Chapter 6

BIOLOGICAL ASSESSMENT OF RADIATION DAMAGE

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INTRODUCTION

Biological dosimetry involves measuring a given physiological response to a known or estimated exposure dose of a toxin (in this case, ionizing radiation). Reliable biological dosimeters of radiation injury are needed *(a)* to perform casualty triage, *(b)* to determine probable exposures when physical dosimeters (external indicators of radiation that are used in the field) are absent, *(c)* to confirm physical dosimetry, and *(d)* to monitor the progress of radiotherapy. This chapter deals with biological dosimetry relating to accidental exposure or nuclear warfare, rather than the clinical monitoring of radiotherapeutic progress, although similarities exist. The armed forces presently issue physical radiation dosimeters to groups rather than to individuals; shielding differences may affect individual doses. Variations in age, gender, health, and genetic background may greatly affect the biological responses to a given radiation dose. Careful monitoring through biological dosimetry can provide more reliable indicators of exposure, which will be used in formulating prognoses on treatment and survival.

Damage from ionizing radiation occurs at both the local and systemic levels. Biological indicators are diverse and may include changes in levels of tissue enzymes,[1-13] metabolites,[14-22] and cell populations (such as lymphocytes[23-25] or sperm[26]); changes in individual behavior;[21,22] and a general onset of malaise.[21,23-27] Both early and late responses to radiation injury may be reflected. Elevations in serum amylase levels may occur within hours to days,[2-8] and elevated levels of lactate dehydrogenase[11,28] or zinc protoporphyrin[29] are observed several weeks after irradiation. The ideal biological dosimeter should be reliable, able to detect 0.1-10.0 Gy, linear in response regardless of dose or quality of radiation, simple to use, preferably noninvasive, sensitive to radiation occurring during the first hours or days after exposure, and preferably radiation specific.

A combination of five indicators (Table 6-1) may provide a reliable gauge of radiation exposure: *(a)* the physical symptomatology of the prodromal stage of ARS, *(b)* lymphocyte numbers, *(c)* serum components (diamine oxidase and serum amylase), *(d)* urinary components, and *(e)* chromosomal aberrations.

PHYSICAL SYMPTOMATOLOGY AT THE PRODROMAL STAGE

The most reliable method of biological dosimetry is the physical symptomatology at the prodromal stage of ARS,[21,24] which occurs within minutes to 1 day after irradiation and includes nausea, vomiting, anorexia, diarrhea, and general malaise. The severity, duration, and time of onset of these symptoms are related to the dose and quality of the radiation received. A dose-dependent latent period occurs between the end of the prodromal symptoms and the onset of later hematopoietic or gastrointestinal complications (Table 6-2). The onset and latency are inversely related to the dose, whereas the duration and severity are directly related and vary

for individuals. The median acute gamma radiation doses have been estimated for anorexia (0.97 Gy), nausea (1.4 Gy), fatigue (1.5 Gy), vomiting (1.8 Gy), and diarrhea (2.3 Gy).[25,27] Protraction of the dose over 1 to 7 days results in doubling the median doses.

Several early physiological responses are associated with exposure to radiation doses in the high-lethality (greater than 5.5 Gy) range (Table 6-3). These symptoms usually reflect an unfavorable prognosis for survival without adequate hospitalization and extraordinary lifesaving measures, such as bone-marrow transplantation.[21,24,25] At still higher radiation doses, even these measures would not be effective.

Erythema and epilation are two other indicators of clinically significant radiation exposure. Erythema has a threshold of 3-4 Gy, depending on the area of irradiated skin, with a median dose estimate of 6 Gy.[25,30] Erythema occurs in two phases: an early phase, appearing within several to 24 hours after irradiation, and a later "main erythema," reappearing 2-3 weeks after irradiation and persisting for several weeks.[31] Erythema also depends on the type of radiation and the skin condition. Epilation occurs approximately 2 weeks after radiation doses larger than 2-3 Gy.[30]

HEMATOLOGICAL DOSIMETERS

Peripheral blood lymphocytes are extremely sensitive to ionizing radiation. They may succumb to interphase death after exposure to a dose of only 0.05-0.15 Gy.[30] The decrease in the number of blood lymphocytes at 24-48 hours after irradiation can be a useful indicator of radiation exposure (Table 6-4).[21,23,24] The radiation doses listed in Table 6-4 are based on exposure to gamma radiation, but the lethality patterns should be similar for a neutron or mixed-field pure radiation exposure as determined from the lymphocyte counts. A lymphocyte count of 1,200-1,500/mm^3 at 24 hours after irradiation is a reduction of 50%. It indicates a potentially lethal exposure, requiring immediate medical attention.[23] Monitoring for the onset of hematological problems at the end of the latency period (which may last several weeks) is advised for persons whose lymphocyte counts reflect a lower but potentially lethal level of exposure. Lymphocyte counts drop to zero in persons who received doses greater than 5.5 Gy.

Granulocytes, platelets, reticulocytes, and erythrocytes are also affected by radiation (Figure 6-1). In particular, lymphocyte and granulocyte counts proved to be valuable biological dosimeters after the 1986 reactor accident in Chernobyl, USSR.[32] Blood cell effects may be detectable in humans after exposure to 0.5-1.0 Gy of gamma radiation. These responses reflect interphase cell death and also the mitotic delay or destruction of the hematopoietic stem cells of the bone marrow.[33] Hematopoietic depression may result either directly from radiation damage to the hematopoietic stem cells, or indirectly from damage to the stromal stem cells that

are responsible for maintaining the microenvironment of the bone marrow. Hematopoietic precursor cells in later stages of development are less sensitive to radiation damage than are the stem cells. In an abnormal process called *maturation-depletion,* these more mature differentiated cells may continue to develop without other precursor cells differentiating to take their place. This same process may be observed in the irradiated testes, where radiation exposure is followed by temporary aspermia.[26]

The length of the latency period between the radiation exposure and the decrease in blood cell numbers depends on the degree of damage and on the normal lifetime of that particular class of blood cells. Human platelets have a life span of 4-5 days, and because they decrease by attrition without replacement, hemorrhage will occur. Radiation-induced mortality after a dose of 2-12 Gy results from hemorrhage and other hematopoietic problems. These symptoms are called the hematopoietic subsyndrome. Physical examination and blood cell counts are readily obtainable, although other technologies may be too time consuming or otherwise not presently feasible for large-scale field use.

BLOOD SERUM DOSIMETERS

Although a number of serum factors may be useful biological dosimeters, none has received widespread verification or acceptance. However, two show potential: serum amylase [2-8] and diamine oxidase (DAO).[1,13] Serum amylase (Figure 6-2) becomes elevated in humans who receive radiotherapy when the parotid gland is included in the field of exposure.[2,4] This response is an early indicator of damage, and elevation can be seen within hours after irradiation. Amylase levels increase almost tenfold, peaking at 24-36 hours after exposure to 1 Gy.[2] A linear correlation exists for the peak serum elevation and the radiation dose received; the peak response occurs slightly earlier with increasing doses.[2,4] Radiotherapeutic fractionated doses of 1-2 Gy/day result in the further destruction of the parotid gland and a reduction in serum amylase by the end of the first week. The response depends on exposure of only the salivary glands (levels of pancreatic serum amylase are not altered in response to radiotherapy).[5] Interestingly, the elevation of serum amylase in response to radiation has been reported only in humans; it cannot be reproduced in rodent research models.[7] The effect of neutron radiation or of combined injury on the elevations of serum amylase has not been determined.

DAO is another serum enzyme that is a potential biological dosimeter of radiation injury.[1,13] DAO is produced primarily by intestinal villi during cell proliferation and differentiation. In humans, DAO levels have been used to monitor the effects of chemotherapy on the gut, but the response following irradiation has not been determined.[34] Plasma levels of DAO (Figure 6-3) increase in a dose-dependent manner in mice on days 2-4 following exposure to cobalt-60 gamma or neutron radiation.[1] This initial increase may not be associated with gut damage but may

reflect a more systemic injury.[1] If the human response is consistent with the mouse model, patients who receive radiotherapy that does not involve the gut should have changes in DAO levels related to the radiation exposure. The mouse model also indicates that the DAO response may not be reliable in combined injuries involving radiation and burns.[1]

Other changes in serum enzymes have been reported; however, they are not as indicative as those in serum amylase and DAO. Serum alkaline phosphatase in rats decreases after irradiation in a dose-dependent manner.[10] The decrease occurs primarily in the intestinal and liver isozymes, and may be useful for dosimetry on days 2-4 after irradiation. The intestinal isozyme decreases on day 3 by 77% after a 5-Gy radiation dose, and by 90% after an 8-Gy dose. The responses of these isozymes in humans have not been confirmed.

Other serum indicators have been examined in humans receiving radiotherapy, but results are sometimes difficult to interpret because of interactions between the chemotherapy, cancer, and long fractionation schedules. Changes in the levels and classes of immunoglobulins in association with immunosuppression after radiotherapy do not appear to be useful in biological dosimetry.[35] Serum lactate dehydrogenase increases during the first week of radiotherapy, with even higher levels at weeks 4-5 in a majority of patients, but these increases may be influenced by other factors, including infection.[11] Levels of plasma hemoglobin and haptoglobin increase up to 40%, but not until week 4 of radiotherapy in cancer patients.[36] Haptoglobin is produced by the liver and hemoglobin is produced by hematopoietic precursors. The elevation of hemoglobin is probably associated with recovery of the hematopoietic system, and the dose dependency (not yet determined) will be associated with increased delay of elevation.

URINARY DOSIMETERS

Lacking metabolic enzymes, urine provides relatively stable biological indicators. It can also be obtained noninvasively. After irradiation, urine contains more creatine,[18,19,28] histamine,[17] taurine,[16] amylase,[5] and prostaglandins.[15]

Postirradiation elevation of creatine occurs during the first 3 days after exposure of rats to less than 0.25 Gy of X radiation.[18] The average urinary creatine-creatinine ratio during this period is dose dependent for 0.25-6.5 Gy. Creatinuria occurs in humans after irradiation, but because it may also be affected by exercise, muscular atrophy, trauma, or starvation,[28,37] it cannot be considered specific to radiation injury.

Elevations of histamine occur in the blood of patients receiving radiotherapy.[20] The maximal elevation of histamine in the urine of rats occurred on the first day after exposure to 9 Gy of cobalt-60 gamma radiation.[17]

Taurine is an amino acid that is excreted in large concentrations from rats in the first 3 days after irradiation.[16] Elevated taurine levels have been reported in exposed humans,[38] and may be attributed to altered excretion patterns from kidney damage, altered synthesis, and increased release from damaged tissues (particularly lymphoid tissue).[39]

Glycine and hydroxyproline are also excreted in increased amounts (up to ten times normal levels) in the urine of humans during the first week after receiving 25-180 rem.[38] Elevations of prostaglandins have been detected in the plasma of patients receiving radiotherapy[14] and in the urine of laboratory mice receiving cobalt-60 gamma radiation or neutron radiation.[15]

The development of biological dosimetry kits based on urinalysis would be hindered by *(a)* biphasic responses, such as occur with prostaglandins[15] and taurine;[39] *(b)* diurnal variations, as with creatine;[37] *(c)* reductions in urinary volume following irradiation;[15] and *(d)* problems with 24-hour urine collection.

CHROMOSOMAL DOSIMETERS

Although the most widely used biological dosimeter of radiation injury is prodromal symptomatology as revealed by physical examination, the most precise method is the determination of chromosomal aberrations in human blood lymphocytes.[40] Such chromosomal analysis has been used to determine the radiation doses received after industrial accidents,[41-43] the occupational exposures received by uranium miners,[44] and the radiation exposure of the atomic-bomb survivors of Hiroshima and Nagasaki.[45] A correlation of chromosomal damage with the radiation dose has been confirmed with *in vitro* experiments and with patients receiving radiotherapy.[46] Response curves have been prepared for the various types of radiation (Figure 6-4), and for the three assay methods in use (Table 6-5): *(a) cultured peripheral blood lymphocyte technique,*[40-47] *(b) premature chromosomal condensed chromosome (PCC) technique* [48-50] and *(c) micronuclei technique* with lymphocytes or erythroid precursors.[51-54]

Cultured Peripheral Blood Lymphocyte Technique

Typically, blood lymphocytes are nondividing cells that have progressed to the G_o stage of the cell cycle. They may be stimulated into mitosis with phyto-hemagglutinin. This culturing process requires about 48 hours to obtain a sufficient number of lymphocytes in mitosis, at which time the chromosomes have become condensed and the damage is visible.[40,55] The number of dicentric aberrations, ring structures, or total number of aberrations per mitotic cell are tabulated and recorded. Dicentric (Figure 6-5) and ring chromosomes are formed from asymmetrical interchromosomal exchanges, in which one of the resulting chromosomes contains two centromeres. The formation of dicentric chromosomes is linearly related to the radiation dose (Figure 6-4), although the response may

vary with the type of radiation (and also between laboratories).[56-58] These differences can be seen in curves D (curvilinear) and G (linear) of Figure 6-4, which are low-dose-rate gamma radiation exposures from two laboratories.

In addition, Figure 6-4 shows a difference between high-dose-rate X radiation (C) and high-dose-rate gamma radiation (E and F), although the RBE for gamma and X radiation is usually similar. Irradiation with high-LET alpha particles (curve A) or medium-LET neutrons (curve B) is more damaging than irradiation with low-LET radiation, such as X rays and gamma rays (curves C-G). To assess the dose accurately, the type of radiation must be known. Beta radiation exposure may produce skin damage of medical consequence, but it would not be accurately reflected in this assay.

An average assay requires one workday for a trained technician to count about 10,000 lymphocytes to obtain 200 cells in metaphase.[58-59] Lower radiation doses produce lower aberration yields, and require that even more cells be counted for statistical accuracy. Several problems are associated with the standardization of this technique, including variations in tissue culture conditions between laboratories, the percentage of cells in first mitosis at 48 hours, variations in health and age of persons examined, whole-body versus partial-body exposure, and radiation effects resulting from different types of radiation (low versus high LET) and dose rates.[47,48,56,58,60-63] The normal incidence of chromosomal aberrations increases with the increasing age of the individual and previous exposure to carcinogens.[52,64] The time interval at which the sample was collected after irradiation can also affect the results because the damage may have a chance to repair.[47] A program coordinated by the International Atomic Energy Agency found a 15%-36% difference in the scoring of a standard test sample among fourteen test laboratories.[56] A previous study found an eightfold difference, which was attributed mainly to differences in culture conditions and in the time of first mitosis.[56,65] When all laboratories examined the same slides, the differences were greatly reduced. A computer system has been applied to the analysis of chromosomal aberrations using parallel image processing,[66] but the preparation of samples still requires a specialized laboratory and is not feasible for a military or civilian field hospital.

Premature Condensed Chromosome Technique

The PCC technique eliminates many of the inherent problems of mitotic peripheral blood lymphocyte cultures.[48-50] In this method, human lymphocytes are fused to mitotic Chinese hamster ovary (CHO) cells using polyethylene glycol. The mitotic CHO cells cause the individual lymphocyte chromosomes to condense and become visible.[62,63] After staining, the human chromosomes and those from CHO cells will be different colors.[62,63] The damage is assessed by determining the number of human chromosomal fragments in excess of the normal forty-six. Samples for PCC analysis require less than 2 hours for preparation time, eliminate variations due to culture conditions, and may be

analyzed quickly because 200 lymphocytes with visible chromosomes are easier to locate and count.[48-50,63] As with chromosomal aberration analyses of cultured lymphocytes, assay-sampling time after irradiation remains a concern, because repair can reduce the aberration yield.[48,49] The effects of dose rate, radiation quality, and LET on the reliability of this technique have not been investigated, nor have the possible alterations from combined injury. Future research may focus on the development of a nuclease-free homogenate from mitotic cells that will induce the premature condensation of chromosomes. This would eliminate the requirement of specialized tissue-culture laboratories for fresh mitotic cells and would permit the development of a standardized assay kit. Even with computer analysis, the assay may still be too time consuming for use with mass casualties.

Micronuclei Technique

Micronuclei are chromosomal fragments that lack centromeres. As a result, they are not incorporated into the new nucleus during mitosis but form a smaller satellite structure. As with other chromosomal aberrations, the rate of micronuclei formation depends on the dose rate and the LET. Therefore, neutrons have an RBE of 4.4 in mouse bone-marrow cells compared to a lower RBE of cobalt-60 gamma radiation.[54] Micronuclei are easier to detect in the bone-marrow normoblasts because the micronuclei remain after the main nucleus has been eliminated. Control values for this assay are about 1-2 micronuclei/1,000 cells, based on an average screening of 2,000 cells.[54] The assay may also be performed on mitotically stimulated peripheral blood lymphocytes, extending the analysis time by the standard 48-hour culture period, or it may be performed on cells obtained from a bone-marrow transplant.

OTHER DOSIMETERS

Fluorometric Immunoassay

Fluorometric assays or immunoassays have been developed for specific biological indicators present in blood or urine. During the late 1970s, increased emphasis was seen in the Soviet literature on fluorescent and chemoluminescent changes in blood cell and serum components after irradiation.[67-70] Fluorescent techniques permit the development of dedicated fluorometers for use in screening blood samples rapidly for changes in specific components. Dedicated field-portable fluorometers are currently used by the U.S. Public Health Service for detection of zinc protoporphyrin (ZPP) from a drop of blood.[71] Elevation of ZPP is a diagnostic indicator for lead poisoning and for iron deficiency anemia.[72] ZPP also becomes elevated in the blood of mice following whole-body exposure to ionizing radiation (Figure 6-6).[29] This elevation is associated with the recovery of the hematopoietic system at 10-18 days after irradiation, and is not observed in animals dying from the hematopoietic subsyndrome. The elevation of ZPP occurs

too late to be useful in general screening or triage; however, it may be feasible to develop a field-portable dedicated fluorometer for other biological dosimeters.

Immunoassay offers another promising technique for field-dosimeter assay kits. A fluorescent immunoassay has been developed at the Lawrence Livermore National Laboratory to detect changes in the determinants of the MN antigens located on the surface of red blood cells.[73,74] The M and N blood-group antigens are heterozygous forms of glycophorin A. Like those antigens of the AB blood group, persons may be homozygous MM or NN, heterozygous MN, or hemizygous NO and MO. The general population is predominantly MN, and MN-antibody-labeled human blood cells fluoresce red and green when exposed to laser light. Blood cells from MN-heterozygous humans exposed to ionizing radiation have a greater variant frequency or proportional alteration in the MN blood antigen (MN, NN, MO, NO).[73] These abnormal cells (nonheterozygous) fluoresce red or green, reflecting the presence of one antigen; they do not reflect both colors as do normal cells. This assay has been used on blood samples from the atomic-bomb survivors in Japan[73] and the more recent victims of the nuclear-reactor accident in Chernobyl.[74] The change in the MN blood-group antigen occurs in the stem cells of the bone marrow, and its earliest appearance in the recovering marrow will be 2-3 weeks after irradiation. It would not be useful as an early triage indicator during the first few days.

Determination of Whole-Body Radionuclides

The degree of internal contamination from most radionuclides can also be determined by the use of physical dosimeters, such as whole-body radiation scanners. Iodine-131, a radioactive isotope with a half-life of 8 days, is usually released in large quantities after a nuclear accident or explosion. It is usually either inhaled or ingested. It enters the food chain when it is deposited on grass, which is eaten by dairy cows who, in turn, produce milk for human consumption. Once internalized, iodine-131 collects in the thyroid, where sufficient concentrations may lead to hypothyroidism, organ ablatement, or increased incidence of thyroid cancer. The levels of iodine-131 can be determined with an external thyroid scan.

A neutron dose can be calculated either by measuring the presence and concentration of sodium-24 in the plasma or by using a whole-body scan. Exposure to neutrons results in the conversion (neutron activation) of sodium-23 (the nonradioactive, abundant isotopic form of sodium) to sodium-24. Sodium-24 is a radioactive isotope with one more neutron than sodium-23. It has a half-life of 15 hours and decays to nonradioactive magnesium-24, releasing both beta and characteristic gamma radiation. Sodium-24 should be determined from plasma samples rather than from whole blood, because it appears mainly in plasma rather than in red cells.[75] The presence of sodium-24 above background levels indicates a neutron exposure,[75] and the radiation dose can be calculated from the levels of sodium-24 present.

PROBLEMS

Problems exist in the use of biological dosimeters, including their potential unreliability in combined-injury situations.[23,47,48,61-63] For example, the value of a lymphocyte count becomes questionable in a combined injury involving infection or burn. Additional problems include the following:

- Collection, contamination, stability, and reproducibility of the sample

- Variable changes in a biological response, according to the dose of radiation received and the time elapsed between exposure and examination

- Length of time required for assay (48-72 hours for some chromosomal analyses), which makes its feasibility for field use questionable

- Lack of an appropriate research model system (Some biological changes observed after radiation exposure in humans, such as serum amylase, do not occur in laboratory animals.)

- Radiation variables: dose, dose rate, quality, type, and mixed-radiation fields (Fission neutrons have an RBE of 18 compared with X radiation for the production of dicentric chromosomal aberrations.[57] The possibility exists for a significant error in measurement if the type of radiation or proportion of mixed-field radiation is not known.)

Many of these problems might be overcome by using more than one biological indicator (for example, using one as a screening agent and another as confirmation). Lymphocyte and granulocyte cell counts, combined with chromosomal aberration assays, provided biological dosimetry for the Chernobyl patients, in order to minimize the unreliability from burn indicators alone.[32] Biological indicators for use during different time periods after irradiation need to be identified. For example, one set would be used at 1-3 days and another set at 1-2 weeks after exposure. Indicators from the different status groupings in Table 6-6 should be used jointly to provide a more complete picture of the damage and the biological response.

SUMMARY

The levels of a number of biological compounds become altered in body tissues after radiation exposure. These changes occur *(a)* as a direct result of the radiation damage, or *(b)* in response to mobilization for repair, regeneration, and cell proliferation. In some cases, the degree of alteration is proportional to the dose of radiation, and those degrees may serve as diagnostic aids in the triage of casualties and in the monitoring of radiotherapy. With the exception of neutron

activation of the elements in tissues, none of the biological indicators is unique to radiation injury.

Several problems are associated with the use of most biological dosimeters, particularly in a mass-casualty situation. Many biological indicators have not been completely characterized in humans. In addition, many indicators (including lymphocyte counts) may not be reliable with injuries involving radiation combined with burn or infection. Combinations of nonlethal doses of radiation, burn, or infection may result in mortality.[76] Immunosuppression induced by radiation exposure may increase the likelihood of succumbing to infection or neoplasm,[77] and the degree of immunosuppression is an important prognostic indicator. At present, we have no reliable measurement of that degree (Table 6-6). Future research needs to address the detection of immunosuppressive factors that are released in blood and tissue fluids after injury.

The most reliable indicator for mass casualties is the characterization of early symptoms of the prodromal stage of ARS: nausea, vomiting, and diarrhea. Greater accuracy may be obtained with a lymphocyte chromosomal analysis that can detect radiation exposure as low as 0.03-0.06 Gy. However, this procedure must be performed by a tissue culture laboratory, and it is time consuming. Table 6-6 shows biological indicators of postirradiation damage in patients. For instance, blood-cell counts may indicate the need for a bone-marrow transplant, but indicators of gut status may show possible gastrointestinal cell death, which would contraindicate the transplant.

Two serum enzymes, amylase and diamine oxidase, show promise as biological dosimeters. Each has drawbacks: the salivary glands must be in the field of exposure for elevations of serum amylase to occur, and the response of DAO has not been characterized in irradiated humans. Portable fluorometers for specific biological compounds and the development of specific immunological assays also hold promise for the future of biological dosimetry.

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