EXPERIMENT 6:

GENOTOXICITY TESTS USED IN THE DETERMINATION OF CHROMOSOMAL DAMAGE

A. GENERAL INFORMATION

Many chemical and physical agents which can affect cell functions are encountered nowadays. One of the most important problems in long-term exposure to these agents is genotoxicity.

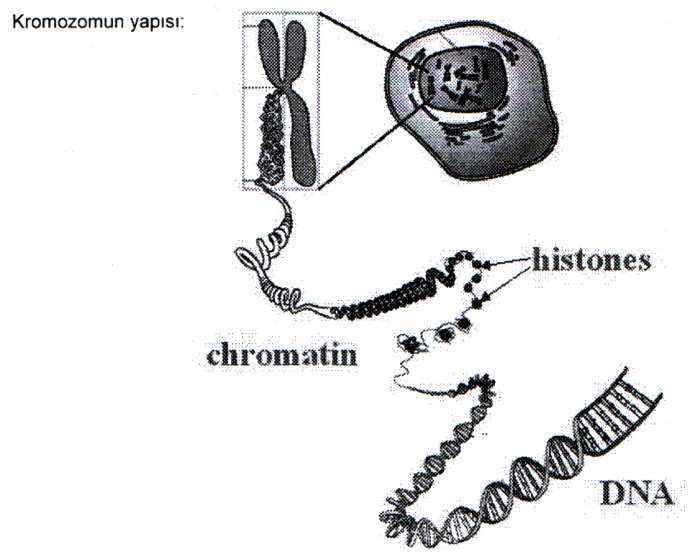
For the determination of genotoxicity, several test systems have been developed. However, "Sister Chromatid Exchange" and "Micronucleus" tests are two of the most commonly used tests due to their applicability.

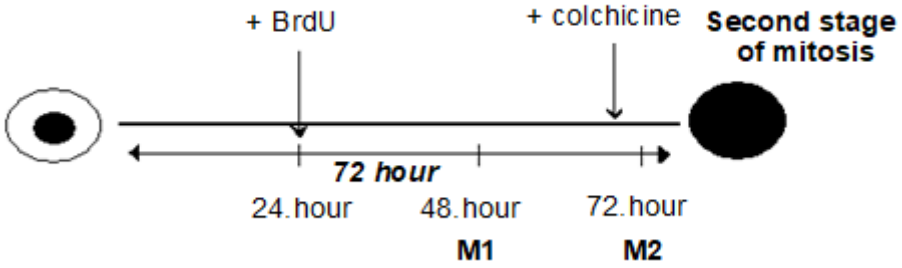
1. Sister Chromatid Exchange (SCE)

SCE is a method based on the exchange of genetic material between two identical sister chromatids.

The significance of this test is controversial currently, because the displacement of the broken sister chromatids is among the identical "locus". However, it is one of the most sensitive methods used to determine exposure to genotoxic substances.

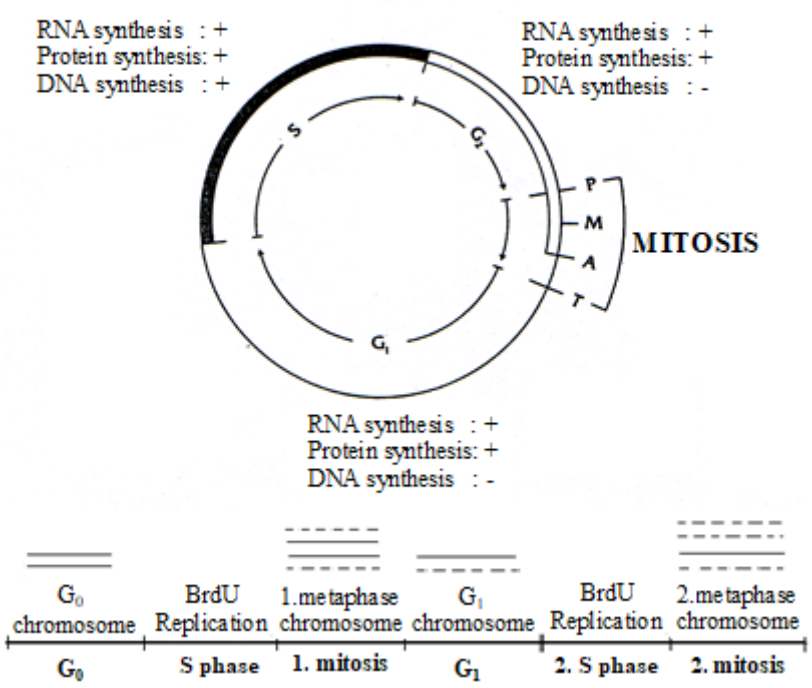
SCE is a method of determining whether a genotoxic substance has been exposed. It is a very sensitive test, which has a great advantage to be able to detect very small exposures. However, it doesn’t give a certain result about mutagenicity. High "SCE/cell" ratio doesn’t mean that the individual will have cancer, but "SCE/cell" ratio is high in all cancer types. This can be explained by DNA repair ability, itself.



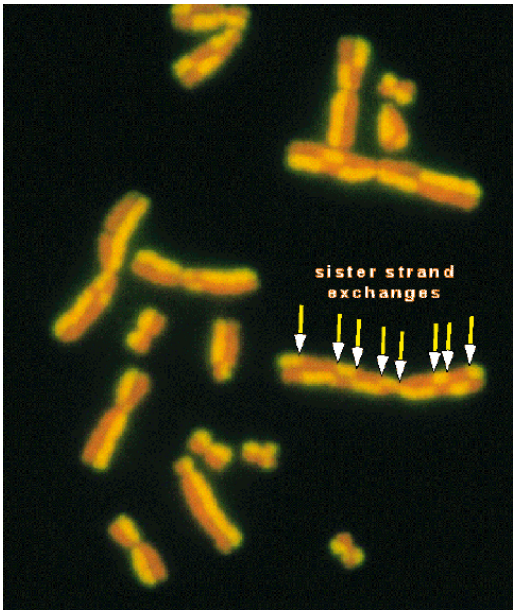


SCE test is usually performed with human peripheral blood lymphocytes. Peripheral lymphocytes are partitioned using ***phytohemagglutinin***, an aspecific antigen, to stimulate them when they are in the G_0 phase of the cell cycle. Immediately before the fixation, colchicine is added to stop cell division at the second mitosis of the metaphase. Different staining of these sister chromatids is possible to distinguish the two chromatids in the chromosomes. For this purpose, ***Bromodeoxyuridine (BrdU)*** is added to the cell culture at 24 hours of division. BrdU is a thymine analogue and has the ability to combine with DNA.

Cell Cycle

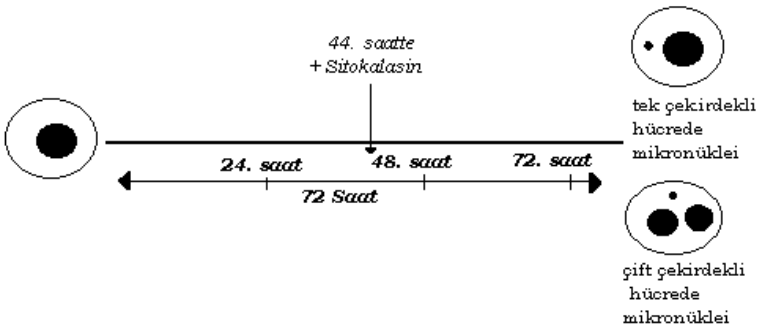


If BrdU enters the structure of a single DNA strand of chromatids, the color seen in the microscope will be darker. However, if both 2 DNA strands contain BrdU, the color will be lighter. If there is a change in the sister chromatids, that means exposure to a genotoxic substance, a piece of dark part will be displaced with a piece of light part.



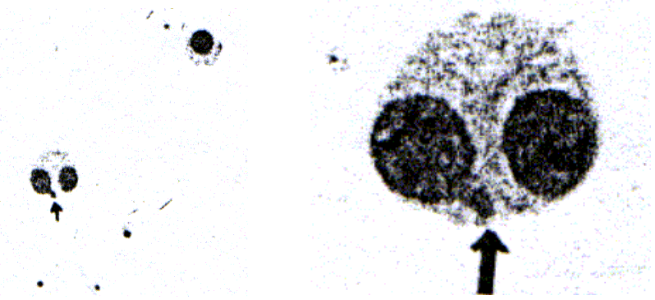
2. Micronucleus test (MN)

Micronucleus test is a method based on the visualization of chromosomes or chromosome fragments that break from cell nucleus.



MN tests are usually performed with human peripheral blood lymphocytes. ***Phytohemagglutinin*** is used to stimulate the division of cells. Under normal conditions, during cell division the nucleus divides firstly, then the cytoplasm divides and two cells form. Cytochalasin prevents the division of cytoplasm in this step, allowing 2 nuclei to be seen in a single cytoplasm. If there is a break from the nucleus, this allows the micronucleus to be seen.

MN is more deterministic than SCE, but not as sensitive as SCE



Micronucleus

B) EXPERIMENTAL PROCEDURE

1. SCE

Incubation:

Blood samples, culture medium containing fetal calf serum (FCS) and phytohemagglutinin place to the test tubes. The mixture is incubated at 37 ° C in incubator, then BrdU is added at 24. hours. Colchicine is added at 68. hours. The cell and culture medium is centrifuged at 72 hours. The supernatant is discarded. All the operations up to this stage are carried out under sterile conditions.

Fixation:

1. Cells are treated with hypotonic KCl.

2. The tubes are centrifuged, the supernatant is discarded.

3. Fixation is performed by the addition of Carnoy's solution (glacial acetic acid:methanol, 1: 3) while the tubes are being shaken.

4. The tubes are centrifuged and the supernatant is discarded.

5. Fix with Carnoy's solution.

6. The tubes are centrifuged, the supernatant is discarded.

7. The slides are prepared by dropping the cell suspension from a high distance.

Staining: Slides are stained with Hoechst and Giemsa, then dried for microscopic examination. Breaks are counted.

Evaluation: The "average SCE/cell" ratio is calculated for each individual. If this value is higher than the control group value, that indicates a person is exposed to a chemical substance that can affect genetic material (DNA).

2. MN

Incubation:

Blood samples, culture medium containing FCS and phytohemagglutinin place to the test tubes. The mixture is incubated at 37°C in incubator, then cytochalasin-B is added at 44. hours. At 72 hours the tubes are removed from the incubator, the cell and culture medium are centrifuged. The supernatant is discarded. All the operations up to this stage are carried out under sterile conditions.

Fixation:

1. Cells are treated with hypotonic KCl.
2. The tubes are centrifuged, the supernatant is discarded.
3. Fixation is performed by the addition of Carnoy's solution (glacial acetic acid:methanol, 1: 3) while the tubes are being shaken.
4. The tubes are centrifuged and the supernatant is discarded.
5. Fix with Carnoy's solution.
6. The tubes are centrifuged, the supernatant is discarded.
7. 7. The slides are prepared by dropping the cell suspension from a high distance.
8. 3-5 drops of supernatant are placed on the clean slides and the slides are prepared by spreading.

Staining: Slides are stained with May-Grünvald Giemsa solution , then dried for microscopic examination. MN are counted.

Evaluation: The number of MN in 1000 binuclear cells is determined for each individual.If the number is higher than the control group value; that means the individual is exposed to a chemical that can affect genetic material (DNA).