referring patients to the infirmary. We are greatly indebted to Professors Robert Cruickshank and D. M. Dunlop for valuable criticism and help in the preparation of this paper.

REFERENCES

Brumfitt, W., Willoughby, M. L. N., and Bromley, L. I. (1957). *Lancet*, **2**, 1306.
Buchanan, J., Buchanan, W. W., Melrose, A. G., McGuinness, J. B., and Price, A. U. (1958). Ibid., **2**, 719.
Edwards, G., and Fear, E. C. (1958). *Brit. med. J.*, **2**, 1010.
Elmes, P. C., Fletcher, C. M., and Dutton, A. A. C. (1957). Ibid., **2**, 1272
English, A. R., McBride, T. J., van Halsema, G., and Carlozzi, M. (1956). *Antibiot. and Chemother.*, **6**, 511.
Fairbrother, R. W., and Southall, J. E. (1957). *Lancet*, **2**, 974.
Garrod, L. P. (1957). *Brit. med. J.*, **2**, 57.
Gould, J. C., and Bowie, J. H. (1952). *Edinb. med. J.*, **59**, 178.
Helm, W. H., May, J. R., and Livingstone, J. L. (1956). *Lancet*, **1**, 775.
Hers, J. F. P., and Mulder, J. (1953). *J. Path. Bact.*, **66**, 103.
Hewitt, D. (1956). *Brit. J. prev. soc. Med.*, **10**, 45.
Hill, A. Bradford (1955). *Principles of Medical Statistics*, 6th ed. Lancet Ltd., London.
Jones, W. F., jun., and Finland, M. (1957a). *New Engl. J. Med.*, **257**, 481.
—— —— (1957b). Ibid., **257**, 536.
*Lancet*, 1954, **1**, 450.
—— 1955, **2**, 601.
Mackie, T. J., and McCartney, J. E. (1953). *Handbook of Practical Bacteriology*, 9th ed. Livingstone, Edinburgh.
May, J. R. (1953). *Lancet*, **2**, 534
—— and Oswald, N. C. (1956). Ibid., **2**, 814.
Moyes, E. N., and Kershaw, R. A. (1957). Ibid., **2**, 1187.
—— and Kalinowski, S. Z. (1959). *Tubercle (Lond.)*, **40**, 112.
Mulder, J. (1956). *Proc. roy. Soc. Med.*, **49**, 773.
—— Goslings, W. R. O., van der Plas, M. C., and Cardozo, P. L. (1952). *Acta med. scand.*, **143**, 32.
Ogilvie, A. G., and Newell, D. J. (1957). *Chronic Bronchitis in Newcastle-upon-Tyne*. Livingstone, Edinburgh.
Oswald, N. C. (1958). *Recent Trends in Chronic Bronchitis*. Lloyd-Luke Ltd., London.
Rawlins, G. A. (1953). *Lancet*, **2**, 538.
—— (1955). *J. med. Lab. Technol.*, **13**, 133.
Reid, D. D. (1956). *Proc. roy. Soc. Med.*, **49**, 767.
Reid, L. M. (1954). *Lancet*, **1**, 275.
—— (1955). *Thorax*, **10**, 199.
—— (1956). *Proc. roy. Soc. Med.*, **49**, 771.
Rountree, P. M., and Rheuben, J. (1956). *Med. J. Aust.*, **1**, 399.
Sinclair, J. D. (1955). *Brit. J. Tuberc.*, **49**, 157.
Stewart, F. S. (1959). *Bigger's Handbook of Bacteriology*, 7th ed. Baillière, Tindall and Cox, London.
Stocks, P. (1959). *Brit. med. J.*, **1**, 74.
Stuart-Harris, C. H. (1959). Ibid., **1**, 1606.
—— and Hanley, T. (1957). *Chronic Bronchitis, Emphysema, and Cor Pulmonale*. Wright, Bristol.
Wallace, A. T., and Duguid, J. P. (1952). *Edinb. med. J.*, **59**, 200.
Welch, H. (1957). *Antibiot. Med.*, **4**, 20.
Williams, E. (1954). *Lancet*, **2**, 999.
Wolf, S., and Pinsky, R. H. (1954). *J. Amer. med. Ass.*, **155**, 339.

A grant to Tanganyika of £2,220 from Colonial Development and Welfare Funds for laboratory research into the sterilization of tsetse flies by irradiation has been approved by the Secretary of State for the Colonies. Experiments elsewhere with irradiation at the pupal stage of another species of fly demonstrated that activated cobalt so affected the reproductive capacity of both males and females that the latter could not reproduce, and, furthermore, normal females when mated with sterilized males produced only sterile eggs and did not usually mate again. Accordingly, if a sufficient number of pupae could be released over a sufficient period of time, the fly population would die out. These results suggest that the same method might be used against these flies, but it is first necessary to discover the stage of the insect's development at which it is most susceptible to sterilization. This entails working in a laboratory with insect pupae of known age, as near as possible to a good supply of tsetse flies, and the Colonial Pesticides Research Unit at Arusha will undertake this research.

# THE CHROMOSOMES IN A CASE OF PURE GONADAL DYSGENESIS

BY

**D. G. HARNDEN,* Ph.D.**

*M.R.C. Radiobiological Research Unit, Harwell, Didcot, Berks*

AND

**J. S. S. STEWART, M.B., Ch.B.**

*Lecturer in Surgery, University of St. Andrews, Queen's College, Dundee*

The normal chromosome number in man is now firmly established to be 46 (see, for example, Tjio and Levan, 1956). There are 44 autosomes, and 2 sex chromosomes which are designated XX in the female and XY in the male. In human intersexes the chromosome constitution may be abnormal, as in chromatin-positive Klinefelter's syndrome, where there are 44 autosomes and XXY sex chromosomes (Jacobs and Strong, 1959), and in chromatin-negative Turner's syndrome, where there are 44 autosomes and only one X chromosome (Ford *et al.*, 1959a). It may, however, be indistinguishable from normal. In hereditary male pseudohermaphroditism (testicular feminization) there is an apparently normal male complement of chromosomes (Jacobs *et al.*, 1959). We report here the chromosomal constitution of a patient with the syndrome which is usually described as pure gonadal dysgenesis (see discussion on nomenclature below).

## Clinical Appearance and Cytological Examinations

The patient is a tall eunuchoid female who presented, aged 19, with primary amenorrhoea. Before treatment, scanty axillary and pubic hair was present, breast development was absent, and daily urinary excretion of gonadotrophins was raised. The external genitalia were of the normal female type, the clitoris being normal in size. There was a normal-sized uterus, but no gonads could be palpated on vaginal examination. Oestrogen-replacement therapy resulted in breast development, and vaginal bleeding followed oestrogen withdrawal. An oral mucosal smear was chromatin-negative, and the leucocytes showed no drumsticks. Full clinical details are given by Stewart (1959).

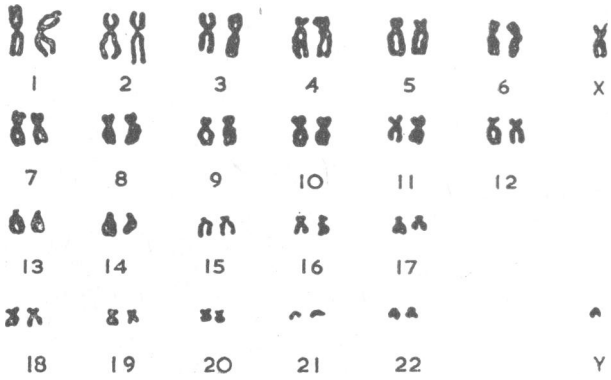
A skin biopsy specimen was taken from the patient's thigh, using a local analgesic, and divided into two parts. One portion was fixed immediately for nuclear sexing and the other placed in sterile Glaxo medium 199 and transferred to Harwell, where a tissue culture was established. After two weeks cells of the actively dividing culture were treated with colchicine, and orcein-stained chromosome preparations were made. The technique is described in detail by Harnden (1959).

| Tissue | Chromosome Counts (No. of Cells) | | | | | | Sex Chromosomes |
|---|---|---|---|---|---|---|---|
| | <45 | 45 | 46 | 47 | 48 | >48 | |
| Skin biopsy 1 .. | 1 | — | 52 | 1 | — | — | XY |
| ,, ,, 2 .. | 1 | 1 | 42 | 1 | — | — | XY |

The cells of the first portion were found to be chromatin-negative, no sex chromatin being present in 100 suitable nuclei. Cultured cells of the second portion contained 46 chromosomes (see Table). Twelve cells were analysed

*Now at Medical Research Council Group, Department of Radiotherapy, Western General Hospital, Edinburgh, 4.

in detail and found to be indistinguishable from the normal male morphology of 46 chromosomes, including one X and one Y chromosome (see Fig.). These findings were confirmed in a second skin biopsy, taken later from the patient's opposite thigh.




Ideogram prepared from a photograph of a skin culture cell showing 46 chromosomes, including one X and one Y chromosome. (×870)

## Discussion

The chromosome number of this patient is 46, the sex chromosomes are those of a normal male, and no structural change is detectable in the chromosomes. We would not exclude the possibility that there may be a structural change, such as a translocation or deletion, too small to be detected by the present technique. It has been suggested that certain human intersexes may be chromosomal mosaics—that is, they are composed of two or more types of cell of differing chromosomal constitution. Ford *et al.* (1959b) report a case of Klinefelter's syndrome which is interpreted as a probable XX/XXY mosaic. We have no evidence that the present patient is a mosaic, but the possibility of mosaicism in some of the tissues not examined cannot be ruled out.

This patient is obviously not a case of classical Turner's syndrome because of the absence of the usual stigmata, and the only condition of importance which must be considered in the differential diagnosis is testicular feminization. Jacobs *et al.* (1959) report several cases of this syndrome in which they have found the sex chromosomes to be XY. Such a diagnosis in the present patient is excluded by the absence of breast development and the presence of axillary and pubic hair prior to treatment, the presence of a normal-sized uterus, and the production of vaginal bleeding following oestrogen withdrawal. There is, however, a strong resemblance between this case and those described by Swyer (1955) and by Hauser *et al.* (1956). Similar chromatin-positive cases have been reported. Laparotomies carried out on three of those (Hauser *et al.*, 1956; Greenblatt *et al.*, 1956; Hoffenberg and Jackson, 1957) and on one chromatin-negative case (Hauser *et al.*, 1956) have confirmed that gonads were absent. Though one cannot be certain without laparotomy that gonads are absent in the present case, it seems highly probable that this and Swyer's cases belong to the same syndrome as do those in which the diagnosis of gonadal dysgenesis was confirmed by laparotomy.

Swyer's cases were originally described as examples of an unusual type of male pseudohermaphroditism, but Hoffenberg and Jackson (1957) proposed the name pure gonadal dysgenesis. This was accepted by Swyer (1957). The question of nomenclature is not made easier by the finding of a normal male chromosomal constitution in our case. If one accepts the presence of a Y chromosome as the criterion of maleness, Swyer would appear to be quite correct in using the term "male pseudohermaphrodite," but this obscures the close similarity between the chromatin-positive and the chromatin-negative cases. Hoffenberg and Jackson are equally correct in describing their case as one of pure gonadal dysgenesis. The chromosome findings, however, preclude the acceptance of the present case as a variant of Turner's syndrome.

A study of this and similar cases may provide much information of value about the way in which genetic and non-genetic factors interact to produce the phenotypic sex of the individual. The recent work on Klinefelter's syndrome suggests that the Y chromosome of man (unlike that of *Drosophila*) has a strongly masculinizing effect (Ford *et al.*, 1959b). In the present case, however, a Y chromosome is present but there is an extensive development of female characteristics. Jost (1953) has shown that rabbits, whether genetically male or female, develop female internal and external genitalia if gonadectomy is performed during early intrauterine development. The present case and other similar cases, whether chromatin-positive or chromatin-negative, can be understood as a group if there has been a failure of gonadal development at a very early stage of embryonic life. In the absence of gonads a female phenotype develops regardless of the chromosomal sex of the individual. The failure of masculinization in the chromatin-negative cases could be attributed to the absence of a male morphogenetic substance which would normally originate in the testes (Grumbach and Barr, 1958). Environmental causes of this developmental error in man must be considered, but a genetic aetiology seems more likely in some instances at least, since familial cases have been reported (Elliott *et al.*, 1959).

Thus the mere possession of a Y chromosome is not in itself sufficient to ensure the development of a male phenotype. This conclusion is supported by the study of Jacobs *et al.* (1959) on cases of testicular feminization and by the case reported by Nilsson *et al.* (1959) of an apparently female child with XY sex chromosomes. In all these cases sex *determination* was apparently normal for a male. It would seem, however, that other factors which may or may not be genetically determined are necessary to ensure that sex *differentiation* is also normal.

## Summary

Chromosome analysis has been carried out in a tall eunuchoid female diagnosed clinically as a case of pure gonadal dysgenesis. The chromosome number is 46 and the sex chromosomal constitution is XY. The significance of this finding is discussed.

We are grateful to Dr. E. G. Oastler for permission to study this patient; to Dr. Bernard Lennox for his report on the nuclear sex; to Dr. C. E. Ford for his assistance in the preparation of the manuscript; and to Miss C. M. Scammell for her skilful technical assistance.

### REFERENCES

Elliott, G A., Sandler, A., and Rabinowitz, D. (1959). *J. clin. Endocr.*, **19**, 995.
Ford, C. E., Jones, K. W., Polani, P. E., de Almeida, J. C., and Briggs, J. H. (1959a). *Lancet*, **1**, 711.
—— Polani, P. E., Briggs, J. H., and Bishop, P. M. F. (1959b). *Nature (Lond.)*, **183**, 1030.
Greenblatt, R. B., Carmona, N., and Higdon, L. (1956). *J. clin. Endocr.*, **16**, 235.

Grumbach, M. M., and Barr, M. L. (1958). *Recent Progr. Hormone Res.*, **14**, 286.
Harnden, D. G. (1959). *Brit. J. exp. Path.* In press.
Hauser, A., Keller, M., and Wenner, R. (1956). *Schweiz. med. Wschr.*, **86**, 299.
Hoffenberg, R., and Jackson, W. P. U. (1957). *Brit. med. J.*, **1**, 1281.
Jacobs, P. A., and Strong, J. A. (1959). *Nature (Lond.)*, **183**, 302.
—— Baikie, A. G., Court Brown, W. M., Forrest, H., Roy, J. R., Stewart, J. S. S., and Lennox, B. (1959). *Lancet*, **2**, 591.
Jost, A. (1953). *Recent Progr. Hormone Res.*, **8**, 379.
Nilsson, I. M., Bergman, S., Reitalu, J., and Waldenström, J. (1959). *Lancet*, **2**, 264.
Stewart, J. S. S. (1959). *Acta endocr. (Kbh.)*. In press.
Swyer, G. I. M. (1955). *Brit. med. J.*, **2**, 709.
—— (1957). Ibid., **1**, 1421.
Tjio, J. H., and Levan, A. (1956). *Hereditas (Lund)*, **42**, 1.

# THE CHROMOSOMES OF A TRUE HERMAPHRODITE

BY

**D. G. HARNDEN,* Ph.D.**

*M.R.C. Radiobiological Research Unit, Harwell, Didcot, Berks*

AND

**C. N. ARMSTRONG, M.D., F.R.C.P., D.P.H.**

*Clinical Sub-Dean, Royal Victoria Infirmary, Newcastle upon Tyne*

The recent work on the chromosomes of human intersexual conditions — for example, Klinefelter's syndrome (Jacobs and Strong, 1959) and Turner's syndrome (Ford *et al.*, 1959)—has led to a re-examination of known cases of other types of intersexuality. The case considered here is of a true hermaphrodite, and, though we can obtain no evidence of chromosomal imbalance, the findings are nevertheless of great interest from the developmental point of view.

The full clinical and pathological details of this case have already been published (Armstrong, 1955; Armstrong *et al.*, 1957), but a few of the most important features are worth repeating here.

The patient, aged 70, has lived as a female, but the general appearance is rather masculine. The hair distribution is male, there is no breast development, and the external genitalia are partially masculinized. The right gonad was found to be predominantly a testis but showed at least one atretic ovarian follicle. The left gonad is an ovary. The skin, the oral mucosa, and the internal genitalia (ovary, ovotestis, uterus, and vas deferens) are chromatin-positive, though in the latter "the typical chromatin mass is smaller and less frequent than usual." Two to three neutrophils in every 500 counted were found to have a typical female "drumstick."

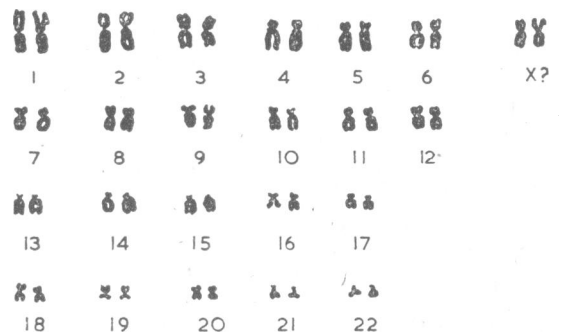
## Results

Chromosome examinations were carried out by the method described by Harnden (1959). Two skin-biopsy specimens, one from the left arm and one from the right arm, were sent from Newcastle to Harwell, and set up in culture about 24 hours after biopsy. Two separate cultures were established from each biopsy, so that there were four cultures in all. A slight yeast infection which appeared in all the cultures was eliminated by adding 100 units of nystatin per ml. of culture medium. The cultures varied somewhat in their rates of growth, but all were slow to start proliferating, and a period of about four weeks elapsed before cytological preparations could be made. The results of the examination of these preparations are shown in the Table.

*Results of Chromosome Examinations*

| Tissue | | Chromosome Counts (No. of Cells) | | | | | | Sex Chromosomes |
|---|---|---|---|---|---|---|---|---|
| | | <45 | 45 | 46 | 47 | 48 | >48 | |
| Left skin Biopsy | L1 | — | — | 32 | — | — | — | XX |
| | L2 | 2 | 1 | 30 | 1 | — | 1 | XX |
| Right skin Biopsy | R1 | 1 | 1 | 31 | — | 1 | — | XX |
| | R2 | 1 | 7 | 55 | — | 1 | — | XX |

All four cultures contained predominantly cells with 46 chromosomes. The sex chromosomes are interpreted in all four cultures to be XX (see Fig.). The few counts other than 46 can be accounted for as technical errors. In three of the cultures the chromosomes were, so far as one can say from this sort of examination, normal female. In the fourth (R2), however, there occurred in about 7% of the cells an abnormally long chromosome. Careful analysis of several of these cells showed that this could be explained as a reciprocal translocation between members of chromosome pairs 3 and 4 (in order of size). All the cells of this culture were in other respects normal female. Had this abnormality been present in the original tissue it is felt that it would also have been observed in the other culture from the same biopsy. This was not found, though several hundred cells were carefully examined. It is considered, therefore, that this aberration had arisen in culture, though the possibility of its presence in the patient




Ideogram prepared from a photograph of a skin culture cell showing 46 chromosomes. The two X chromosomes cannot be identified with certainty, but only as two of a group of six chromosomes. (×870.)

cannot altogether be ruled out. It can be concluded that the cells of these two skin biopsies from widely separated sites contained chromosomes characteristic of the normal female. In corroboration of this Dr. J. E. Gray, of the Department of Anatomy, King's College, Newcastle, has examined the skin adjacent to the biopsy sites and found both to be chromatin-positive.

Miss P. A. Jacobs, of the Medical Research Council Group, Western General Hospital, Edinburgh, has examined the chromosomes of cells from a bone-marrow culture and agrees with the finding that they are of the normal female pattern.

## Discussion

From the aspect of technique two points arise from these results. It is of interest that successful cultures can be established with regularity from the skin of a

*Now at Medical Research Council Group, Department of Radiotherapy, Western General Hospital, Edinburgh, 4.