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Biodosimetric Diagnostic Profile

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1. – Introduction

Dosimetry is a fundamental tool in radiopathology and radiation protection. The absorbed dose due to external irradiation, in normal conditions as well as in the case of accidental exposure, is usually measured using instruments that are sensitive to the physical effects of ionising radiation (film badges, thermoluminescence dosimeters, etc.).

In addition, for the purposes of dosimetry, internal contamination from radionuclides emitting α, β and γ rays can be directly diagnosed by special detectors (whole-body counters; local measurements on the thyroid, lungs, etc.) or by indirect methods. The latter consist in searching for the contaminating radionuclide on biological specimens (nasal mucus, urine and faeces) and then making dosimetric estimates of the internal irradiation, using mathematical models based on the metabolic characteristics and physico-chemical properties of the incorporated radionuclide.

Biological dosimetry, as well as physical dosimetry and radiotoxicology, is used in many conditions—mainly accidental. It consists basically of a study on the indicators of radio-induced biological phenomena when expressed according to a suitably correlated dose-dependent relationship.

Pasquier and Masse [1] clearly stated that: "*Dans les deux cas des dosimetries biologique et clinique, à l'opposé de la dosimétrie physique, l'individue irradié 'est son propre dosimétre'...*".

On an institutional and operational level, we recall the organisational strategy of the "Centre International de Radiopathologie" (CIR) of Paris (attached to WHO), which incorporates the Curie Institute [2, 3], and the criteria and methods adopted by the biological dosimetry laboratories of the National Radiological Protection Board (NRPB) at Harwell, Oxon, Great Britain [4, 5]. In a European context, likewise interesting is the scientific initiative undertaken (at the time) by the Commission for Radiological Protection of the Ministry of Internal Affairs of the Federal Republic of Germany to evaluate state-of-the-art biological methods for calculating absorbed dose for the purposes of practical applications, standardisation of criteria and choice of appropriate research programmes [6]. In the United States, the "Radiation Emergency Assistance Centre and Training Site" (REAC/TS) of Oak Ridge, Tennessee has become important in the field of biological dosimetry [7, 8].

In Italy biodosimetric research is carried out at only a few scientific institutes and in any case outside any organic programme. Although any permanent, dedicated facility is to be excluded, since it would not be realistically justifiable, to date no regulation exists capable of converting scientific expertise into immediate and efficient operative availability and of coping with a huge range of particular requirements (geographical, technical, temporal and quantitative).

Hence, biological dosimetry studies on ionising radiation are of great topical interest in view of the use of radiation in industry, medicine and research. Another fact worth underlining is the existence in Italy of nuclear power and research plants: although closed down, they still require constant surveillance and will constitute a complex problem *vis-à vis* radiation protection during their decommissioning. The Chernobyl [9] and Goiânia [10, 11] accidents show how entirely different events (the former due to fusion of a reactor, the latter to imprudent handling of a source of radioactive cesium) involved not only people occupationally exposed to risk but also members of the public, who unfortunately were unaware of the exposure they had undergone for quite some time. So we cannot exclude having to use biodosimetric systems to cope with accidental events involving a large number of people.

Some international research laboratories have also studied the delicate problem of individual response to radiation using different methods [12-15]. Their main concern has been that individuals or population subgroups who could be more radiosensitive might not be sufficiently protected by current radiation protection regulations.

Worth pointing out is that biological dosimetry studies can be extended to the effects of agents other than ionising radiation: radiofrequency, microwaves, extremely low frequency, and chemicals of industrial, environmental or pharmacological interest.

2. – General features of biological effects induced by ionising radiation

For a better understanding, let us first define the terms interaction, biological effect and harm, which are commonly used in the literature on radiopathology and radiation protection.

In this connection, it should be noted that *a*) when ionising radiation interacts with matter the electric equilibrium is perturbed, without this being automatically translated into an appreciable biological effect and less so into harm; *b*) to say a biological effect has taken place requires the presence of morphological and/or functional variations, in higher level structures than molecular; *c*) the occurrence of a biological effect does not necessarily cause harm to health: for this the biological effect has to exceed the limits of efficiency of the adaptive mechanisms of the organism, which vary according to age, sex, health, lifestyle, etc. [16].

The effects to be expected under the different conditions of exposure to ionising radiation will in any case be connected with the dose absorbed by the whole body or by individual organs, as the expression of the energy from the different types of radiation. Harm to man from ionising radiation can be divided into three main types: *a*) deterministic (nonstochastic) somatic; *b*) stochastic somatic; *c*) hereditary or genetic (stochastic) (fig. 1) [17].

According to ICRP publication 41 [18] deterministic effects are those whose frequency and severity vary with dose and for which a threshold dose can be identified (fig. 2). In particular, deterministic effects have the following characteristics: *a*) they appear only when the threshold dose characteristic of each effect is exceeded; *b*) when the threshold dose is exceeded, an effect occurs in *all* irradiated subjects (the value of the threshold dose is also a function of dose rate); *c*) the latency period is usually brief (a few days or weeks); *d*) the severity of clinical signs increases with increasing dose [18-20].

Hence, deterministic effects can be totally prevented by keeping doses below threshold. In this connection, the threshold values given in ICRP 41 [18], and

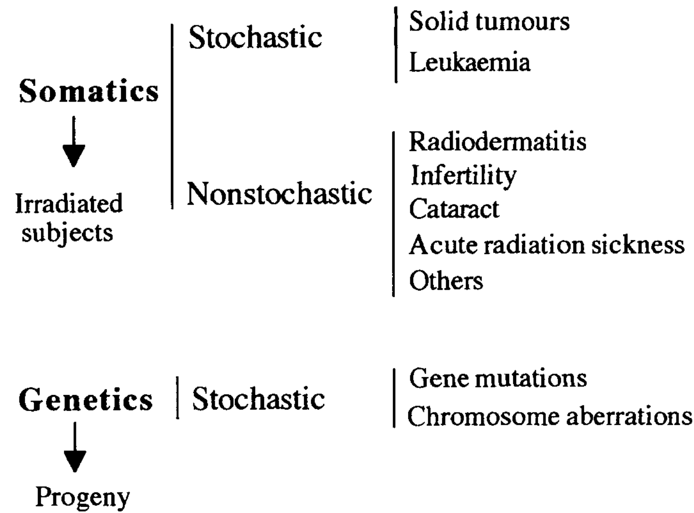

Fig. 1. – Somatic and genetic effects induced by ionising radiation.

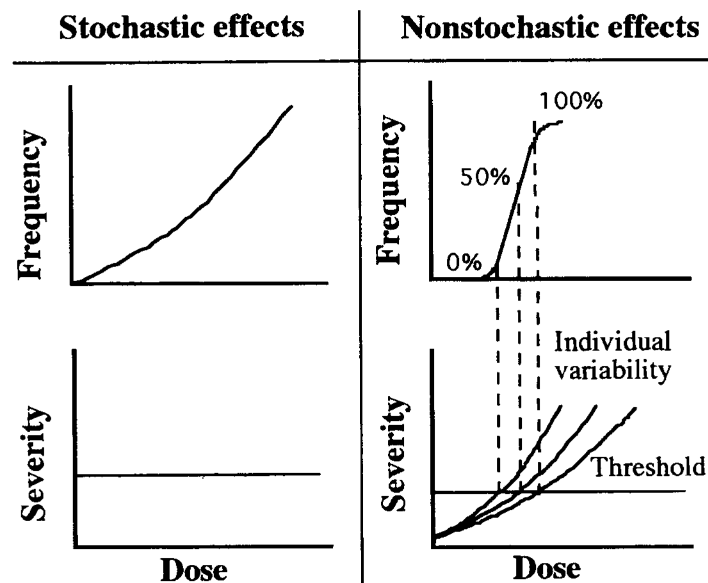

Fig. 2. – Dose dependence of stochastic and nonstochastic effects.

confirmed in ICRP 60 [20], for single short exposures and fractionated and protracted exposures (both annual and total) are very important (table I).

On the other hand, for somatic stochastic effects (fig. 2) (solid tumours and leukemia) only the probability of occurrence, and not the severity, is a function of dose and a conservative hypothesis excludes the existence of a threshold dose.

These effects have the following characteristics: *a*) it is supposed that they can occur without the threshold dose being exceeded; *b*) they are probabilistic in the exposed individual; *c*) they have a random distribution in the exposed population; *d*) they have been demonstrated by radiobiological experiments and epidemiological investigations (statistical causal association); *e*) the probability of their occurrence increases with increasing dose; *f*) they can appear years, and even tens of years, after irradiation; *g*) they do not appear gradually in relation to the dose received but are an "all-or-nothing" type, whatever the dose; *h*) radio-induced tumours cannot be

TABLE I. – *Threshold dose for nonstochastic effects.*

Tissue and effect	Thresholds		
	Total dose equivalent received in a single brief exposure (Sv)	Total dose equivalent received in highly fractionated or protracted exposures (Sv)	Annual dose rate if received yearly in highly fractionated or protracted exposures for many years (Sv yr^{-1})
Testes			
Temporary sterility	0.15	NA	0.4
Permanent sterility	3.5	NA	2.0
Ovaries			
Sterility	2.5–6.0	6.0	> 0.2
Lens			
Detectable opacities ([a])	0.5–2.0	5.0	> 0.1
Visual impairement (cataract)	5.0	> 8.0	> 0.15
Bone marrow			
Depression of hemtopoeisis	0.5	NA	> 0.4
Fatal aplasia	1.5	NA	> 1

NA: not applicable, since threshold is dependent on dose rate rather than on total dose.
(*a*) Minimum detectable lens opacities.

distinguished from neoplasias caused by other cancerogenic agents (molecular biology has not yet identified a genetic signature in radio-induced tumours [19-23].

For stochastic somatic effects, a linear-type dose-effect curve with extrapolation through the origin of the coordinates (absence of threshold) is admitted as a conservative hypothesis. Alternatives to this model are: linear with threshold, linear-quadratic and quadratic with and without threshold. The linear no-threshold model is processed on the basis of epidemiological observations concerning exposure to intermediate and high doses (the Japanese survivors of the atomic explosions, patients undergoing medical irradiation, exposed workers). Epidemiological data are fairly numerous for high doses, but somewhat rare for intermediate doses and lacking for small doses (silent zone). For doses of the order of 1 Gy the occurrence of tumours in a wide group of exposed people (thousands) is sufficiently high to establish beyond doubt that radiation has caused the tumours. To measure the effect of 10 mGy with the same accuracy as for 1 Gy requires a 10 000 times wider sampling [24].

The lack of epidemiological observations at low doses can be correlated to the possible absence of radio-induced effects, or to their being masked; although present they might not be observable at an epidemiological level as included in the statistical fluctuations of the "natural" or "spontaneous" incidence of tumours.

After exposure to radiation, a minimum period of response (asymptomatic) has to be taken into consideration, followed by a period at risk during which the appearance (at a diagnostic level) of radio-induced tumours is expected [25]. For all forms of leukaemia (except chronic lymphatic leukaemia for which no radio-epidemiological evidence is available) and for bone cancer (from α-radiation of radium-224), epidemiological data

TABLE II. – *Grades of radio-induced tumour (decreasing scale).*

Grade	UNSCEAR (1988)		ICRP60 (1991)
	additive model	multiplicative model	
1	bone marrow	lung	stomach
2	stomach	stomach	colon, lung
3	lung	bone marrow	
4	breast	colon	bone marrow
5	colon	breast	oesophagus, bladder
6	ovary	bladder	
7	bladder	oesophagus	breast
8	oesophagus	ovary	liver
9	myeloma	myeloma	thyroid
10	other	other	other

indicate a waveform-type of time behaviour, beginning about 2 years after exposure (minimum latency) and reaching a peak after 5–8 years, followed by a slow decrease to the values of natural incidence 30 years or less after irradiation. For the remaining tumours a minimum response time of 5 years has been established, followed by a gradual and slow increase in the probability of appearance up to 10 years. This increase, again probable, is constant in the subsequent period [25]. A conservative hypothesis extends the risk period to a lifetime. Each type or istotype of tumour shows a different grade of radio-induction (table II).

Rather surprisingly, in radiopathology uncertainties exist on the definition of the dose amount. Emblematic of this situation is the title of the work by V. P. Bond, L. E. Feinendegen and J. Booz: "What is a *low dose* of radiation?" opening the Workshop on "Low Dose Radiation and the Immune System" in May 1987, Dreieich-Frankfurt (Germany) [26].

Apart from complex microdosimetric analyses, which can be found in the proceedings of the Workshop, a more practical reference is the UNSCEAR publication of 1988 [23], classifying dose quantities as follows: *a*) Low-LET radiation ("sparsely ionising radiation"): low 0–0.2 Gy; intermediate 0.2–2.0 Gy; high 2–10 Gy; very high 10 Gy. b) High- LET radiation ("densely ionising radiation"): corresponding dose equivalent in Sv. *c*) Dose rate for all types of radiation: low 0–0.5 mSv/min; intermediate ~ 25 mSv/min; high > 0.05 Sv/min (50 mSv/min).

3. – General aspects of biological dosimetry

Hence, the term *biological dosimetry* means the set of methods that enables a measurable correlation between absorbed dose and radio-induced biological events (fig. 3). Biological dosimetry is mainly applied in cases of certain or presumed accidental overexposure to radiation, in order to acquire useful data for prognosis and therapy.

Development of biodosimetric methods, which support or integrate physical dosimetry in the case of it being doubtful or absent, is justified by the large number of

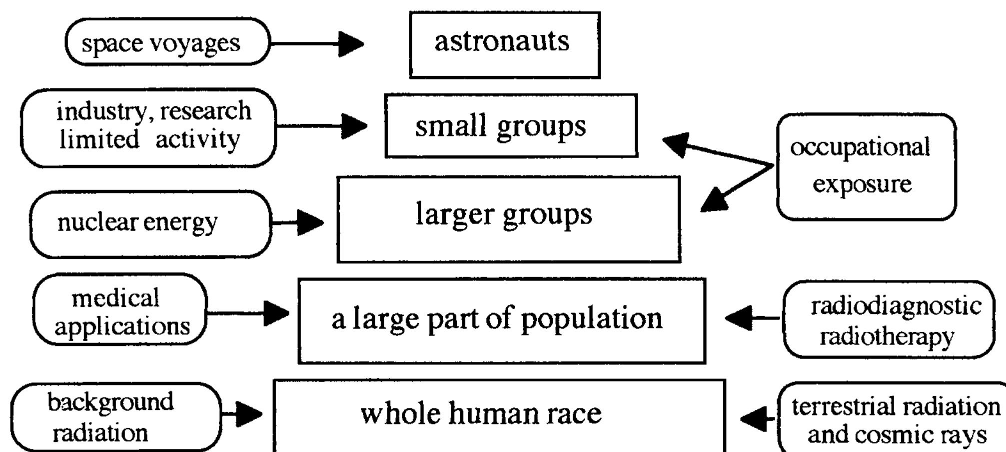

Fig. 3. – Radioexposure of groups of individuals [27].

people occupationally "exposed" to the risk of ionising radiation (in Italy an estimated value of 100 000 individuals in industry, research, health services, etc.).

As demonstrated by the Chernobyl accident, these methods are applicable in the case of the population being accidentally exposed to ionising radiation. Also, as biological dosimetry is an important diagnostic tool in radiopathology and radiation protection, it can be used to obtain significant information on the dose response to radiation at individual level or on groups at risk. The studies on the variability of patients' responses to radiotherapy are equally important.

Biodosimetry can be used in the following areas:

- accidental exposures;
- medical surveillance (individuals or groups) of workers exposed to the "risk of greatest dose";
- individual radiosensitivity;
- multiple risk exposure;
- challenge assay for revealing congenital or acquired defects in DNA repair;
- space or high-altitude flights;
- oncological radiotherapy;
- forensic medicine (*e.g.*, demonstration of causality in radio-induced neoplasms);
- animal models in environmental surveillance.

On an organisational level, the methodology chosen should be well validated and able to provide useful results in terms of feasibility, significance and cost acceptability [28, 29]. Training and information activities, therefore, are absolutely necessary to guarantee correct interpretation of the significance of dose-dependent clinical and cytological data. Facilities able to provide cytogenetic-based dose estimates (cytogenetic dosimetry) should be identified.

Any plan of organisation should take into account not only the cases selected according to "cold" medical criteria, but also the subjects proposed by "hot" socio-political pressures. It follows that biodosimetric competence, particularly cytogenetic dosimetry, must be able to count on a precise identification of structures and means [29].

TABLE III. – *Biological dosimetric indicators.*

Clinical	prodromal symptoms (nausea and vomiting), skin radiolesions, lens opacity
Cytological	lymphocytopenia and early hypergranulocytosis, thrombocytopenia and leukopenia
Cytogenetic	chromosome aberrations and micronuclei
Biochemical	amylase and thymidine concentration in blood serum, electron spin resonance, lectin action on platelets, erythrocytes and lymphocytes
Immunological	modification of lymphocyte populations
Genetic	mutation monitoring such as resistence to 6-thioguanine and mutations of glycophorin A

TABLE IV. – *Requirements of biodosimetric assays.*

accessibility of the biological material (stress and/or pain)
sampling feasibility (time and difficulty)
sensitivity of the method (dose-range)
knowledge of the dose-effect relationship
interference factors
time for results
time of application after exposure
experimental feasilibity (individuals/day/operator)

Having synthesised the structural scenario in which biodosimetric competence is articulated, it is important to note that the phenomena which can be suitably correlated with the amount of irradiation and thereby act as biological indicators for evaluating dose are multiple (clinical, cytological, cytogenetic, biochemical, immunological, mutational) (table III) [6]. Requirements of biodosimetric assays are reported in table IV [6].

Biological dosimetry, particularly cytogenetic, is applied in the following anomalous situations:

– verification and evaluation of certain or suspected accidental overexposure in the absence of physical dosimetry data due to non-use of individual dosimeters in controlled zones;

– verification and evaluation of certain or suspected accidental overexposure of persons operating in zones where only environmental, not individual, dosimetry is carried out;

– checking of uncertain results of physical dosimetry for various reasons, including "spurious" exposure and exposure of the dosimeter to chemical agents or other physical agents;

– biological-type dosimetric integration for diagnostic, prognostic and therapeutic purposes;

– evaluation of population involvement after a nuclear emergency.

As a final remark, cytogenetic biodosimetric investigations not only have a reassuring psychological function, but also act as a deterrent against false claims of exposure.

4. – Clinical biodosimetry

From a clinical viewpoint, the prodromic symptoms (particularly nausea and vomiting) of acute radiation sickness (ARS) are normally considered biodosimetrically significant.

Their scale of values (semiquantitative) as regards the gravity of the prognosis is based on their latency period, their intensity and persistence [17, 18, 20, 30].

The parameters used in a first diagnostic and dosimetric approach to ARS are, in fact, the time of appearance and the intensity of general prodromic and local (cutaneous reactions) symptoms, as well as the gravity of the early haematological symptoms (lymphocytopenia and transient hypergranulocytosis). Vomiting and nausea due to irradiation usually appear between 20 min and 3 h after exposure.

The onset of symptoms having radiopathological significance 5 to 6 h after exposure is highly unlikely [31]. The haematological, gastrointestinal and neurological syndromes of ARS are correlated with different induction thresholds (fig. 4) [17].

The Soviet physicians recommended that many of the "traditional" thresholds should be scaled up (table V), following their clinical observations after the Chernobyl accident [32].

Likewise, for cutaneous radiolesions, in the absence of physical dosimetry, dose estimates can be performed on the basis of clinico-dosimetric criteria. Effects induced on the skin by external irradiation are typically deterministic symptoms (*i.e.* epilation, erythema, oedema, blisters and necrosis). These manifestations can be used for dosimetric interpretation, also as a function of latency. In the precocious phase, for doses of 1.5 Gy and over, conjunctival hyperemia can appear; for 5 Gy and above, skin erythema, which is often transient.

Particularly interesting are blisters, whose onset 21 days after irradiation is correlated with a dose between 12 and 20 Gy [17]. A quicker appearance implies higher doses. As for chronic exposure, the so-called "radiologist's skin", characterised by dry thin skin, with verrucas, hypercheratosis, telangiectasis and onicopathy, can be

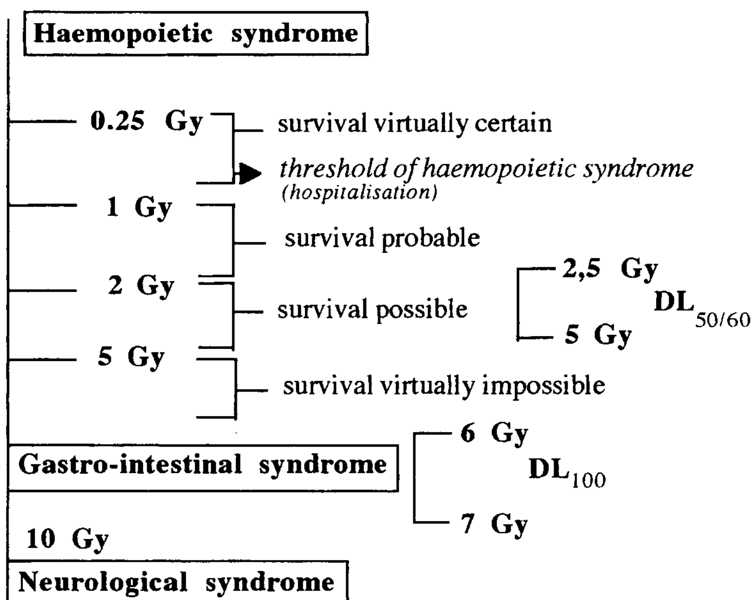

Fig. 4. – Acute radiation sickness (ARS) syndromes.

TABLE V. – *Dosimetric correlation in* ARS *according to Soviet Physicians.*

Clinical observations	Dose (Gy)
Haemopoietic syndrome	1–10
Gastrointestinal syndrome	10–20
Cardiovascular syndrome or toxaemia	20–50
Neurological syndrome	> 50

correlated with doses of the order of some tenths of a Gy/week over long periods (several months/years).

From a biodosimetric viewpoint, it should also be noted that subclinical alterations in dermal vessels, in the absence of clinical signs on the skin, can be revealed by a capillaroscope only for exposure to doses of the order of 10–30 Gy of low-LET radiation accumulated over 8–25 years [18].

Irradiation of the crystalline lens causes a cataract associated with a visual deficit with a threshold dose of about 5 Sv received in a single brief exposure, or a total dose equivalent exceeding 8 Sv received after strongly fractionated or protracted exposure [18, 20].

The latency period varies from a few months to some years as a function of the quantity and distribution of dose. Dosimetric data are also available for slight opacity of the lens. Specifically, the threshold dose for inducing "minimum detectable lens opacity" is estimated to be of the order of 0.5–2.0 Sv for a single brief exposure and of 5 Sv for strongly fractionated or protracted exposures (table I).

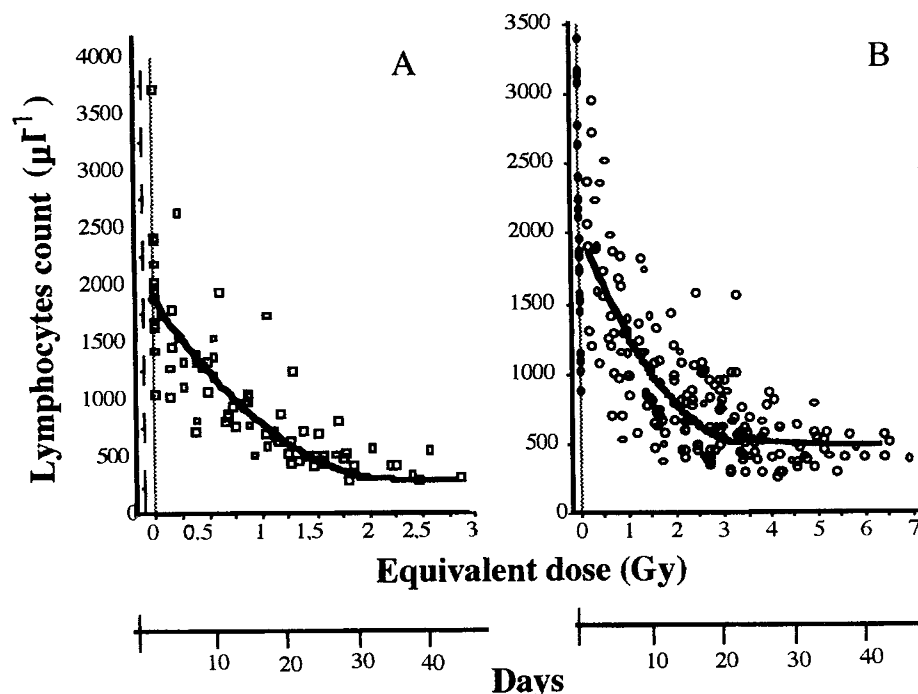

Fig. 5. – Lymphocyte decrease in patients undergoing radiotherapy in head/neck (A) or pelvic (B) sites.

5. – Cytological biodosimetry

The haematological syndrome of ARS provides valuable semiquantitative dosimetric information via the behaviour of the different cellular series [30].

In the early phase (the first two days after whole-body irradiation) the lymphocyte decrease acquires mainly diagnostic and prognostic value [17]. Lymphocyte decrease can also be significant following partial-body irradiation. We have observed this decrease in patients undergoing radiotherapy for head/neck and pelvic tumours (fig. 5) [36].

TABLE VI. – *Grades of* ARS *in the Chernobyl patients* (1986).

Grade I, 105 patients	outcome: non-lethal.
Dose	(0.8)–2.1 Gy γ radiation (biological dosimetry).
Prodromal symptoms	2 h after exposure
Latency period	over 30 days
Climax (critical period)	not associated with "significant" radiodermatitis
Hematological data	lymphocytes 600–1000/μl in the first days, leucocytes 4000–3000/μl 8–9th days and 3500–1500/μl at climax, platelets 60–40 000/μl at 25–28th days
Grade II, 53 patients	outcome: non-lethal
Dose	2–4 Gy γ radiation (biological dosimetry).
Prodromal symptoms	within 1–2 h
Latency period	15–20th days
Climax	infectious complications, moderate bleeding (absence of "significant" radiodermatitis)
Hematological data	lymphocytes 500–300/μl within 3–6 days, neutrophyles up to 1000/μl 20–30th days, platelets up to 40 000/μl 17–24th days.
Grade III, 23 patients	outcome: 7 patients dead between 2nd and 7th week
Dose	4.2–6.3 Gy γ radiation (biological dosimetry)
Prodromal symptoms	within 30 min–1 hour vomiting, headache, slight temperature, transient erythema
Latency period	8–17 days
Climax	epilation, high fever, infectious complications, bleeding; serious radiodermatitis in 6 patients
Hematological data	lymphocytes 200–100/μl within 3–6 days, neutrophyles $< 1000/\mu$l 8–20th days, platelets $< 40\,000/\mu$l 10–16th days
Grade IV, 22 patients	Outcome: lethal between 10 and 50 days for 17 patients; another 2 patients dead at Kiev Hospital on 4th and 10th day due to thermal burns and radiodermatitis
Dose	> 6 Gy (up to 12–16 Gy) γ radiation in 18 patients (biological dosimetry)
Prodromal symptoms within half an hour	vomiting, headache, fever
Latency period	within 6–8 days
Climax	enteritis, serious toxic state, fever, lesions of the oral cavity and salivary glands, radiodermatitis over 40-90% of the body; pneumonia
Hematological data	lymphocytes $< 100/\mu$l within 3–6 days, granulocytes 500/μl 7–9th days, platelets $< 40\,000/\mu$l 8–10th days

TABLE VII. – *Outcome and grades of* ARS *of Chernobyl patients.*

Total number of patients		237
Survival of acute phase		209
Died in acute phase (1986)		28
Survivors (to February 1996) of the acute phase		
ARS	Grade I (mild)	41
	Grade II	49
	Grade III	15
	Grade IV (severe)	1
ARS not confirmed		103
Total		209

The transient hypergranulocytosis observed 36–48 h after irradiation is also indicative in this sense; however, the dose-dependence of this response is lower than the decrease in lymphocytes [17]. Lymphocytopenia and hypergranulocytosis in association with nausea and vomiting constitute the screening generally used for a first selection of irradiated subjects following an accident [32, 34]. Although the thrombocytopenia and granulocytopenia observed at the climax (critical period) of ARS are undoubtedly of biodosimetric significance, they are much more important in guiding the choice of therapy.

In table VI the subjects irradiated at Chernobyl are classified according to four grades of severity as a function of clinical, haematological and biodosimetric data [17, 32].

The characteristic behaviour of the radiosensivity of different cells can be explained by radiation haematology [30, 35, 36].

A strong radioresistance characterises the peripheral blood cells (segmented granulocytes, erythrocytes, reticolocytes, platelets), which show signs of damage from doses of some tens of Gy.

Cells of the maturation compartment of bone marrow, which in this phase lose their replicative capability, present a low radiosensitivity. A total block of hemapoietic activity, such as can occur for a whole-body dose of 10 Gy, works through the total mitotic inhibition of the cells of the staminal and proliferative compartments of the bone marrow and lymphopoietic-tissue cells. Lymphocytes are, however, an exception in that they are extremely radiosensitive even in peripheral blood.

Table VII shows data on the survival of Chernobyl patients with ARS, updated to 1996 [37].

6. – Cytogenetic biodosimetry

Cytogenetic dosimetry, a branch of biological dosimetry, is an important diagnostic tool in the field of medical radiation protection. A knowledge of the biological basis as well as of the limitations and contra-indications of this methodology is desiderable for its application. Cytogenetic dosimetry also constitutes a further scientific and technical contribution to the specialistic quality of medical radiation protection.

6·1. *Chromosomes and their aberrations.* – The genetic material of all organisms is the double helix macromolecule—DNA—composed of a pair of intertwined polynucleotide chains. In most organismss, including humans, the DNA is organized into discrete "packages" called "chromosomes" that can be visualized and studied only during cell division (mitosis or meiosis).

During the cell reproductive cycle we can distinguish the interphase and the mitosis. In the interphase nucleus, the chromosome appears, in the G1 phase such as a unique DNA double helical, that is duplicated in the synthesis phase (S). In the G2 phase the chromosomes appears such as two DNA double helices.

During metaphase, chromosomes present a definite morphology. Each one is composed of two parallel identical structures, called "chromatids", attached to each other at a specific point along their length, called "centromere". Each chromatid consists of a unique double helix of DNA associated with histonic and non-histonic proteins.

Each species is characterised by chromosomes of a specific number, size and form. The location of the centromere is also characteristic. When the centromere is located at or near the middle of the chromosome length, the chromosome is called "metacentric"; when it is near one end, it is termed "acrocentric"; when it is somewhere in between, the chromosome is termed "submetacentric" (fig 6).

If cells are irradiated at the beginning of the cell cycle (G1 stage) the chromosomes behave as though composed of a single unit. The chromosome breaks and their eventual anomalous rearrangement produce the aberrations. Each break or aberration seen at subsequent mitosis will involve the entire chromosome (*i.e.*, both chromatids will be affected in the same way at the same point along their length). The class of aberrations involving both chromatids identically is known as "chromosome-type". When the cells are irradiated in the S and G2 phases of the cell cycle, the chromosomes behave as though they have become double structures, composed of two parallel units, either of which can be affected independently of the other [38].

The chromosome and chromatid exchanges that occur between sites located on different chromosomes are termed *interchange;* the exchanges that occur between sites located on the same chromosome are termed *intrachanges*. All types of exchanges can be *complete* (when all the "broken ends" join to give rise to the exchange) or *incomplete* (some "broken ends" do not interact and remain "free broken ends").

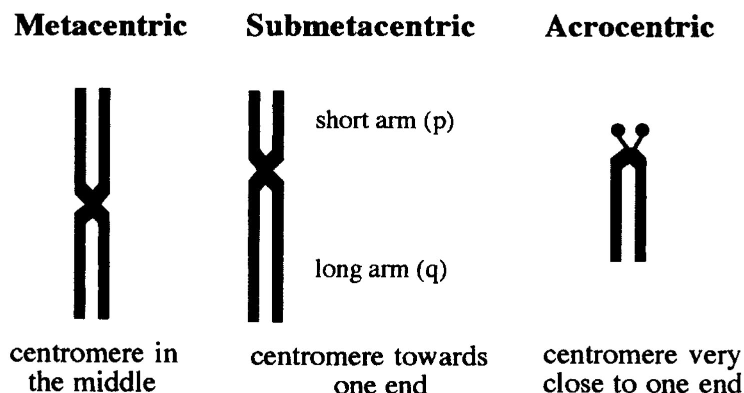

Fig. 6. – Basic shapes of human metaphase chromosomes according to the location of the centromere.

Chromosome intrachanges

Terminal deletion:	indicated by the presence of acentric fragments.
Interstitial deletion:	appears as paired dots (minute).
Acentric ring:	ring formed by paired chromatid segments without centromere.
Centric ring:	ring formed by paired chromatid segments with centromere and accompanied by acentric fragments.
Inversion:	results from interaction of "broken ends" located in the same chromosome before its duplication.
Paracentric inversion:	occurs when the broken ends are in the same chromosome arm.
Pericentric inversion:	occurs when the broken ends are located on opposite sides of the centromere

Chromosome interchanges

Reciprocal translocation:	results from a symmetrical exchange of segments between two different chromosomes.
Dicentric:	results from the exchange between (polycentric chromosomes) two (or more) chromosomes that unite to form a chromosome with two (or more) centromeres accompanied by one (or more) acentric fragments.

Therefore, the exchanges can be *asymmetrical* when one or more acentric fragments are formed, or *symmetrical* without the formation (except for incomplete exchanges) of acentric fragments. The acentric fragments resulting from breaks of the chromosomes (not associated with exchanges) are called *terminal deletions.* It is not possible to distinguish between chromosome-type terminal deletions and non-sister union isochromatid deletions, so in cases of radiation exposure it is appropriate to classify all paired acentric fragments as terminal deletions [9].

Early studies on radiation cytogenetics (1930-1940) established both the types of chromosomal aberrations induced by ionising radiation and the quantitative relationships relating the frequency of aberrations to the level of radiation exposure. It was clear that chromosomal aberration could be used as a biological dosimeter [39].

Human peripheral blood lymphocytes are normally present in a non-dividing state (G0), but after treatment with certain plant lectins (*e.g.*, phytohaemoagglutinin [PHA]) they are able to proliferate *in vitro*. This discovery gave access to samples of dividing cells from human subjects, permitting a rapid development in cytogenetic analysis, even as a clinical procedure to be used for many diseases.

It was demonstrated that ionising radiation is able to induce chromosomal aberrations in human cells after *in vitro* (of the culture) or *in vivo* (of the body) exposure. *In vitro* human lymphocyte calibration curves were then developed and during the early 1960s the cytogenetic dosimetry technique was tested on a number of cases of accidental human radiation exposure. By the late 1960s it could be said that the technique was well established [39].

Determination of the dicentric (plus centric rings) frequency in human peripheral blood lymphocytes at present is the main cytogenetic methodology (see section "cytogenetic tecniques") applied in the case of acute accidental whole-body overexposure, because their production is nearly specific to ionising radiation and their background level is low [9].

6·2. *Micronuclei.* – Micronuclei (MN) were observed for the first time more than a century ago in the cytoplasm of erythrocytes as small round-shaped structures stained like the cell nucleus. These "fragments of nuclear material" were also observed in other cellular types such as mouse and rat embryos and *Vicia faba* and called "fragment nuclei" or "micronuclei". Evans in 1959 made the first count of MN to estimate cytogenetic damage in *Vicia faba* roots after neutron and gamma-ray exposure [40].

The acentric fragments contribute to the formation of MN to a greater frequency (fig. 7). About 60% of acentrics end up as MN [40]. The acentric fragments, lacking a centromere, are not integrated in the daughter nuclei and generate MN. Acentric formation is a frequent process whenever chromosome aberrations are induced, because all aberrations, with the exception of inversions and translocations, are accompanied by one acentric fragment. Many studies demonstrated that after ionising radiation and clastogenic chemical exposure, the DNA content and the size of most MN are compatible with the assumption of acentrics being the cause of MN. Another hypothesis is that anaphase bridges caused by multicentric chromosomes disrupt and leave behind one or more fragments, forming MN [41].

The kinetochore, a structure present in the centromere of each chromatid, permits the adhesion of spindle fibres, so the chromatids can be guided to the spindle poles in anaphase. If the kinetochore is damaged, this process cannot take place and the chromatid, not integrated in the corresponding daughter nucleus, will form a micronucleus.

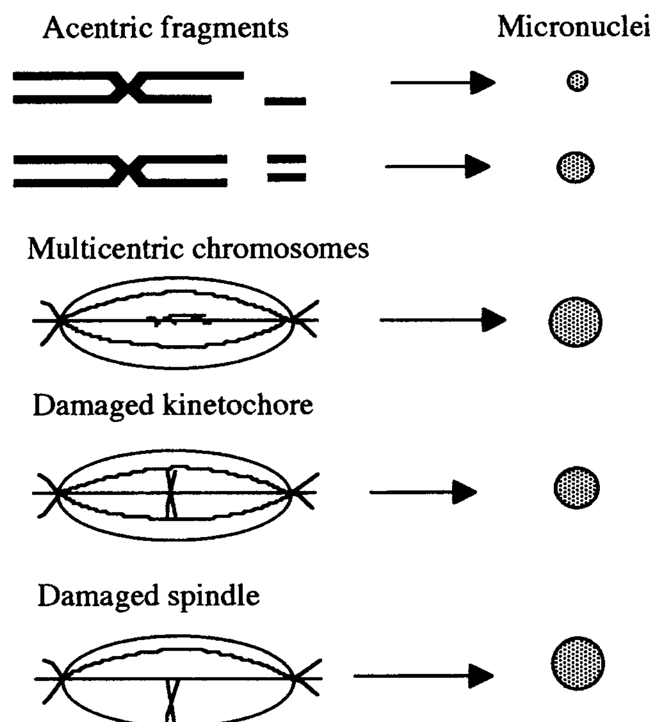

Fig. 7. – Origin of micronuclei.

Many authors who have long studied micronuclei have formulated precise criteria for their identification. The micronucleus:

– looks like a cell nucleus but is smaller;

– is DNA positive;

– is round or oval-shaped with distinct borders;

– is non-refractile;

– is coplanar with the cytoplasm;

– has size depending on cellular type and agent applied (clastogen or poison spindle); in general it ranges between 1/50 to 1/3 of the diameter of the cell nucleus [41].

Fenech e Morley [42] have attempted to improve this methodology by introducing two analytical techniques. Both are based on identification of the cells at the first mitotic division. The first, autoradiographic, consists in marking the PHA-stimulated lymphocytes with impulses of tritiated thymidine 48 h after the start of culture. By this method micronuclei can be studied in parasynchronous subpopulations at phase S. In the second technique micronuclei are observed only in cells in which the cytokinesis process has been blocked using B cytochalasin. These proliferating cells, which have completed their nuclear but not their cytoplasmic division, are easy to distinguish because they appear binuclear. Since it has been demonstrated that B cytochalasin, at least at the doses used, neither increases the micronucleus frequency nor disturbs cell kinetics, it is clear that the second method reaches two objetives: *a)* the newly formed micronuclei remain "trapped" in the cytoplasm, which has not divided; *b)* the binucleate cells (BCs) are clearly in their first metaphase. Since observation of micronuclei is limited to just binucleate cells, sample readings are easier.

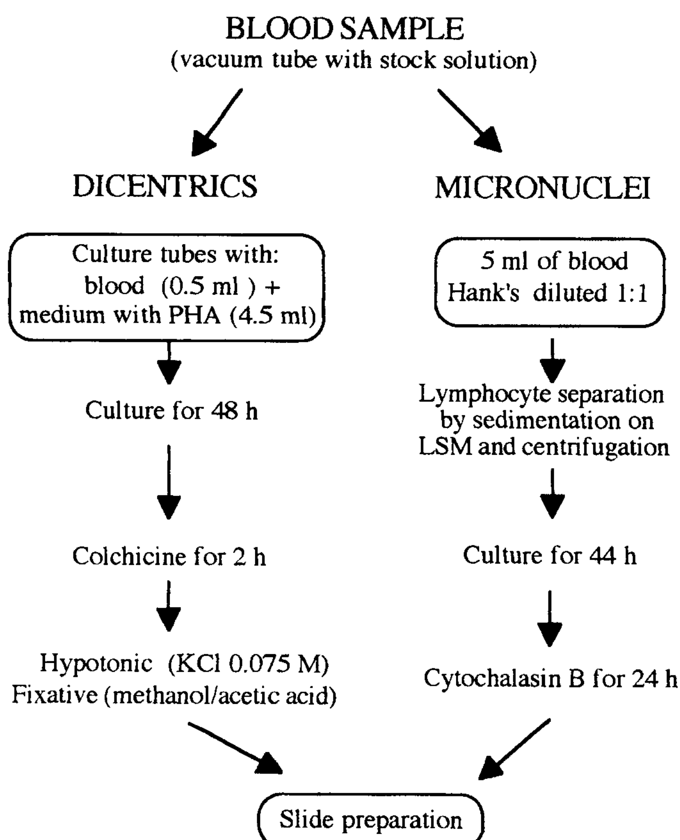

Fig. 8. – Flow chart of slide preparation for dicentric and micronucleus count.

6·3. *Cytogenetic techniques.* – Cytogenetic techniques regard the procedure for preparing slides for optical microscopy. In biodosimetry these procedures use peripheral blood lymphocytes cultivated and phytostimulated *in vitro*.

This technique has permitted strong development in human radiation cytogenetics and its application in biological radiation dosimetry [39]. Lymphocytes have many advantageous biological properties, *e.g*: 1) they are easy to obtain and culture; 2) they are ubiquitous in the body; 3) they are in the G0 phase of the interphase (resting cell), which means that they can "memorise" the damage induced by irradiation and then express it after *in vitro* culture as chromosome aberrations or micronuclei; 4) a fraction of the lymphocyte population has a long life (about 3 years) expectancy [43].

A sterile sample of a few millilitres of venous blood is collected in a vacuum tube containing heparin to prevent coagulation. Whole blood is generally used to inoculate tissue culture medium containing 20% fetal calf serum, penicillin/streptomicin (fig. 8). The lymphocytes should be stimulated to pass from their G0 phase and enter the active cell cycle. This is generally done with 1% PHA, which preferentially stimulates the T lymphocytes (fig. 9). Several different culture media can be used, including Eagle's

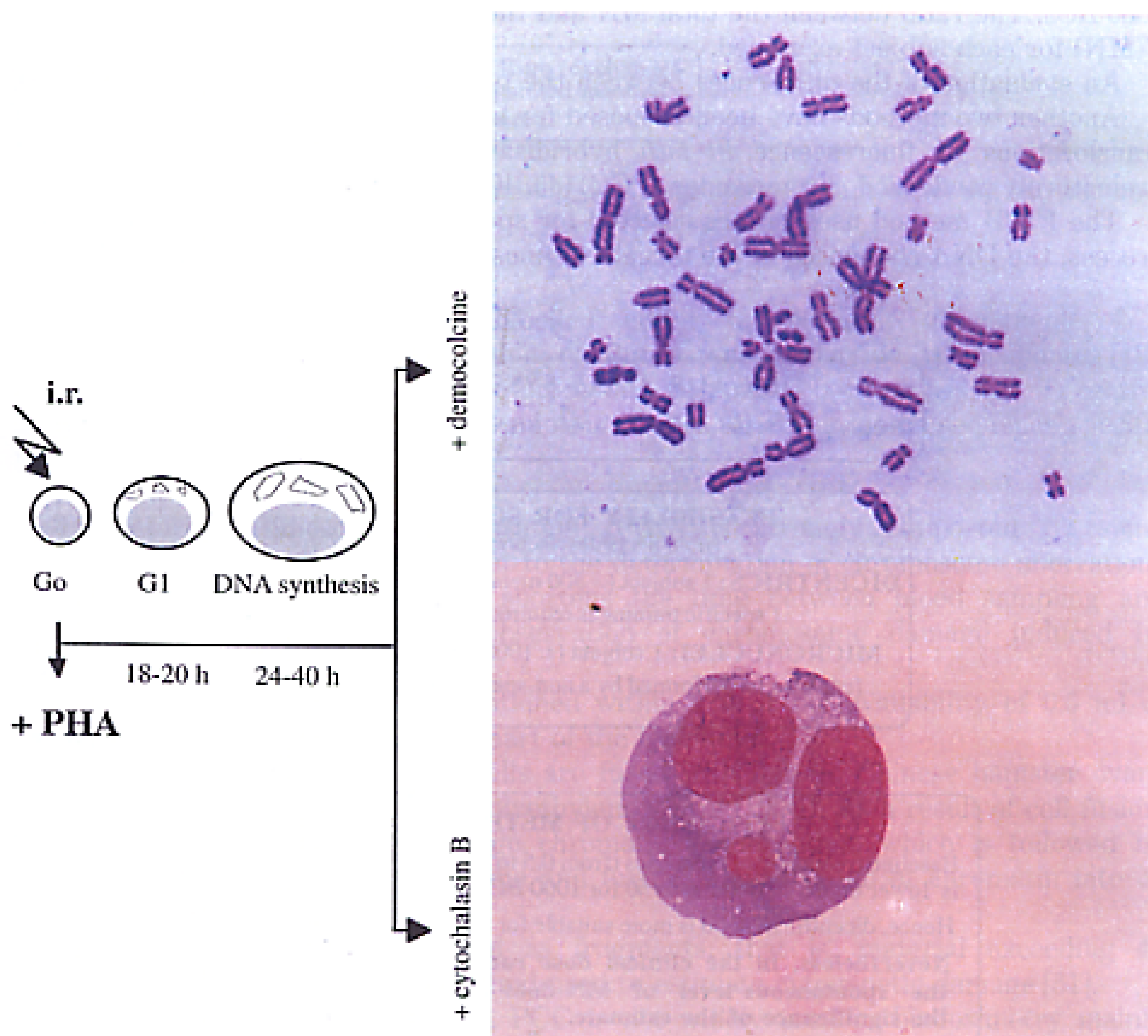

Fig. 9. Lymphocyte PHA-blastization with metaphase (above) and binucleated cells (below).

Minimal Essential Medium, McCoy's 5A, Ham's F10 and RPMI 1640. Generally, RPMI 1640 is preferred because it lowers culture time. Cultures are incubated at 37 °C in an atmosphere containing 4–5% carbon dioxide.

To select suitable metaphases, the slides are observed with an optic microscope at low magnification (usually 100–200 ×). The better spreads are then scored with an oil-immersion lens at 1000 ×. At this magnification, the scorer should count the number of individual pieces and note the presence of aberrations. Usually, metaphases outside the range 46 ± 1 centromere are rejected (Bender *et al.*, 1988). If the cell contains unstable aberrations, then it should balance. For example, a spread containing a dicentric and an acentric fragment will count 46 pieces. But, a centric ring with its fragment, will count 47 pieces. The X- and Y-stage coordinates of all complete cells analysed, including those free from aberrations, should be recorded for future reference [9].

Optical microscope observation of micronuclei is carried out at 400 × magnification. Mononucleated and binucleated cells are noted separately for a total of 200 cells; also recorded for every 100 cells is the number of BCs without micronuclei (0 MN), and with 1 (1 MN), 2 (2 MN), 3 (3 MN) and 4 or more (≥ 4 MN) micronuclei for a total of 1000 BCs. The ratio between the total MN and the total BCs gives the MN frequency F(MN) for each subject examined.

An evaluation of the differences between the two methods is synthesised in fig. 10.

Another two methods have been proposed for biological dosimetry: 1) the scoring of translocations by fluorescence *in situ* hybridisation (FISH) [44]; 2) the scoring of prematurely condensed chromosomes (PCC) [45, 46].

The FISH method uses probes selected for specific regions of the genome. In this process, the DNA sequences in the target chromosome and probe are denatured.

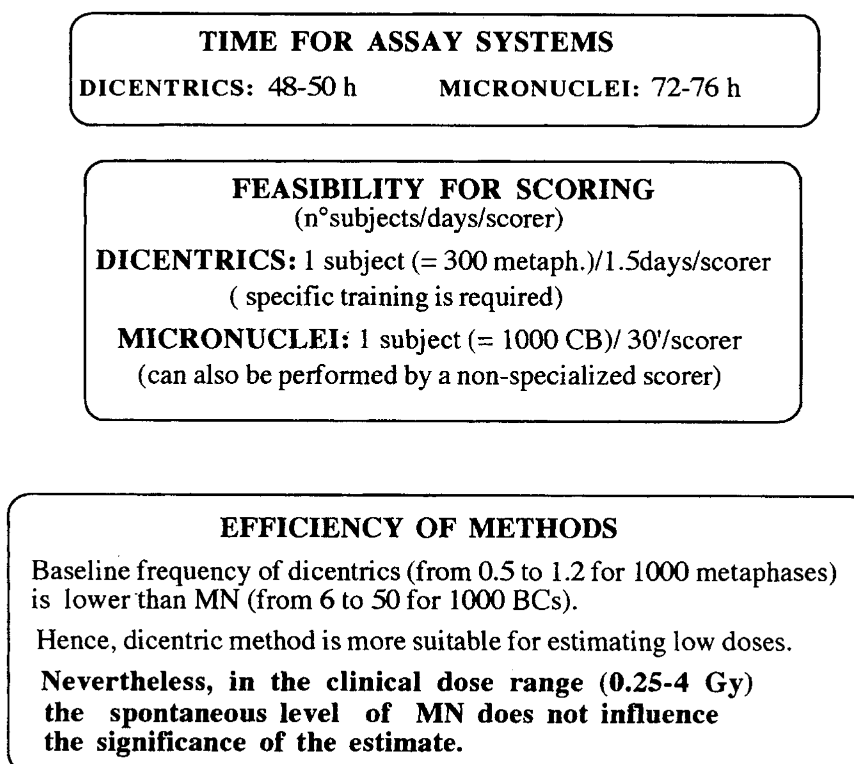

Fig. 10. – Comparison between two cytogenetic methods.

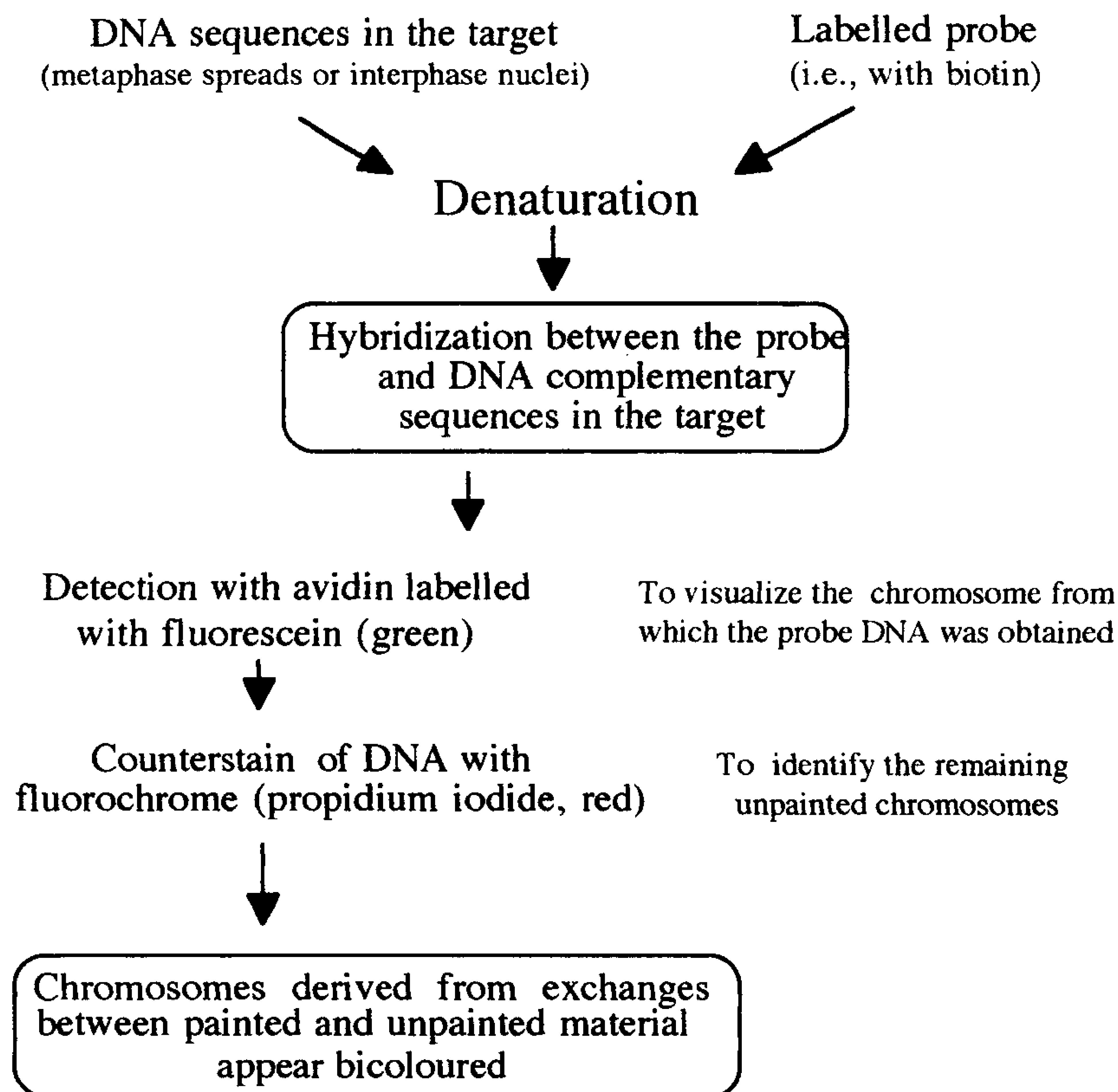

Fig. 11. – Flow chart of slide preparation for translocation count following chromosome painting.

The probes themselves are labelled either by covalent attachment of a fluorochrome [47] or by incorporation of fluorescent nucleotide triphosphates [48], hence speeding up and facilitating the procedure. Commonly used probes are: 1) centromeric probes, 2) whole chromosome painting probes, 3) chromosome sequence specific probes, 4) telomeric probes [49].

Chromosome painting preparation is reported in fig. 11 [50].

Premature chromosome condensation (PCC) is strongly supported by some international laboratories. In contrast to the determination of chromosome aberrations or MN, this method considerably shortens the time between blood sampling and analysis and enables immediate observation of chromosome damage induced by physical or chemical agents [46].

Furthermore, the results are obtained within 2 h of the availability of 0.5 ml of blood [45]. Figure 12 shows a flow chart of the technique.

Peripheral blood mononuclear cells are fused with mitotic Chinese hamster ovary (CHO) cells by means of chemical polyethylene glycol (PEG). As a result of cell fusion, the mononuclear blood cells undergo chromatin condensation, which is followed by dissolution of their nuclear membrane and further condensation of chromatin into 46 single interphase chromosomes [45].

Radiation effects are expressed as fragments, that is, material exceeding 46 interphase chromosomes is counted and used to estimate cytogenetic damage [51].

Despite the obvious advantages, PCC is not widely used in biodosimetry mainly because: *a*) its application requires the use of established cell lines, which means a constant laboratory practice for routine maintenance of the cultures; *b*) scoring of

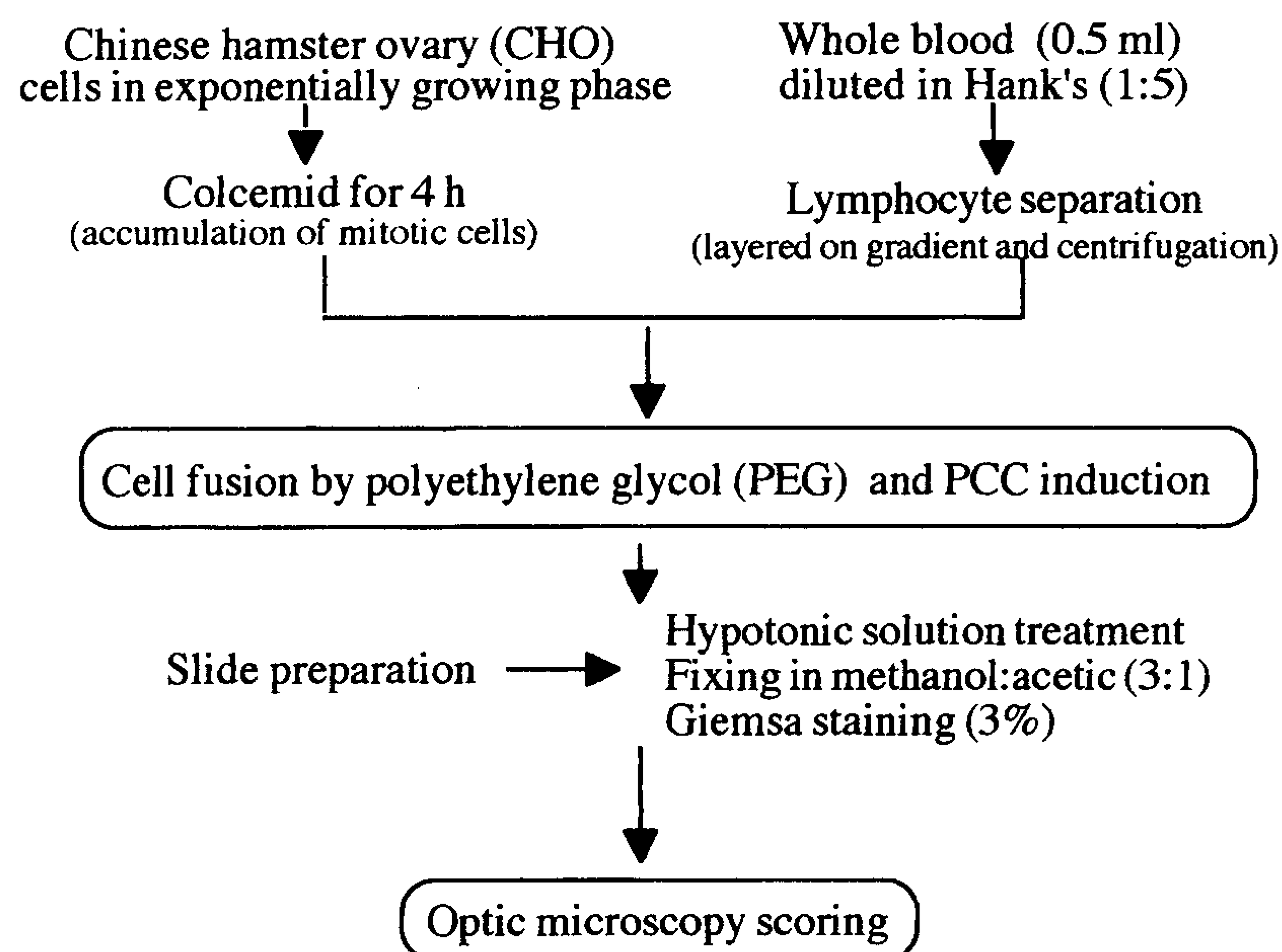

Fig. 12. – Flow chart for chromosome scoring using the PCC method.

peripheral blood mononuclear cell PCC is more difficult than for metaphase chromosomes; *c*) some factors may interfere with the fusion process, resulting in an extremely variable PCC yield, which influences the quality of the slides.

6·4. *Calibration curves.* – Ionising radiation dosimetry, consisting in counting chromosome aberrations or micronuclei in human lymphocytes, is feasible if we assume that the phenomenon follows dose-dependent function and that there is comparison between *in vivo* and *in vitro* irradiation. Since the fundamental work by Bender [52] many studies have confirmed that the dose-response curve obtained after whole-body *in vivo* irradiation with single short doses is very similar to *in vitro* irradiation.

Between 1963 and 1975 several experiments, correlated with accurate physical dosimetry, were carried out on the topic, both on laboratory animals as well as on individuals irradiated during accidents or therapy. Experimental data widely confirmed the correspondence between cytogenetic effect and dose [39, 53].

Demonstration of the fundamental theorem of cytogenetic dosimetry (correspondence between *in vivo* and *in vitro* irradiation) permits us then to develop biodosimetric calibration curves by means of *in vitro* irradiation of blood by progressive and sequential doses.

The calibration curve for cytogenetic dosimetry is a basic indispensable tool that every biodosimetry laboratory should acquire using well-defined processing criteria. Using curves belonging to other laboratories involves the risk of invalidating the biodosimetric estimate due to numerous and often unpredictable confusing factors with regard to methodology. The curves that better represent the behaviour of cytogenetic phenomena have been constructed by interpolating experimental data by means of a second-degree polynomial regression described by the equation:

$$Y = A + \alpha D + \beta D^2 , \tag{1}$$

where Y is the frequency of the cytogenetic effect at dose D; A, α and β are coefficients estimated by the least-squares method [9].

TABLE VIII. – *Cytogenetic background observed in healthy human subjects.*

Dicentric test		Micronucleus test	
Subjects	29	Subjects	150
Age range (years)	16–61	Age range (years)	18–65
Methaphases observed	7099	Binucleated cells	125 518
		F(CBwMN)	0.0238 (0.0127) (*)
Dicentric yields	0	F(MN)	0.0267 (0.0147) (*)

(*) Standard deviation.

Reference values for dicentrics and micronuclei of healthy non-irradiated subjects (background) (table VIII) as well as calibration curves for low-LET irradiation for doses up to 3 Gy and 6 Gy (fig. 13) are available at the National Laboratories of Frascati, INFN [28, 50].

With regard to interpreting the variability of the phenomenon, for a useful comparison, table IX lists basic data on the dicentric frequency reported by other authors [28].

In the first place, comparison clearly shows the inhomogeneity of data regarding the "spontaneous" dicentric frequency. The data pass from a value of zero for newborn subjects [39] to a rather high value of 0.005 for 21 adults [54]. It is also true that a value of 0.00078 obtained from 2000 individuals [55] has notable weight. Consequently, an acceptable estimate seems to be one event (dicentric) per 1000 metaphases for adults.

Continuing with our comparison between authors, the variability found in the parametric coefficients suggests further considerations: *a*) in terms of behaviour of the dose-effect curve, the polynomial model correlates with good approximation with the experimental data of each author; *b*) the difference between laboratories could be due

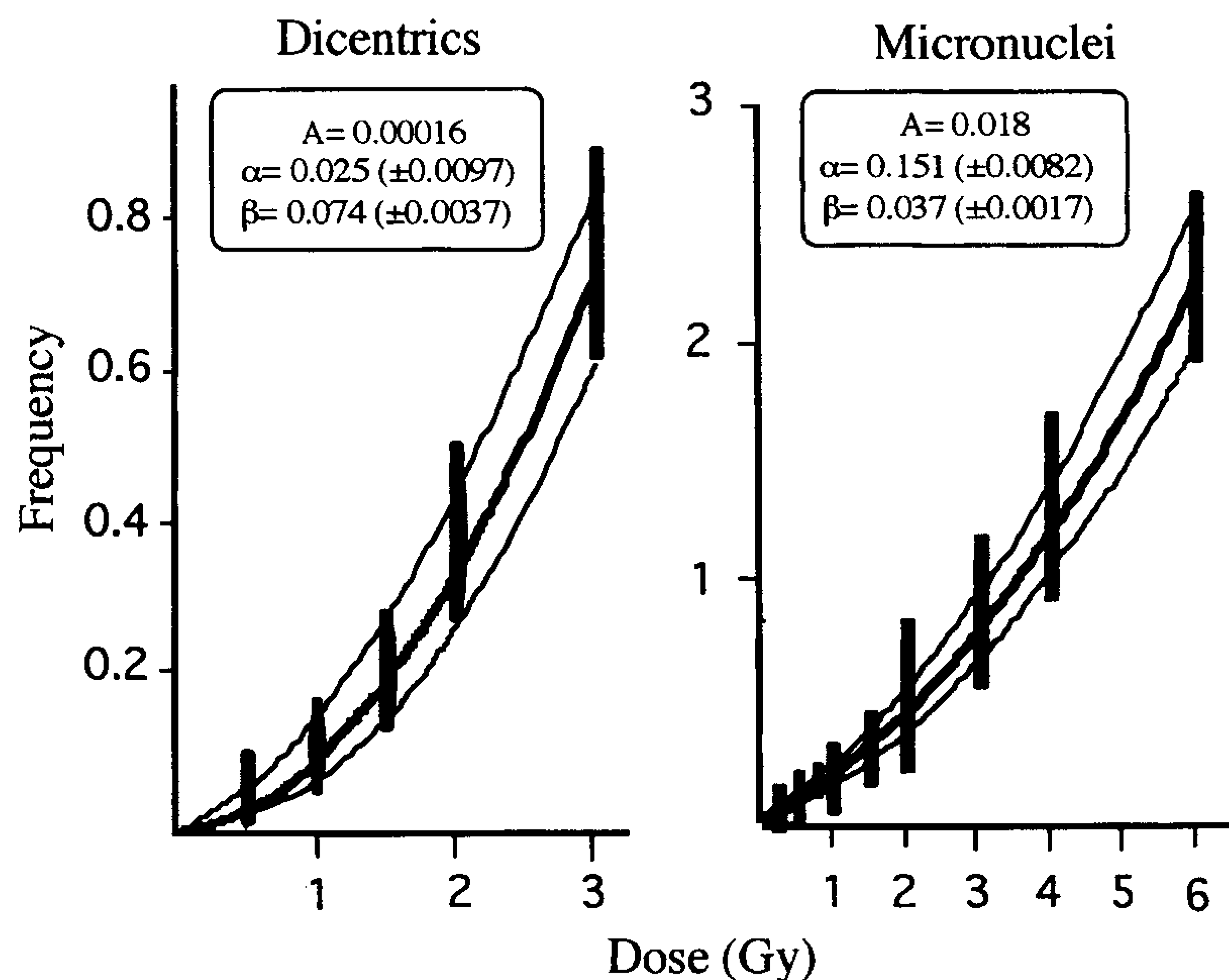

Fig. 13. – Dicentrics and micronucleus frequency in human lymphocytes after *in vitro* X-irradiation (the outside curves indicate the 95% confidence interval).

TABLE IX. – *Dicentric data and interpolation curves of different authors.*

Authors	Subj.	Background frequency	Dose range (Gy)	Linear-quadratic coefficients		
				A	alpha	beta
Evans '67	—	—	1.2–4.0	—	0.290	0.007
Bender *et al.*, '69	—	—	1.0–3.0	—	0.056	0.055
Sasaki '71	—	—	0.3–4.0	—	0.075	0.071
Buckton *et al.*, '71	21	0.005	—	—	—	—
Linieki *et al.*, '73	—	—	0.5–4.5	—	0.040	0.026
Lloyd *et al.*, '75	—	—	0.05–8.0	– 0.02	0.116	0.046
Linieki *et al.*, '77	2	—	1.0–6.0	– 0.03	0.025	0.031
Edwards *et al.*, '80	—	—	0.05–4.0	0.0022	0.037	0.028
Lloyd *et al.*, '80	2000	0.00078	—	—	—	—
Schmidt *et al.*, '76	—	—	0.25–4.0	—	0.054	0.079
Fantes *et al.*, '83	4	—	0.5–4.0	0.0024	0.120	0.118
Bauchinger, '84	—	0.00038	0.5–4.0	—	0.040	0.060
Fabry *et al.*, '86	—	—	0.05–2.0	—	0.060	0.044
IAEA '86	4	0.001	—	0.0005	0.016	0.049
Bender *et al.*, '86	431	0.0017	—	—	—	—
Lloyd *et al.*, '88	—	—	—	—	—	—
Bender *et al.*, '88	44	0.0028	—	—	—	—
Bender *et al.*, '88	4501 (*)	0	—	—	—	—
Mean	—	0.00167	—	—	0.077	0.051
SE	—	0.00066	—	—	0.022	0.008
Our data	29	0.000	0.5–3.0	0.00016	0.025	0.074
SE	—	—	—	—	0.0097	0.0037

(*) Newborn.

to different *modi operandi* (culture medium, type of PHA, irradiation modality, etc.), which are difficult to compare; *c*) the variation in response at individual level, recently re-evaluated, could strongly depend on the sample of individuals examined and could be significant in causing inhomogeneity.

This diagnostic methodology has been thoroughly tested internationally (see IAEA document on the basic aspects of application of the methodology) [9]. The IAEA has also underlined the need to find more ways to accelerate the observation and count of radio-induced metaphases and chromosome aberrations. Attempts have been made by some authors to automate reading of metaphase plates and observation of chromosome aberrations [56, 57].

Worth mentioning in this context is the contribution of the CARIOPEPR (INFN-ENEA-SSN) research group, which has tackled the problem with a Precision Encoding and Pattern Recognition (PEPR) system constructed and already used at the National Laboratories of Frascati (INFN) for automatic measurement of event images at high energy (fig. 14) [58].

The idea to develop this system came from a similar experiment carried out the University of Nijmengen in Holland with the BIOPEPR system developed for

TABLE X. – *Comparison between our micronucleus data and data reported in the literature.*

Authors	Subj.	Background frequency	Dose range (Gy)	Linear-quadratic coefficients		
				A	alpha	beta
Fenech *et al.*, '85	8	0.0088	1.0–4.0	0.010	0.075	0.007
Fenech *et al.*, '86	6	0.0100	0.05–0.4	0.010	0.070	0.026
Mitchell *et al.*, '87	?	0.0080	0.05–4.0	0.002	0.126	0.032
Fenech *et al.*, '87	?	0.0110	1.0–2.0	0.005	0.143	0.008
Kormos *et al.*, '88	4	0.0140	0.5–4.0	0.011	0.139	0.037
Ramalho *et al.*, '88	2	0.0360	0.5–3.0	0.056	0.094	0.069
Prosser *et al.*, '88	14	0.0140	0.1–5.0	0.013	0.117	0.009
Huber *et al.*, '89	14	0.0160	—	—	—	—
Littlefield *et al.*, '89	2	0.0060	0.25–3.85	0.006	0.095	0.050
Ban *et al.*, '91	3	0.0230	0.5–3.0	0.013	0.104	0.012
Gantenberg *et al.*, '91	12	0.0200	1.25–5.0	0.008	0.120	0.023
Balasem *et al.*, '91	6	0.0160	0.05–6.0	0.033	0.126	0.016
Huber *et al.*, '92	20	0.0153	3.0	—	—	—
Fenech *et al.*, '92	6	0.0115	—	—	—	—
Vral *et al.*, '92	4	0.0180	1.0–4.0	—	—	—
Surrales *et al.*, '92	10	0.0170	—	—	—	—
Lee *et al.*, '94	5	0.0099	0.5–4.0	0.079	0.025	0.032
Krishnaja *et al.*, '94	15	0.0125	0.5–5.0	—	0.055	0.013
Bender *et al.*, '94	57	0.0107	—	—	—	—
Di Giorgio *et al.*, '94	200	0.0099	—	—	—	—
Vral *et al.*, '94	6	0.0248	0.1–3.0	0.025	0.049	0.038
Mean	—	0.0149	—	0.021	0.096	0.027
ES	—	0.0015	—	0.006	0.010	0.005
Our data	150	—	—	—	—	—
Mean	—	0.0263	0.1–6.0	0.018	0.151	0.037
SE	—	0.0013	—	—	0.0082	0.0017

automated oncocytological screening of cancer of the neck of the uterus. Several computerised system for semi-automated analysis of chromosomes are now commercially available. Better prospects seem to be offered by the systems based on neural networks, which are under study [28].

Table X shows a comparison between our MN data and data by other authors obtained using similar methodological approaches. It should be noted that the results of the other authors were obtained for a limited number of persons (column 2), so the individual variability in response is not very reliable. There is a large difference, about a factor of 6, between the maximum value of 0.036 and the minimum of 0.0060 (column 3), in the spontaneous frequency of micronuclei.

This variability, which has a mean value of 0.015, is probably due more to the different experimental conditions used by the authors than to the variability between the examined individuals. It should be noted that in most cases the slides were prepared using a fixation method [28]. In such conditions it is easy to underestimate

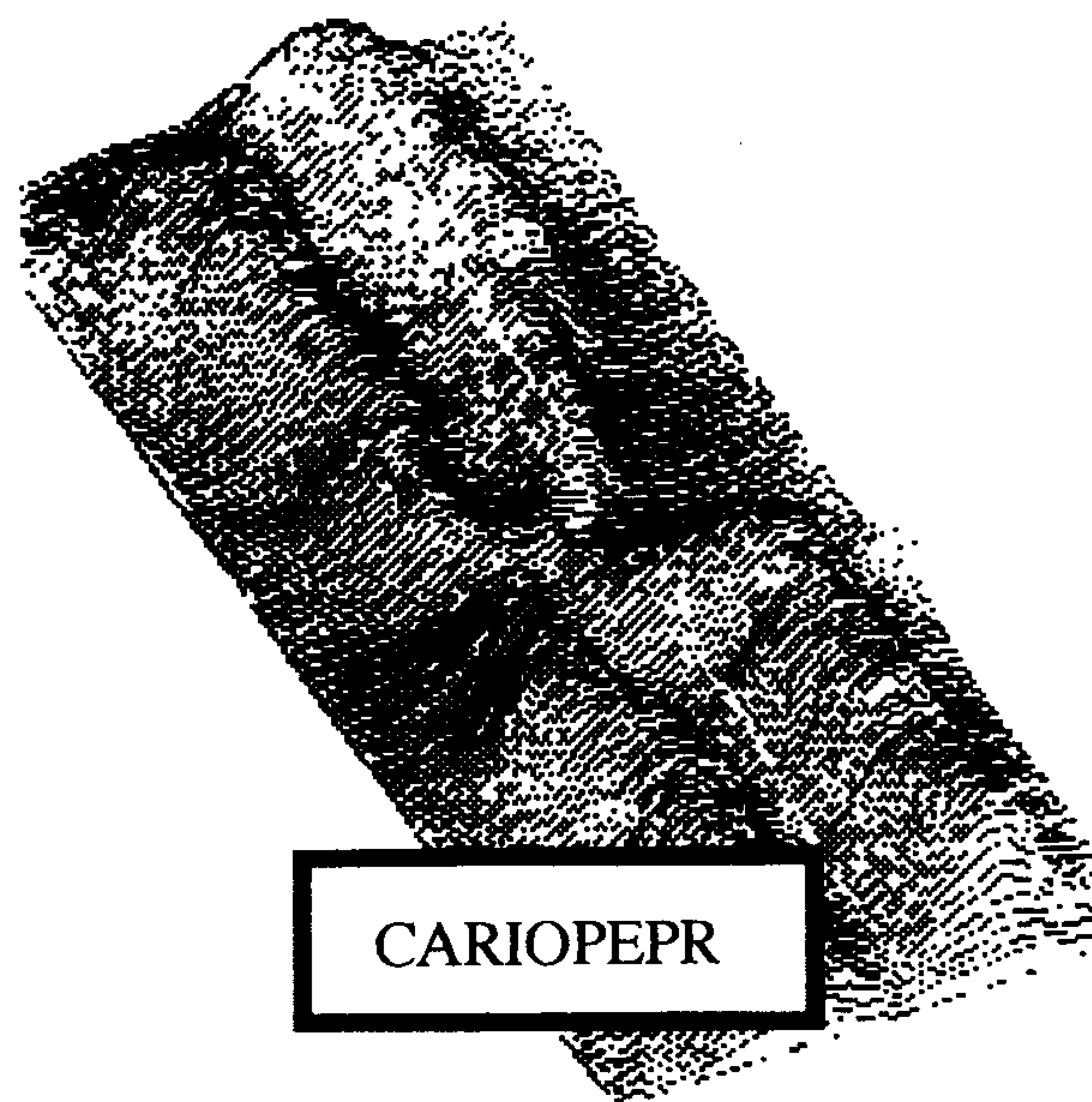

Fig. 14. – Profile of chromosome in metaphase (CARIOPEPR).

the cytogenetic event because binucleated lymphocytes are extremely delicate and can be easily changed by the fixation. Instead, the use of direct cytocentrifugation on the cellular suspension gives better preparations and easier reading. Although the variability in the observations carried out after *in vitro* irradiation continues to exist, it tends to attenuate quite significantly. With regard to the effect of single scaled doses, the frequency values appear fairly homogeneous apart from a few isolated cases.

7. – Other biodosimetric methods

Other biological methods are available for estimating absorbed dose. They are based on non-cytogenetic modifications that have a biological base, such as the glycophorin-A test and amaylasemia assay, or a physical base, such as the test on the concentration of long-lived free radicals using electron spin resonance (ESR).

7·1. *Glycophorin-A.* – Glycophorin-A (GPA) is the major red blood cell transmembrane sialoglicoprotein present in about $(5–9)\cdot 10^5$ copies per cell. It occurs in two allelic forms, GPA^M and GPA^N, of the MN blood group, which are differentiated only by two amino acids at positions 1 and 5 from the amino terminus. Because of their high sialic acid content, GPA and the other transmembrane glycophorins account for approximately 60% of the red blood cell negative surface charge. As such, they play a role in modulating the interaction between erytrocytes, as well as the red cell interaction and vascular endothelium and other circulating blood elements [59].

Glycophorin-A is the product of two co-dominantly expressed alleles mapped to human chromosome 4q28-q31. Approximately 50% of the human population is heterozygous at this locus. Mutations at the GPA locus are likely to be neutral because this protein has no essential function. Individuals totally lacking GPA on their erytrocytes do not show any apparent effect on the red cell lifetime or function [60].

Variant cells with a gene-expression-loss phenotype at one allele of this locus ($GPA^{M/0}$, $GPA^{M/M}$ or $GPA^{N/0}$ $GPA^{N/N}$) can arise through various mechanisms, so the GPA method is sensitive to different mutagenic lesions, such as point mutations in the

gene, or to chromosomal events. Glycophorin-A is expressed on proerytroblasts, so that a mutation is fixed in early precursor cells and the resulting mutants are be able to proliferate and to differentiate into variant erythrocytes in peripheral blood [59].

The GPA locus mutation assay is an indirect but quantitative method for identifying somatic allele-loss mutations in the GPA gene of bone marrow erythroid precursor cells detected as variant peripheral blood erythrocytes that fail to express an allelic form of GPA. For this purpose specific monoclonal antibodies for two allelic forms are used; they are then conjugated with different fluorescent dyes and used to label fixed erythrocytes from heterozigous MN donors. Flow cytometry and sorting are used to enumerate and purify rare, single-colour cells that lack the expression of one of the two alleles (GPA-allele-loss variant phenotypes). The GPA assay was originally developed using a dual-laser flow sorter. Later, the method was modified (BR6 assay) and performed with a simpler flow cytometry [61].

Several investigations of human populations suffering from acute whole-body exposure to ionising radiation have been conducted by means of the GPA locus mutation assay. These studies included Hiroshima atomic bomb survivors [62], ^{137}Cs exposed individuals in Brazil and Estonia [63-65] and Chernobyl accident victims [66]. They have demonstrated an increase in long-term persistent elevation of the frequency of GPA allele loss variants that is associated with the radiation dose in these populations. The GPA allele-loss variant frequencies as a function of radiation doses are interpolated by a linear regression with a slope of approximately 25 radiation-induced allele-loss erythrocytes/10^6 cells/Gy.

Results of these studies have also demonstrated that exposure to ionising radiation induces long-lived GPA locus mutations in bone marrow progenitor red cells and that these cells continue to produce circulating erythrocytes, expressing these radiation-induced GPA allele-loss mutations years after exposure. That is, this cell system represents a "biological memory" of past radiation exposure and can provide a useful tool for retrospective biodosimetric evaluation of individuals acutely exposed to relatively high radiation doses [65].

Other applications of the GPA locus mutation assay are: samples from cancer-prone individuals with known DNA repair/metabolism abnormalities (ataxia telangiectasia homozygotes and Bloom's syndrome) [67, 68] and patients undergoing chemotherapy [69].

7·2. *Amylasemia.* – Amylase is an enzyme secreted by the salivary glands (parotid) and by the pancreas; it catalyses the hydrolisis of starch and glycogen in assimilable sugars. If the amylase in the blood is measured during the first few days after

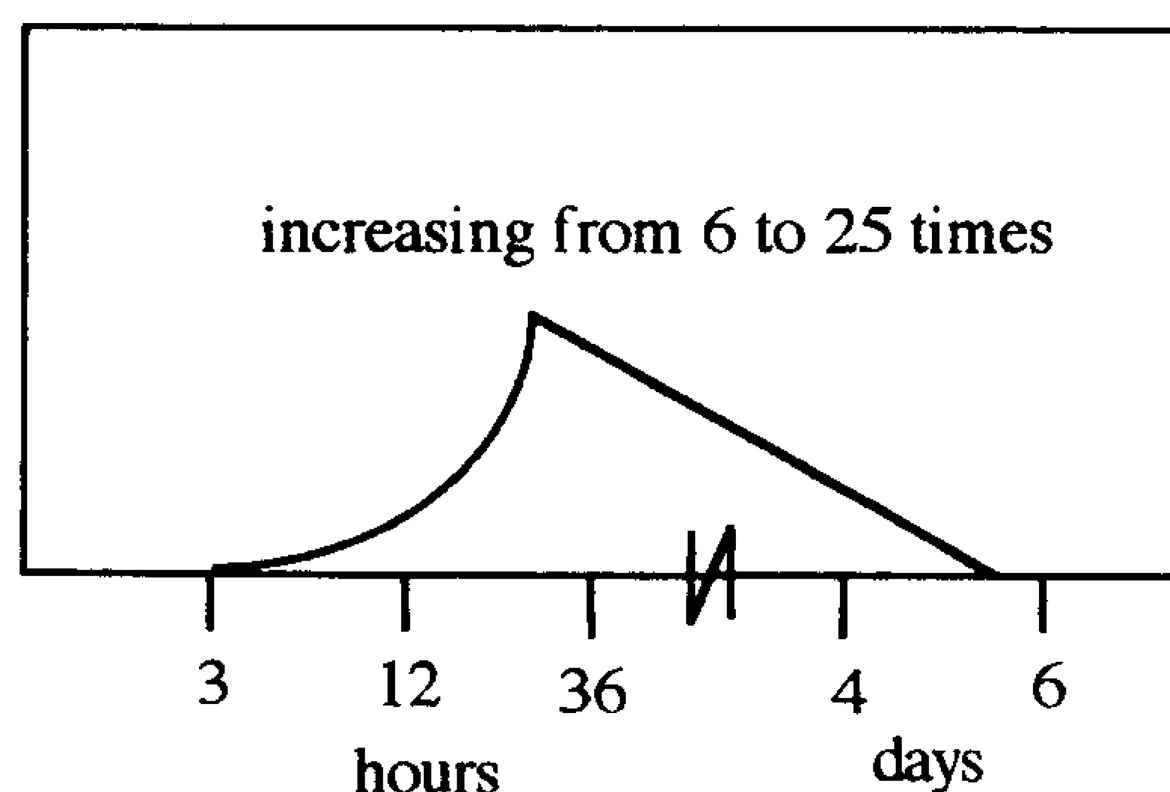

Fig. 15. – Amylasemia increase after irradiation.

irradiation, it contributes to biodosimetric analysis both as a semiquantitative indicator of dose as well as a topographic indicator (irradiation of the head) (fig. 15) [70].

No amylase increase 24 h after irradiation means the dose is lower than 1 Gy (threshold dose). The amylase measurement can then be compared with the electroencephalogram (EEG), whose radio-induced variations have the same threshold [71].

The shielding of the salivary glands prevents an increase of the enzyme in the blood. The pancreas has a much lower sensitivity also because of its deep anatomical position: no increase in amylasemia has been observed for doses up to 2 Gy [72].

7·3. *Electron spin resonance.* – Ionising radiation passing through a material gives rise to interactions with atomic orbitals and produces mainly ionisation. Whenever this elementary interaction involves an aggregate of molecules, it can cause breakage of the binding and hence splitting of the molecules, resulting in the formation of radicals. Some significant aspects of this specific process of absorption of ionising energy by a substance are: 1) The number of free radicals that form in a given substance depends on the absorbed energy, that is dose. 2) The free radicals have paramagnetic properties and hence can be detected with physical methods based on identification of uncoupled electron spin in the radical. 3) As the radicals are strongly reactive, they tend to disappear quickly from the system, giving rise to more or less disordered recombinations (including aducts on the complex DNA molecule). However, there are crystalline or plastic waterless structures (and hence also particular biological structures) that can conserve radiation-induced radicals for years or even indefinitely. 4) For some materials, irradiation gives rise to only one type of radical, but in general two or more radical species form.

As measurement of ion couples provides an efficient means of measuring dose absorbed by a material, so can measurement of free radicals. Taking into account the above points, it is possible to develop a technique for measuring the energy released by "counting" the number of free radicals induced by the radiation itself. The physical principle that enables this objective to be reached is measurement of the resonance of the magnetic moment associated with the spin of the uncoupled electron. In fact, this electron generates a magnetic field that can be resonated and hence measured by means of electromagnetic fields of suitable frequency.

Let us suppose that a material containing only one molecular type be exposed to ionising radiation and that it absorbs a dose D. The relationship between dose and number of molecules that undergo radical breakdown, similarly to what happens for radioactive decay, can be modelled and then the following differential equation imposed:

$$\mathrm{d}N/\mathrm{d}D = -k \cdot N \cdot D \,, \tag{2}$$

where N is the number of molecules of the material, D is the dose and k is a constant depending on the specific type of molelcule. If we suppose that only one free radical is formed for each induced division, the number of radicals, that is the number of resonance centres for the objectives of the measurement, is given by

$$R = N_0(1 - e^{-kD}) \,, \tag{3}$$

where N_0 is the initial number of molecules of the type present in the exposed material.

If various chemical species are present in the irradiated material, there will be several different centres of resonance from such species.

The formation of free radicals has been experimentally measured in the ascending line of the curve. The curve is practically linear within a range from 0 to 10^3 Gy.

Since free radicals are a particularly reactive chemical species, they tend to form compounds with the substance of the material; hence the centres of resonance decrease with time according to, at least experimentally, a law of the type:

$$R(t) = a \cdot \ln(t) + b \,, \tag{4}$$

where a and b are constants characteristic of the compound in question. The disappearance of radicals from the material is fairly rapid, particularly in the presence of water; however, as already said, there are compounds in which the radicals formed are nearly stable with time in normal ambient conditions. Dosimetry based on this technique and applied to biological compounds is therefore limited to materials for which the radicals are sure to be stable.

Detection of the presence of free radicals is based on the principle of magnetic resonance, which consists in exposing the material studied to an intense magnetic field and superimposing a variable electromagnetic field of suitable frequency on the material in a perpendicular direction [73, 74]. Spectroscopy can be used to measure the variable field because of the energy absorbed by the material in resonance conditions. While in the case of the atomic nucleus (and hence in nuclear magnetic resonance—NMR) the resonance frequency lies in the radiofrequency region, in the case of electron spin, it lies in the microwave region; hence, since ESR spectroscopy is correlated with paramagnetic material (which has one or more uncoupled electrons), it is also termed electron paramagnetic resonance (EPR).

The precession of the magnetic moment is the physical principle used; namely, in the presence of an external magnetic field H, the magnetic moment of the electron circulates around H with the Larmor frequency

$$\nu = g \cdot \gamma \cdot H \,, \tag{5}$$

where g is the Landè factor and γ is the gyromagnetic coefficient. If a variable field of frequency equal to the Larmor frequency is superimposed in a perpendicular direction to H, resonance is reached, with transition of the magnetic dipole to a higher energy level.

During the transition an amount of energy

$$\Delta E = h \cdot \nu = h \cdot g \cdot \gamma \cdot H \tag{6}$$

is subtracted from the microwave beam. Due to this absorption the number of resonance centres can be detected, and hence the dose absorbed by the material.

This is clearly a very sophisticated technique with numerous parameters involved, which have to be fixed material by material, even relative to the preparation of the compound to be studied and to the stability in time of the free radicals produced in the material. It is therefore necessary to prepare calibration curves giving the correct dosimetric values. For this purpose the Commission of the European Community has set up a programme on intercalibration and intercomparison between laboratories in order to verify the consistency and accuracy of retrospective EPR dosimetry [75].

We have already mentioned the fact that this technique, which is widely used for specific purposes in the field of chemistry, is applied in dosimetry when the irradiated

material guarantees sufficient stability in time of the free radicals formed after exposure. Numerous inert materials, and even biological materials, can be used to reconstruct doses due to particular events. Dental enamel has proved suitable for the purpose to [76]. In this case ionising radiation leads to the formation of the CO_3^{3-} radical because of the breakdown of the hydroxyapatite molecule; but other compounds, such as bone, hair(s), nails, esoskeletons and shells (particularly the mother of pearl used to make shirt buttons) have been studied and used.

The technique is also used to date fossils, to evaluate the preservation of irradiated foodstuff, etc. [77], but our interest here lies mainly in its application in accidental situations. For instance, it has been used to evaluate the absorbed dose in Japanese exposed to the nuclear explosions [78-80]; also worth recalling are two applications in actual radiation accidents, one described by Sagstuen [81] and the other used after Chernobyl [82-84].

The first regards exposure of a worker to about 2.5 PBq (65,7 kCi) of ^{60}Co in a γ-irradiation facility in Norway; the dose in this case was evaluated on the basis of ESR dosimetry carried out on the nitroglycerol tablets that the worker (coronary patient) normally carried. It was possible to confirm an estimate (10 Gy) based on the numerous chromosomal aberrations in a specimen containing, however, too few lymphocytes for a reliable estimate.

The Chernobyl experience regarded application of this technique to individuals acutely or chronically exposed due to their activities or to environmental contamination. Results confirmed the high levels of exposure of some categories of people and also allowed retrospective correlation of health with the pathologies found. It was shown that doses between 1 and 15 Gy had been received by six liquidators who for several years had carried out a variety of tasks inside the sarcophagus covering the Chernobyl reactor. The dose received was so protracted that deterministic effects were not apparent [85, 86].

8. – Individual radiosensitivity

The biological relevance of individual radiosensitivity was well summarised at the Second L.H. Gray Workshop, 1992: "The primary aim was to evaluate the usefulness of chromosome damage assays for identification of radiosensitive individuals and the relationship of this sensitivity to cancer predisposition" [87].

Individual response to ionising radiation is an extremely interesting radiobiological parameter in evaluating the effects of exposure both for clinical (radiotherapy) as well as for professional (occupational medicine) reasons. In short, radio-induced damage to DNA can produce notable radiobiological effects such as cell inactivation and neoplastic processes. However, in certain conditions a cell is able to modulate radiobiological damage by means of enzymatic-type repair mechanisms until re-establishment of the DNA molecular structure. It is, therefore, particularly interesting to be able to evaluate the repair capability of a single biological system or a single individual.

8·1. *Scientific references.* – Individual radiosensitivity, that is the susceptibility to exposure typical of a single subject, is well qualified to be considered in the context of radiation protection since it has assumed a position of prime importance in the last few

years in predicting deterministic effects and, to a greather extent, stochastic effects induced by radiation. In this regard the following references are interesting:

– BEIR III (1980): "...To the extent that substantial population subsets can be identified in the future as being at particularly greater risk of radiation carcinogenesis their risk will require separate consideration ..." [21].

– UNSCEAR (1986): "...Progress in cancer genetics has shed some light on possible mechanisms of differential susceptibility to cancer development in individuals with various inherited traits. The relevance of these phenomena to radiation-induced cancer requires attention ..." [88].

– UNSCEAR (1988): "...Given the genetic variability that exists between individuals within a group and between groups, it is reasonable to presume that the risk of cancer may vary among individuals of the same sex, age and apparent life-styles when exposed to the same amount of ionising radiation. A number of relatively rare, largely recessive disorders are known in which fibroblasts from trait-bearers are deficient in the repair of some radiation damage *in vitro*; it is also known that these individuals are at increased risk of a variety of malignacism, especially malignancies lymphoma and leukaemia ... The gene for A-T is relatively common in Israel, where it is expected that at least some children irradiated for *Tinea capitis* would be carriers of the gene ... the thyroid cancer pattern may also reflect this fact ..." [23].

– ICRP 60 (1991): "...There are no epidemiological data currently available which identify adult subpopulations that are hypersensitive to the induction of cancer by ionising radiation although such groups are known to exist. In the case of exposure to UV light, patients with the DNA repair deficient genetic disorder, xeroderma pigmentosum (XP) show substantially increased susceptibility to sunlight (UV) -induced skin carcinoma. In general, *in vitro* studies show that cells from XP patients are not hypersensitive to ionising radiation. Patients with the leukaemia-prone genetic disorder ataxia-telangiectasia (A-T) are, however, extremely sensitive to the effects of low-LET radiations. Cellular studies implicate DNA repair deficiency as the cause (Cox, 1982; Debenham *et al.*, 1987; Arlett *et al.*, 1989). It is important to recognise that even if all A-T patients were more likely than healthy persons to develop leukaemia, the very low frequency of the homozygous A-T mutation in the population implies an extremely small contribution to population risk. Additionally, the A-T mutation in the more frequent heterozygous form has also been suggested to confer a degree of spontaneous cancer susceptibility, particularly that of breast (Swift *et al.*, 1987). Consequently A-T heterozygotes could, in principle, constitute a small but possibly significant sensitive subpopulation, although this has not yet been established. Other human genetic disorders such as retinoblastoma where tissue specific cancers may be associated with heterozygosity for so-called "cancer suppressor genes" could also be considered to carry increased risk (Knudsen, 1986; Reik and Surani, 1989). Our current lack of knowledge on the frequency of all such mutations and their implications for induced cancers preclude, however, any quantitative estimate of their cancer yield in an irradiated human population ..." [20].

Recently (1994), the Nuclear Energy Agency (NEA), of the Organisation for Economic Co-operation and Development (OCDE) published an interesting document titled "Radiation Protection Today and Tomorrow"; among the scientific and technical reasons for making fundamental changes in the concepts and applications of the principles of radiation protection, the document reports: "...a better picture of the

mechanisms of tumour induction at the gene level from results of biological research. Recent research seems to indicate that it might be possible to identify certain radiation-induced cancers as well as individuals who are significantly more radiosensitive than normal. If this is confirmed, it would have important implications for the management of the system of protection ..." [27].

In the same document it is added that "...For example, research leading to better understanding of biological mechanisms affecting sensitivity to radiation as related to specific genetic conditions, while of great benefit, could create ethical problems in the field of radiation protection. If science were to develop the ability to determine that certain individuals are much more sensitive to radiation than normal, or at greater risk than the populations in general, ethical questions about the need for additional protection and work limitations for such persons could arise. Thus, workers might be sorted following criteria of genetic predisposition and assigned to specific posts. Specific dose restrictions might be established for those with significantly greater sensitivity to radiation than normal. Even if such an approach were socially acceptable, there would still be cause to question the real ethical and economic benefits of such a selection. It is noted, however, that health predispositions, such as allergies, lead to such selections and pose similar problems in many industries ..." [27].

It should be underlined here that Italian law makes it compulsory to evaluate the "individual sensitivity of the worker" to agents other than ionising radiation. This applies in the case of exposure to lead (Public Law n. 277/1991) and to cancerogenic agents (chemicals) (Public Law n. 626/1994).

It has also been demonstrated in *in vitro* assays that patients with lung or colon neoplasia can present a higher frequency of chromosome breakage following bleomycine treatment of lymphocyte cultures [89]. The same phenomenon of chromosome fragility (genomic instability) has also been found in young adults suffering from malignant neoplasia (squamous cell carcinoma) of upper portions of the respiratory and digestive systems (nose-pharynx, oral cavity, pharynx and larynx) [90].

In 1997 the ICRP Committee 1 (Task Group on Genetic Susceptibility to Cancer) carried out a review of genetic predisposition to cancer. It included a preliminary evaluation of the possible implications in the estimation of the risk of cancer following irradiation.

8·2. *3AB-index*. Lymphocytes after *in vitro* irradiation demonstrate that there exists a large interindividual difference as measured by the MN test (fig. 16).

In order to measure individual variability in the biological response to ionising radiation, our research group used the MN test on BCs integrated with a double estimate of the cytogenetic effect with and without 3-aminobenzamide (3AB) [91]. The 3AB compound can increase the effect of *in vitro* irradiation on lymphocytes, thus also increasing the radiation-induced cytogenetic response (increase in MN frequency). This result can be attributed to the 3AB inhibiting the activity of poly(ADP-ribose)-polymerase (fig. 17), an enzyme involved in the DNA repair process following damage (fig. 18).

Poly(ADP-ribose) polymerase is a nuclear protein of 116 kd (1014 amino acids), capable of synthesising poly(ADP-ribose) starting with the nicotinamide-adenindinucleotide (NAD) molecule. This enzyme is bound to the histone protein H1 of DNA. Newly formed poly(ADP-ribose) is able to open and distend the nucleosome structure by acting on the chromosomal protein and modifying the degree of super-coiling of

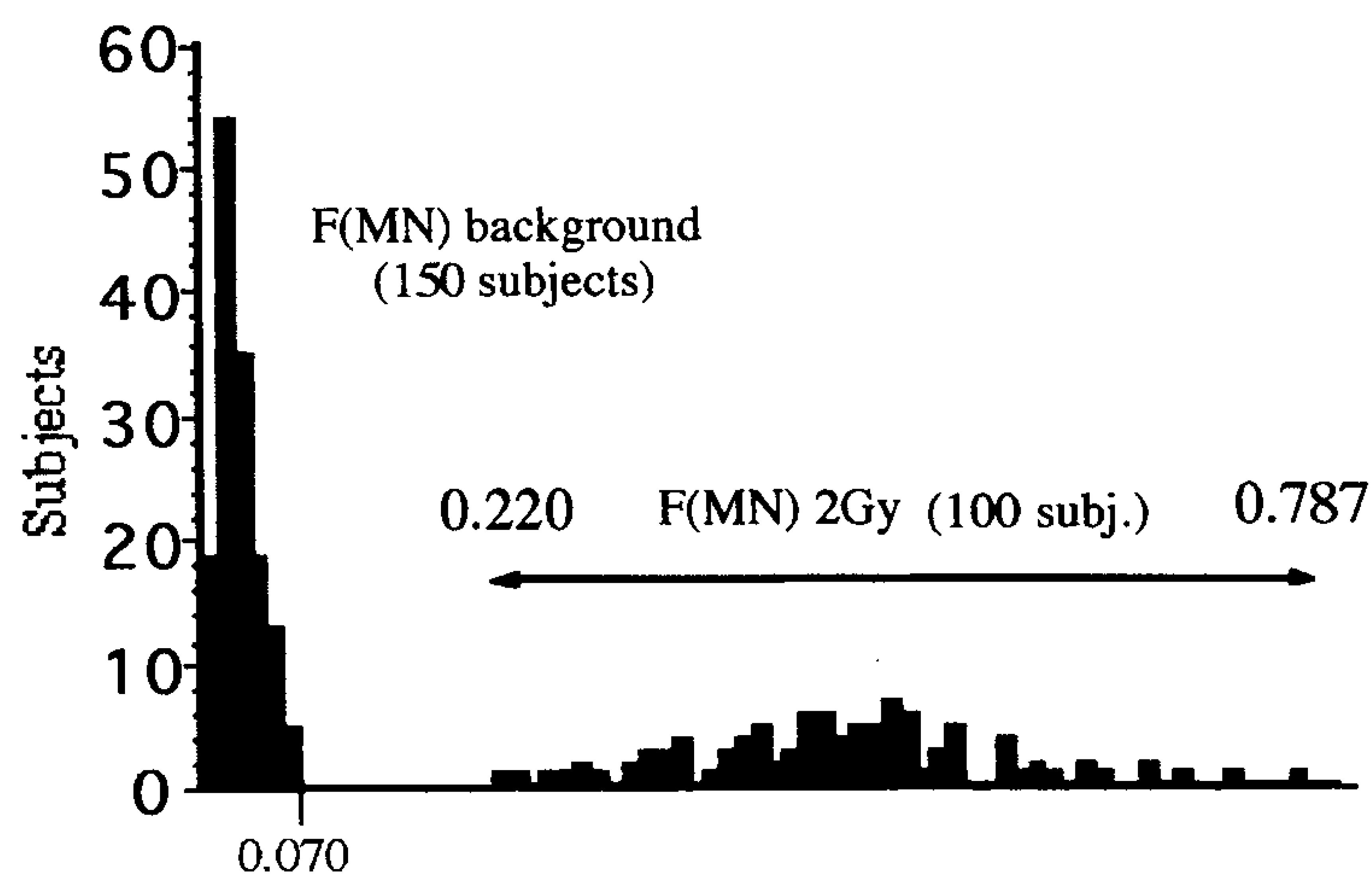

Fig. 16. – Micronucleus frequency of *in vitro* irradiated lymphocytes from healthy individuals.

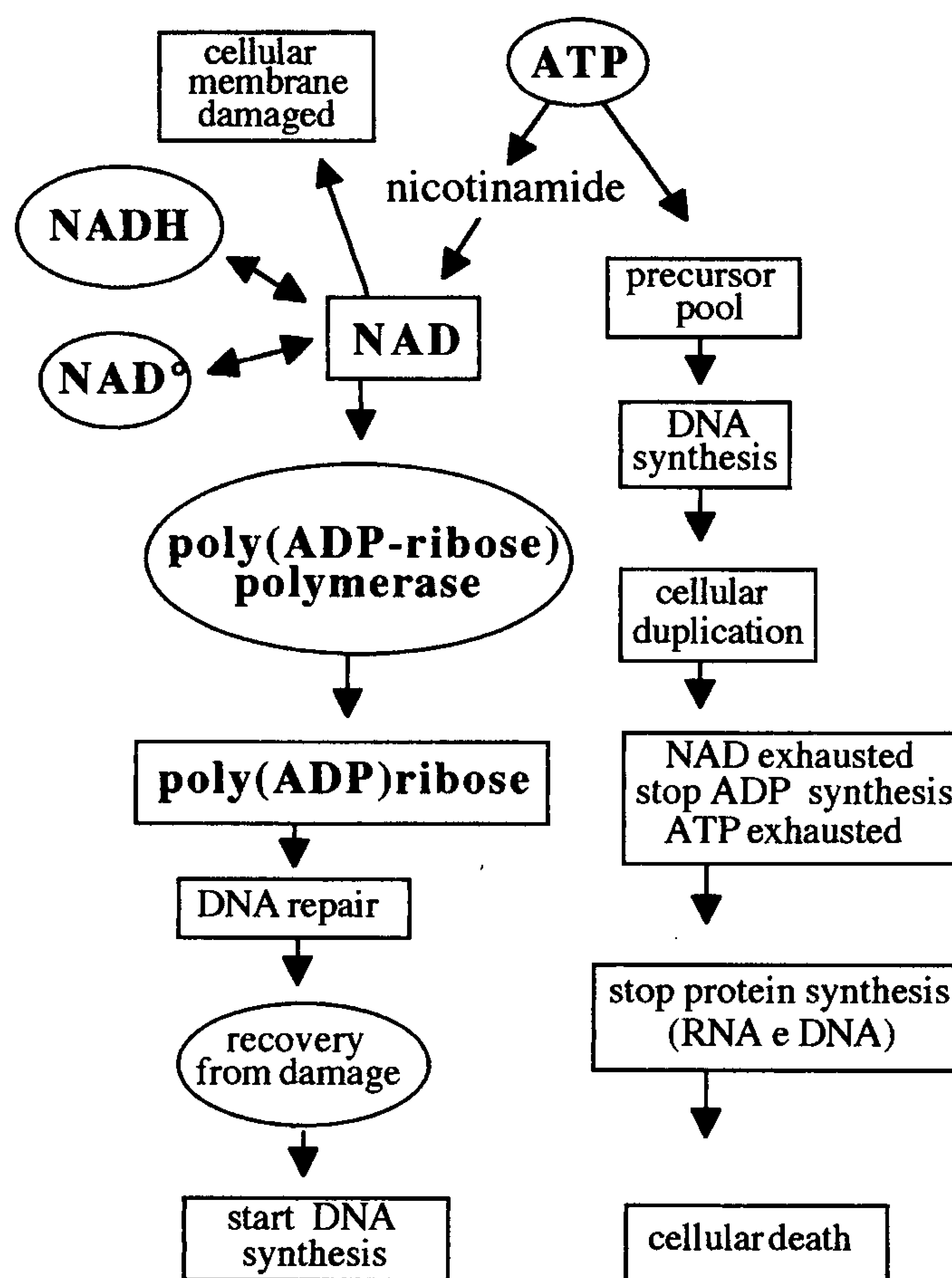

Fig. 17. – Biochemical pathway of poly(ADP-ribose)polymerase. ATP: adenosin-three phosphate. ADP: adenin-diphosphate. NAD: nicotin-adenin-dinucleotide (NADH, reduced; NAD°, oxydised)

DNA. This operation is necessary to create suitable conditions for effective DNA repair.

Further tests on the involvement of poly(ADP-ribose)polymerase in DNA repair have been carried out, using measurements of the incorporation/embedding of labelled NAD, on isolated nuclei or on cells whose membrane has been made permeable [92].

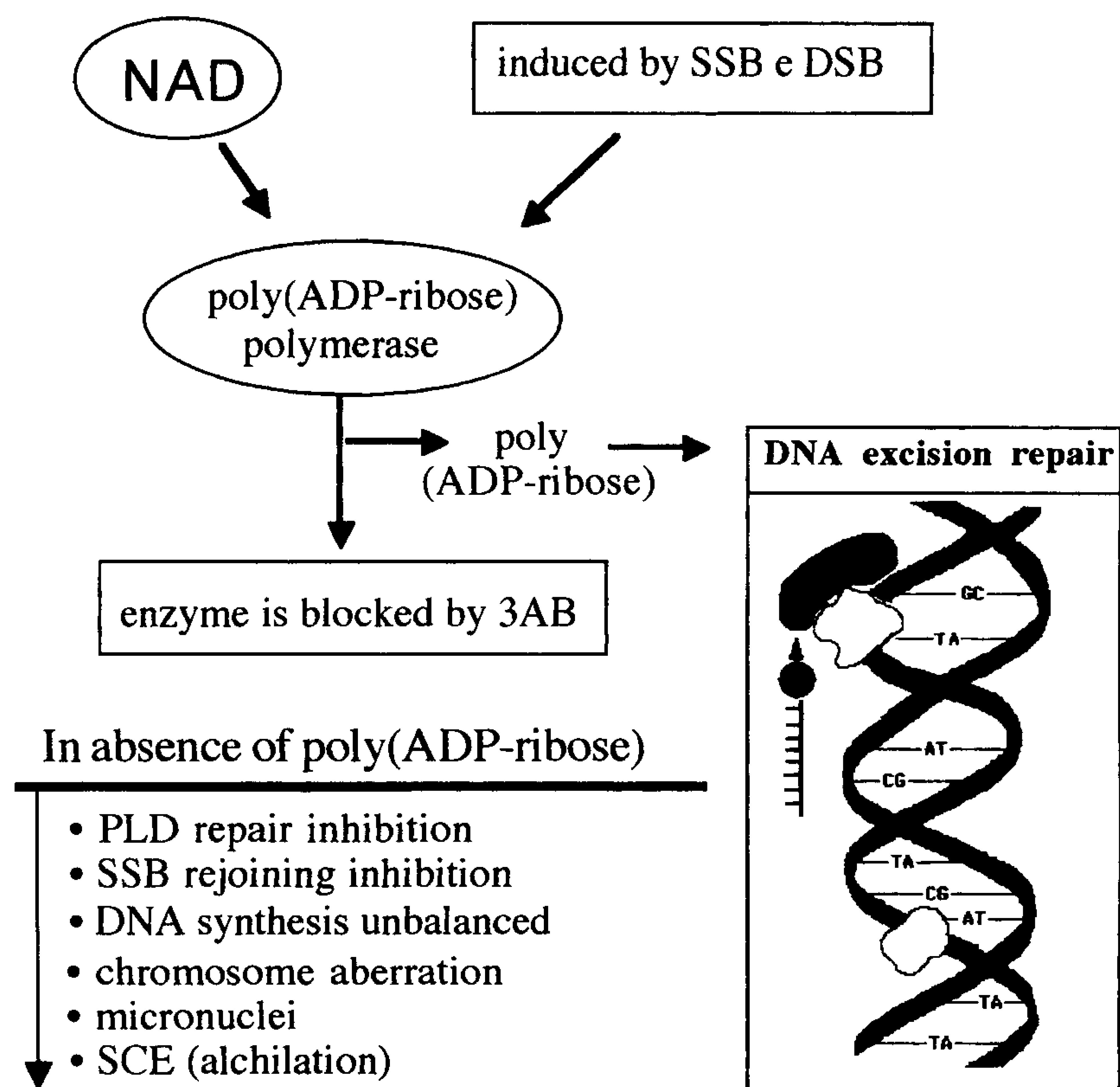

Fig. 18. – 3AB action on poly(ADP-ribose)polymerase. SSB: single-strand break. DSB: double-strand break. PLD: potentially lethal damage. SCE: sister chromatid exchange.

It has also been demonstrated that treatment with clastogenic agents, ionising radiation included, increases stimulation by poly(ADP-ribose)polymerase [93, 97]. Since this enzyme's activity is stimulated by DNA single- (SSB) or double-strand (DSB) breaks, this confirms its importance in the process of repair phenomena [94, 97-99].

Among the inhibitors of poly(ADP-ribose)polymerase are the analogs of pyrimidine (nicotinamide), purine (caffeine) and benzamide (amino derivatives). From experimental results, the benzamide analogs seem to have a better inhibitory capability; one of them, 3AB, is the least toxic at cell level [95].

After ionising-radiation-induced damage, the inhibition of poly(ADP-ribose) polymerase activity, by a specific inhibitor in non-cytotoxic conditions, fixes potentially lethal damage and delays (or inhibits) DNA strand-break rejoining [100, 101].

In our experiments on human lymphocytes, 3AB treatment induced a significant increase ($p = 0.0005$) in MN yields at 2 Gy. The effect of 3AB on the irradiation response (MN yields) can be estimated by the relation:

$$I_{3AB} = \frac{MN_{+3AB} - MN_{-3AB}}{MN_{+3AB}}, \tag{7}$$

where I_{3AB} is the index of 3AB action; MN_{+3AB} and MN_{-3AB} are the MN frequencies obtained respectively with and without 3AB. This index has zero value when there is no

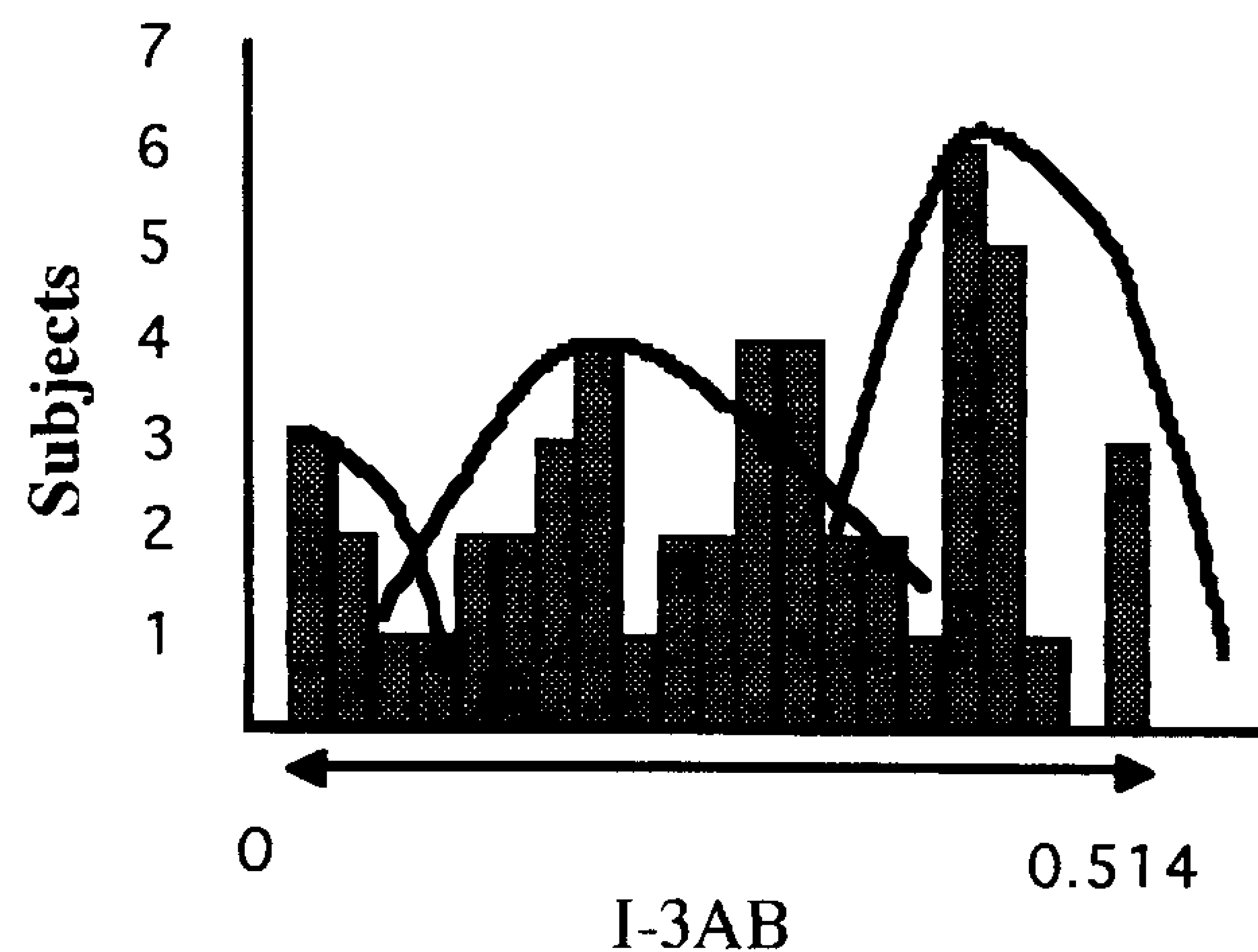

Fig. 19. – Distribution of the 3AB-index in 51 healthy individuals.

3AB effect (DNA repair absent or not poly(ADP-ribose)polymerase sensitive?), while it achieves values close to one when the irradiation damage is particularly 3AB sensitive (DNA repair in progress with maximum level of poly(ADP-ribose) synthesis?). The standard error (SE), calculated by the square root law, also allows a more accurate evaluation of the individual level of radiosensitivity (or radioresistance) within a group or population [91].

Hence, we can get a better understanding of the chromosomal fragility of the subject examined (genomic instability of the individual). The biological response (MN frequency) obtained from combined treatment (ionising radiation and 3AB inhibitor) and properly integrated with $\mathrm{I}_{3\mathrm{AB}}$ can, therefore, provide useful information on the capacity of lymphocytes (and hence the donor) to respond to irradiation.

Evaluation of the influence of 3AB on DNA damage (MN yields) induced by irradiation has revealed the existence of different interindividual response levels.

As shown in fig. 19, the distribution of $I_{3\mathrm{AB}}$ in 51 subjects can be divided into 3 subgroups: low, middle and high radioresistence. A similar distribution of radiosensitivity, even with other endpoints, has been hypothesised in some radiobiological works on cell radiosensitivity. A clear example comes from experimental data on complex chromosomal rearrangements (CCRs) observed in lymphocytes of patients after radiotherapy [102]. The authors demonstrate that the 47 patients examined can be divided into 3 subgroups having different mitosis frequencies with CCRs: 20, 14, and 13 subjects with mean values (CCRs) of 0.036, 0.079 and 0.091, respectively. This subdivision can also be correlated with the clinical signs of hyperreaction to radiation exposure.

While the above observations apply only after radiotherapy, a very suggestive hypothesis is that our $\mathrm{I}_{3\mathrm{AB}}$, measured before treatment might serve as a predictive assay for evaluating individual radiosensitivity [103].

9. – Biological dosimetry: guidelines

The choice of *modus operandi* has to take into account all the problems involved in a request for biodosimetric consultation: *a*) strategy of availability and communication;

b) shipment of blood samples and their proper conservation up to delivery to the laboratory; *c*) preparation of cytological specimens for microscope investigations according to technically consolidated methods; *d*) dose estimate by means of available calibration curves, or other biodosimetric evaluations based on well consolidated extrapolations [104]. Hence, coherent and feasible guidelines based on different accident situations are necessary.

9·1. *Acute exposure with blood sampling within* 5 *weeks from the accident.* – The best conditions for estimating dose with the standard dicentric (and MN) method are represented by acute (< 0.5 h) and uniform whole-body irradiation. A further diagnostic advantage is given by timely blood sampling: in fact, due to the progressive elimination of cells with unstable aberrations, the IAEA [9] has estimated that the dicentric frequency remains more or less the same for about 5 weeks after irradiation. Similar considerations apply for the MN method.

The MN method can be considered as a kind of prescreening and used to quickly select the cases that could be clinically significant (deterministic effects). Subsequently, the priority cases are selected on the basis of: their having a higher MN frequency; the number of subjects to examine; the operative capacity of the biodosimetric laboratory.

The Giemsa stain method (used for micronuclei and dicentrics) is more economic than the fluorescent probe technique. The FISH method facilitates observation of a wider spectrum of aberrations (particularly translocations), but requires simultaneous observation of a larger number of metaphases. It is also more costly compared to the Giemsa stain method with dicentrics, and in the conditions considered here (blood sampling within 5 weeks of acute exposure) the diagnostic advantages are limited. We have not yet developed *our own* calibration curve for the FISH method, however, a *provisional comparison* could be made with the calibration curves developed by other authors [105-108].

A useful comparison can also be made with the values of controls, taken from the literature or directly determined in the laboratory. Whenever dicentrics are sufficiently numerous, the *equivalent whole-body dose* is validated through calculating the Poisson distribution:

$$PY(n) = e^{-Y} \frac{Y^n}{n!}, \tag{8}$$

where Y is dicentrics/cell (observed in the sample); $n = 0, 1, 2, \ldots$ dicentrics distributed in the cells and e is the base of the neper logarithms.

When the observed values show overdispersion compared to those theoretically calculated, the coefficient of dispersion (CD) is analysed to estimate the fraction of body irradiated and assign the corrected dose to partial-body irradiation, using the equation of the Dolphin method as reported by IAEA [9].

Once the confidence limits (upper and lower) of the biodosimetric evaluation are established by means of dicentrics, the quality of the response of the subject (individual radiosensitivity) can be verified using the index of the effect of 3AB on micronuclei (I_{3AB}) [91].

Hence, the biodosimetric investigations that can be proposed in this accident situation are:

Micronuclei (Giemsa)
Dicentrics (Giemsa)
Poisson analysis for dicentrics and micronuclei to estimate partial-body irradiation
I_{3AB} (individual radiosensitivity)
Amylasemia (head irradiation)
EEG (head irradiation)
Dicentrics and translocations (FISH).

Biodosimetric use of the haematologic examination is indicated in the INFN report: *Procedure biodosimetriche in caso di sovraesposizione accidentale alle radiazioni ionizzanti*, November 1995 [104].

9'2. *Acute exposure with blood sampling more than* 5 *weeks after the accident.* – According to data published by IAEA [9], unstable radio-induced chromosome aberrations remain constant for a few weeks and then decrease rapidly. For acute whole-body irradiation, dicentrics remain constant for about 5 weeks and then gradually decrease until about the tenth week they are about 50% of the initial value. This decrease can remain constant for a few years (3 years in the case observed) (fig. 20). The NRPB suggests that unstable aberration yields, induced by high dose, decrease by about a factor of 2 in the first 6 months and subsequently more slowly, so that over a long period the average rate of reduction is a factor of 2 in about 3 years [109].

From this basis, it is possible to proceed initially as for the foregoing accident situation, using the MN and dicentric methods. With the former, generally significant and useful cytogenetic data can be obtained fairly economically and without any particular difficulty. The latter gives useful indications for reconstructing dose using the IAEA [9] curve or others obtainable from the literature [71, 109-113]. The advantage in calculating I_{3AB} (individual response) has been confirmed also in this case.

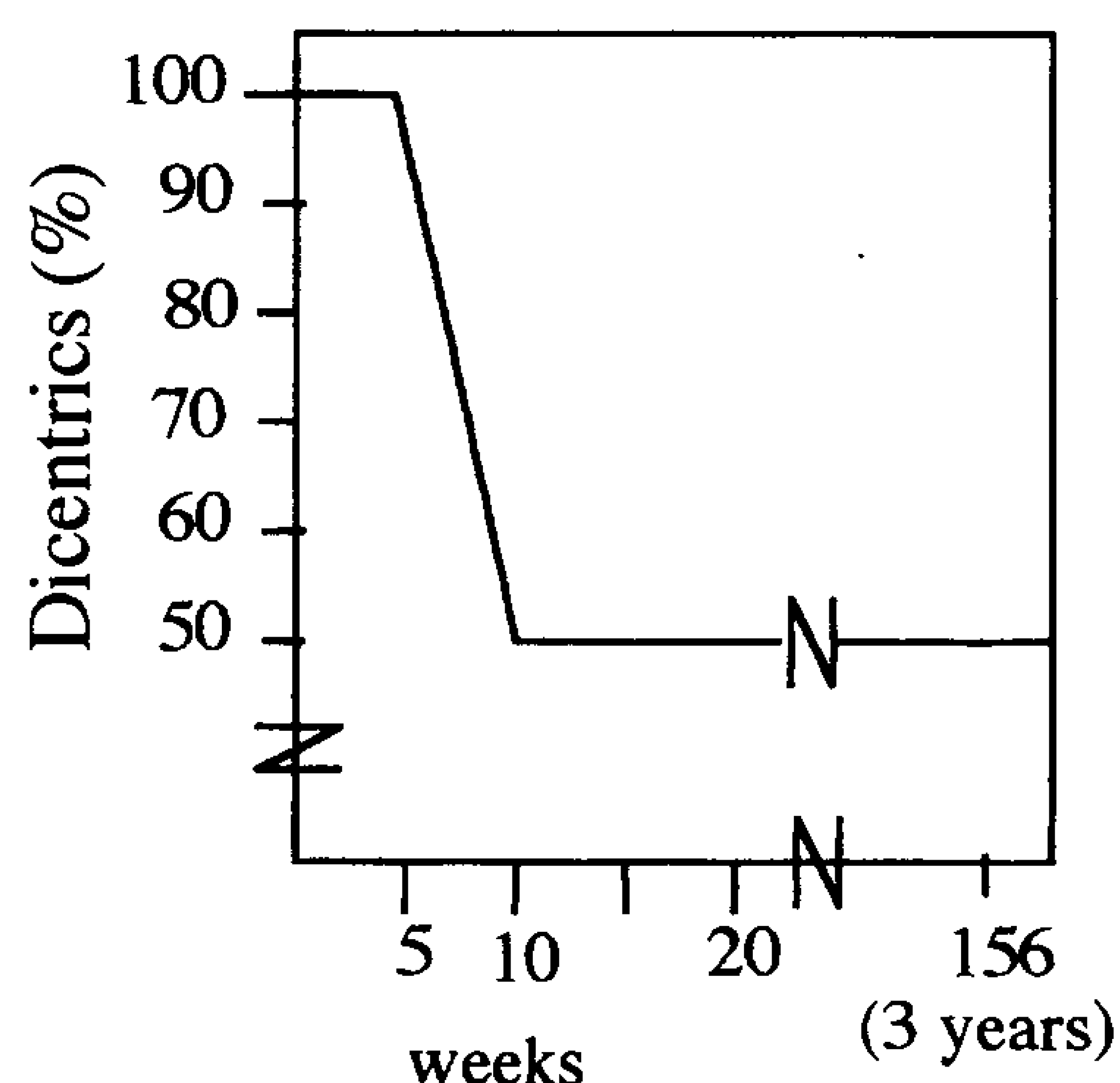

Fig. 20. – Estimate of dicentric decrease after *in vivo* irradiation [9].

However, when blood has been sampled late, translocation (stable aberrations) counting using FISH is clearly important. If the laboratory has not developed its own calibration curve for translocations, it is logical (and advantageous) that it proceed as quickly and economically as possible with evaluating the frequency of spontaneous translocations using a properly selected control group. This study can be carried out by applying chromosome painting to the specific chromosomes indicated by literature data as being more radiosensitive (chromosomes 1 and 7 due to breakage, or 8 and 14 to translocation) [114, 115]. As well as calculation of I_{3AB}, the investigation should be integrated with an evaluation of the translocation frequency after an *in vitro* dose of 2 Gy, in order to measure the level of individual response (radiosensitivity). In a preliminary evaluation at this stage of development, a translocation frequency outside the normal range represents a significant *contact index* with ionising radiation, even when blood has been sampled long after exposure.

In this case and in the absence of a tailored calibration curve, the following procedure can be used:

– *provisional comparison*, using the calibration curve for translocations with the FISH developed by Bauchinger [105, 106] and Lucas [107, 108] and taking into account the differences in the chromosome probes and the importance of the *equivalent genome* obtainable through the equation of Lucas;

– *reconversion* of the translocation frequency into dicentric frequency and reading of the dose on the dicentric calibration curve; this reconversion is done as a precaution, since for equal dose the number of dicentrics is the same as or lower than the number of translocations.

Whenever the two procedures described above are coherent with current biodosimetric criteria, they should lead to substantially similar conclusions. Therefore, the biodosimetric investigations that can be proposed in the exposure conditions considered here (blood sampling more than 5 weeks after exposure) are

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Micronuclei (Giemsa)
Dicentrics (Giemsa)
I_{3AB} (individual radiosensitivity)
Dicentrics and translocations (FISH)

9·3. *Protracted exposure.* – As established by IAEA [9], for exposure lasting a few hours or days and for doses not considered low (> 0.3 Gy), a time-dependent correction function termed the *G function* can be introduced in the calculation of dose by the dicentric method [116].

In this case the linear quadratic equation is modified as follows:

$$(9) \qquad Y = aD + bG(x)\,D^2, \qquad \text{where} \qquad G(x) = \frac{2}{x^2}\left[x - 1 + e^{(-x)}\right],$$

for $x = t/t_0$, where t is the duration of irradiation and t_0 the average lifetime of the breaks (~ 2 h). For very protracted exposure (weeks) $G(x) \sim 0$, therefore $Y \sim aD$. At low doses (< 0.3 Gy) and low dose rates the dose-response relation of the dicentrics is basically linear ($Y = aD$).

In the exposure situation considered here the following biodosimetric investigations are proposed:

Micronuclei (Giemsa)
Dicentrics (Giemsa)
I_{3AB} (individual radiosensitivity)
Dicentrics and translocations (FISH)

9·4. *Fractionated exposure.* – As established by IAEA [9], for fractionated exposure with intervals between doses of over 6 h the biodosimetric estimate with the dicentric method is equal to the sum of the single fractions of dose obtained from

$$Y = \alpha D + \beta D^2 \tag{10}$$

For a more accurate estimate a correction factor—the *cytogenetic recovery factor* k—can be introduced [33].

If the interval between doses is less than 6 h the linear-quadratic equation is modified:

$$Y = \alpha D + \beta e^{(-t_1/t_0)} D^2 , \tag{11}$$

where t_1 is the time between fractions of dose and t_0 is the average lifetime of the break (about 2 h).

During the first 5 weeks of exposure the same procedure as for acute exposure can be used, with blood sampled within this period. A tentative reconstruction of the dose in fractionated exposure can also be performed using the curve obtained from IAEA data [9].

For this type of exposure the following biodosimetric investigations are proposed:

Micronuclei (Giemsa)
Dicentrics (Giemsa)
I_{3AB} (individual radiosensitivity)
Dicentrics and translocations (FISH)

Our laboratory is developing the glycophorin-A method, to be used mainly in biodosimetric evaluations for acute exposure with late blood sampling and for chronic exposure (protracted or fractionated over a long period).

9·5. *Modus operandi.* – Biodosimetry is articulated as follows:

Delivery of samples in double test tubes
Cryopreservation of some samples for any subsequent studies
Blastization of lymphocytes by *in vitro* culture
Slide preparation → MICRONUCLEI-DICENTRICS-TRANSLOCATIONS
MN frequency reading in BCs
Dose estimation with MN test
Dicentric frequency reading in euploid metaphases
Dose estimation (with dicentric test)
Translocation count and comparison with normal range

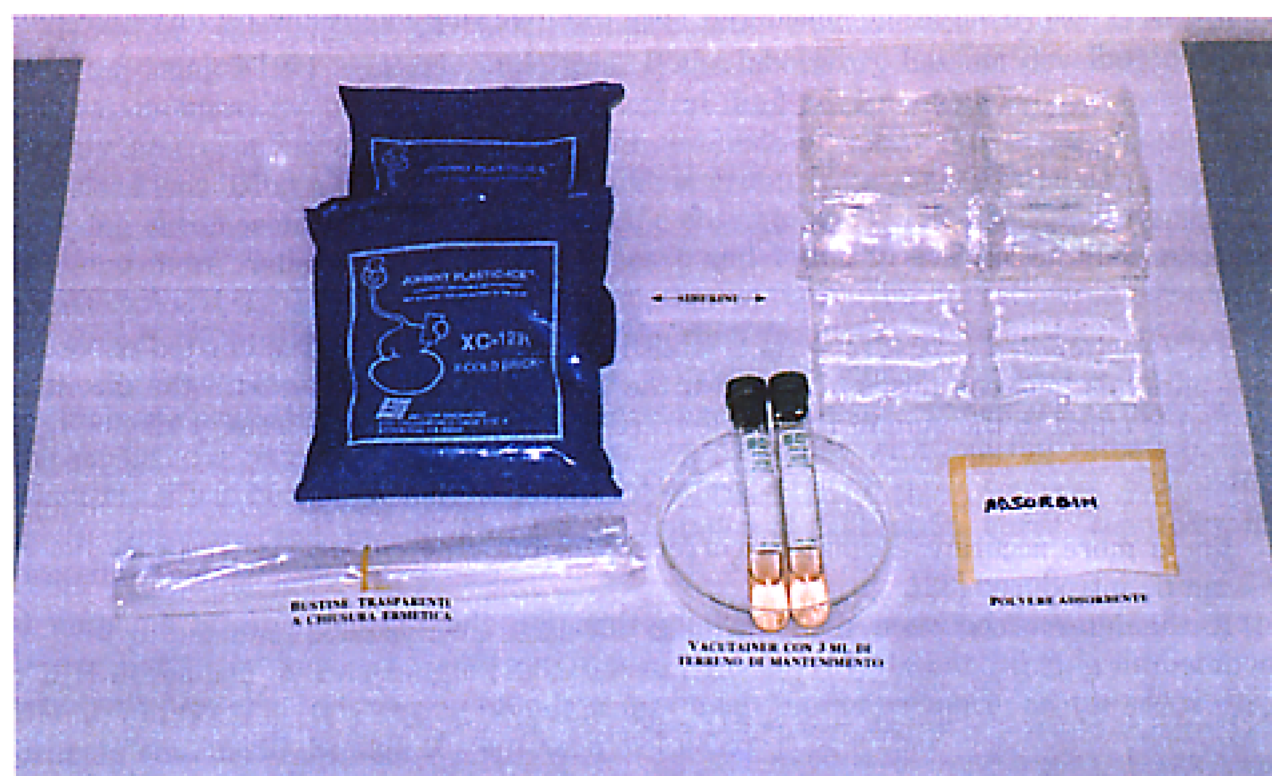

Fig. 21. – Transport kit for blood samples.

A special transport kit provided with all the necessary for blood sampling and blood conservation during shipment has been developed (fig. 21). It consists of 10-ml-capacity glass test tubes in vacuum (Vacutainer), containing 3 ml of conservation solution that we developed to keep peripheral blood lymphocytes vital for more than 3 days, *i.e.*, delivery time. The kit contains transparent hermetically closed bags, absorbent material and cold bricks. Also included is a form headed "private and confidential" to be filled in by the physician applying for biodosimetric consultation. The complete kit is contained in a thermostatic box ready for delivery via carrier, in conformity with the regulations for the transport of *potentially infective biological specimens*. Once the blood samples reach the laboratory they are used for preparing slides according to standard procedures.

The MN slides are then read according to the procedure reported in subsect. 6·3. The MN frequencies of the subject and of the range of controls are compared. If the values of the subject are higher than those of the controls, dose is estimated using a calculation programme based on the calibration curve (DOSIME) [117].

To count the dicentrics, metaphases are observed with the optical microscope at two magnifications: 200× to search for the metaphases and 1000× for detailed observation of the chromosome morphology. For each metaphase observed, the X,Y coordinates characterising its position on the slide and its chromosome number (CN) are noted. Then any unstable chromosome aberrations are recorded: dicentrics (DIC), acentric fragments, associated and not (AF), and centric rings (CR). All other aberrations such as chromatid breaks, acentric rings, and gaps are noted, even though they are not included in the biodosimetric evaluation. The dicentric frequency F(DIC) is obtained from the ratio of the total number of dicentrics to the total number of euploid metaphases.

The IAEA [9] propose that 500, with a minimum of 200, metaphase plates must be observed; Oak Ridge Laboratories suggest 300 for all practical purposes [8]; and NRPB, 500 [109, 118].

The biodosimetric report for both micronucleus and dicentric investigations can be completed with the 3AB-index. It should also be reported whether the translocation values are within the normal range or not, and in either case what they are after an *in vitro* dose of 2 Gy.

Calculations for reconstructing dose in the case of delayed blood sampling after acute irradiation and for protracted or fractionated irradiation should be reported in specific documents.

10. – Importance of biodosimetry in accidents

10'1. *Brescia* 1975. – The Brescia accident is very interesting as a typical example of very acute irradiation in the lethal range. The accident occurred in northern Italy on 13 May 1975 in a small installation for cereal irradiation. The only subject involved was irradiated during a few minutes by a source of cobalt 60. The nuclear nature of the accident was not evident in the first hours after the accident; the patient landed in Paris on 17 May, four days after exposure, when the seriousness of his disease was recognised at a local hospital. Whole-body but not uniform exposure was ascertained. The subject survived for only 12 days.

The most exposed parts were the left shoulder and arm, the front and lateral portions of the neck, the top of the skull, and the face.

Reconstruction of the accident was carried out 4 days after its occurrence using phantoms (Rando-type phantoms) and different types of dosimeters: film badges such as those used for routine monitoring, and different types of lithium fluoride thermoluminescent dosimeters for gamma rays. Only the dose rates could be measured: 12 Gy/min on the left shoulder, 8 Gy/min on the anterior left side of the neck and 6 Gy/min at the top of the skull. It was certain that high doses had been delivered to this patient, since the brain dose ranged from 12 to 24 Gy. The doses delivered to various organs were also calculated. Most doses to the organs exceeded 10 Gy. The mean bone marrow dose was estimated to be about 12 Gy [119].

Nevertheless, a complete reconstruction of the accident was impossible because the exact duration of exposure was not known. Therefore, chromosome analysis was of prime importance. The mean value of dicentrics led to an estimate of a whole-body dose equivalent of 12 Gy of γ-radiation dose. Moreover, the distribution frequency histogram showed a bimodal aspect with one of the modes giving an estimated dose of 8.7 Gy and the other over 30 (dicentrics did not follow a normal Poisson distribution) [120].

10'2. *Ciudad Juarez* 1983. – In December 1983 an electrician from the *Casa Medica* of Ciudad Juarez was given the job of clearing out unutilised equipment from a warehouse belonging to the clinic [121]. Among the things he loaded into his van was a stainless steel container the size of a milk bottle. This container in fact was a capsule for radiotherapy containing about 6000 microspheres of cobalt 60 with a total radioactivity of 1000 Ci, equal to 37 TBq.

While transporting the material to the scrap yard, the capsule broke, and about 700 radioactive microspheres spilt onto the floor of the van (which remained parked for two

months in a Juarez street), other microspheres spread over the ground in the scrap yard, and yet others spread among the scrap. The latter was then transported in part to a steelworks at Ciudad Juarez where it was used to make restaurant-table supports and in part to a steelworks at Chihuahua where it was transformed in steel joists for buildings.

The accident was only discovered halfway through January 1984 when lorries transporting material from the Chihuahua steelworks were inspected near the Los Alamos military base. The accident involved four North American states and numerous areas in Mexico. Roughly 20 000 houses had to be inspected, with about 800 requiring demolition. About 4000 people had been exposed with the following doses attributed: 80% < 5 mGy; 18% 5–250 mGy; 2% > 250 mGy. Of the latter five persons had absorbed from 3 to 7 Gy in two months. The predominant contribution in estimating doses was made by cytogenetic dosimetry. For a long time the symptoms suffered by the most irradiated individual were blamed on other causes (*e.g.*, skin lesions on the hands and feet of some of the scrap-yard employees were thought to be "injuries due to sport").

10·3. *Chernobyl* 1986. – The accident on 26 April 1986 at the Chernobyl nuclear power plant in the Ukraine caused acute radiation sickness (ARS) and deaths among the plant workers and the firemen immediately called there. Thousand of people involved in the emergency actions on the site during the accident and in the clean-up operations were exposed.

Radioactive contamination spread to many countries (Belarus, Ukraine, and Russia) and necessitated evacuation of more than 100 000 people in a radius of 30 km around the accident site. Besides the biological dosimetry investigations performed on patients who in the first-phase showed certain signs of ARS or who were suspected to suffer from it (table VI) [17, 32], many cytogenetic studies were performed by various clinics and scientific institutes on representative samples from the most contaminated groups or populations.

By way of example, we report the results of cytogenetic studies of important scientific institutes of the ex URSS, presented at the International Conference (EC, IAEA, WHO) "One Decade after Chernobyl", Austria Center Vienna, Austria, 8-12 April 1996.

At the Central Research Institute of Roentgenology and Radiology of St. Petersburg, Russia, cytogenetic investigations were carried out on the liquidators who had participated in clean-up operations in the 30-km radius around the nuclear power station [122]. The samples examined were composed of:

- 1st group: 19 persons examined one month after the accident
- 2nd group: 50 persons examined from 1990 to 1993
- 3rd group: 50 persons examined in 1995
- control group: 50 healthy volunteers.

Chromosome analysis involved observation of dicentrics, rings and other aberrations. The MN test with the cytokinesis block method was also applied after *in vitro* irradiation at 1.5 Gy in order to evaluate the chromosome fragility.

The study showed that:

- 1st group: the total number of chromosome aberrations was three times greater (single-strand fragments 1.3 times; double-strand fragments 5.2 times). Dicentrics were 0.12% (0 dicentrics in the control group).

- 2nd group: an increase in dicentrics was found, while the total number of chromosome aberrations was low. The MN frequency was close to that of the control group, but each binucleated lymphocyte contained 3, 4 or more micronuclei as a specific effect of radiation dose.

- 3rd group: an increase in dicentrics was observed (about 2.5 times relative to the mean value measured in the 2nd group). The increase in the total number of chromosome aberrations was similar to that of the 1st group. An increase in MN frequency was observed.

In the 2nd and 3rd groups of liquidators the *in vitro* irradiation test (genomic instability) showed a decrease in chromosome aberrations and micronuclei compared to the control values. This result suggested the possibile presence of an "adaptive response".

At the "Institute for Nuclear Research" National Academy of Science of Ukraine, Kiev (Interdisciplinary Scientific Technical Center "Shelter", Chernobyl) cytogenetic investigations were performed on:

- 20 liquidators involved in the clean-up operations for some years,

- 26 farmers working in the evacuated area for 10 years and 20 people living in Kiev but not involved in nuclear or chemical industries, to act as controls.

The level of DNA instability in peripheral blood lymphocytes and the MN frequency were investigated [123]. In the liquidators both DNA instability and the MN frequency in BCs had increased.

At the Institute of Genetics and Cytology, Academy of Sciences of Belarus, Minsk, cytogenetic investigations were performed on two groups of children, with the following results [124].

Group A: 60 children living in 10 villages of the evacuated 30 km area of the Bragin district (cytogenetic study in August 1986; evacuation on 7-8 May 1986). A significant increase in chromosome aberrations (dicentrics and rings) was observed. The mean value of dicentrics and rings per cell was 0.0095 (0.0006 in the control group).

Group B: Children evacuated from Bragin one month after the accident. Cytogenetic study performed on 26 August 1986: the chromosome aberrations were three times greater in comparison with the control group. Cytogenetic study performed on 5 December 1986: a further increase in chromosme aberrations was measured. Cytogenetic study performed in 1988: the frequency of chromosome aberrations appeared stationary relative to the preceding study. Cytogenetic study based on MN test with cytokinesis block method in 1991: the number of BCs containing MN was 5.3 times greater than in the control group. The same chromosome-aberration frequency was found in lymphocytes after *in vitro* irradiation at 0.3 Gy and in the control group after *in vitro* irradiation at 1 Gy (increase in radiosensitivity).

The researchers specify that: "...the count of chromosome aberrations in circulating lymphocytes of people living in particular radio-ecological conditions is at present the most objective biological indicator of the effect of radiation. The poor organisation of radiation monitoring the first few months after the Chernobyl accident increases the

importance of biological dosimetry ... The biodosimetry based on our research, and carried out using various standard curves to evaluate the absorbed dose, showed that the chromosome aberration frequency in children from the 30 km radius evacuated area of the Bragin district and from town of Bragin corresponds to a dose di 200–500 mSv. According to the data of the Institute of Radiology (Belarus), based on physical dosimetry methods, the dose received by the people living in these areas between 1986 and 1988 does not exceed 155 mSv. The reasons for the discrepancies in the estimated doses require further thorough investigation ...".

Studies on genomic stability in peripheral blood lymphocytes of children living in the contaminated areas of Brest, Gomel and Minsk regions (1993-1995) have pointed out:

– an anomalous response of the irradiated lymphocytes in the G2 phase at doses ranging from 5 to 60 cGy,

– an interindividual variability in the genomic response after exposure to chemical agents (cicloheximide and actinomycin D), capable of modifying the radio-induced DNA damage,

– the role of cell lethality in determining cytogenetic effects after *in vivo* chronic and acute exposures.

The results obtained after 10 years of studies performed on children living in the regions of Gomel, Mogilev and Brest (Belarus) demonstrated the importance of evaluating a complete spectrum of genetic damage in peripheral blood lymphocytes (the biodosimetric profile).

10·4. *Goiânia* 1987. – In September 1987 a radiation accident occurred in Goiânia (Brazil): a 1375 Ci (50 TBq) radiotherapy cesium 137 source was removed from its housing and damaged. Many individuals incurred external and internal exposure. In total, some 112 000 persons were monitored, of whom 249 were contaminated either externally or internally [10, 125]. Because of the complex nature of the exposure it was not possible to accurately estimate the doses received using physical dosimetry approaches.

Moreover, exposure conditions in this accident were complicated since two weeks had elapsed between breaching of the source and the discovery of the accident. Among the affected persons 20 were identified as needing hospital treatment, 4 of whom died within four weeks of their admission to hospital. Cytogenetic analysis was very helpful in distinguishing the seriously irradiated persons. More than 110 blood samples were analysed by cytogenetic dosimetry. The estimated dose ranged from zero up to 7 Gy. The Poisson statistical distributions of the cytogenetic data of some affected persons showed non-uniform exposures. Urine and faecal samples collected daily from patients were used as a screening method to evaluate the radioactive intake and the committed dose with mathematical models.

10·5. *San Salvador* 1989. – During routine operation of a gamma-ray facility used for sterilising plastic products for medical use, an unshielded 666 TBq ^{60}Co (18 000 Ci) source became lodged in the "up position" and did not return to the protective pool of water under it. Three workers at the facility entered the sterilisation unit and attempted to disengage the source and correct the malfunction. In a preliminary reconstruction of the accident, it was estimated that the men had been exposed for 3 to

4 minutes at various distances from the source and that they had received doses in a range from 3 to 10 Gy with localised exposure in excess of 200 Gy to the feet of two of the three people [126].

Cytogenetic dosimetry evaluations with the dicentric method were performed for the three operators. Based on the observed frequencies and on the cellular distribution of dicentric chromosomes 35 days after the accident, it was estimated that the men received irradiation to over 90% of their bodies and that the doses ranged from 3 to over 8 Gy.

10·6. *Estonia* 1994. – A ^{137}Cs source, probably originating from a sterilisation device in Estonia, was stolen by three brothers on 21 October 1994 and kept in a private house for four weeks. This resulted in the death of one person, acute radiation injuries to four people and exposure of several other people to lower doses of radiation. Conventional chromosome aberration analysis was performed to estimate radiation exposure for eight persons who had been or were suspected to have been near the source. The same cytogenetic analysis was carried out on 10 people consisting of medical staff who had visited the house and rescue personnel involved in removal of the source from the house.

Dose estimate based on the frequencies of dicentric chromosomes was performed both by the standard method (calibration curve produced *in vitro*) and by considering possible dose protraction (G function of Lea and Catchside) and non-uniform exposure (Poisson distribution). Considerable differences in dose estimates were obtained, depending on the approach used, ranging from about 1 Gy to almost 3 Gy in the patient most heavily exposed. On the basis of the deterministic health effects observed in some of the individuals, it was evaluated that the dose estimates obtained utilising the information on dose protraction were more realistic than those obtained by comparison with the *in vitro* calibration curve.

Chromosome painting analyses using FISH for chromosomes 1, 2 and 4 and centromeres were performed in parallel. The dose estimates performed on the basis of FISH translocation frequencies were consistent with dicentric analysis [127].

10·7. *Vicenza* 1996. – On 17 February 1996 a train consisting of 12 wagons transporting scrap iron to the Beltrame steelworks at Vicenza entered Italy through the Tarvisio pass. One of the wagons from the Czech Republic contained radioactive material. The train remained for 36 hours on platform 8 of Vicenza station, a good distance from the area reserved for passengers. In this time four shifts of drivers plus a group of switchmen had worked in the neighbourhood of the wagon containing the radioactive material, at varying distances and for different times.

On 19 February 1996 during a routine inspection to clear the wagons, higher than normal-background ionising radiation was measured around one of the wagons. It was then ascertained that it was due to the presence of cobalt 60 in the scrap iron. The exposure around the wagon was measured and doses to the pesonnel most involved in work near the wagon and to the population near the route taken by the train were evaluated.

However, in order to estimate the doses to 7 workers who had been near the wagon as accurately as possible, in addition to a reconstruction based on physical measurements, cytogenetic dosimetry, using the micronucleus and dicentric analysis, was carried out. Cytogenetic dosimetry indicated a normal response in the subjects observed.

11. – Retrospective biodosimetry and probability of causation

In refs. [50, 104] different accidental exposure conditions have been considered and classified according to time parameters. In this context it is specified that biodosimetry by micronuclei and dicentrics (unstable indicators) can be performed properly after recent exposure. Instead, chronic exposure needs stable biodosimetric indicators, that is they should be capable of integrating in time the radio-induced damage [63]. Actually, the late indicators are represented at an applicative level by GPA, chromosome translocations and ESR. Therefore, stable indicators may reveal acute irradiation that took place in the past.

However, for chronic exposure both micronuclei and dicentrics can still be used as diagnostic tools, because frequencies that differ from the control values can point towards conditions of chromosome fragility or in any case to defects in DNA repair. The use of a challenge assay (*e.g.* additional *in vitro* irradiation at 2 Gy) and the I_{3AB} increase the expression of this disorder and could provide a better explanation of an abnormal situation.

The possibility of retrospective biodosimetry, both for chronic and for acute remote exposure, could be useful in medico-legal evaluation of radio-induced oncological events whenever individual physical dosimetry is absent or precarious or when just biological confirmation of physical dosimetry is required.

A tool in insurance, civil and penal claims is the Probability of Causation (PC). This criterion, which evaluates the likelihood level of the causal hypothesis, linking exposure and tumor, is based on scientific knowledge. Synonyms are Assigned Share or Attributable Risk Ratio [25, 128].

Evaluation of the connection between the two is translated into a mathematical instrument by defining probability through the following expression:

$$\text{PC} = \frac{\text{radiation risk}}{\text{radiation risk} + \text{“natural cancer” risk}}\,. \tag{12}$$

The radiation risk is related to dose and to the risk coefficient, which must be tailored to the specific situation. So the radiation risk is given by

$$\text{radiation risk} = f(D)\cdot a(T, E, S, P, O)\,. \tag{13}$$

The $f(D)$ function is linear or linear-quadratic, depending on the specific tumour, and the tailored risk coefficient depends at least on the following parameters: age at exposure (T), age at diagnosis or at death (E), sex (S), the pertinent population (P) and the affected organ or tissue (O). Since the relative excess risk R due to the exposure is quantitatively defined as

$$R = \frac{f(D)\cdot a(T, E, S, P, O)}{\text{“natural cancer” risk}}\,, \tag{14}$$

the value of PC, with simple transformations, is given by

$$\text{PC} = \frac{R}{1+R}\,. \tag{15}$$

Using the cytogenetic method for estimating dose, $f(D)$ can be changed into biological dose $f(D_{\text{bio}})$. Also, tailoring of PC can be enhanced by introducing a

corrective parameter (I) that expresses the radiosensitivity of the subject. This corrective factor, after suitable mathematical manipulation, can be derived from I_{3AB} and/or from the challenge assay [129].

This new parameter can then be introduced in the equation

$$\text{radiation risk} = f(D)\cdot a(T, E, S, P, O, I). \tag{16}$$

Whenever $f(D)$ is linked to D_{bio}, the relative excess of risk R is defined as

$$R_I = \frac{f(D_{bio})\cdot a(T, E, S, P, O, I)}{\text{"natural cancer" risk}}, \tag{17}$$

then the value of PC is

$$PC_I = \frac{R_I}{1 + R_I}. \tag{18}$$

Another possible use of recent or retrospective biodosimetry, either for integration with the physical dose or for use in its absence, is to project the lifetime risk of radio-induced tumours, calculated according to the criteria adopted by ICRP 60 [20].

12. – Biodosimetry in oncology

Side by side with the concern that the more radiosensitive inviduals or sub-groups of populations may not be fully safeguarded by standard protection measures is the very real requirement of being able to measure the radiosensitivity of patients who have to undergo radiotherapy, nuclear medicine treatment or chemotherapy. Hence, an interest in thoroughly studying biodosimetry with regard to these specialised fields is strongly justified.

12·1. *Nuclear medicine and oncology.* – We have estimated (using the MN test) the dose received by lymphocytes from patients undergoing ^{131}I therapy, following total thyroidectomy for thyroid carcinoma, and the radiobiological response as acquired radiosensitivity (3AB-index). From a global examination of the cytogenetic effect observed in the patients, we also attempted to evaluate at individual level the attenuation of the radiobiological response induced by ^{131}I, as a function of the time passed after administering a single dose, using a mathematical model of exponential decay [130].

In 28 blood samples from 21 patients undergoing ^{131}I treatment after surgical thyroidectomy for cancer, the MN frequency observed in peripheral blood lymphocytes (MN test on CBs) had a weighted mean of 0.044 ± 0.006, which was significantly different ($p < 0.0001$) from that observed in 93 healthy individuals (0.025 ± 0.001). The MN frequency ($F_{(MN)}$) of the patients correlated fairly well ($R = 0.736$) with the modified activity (A_{mod}), calculated by the following equation:

$$A_{mod} = \sum_{i=1} (A_i \cdot e^{-(d_i \cdot k)}), \tag{19}$$

where A_i is the ^{131}I activity on a determined day, e the logarithmic base, d_i the number of days that have passed between that day and blood sampling, and k is a day coefficient, defined in this context as the "daily attenuation factor". In (18), k has been

given increasing values (from zero to one) to identify the corresponding values of A_{mod} that allow the best linear regression between the values of the radioactivity administered and the MN freqeuncy. Thus, calculating the regression coefficient for each series of values of A_{mod}, it was found that with $k = 0.0003$, A_{mod} values were obtained that indicated 0.736 as the best possible regression coefficient. So, for $k = 0.0003$, we can establish the following relation:

$$F_{MN} = 7.9 \cdot 10^{-5}(\pm 1.4 \cdot 10^{-5})\, A_{mod} + 0.014; \qquad R = 0.736\,. \tag{20}$$

We then used the MN frequency to estimate, by means of our DOSIME program, the dose (Gy) received at individual level during ^{131}I treatment. In these subjects the calculated dose showed good correlation with A_{mod}:

$$D_{bio} = 0.0009(\pm 0.0002) \cdot A_{mod} + 0.0675 \qquad (R = 0.755)\,. \tag{21}$$

The weighted average of I_{3AB} was 0.320 (SE: 0.046) for 15 patients and 0.217 (SE: 0.032) for 38 healthy subjects.

On the basis of these preliminary investigations we were able to use the MN frequency to evaluate absorbed dose at individual level and to determine the significance of the 3AB-index as an adaptive response to ^{131}I therapy [131, 132].

12·2. *Radiotherapy and oncology.* – In the field of oncological radiotherapy, the discovery of indicative and predictive biological parameters in individual response to treatment has stimulated studies and research, whose results, however, are still the subject of controversy.

A fundamental contribution to investigations on the radiosensitivity of tumour cells was made by Fertil and Malaise [133]. The two authors demonstrated that the radiosensivity of cell lines resulting directly from human tumours is peculiar to each tumour. The parameter they considered predictive is the fraction of cells that survives after a dose of 2 Gy (SF2). This observation was subsequently confirmed by the studies of many authors [134-140]. Bristow and Hill [141], in experiments involving fractionated treatment on rat tumours to study different radiosensitivities and the survival of tumour cells after explant, demonstrated that the level of cell killing in each tumour treated *in vivo* was predicted by the SF2 measured *in vitro*. However, not all the later studies confirmed this correlation between tumour response to radiotherapy and the *in vitro* response of tumour cells [142, 143].

Similar considerations apply to the use of other biological parameters, such as the level of ploidia and the fraction of tumour cells in the S phase as determined with different methods (tritiated thymidine, bromodeoxyuridine, cytofluorimetry). Such parameters have been found promising in predicting the evolution of tumours and their radiocurability but are not extendible to all neoplasias [144-147].

Our studies on measuring the cytogenetic effect *in vitro* via the MN test on recently collected human lymphocytes provided valid information on the radiosensitivity of lymphocytes (and hence of the individual donor). Therefore, it seemed logical to transfer our experience in this sensitive area of radiobiology to oncological radiotherapy, especially in consideration of the real possibility of measuring the radiosensitivity of patients and tumours via the MN method [148-151].

During our research in collaboration with two hospitals (*Niguarda Cà Granda* of Milan and *Casa Sollievo della Sofferenza* of San Giovanni Rotondo, Foggia), we observed the MN frequency in lymphocytes taken from patients undergoing

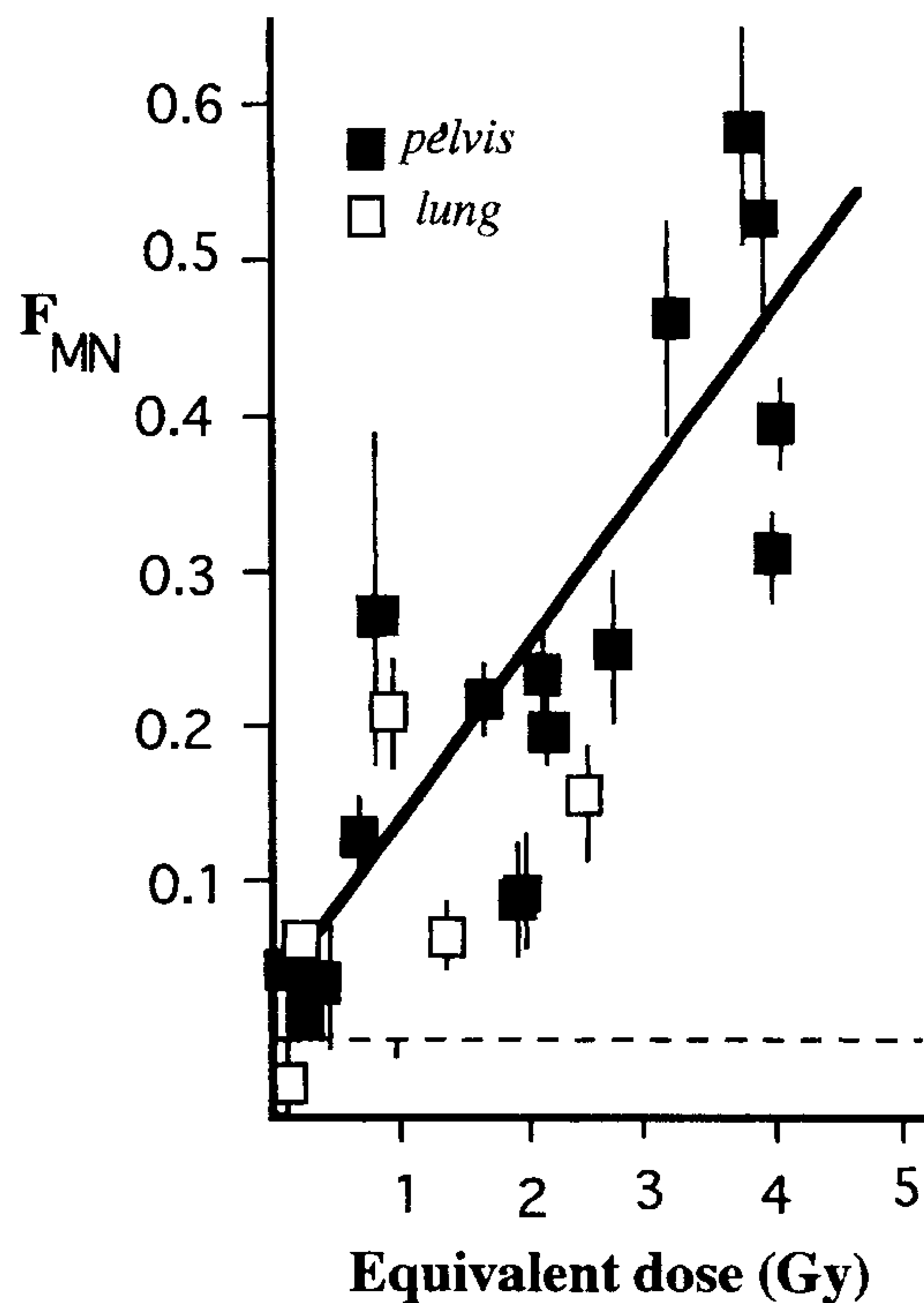

Fig. 22. – Micronucleus frequency in lymphocytes of patients undergoing radiotherapy in pelvic and pulmonary sites. Bars indicate standard errors.

radiotherapy (fractionated exposure) for lung tumours (6 patients), pelvic (15 patients), breast (7 patients), head and neck (4 patients) tumours and for Hodgkin lymphoma (4 patients). The objective of our research was to evaluate 1) the biodosimetric capabilites of the test in different exposure conditions; 2) the absorbed biological dose (whole-body) at individual level; 3) the attenuation of the cytogenetic effect as a function of time between exposure and blood sampling [33, 103].

Regarding the patients irradiated for breast tumours, the MN method did not reveal any dose-effect response for low and homogeneous cytogenetic response. Such a result could be explained both by the modality of exposure and by the low blood flow in the irradiated site. For the patients irradiated for Hodgkin lymphoma and head-neck tumours, there seemed to be a dose-dependent correlation. In the patients undergoing radiotherapy in lung and pelvic sites, the MN method showed that the MN frequency increased with increasing total physical dose (fig. 22).

Therefore, the MN method seems suitable for biodosimetric measurements on subjects undergoing radiotherapy in sites with a higher blood flow. However, with increasing total physical dose, the MN frequency in the patients was lower than the frequency observed in our calibration curves (fig. 23).

This phenomenon can be attributed to fractionation of the dose rather than to the condition of partial-body irradiation. In fact, for fractionated treatment on parts of the body having a large blood volume and flow (pelvic and pulmonary sites), we can hypothesise that "lymphocyte remixing" contributes to a final homogenisation of the total absorbed dose.

Thus, in radiobiological terms we can suppose that with fractionated irradiation there exists a partial cytogenetic recovery from the effect induced by a single exposure as a function of time between exposure and blood sampling. It is important to note that

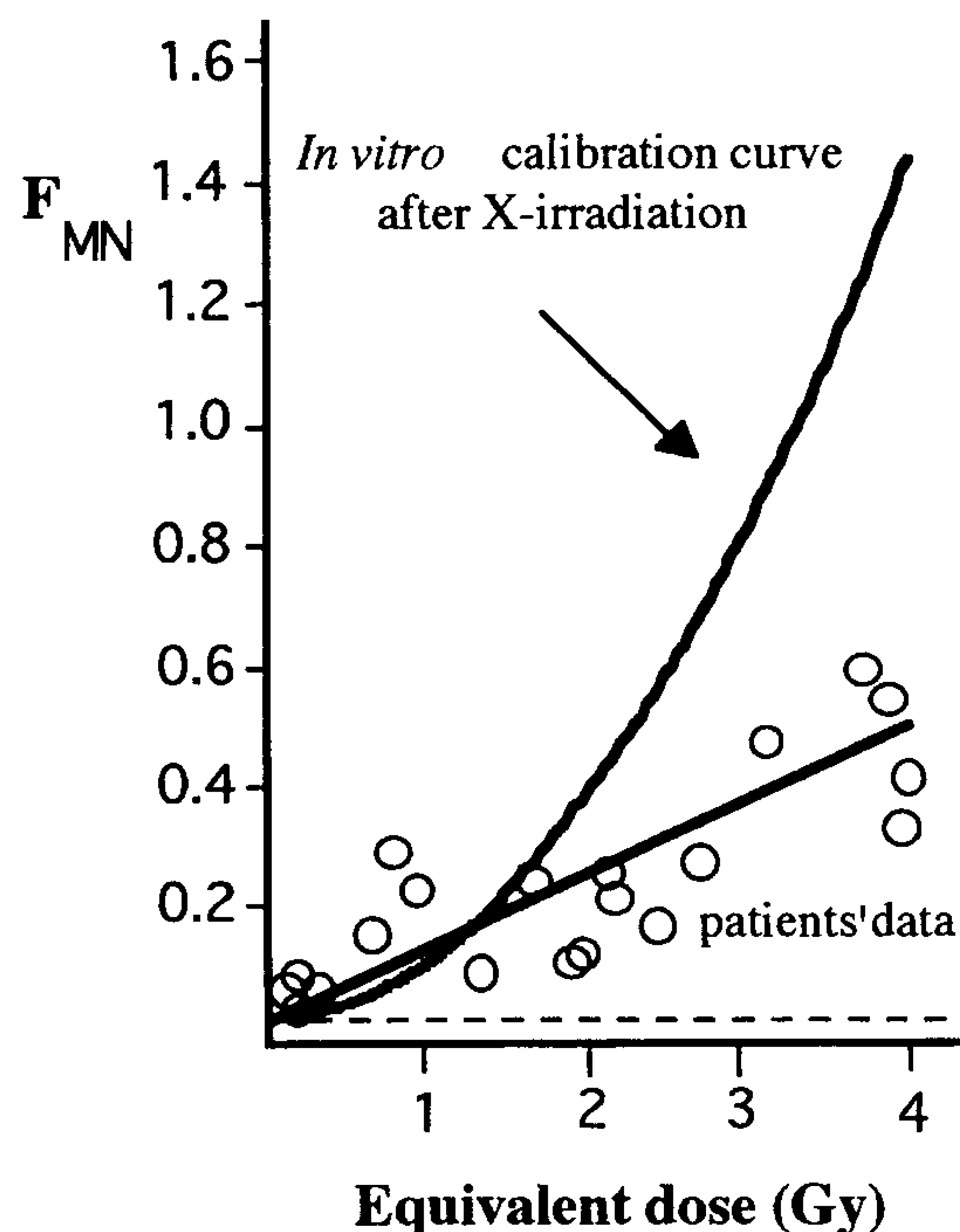

Fig. 23. – Comparison between MN frequencies observed in lymphocytes of patients undergoing radiotherapy and obtained after *in vitro* X-irradiation (calibration curve).

factors such as the amount of cells that recirculates after exposure and the effect of blood remixing with unirradiated lymphocytes are involved in individual recovery from the cytogenetic effect.

We have applied a decay formula, as a function of time (*e.g.*, radioactive decay). In our case, the sum of the decreases observed in micronuclei after each dose can be expressed as follows:

$$F(MN) = \sum_{i=1} (MN_i \cdot e^{-(d_i \cdot k)}) . \tag{22}$$

Here, the cytogenetic effect and the time are exponentially correlated by the coefficient k (cytogenetic recovery factor); $F(MN)$ is the cytogenetic effect measured in lymphocytes of patients during therapy; MN_i is the cytogenetic effect, calculated daily, at blood sampling and d_i is the number of days from an exposure to blood sampling. In agreement with the above formula: *a*) k can have only positive values between 0 and 1; *b*) if k tends to zero, the $F(MN)$ value approaches the *in vitro* calibration curve, which means decreasing recovery from the cytogenetic effect; *c*) increasing values of k are directly proportional to increasing recovery from the cytogenetic effect as a function of time.

We can suggest that after therapeutic irradiation a decidedly low value of k demonstrates high radiosensitivity of the lymphocyte pool, *i.e.* a subject with difficulty in recovering from the cytogenetic effect. On the other hand, a definitely high k demonstrates low radiosensitivity of the lymphocyte pool, *i.e.* a subject with rapid recovery from the cytogenetic effect.

In this preliminary experience on patients undergoing radiotherapy for lung and pelvic neoplasias (21 subjects), the values of k range between 0.030 and 0.307. This individual variability could serve as a reference for carrying out a comparison with the clinical response to treatment (clinical indicators of radiocurability).

In a recent research we showed that there is an interesting correlation between the decrease in peripheral blood lymphocytes and the cytogenetic data (MN frequency, cytogenetic recovery factor and 3AB-index) in patients undergoing radiotherapy in pelvic and head/neck sites [103].

Studies on the use of the MN method and its development in model form (k factor) could be useful in some problems connected with radiotherapy.

Also acute or delayed effects clinically observed after radiotherapy could be partly explained by means of the cytogenetic recovery factor. Concerning acute damage, previous knowledge of the patient's radiosensitivity could give some indication as to whether the subject should undergo accelerated fractionation or combined radio-chemotherapy, which is known to lead to greater harm. Long-term clinical observation could confirm the validity of k in expressing any predisposition the subject may have to develop delayed harm.

12·3. *Chemotherapy and oncology.* – Even though most of the research has so far been dedicated to cytogenetic dosimetry in radiopathology and radiation protection, our studies have also concerned chemotherapy in oncology [152].

Our cytogenetic observation concerned two groups of patients: 1) subjects undergoing cisplatin (CDDP) and vinorelbine (VNB) treatment for lung carcinoma (9 subjects) (table XI); 2) patients undergoing 5-fluorouracil (5FU) treatment alone or combined with folinic acid for adenocarcinoma of the large intestine (9 subjects).

The aims of the study were to *a*) evaluate the clastogenic capacity of the therapeutic treatment relative to the dose administered, therapeutic schedule and individual sensitivity; *b*) verify the predictive capability of the method with regard to the efficacy and toxicity of the therapy, by means of correlation between cytogenetic and clinical

TABLE XI. – *Micronucleus frequency after cisplatine and vinorelbine treatment.*

Gr.s	Patients (age)	Dose (mg)		Days	NM frequency			
					chemotreatment		2 Gy *in vitro*	
		CDDP	VNB		before	after	before	after
A	01 (73)	180	100	56	0.044	0.034	0.455	0.311
	02 (71)	270	150	7	0.081	0.060	0.403	0.541
	02	270	150	58	—	0.024	—	0.198
	03 (71)	150	60	29	0.046	0.063	0.302	0.306
B	04 (72)	170	100	29	0.120	0.064	—	0.268
	05 (53)	140	80	8	0.040	0.118	0.472	0.361
C	06 (46)	520	280	49	0.082	0.055	0.643	0.571
	06	520	280	110	—	0.410	—	0.745
	07 (70)	170	100	29	0.022	0.421	—	0.622
	07	170	100	54	—	0.062	—	—
	08 (63)	280	180	35	0.050	0.599	0.583	0.804
	09 (70)	240	160	29	—	0.653	—	0.846
	09	240	160	95	—	0.091	—	0.421
	09	240	160	215	—	0.132	—	0.534

data. The MN method was carried out on peripheral-blood lymphocytes of the patients before (baseline sampling) and during (after 2 cycles and again after 4-5 cycles) therapy.

The baseline values of the MN frequency were higher than those observed in healthy individuals and also showed a notable individual variability. They ranged between 0.022 and 0.120 with a mean of 0.061 (±0.011) in the patients with non-small-cell lung carcinoma and between 0.050 and 0.113 with a mean of 0.076 (±0.008) in those with adenocarcinoma of the large intestine. The two means are statistically different from the mean of 0.026 (±0.0013) obtained on 134 healthy control subjects. With regard to the MN frequency observed during therapy, the results for the two treatments were divergent.

For the CDDP+VNB treatment, the values obtained allowed us to divide the patients into three groups as a function of the cytogenetic effect observed (table VIII): 1) group A with a decrease in MN frequency during therapy; 2) group B with a higher MN frequency than the baseline values; 3) group C with a significantly larger MN frequency than the baseline values and a notable inter- and intraindividual difference (table XI).

In the treatment with 5FU the results mainly pointed out the incapacity of the drug to produce micronuclei *in vivo* (clastogenic effect absent?). *In vitro* treatment with a 2 Gy dose of irradiation to lymphocytes of patients in 5FU therapy showed (in 5 cases out of 7) a notably lower MN frequency. This phenomenon, which is dose dependent, is particularly evident when the chemotherapeutic dose exceeds 4100 mg [28].

Based on the data collected so far, we can make the following general considerations. The increase in MN frequency in the baseline sampling of the patients with neoplastic pathologies, compared to the healthy control subjects, can be taken as an indication of "cytogenetic fragility", and merits further studies. This condition, if confirmed by analyses on a larger number of individuals, could have important diagnostic implications. The MN increase produced in lymphocytes by combined CDDP-VNB treatment varies from patient to patient.

TABLE XII. – *Comparison between* MN *frequency and outcome of the tumour after chemiotherapy.*

Groups	Patient	MN frequency			Outcome
		a	*b*	*c*	
A	01	0.034	—	—	PRD
	02	0.060	0.024	—	NC
	03	0.063	—	—	PRD?-NC
B	04	0.064	—	—	NC
	05	0.118	—	—	NC
C	06	0.055	0.410	—	PR < 50%
	07	0.421	0.062	—	PR < 50%
	08	0.599	—	—	NC
	09	0.653	0.091	0.132	PR < 50%

a, *b* and *c*: different sampling; PRD: progress of disease; NC: no change; PR: partial remission.

The clastogenetic effect is in perfect agreement with the mode of action of CDDP which binds stabily to DNA with the formation of intra- and interstrand bridges, thereby causing breakage of the molecule. Table XII reports a comparison between the cytogenetic effect and the outcome of the tumour after combined CDDP-VNB treatment. If we correlate the outcome of the tumour after chemotherapy with the MN frequency, we could suggest that the cytogenetic response indicates the course of the pathology at individual level. The hypothesis of a relationship between MN frequency and efficacy of treatment is particularly encouraging for the purposes of prognosis.

The treatment with 5FU (alone or combined with folinic acid) does not produce any increase in MN in lymphocytes of the patients, so it is impossible to carry out a cytogenetic evaluation.

However, it would be worth carrying out further studies on the biological effect observed in these cells, which after *in vivo* contact with 5FU present a lower production of MN after *in vitro* irradiation at 2 Gy.

Two hypotheses can be proposed for this phenomenon: 1) there is probably an adaptive response from the lymphocyte cells after treatment with the drug; 2) turnover of the lymphocyte pool stimulated by the action of the drug, with consequent release into the circulation of the most radioresistant lymphocyte cells. The cytogenetic effects associated with the administration of anti-blastic drugs can be transferred onto the calibration curve and usefully compared with the effects of radiation doses; in this way we can obtain a kind of equivalent dose, which can be applied in risk estimation.

13. – Conclusions

The United States Congress Public Law 97-414 (4.1.1983), known as the “Orphan Drug Act”, is intended to regulate the research and production of rare drugs that are used for serious or very serious illnesses and not sufficiently promoted for study and development due to their cost and low demand. There is clear similarity with the normally complex, costly and often unusual diagnostic methods used in certain fields of medicine that are limited.

Some of the diagnostic investigations that are now included in the protocol of the biological indicators for evaluating doses of ionising radiation can be numbered among these methods and almost termed “Orphan dosimetric bioassays”. However, this definition must consider an exception, that is, an unexpected and heavy request for examinations. Such a situation could occur after a nuclear emergency involving a large number of people . In this case, a hurried “adoption of the orphan” is usually attempted. Dramatic confirmation comes from the Chernobyl accident, where, indeed, biological dosimetry played a leading role, given the complete absence in the first phase of individual physical dosimetry. Of note also is that only some (initially 154 out of 203) of the people suffering from acute irradiation sickness were able to undergo cytogenetic dosimetry analysis owing to its relative complexity.

Very appropriately the IAEA have underlined the need both to have wider means available for quicker observation and counting of metaphases and radio-induced chromosome aberrations as well as to identify faster and easier methods.

On an operational level, health departments should be able to assure the most efficient diagnostic services, speed and organisational flexibility, in relation to the variability in the number of subjects to be controlled. Another important point to be

taken into account is that population groups can include children, pregnant women, the old and the sick.

To avoid the institution of "empty cathedrals of health" that serve no purpose, we suggest that any hospital with a 24 h clinical laboratory, adapted to meet the needs of radiation protection, would be suitable for "screening irradiated people". Blood sampling for cytogenetic dosimetry should concern *all* the inviduals who can be labeled as "irradiated" (for medical histories and/or haematological picture). The hospital should be closely linked to a research institute that is competent and up to date in biodosimetry.

In conclusion, what is clear is the contradiction between the low probability of the accidental events considered and the need to have adequately prepared health measures able to cope with an emergency involving any number of people.

The health services should therefore be characterised by precisely identified structures and means, clear organisation and continuous validation. Such a technico-organisational policy should prevent adding the devastant effects of improvisation to the harm caused by the accidental event itself.

Continuity of competence can be guaranteed through a scientific program that produces concrete contributions in numerous fields of interest (nuclear medicine, radiotherapy, chemotherapy, space radiobiology, comparative cytogenetics). In return, the results of the program would contribute to improving the applications of diagnostic biodosimetry in radiation protection.

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B. CROSIGNANI, P. DI PORTO, M. SEGEV, C. SALAMO and A. YARIU – Nonlinear optical beam propagation and solitons in photorefractive media.
C. FURETTA – Thermoluminescence.
E. GALLO – Proton structure functions at HERA.
R. GIRLANDA, S. SAVASTA and B. AZZERBONI – Quantum description of the electromagnetic field in a confined polarizable medium.
D. GIULIETTI and L. A. GIZZI – X-ray emission from laser-produced plasmas.
G. MONTAGNA, O. NICROSINI and F. PICCININI – Precision physics at LEP.
V. PRIVITERA, S. COFFA, F. PRIOLO and E. RIMINI – Migration and interaction of point defects at room temperature in crystalline silicon.