The present study describes in detail the autoradiographical technique for the study of human chromosome replication. The procedure is as follows.
Leukocytes in the peripheral venous blood taken from human subjects, freshly isolated from the individual using phytohemagglutinin, were cultured in suspension for 72 hours. Tritiated thymidine was added to the cultures at various times to a final concentration of 1μc per ml. After incubation for 10 minutes with the labeled thymidine, the cells were washed and reincubated in a fresh culture medium containing a large excess of unlabeled thymidime. Slides were prepared according to the air-drying technique. The cells were stained with acetic orcein. After staining, all slides were examined under the microscope. In metaphase cells suitable for analysis the chromosomes were counted, analyzed, and photographed.

The slide location of each metaphase was recorded so that they could be relocated following the autoradiography. The slides were dipped in NTB-3 liquid emulsion (Kodak) for 1 to 2 seconds. After 2 weeks of exposure at 4°C to a dry atmosphere, the slides were developed in D-19 (Kodak) for 4 minutes at 18°C. The autoradiographs corresponding to the previously selected well-spread metaphases were relocated and photographed. Photomicrographs of autoradiographs and the corresponding set of chromosomes were analyzed comparatively. Heavy grain content following autoradiography may make obscure chromosomal morphology, especially the fine structure, and make the exact identification of chromosomes difficult. In addition, the preparation of karyotypes before autoradiography introduces an element of objectivity into the matching of the corresponding chromosomes after autoradiography. Most importantly, the statements regarding light or heavy labeling of a chromosome and the chronology of chromosomal replication are based on the matching of the autoradiographs with previously determined karyotypes of the metaphases examined.

Culture of peripheral blood lymphocytes for chromosome analysis

The blood cell karyotyping method was developed to provide information about chromosomal abnormalities. Lymphocyte cells do not normally undergo subsequent cell divisions. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

Test procedure

1. Inoculate approximately 0.5 mL of heparinized whole blood into a glass or plastic tube with 10 mL of medium.
2. Incubate the culture at 37°C in 5% CO_2 atmosphere for 72 hr.
3. Add 0.5 µg/mL of KaryoMAXColcemid Solution (Cat. No. 15212012 or 15210040) to each culture tube.
4. Incubate the culture for an additional 15–30 min.
5. Transfer the culture to a centrifuge tube and spin at 500 x *g* for 5 min.
6. Remove the supernatant and re-suspend the cells in 5–10 mL of hypotonic 0.075M KCl (Cat. No. 10575090).
7. Incubate at 37°C for 10–12 min.
8. Spin at 500 x *g* for 5 min.
9. Remove the supernatant, agitate the cellular sediment and add drop-by-drop 5–10 mL of fresh, ice-cold fixative made up of 1 part acetic acid to 3 parts methanol. Leave in 4°C for 10 min.
10. Repeat steps 7 and 8.
11. Spin at 500 x *g* for 5 min.
12. Resuspend the cell pellet in a small volume (0.5–1 mL) of fresh fixative, drop onto a clean slide, and allow to air dry.