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TITLE

Radiation cytogenetic *in vitro* studies on human donors in the development of a suitable biological dosimeter

FINAL REPORT FOR THE PERIOD

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FINAL REPORT IN CONNECTION WITH THE WORK CARRIED OUT UNDER RESEARCH CONTRACT 3173

Regulations in our country treat work with ionizing radiation as work under special conditions. Among the technical, medical, dosimetry and other controls, chromosomal aberrations in the peripheral blood lymphocytes should be examined every five years if the dose received is greater than 50mGy (according to the dosimeter). For personel working in nuclear power stations, this limit is 100mGy, and the accepted number of chromosomal aberrations twice that of normal population.

During last 15 years, since the beginning of our Cytogenetic Unit in Clinical Hospital Center "Zvezdara", we examined a number of different groups of individuals who had been, or have been permanently in contact with ionizing radiation; patients with hyperthyreosis before and after the treatment with ^{131}I, patients with kidney diseases receiving Hipuran (with ^{131}I), patients with routine examinations of intestinum, group of patients with Ca uteri treated either by Katetron or clasic intracavitary applicators, etc, etc.

Occupationally exposed people are under our medical and cytogenetical control periodically all this time. There is a number of them who have been working 10-20 years on nuclear reactor in Vinča. Four survivors who were irradiated in the accident in 1958. with doses of Neutron and gamma rays between 323-426 rads, have also been under our regular check-ups.

Beside these analyses, we have also established various dose-response curves, for X-rays, gamma rays, electrons (various energies 10-42 MeV) and 42 MeV Neutrons. These investigations were done by means of clasic cytogenetic analysis, harvesting of cells done after 48 hours of culture.

Experiments and results became more accurate by introducing FPG technique, and scoring first division cells only. According to the IAEA programme the agreement was to establish new dose response curve for low-LET radiation. In our laboratory the irradiation of blood samples was done with gamma-rays from Co-bomb, with doses of 0,5 1,00 2,00 3,00 and 4,00 Gy, and cultures set up in the presence of BUdR. We analysed cells until 100 dicentric chromosomes were found per dose. For 0,5 Gy we analysed 1015 cells, for 1,0 Gy 557 cells, for 2,00 Gy 283 cells, for 3,0 Gy 155 cells and for 4,00 Gy 91 cells. According to the model $y= a+bx+cx^2$, curve parameters were:

$$y = 0,03 + 7,05x10^{-4}X + 4,81x10^{-5}X^2$$

with the coefficient of determination $R^2 = 1,00$.

This new curve is in good agreement with our previously established one, without BUdR, but harvest done after 48 hours.

Additional couple of observations from this experiment were, that there was no difference in scoring between the two concentrations of BUdR used: 5 microgr and 10 microgr./ml of culture; and that there were no culture differences when using different types of PHA (Welcome, or PHA-M,Difco).

In this experiment, 1000 cells were scored for unirradiated control. The overall percentage of structural aberrations was 1,5%. Only one dicentric chromosome was found.

This particular curve for gamma-rays was completed later on with low-dose data. Samples were irradiated with the same Co-bomb with doses of 0,1 0,2 and 0,3Gy gamma rays. Three thousand cells were analysed per dose. The fit done in the same way as before gave the coefficients:

$$y = 1x10^{-3} + 3,33x10^{-4}.X - 3,32x10^{-6}.X^2$$

Combining these two curves for high and low doses, we got:

$$y = 2,75x10^{-3} + 9,22x10^{-4}.X + 4,43x10^{-6}.X^2$$

Since we were not particulary happy with the fit of these two curves, results were sent to NRPB. The suggestion was that data for 0,5 and 1,0Gy were too high, and Dr Lloyd and Dr Edwards fitted a curve through o, o,1 o,2 o,3 2,o 3,o and 4,o Gy data and got:

$$y = (0,53 \pm 1,06x10^{-4})D + (7,24 \pm 7,43x10^{-6})D^2$$

The data for 0,5 and 1,0Gy are going to be repeated in a new experiment.

The practical use of this curve came into life in the experiment with simulated nuclear accident at NRPB, when samples of irradiated blood were distributed to different laboratories. The organisation and airport formalities were finished in no time, and lymphocytes grew very well indeed. Results from these experiments are already published in Mutation Research.

One importaint question in post-Chernobyl period was establishing new laboratory control level for structural chromosomal aberrations. This was done both for newborn and adult population. Every month on the same date, beginning with 27. May 1986., 10 healthy babies were selected, born after uneventful pregnancies, and blood taken 24 hours after birth. At the same time blood was drawn from their mothers as well. One hundred cells per infant/mother were analysed. These analyses were done in the same manner for 10 months, until February 1987 (T_1-T_{10} on the graph, to cover one whole pregnancy of nine months).

Immediately after the accident in Chernobyl the measurements of radioactivity in our country showed that overall beta-activity was 100-10.000 times higher than normal ("normal" being couple of mBq/m^3 of air in Belgrade area), and mean exposition doses of gamma irradiation 10-55 times more (normal being 12,8 microR/ /h). These values decreased in time, and since June 1986 are in the limits of those recomended by WHO.

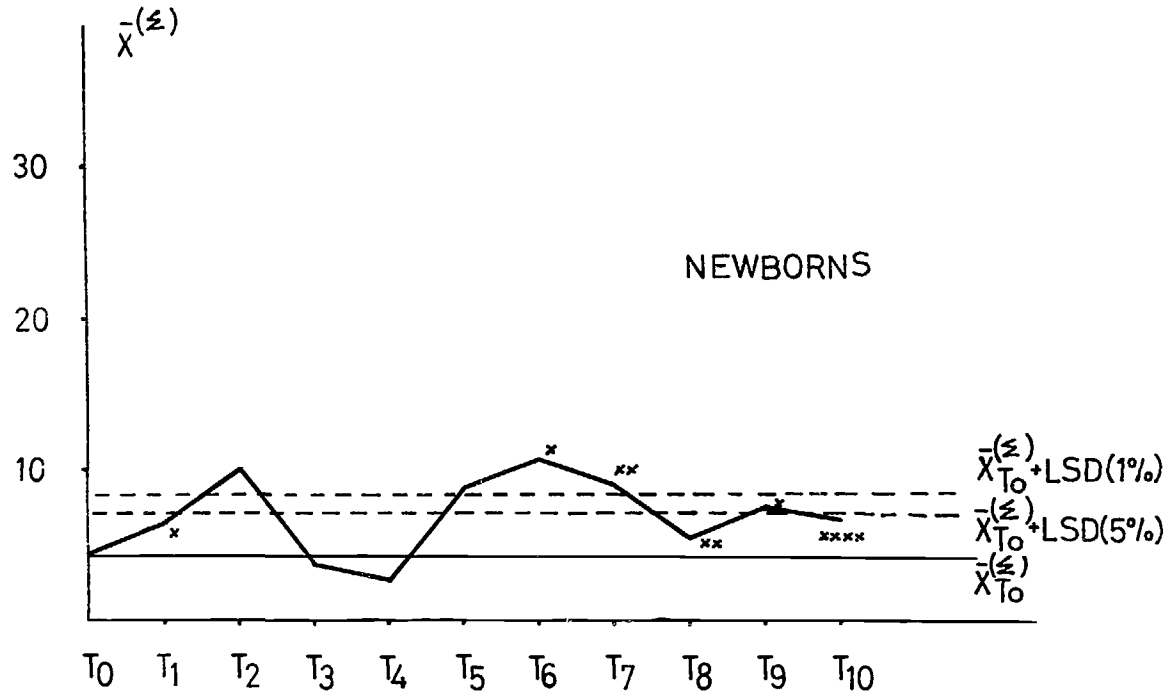
The results of cytogenetic analyses are shown in the graph, and both analysis of variance and LSD model

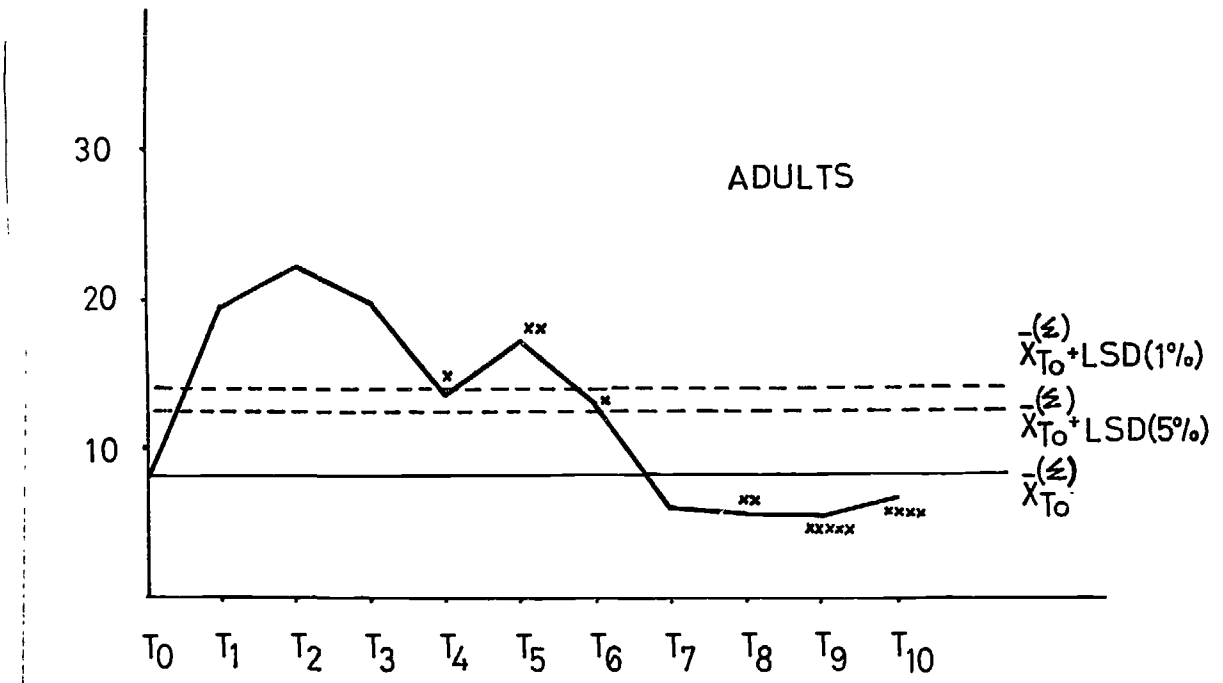
showed highly significant differences in comparison to the background level ($\bar{X}_{To}^{(i)}$) of structural chromosomal aberrations.

Dicentric chromosomes are put separately (x) on the graph (the exact number of them per 1000 cells analysed).

These investigations are continuing, but in intervals of 2-3 months, because of the capacity of the laboratory.

What is left now, is to introduce micronucleus technique. First steps are already done and in the course of next year we hope we will make a useful and qiuck routine model system out of it.


$\bar{X}^{(\leq)}$
30
20
10
NEWBORNS
$\bar{X}^{(\leq)}_{To}$+LSD(1%)
$\bar{X}^{(\leq)}_{To}$+LSD(5%)
$\bar{X}^{(\leq)}_{To}$
T_0 T_1 T_2 T_3 T_4 T_5 T_6 T_7 T_8 T_9 T_{10}


30
20
10
ADULTS
$\bar{X}^{(\leq)}_{To}$+LSD(1%)
$\bar{X}^{(\leq)}_{To}$+LSD(5%)
$\bar{X}^{(\leq)}_{To}$
T_0 T_1 T_2 T_3 T_4 T_5 T_6 T_7 T_8 T_9 T_{10}