Anhang/Appendix T 1

CELLULAR AND EXTRACELLULAR ASPECTS OF RADIATION RESPONSE

Bozidar Djordjevic
Department of Radiation Oncology
SUNY, Health Science Center at Brooklyn
450 Clarkson Avenue
Brooklyn, New York 11203, USA

Introduction. There is overwhelming evidence that the principal target of the biological action of ionizing radiation are cellular nuclei. This was most elegantly shown with nuclear transfer experiments in amoebae, where irradiated animals could be rescued if their nuclei were replaced with those from unirradiated animals, while replacement of the cytoplasm, did not have that effect (82). Similarly, experiments with electron beams and alpha particles with a limited range revealed that irradiation was effective in inactivating protozoa only when the beam reached the nucleus; with more energetic (longer range) beams, energy per lethal events was actually wasted (because it was spent in already doomed cells) (18,74,135). Nevertheless, damaging the cytoplasm was also deleterious, in that heavily irradiated (or otherwise damaged) cytoplasm in the amoebae transplantation experiments was incompatible with vital functions (81,82). These and similar experiments provide evidence that damage clearly outside the cellular nucleus may in some indirect fashion contribute to cell inactivation. We are still far removed from a full understanding of molecular mechanisms involved in radiation response, but we will presently try to elucidate interactive processes determining survival. In particular, we will introduce the concept of a metastable state in irradiated cells, similar to, but not identical to the state leading to apoptosis (interphase cell death, 124).

How radiation may act in an indirect fashion is perhaps most vividly illustrated by the so called Abscopal Effect (24). This term, presently not in common use, implies that parts of an organism not exposed to radiation may be influenced by parts exposed to radiation, such as sometimes seen in whole body irradiation (66,103). More recently, the notion that the effect of radiation (and other agents) may be modified by epigenetic factors has resurfaced: it appears that an important component of the multistep-carcinogenesis process is a disruption of intercellular gap junctions (123,134). This points to a complex organismal response to irradiation, where tissues respond to irradiation both in a direct and in an indirect way. In this view, not only cells directly targeted by irradiation, but the cellular microenvironment is of paramount importance in radiation response. How microenvironment may affect radiation response, is elucidated below.

The Extracellular Matrix and Radiation Response. The current paradigm in radiation biology is that clonogenic inactivation of cells in general, and DNA damage in particular, are the primary events in organismal radiation response. While this postulate is

basically valid, a more balanced view invoking the interplay of various intra- and extracellular structures in radiation response, is in order. The extracellular matrix (ECM) is part of the cellular microenvironment, and consists of an organized meshwork of protein and polysaccharide molecules, categorized in three major fiber forming proteins, collagen, elastin and fibronectin, interwoven with glycosamine chains (1,14,65). These molecules are exuded by cells and surround their outer surface, performing not only a structural role, but also a variety of regulatory functions actively participating in cellular behavior in an epigenetic fashion. These molecules interact with each other, and the structure as a whole interacts with the plasma membrane (13). As an example, migration, proliferation, development, wound healing and carcinogenesis, among others, are all modified by the ECM (11,12,13,14,61,107). While it is not entirely clear how order in ECM is enacted and then maintained (1,65), fluorescent antibody staining enables visualization of arrays of these molecules on the surface of cultured fibroblasts (118). Whatever the mechanism of this ordered formation is, it seems to be propagated from cell to cell, thus leading to a highly organized superstructure of cells (122). Moreover, order is recognized in a transmembrane fashion, with e.g., fibronectin influencing the cytoskeleton (arrayed polymerized microtubules) to assume an ordered structure (49). It is such interaction which allows fibroblasts to flatten out and proliferate on fibronectin-coated surfaces (61,130). Conversely, the cytoskeleton may influence the extracellular matrix, also in a wave of orderly propagation (49). Thus, there is a feedback interaction, largely responsible for tissular function; how these structures may be affected in their morphology and function by ionizing irradiation remains to be determined, but a coherent picture is slowly emerging. We will first present an overview of direct effects of radiation on ECM, and then how overall viability of irradiated cells may be influenced through changes in the ECM.

In a case where various components of the ECM were irradiated with low LET gamma and high LET iron particles, a differential effect of the two types of irradiation was obtained (8,12). While a decrease in collagen III was observed in the periepithelial stroma of mouse mammary glands, iron particle irradiation had no such effect, as determined by immunohistological staining. The glycoprotein tenescin, normally absent in the mammary gland, was found after irradiation with 0.8 Gy of iron particles. Laminin, normally localized to the basement membranes of vessels and epithelium, changes immunoreactivity after exposure to fast heavy particles (33). In general, irradiation, especially high LET, causes a rapid remodeling of the ECM in the mammary gland (8). In other tissues, too, irradiation causes changes in the ECM: in mice lung tissue, the condensed, highly anionic portion of the proteoglycan molecule is decreased after low LET irradiation (83,101).

The effect of ECM disturbance by irradiation on clonogenic survival is not yet adequately demonstrated, but indirect evidence points to the existence of a relationship. Notably, the shape of radiation dose-clonogenic inactivation plots (survival curves) is dramatically affected by agents known to be specifically involved

with the ECM. These survival curves are obtained by comparing clonogenicity (colony forming ability) before and after irradiation, and it was observed that in an aortic epithelial cell line (BEAC), the shoulder portion of the survival curve was substantially enlarged after treatment of irradiated cells with the basic fibroblastic growth factor (bFGF) (38). The increased shoulder width changes the survival curve parameter Dq from 34.4 Gy, to 205 Gy, a change with a very pronounced cumulative effect when multifraction irradiation (as practiced in radiation therapy) rather than a single dose was used (38). On the other hand, bFGF is also capable of rescuing endothelial cells from apoptosis initiated by the tumor necrosis factor (TNF). It has been shown that TNF is regulated by a cascade of events involving cytokines initiated at the plasma membrane, as evidenced in co-incubation experiments with nucleus-free extracts (46,54). However, as much as this information is alluring, direct evidence for the possible extranuclear initiation of radiation death is not firmly established (see below).

The Cytoskeleton and the Nuclear Matrix. In addition to the extracellular matrix, the cytoskeleton and the nuclear matrix also modulate radiation response. This is a consequence of the interconnectedness of all the cellular organelles, so that even compartmentalized radiation damage may be affected by the other cell components. To better appreciate the various ramifications of cellular response, we will review briefly the nature of this structure, its function and the changes it undergoes following irradiation. The cytoskeleton is composed of 3 classes of structures: 1) microtubules, made of the protein tubulin, 2) microfilaments, made of the protein actin which can cause contraction with the protein myosin, and 3) intermediate filaments which are elastic, flexible and strong. These structural proteins may differ from tissue to tissue, and in the same tissue of different animals. These structural proteins are in a dynamic equilibrium responsible for the shape of cells, and genetic or toxic agents may cause a collapse of the rigid system. A variety of cytoskeletal disorders have been associated with particular diseases, but we will mention only those conditions related to radiation effects. Suffice it to say that without a functional cytoskeleton, the effect of Brownian movement inside the cell becomes evident, since no internal anchorage supports various particulate cellular elements. The dynamic nature of the structural proteins comprising the cytoskeleton becomes visible with special techniques, such as localized laser labeling and immunochemical staining (99). For instance, it was shown that microtubules move either by transport (microtubular-dependent motors bound to a cytoplasmic matrix) or by treadmilling (polymerization at the plus end and depolymerization at the minus end) (99). The interaction of the cytoskeleton with other cell organelles is evident on several levels. The link with cellular membranes, both within the cell and at points of cell-to-cell contact, determines the cell shape and tissue integrity. These linkages are not simply mechanical, but have clearly more complex functions, such as signal transduction during morphogenesis (13,49). Within the plasma membrane itself, a skeletal structure

exists which provides both confining and binding, playing a pivotal role in the molecular organization of that organelle (56,73,75,121). Other interactive molecules such as CD44 glycoprotein may be also found in the plasma membrane extending to both the extracellular matrix and to the cytoskeleton. This accounts for the crucial difference between tumor and normal cells, and for the metastatic process (42,67,107). Similarly, mechano-sensitive properties of cell membranes play variety of other roles, such as the auditory reception and transmission processes (20). It is therefore not surprising that ionizing irradiation affects the intricate balance of the cytoskeleton: In confluent cultures of HT29 cells exposure to 0.5 to 1.0 Gy of x-rays, actin and intermediate filaments were disrupted, while microtubules were resistant (112). On the other hand, CHO-KI cells irradiated with up to 20 Gy of 137 Cs gamma rays, induced a G2 phase progression delay, which was not evident in cytochalasin B-induced polykaryon formation, presumably because the latter system dispensed with the need for microtubule participation in cell progression (19). From these findings it was concluded that among others microtubule functioning was inhibited by irradiation (126). Congruent with these findings are reports that clonogenic survival is also affected by disturbances of cytoskeletal architecture, such as the survival enhancing effect of non-toxic treatment with cytochalasin B (113-117), and the radiosensitizing effect of trypsinization (through attendant cytoskeletal disturbances (94-97)). Thus a cause-and-effect relationship between the cytoskeletal structure and clonogenic cell inactivation by irradiation is established.

Evidence is accumulating that the nuclear matrix plays a contributing role in radiation response. Work of Oleinick and associates is most informative in this regard: of the various types of damage to DNA by ionizing radiation, DNA double strand breaks (DSBs) and DNA-protein crosslinks (DPC) were investigated (7,76). It was found that especially prone to DPC were matrix-associated regions of DNA (MARs) during the replication process. In an <u>in vitro</u> assay involving isolated murine erythroleukemic nuclei (129), it was found that binding of MARs to the matrix proteins was increasing with the irradiation dose. These results indicated that the specific interaction of MARs with proteins of the nuclear matrix provides a radiation sensitive substrate for the formation of DNA-protein cross links. Another class of radiation damage, DSBs, are distributed more randomly along the length of the molecule, unlike the case of DPCs, which are restricted to MARs (76).

<u>Effects of Intercellular Contact</u>. When debating the possible extracellular contribution to radiation damage, there is a need to clearly separate the site of the initial damage and its relation to clonogenic inactivation. One line of evidence comes from experiments where feeder cells were added to sublethally irradiated test cells, resulting in a decrease in survival of test cells. In this particular situation, the survival-diminishing agent was tentatively identified as TNF released from feeder cells (39). A similar effect was seen when supralethally irradiated HeLa feeder cells were co-agglomerated with live HeLa forming the so called

hybrid spheroids (29,31,57). It became evident that upon prolonged incubation and repeated irradiation of hybrid spheroids, the constituent HeLa feeder cells diminish survival of test cells, especially of fibroblast test cells, when hybrid spheroids were dispersed and plated for colony formation (31). (Note, however, that fibroblasts used as feeder cells had an opposite effect, increasing survival of test carcinoma cell lines (40)) . Thus the possibility must be considered, that radiation may act not only by targeting cells ultimately inactivated, but also through a larger volume than actually is the physical space of these cells (93). Beside that such findings reflect on the notion of extracellular sources of radiation lethality, they have also obvious implications for tumor radiotherapy, in that they explain the often observed cure of localized tumors, when such cure was not expected from the radiation doses delivered to tumors of certain sizes.

The overall complexity of cellular contact and the presence (or in absence) of growth factors on radiation response is illustrated on another type of spheroids (77-79,100). It has been shown by Durand and co-workers (32), that Chinese hamster ovary (CHO) cells survive to a larger extent when irradiated in the form of agglomerates comprised of 30-50 cells. Also, irradiating other cell types in spheroids with single doses increases survival slightly (57, 92) (contrary from what was observed with multiple dose irradiation - see above). This state of enhanced resistance to irradiation persist for a period of time following dispersal of spheroids by trypsinization, and therefore could not be attributed to trivial physiological conditions such as hypoxia. This physiological state generally diminishes following spheroid disaggregation, to disappear completely after about 8 hours (32). Significantly, this state is not marked by changed population composition, a factor which could alter radiation response in its own right. Inasmuch the cause of this phenomenon is not known, it should be noted that several factors may be involved in the response of spheroids to irradiation, some of which may be acting in opposite direction. Indeed, as we argue elsewhere in this review, cell roundedness in spheroids of V79 cell may cause decreased, not increased survival after irradiation, but suboptimal growth conditions may have an opposite effect (96). This last condition may be operative in Durand's system too, as spheroids were maintained in medium supplemented with 5% serum, while monolayer cultures were maintained in 10% serum (32). Other, more specific changes may also occur in spheroids, such as junctional gap formation (77-79,129,134) which may or may not influence radiation response. It should be noted that gap junction formation is implicated in another phenomenon involving close cellular contact, the so called "Bystander Effect". In this phenomenon, cells not able to process a chemical into to a metabolite with chemotherapeutic action, acquire the active metabolite from other cells capable of such processing, inactivating the former cells (47,102). More pertinent to radiobiological phenomena, is the finding that destruction of gap junctions is instrumental in fostering carcinogenesis (123). Namely, cells initiated to become neoplastic are thought to be inhibited on that pathway by surrounding normal cells via gap junctional intercellular communication (134). Only after

downregulation of such intercommunication can initiated cells proliferate (134). Hence, epigenetic, in addition to genetic alterations are involved in carcinogenesis.

In addition to the above changes, chromatin alterations which take place during spheroid formation, could possibly contribute to the altered radiation response in mammalian cells (41,51,93). Other experimental evidence points in the same direction, albeit operating in different systems: Neoplastic transformation decreases radiosensitivity in rat embryonal cells, presumably via changed nuclear matrix mediated DNA organization in transformed cells (68). In mouse leukemic cells of varying radiosensitivity, the differential loss of DNA supercoiling ability could be due to differences in DNA-nuclear matrix anchor points. Reproducible differences in nuclear matrix protein configuration were found in these cell lines: a total of 9 proteins were found in the radioresistant cell line, but were absent in the radiosensitive cell line (69). However, in 4 human tumor cell lines of differing radiosensitivity, no correlation of radiation induced DNA double strand breaks and survival was observed (80). All this points to the complexity of cell organelle interactions in radiation response. A corollary of these findings is that the initial DNA damage after irradiation is most likely not the only cause of the survival pattern of cells in spheroids, but events subsequent to irradiation, in what we, somewhat imprecisely, term repair processes.

DNA as Primary Target of Irradiation. The notion that the cell nucleus in general, and DNA in particular, are the primary targets of irradiation, received a boost when it was demonstrated that specific changes in cellular DNA increased sensitivity to irradiation. Since thymine is a specific DNA constituent, incorporation of its chemical analogs (proffered in the form of its nucleoside derivatives, for ease of metabolic processing) presumably changed DNA molecules only. This was achieved with pyrimidines halogenated in the position 5, thereby substituting the methyl group of thymine. The ease of incorporation, and to some extent functional similarity, depend on the similarity of the diameter of halogen atoms to the methyl group of thymine which are attached to the position 5 of the pyrimidine ring. Thus, the closest approximation to the methyl group (2.00 A) being that of the bromine atom (1.95 A), corresponds to the ease of substitution of thymidine by 5-bromodeoxyuridine (BUdR). The phenomenon of radiosensitization has been first demonstrated with BUdR in ultraviolet irradiated bacteria (44) and in mammalian cells in culture (21), and later with ionizing irradiation in mammalian cells (35). We will not dwell how BUdR substitution became a tool to discern the mode of DNA replication (21), and still later how it revolutionized this field in conjunction with flow cytometry (104,105), but only point out how a technique initially hailed as a decisive proof of DNA being the primary target of irradiation, later also provided evidence of a more complex response. Even in the very beginning of radiosensitization research, it was recognized that BUdR did not always cause increased response to irradiation: in those mammalian cells (lymphomas) which were

extremely sensitive to irradiation, no increased sensitivity was obtained following analog incorporation (59), as if radiosensitization occurs only in (normally) radioresistant cells. That changing the target molecule (DNA) at the time of irradiation is not a prerequisite for radiosensitization was shown in experiments where BUdR was supplied after irradiation, diminishing survival (25). Another interesting effect of BUdR incorporation, is its modifying effect on cell-cycle fluctuation in radiation response (27, Fig. 1, also see below). More recently it has been shown that the extent of radiosensitization by halogenated pyrimidines depends not only on the amount of substitution of thymine by the analog, but also by the proliferative status of cells. In a case where the radiosensitivity of BUdR substituted cells was investigated, it was found that when cells in a monolayer where in contact with each other, a smaller radiosensitization factor was obtained than when cells were not touching each other (50). This indicated that for a given amount of substitution with the analog, other factors were playing a role, too. This other factor was most likely associated with an induced state of the so called contact inhibition of cell proliferation. Namely, it has been shown that BUdR incorporation confers to neoplastically transformed cells the ability of inhibition of cellular proliferation upon contact with each other (108), an ability normally seen only in untransformed cells. Thus, an interplay of various factors determines radiosensitivity, most prominent among these being the physical property of DNA molecules and the proliferative state of cells.

From the foregoing, one could deduce that cellular DNA may well not be the only critical target of irradiation. However, it is not clear what these additional targets may be. In experiments involving controlled decay of radioactive iodine in DNA of frozen CHO cells, Hofer (48) has shown that a nuclear structure becomes more radiosensitive with a prolonged chase of the radioactive label, and that damage to a higher order structure, rather than to DNA molecules proper, may substantially contribute to cell death. Therefore, we must seek to understand both the nature of cellular structures to be repaired, and the process of repair itself; only then will we be able to successfully manipulate these processes and structures to a beneficial outcome of tumor radiotherapy.

Cell Survival and Repair Processes. There is a longstanding awareness that survival of irradiated mammalian cells is influenced by their postirradiation experience. This in itself was a radical departure from the original postulate of the target theory - that the effectiveness of radiation is determined by the physical impact of radiation alone (58). After the realization that many events take place between the impact of radiation and the expression of damage, the introduction of the concept of radiation damage and its repair took place (62-64). This concept alleviated the original quandary of the unsteady radiation response, but it did not solve the problem of how the repair capacity relates to the cellular machinery. We are beginning to understand the molecular mechanisms involved in repair, such as restoration of double strand breaks, but crucial questions about cell viability following irradiation,

and how postirradiation phenomena may be manipulated to improve radiotherapy of tumors, remain.

One condition which affects survival of irradiated cells is their mode of proliferation. This is perhaps best exemplified with the periodic irradiation of synchronized mammalian cells in culture. Here, radiosensitivity varies according to the stage in the cell cycle. Cells in mitosis are most radiosensitive, followed by cells in the G2 phase and cells in the G1-S transitional phase in mammalian systems (22,23,91). Cells in the mid G1 phase (except those without a prolonged G1 phase (109)), and in the S phase are most resistant. This response can be modified by the use of metabolic inhibitors <u>after</u> irradiation, especially after irradiation in the G1-S phase transition (22,24,91). Since the largest increase in survival was achieved by the inhibition of subsequent DNA synthesis in cells irradiated in the G1-S transitional phase, it was surmised at one time that radiation damage has to be repaired before DNA replication, lest the radiation induced error be copied and transferred to subsequent generations (53). Alternatively, it has been postulated that the process of DNA replication itself is somehow deleterious to damaged DNA, implying that damage must be repaired first, before replication can safely proceed (62-64). It turned out that neither explanation withstood more careful scrutiny, in that sparing of cells could be disassociated from any inhibition of DNA synthesis: much of the rescuing in survival could be achieved in spite of postirradiation DNA synthesis. It had been shown by Lange and associates (96) that an increased survival, without a concomitant decrease in progression through the S phase, as revealed by flow cytometry could be achieved by subjecting cells to medium isotonically diluted in saline. Nutrient dilution does however increase cell spreading and changes chromatin conformation, as seen by the nucleoid halo assay (52). It has also been shown by Lange et al. (98) that in V79 cells some potentially lethal radiation damage is neither fixed nor repaired for long periods of time. Apparently, after obtaining a given level of survival using one assay system, more repair may be obtained using another system. This is made evident when V79 cells exposed to 10 Gy of x-rays were treated in hypertonic saline for 20 min and then incubated in either growth medium, or conditioned medium before trypsinization and plating: only with the intermittent treatment with the second medium, was increased survival obtained. When hypertonic saline was given after incubation in growth medium, increased survival was obtained, plateauing after 70 min. However, an extra increase in survival was obtained when incubation in conditioned medium intervened (98). What seems to affect survival, is the shape cells assume during and after irradiation: Cellular roundedness, obtained either by trypsinization (94,95), or by maintaining cells as suspended spheroids (97), decreased survival in irradiated cells. Thus cellular and/or chromatin configuration appear to affect cellular recovery from radiation damage (41,51,93).

<u>Cell Membrane Effects</u>. For years, radiobiologists have been considering that the cell membranes, including the plasma membrane, may be a cellular target of irradiation. Bacq and Alexander (6)

proposed the "enzyme release" hypothesis, whereby membranes, especially lysosomal membranes are damaged by irradiation, so that lytic enzymes are released inside the cells, killing them. Indeed, a number of experiments (73,75) showed that enzymes are released following irradiation from specific cellular compartments. Even interphase death was at one time attributed to an indirect effect of irradiation on cell membranes, but now we know that interphase cell death (Apoptosis) has a different etiology (124), and in general, the membrane hypothesis lost its initial allure. Still, in the light of the interrelatedness of different cell organelle injury to survival of clonogenic ability, it is instructive to review relevant findings of the effect of irradiation on membranes, and on the plasma membrane in particular. Most of these findings pertain to permeability changes, vascular changes, cell surface blebbing following irradiation (16,119,120,125), and other effects not immediately related to clonogenic survival. However, a convincing argument in favor of clonogenic effects of plasma membrane changes after irradiation, is the oxygen effect, whereby the dose of irradiation to inactivate a cellular component or whole cells, is reduced in the presence of oxygen. Since this phenomenon is most pronounced in systems containing phospholipids and other derivatives of fatty acids, it was proposed by Alper (3,4), that under oxic conditions, irradiated cells suffer the so called "O" damage, presumably associated with membranes, while under anoxic conditions, the "N" damage, mostly involving non-membrane damage, is predominant in cell inactivation. A further corollary of this postulate is the inferred role of antioxidants in both survival of irradiated cells, and the stability of isolated membranes after irradiation (2,15). Here, increased survival and membrane stability was observed when tocopherol (vitamin E) was added to the respective systems, and it was inferred that such addition saved the substrate from oxidative damage. Oxidative damage was also invoked in the differential effect of lidocaine (a membrane specific anesthetic), which was found to be an effective radiosensitizer in hypoxic mouse lymphoma cells, but a protector in oxygenated cells (131,132). A differential effect of another category was found by Djordjevic in HeLa cells, where procaine, a moderate protector when given to oxic cells during irradiation only, was a potentiator of cell killing when administered postirradiation (26,28, Fig. 2). A further quandary, rather than clarification of the role of plasma membranes in radiation response, was obtained with artificial alteration of the plasma membrane composition by the introduction of unsaturated fatty acids. Such alteration increase membrane fluidity (131), and increase radiation sensitivity in isolated membranes and some bacterial systems, but will not cause increased radiosensitivity in intact mammalian cells (133). In this case, the presence of natural antioxidants seemed to have an ameliorating effect, countering any effect due to membrane composition on radiation sensitivity (55,106). Nevertheless, even as specific alteration in the plasma membrane did not bring about unequivocal confirmation or refutation of a model of (alternate) cellular targets of irradiation, changed radiosensitivity, when found, may prove to be of clinical significance.

It is well known that free radicals play an important role in cellular metabolism and tissue injury (10,17,111). They are also produced as a consequence of the indirect effect of irradiation, accounting for most of the lethal effect (6). A plausible approach to radioprotection is by inactivation of deleterious long lived oxygenated organic radicals located in membrane lipids, achieved by the application of the enzymes Superoxide Dismutase (SOD) and catalase to various systems. This has been extensively studied by a number of workers, notably by Petkau and Associates (9,37,88,90). Especially suitable for this type of intervention are artificial membranes, in which constituent fatty acids, when irradiated, become oxidized into alkyl radicals (87). This reaction can be reversed in the presence of SOD (9). While this process is clearly associated with membranes, radioprotection is obtained also in cellular systems and even in whole animals. Inasmuch the detailed pathway of lipid peroxidation, and of SOD intervention presents a rather complicated picture (85,89), the following may highlight important steps involved in the phenomenon of lipid peroxidation and neutralization by SOD. Radioprotection was achieved in the mycoplasma Acholeplasma laidlawii, amounting to an increase of 7.5 to 18.8% of D_0 on the fast component of the survival curve, depending on the buffer used (84). Female Swiss mice irradiated with 5.5 and 6.75 Gy of x-rays and injected with 35 ug/ml of bovine SOD hastened the recovery of erythrocyte count so that 22 days postirradiation the erythrocyte count and percentage reticulocytes significantly improved over the control series (86). The $LD_{50(30)}$ in treated mice was 7.0 Gy, compared to 6.27 Gy for the saline injected control group (85). Moreover, constituent SOD was found to be higher in humans previously exposed to radiation sources (90), giving rise to the notion of measuring enzyme levels as an internal dosimeter.

Perhaps the most interesting aspect of these studies is the increased efficiency of superoxide formation at very low doses of radiation. This phenomenon takes place when individual foci of superoxide formation are so thinly distributed after low doses of radiation, that their inactivation through mutual combination is diminished (89). This is especially pronounced at physiological (7.4) pH, which fosters superoxide radical stability (60), thus prolonging their availability for interaction with biological molecules. The overexpression of low doses of radiation in superoxide formation may conceivably be correlated with the now familiar pronounced clonogenic inactivation after small doses (70,71,128). Another area where superoxide overexpression may be instrumental is neoplastic transformation, where the redox state may have an epigenetic effect: the normal unstable p53 tumor suppressor protein can be converted under a favorable oxygen regimen, to a more stable configuration, mimicking the mutant protein (134). Thus carcinogenicity of low radiation doses may have been underestimated.

Concluding Remarks. It can be deduced from the foregoing that the biological effects of radiation may not be as straightforward as even very recently assumed. It is evident now that radiation may

act on different levels in the cellular and organismal hierarchy, and through different interacting processes. Besides the direct effects on genetic material, epigenetic processes are also taking place. Thus DNA, considered to be the primary target of radiation, may be subjected to a variety of modifying processes after irradiation, before its final disposition in a (mal)functioning state. Such modifying processes may often be initiated from outside the target area, leading to either restoration (recovery) or to self-destruction (suicide). Whether in extreme but presently undocumented cases such self-destruction may conceivably involve genetic material not initially targeted by irradiation is an open question, but it probably would be very rare. More important is the consideration that for extended periods of time the biological consequences of radiation are amenable to manipulation, pointing to the possibility of improved radiation therapy of cancer (45).

Of special interest is the possibility that very low doses of radiation have a disproportionate biological effect. This counters the accepted wisdom of the corrective effect of repair processes, but such notions are based on the effect of larger doses, not the very small ones. Yet we know that lethality after very small doses may be considerable (see above), and we saw how the Petkau Effect is disproportionately manifested after small radiation doses. Such considerations are important in carcinogenesis, the principal hazard of low radiation doses. Unfortunately, it is difficult to distinguish between newly induced and a background of preexisting neoplastically transformed cells (72,110); in order to approach this problem, better methods of detecting initial stages of carcinogenesis must be devised.

FIGURE LEGENDS

1. Upper panel: Survival of BUdR containing synchronous HeLa cells x-irradiated with 3 Gy (empty circles), or of unsubstituted cells x-irradiated with 6 Gy (full circles) at different times after starting cultures from mitotic cells. Lower panel: DNA synthetic profile obtained in these cultures by pulse labeling with tritiated thymidine (ref. 27).

2. Effect of incubation of synchronous (early G1) HeLa cells with or without (postirradiation) treatment with 1 mM procaine at 37°C or 41°C for different periods of time (ref. 28).

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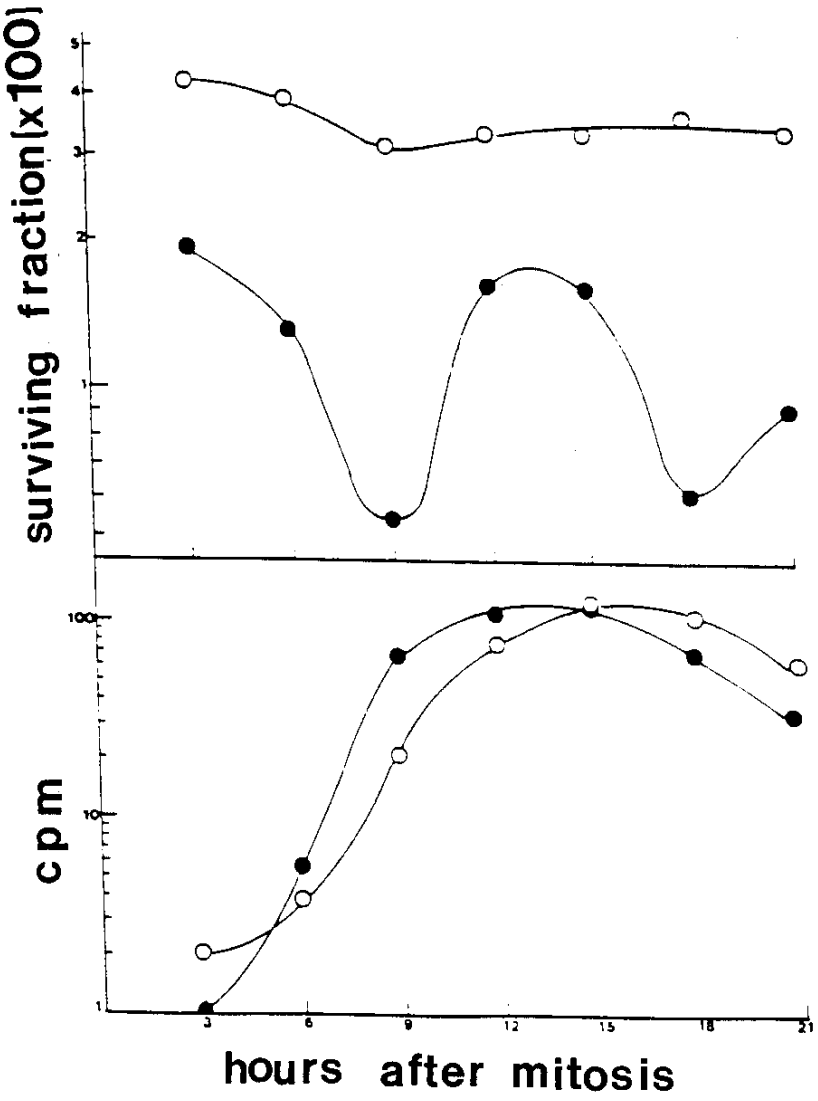
surviving fraction (x100)
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Anhang/Appendix T1