10.1 Preparation of chromosomes

The chromosomes only become visible and suited for the classical chromosome analyses during the metaphase stage of the cell cycle. As the metaphase stage is very short, cells in strong growth are necessary in order to prepare chromosome slides for microscopic observation.

Lymphocyte culture is the most common method for obtaining many cells in the metaphase stage simultaneously. A mitotic rate of 1 % is common in good cultures. Normally the lymphocytes are inactive cells, therefore they have to be stimulated to divide. The most commonly used mitogene (mitose stimulating agent) is PHA (phytohaemagglutinin) which is a bean extract.

The following procedures are used for 'whole blood' culture and chromosome preparations:

- Use blood stabilized with heparin, which can be stored at room temperature (20 C^0) in up to five days before initiation of the cultivation.
- 0.3-0.5 ml full blood in 5 ml growth medium (RPMI) added 10% foetal calf serum and PHA is grown for 60 hours at 38 C^0
- the last two hours with added colcemid (then centrifuge and remove the supernatant)
- the cells are treated in half physiological salt solution (0,075M KCl) for 10 min. --- do
- the cells are treated in fixative (methanol acetic acid 3:1) added slowly while shaking, after 15 min. ---------- do
- wash in new fixative three times, getting the cells in suspension every time ---------- do
- store in fixative at minus 20 C^0. The cells can now be stored for years before use
- drop cell suspension on slides and let it dry while blotting off the surplus fixative at the edges of the slides
- stain with Giemsa or another nucleus stain
- microscopic observation and photo

Chromosomes can also be made from fibroblast cell cultures, which can be established from an ear clip or subcutaneous muscle tissue. After a post mortem examination tissue from the lung or the kidney can also be used to establish a primary cell culture. The culture must be established within the first few day after the sample has been obtained. The cell culture is established in a Falcon tube by adding media and a few small lumps of tissue, which have been minced by a pair of scissors. Normally it has to grow for more than 10 days before a sufficient amount of cells for chromosome preparations have developed. The growth media are the same as mentioned under 'blood' culture, but here no PHA has to be added.

To identify the individual pairs of chromosome specific staining is needed. This is done by adding a thymidine analogue Bromo-deoxy-Uracil (BrdU) to the growing cells 6-7 hours before harvest, depending on growth rate. After staining with Acridine orange the BrdU incorporation should give faint cromatids in the late replicated areas (after addition of BrdU), whereas the earlier replicated areas give a strong bright colour. By means of this staining the inactive X-chromosomes in the normal female can be demonstrated, as the inactive X-chromosome has an extremely late replication,