A RAPID DIRECT METHOD OF OBTAINING METAPHASE AND PROMETAPHASE CHROMOSOMES FROM CELLS OF CHORIONIC BIOPSY MATERIAL AND HUMAN EMBRYOS IN THE FIRST 3 MONTHS OF PREGNANCY

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Cytogenetic analysis of chorionic biopsy samples in the early stages of pregnancy is widely used for the prenatal diagnosis of chromosomal diseases [1, 3]. The "shake-imprint" method [2], developed in the writers' laboratory previously, enables the fetal karyotype to be analyzed on the day the sample is obtained. However, just as in other modifications of the direct method [6, 7], most of the chromosome spreads obtained by this method are of comparatively poor quality and are not suitable enough for differential staining. In order to obtain better results the present practice is to incubate samples of chorion for 24-36 h in a special medium (Chang's) of complex composition, with the addition of expensive mitogens [7].

We describe below a simple and rapid method of preparing chromosome spreads from cells of the chorionic villi, which is capable of providing high-quality preparations of metaphase and prometaphase chromosomes and enables karyotyping to be carried out as early as 1.5 h after chorionic biopsy.

EXPERIMENTAL METHOD

Experiments were carried out on 55 chorionic biopsy samples (17 diagnostic, 38 therapeutic) at the 8th-11th weeks of pregnancy and on 15 chorionic samples from artificial abortions performed at the same times. Transcervical chorionic biopsy was undertaken by means of a flexible plastic intravenous injection catheter (Borum) with the metal obturator of a "Trophocan Portex" catheter, under the control of an ultrasonic apparatus (Toshiba). Samples (villi or small pieces of tissues) were taken directly from the biopsy material, which had previously been washed thoroughly with Ranks' solution at 37°C to remove blood, under the control of an MBS-2 binocular loupe; the samples were at once transferred into 5 ml of hypotonic 0.9% trisodium citrate solution with the addition of 2 drops of 0.01% colchicine. After incubation for 30 min at 37°C an equal volume of fixing mixture (3:1) of methanol and glacial acetic acid was added drop by drop to the hypotonic solution. The mixture was exchanged 30 min later for standard fixative at −10°C, which was changed twice. The sample was allowed to stand at −10°C for 30-40 min. An equal volume of distilled water was added to the samples 5-10 min before preparation of the specimens. The specimens were removed by means of fine forceps on to filter paper and then transferred to a clean' defatted slide in 3-4 drops of 60% acetic acid. Maceration of the tissue and release of the cells were observed under the dark field of the MBS-2 microscope. Usually after 3-4 min, on rocking the slide, a uniform distribution of the cell suspension could be obtained over its surface. The excess of suspension was poured on to a clean slide, heated to 45°C. Remains of the test specimen were transferred to the same slide. This procedure was repeated several times. After the suspension had dried a few drops of fixative were applied to the slide, which was dried and stained with 5% Giemsa solution and examined under the microscope. For differential staining, freshly made preparations were placed in 0.15 M Na_2HPO_4 (1 h, 45°C), dried, and kept on a heating stand at 45°C for 24 h.

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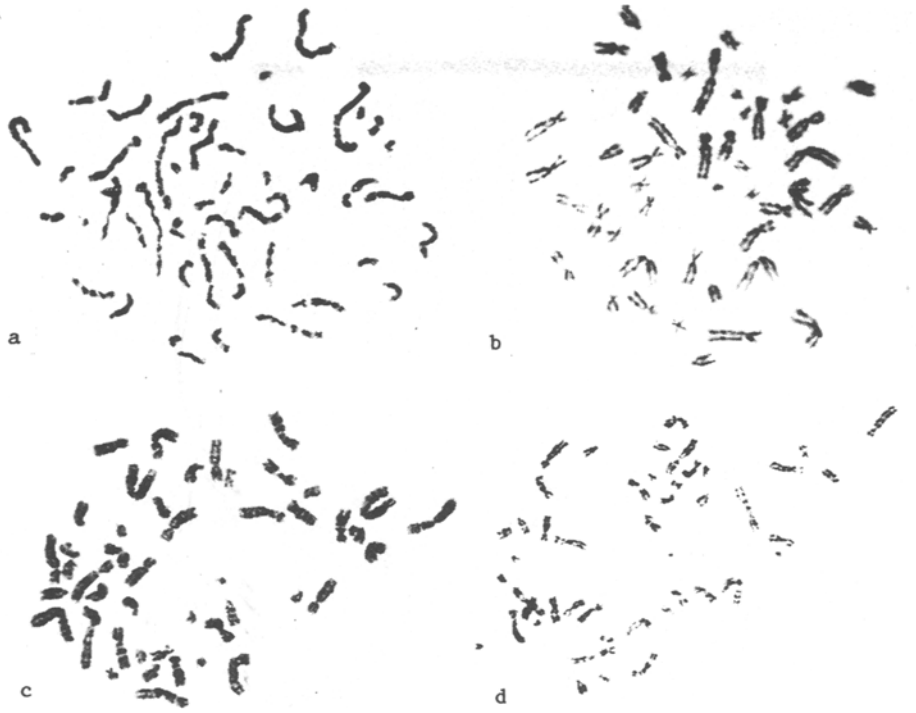

Fig. 1. Prometaphase (a) and metaphase (b, c, d) plates from cells of diagnostic chorionic biopsy material at 8-10 weeks of development. Rapid method of chromosomal analysis. Giemsa staining after treatment with phosphate buffer (b), with phosphate buffer and 0.01% trypsin (a, c), and phosphate buffer and a mixture of 0.25% trypsin and Giemsa stain (d).

Two modifications were used to detect G-staining: 1) with 0.5% trypsin (Difco) in phosphate buffer, pH 6.8 (exposure was determined empirically and was about 10-40 sec); ethanol 70 and 96%; Giemsa staining; 2) a mixture (1:1) of the Giemsa mother solution and 0.25% trypsin at 37°C was applied to the preparation (duration of staining 7-15 min).

To detect s banding the preparations were stained with quinacrine mustard by the standard method [4]. To detect C staining the preparations were treated with a solution of barium hydroxide [5] and stained by Giemsa's method.

Chromosomal analysis was carried out with the NU microscope (East Germany) and Opton photomicroscope (West Germany). The number of metaphase plates suitable for analysis was counted on each slide, the number of chromosomes was recorded, and their morphology was analyzed in high-quality plates.

EXPERIMENTAL RESULTS

Karyotyping was carried out on 67 of the 70 specimens of chorion used. In 3 cases (2 stillbirths, 1 with degenerating villi of an 11-week fetus) mitoses were absent. The number of metaphase plates suitable for analysis varied from 7 to 20 per slide (average 12-15) and it was appreciably greater (average about 20) from biopsy material from chorionic villi than from abortive material (average 9-13). About 30% of all metaphases analyzed had greatly elongated chromosomes and were at the prometaphase stage (Fig. 1a), whereas the rest were at the early and middle metaphase stage (Fig. 1b-d). The number of plates with artefactual loss of chromosomes varied from 2 to 5% and was much smaller than after incubation for 2 h in medium with colchicine [8]. Another important advantage of the suggested method is the high quality of the metaphase plates: a weak degree of spiralization and good scattering of the chromosomes, and unseparated or only slightly separated chromatids. Such chromosomes are suitable for differential staining. Modifications of G staining which we used are highly reproducible. Incubation of fresh preparations in Na_2HPO_4 solution itself leads to the appearance of bright spots on the chromosomes, whose arrangement is quite comparable with the ordinary pattern of G staining and allows demonstration of the Y chromosome (Fig. 1b). The typical differential banding can be obtained by treating the chromosomes with trypsin solution (Fig. 1c), with a mixture of

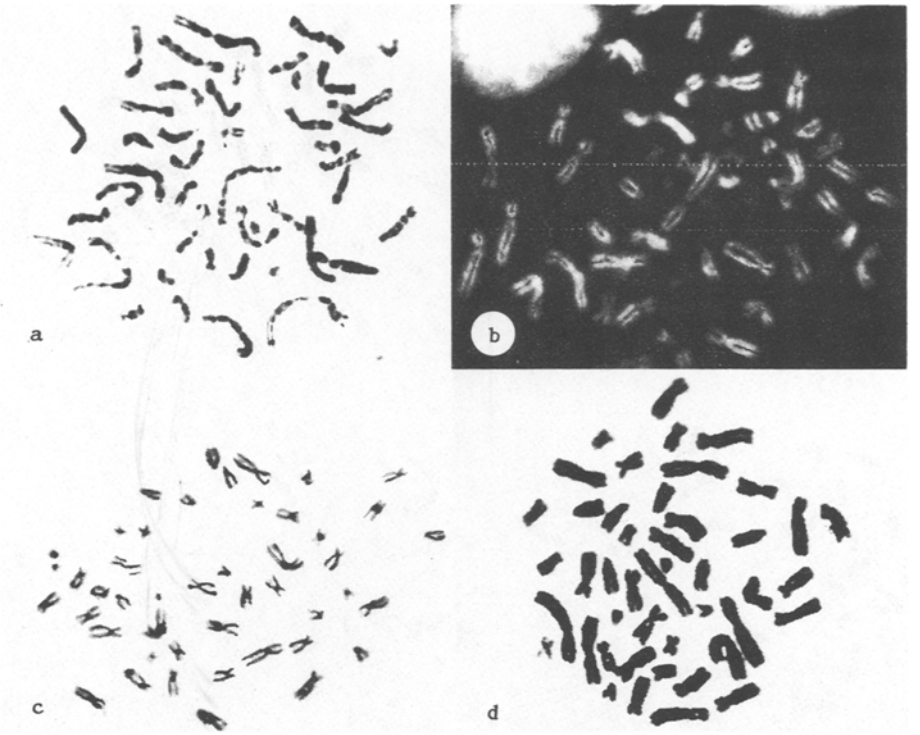

Fig. 2. Metaphase plates from cells of abortive chorion at 10-11 weeks of development (a, b, c) and embryonic brain cells (d). Rapid direct method of chromosomal analysis, Q staining after cesium chloride treatment (a), Q staining with quinacrine mustard (b), C staining after treatment with barium hydroxide (c), and routine Giemsa staining (d).

Giemsa stain and trypsin solution (Fig. 1d), or cesium chloride (Fig. 2a). Chromosomal preparations from chorionic biopsy material, stained with quinacrine mustard and for C heterochromatin, are illustrated in Fig. 2b, c.

Because of the high natural mitotic activity the above-mentioned method can be used not only for chromosomal analysis of the chorion, but also for similar analysis of cells of the fetus itself and, in particular, its brain and meninges, which are macerated particularly easily by acetic acid (Fig. 2d). The possibility of rapid karyotyping of cells of the fetus itself by the direct method does away with the need to use the longer and more laborious method of culture of fetal fibroblasts for this purpose, requiring special equipment and media and appropriate training [7].

Thus the new version of the direct method of chromosomal analysis of chorionic biopsy material developed by the writers, combined with the modification of & staining, can yield a sufficient number of metaphase plates suitable for differential staining [1]; cells not only of the chorion, but also of the fetus itself, can be karyotyped [2]; chromosomal aberrations can be studied in both metaphase and prometaphase chromosomes [3]. The method shortens the time of processing the material substantially and it does not require special culture media or equipment [5].

All these evident advantages, coupled with simplicity in use, enable this method to be recommended for large-scale use in centers of medical genetics for the diagnosis of chromosomal diseases of the fetus during the first 3 months of pregnancy.

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