A CASE OF HUMAN INTERSEXUALITY HAVING A POSSIBLE *XXY* SEX-DETERMINING MECHANISM

By PATRICIA A. JACOBS and Dr. J. A. STRONG

Medical Research Council Group for Research on the General Effects of Radiation and Department for Endocrine and Metabolic Diseases, Western General Hospital and University of Edinburgh

RECENT improvements in techniques for the examination of human somatic chromosomes have made possible the study of the chromosome complement of human intersexes; consequently, it is now practicable to investigate the relationship in these cases between sex as determined by direct chromosome study, and sex as inferred from the study of 'nuclear sex chromatin' of the type described by Barr and Bertram[1]. This report is concerned with one of a series of patients with gonadal dysgenesis who are under investigation, and the particular feature of interest is the occurrence of 47 somatic chromosomes in contrast with the normal number of 46 in man.

In recent years the diploid chromosome number of 46 has been recorded in a large number of instances. In addition to the 60 cases cited in a previous publication[2] we have recorded a diploid number of 46 in bone marrow preparations from a further 40 European subjects. Kodani, on the other hand, has recently published results of counts made on testicular material from 36 Japanese and 8 American white males[3,4]. He claims that in 16 Japanese and 1 American there was a diploid number of 48; in 2 Japanese a diploid number of 47, and that in the remaining 13 Japanese and 7 whites the number was 46. He suggests that 46 is the basic diploid chromosome number for man, but in some instances there are additional "supernumerary chromosomes". The occurrence of this type of supernumerary chromosome, however, has not been reported previously among the vertebrates and awaits confirmation by other workers.

Our patient, an apparent male aged twenty-four, was presented as a case of gonadal dysgenesis with gynæcomastia and small testes associated with poor facial hair-growth and a high-pitched voice. Biopsy examination of testicular tissue showed the seminiferous tubules to be extremely hyalinized and atrophic, and also an apparent increase in the number of interstitial cells. Chromosome studies were attempted on part of this material, but no spermatogonial mitotic or meiotic divisions were seen. Smears made from both the buccal mucosa and the blood were examined by Dr. B. Lennox of the Department of Pathology, Western Infirmary, Glasgow, and found to demonstrate typical female morphology with regard to their nuclear sex chromatin.

Material obtained by sternal marrow puncture was used for investigating the somatic chromosomes. The technique used for culturing the material in the presence of colchicine and for making squash preparations has already been described[2].

The chromosomes were counted in 44 cells in metaphase and the results are shown in Table 1.

Table 1

Chromosome No.	45	46	47	48	49
No. of cells	2	7	29	5	1

The majority of the cells contained 47 chromosomes, and in all those cells where the chromosomes were well fixed and spread, the count was undoubtedly 47 (Fig. 1). The apparent variation is in all probability due to technical errors. Fragments of cells containing chromosomes may become lost during the squashing process so that counts lower than the diploid number are obtained; and occasionally chromosomes split at the centromere and individual chromatids may be counted as chromosomes, giving a count higher than the diploid number[2].

A study of the chromosome morphology in 8 cells of a suitably high standard showed that each of these had a normal male complement with the *Y* chromosome present, but that there was also an extra chromosome having a sub-median centromere occurring in the medium size range. In the normal male there are 15 chromosomes in this range, and in the female 16, all having sub-terminal or sub-median centromeres[2]. Owing to the slight variations in their size and morphology these chromosomes have proved difficult to pair, and it is in this category that the *X* chromosome is to be found.

There are strong grounds, both observational and genetic[5,6], for believing that human beings with chromatin-positive nuclei are genetic females having two *X* chromosomes. The fact that this patient is chromatin-positive and has an additional chromosome within the same size range as the *X*, as well as an apparently normal *Y*, makes it seem likely that he has the genetic constitution *XXY*. The possibility cannot be excluded, however, that the additional chromosome is an autosome carrying feminizing genes.

The presence of the extra chromosome might have been due to one or other of the parents having 47 chromosomes, and, therefore, chromosome studies were made on marrow specimens from both parents. Both were found to have a diploid number of 46 (Table 2), and analysis of cells of suitable quality showed the morphology of the chromosomes to be normal.

The occurrence of the extra chromosome therefore may be due to non-disjunction at either mitosis or meiosis during gametogenesis in one or other parent. Alternatively, it may be due to non-disjunction

Fig. 1. Metaphase plate showing 47 chromosomes

Table 2

Father	Chromosome No.	44	45	46	47	48	49
32 cells counted	No. of cells	2	3	26	—	—	1
Mother	**Chromosome No.**	**44**	**45**	**46**	**47**	**48**	**49**
39 cells counted	No. of cells	1	3	33	2	—	—

occurring during the patient's very early embryological development, in which case there is a possibility that the patient may be a mosaic. Unfortunately, it is not possible with the techniques at present available to examine the chromosomes of tissues arising from different germ layers of the embryo.

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A further report of this and other cases will follow.

[1] Barr, M. L., and Bertram, E. G., *Nature*, **163**, 676 (1949).
[2] Ford, C. E., Jacobs, P. A., and Lajtha, L. G., *Nature*, **181**, 1565 (1958)
[3] Kodani, M., *Proc. U.S. Nat. Acad. Sci.*, **43**, 285 (1957).
[4] Kodani, M., *Amer. J. Human Genetics*, **10**, 125 (1958).
[5] Grumbach, M. M., and Barr, M. L., "Recent Progress in Hormone Research", **14**, 255 (1958).
[6] Polani, P. E., Bishop, P. M. F., Lennox, B., Ferguson-Smith, M. A., Stewart, J. S. S., and Prader, A., *Nature*, **182**, 1092 (1958).

DISTRIBUTION OF NEWLY SYNTHESIZED DEOXYRIBONUCLEIC ACID IN DIVIDING CHROMOSOMES

By PHILIP S. WOODS and MARIE U. SCHAIRER
Biology Department, Brookhaven National Laboratory, Upton, Long Island, New York

FROM autoradiographic studies with tritiated thymidine, newly synthesized deoxyribonucleic acid was shown to be equally distributed between both daughter chromosomes at anaphase by Taylor, Woods and Hughes[1] (Fig. 1*A* and *B*). When the labelled chromosomes were allowed to duplicate a second time in a medium containing no radioactive tracer, the isotope appeared in only one daughter chromosome of each sister pair (Fig. 1*C*). These findings differed markedly from the earlier conclusions of Plaut and Mazia[2], whose interpretations were based on the analysis of deoxyribonucleic acid labelled with carbon-14 in whole sets of daughter chromosomes in anaphase and telophase. Since they observed significant differences in the amount of label between the two daughter nuclei, they concluded that individual chromosomes were also labelled unequally. Recently, La Cour and Pelc[3] reported that unequal labelling could occur as a result of treatment with colchicine. Since in the experiment of Taylor *et al.* colchicine was present during the second synthesis and absent during the first, they suggested that colchicine may produce the unequal label.

In order to clarify the situation, the experiments of La Cour and Pelc were repeated. Since critical quantitative data were lacking in their experiments, information in the form of grain counts over a number of daughter chromosome pairs were collected and the chi-square test applied to determine any significant deviations between daughter halves.

The experiment went as follows: two seedlings of *Vicia faba* were grown in separate nutrient solutions containing tritiated thymidine (1·4 μgm./ml., 2·0 μc./ml.). One solution contained in addition 0·03 per cent colchicine. After 8 hr. the seedling which was treated with thymidine labelled with tritium alone was removed and placed in a non-labelled solution containing 0·03 per cent colchicine. After 6 additional hours two roots of this seedling were fixed, stained by the Feulgen squash procedure, and covered with autoradiographic stripping film. The other

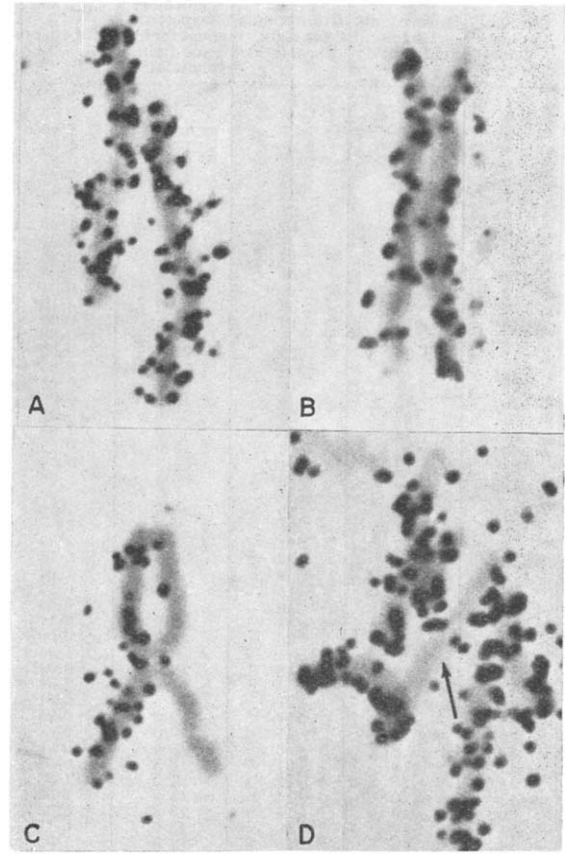

Fig. 1. *A*, autoradiograph of chromosome number 9 of second root of Table 2, colchicine present during synthesis; *B*, autoradiograph of chromosome number 4 of second root of Table 1, colchicine absent during synthesis; *C*, autoradiograph of chromosome from root treated with tritiated thymidine for 8 hr. followed by colchicine solution without tritiated thymidine for 30 hr.; *D*, autoradiograph of chromosomes from lightly squashed root from same seedling as that used in *B*