX. The clue to the possibility of a particularly unusual complex was in the sex chromatin pattern of nuclei in the buccal smears. In earlier work, we had encountered duplication of the sex chromatin in 20 to 40% of nuclei in patients with three X chromosomes (XXX or XXXY),[11, 23] but we had not previously found buccal smears in which a proportion of nuclei had *triplicated* sex chromatin.

CASE 1.—*History; physical and laboratory findings*: This patient, for whom the diagnosis was mental deficiency without psychosis, was born on January 13, 1947. The parents were of borderline intelligence and two maternal aunts were mentally defective. The patient was the eldest of three siblings and was followed by a normal brother and a normal sister. The maternal and paternal ages at the time of the patient's birth were 21 and 19 years, respectively; the parents were not consanguineous.

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The patient weighed 112 lb., height was 62½ in., span 60 in., and measurements pubis to crown and pubis to sole were 30½ in. and 32 in., respectively (Fig. 1). Breast development was normal for her age and there was a small supernumerary nipple on the left side. Pubic hair was scanty and there was no growth of axillary hair. Other than a slight bilateral internal strabismus, the remainder of the physical examination showed nothing abnormal.

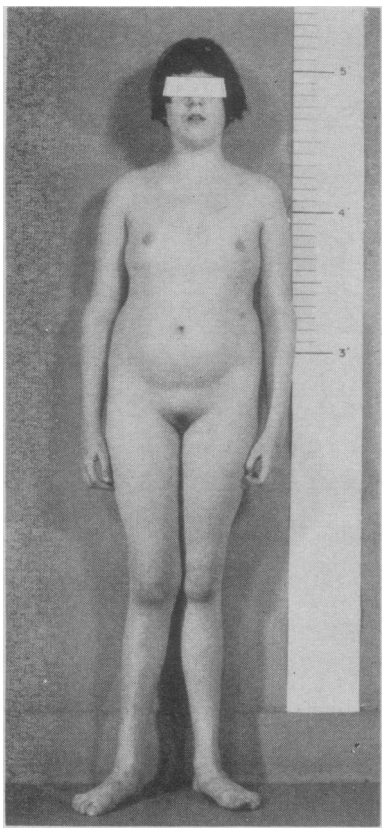

Fig. 1.—Case 1. Photograph taken when patient was 12 years and 4 months of age.

On gynecological examination (by Dr. Lillian M. Beattie, Department of Obstetrics and Gynecology, University of Western Ontario) the labia majora and minora were found to be normal for early adolescence and the clitoris was small, perhaps a little smaller than normal. The introitus, vagina and cervix were normal for the patient's age, and a uterus of average dimensions was palpable on rectovaginal and bimanual examination. An ovary, prolapsed in the posterior cul-de-sac, could also be felt. Normal menses began at the age of 12 years and 3 months. Thus, the reproductive system appeared to be normal for a girl in early adolescence.

The urinary 17-ketosteroid excretion was low, measuring 1.4, 1.5, 2.2 and 1.9 mg./24 hrs. in four specimens. The excretion of total 17, 21 dihydroxy-20-ketosteroids was 6.2, 7.1 and 5.0 mg./24 hrs. on three occasions, or within the normal range. The urinary excretion of gonadotrophins was also normal, being > 5 and < 10 rat uterine units. I^{131} studies (done by Dr. F. C. Heagy, Ontario Cancer Foundation London Clinic) showed a normal I^{131} pickup by the thyroid of 17% after 24 hrs. However, there was little or no thyroid reserve function, for the repeat pickup of I^{131}, 48 hrs. after injection of 4 units of TSH, was only 15%.

Psychological Examination

The following report was submitted (by Mr. W. R. Watson, psychologist, Ontario Hospital, London):

"The patient sat rigidly and silently throughout testing but in general responded fairly appropriately when asked single questions. She attempted almost everything she was asked to do. Her voice was low, at times inaudible, and her enunciation was not clear. Her level of speech was similar to that of a child first learning to speak; that is, she spoke in phrases rather than sentences. She initiated almost no behaviour on her own during the session and displayed no outgoing affect. Her manner remained passive and withdrawn and it was impossible to tell whether or not she had any grasp of her surroundings until she uttered a reasonably correct answer to simple questions.

"Intelligence Test Performance suggested an I.Q. of about 30 (mental age about 4 years). At this level she could be expected to do only very simple tasks and follow only very simple instructions. Such tasks as reading and writing require a level of conceptual functioning that is a good deal beyond her capacity. Even drawing or copying simple figures is difficult for her because of the visual-motor co-ordinations involved. However, she can count simple objects when arranged together, counting accurately as high as six or seven. She can conceive of such an instruction as to go to a store and buy bread. Her self-concept remains at a simple body image level where she realizes that people have or at least should have two ears, two arms, two thumbs, etc. Her grasp of reality or the world around her parallels this; for instance, she can name and recognize common objects and can realize if they are damaged. She knows that she has a father, mother, brother and sister, but has difficulty remembering their names."

TABLE I.—SEX CHROMATIN STUDY FOR CASE I.

Preparation	*Proportion of nuclei (%) with 1, 2 or 3 masses of sex chromatin*			
	1	*2*	*3*	*Total (%)*
Buccal smear.....	17	35	43	95
Vaginal smear.....	22	43	30	95
Skin biopsy.....	23	59	17	99

Sex chromatin studies: The sex chromatin was examined in smears from the buccal and vaginal mucosae, stained with thionin, and in sections of a skin biopsy specimen, stained with hematoxylin-eosin and by the Feulgen method. Table I, which contains data obtained from a study of 200 nuclei in each preparation, shows the proportion of nuclei that contained one, two or three masses of sex chromatin. The three kinds of nuclei are illustrated in Figs. 2 and 3. In some nuclei that were recorded as having two sex chromatin masses, one of them was unusually large and probably represented two masses of sex chromatin that had fused together. The extra sex chromatin in the interphase nuclei led to an unusually large proportion of cells (approaching 100%) that were chromatin-positive.

Nuclear morphology in the neutrophil leukocytes had a typical female pattern. There was a drumstick nuclear appendage in 42 out of 1000 cells, the 6th one being found in the 139th neutrophil leukocyte examined. Cells with two drumstick nuclear appendages were not seen. The Arneth Index was 2.7.

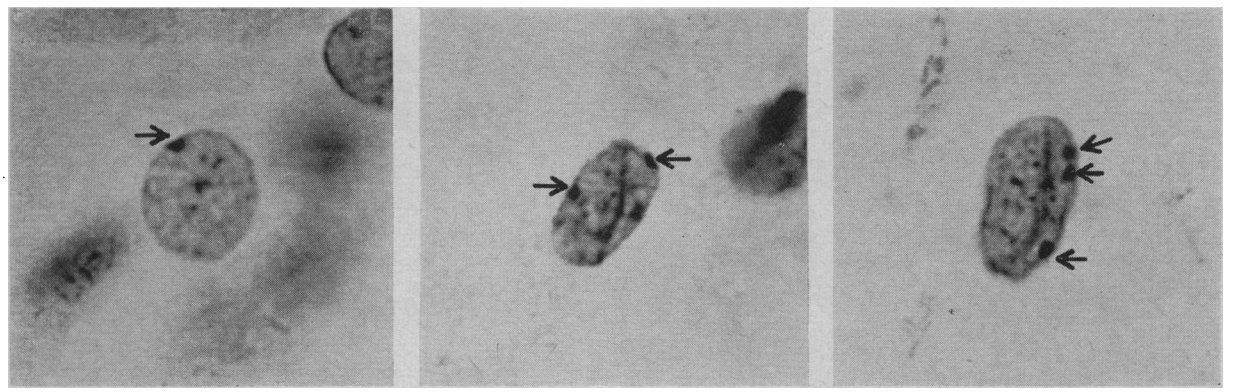

Fig. 2.—Nuclei with one, two and three masses of sex chromatin in a vaginal smear. Thionin stain. ×2000.

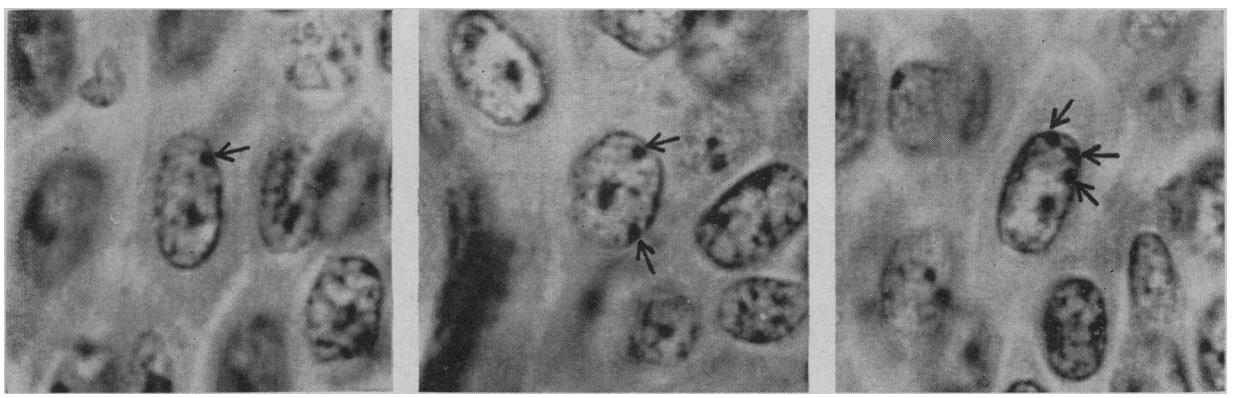

Fig. 3.—Nuclei with one, two and three masses of sex chromatin in a skin biopsy specimen. Hematoxylin and eosin stain. ×2000.

Chromosome study: Chromosomes were studied in cells derived from cultures of leukocytes in the peripheral blood, using a modification of the method of Moorhead *et al.*[24, 25] Among 110 metaphase plates, 102 of them contained 48 chromosomes (Table II). In the two cells with 49 chromosomes, there were indications that a chromosome had been broken, and the 6 cells with 47 chromosomes showed the deleted chromosome to be from various groups. The few counts of 47 and 49 were probably technical artefacts. In any event, the prevailing chromosome number was 48, or two above the normal diploid number for man.

TABLE II.—RESULTS OF CHROMOSOME ANALYSIS

	Number of cells with chromosome counts of:					*Total cells*	*Sex chromosome*
Case	*<47*	*47*	*48*	*49*	*96*	*counted*	*complex*
1		6	102	2		110	XXXX
2	9	15	99		1	124	XXXX

Twenty-one cells containing 48 chromosomes were examined in detail and karyotypes were prepared for 7 of them (Fig. 4). In each cell, the two extra chromosomes were in the 6-12 group. They were interpreted as X chromosomes, giving a karyotype of 44 autosomes and an XXXX sex chromosome complex, which is consistent with the sex chromatin pattern of the interphase nuclei. The disparity in length between the two members of the second pair of chromosomes (Fig. 4) was not considered significant, since it happened to occur in this particular cell and not in others that were analyzed in detail. There is frequently a difference of up to about 15% in the length of homologous chromosomes, as seen in preparations made by procedures currently in use. This is caused by such factors as a difference in the degree of contraction between the two chromosomes, excessive mechanical extension of one of them on the slide, or foreshortening of a chromosome by a bend along its length and in the plane of the optical axis of the microscope.

CASE 2.—*History; physical and laboratory findings*: This patient, whose clinical diagnosis was mental deficiency without psychosis, was born on August 1, 1928. She was the youngest of six siblings and had three brothers and two sisters. The mother's age was 41 years and the father's age 39 years at the patient's birth; the parents were not consanguineous. So far as could be learned, there was no history of mental deficiency or mental illness among members of the immediate family. There was a record of prolonged breech delivery, and pneumonia and mastoiditis at the age of 9 years.

The patient weighed 106 lb. Her height was 64¼ in., span 64½ in., measurements crown to pubis 30½ in. and pubis to sole 34¾ in. (Fig. 5). The breasts were small. There was a moderate amount of pubic hair with a feminine distribution and a small amount of axillary hair. Other than a slight enlargement of the right lobe of the thyroid gland, the remainder of the physical examination was negative.

Gynecological examination showed normal external genitalia and a clitoris of average size. The vagina was normal and the cervix was perhaps a little smaller than

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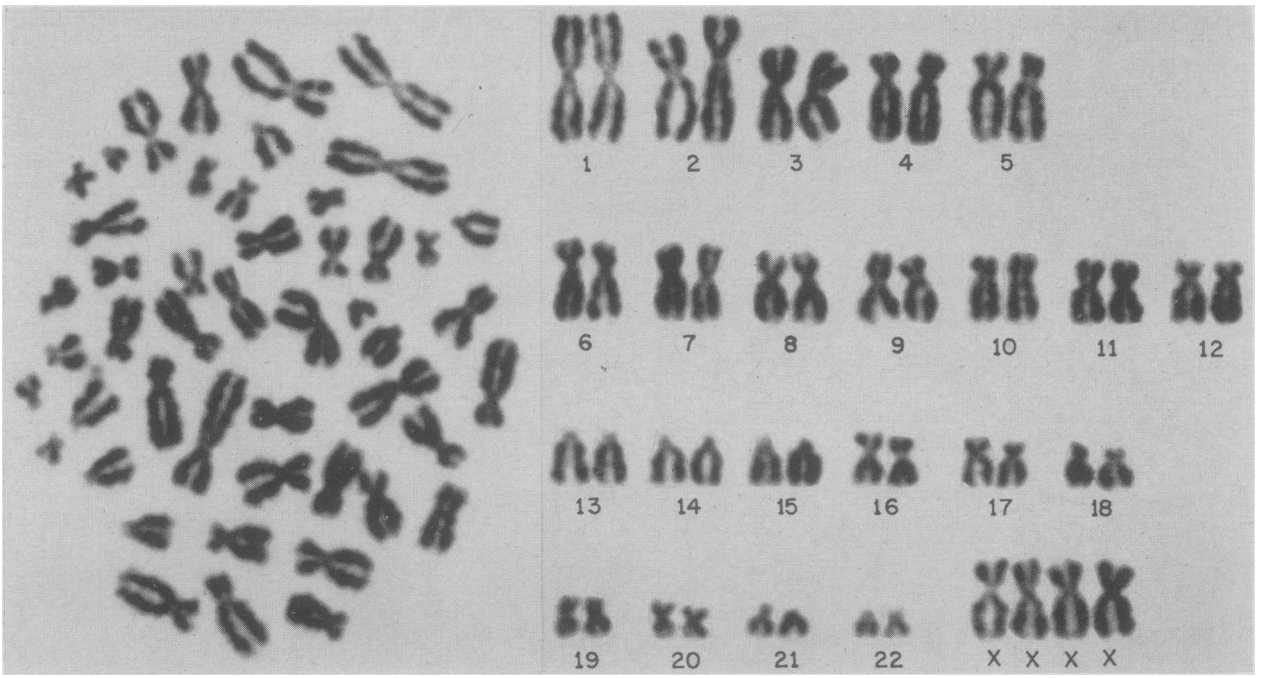

Fig. 4.—Metaphase plate and the corresponding karyotype of a cell containing 48 chromosomes (Case 1). The complement consists of 44 autosomes and an XXXX sex chromosome complex.

usual for the patient's age. Ovaries could not be felt, but there was a normal menstrual history. Thus, the reproductive system had no unusual features, so far as could be ascertained.

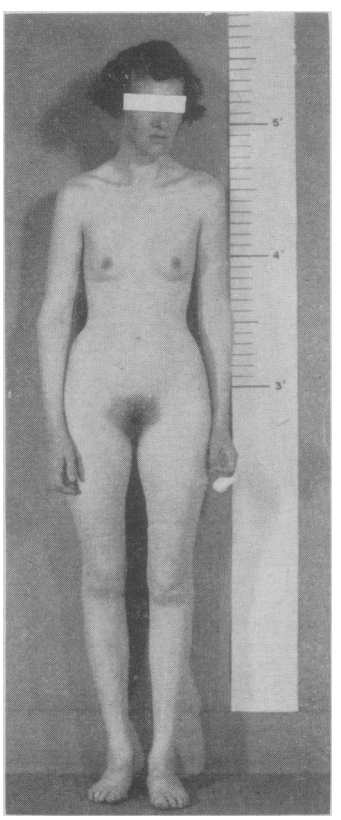

Fig. 5.—Case 2. Photograph taken when patient was 30 years old.

The urinary level of 17-ketosteroid excretion on five separate tests was 3.8, 3.3, 4.5, 5.1 and 2.8 mg./24 hrs., or at the lower limit of the normal range. The excretion of total 17, 21 dihydroxy-20-ketosteroids was normal, values of 6.3, 4.7 and 4.9 mg./24 hrs. being obtained on three occasions. Gonadotrophin excretion was > 2 and < 5 rat uterine units. I^{131} pickup by the thyroid was 12% after 24 hrs. and 52% 48 hrs. after injection of 4 units of TSH. A photoscan of the thyroid region after TSH administration showed a diffuse distribution of increased activity in the enlarged right lobe.

Psychological assessment: A psychologist (Mrs. C. L. Crone, Ontario Hospital, London) reported as follows:

"The Wechsler Adult Intelligence Scale places this patient on the borderline between moron and imbecile intelligence ranges. Her scores are as follows: Verbal I.Q., 54; Performance I.Q., 51; Full Scale I.Q., 50. Although there is little subtest scatter, the comprehension test (considered to measure judgment) tends to be slightly elevated in relation to other tests.

"While her performance on personality tests is in many ways in accordance with the response expected from a person of defective intelligence, in certain areas her test behaviour is above what would be expected from a person with an I.Q. of 50. One such area is her rather more adaptive approach to the world than one would anticipate. It appears that she would be capable of doing some tasks without supervision and also of modifying her behaviour in terms of her experience. In addition, the patient is capable of differentiating human figures on the Rorschach test. This suggests a perception of herself in relation to other humans. Also, her self-concept appears relatively well established and even sex-role seems more clearly delineated than would be expected.

"While the patient appears to be fairly resilient to emotional stress and childlike aggressive symbols appear to be organized and under control within her own limited ability, her lowest threshold is in the area of

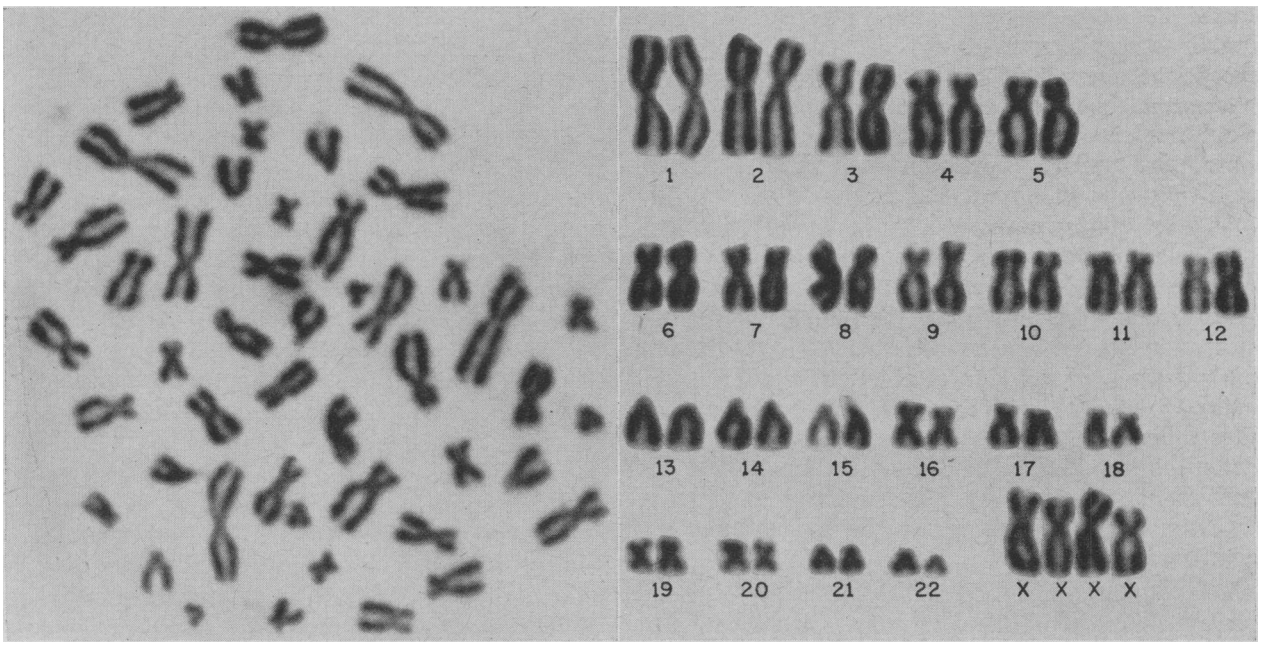

Fig. 6.—Metaphase plate and karyotype from a cell with 48 chromosomes (Case 2). There are 44 autosomes and an XXXX sex chromosome complex.

experienced hostility. Tests indicate that she is likely to become easily angered and one would expect some uncontrolled 'acting out' behaviour.

"In summary, the ego-functioning is decidedly limited by the I.Q. level. Interestingly enough, however, she does produce some test responses of a level rather uncommon for a person of her I.Q. range. These are specifically in the areas of adaptive ability, some limited empathic qualities, sex-role identification and emotional resiliency."

TABLE III.—SEX CHROMATIN STUDY FOR CASE 2

Preparation	*Proportion of nuclei (%) with 1, 2 or 3 masses of sex chromatin*			
	1	*2*	*3*	*Total (%)*
Buccal smear.....	21	45	23	89
Vaginal smear.....	20	44	33	97
Skin biopsy	24	63	7	94

Sex chromatin studies: The sex chromatin counts are recorded in Table III. In the buccal smear and skin biopsy specimen, the proportion of nuclei with three masses of sex chromatin was lower than in Case 1, but the presence of such nuclei in addition to those with two masses was still an unusual feature of the preparations.

There were 19 neutrophils with drumsticks in 1000 neutrophil leukocytes examined, the 6th such cell being the 433rd neutrophil studied. One eosinophil with a typical drumstick nuclear appendage was seen. No neutrophil was found in which there was more than one unequivocal drumstick. The Arneth Index was 2.4.

Chromosome study: Chromosome counts were made on 124 metaphase plates in preparations from leukocytes of peripheral blood (Table II). Of the 99 cells with 48 chromosomes, 23 were examined in detail and karyotypes were constructed for 8 of them (Fig. 6). The two extra chromosomes were consistently in the 6-12 group. In view of the sex chromatin pattern in the interphase nuclei, they can most logically be interpreted as X chromosomes. Specific identification of the X chromosome, in relation to other chromosomes of comparable size and morphology, is difficult. Even among experienced cytogeneticists, there is considerable variation in data recorded for length and arm ratio of the X chromosome.[26] This may reflect the difficulty in identifying the X chromosome or a true biological variation in its size and the position of its centromere.

Since there were 15 metaphase plates with 47 chromosomes, the possibility of an XXXX/XXX mosaicism was considered. Thirteen of the 15 cells were suitable for detailed analysis. The chromosome missing, in relation to cells with 48 chromosomes, varied from one cell to another and in only 4 of the metaphase plates was the missing chromosome in the 6-12 group. Considering that this is the largest chromosome group in the Denver system,[26] it is likely that the counts of 47 were either artefacts or inconstant biological variants in a population of cells that was being rapidly renewed. The cell with 96 chromosomes was clearly a tetraploid cell, such as is found occasionally in preparations from blood and other sources. The appearance of the 9 cells with < 47 chromosomes suggested that the cells had been broken, with loss of chromosomes as a technical artefact, although a biological factor may have been responsible for some of them.

In spite of difficulties in accounting with certainty for all cells that departed from the modal chromosome number, the basic chromosome complement in this patient consisted of 48 chromosomes. The most plausible interpretation of the findings, especially in view of the sex chromatin pattern, is that the additional two chromosomes were part of an unusual tetra-X sex chromosome complex.

DISCUSSION

Aside from occasional nuclei that are polyploid, which occur in almost any tissue, the nuclei of

normal females are exactly diploid (2n: 46) and contain a single mass of sex chromatin. However, exceptions occur at a few sites where polyploidy is a normal feature of the cells. For example, polyploid nuclei with more than one sex chromatin mass (the number depending on the degree of ploidy) occur in abundance in the liver,[27-29] the bronchial epithelium[30] and the amnion.[31] Inconstant chromosome complements, including hyperdiploidy, are not uncommon in malignant cells, and a few nuclei contain two or three sex chromatin masses in some tumours in female hosts.[32-34] But the presence of an appreciable number of cells with two and three masses of sex chromatin, at sites where the nuclei are predominantly diploid under normal circumstances, is distinctly unusual and probably results from the nuclei having two X chromosomes in excess of the normal female complement.

The observations recorded in this paper raise several difficult problems related to the origin of the sex chromatin, the role of the X chromosomes in gonadal differentiation, and the source of the additional X chromosomes in the nuclei of these patients.

The precise relationship of the sex chromatin to the chromosomes is still a matter that requires further investigation. The weight of evidence favours the derivation of the sex chromatin from elements of the sex chromosome complex rather than from autosomes. This rather crucial point was discussed in some detail elsewhere,[35] but it must be touched on again, for the concept of a sex chromosome origin for the sex chromatin strongly influences the interpretation of extra chromosomes when they are in a certain size range.

The only known difference between the chromosome complements of the two sexes is in the sex chromosome complex (XX and XY in nearly all mammals). The sex chromosomes have characteristics that differ from those of the autosomes in male germ cells, in which the XY complex tends to be more condensed and deeply staining (positive heteropyknosis). In many invertebrates, the interphase nuclei have chromatin masses (chromocentres) which are sex-specific and which represent elements of the sex chromosome complex. Chromocentres of sex chromosome origin have been demonstrated in nuclei of some tissues of the bank vole.[36] For these and other reasons, it seems very likely that the sex chromatin is of sex chromosome origin.

To carry the analysis a step further, there is increasing evidence that the sex chromatin represents a single X chromosome, part of which is in a state of positive heteropyknosis. Ohno and his collaborators, from their study of prophase nuclei, feel that only one X chromosome is positively heteropyknotic in females of the rat,[29, 37] mouse[37] and opossum,[38] and that this X chromosome forms the sex chromatin at interphase. The extension of the foregoing findings to man seems to be legitimate in view of the recent discovery, by Grumbach, Morishima and Chu,[18,19] of two females with gonadal dysgenesis, chromatin-positive nuclei and an XO sex chromosome "complex". The nuclei of these unusual patients are reminiscent of the nuclei of the spruce budworm,[39] the mulberry silkworm,[40] and the domestic chicken,[41,42] for in these species the female has an XO or XY sex chromosome constitution and a sex-specific chromocentre in the interphase nucleus. In man, on the other hand, and perhaps in mammals generally, an X chromosome is not as a rule positively heteropyknotic when there is only one X chromosome in the nucleus.

Because of the studies that were summarized above and the finding of three sex chromatin masses in a proportion of our patients' nuclei, we have interpreted the two additional chromosomes as X chromosomes, rather than autosomes of a similar size and morphology. The findings in patients with an XO/XXX mosaicism make the validity of this interpretation virtually certain. In these patients, in whom duplication of the sex chromatin is clearly demonstrable in buccal and vaginal smears, the extra chromosome in some cells can hardly be other than an X chromosome, for one of the X chromosomes is missing in other cells. The mosaicism probably originates through non-disjunction of an X chromosome during an early mitotic division of the fertilized ovum.

In nuclei with three masses of sex chromatin, it would appear that three X chromosomes display positive heteropyknosis and are thus visible at interphase, while the remaining X chromosome is euchromatic and thus not in evidence. Further, the number of X chromosomes in positive heteropyknosis seems to be variable from one cell to another, for the 35 to 69% of nuclei with two masses of sex chromatin and the 17 to 24% of nuclei with one mass of sex chromatin are unlikely to have resulted entirely from difficulties of technical and interpretative origin. Perhaps individual cells change from time to time or go through cycles, in this respect, the dynamic aspect being concealed in fixed preparations. There is no doubt much to be learned about the sex chromosomes (for example, see Geerts);[43] new facts concerning them will be of value to both basic science and clinical investigation.

The discovery of abnormal sex chromosome complexes gives some inkling as to the role of the X and Y chromosomes in gonadal differentiation. In females, there is usually failure of ovarian maturation when there is a deficiency of X chromosome material, as in XO and Xx individuals. Development of ovaries proceeds normally when there are one or two X chromosomes in excess of the normal female complement. But, as a general rule, inclusion of a Y chromosome in the complex causes the indifferent gonads of the early embryo to develop into testes. With rare exceptions, an XY complex results in normal male development, whereas the

infertile male with Klinefelter's syndrome is associated with XXY,[44, 45] XXXY[11, 46, 47] or XXYY[48, 49] sex chromosome complexes. There is thus demonstrated the potent testis-promoting influence of the small Y chromosome of man, even though the genetic imbalance produced by combinations of one or two Y chromosomes with two or three X chromosomes causes regressive changes to occur in the testes at puberty.

There are several mechanisms that could, in theory, be responsible for the tetra-X complex.

1. Non-disjunction of the X chromosomes at the reductional division of oögenesis could result in a secondary oöcyte with two X chromosomes. Another non-disjunction in the equational division, involving one of these chromosomes, could produce a mature ovum with three X chromosomes. Fertilization of such an ovum by an X-bearing sperm would produce a tetra-X zygote. If the fertilizing sperm were Y-bearing, the zygote would contain an XXXY complex, such as occurs in an occasional patient with the Klinefelter syndrome.

2. The triplo-X female is fertile and could produce XX-bearing ova. But non-disjunction must occur in either the first or second meiotic division to produce a mature ovum with three X chromosomes. In view of the apparent rarity of the XXX female and the necessity of meiotic non-disjunction, this possibility seems to be remote. It can easily be tested for by studying buccal smears from the mothers of such patients. We were unable to obtain this material in connection with our two patients.

3. Starting from an XX-bearing fertilized ovum, non-disjunction involving both X chromosomes in the first mitotic division would give rise to a blastomere with four X chromosomes and one with no X chromosome. The blastomere without X chromosomes would not likely survive. A similar mechanism, but starting from an XY-bearing zygote, was suggested as a possible explanation for an XXYY complex found recently in a patient with Klinefelter's syndrome.[49]

Thus, the origin of the XXXX complex appears to be a complicated form of non-disjunction of the X chromosomes during oögenesis or division of the fertilized ovum. Perhaps a reduced vitality of the ovum (blastophthoria), such as might result from delayed fertilization with over-ripeness of the ovum or from advanced maternal age, increases the likelihood of abnormal chromosome behaviour in cell division.[50]

SUMMARY

Two mentally defective but otherwise normal females had an unusual sex chromatin pattern in their interphase nuclei. In addition to typical sex chromatin-positive nuclei, some contained two and others three, masses of sex chromatin. A chromosome analysis, using the leukocyte method, showed that both patients had 48 chromosomes, or two in excess of the normal complement for man. The extra elements were considered to be X chromosomes, giving a tetra-X sex chromosome complex.

The additional X chromosomes probably arose through non-disjunction of X chromosomes during oögenesis or an early division of the fertilized ovum. X chromosomes in excess of the normal female complement are apparently compatible with normal maturation of ovaries and other components of the reproductive system, but the tetra-X complex is perhaps an etiological factor in the mental deficiency.

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