Effect of G-Banding Techniques on the Ultrastructure of Human Chromosomes

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Received October 22, 1973

Summary. The ultrastructure of human chromosomes is studied after each step of the G-banding technique. Without staining the primary coils are shown. After the first and second G-band staining steps, an apparent destruction of the original structure takes place. Dense loops of fibrils, partly arranged in bundles, in G-band regions can only be seen after the final Giemsa staining. The rest of the chromatid also consists of fibrils, but not so compact. The diameter of the fibrils is 300—600 Å. The centromere region consists of parallel fibrils. Identical chromosomes were studied with light and electron microscope, threedimensionally depicted and evaluated by means of the equidensity method.

Several methods which were recently developed produce bandlike structures within chromosomes. These methods are partly based on the effects of NaOH, followed by SSC treatment and Giemsa staining (Arrighi and Hsu, 1971; Sumner et al., 1971; Schnedl, 1971) and partly on the treatment of chromosomes with proteolytic enzymes followed by Giemsa staining (e.g. Seabright, 1971). The effects of NaOH, phosphate buffer and Giemsa staining on the ultrastructure of human chromosomes are reported here.

Material and Methods

Peripheral blood cells from one male and one female donor were cultured according to Moorehead et al. (1960). After 69 hrs incubation at 37°C 0.2 ml of 0.04% Colcemid solution was added and incubation continued for 3 hrs at 37°C. After hypotonic treatment with 1 part physiological saline to 3 parts distilled water incubated at 37°C for 10 min the suspension was fixed twice with methanol-acetic acid (1:3) and then dropped on to cooled (4°C) glass slides. This procedure is referred to in the description of the results as step 1.

The air-dried chromosomes were then treated according to the methods described by Schnedl (1971); the slides were immersed for 90 sec on 0.002 N NaOH (step 2) after which
they were incubated for 24 hrs in 0.15 M phosphate buffer (pH 6.8) at 50°C (step 3). After dehydration in ethanol (3 × 70, 96, 100%) and air drying, the slides were stained with Giemsa solution for 30 min (step 4).

Some of the slides were stained with Giemsa after step 1 to determine the effects of Giemsa alone.

After each step some samples were prepared for electron microscopic investigation according to our recently published method (Ruzicka, 1973). The slides were coated with carbon using a Leyboldt EPA 100 evaporator. Cooling was with liquid N₂, and the process was carried out in a vacuum of at least 5 × 10⁻⁶ Torr until a gold-coated aluminium foil charged its color from red to blue, indicating the correct thickness of the carbon layer. Suitable mitotic figures were photographed and marked with a marker diamond under the light microscope. The coated chromosomes were floated off from the glass slides with 0.3 N fluoric acid, washed in distilled water and stained with 1% uranyl acetate for 15 min. The carbon film with the chromosomes was then fixed on grids and studied with a Zeiss EM 9S-II.

Results

Chromosomes Prepared Up to Step 1 (air-dried preparations). In these preparations the chromosomes show primary coils in most cases (Fig. 1A). At higher magnification the marginal zone of the chromosomes reveals small granular dense regions and spread fibrils with a diameter of about 250 Å (Fig. 1B).

![Fig. 1. A Chromosomes (prometaphase) after hypotonic treatment (step 1), stained with uranylacetate. Magn.: 6500:1.](image)

![B Part of a chromosome after step 1. Magn.: 91000:1](image)