examined. GTG banding⁴ showed a deletion of the long arm of the X chromosome at band q23. Six hours before the harvest, 100 mg/l of 5-bromodeoxyuridine was added to the culture. RBA banding⁵ showed that in 50 cells analysed the aberrant X chromosome was inactivated (figure). The chromosomes of the patient's parents could not be examined.

Discussion

A critical role of the X chromosome in primary determination of sex and fertility has recently been revealed. Studies of deletions of the X chromosome showed that in most cases of Xq- aberrations the breaks occur within the critical region Xq13-q27,⁶ which is responsible for normal ovarian and female sex development. Female patients with deletions of the long arm of the X chromosome have two different phenotypes: one with characteristics of Turner's syndrome, and the other with a picture of pure gonadal dysgenesis.^{1-3 7} The critical point for these two phenotypes lies within the band q22 or q21.⁸⁻¹⁰ Female patients with deletions distal to the critical band have amenorrhoea without any stigmata of Turner's syndrome.^{8 9 11}

In our patient, as in most cases of deleted Xq, the proximal part of the long arm of the X chromosome (with its inactivation centre)^{12 13} was left intact and the deleted X chromosome was inactivated.

In our patient, the breakpoint occurred at band q23 within the critical region of the X chromosome, resulting in infertility. The deletion of genetic material of one X chromosome is a frequent cause of hypergonadotrophic amenorrhoea and ovarian dysfunction. In spite of normal laparoscopy findings the high level of serum gonadotrophins in our patient and progressive oligomenorrhoea showed altered ovarian function. Serum oestradiol was high owing to the excessive pituitary stimulation.

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Cd banding studies in a homologous Robertsonian 13;13 translocation

SUMMARY A phenotypically normal female with a history of two miscarriages was found to have the karyotype 45,XX,t(13p:13p). C banding showed the translocation to have two regions of centromeric constitutive heterochromatin, silver staining showed an active NOR in 60% of the cells screened, and Cd banding studies showed a single Cd band with absence of the Cd band at the suppressed centromere.

Eiberg¹ produced a method which clearly showed in metaphase two dot-like bodies situated in the area where the centromere should be. These centromeric dots are considered to be the kinetochores and appear to be equal in size.^{1 2}

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In cases where dicentric chromosomes have occurred owing to translocation or isochromosome formation,^{3,4} C banding has shown two regions of darkly staining centromeric heterochromatin. It has been suggested that the stability of these dicentric chromosomes may result from the closeness of their centromeres and the suppression at times of one or the other of them. Centromeric suppression is thought to be expressed by chromatid separation at one of the centromeric heterochromatin regions (fig 1). When Cd banding has been applied to

dicentrics with chromatid separation at the centromere, the Cd bands were absent.^{3,5,6}

We were interested in a detailed Cd banding study of those metaphases which not only showed chromatid separation at the centromere but, more importantly, those which did not (fig 1). Lau and Hsu⁷ have suggested that there is a possibility that centromeres of a dicentric chromosome, when close together, may function as one unit. However, if the centromeres were too close together, their presence might be represented by one Cd positive band and not two.

In our case, the dicentric translocation product was possibly formed by fusion at both nucleolus organiser regions, or else a fusion between the nucleolus organiser region of one chromosome and the short arm of the other. It is noteworthy that one

FIG 1 C banded examples of the dicentric chromosome showing polymorphisms of the centromeric constitutive heterochromatin.

FIG 2 Combined Ag/G banded chromosomes of 13-15 group from proband and her parents showing NORs and example of one maternal pair of chromosomes 13 stained by Giemsa to illustrate elongated stalk (NOR).

of the maternal chromosomes 13 possesses a large NOR (fig 2) and that, in the proband's translocation, a large NOR does separate the two centromeres adequately for study (fig 2).

Materials and methods

Cd banding was performed by the original procedure of Eiberg,¹ except that air dried spread slides were seen to band best not at 7 days old, but at 9 days old, when stored at room temperature.

Discussion

During the study, slides were examined between 10 and 45 minutes after incubation in Earle's salts, pH 8.8, at 85°C. Metaphases which began to denature first were those which were less spiralsed, producing clearly defined Cd bands after only 10 minutes' denaturation. Cells in early metaphase which showed no chromatid separation did not begin to show definite Cd bands until 25 minutes after incubation had taken place. It was observed that C band regions of the C group 17, 18, 2, 3, 4, 5, 19, and 20 faded first, to be followed in sequence by 16qh, 1qh, and 9qh. The D group chromosomes were seemingly inconsistent, some not denaturing at the C band region until after the 1qh. The loss of staining in the suppressed centromere began to take place at the same time as the 16qh when it could be seen to be 'melting' away to the extremities of each side of the chromatid body (fig 3), finally to disappear.

As far as the technique allows, we are of the same opinion as Eiberg¹ that the Cd bands are equal in size. This was of particular assistance because in 40% of the cells examined the NORs in the dicentric chromosome stained positive to give the impression of having double Cd bands (fig 4) instead of single, which appeared in the other 60% of metaphases (fig 5). During the study, 500 cells were analysed and in none were double Cd bands observed, including those cells which did not show chromatid separation at the centromere. It was not possible to detect whether or not there was alternative constriction of the two centromeres as the translocation was homologous.

FIG 3 Partial metaphase showing 'melting' away effect of the heterochromatin at the suppressed centromere.

FIG 4 Cd banded partial karyotype showing active NORs and Cd bands.

FIG 5 Cd banded partial karyotype showing single Cd bands.

The interesting observations of dual centromeric activity or suppression of one centromere in dicentric Robertsonian translocations, and whether or not this can be demonstrated by the presence of double or single Cd bands, is no doubt still open for further research, but in this study we have only been able to demonstrate single Cd positive bands, indicating suppression at one of the centromeres in all cells analysed.

The likelihood of persons with a homologous translocation, such as ours, producing a normal fetus is extremely remote. As there was no evidence of mosaicism, it would suggest that the chromosomal rearrangement occurred very early in the development of the zygote, immediately following association at the pronuclei at fertilisation.^{8 9}

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Addendum

Since completion of this paper, we have reviewed three cases of dicentric bisatellited marker chro-

mosomes resulting from Robertsonian translocations and all show an absence of the Cd band at the suppressed centromere.

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