REPORT NO. IAEA-R-2055-F

TITLE

Alteration of cellular and subcellular electrophysiological parameters in mammalian cells by high- and low LET irradiation at low dose-levels, (part of a coordinated programme on cell membrane probes as biological indicators in radiation accidents)

FINAL REPORT FOR THE PERIOD

1977-11-01 - 1980-12-14

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INTERNATIONAL ATOMIC ENERGY AGENCY

DATE December 1980

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International Atomic Energy Agency

3rd Research Coordination Meeting on Cell Membrane

Probes as Biological Indicators in Radiation Accidents

10-13 November 1980, Salzburg, Austria

ALTERATION OF CELLULAR MEMBRANE PARAMETERS IN MAMMALIAN CELLS AND ITS APPLICATION AS BIOLOGICAL DOSIMETER FOR HIGH- AND LOW-LET IRRADIATION

(Research Contract No. 2055/RB)

FINAL REPORT

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1. INTRODUCTION

Although several radiation induced biological effects have been found for high-level exposure, there is still insufficient knowledge of biological indicators after low-level radiation exposure of man. However, it is the dose range below several hundred rad[1)] that is of importance for the use of a "biological dosimeter" in accidental radiation exposure.

In order to be applicable in practical health physics the following optimization conditions should be met:

1. Statistically significant magnitude of the radiation-specific biological effect at dose levels D below the lethal dose, i.e. D << 600 rad.
2. Manifestation of the radiation induced effect preferably already shortly after the accident (minutes to hours), but in any case at least one to two days afterwards.
3. Minimum additional physiological stress to the accident victim due to the application of the biological dosimetric method.
4. High cost effectiveness of the methodology in order to promote wide-spread use, e.g. at clinical centers rather than application limited to specialized research establishments only.

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1) 1 rad ≙ 0.01 Gy

Since the primary interaction of ionizing radiation with any form of living matter occurs at the cellular level, the component with the highest hit probability, i.e. the cell membrane, represents a potential biological indicator in samples from accidentally radiation exposed subjects. Apart from its function as boundary layer between extra- and intracellular regions the cell membrane is also involved in important processes of regulation and information necessary for metabolic and synthetic activities. Closely related to these cellular activities are bioelectric properties, such as transmembrane resting potential (MRP) and surface charges. Extensive studies of the relationship between the electrical potential of the cell membrane and various physical, chemical and biological stimuli revealed that this potential is a highly sensitive indicator for cellular reactions, especially whenever phenomena of transport and ion gradients as well as permeability are involved (1-4).

In this project the MRP was studied for its suitability as biological indicator of the level of accidental radiation exposure. The research was carried out at the Division of Biophysics at the Institute of General Biology, Biochemistry and Biophysics, University of Salzburg under the IAEA-contract 2055/RB for the period of 1977 to 1980. During this period the following research topics were dealt with:

a) Development of methodology and installation of a low-cost test-chamber for intracellular MRP-recordings of human cells (total cost: $ 8000 for minimum configuration).

b) Dose-response studies of MRP-changes of human cells after irradiation with low- and high-LET radiation.

2. EXPERIMENTAL METHODS

A detailed description of the materials and methods applied is given in the Second Progress Report (5).

2.1. Intracellular electrical measurements

A test chamber was constructed, providing optimum insulation against mechanical vibrations in the nanometer range and electrical noise from stray fields and power supply-line interferences. The former is achieved by a table construction of high dynamic stability and low resonance frequency. Electrical noise reduction is provided by a semi-closed Faraday-cage connected to a low-resistance laboratory ground, an electronically stabilized power supply system and an additional notch-filter. Thereby a reduction of electronic noise in the pick-up system for the MRP-signal is achieved by more than a factor of 4 as compared to the unprotected system.

The system for cellular MRP-measurements consists of (Fig. 1):

a) Recording glass microelectrode, reference electrode and electrometer amplifier.

b) Headstage coupled with motorized micromanipulator and remote control.

c) Inverted light microscope with video- and film-camera system

d) MRP-signal as input for storage oscilloscope and optional recording on dual-channel strip chart recorder.

The complete system of test chamber and associated electronics is shown in Fig. 2.

All measurements were carried out temperature-controlled at 37°C. Upon insertion of the microelectrode (resistance: 10-20 MΩ, tip potential: < 5 mV) into the cell the MRP reaches a maximum value (Fig. 3, "IN") which is maintained typically for about 20 s until the electrode is withdrawn (Fig. 3, "OUT").

2.2. Human cells

Monolayers of human lung cells (WI38, FLOW 2002) were grown in plastic petridishes, using conventional cell culture techniques and incubated at 37°C. For alpha-irradiation experiments cells were grown on pre-treated solid state particle detectors (Kodak-Pathé LR 115). A sterilization method was developed which minimizes physical detector damage. Growth tests with human embryonic lung fibroblasts showed that the cells adhered to the treated films in a normal manner. Test exposures of the film to alpha particles revealed no significant differences in the response of the sterilized detector as compared to the untreated sample other than smaller track dimensions due to annealing processes (Fig. 4).

In order to faciliate extrapolation from in vitro-studies to more realistic in vivo-conditions human lung biopsy samples were also used. In collaboration with the Lung Department of the Salzburg Clinics tissue samples of epithelial cells of morphologically healthy appearance were taken from randomly selected patients. Upon removal of the sample from the lung the tissue samples were stored in 1 % physiological saline solution.

2.3. Irradiation and dosimetry

Cell cultures were irradiated as monolayers in petridishes at 37°C. For the low-LET irradiation a Co 60-source (activity: 1 Ci[1]) was used. During irradiation the cells were covered by a thin layer of medium. For the experiments of simultaneous MRP-measurement and gamma irradiation the cells were exposed at a dose rate of 2 rad/min. All other experiments with gamma irradiated cell cultures or tissue samples were carried out at a dose rate of 20 rad/min.

For high-LET irradiation experiments an Am 241-source (activity: 500 µCi/cm) was applied. This alpha source (alpha particle energy: 5.5 MeV) consisted of americium deposited on metal foil and was enclosed in a shutter with electronically controlled shutter speed to ensure high precision of the radiation exposure time. During alpha particle exposure the medium was decanted. The remaining thin medium layer on top of the cells had a mean thickness of 8 µm. After traversal of the air-medium layer between source and cell surface the mean LET of the alpha particle impacting at the cell surface was 160 keV/µm. Due to inhomogeneous variations the conventional macroscopic dose concept is insufficiently accurate for the description of pure membrane based effects. A detailed discussion of the microscopic dose description and calculation of critical dose calues for different cell organelles was given in the Second Progress Report (5). There-

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1) 1 Ci = 37 GBq

fore in the following alpha radiation exposure is described as the number of alpha particles traversing the cell. Based on measurements of the geometrical dimensions of the irradiated cell and the irradiation conditions each cell was hit on the average by 2 alpha particles in all alpha exposure experiments.

2.4. Data analysis

In the literature radiation induced biological effects are often described as the relative change observed at the irradiated sample in percent of the unirradiated control. This approach is valid wherever basic radiobiological research problems are addressed. However, for any practical application of a biological dosimeter in radiation protection it is not possible at present to compare the value of a given biological parameter observed after accidental radiation exposure with the control value of the unirradiated victim. Since biological variability limits the validity of the application of a single "control-value" of the biological indicator for a whole population group, two solutions seem reasonable:

1. Installation of a data base of the individual "control value" of the biological parameter for each subject concerned. This could be part of the personal data like blood group, rhesus factor, etc.
2. Assessment of absolute values of the selected parameter and their changes in dependence of the absorbed dose.

Due to the lack of appropriate data bases in the following the second possibility was chosen for the data analysis. All data are presented as absolute values, thereby comprising both types of fluctuations, i.e. biological variability between the samples as well as statistical variation of the biological parameter.

3. RESULTS

Altogether more than 500 MRP-measurements were carried out on unirradiated cell cultures and over 1800 measurements on radiation exposed cells.

Biopsy samples were taken from 8 subjects and altogether 300 MRP-measurements were performed.

3.1. Simultaneous MRP-measurements and gamma irradiation in cell cultures

As an example Fig. 5 shows the typical MRP-change of an unirradiated human lung cell in logarithmic growth state (FLOW 2002) as a function of time after impalement with a microelectrode (curve A). The MRP decreases steadily within several minutes to approach zero, thereby indicating cell death because of membrane damage from the electrode. For comparison, the complete set-up with both electrodes in the medium, but without cell impalement, was irradiated under the same conditions with gamma rays and neutrons. However, the influence was neglegible and resulted in a DC-offset of only $\pm$ 0.5 mV (Fig. 5, curve B).

The temporal MRP changes before and during gamma irradiation (dose D = 2 rad) of a human lung fibroblast (FLOW 2002) is shown in Fig. 6. MRP increases within seconds after the beginning of the irradiation and reaches a maximum value (V_γ) within about 30 s. V_γ is more than 25 % higher than the corresponding value of the unirradiated cell (V_o). Then the MRP declines to zero in a similar way as the control in Fig. 5. This temporary hyperpolarization can be observed within seconds after low-level in vitro-irradiation.

3.2. Temporal MRP changes in cell cultures after gamma irradiation

In order to determine the statistical variation and the degree of reproducibility for a given biological target the frequency distribution of MRP-values was determined for unirradiated human lung cells (WI38) from different cultures in stationary growth phase. From Fig. 7 it can be seen that extreme MRP values differ by a factor of 4.

The values are approximately normally distributed, which is also indicated by a skewness (s_k) of 0.5 and a kurtosis (k) of less than 3 (Tab. 1).

Figs. 8, 9 represent the short-term MRP changes during the first 30 minutes after gamma irradiation with 60 rad, respectively 600 rad. Measurements were carried out at one-minute-intervals. Standard deviation of the unirradiated control mean value ($\pm$ 20 %) is indicated by the dashed lines; about the same standard deviation is associated with each MRP-value but it has been omitted to faciliate the graphical presentation. The 60 rad-gamma exposure resulted in large oscillations around about the same mean value as the control. This is in contrast to the relatively small variations around a reduced mean value (about 50 % compared to the control) in case of the 600 rad exposure. FOURIER-analysis of the oscillations reflects the significant differences of the coefficients a_i, b_i for the two groups of experiments (Tab. 2).

MRP-measurements were carried out repeatedly at certain times after gamma irradiation (Tabs. 3, 4). Already 24 hours after 60 rad gamma exposure there is no statistically significant difference between the

mean MRP-value of unirradiated control and irradiated cells. Also 2-3 days afterwards no difference can be detected.

A dose of 600 rad results in a large reduction of the mean MRP-value 3 hours after irradiation. However, this is followed by a fast recovery within the next 24 hours up to almost the control value.

3.3. Temporal MRP-changes in cell cultures after alpha irradiation

Fig. 10 shows the frequency distribution of MRP-measurements with human lung cells 24 hours after alpha particle exposure (2 alpha particles per cell). The range of values is lower than for the unirradiated controls as it can be seen also by the smaller variance. The MRP-histogram is symmetrical ($s_k < 0.5$, $k < 3$) around a slightly reduced mean value (Tab. 5).

Results of MRP-measurements at different times after alpha exposure are contained in Tab. 6. Shortly after irradiation mean MRP-values are lower by about 30 % than the control. However, no large oscillations occur as with the 60 rad-gamma exposure. Again the following 24 hours are characterised by an almost complete recovery of the control MRP value. No significant changes have been observed during the following 6 days.

3.4. MRP changes of human tissue samples after gamma irradiation

MRP-measurements were carried out on unirradiated human lung tissue samples within 30 minutes after biopsy and 24 hours later (Fig. 11). Despite storage at 37°C in physiological saline solution the cells in the sample suffered severe damage and mean MRP-values were greatly reduced. Therefore all measurements were carried out within 2 hours after biopsy.

MRP (mV)

Fig. 12 shows the mean MRP of lung biopsy samples 30 minutes after gamma irradiation with 60 rad in comparison to controls. The mean MRP of the irradiated tissue is lower than the control but it is not statistically significant due to the large standard deviation.

SUMMARY AND CONCLUSIONS

The time-dependent change of the intracellular transmembrane potential (MRP) of human cells was investigated for its suitability as a biological indicator in radiation accidents. It proved to be a highly sensitive parameter for indicating fast cellular changes within seconds after onset of gamma irradiation already at a dose of 2 rad. Increasing the gamma dose to 60 rad resulted in an oscillating fluctuation of the MRP directly after irradiation. However, this effect is only transitional and completely repaired after 24 hours. Application of high gamma doses (600 rad) causes a depolarizing effect of reduced MRP without large oscillations. After 1-2 days also this effect can no longer be discriminated against MRP-values of unirradiated controls.

High LET-irradiation with alpha particles (2 hits per cell) causes a general lowering of the mean MRP immediately afterwards without pronounced oscillations. After 24 hours the MRP of unirradiated controls is reached again.

Gamma irradiation (60 rad) of human lung biopsy samples within minutes after the exposure resulted in lower mean MRP values than the controls. However, statistical significance of this effect is reduced due to pronounced MRP-fluctuations, resulting in a large standard deviation.

With regard to the optimum criteria mentioned in the introduction it can be concluded that the conditions 1 (high sensitivity of method), 3 (minimal additional stress) and 4 (low cost) are mostly met by the method investigated. However, it is only suitable for observations shortly after the accidental exposure, since repair effects cause an almost complete compensation of the effect within a relatively short period of several hours at low dose levels.

REFERENCES:

(1) Redmann, K., Burmeister, J. and Jenssen, H.-L.: The influence of hyperthermia on the transmembrane potential, zeta potential and metabolism of polymorpho-nuclear leukocytes. Acta biol. med. germ. 33 (1974) 187-196.

(2) Redmann, K., Peker, J. and Wohlrab, W.: Der Einfluß von Methylkatechol auf Transmembranpotential und Zetapotential kultivierter Hamstermelanomzellen. Dermatol. Monatsschr. 161 (1975) 585-596.

(3) Redmann, K., Walliser, S., Kalkopf, W. and Weuffen, W.: Zum Membraneffekt von Kaliumrhodanid und Methotrexat. Acta biol. med. germ. 35 (1976) 1493-1498.

(4) Redmann, K. and Reichel, G.: Die Wirkung von Röntgenstrahlen auf das Transmembranpotential und die elektrophoretische Beweglichkeit polymorphkerniger Granulozyten und FL-Zellen. Rad. and Environm. Biophys. 14 (1977) 21-30.

(5) Pohl-Rüling, J., Steinhäusler, F., Hofmann, W. and Pohl, E.: Alteration of cellular membrane parameters in mammalian cells and its application as biological dosimeter for high- and low-LET irradiation. 2nd Progress Report, IAEA-Res.-Contr. No. 2055/RB (1979).

Tab. 1 MEASURES OF CENTRAL TENDENCY FOR THE TRANSMEMBRANE RESTING POTENTIAL (MRP) OF UNIRRADIATED HUMAN LUNG CELLS (WI38)

QUANTITY	VALUE
MEAN MRP (mV)	15
VARIANCE	9
SKEWNESS	0.5
KURTOSIS	2.6

Tab. 2 COEFFICIENTS a_n, b_n OF FOURIER-SERIES FOR OSCILLATING TRANSMEMBRANE RESTING POTENTIAL (MRP) OF GAMMA-IRRADIATED HUMAN LUNG CELLS (WI38)

GAMMA DOSE	FOURIER-COEFFICIENTS							
	a_0	b_0	a_1	b_1	a_2	b_2	a_3	b_3
60 rad	31	0	2.1	-2.0	-3.6	-5.7	1.4	-0.9
600 rad	19	0	0.5	$\ll 0.01$	<0.5	<0.5	<0.1	-1.0

$$f(t) = \frac{a_0}{2} + \sum_{n=1}^{\infty} (a_n \cos n\omega t + b_n \sin n\omega t)$$

where:

$$a_n = \frac{2}{T}\int_0^T f(t) \cos n\omega t \, dt \qquad n = 0,1,2,\ldots$$

$$b_n = \frac{2}{T}\int_0^T f(t) \sin n\omega t \, dt \qquad n = 1,2,\ldots$$

$\omega = 2\pi f$ = radian frequency

$T = 1/f$ = period of f(t)

Tab. 3 MEAN TRANSMEMBRANE RESTING POTENTIAL (MRP) OF HUMAN LUNG CELLS (WI38) AT DIFFERENT TIMES (t) AFTER GAMMA IRRADIATION (D = 60 rad)

POST-IRRADIATION TIME (t)	MEAN MRP (mV)
30 MINUTES	17 ± 8
24 HOURS	14 ± 3
48 HOURS	16 ± 3
72 HOURS	14 ± 2
UNIRRADIATED CONTROL	15 ± 3

Tab. 4 MEAN TRANSMEMBRANE RESTING POTENTIAL (MRP) OF HUMAN LUNG CELLS (WI38) AT DIFFERENT TIMES (t) AFTER GAMMA IRRADIATION (D = 600 rad)

POST-IRRADIATION TIME (t)	MEAN MRP (mV)
3 HOURS	8 ± 2
24 HOURS	13 ± 2
96 HOURS	14 ± 3
UNIRRADIATED CONTROL	15 ± 3

Tab. 5 MEASURES OF CENTRAL TENDENCY FOR THE TRANSMEMBRANE RESTING POTENTIAL (MRP) OF HUMAN LUNG CELLS (WI38) 24 HOURS AFTER ALPHA IRRADIATION (FLUX: 2 α/CELL)

QUANTITY	VALUE
MEAN MRP (mV)	13
VARIANCE	9
SKEWNESS	-0.2
KURTOSIS	2.8

Tab. 6 MEAN TRANSMEMBRANE RESTING POTENTIAL (MRP) OF HUMAN LUNG CELLS (WI38) AT DIFFERENT TIMES (t) AFTER ALPHA IRRADIATION (FLUX: 2 α/CELL)

POST-IRRADIATION TIME (t)	MEAN MRP (mV)
30 MINUTES	10 ± 3
24 HOURS	13 ± 3
144 HOURS	14 ± 4
unirradiated control	15 ± 3

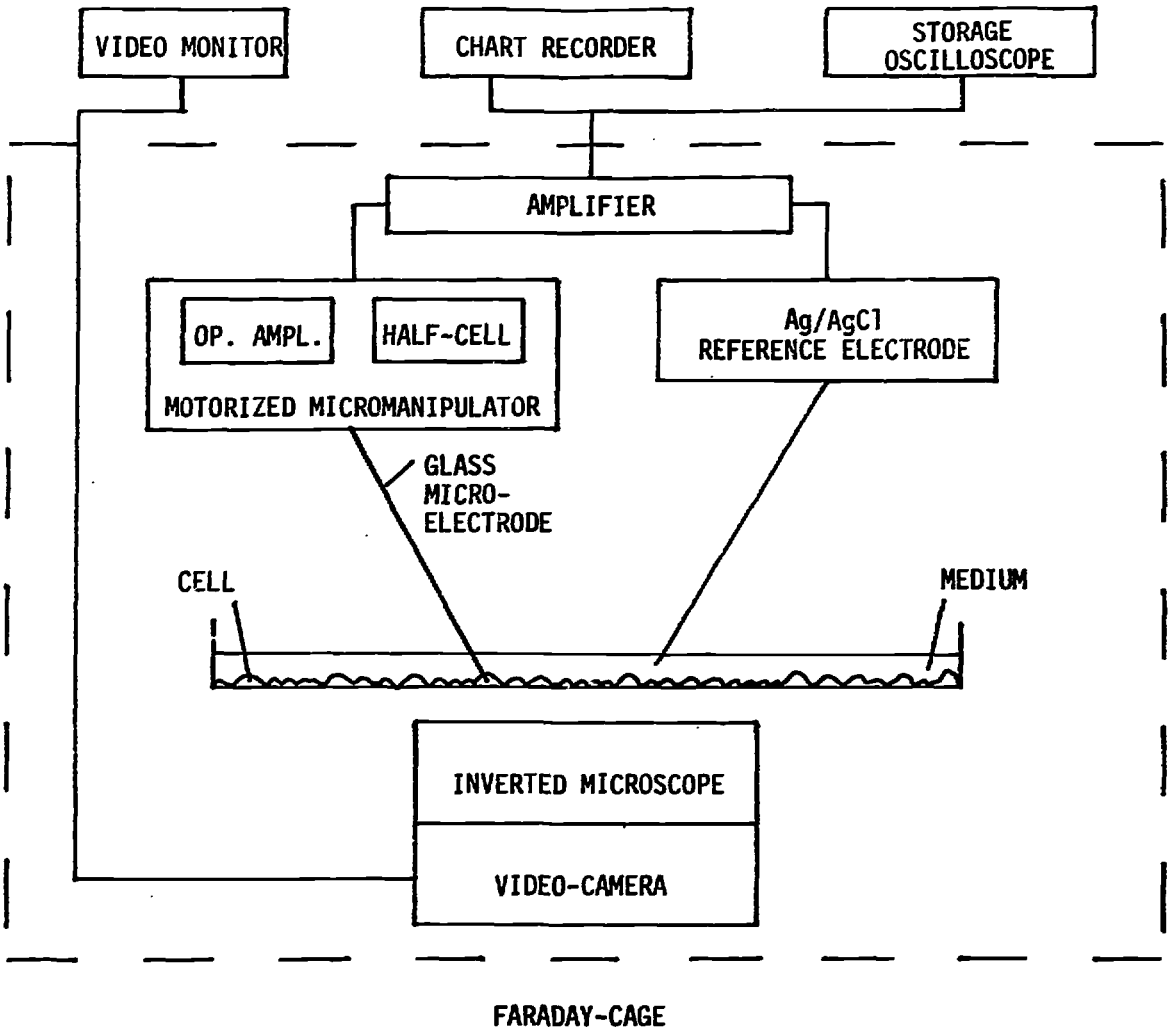


Fig. 1 EXPERIMENTAL SET-UP USED FOR INTRACELLULAR ELECTRICAL MRP-RECORDINGS FROM HUMAN CELLS

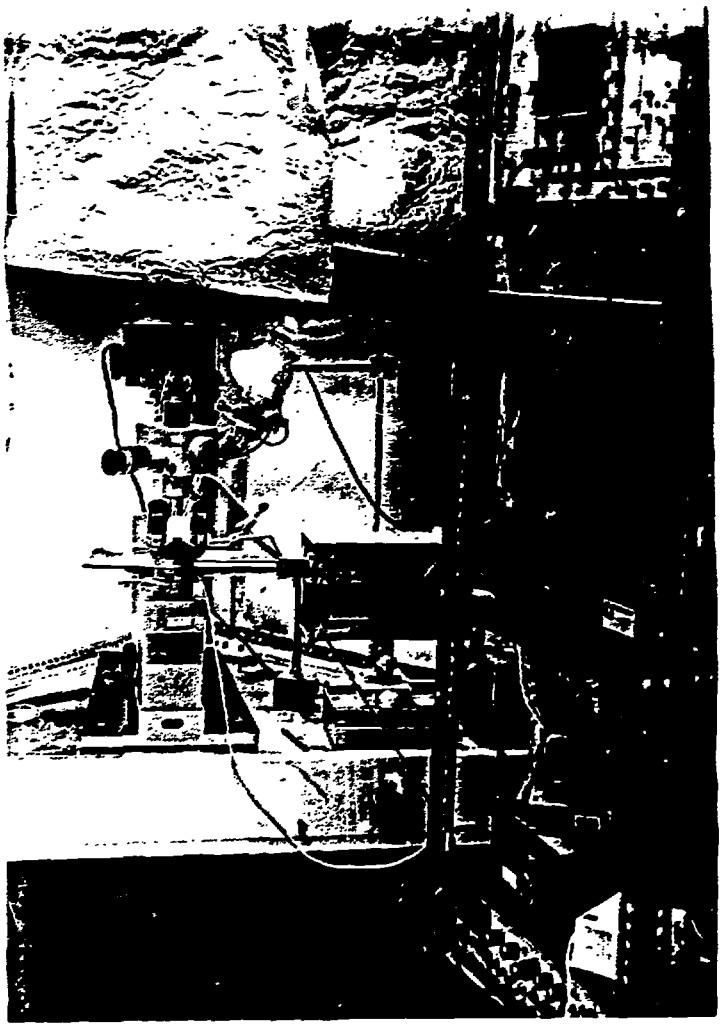


Fig. 2 TEST CHAMBER AND SYSTEM FOR CELLULAR MRP-MEASUREMENTS WITH ASSOCIATED ELECTRONICS

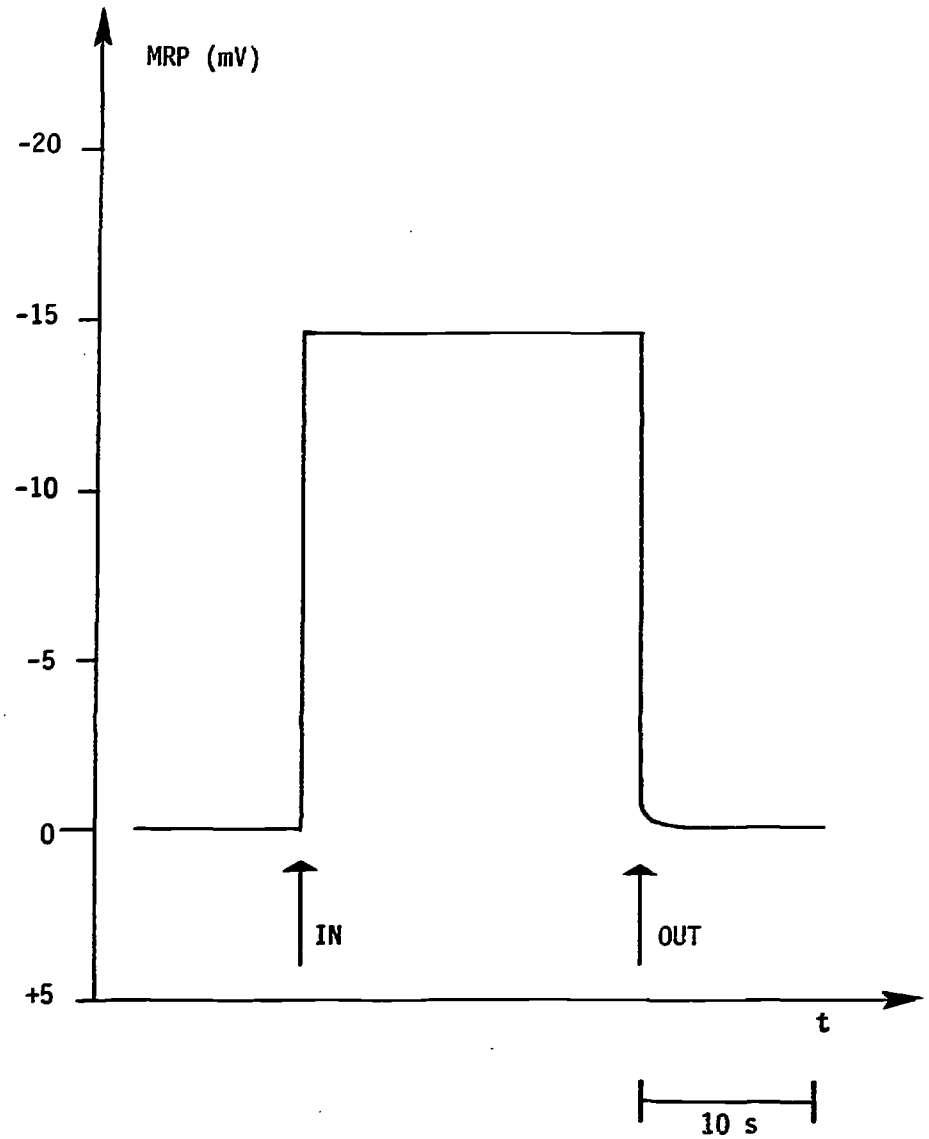
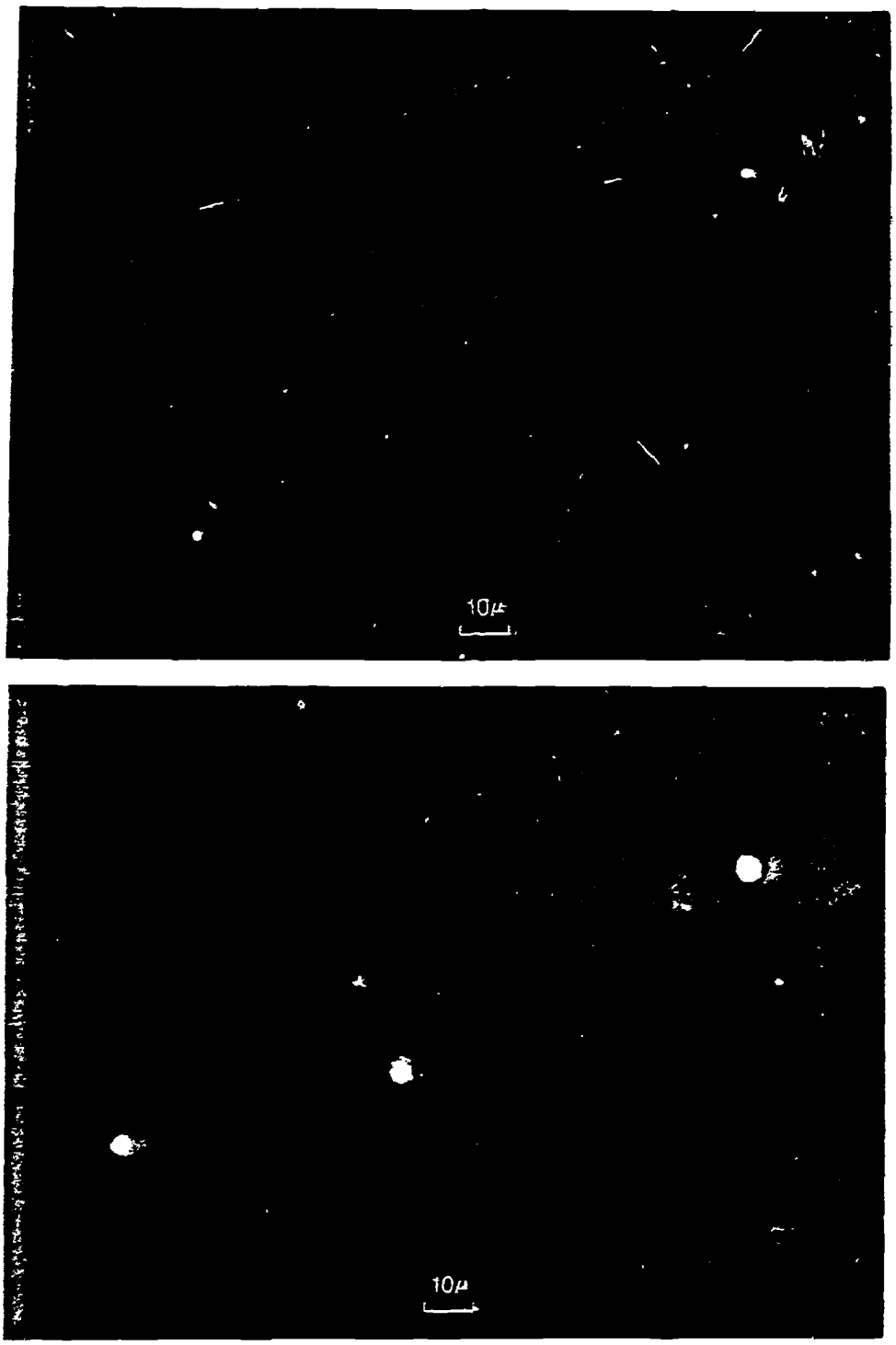


Fig. 3 MEASUREMENT OF TRANSMEMBRANE RESTING POTENTIAL (MRP) OF NON-EXCITABLE HUMAN LUNG CELLS (WI38) WITH GLASS-MICROELECTRODES



(a)

(b)

Fig. 4 ETCHED ALPHA TRACKS OF STERILIZED (a) AND UNTREATED (b) SOLID STATE PARTICLE DETECTOR (Kodak-Pathé LR 115) AFTER EXPOSURE TO 5.5 MeV ALPHA PARTICLES

(impact energy on detector surface: 2.5 MeV)

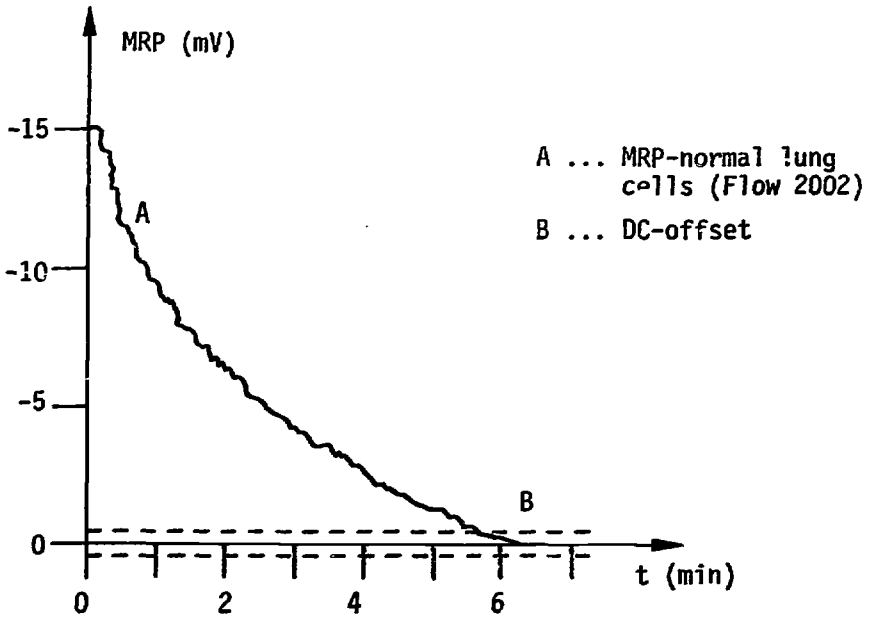


Fig. 5 EXAMPLE FOR TEMPORAL MRP CHANGES OF UNIRRADIATED HUMAN LUNG CELLS AND DC-OFFSET OF EQUIPMENT DURING IRRADIATION WITH GAMMA-RAYS AND NEUTRONS

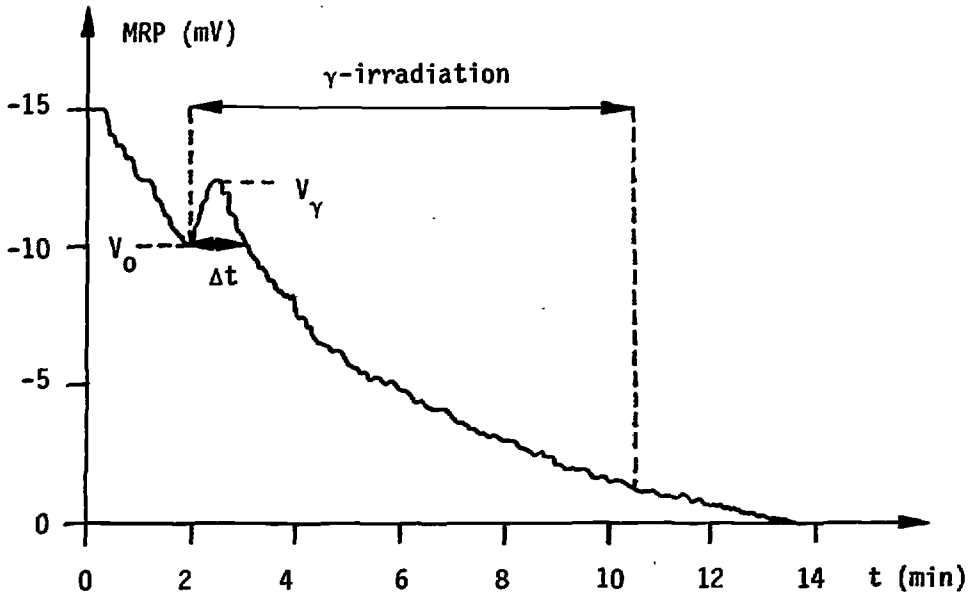


Fig. 6 EXAMPLE FOR TEMPORAL MRP CHANGES OF GAMMA-IRRADIATED NORMAL LUNG CELLS (FLOW 2002)

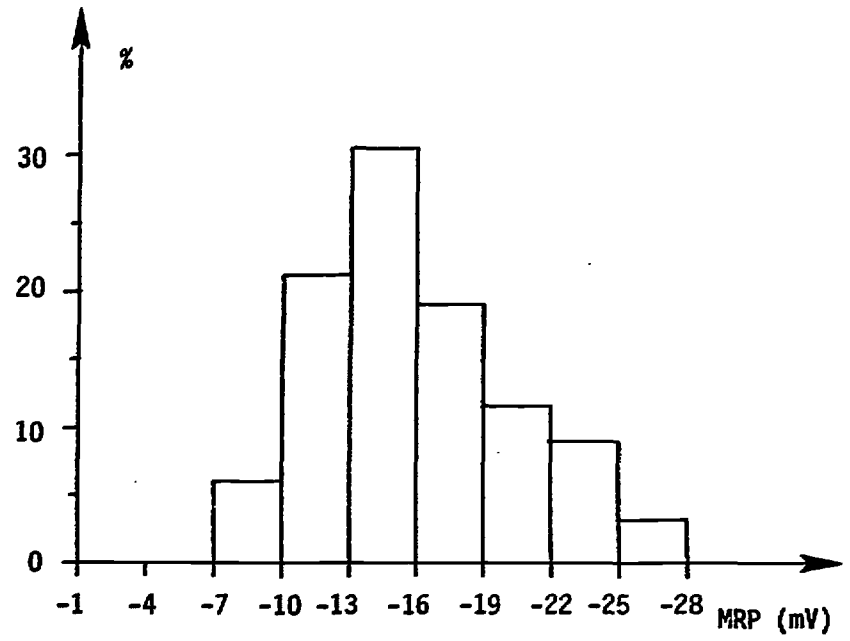


Fig. 7 FREQUENCY DISTRIBUTION OF THE TRANSMEMBRANE RESTING POTENTIAL (MRP) FOR UNIRRADIATED HUMAN LUNG CELLS (WI38)

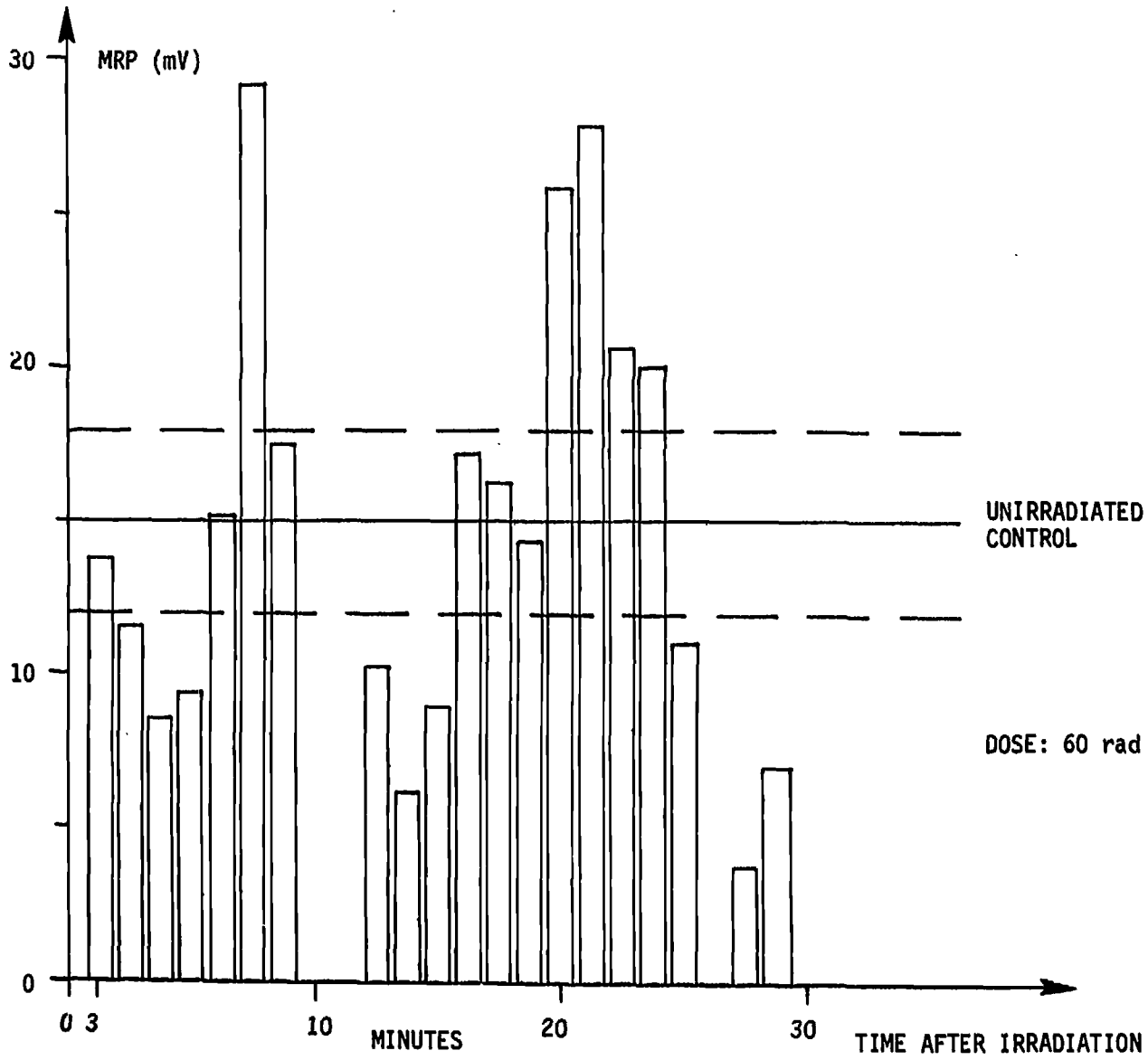


Fig. 8 TRANSMEMBRANE RESTING POTENTIAL (MRP) OF IRRADIATED CELLS (WI38) AT DIFFERENT TIMES AFTER GAMMA IRRADIATION

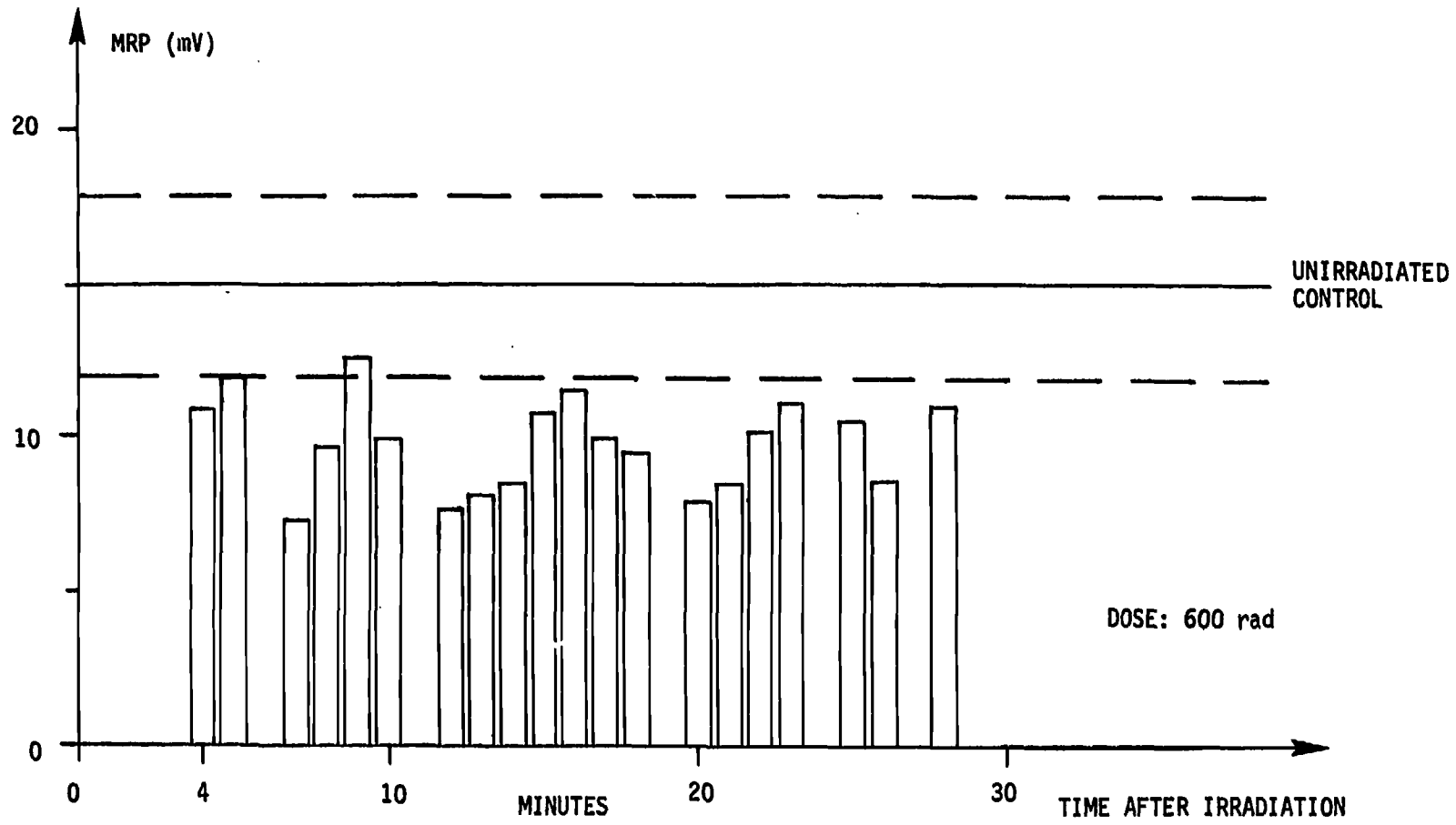


Fig. 9 TRANSMEMBRANE RESTING POTENTIAL (MRP) OF IRRADIATED CELLS (WI38) AT DIFFERENT TIMES AFTER GAMMA IRRADIATION

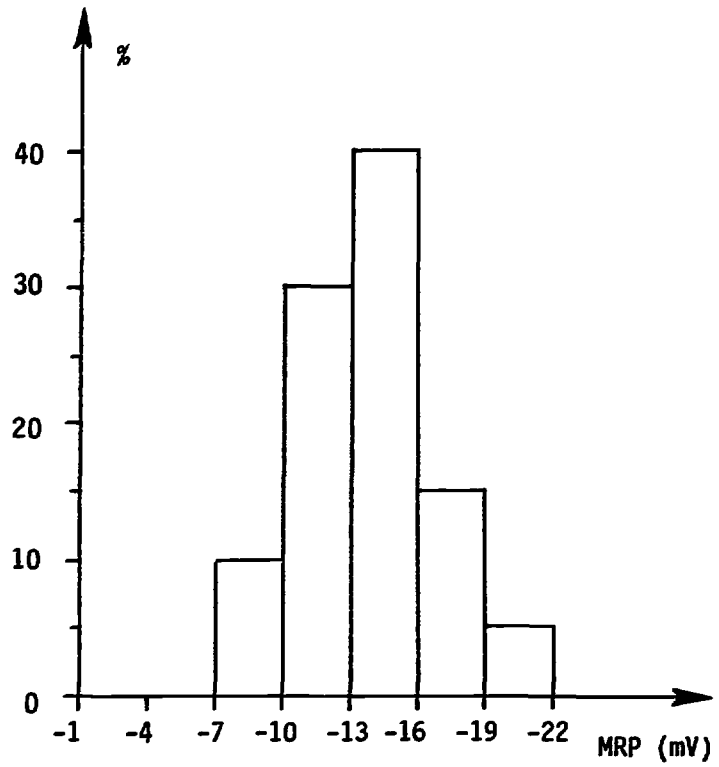


Fig. 10 FREQUENCY DISTRIBUTION OF THE TRANSMEMBRANE RESTING POTENTIAL (MRP) FOR HUMAN LUNG CELLS (WI38) 24 HOURS AFTER ALPHA IRRADIATION (FLUX: 2 α/CELL)

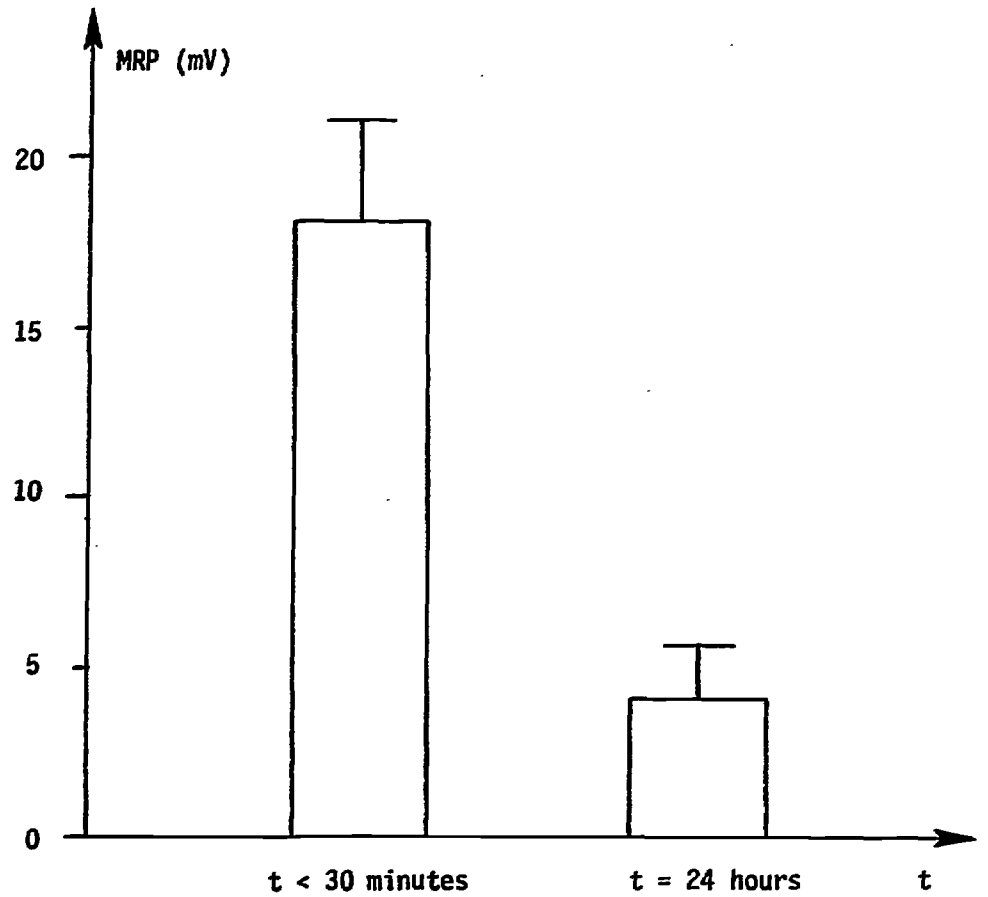


Fig. 11 MEAN TRANSMEMBRANE RESTING POTENTIAL (MRP) OF UNIRRADIATED HUMAN LUNG TISSUE SAMPLES AT DIFFERENT TIMES (t) AFTER BIOPSY

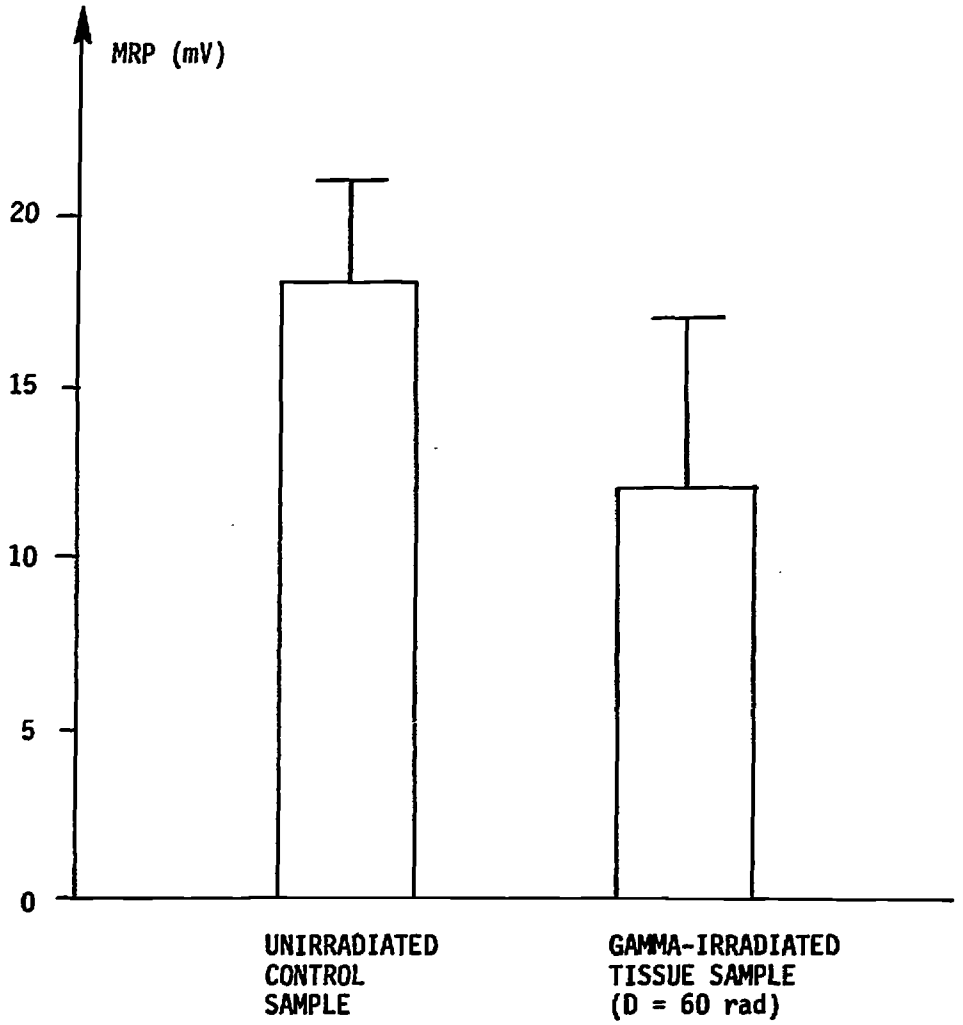


Fig. 12 MEAN TRANSMEMBRANE RESTING POTENTIAL (MRP) OF HUMAN LUNG TISSUE SAMPLES 30 MINUTES AFTER GAMMA IRRADIATION (D = 60 rad)

Relevant literature references reporting work done under this contract:

1. Hofmann, W., Steinhäusler, F. und Bernroider, G.: Computerunterstützte Zellklassifizierung und Mikrodosimetrie für inhalierte alphastrahlende Radionuklide. Proc. 2. deutschspr. DECUS-Symposium, Bonn (1979).

2. Steinhäusler, F., Hofmann, W., Pfligersdorffer, P. and Pohl, E.: Study of track ultra-structure for high LET-particles under varying environmental conditions. 10th Int. Conf. on Solid State Nucl. Track Det., Lyon (1979).

3. Hofmann, W. and Steinhäusler, F.: Cellular lung dosimetry for inhaled alpha-emitting radionuclides. Book of Abstracts of the 6th Int. Congr. of Rad. Res., Tokyo (1979).

4. Hofmann, W. und Steinhäusler, F.: Die Mikrodosimetrie der Alphastrahlung des Radons und seiner Zerfallsprodukte zur Erklärung biologischer Strahlenreaktionen auf zellulärer Ebene. Int. Symp. "Grundlagen der Radontherapie", Bad Münster, FRG (1979). Z. angew. Bäder- und Klimaheilk. (in press).

5. Steinhäusler, F., Hofmann, W. and Pohl-Rüling, J.: Dosimetric applications of cellular electrophysiological changes under high- and low-LET irradiation in Health Physics. 5th IRPA Internat. Congr., Jerusalem (1980), Vol. II:201-204.

6. Hofmann, W. and Steinhäusler, F.: Methodology of cellular lung dosimetry for inhaled alpha-emitting radionuclides. Ber. nat.-med. Ver. Salzburg 5 (1979) (in press).

7. Steinhäusler, F., Hofmann, W. and Pohl, E.: Problems related to radon in dwellings. Workshop-Report Nordic Soc. Rad. Prot. Meeting on "Natural Radioactivity in Our Environment", (Ed. F. Devik, Statens Institutt for Strahlehygiene, Østeras, Norway), Geilo, Norwwy, Jan. 1980.

8. Hofmann, W., Bernroider, G., Steinhäusler, F. and Pohl-Rüling, J.: Spatial dose distribution in the human lung from inhaled plutonium particulates. Book of Abstracts, 25th Annual Meeting of the Health Physics Society, Seattle, USA, July 1980, P/230:59.

9. Hofmann, W., Steinhäusler, F. and Bernroider, G.: Energy absorption at cellular levels from Curium isotopes deposited in the lung tissue. Proc. 1st National Congress on Nuclear Sciences, Izmir, Turkey, May 1980 (in press).

10. Hofmann, W.: Microdosimetry of inhaled alpha-emitters in the human lung. Proc. 7th Symp. on Microdosimetry, Oxford, England (1980) (in press).