Meetings

Radiosensitivity in Animals

Differences in radiosensitivity exist in animals. Possible reasons for such differences include dissimilarities in: cell kinetics in organ systems of the different species, the proportions of cells that are in different stages of the cell cycle; the size of stem-cell pools; the kinetics of cell proliferation; the susceptibility to various degrees of depletion of mature cell populations; physiological systems; and genetic constitution. In order to explore reasons for differing radiosensitivity, particularly among mammalian strains and species, a symposium was held in Kyoto, Japan, 20–23 May 1968. The meeting was sponsored by the National Science Foundation and the Japanese Ministry of Education, under a United States-Japan cooperation program. The conference dealt with basic questions relating to genetics, physiology, the kinetics of cell proliferation and factors that control cell proliferation in the normal and stressed animal.

Bond (Brookhaven) presented data showing the $LD_{50/30\ day}$ values for mammalian species vary from approximately 150 rads to 1500 rads, and that valid comparisons of radiosensitivity can be made only if essentially uniform whole-body exposure to radiations of comparable quality are used in the determinations. The LD_{50} for large animals in general is low (150 to 350 rads), and that of small mammalian species high (650 to 1500 rads).

Sinclair (Argonne) used tissue culture systems to show that various factors including linear energy transfer, dose rate, ploidy, temperature, oxygen tension and sensitizing or protective agents affect the slope or the shoulder of the survival curve for mammalian cells in vitro. The interrelationships among all of these factors is not known but is in the process of evaluation. A large effect of temperature on the degree of recovery from sublethal damage was also shown in L cells by Terasima [National Institute of Radiological Sciences (NIRS), Chiba], prompting him to describe recovery as metabolic and not physicochemical in origin. Recovery, however, was unaffected by most inhibitors (especially if toxicity was properly allowed for) but was modified by a period of protein inhibition (with cycloheximide) prior to the first dose. This would suggest that presynthesis of certain enzymes is needed for recovery. A temperature dependence of recovery from sublethal damage was also shown by Hyodo-Taguchi and Egami (NIRS, Chiba) to hold in whole-body irradiated goldfish, lending further support to the thesis that the recovery process is metabolic in nature.

Differences in sensitivity of cells as a function of stage in the cell cycle were shown by Sinclair (Argonne) to be at least a factor of 3. The cell age response was shown clearly not to depend on DNA synthesis solely; another factor must be involved which was also shown not to depend on RNA and protein synthesis. It was also shown that the effect of hypoxia appears to be independent of stage of cell cycle, whereas the protective effect of cysteamine has a marked differential effect through the cycle.

The work on goldfish (supported by earlier work of Hornsey and of Withers, and that of Matsuzawa in this meeting) indicates that the shoulder of the survival curve of bowel epithelium stem cells (and thus the capacity to repair sublethal damage) is much larger than that for bone marrow stem cells. This may explain the higher doses required for death from the G.I. syndrome compared to the bone-marrow syndrome. If such differences in the capacity for repair are demonstrated for similar tissues in different species it could in large part explain the observed differential sensitivity of mammalian species.

Fliedner (University of Ulm) used labeling of all cells in the fetal mouse by injecting the mother with tritiated thymidine to support the thesis that bone-marrow stem cells are "resting" in normal marrow. The phase of the cell cycle in which "resting" stem cells remain inactive would have a strong bearing on the radiosensitivity of the species.

Sugahara (Kyoto University) showed that an increase in radioresistance after heavy bleeding could not be due to an increase in hematopoietic stem cells. Tsuchiya *et al.* (NIRS, Chiba) showed that the approximately 100-roentgen difference in radiosensitivity between two strains of mice appeared to be related to the absolute count of nucleated bone-marrow cells. Van Bekkum [Central Organization for Applied Scientific Research in the Netherlands (TNO), Rijswijk] indicated that dissimilarities in numbers of stem cells among various species differ, and that the proliferative capacity of a given number of stem cells depends on whether the stem cells are protected by shielding, or are taken from an unirradiated donor.

Schooley (Berkeley) presented data showing that erythropoietin acts on an intermediate cell population rather than on the colony-forming unit. Stohlman (St. Elizabeth's Hospital, Tufts) noted that the response to erythropoietin is not accomplished at the expense of depletion of the pluripotential stem-cell pool. His conclusions were based on megakaryocytic precursors, and on the fact that splenic colony-forming units increase after the primary splenic erythroid response to erythropoietin. This suggests a migration of stem cells to the spleen and an influx into an intermediate committed compartment partially depleted by erythropoietin during the initial erythroid response.

Patt (University of California) showed that the initial "degenerative" phase of neutrophil depletion following irradiation depended with species on the sensitivity of the constituent cells, and on the turnover rate of mature functioning elements. The recovery phase depends additionally on the capacity of stem cells for turnover and differentiation and on the integrity of the supporting matrix. The mouse and rat may have greater capacities for stem-cell diversion to granulocytopoiesis than does the dog, which may account in part for the marked difference in radiosensitivity.

Nakamura *et al.* (NIRS, Chiba) showed that platelet deficiency and bleeding may play a much larger role in the hematopoietic death in irradiated mice than previously thought. His studies indicate that in some species essentially no loss of red cells from the blood vessels occurs until extreme thrombopenia is present. In other spe-

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cies significant loss of red cells into the tissues may occur with moderate thrombopenia in the absence of overt hemorrhage. An apparent interaction between neutropenia and platelet deficiency was pointed out by Fliedner and Heit (University of Ulm).

A model relating events at the cellular level to the mortality rate in irradiated mammals was presented by Robinson (Brookhaven). The animal's LD_{50} was expressed in terms of five parameters: the n and D_0 for stem cells, their original number, a mature-cells-per-stem-cell or proliferation factor, and a parameter for the animal's mature cell requirement. It was inferred that the shape of the animal dose-survival curve sets an upper limit on the possible interindividual variation of each of the five parameters, and in particular a relatively low limit for that of the stem-cell D_0. It was pointed out (Sinclair, Argonne; Patt, University of California) that additional refinements would be desirable, especially that of making the proliferation rate after exposure dose-dependent. The finding of "small colonies" in tissue culture by Sinclair, confirmed in studies with splenic colony formation units, indicates that this would be a more realistic assumption.

Sugahara (Kyoto) showed that physiological stresses such as hypoxia, exercise, and bleeding, which may or may not be related to changes in size of cell populations, increase resistance. The work of Schooley and of Stohlmann referred to previously, emphasizes the importance of mechanisms that control cell proliferation in the normal and stressed animal.

Microorganisms normally nonpathogenic can be quite pathogenic in the heavily irradiated animal, for example, the presence of pseudomonas is known to greatly decrease the LD_{50} of mice. Wild rodents, however, that would be expected to harbor a number of parasites, are among the most radioresistant of mammals. Van Bekkum (TNO, Rijswijk) showed that the $LD_{50/30\ days}$ of pathogen-free guinea pigs is more than twice that of nonpathogen-free animals. The increase in resistance of gnotic animals may be related to the absence of actual infection (Wilson, Van Bekkum); to an increased rate of utilization of mature elements (Nakamura, Fliedner); to difference in the metabolism of normal substances, for example, bile (Fry, Argonne); to the presence of different flora (several authors); to toxins; or to as yet poorly defined additional factors. Wilson (Notre Dame) showed, using mono-contaminated, previously germ-free animals, that radioresistance may be primarily or secondarily related to the activities of intestinal microorganisms. Antibodies increased survival even in germ-free animals, perhaps because recovery is stimulated by them in some as yet undefined fashion.

"Recovery" in the intact mammal over a time period of days to weeks, undoubtedly including both repair of sublethal cellular damage and cell proliferation, was described by Casarett (University of Rochester). Split-dose experiments in the mammal show that recovery rates so determined are markedly dependent on a number of factors including species, strain, and size of conditioning dose. An exponential decay curve represents a rough approximation to much of the recovery data. The large differences in recovery rates among species do not correlate with factors such as body size, life span, or metabolic rate.

Kondo (University of Nagoya) differentiated inbred strains of mice on the basis of differences in original gene pool and on differences in natural and artificial selection through sister-brother inbreeding. His studies using ten inbred strains indicate that the variability in lethal dose among mice selected from a given strain was not distinguishable from that among mice from different strains.

Tazima (National Institute of Genetics, Nirsima) showed that two fractionated doses of 500 roentgens produced a greater effect than 1000 roentgens (single dose) with respect to mutation frequency in the silkworm. He suggested that a demonstrated pileup of cells in G_2 might account for the differences. Sinclair (Argonne) indicated that there must also be a pileup in G_1, which might be even more likely to show a higher mutation frequency.

Bond (Brookhaven) showed that species differences in LD_{50} values for the gastrointestinal syndrome are considerably less than the variation in LD_{50} for the bone-marrow syndrome (a factor of less than 2 versus a factor of 10). At the high-dose levels involved, severe bone-marrow damage and resultant depletion of blood neutrophils is severe even at the early time associated with the G.I. syndrome. Irradiation of only the bowel or a large segment of the bowel requires higher doses to kill, and the survival time is longer. These findings show a signifi-

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cant contribution of neutropenia and infection to the G.I. syndromes seen with whole-body radiation.

Lushbaugh (Oak Ridge Associated Universities) indicated that while changes in underlying cell populations probably are of cardinal importance in mortality among animals exposed to doses in the "hematopoietic death" range and somewhat above, additional factors assume importance as the dose is increased (above perhaps 1000 roentgens in most species). Direct damage to the small vessels and capillaries become increasingly important. Thus, while the term "hematopoietic syndrome" may be appropriate, the terms "gut syndrome" and "central nervous system syndrome" may in a sense be misnomers. Damage to the principal cell populations presumably involved may not be the critical factor that determines mortality.

Matsuzawa (Aichi Cancer Center Research Institute) counted surviving crypt stem cells after exposure of mice to graded doses of radiation. He obtained a D_0 of 220 rads and an extrapolation number of 4.8. (Earlier estimates by Hornsey *et al.* and by Withers showed a similar broad "shoulder" but a lower D_0). Fry (Argonne) studied the relationship between the LD_{50} for the G.I. syndrome and the parameters of gastrointestinal epithelial cell proliferation in eight mammalian strains and species. Differences in radiosensitivity appeared to be related to the ratio of proliferating to functional cells; the transit time in proliferating and functional compartments; the cell cycle time; the relative rapidity of feedback mechanisms and cell turnover; and the time required for differentiation. That sensitivity and survival time depend on the kinetics of cell proliferation of gastrointestinal cells was indicated by the data of Wilson (Notre Dame), Matsuzawa (Aichi Cancer Center Research Institute) and Fliedner (University of Ulm). The transit time of cells from the crypts to the tips of the villi is prolonged in germ-free animals, and the time for death from the G.I. syndrome is delayed proportionately.

Lushbaugh (Oak Ridge Associated Universities) presented data of Casarett *et al.* (University of Rochester) showing that appreciable capillary damage in the villi probably is present at dose levels corresponding to the G.I. syndrome, which may be in part responsible for denudation of the villi. He also indicated that actual fluid loss from the body during the G.I. syndrome in

rodents is minimal, but that loss of fluid into tissues (edema) may be an important factor. His data indicate that sodium loss (as determined by studies using radioactive sodium) may be minimal, at least prior to the time that the animal approaches the moribund stage. Hyodo-Taguchi and Egami showed a distinct increase in loss of sodium from irradiated goldfish suffering from gastrointestinal syndrome. Lushbaugh pointed out that such loss may well be from vessel damage particularly in the gills of the fish.

In summary of the conference, the problems involved in differential species radiosensitivity were brought into sharper focus, but a great deal of work needs to be done before the differences can be understood. Detailed work is required on differences in pool sizes, the kinetics of cell proliferation, and control mechanisms of cell proliferation in the different species. Physiological variables must be better defined, particularly the rate of utilization of mature functional cells in the irradiated versus normal individual. The possible role of the reticuloendothelial system deserves a great deal more attention, as do changes in blood vessels and the supporting matrix for proliferating tissues, particularly at dose levels high in the lethal range for the bone-marrow syndrome and above.

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