

FURTHER APPROACHES TO BIOLOGICAL INDICATORS
OF RADIATION INJURY

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Despite of the decades-long investigations, the search for proper biological indicator of radiation injuries did not result in techniques fulfilling all the requirements as yet (6). So far, the most reliable assay is the dicentric chromosome aberration analysis (9,10). New developments have been made recently on a cytogenetic technique, i.e. the micronucleus assay, and for local injuries on the application of thermography. Our own results as presented below suggest that these methods are promising.

MICRONUCLEI IN X-IRRADIATED HUMAN LYMPHOCYTES

The detection of micronuclei in lymphocytes has come into the limelight a few years ago. The fairly consequent dose-effect relationships obtained by several laboratories made the technique rather promising (ref. 1), especially after the modification recommended to recognize interphase cells after their first mitotic divisions (2,3,11).

On Fig. 1. our data are shown on dose-response relationships of appearances of micronuclei in human lymphocytes from x-irradiated whole blood as detected in traditional lymphoblasts and cytokinesis-blocked ones.

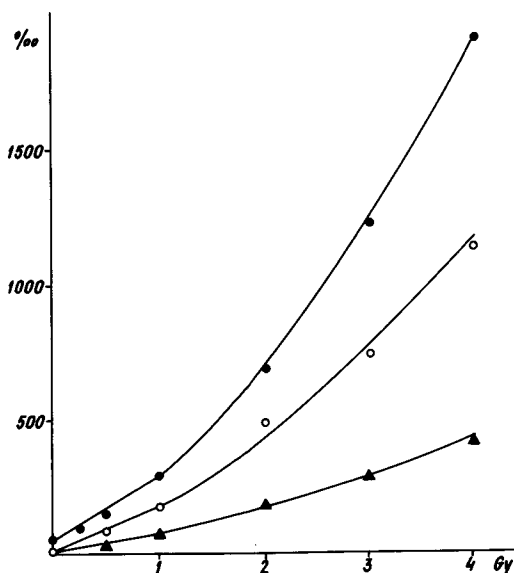


Fig.1. Dose-effect curves of appearances of micronuclei after x-irradiation of human blood in mononuclear lymphoblasts (\blacktriangle), binuclear cytokinesis-blocked cells (\circ) both after 72 hrs and the frequency of acentric fragments (\bullet) after 48 hours incubation.

The points represent data obtained from 3 donors. Cytokinesis-blocking was performed by 3 μ g per ml cytochalasin B through the last 28 hrs of incubation.

In addition, for comparison, the yield of radiation-induced acentric fragments is shown as well. The data from 3 donors per test and per dose could be best fitted to linear-quadratic relationship. The yield-equations for micronuclei in mononuclear lymphoblasts (MN) and for cytokinesis-blocked (CB) cells are as follows:

$$Y_{MN} = (6.9 \pm 2.8) + (38.9 \pm 5.8)D + (16.3 \pm 2.1)D^2$$

$$Y_{CB} = (11.3 \pm 4.3) + (139.0 \pm 44.0)D + (36.8 \pm 18.6)D^2$$

When comparing the three curves on Fig. 1. it is obvious that in CB cells more micronuclei can be detected by appr. 40-50 per cent than in mononuclear lymphoblasts but the frequency of acentric fragments is still higher.

Based on the yield equations presented, if the "c" value is properly low and the frequency of micronuclei in CB-cells is at least double of the background level, an estimate of a dose of appr. 0.1 Gy can be made. The "c" value, however, is somewhat dependent on age (1,3,4) which fact has to be considered in dose assessment through micronucleus assay. Therefore, further collection and comparison of background data by various laboratories will help the wide acceptance of this technique as a reliable biological indicator or even dosimeter of radiation injury.

It has to be noted, however, that further studies are needed also to reveal the origin and mechanism of formation of micronuclei appeared in mononuclear and CB lymphoblasts that the size distribution in the latter is wider, i.e. a push toward the larger diameters were found (5).

THERMOGRAPHY FOR LOCAL INJURIES

In cases of partial body irradiation including extremities the usual tests applied most commonly have only limited values in assessing the severity of local tissue damages. For such situations a few laboratories including our one have introduced various techniques of thermography. We have reported on a clinical case when an industrial radiographer has suffered an accident with Ir-192 gamma radiation source (7). Especially three fingers of his left hand were seriously irradiated. Although clinical symptoms were restricted to the distal parts of the fingers, by contact thermography (Flexi Therm^R) it was indicated that larger areas of the thenar and hypothenar were also involved. Telethermogrammetry has indicated even larger areas involved (8). The case raised several problems on detectability of dose- and time-dependent tissue damages. Investigations on mini-pigs revealed thermographically detectable alterations even in phases without clinical symptoms. The Figs. 2. and 3. present data obtained after x- and gamma-irradiations of thighs of mini-pigs in the dose range between 12.5 and 75 Gy.

We could conclude from our clinical and experimental experience collected so far that thermography is a sensitive and useful technique to detect local radiation injuries also in the phases when clinical symptoms are not evident, i.e. in the early and latent periods.

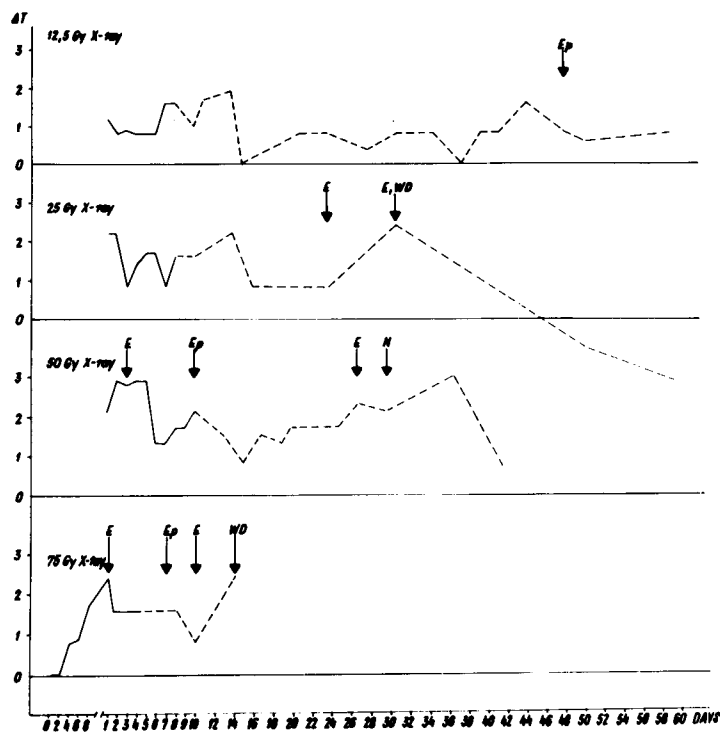


Fig.2. Thermal alterations in time (hours then days) on surfaces of mini-pig thighs after x-irradiation with 12.5, 25, 50 and 75 Gy as detected by NovaTherm^R contact thermography. The ΔT expresses differences compared to the temperature of unirradiated surrounding areas. Letters and arrows indicate time-points when clinical signs appeared, i.e. Ep: epilation, E: erythema, WD: wet desquamation, N: necrosis.

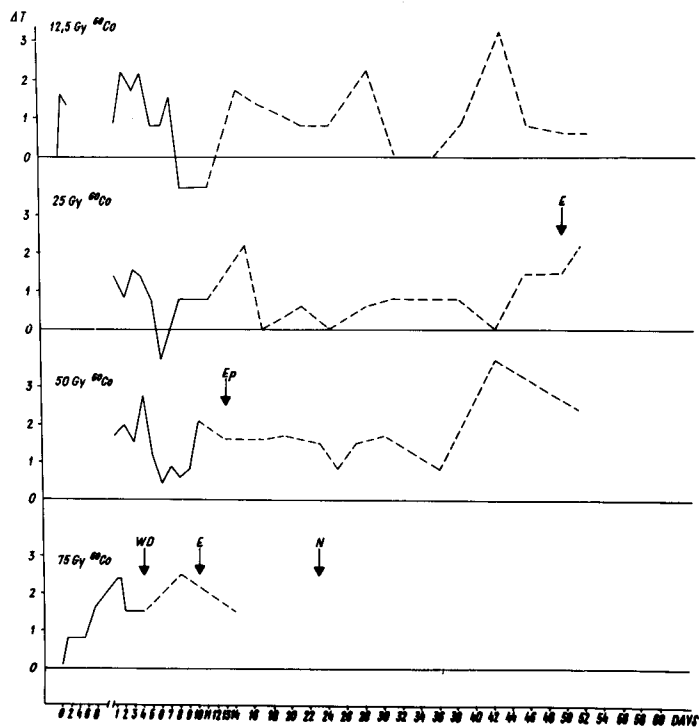


Fig.3. Thermal alterations in time (hours then days) on surfaces of mini-pig thighs after ⁶⁰Co gamma-irradiation with 12.5, 25, 50 and 75 Gy as detected by NovaTherm^R contact thermography. Abbreviations as on Fig.2.

REFERENCES

- 1) Almássy Zs., Krepinsky A., Bianco A., Köteles G.J. (1987)
The present state and perspectives of micronucleus assay technique in radiation protection, *Int.J.Appl.Radiat.Isotop.*, 38, 241-149.
- 2) Fenech A., Morley A.A. (1985) Measurement of micronuclei in lymphocytes, *Mutat.Res.*, 147, 29-36.
- 3) Fenech M., Morley A.A. (1986) Cytokinesis-blocked micronucleus method in human lymphocytes: effect of in vivo ageing and low dose x-irradiation, *Mutat. Res.*, 161, 193-198.
- 4) Kerekes J., Novák J., Köteles G.J. (1987) Micronucleus incidence in lymphocytes of burned patients, *Honvédervos (Military Physician)*, 39, 17-21.
- 5) Kormos Cs., Köteles G.J. (1987) Micronuclei in x-irradiated human lymphocytes, *Mutat.Res.*, in press
- 6) Köteles G.J. Bianco A. (1982) The need for and importance of biological indicators of radiation accidents. In "Cell Membrane Probes as Biological Indicators in Radiation Accidents" International Atomic Energy Agency, TECDOC-273 pp. 7-22.
- 7) Köteles G.J., Lelik F., Sztanyik L.B. (1987) Use of thermography in diagnosis of radiation injury. A case report, *Thermology*, in press
- 8) Köteles G.J., Benkő I. (1986) Tracing the thermic symptom of radiation injuries by infrared image forming, *Mérés és Automatika (Measurement and automatics)*, 34, 383-389.
- 9) Lloyd, D.C., Edwards, A.A. (1983) Chromosome aberrations in human lymphocytes: effect of radiation quality, dose and dose rate, in "Radiation-Induced Chromosome Damage in Man" eds. T. Ishihara, M. S. Sasaki, Alan R. Liss., New York, pp. 23-50.
- 10) Lloyd D.C., Edwards A.A., Prosser J.S., Barjaktarovic N., Brown J.K., Horvat D., Ismail S.R., Köteles G.J., Almássy Zs., Krepinsky A.B., Kucerova M., Littlefield L.G., Mukherjee U., Natarajan A.T., Sasaki M.S. /1987) A collaborative exercise on cytogenetic dosimetry for simulated whole and partial body accidental irradiation, *Mutat.Res.*, 179, 197-208.
- 11) Pincu M., Bass D., Norman A. (1984) An improved micronuclear assay in lymphocytes, *Mutat.Res.*, 139, 61-65.