A STUDY OF
EARLY RADIATION-INDUCED BIOLOGICAL CHANGES
AS INDICATORS OF RADIATION INJURY

MARCH 1969

Prepared for

UNITED STATES ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY
WASHINGTON, D.C. 20315

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LIFE SCIENCES RESEARCH OFFICE
OFFICE OF BIOMEDICAL STUDIES
FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY
BETHESDA, MARYLAND
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FOREWORD

This technical report is the second of three reviews of promising research opportunities to improve the treatment of radiation injury in the soldier. A Study of Early Radiation-Induced Biological Changes as Indicators of Radiation Injury, complements the first report on A Study of the Immunologic Aspects of Therapy of Radiation Injury in the Soldier (1), and a future report, A Study of the Metabolic Aspects of Therapy of Radiation Injury in the Soldier (2).

Part I of this report was prepared by the Staff of the Life Sciences Research Office, Office of Biomedical Studies, Federation of American Societies for Experimental Biology (FASEB). The text reflects the opinions of an ad hoc study group that met at Beaumont, FASEB, on May 28 and 29, 1968. The report has been approved by the majority, not necessarily by all, of the participants. Part II of this report is a critical review with an annotated evaluative bibliography. This study was made for the United States Army Medical Research and Development Command, Office of The Surgeon General, Department of the Army, in accordance with the provisions of Contract No. DADA-17-67-C-7180.
SUMMARY

This study is a part of a broad review of current research on the biological effects of radiation to explore the possibility of developing improved treatment measures. Early biological changes that reliably indicate the degree of radiation injury a soldier has received would simplify the triage of radiation casualties and facilitate treatment regimens. Prompt diagnosis of the radiation-induced injury the individual has experienced and his prognosis are important. In military operations it is not only necessary to save lives, but it is desirable also to preserve and use available manpower. Early radiation-induced biological indicators of the degree of injury would be extremely useful in meeting such manpower demands. In addition, dependable indices would have potential use in the assessment of the severity and extent of radiation injury in civilian populations.

Criteria were selected for suitable biological indices that would be appropriate for the military requirements. Men receiving radiation doses below 1,000 rad were considered most amenable to treatment. Biological indicators of radiation-induced injury should provide results within one to two days after exposure. The techniques of measurement should not require exotic equipment. The radiation-induced biological changes should have a high probability of occurring only after radiation exposure. The biological samples should be easy to obtain and available for sequential studies.

The scope of the study included: the use of physical dosimetry; early clinical manifestations of radiation-induced emesis; radiation-induced cytological changes in the testes; urinary constituents as predictors of radiation injury; hematologic changes; serum iron levels as indicators of hematopoietic dysfunction or injury; etiocolcholanolone in estimating bone marrow granulocyte reserves; total protein-bound neutral hexoses in the plasma as related to radiation sensitivity; effects of radiation on detoxification enzymes, drug metabolism, and biochemical changes; and chromosome aberrations as indices of radiation injury.

Suggested areas for future research emphasis are summarized in Section VII. The developments in the field of thermoluminescence may have applications in the personal dosimetry of the soldier. Post-irradiation vomiting is a reliable clinical symptom indicative of radiation exposure and there is need to understand the mechanisms involved to enhance early diagnosis and treatment measures of radi-
ation casualties. The cells of the testes are highly radiosensitive; however, it is not practical to use testicular cytological changes as a measure of radiation-induced injury. Excretion of certain urinary constituents has been shown to be increased following radiation exposure of animals but the value of these changes has not been proved in man. The hematologic changes that follow a single exposure to radiation have been documented and provide a basis for the current understanding and predication of the degree of radiation injury. There is a pressing need for more information on all types of hematologic alterations that will be produced by the anticipated multiple radiation exposures to gamma and neutron radiations in combat. Additional research is necessary to explore the potential usefulness of the dynamics of monocyte changes as predictors of radiation injury, and to evaluate the capacity of the bone marrow of radiation casualties to recover. Radiation exposure as reflected in decreased bone marrow erythropoiesis is usually mirrored by a subsequent increased serum iron level. It may be possible to utilize this serum iron change as a prognostic measure of the degree of radiation injury. The steroid metabolite, etiocholanolone, has proved a useful tool in assessing the bone marrow granulocyte reserves in radiation therapy of cancer. Additional research emphasis on granulocyte kinetics with etiocholanolone may be rewarding in developing a test to measure granulocyte reserves in determining the effects of multiple radiation exposure damage in the soldier. Total protein-bound neutral hexoses in the plasma have been found to be modified after radiation exposure of animals. These preliminary observations should be extended to man using the newer physicochemical analytical methods. In a similar manner, radiation-induced alterations in the metabolism of drugs or chemical test substances may provide a measure of some aspects of radiation injury. Preliminary evidence of biological changes of this character is sufficiently encouraging to warrant research emphasis on this aspect of the subject. The scoring of radiation-induced leukocyte chromosome aberrations is a useful and reliable technique to determine the approximate degree of radiation injury. Unfortunately, the test requires a number of days to conduct and the method in its present state is not suitable for military field use. Computer technology will simplify the scoring techniques.

A critical review entitled **Clinical and Laboratory Observations Useful in Estimating Degree of Radiation Injury** and an annotated evaluative bibliography constitute Part II of the report.
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PART I

I. THE PROBLEM

The treatment of ionizing radiation injury (radiation injury) in the soldier is the obligation of the United States Army Medical Service. To assist in fulfilling this duty the U.S. Army Medical Research and Development Command, Office of the Surgeon General, Department of the Army, conducts a research program in military nuclear medicine. Pursuant to a broad review of the subject of treatment of ionizing radiation injury in the soldier, the military value of the early detection of radiation-induced biological changes is evident. These changes may indicate the degree of radiation injury the individual has experienced, estimate his military effectiveness, predict his future clinical state, and suggest subsequent medical treatment regimens. A previous report on the immunologic aspects of radiation injury in the soldier was based on an exploration of new approaches to therapy (1).

Consideration of therapy for a number of radiation casualties imposes the problems of priorities (3). Reliable biological indicators of radiation injury would significantly simplify the triage of radiation casualties and would facilitate diagnosis, treatment, and prognosis of individual casualties. Information on the extent of the injury in irradiated troops would be valuable as a guide to therapy and would permit the optimal use of available manpower for duty assignments. This knowledge could also be applied in assessing the degree of radiation injury in civilians exposed to radiation.

A number of clinical criteria are used in the diagnosis and treatment of disease and injury and there is no reason to believe that a single biological test would be a satisfactory indicator of radiation injury. There has been the implication that a single, specific test for radiation injury may be found. This is unfortunate because radiation injury is a complex clinical problem that requires many clinical and laboratory observations that, taken together, have diagnostic value. The perspective in this review has been to make a fresh assessment of the potential usefulness of long-recognized or recently discovered radiation-induced biological changes. The impact of this approach on the military medical aspects of the subject, including research, will result in a more thorough understanding and exploitation of existing knowledge. The reports of these studies will serve to encourage and assist military scientists working in this field and academic scientists working in nuclear medicine research. The eventual therapy of radiation injury under military constraints, will evolve from a successful long-term research program that profits from broad scientific discoveries.
II. BACKGROUND AND PRELIMINARY CONSIDERATIONS

Symptoms, signs, and laboratory observations that characteristically follow single, whole-body exposure to doses of ionizing radiation in the lethal range have been studied in many mammalian species, including man. As a result of these studies, the major patterns of response to whole-body irradiation have been described, and systems for classifying radiation-injured casualties according to severity of injury (triage) have been developed. The studies have also resulted in major advances in our fundamental knowledge of the cellular and biochemical causes of radiation injury.

In mammals, death as a consequence of acute radiation injury following exposure to doses in the lethal range may properly be considered a result of disturbed cellular proliferation (4) and derangements of other biological processes. In fact, following supralethal doses, when death occurs within hours or days, many non-proliferating tissues are injured and their function is seriously disrupted. It has long been recognized that active cellular proliferation and cell differentiation are characteristic of radiosensitive tissues (5) and at the present time are considered the primary basis of diagnosis and therapy. Modern concepts of radiation injury have developed directly from application of newer techniques, especially the use of $^{3}$H thymidine in the study of the kinetics of cell proliferation in the more radiosensitive tissues, e.g. germinal epithelium, hematopoietic tissues, epithelium of the small intestinal crypts, epithelium of the stomach and colon, and basal layers of the skin.

The biochemical changes that follow radiation exposure have been studied in an attempt to relate these to the type and extent of cellular and tissue damage induced by radiation. This search has been rewarding under the controlled laboratory conditions with inbred strains of experimental animals. Unfortunately, these data, while useful, cannot be directly extrapolated to man. The diversity of the diet of man and his genetic background complicate the problem.

Radiation causes an early increase in catabolic and anabolic processes in the reticuloendothelial system (6) with the release of deoxyribonucleic acid (DNA) breakdown products. Employing ion-exchange and paper chromatography, increased urinary excretion of the metabolic products of DNA have been observed. Compounds that have been related to the degree of injury include: $\beta$-aminoisobutyric acid and taurine (7). It may prove profitable to identify the significance of these urinary constituents in radiation-induced injury.
Inhibition of incorporation of thymidine $^{14}$C in the DNA of various tissues of the rat has been observed 1 to 24 hours after total-body radiation (50-800 rad). The total incorporation of tagged thymidine into DNA in the spleen and small intestine decreases with exposure to increasing doses of radiation (8).

The protein-bound carbohydrate concentration (PBC) in the plasma of C$_3$H mice and beagles has been quantified and related to the changes observed after radiation (9). The pre-irradiation PBC levels appeared to constitute a crude index of radiosensitivity prior to radiation exposure and the post-irradiation concentration changes provided a clue to the prognosis of the individual.

Chromosome aberrations offer possibilities as early indicators of radiation-induced biological changes. Chromosome aberrations induced in the lymphocytes of peripheral blood have been suggested as a measure of the degree of radiation injury (10).

A novel way to assess radiation-induced cytological changes may evolve from the use of agents that mobilize granulocytes directly from the myeloid reserves of bone marrow, e.g. the steroid metabolite etiocholanolone (11, 12). This metabolite has been proposed for the estimation of the bone marrow granulocyte reserves in patients receiving cytotoxic agents. One study has been reported on the use of diagnostic tests of this type in the measurement of the degree of radiation damage (13). This is an example of how the studies in the control of malignancy with ionizing radiation, radioactive isotopes, or the cytotoxic agents may be employed in a search for a suitable biological indicator of radiation injury.

There is evidence that iron turnover is markedly decreased following whole-body exposure to 50-100 rad or greater. These observations reflect a diminished erythropoietic function mirrored by the serum iron levels at doses of about 50 rad or higher. Serum iron, therefore, might be useful as a possible indicator of the degree of injury following radiation exposure.

The etiology of the early clinical symptoms, such as nausea and vomiting, that are used to estimate the degree of radiation injury is poorly understood. At present, these signs and symptoms have diagnostic and prognostic significance, largely with respect to their presence or absence, time of onset, and duration. If the underlying physiological mechanism of radiation-induced nausea and vomiting could be identified, it would provide a better understanding of the basic character of radiation injury.

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III. SCOPE OF THE STUDY

Past experiences in treating radiation accident victims were most valuable in this review of biological changes that may be indicators of the degree of radiation injury. From the reports of these clinical cases and from data obtained in experimental animals it was possible to explore approaches to the subject. A principal concern has been to evolve guidelines for future research that would be useful in advancing better techniques for treating radiation injury in the soldier. The ability to detect early radiation-induced biological changes is of central importance to therapy and would enhance the entire treatment regimen. The soldier may be exposed to radiations of variable penetrating power and uneven doses to different areas of the body. A consideration of these variables underscores the need to evaluate the degree of radiation injury produced rather than an estimation of the dose received.

To embrace the subject for adequate analysis in this review and to limit the scope of the study, the following generalizations were accepted:

- Radiation-induced biological injury most likely responsive to treatment will involve doses of less than 1000 R.

- The biological indicator of radiation-induced injury should provide an answer within one to two days after exposure.

- The technique of measurement preferably should not require exotic equipment.

- The radiation-induced biological changes should have a high probability of occurring after radiation exposure.

- The biological sample should be easy to obtain, e.g., blood, urine, hair, or skin, and be available for sequential studies.

- It should be possible to develop and apply the indicator or test within the foreseeable future.

A critical review entitled Clinical and Laboratory Observations Useful in Estimating Degree of Radiation Injury and an annotated evaluative bibliography constitute Part II of the report. Certain review topics are discussed in greater detail in this part of the report.
IV. AD HOC STUDY GROUP AGENDA

The agenda for the ad hoc study group meeting held at Beaumont, Federation of American Societies for Experimental Biology, Bethesda, Maryland, on May 28 and 29, 1968, included the following topics:

- Total radiation dose and its relationship to the degree of radiation injury.
- Ideals of a useful test that is applicable to the military needs.
- Hematologic changes.
- Blood serum iron changes.
- Use of etiocholanolone in estimating bone marrow granulocyte reserves.
- Chromosome aberrations and human radiation cytogenetics.
- Urinary constituents as predictors of radiation injury.
- Total protein-bound neutral hexoses in the plasma.
- Effects of radiation on detoxification enzymes.
V. LIST OF ATTENDEES

AD HOC STUDY GROUP MEETING, MAY 28 AND 29, 1968

ON

A STUDY OF EARLY RADIATION-INDUCED BIOLOGICAL CHANGES AS INDICATORS OF RADIATION INJURY

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VI. REVIEW DISCUSSIONS

A. PHYSICAL DOSIMETRY

In military combat situations the conventional film badge is seldom applicable. A principal disadvantage of the current physical dosimeters to the military is the possibility of partial body shielding from incident radiation. Thus, a dosimeter worn by a particular soldier might not reflect accurately the amount of radiation injury he experienced. However, if dosimeters were carried by representative members of a group distributed throughout the battle area these dosimeters might be useful in estimating the maximum exposure in a particular region of the battlefield.

Photographic dosimetry devices have been used for many years and improved physical dosimeters are under study. Thermoluminescent dosimetry (TLD) has been developed during the last decade (14). TLD is based on the phenomenon that crystalline LiF, CaF₂, BeO, or CaSO₄ emit light when heated after exposure to radiation. The luminescence is proportional to the energy absorbed. A measurable response is obtained from only small amounts of thermoluminescent phosphor, usually 25 mg or less. The accuracy and reproducibility of TLD between 50 mR to 10³ R is ± 3 percent (15), and well within the range of interest to the military. The most suitable form of TLD for military applications appears to be a teflon matrix containing the sensitive phosphor. The devices are easy to handle and impervious to adverse environmental conditions such as temperature and humidity, and mechanical shock extremes. A small teflon insert could be embedded on the soldier's identification dog tag. However, several TLD inserts, a few millimeters in size, placed over different sites of the body, would provide a more accurate measurement of the radiation exposure. The TLD material is "erased" upon readout. Readout devices for TLD consist of a 300° C heating element, a light measuring system, and a calibrated counter. Measurement can be made in 10 to 15 seconds. Portable instrumentation would be needed for military applications to provide rapid readout in remote areas.

Although physical dosimetry accurately measures the impinging radiations, the dose recorded is of limited value in determining the radiation injury sustained by the soldier.

(See Section VII, Suggested Areas for Future Research, p 61)
B. RADIATION-INDUCED EMESIS

Nausea and vomiting are the earliest and most easily recognized clinical manifestations of acute radiation injury. In fact, it has been suggested that the rapidity of onset (an hour or two) and the duration (one or two days) of vomiting are proportional to the degree of injury. Following radiation exposure the symptom is: (a) mild or absent below \( \approx 100 \) rad, (b) noticeable between \( \approx 125 \) and 175 rad, (c) regular at \( \approx 200 \) rad, and (d) virtually certain to occur at higher doses. Furthermore, the presence of diarrhea during the 2 to 3 days post-irradiation suggests doses of radiation in excess of \( \approx 200 \) to 300 rad.

Cobalt irradiation of renal transplant patients with total doses of 450 to 600 rad administered over one and one-half hours produced vomiting when the accumulated absorbed dose exceeded \( \approx 150 \) rad. In the Pittsburgh and Lockport radiation accidents, the first indication of radiation injury was persistent vomiting. It is of historic significance that following the Lockport incident the exposed men returned to their homes erroneously believing they had received an insignificant dose of radiation. The subsequent vomiting caused them to return to the hospital for a more thorough examination.

Psychological factors may play an important role in radiation exposures on the battlefield. Following a nuclear attack, the relatively uninjured survivors are the primary source of manpower for regrouping, aiding the injured, and effectively defending against enemy assault. Therefore, the emotional and mental state of the survivors is important. Adequate training of the soldier for nuclear warfare will probably prevent battlefield panic. However, psychologically-induced vomiting could incapacitate even the men unexposed to radiation. The relatively uninjured individual (e.g. \(< 100 \) rad) will not vomit continually. Of the Marshallese receiving a midline dose of 175 rad, who were not cognizant of the nuclear fallout, about one-third vomited and 10 percent of these experienced subsequent diarrhea. Unfortunately, no evidence is available to determine the extent of associative vomiting that occurred. Probably, the most effective measure to combat anxiety and reflex type emesis is awareness that vomiting may occur following radiation exposure.

Vomiting may be stimulated by a variety of causes and the physiology and pharmacology of vomiting has been reviewed (16). The vomiting syndrome is manifest by the following series of events: salivation and a sensation of nausea; the glottis closes, preventing aspiration of the vomitus into the trachea; the breath is held in mid-inspiration; the muscles of the abdominal wall contract, and
because the chest is held in a fixed position the contraction increases intra-abdominal pressure; the esophagus and the gastric cardiac sphincter relax and reverse peristalsis begins, with the ejection of the gastric contents.

A number of mechanisms inciting the vomiting syndrome have been hypothesized. These include: direct stimulation of the chemo-receptor trigger zone (CTZ), located in the medulla oblongata (e.g. by apomorphine, morphine, and digitalis glycosides); irritant action on the gastrointestinal tract (e.g. by copper sulfate, mustard, and zinc sulfate); stimulation of the nodose ganglion of the vagus (e.g. by veratrum); and excitation of receptors in the heart, central nervous system, or other organs. In experimental animals bilateral destruction of the CTZ prevented the early (2 hours) emetic effect of irradiation and abdominal visceral deafferentiation was required to prevent the delayed (several days) vomiting (17, 18, 19). It appears that the delayed vomiting following lethal whole-body irradiation is mediated through two mechanisms: the centrally located CTZ, and the peripheral visceral afferent receptors of the vagus and sympathetic trunks (19). The precise nature of the mechanisms involved in radiation-induced vomiting in man may be determined as a result of these animal observations. This knowledge may enhance the diagnostic significance of nausea and vomiting in radiation casualties and lead to improved therapeutic measures. Chlorpromazine, for example, is a selective CTZ depressing agent and partially protects against radiation-induced emesis (20).

(See Section VII, Suggested Areas for Future Research, p 61)
C. CYTOLOGICAL CHANGES IN THE TESTES

Testicular epithelium is highly radiosensitive. For example, lethal effects in spermatogonia may be produced by X-ray doses as low as 3 rad (21) and are most sensitive in the premitotic stage (22). The testicular effects in animals are influenced by the intensity, frequency, and nature of the radiation exposure (23).

Cytological effects of irradiation of the testes in man are similar to those observed in experimental animals. In one controlled experiment in humans, the sperm count reduction was dose-related but effects were not manifested for months. Moreover, the histologic examination of biopsy sections taken 4 to 6 hours after radiation exposure revealed that the spermatogonia were sensitive to radiation and showed morphologic changes.

Skillful cytological analyses are required to perform these tests. In addition, obtaining testicular biopsies imposes practical difficulties. These considerations restrict the use of radiation-induced cytological changes in the testes as a measure of radiation exposure dosage.

(See Section VII, Suggested Areas for Future Research, p 61)
D. CHANGES IN URINARY CONSTITUENTS

Among the urinary constituents that have been identified from irradiated animals and man are certain amino acids, β-aminoisobutyric acid, creatine, and taurine; pyrimidine deoxyribosides, deoxycytidines and thymidine; cyclic and heterocyclic biogenic amines; and 5-hydroxyindoleacetic acid.

Excretion of deoxycytidine (CdR) in the urine of rats exposed to radiation has been extensively studied. For example, Kereiakes and associates (24) reported that the increased level of CdR in the 24-hour post-irradiation urine exhibited first order kinetics up to 200 R. The peak of CdR excretion was approximately 15 hours post-irradiation returning to pre-irradiation values at 24 hours.

In measuring the urinary excretion levels of human cancer patients receiving whole-body doses of 60Co radiation, it was found (25) that CdR was excreted in the post-irradiation urine, whereas lower levels of CdR could be detected in the urine collected before irradiation. Elevated urinary levels of CdR may not be specific for radiation injury because similar observations have been made on patients with various stresses such as burns. A notable advance has been the development (26) of a more sensitive (1.0 μg CdR/10 ml urine) and rapid method for the detection and isolation of urinary CdR that utilizes successive exchange resin chromatography.

Increases (25-50%) in plasma CdR in rats following low doses of X-rays (< 50R) suggested that the quantitative determination of CdR might be an indicator of radiation injury (27). A dose-response relationship was found in the range of 50 to 200 R. However, an increase in urinary excretion of various pyrimidine derivatives has been found and there is a possibility that the elevated CdR levels may result from an increased synthesis of pyrimidines (28). Radiation may affect the control mechanism responsible for pyrimidine metabolism and the observed changes could then be related to this action.

Alterations in the excretion patterns of fluorescent urine products by rodents and nonhuman primates were reported (29) after whole-body sublethal 60Co irradiation exposure. The measurements can be made accurately and rapidly 2 to 36 hours post-irradiation. The effects of irradiation injury to the rats were reflected by increased urinary excretion of certain fluorescent products, urinary phosphorus, and the urine volume. The increased phosphorus excretion rates for primates after irradiation appear directly related to the dose while the total fluorescent changes may not be dose-related.
Creatinuria has been observed in animals following exposure to radiation. The radiation-induced creatinuria results from a failure of muscle tissue to utilize creatine synthesized at a normal rate (30). No alterations in rate of creatine synthesis or in its release from muscle were observed after irradiation. The creatinuria is dose-dependent and is maximal between 2 and 4 days after a single whole-body exposure. Koszalka and Andrew (31) reported that insulin completely suppressed creatinuria in rats irradiated with a dose of 500 rad. This phenomenon may extend to other drugs and should be studied.

Excessive urinary excretion of taurine has been observed in the rat after whole-body irradiation. There is an increase in taurine excretion with increasing dose, up to 250 R, but above 250 R the amount of excess taurine excreted by the rat is independent of the dose and, in addition, the bulk of the excess taurine is excreted within 8 hours (32). The hourly urinary excretion of taurine after a single dose of whole-body irradiation is shown in Figure 1. Evidence has been presented that the irradiated rat gives rise to a limited excretion of taurine. A second radiation exposure within approximately 7 days following a maximal response does not elicit a similar excretion of taurine (33). Therefore, this index of radiation injury could be used only if single acute doses of radiation were suspected. The presence of the pancreas is necessary for increased excretion of taurine post-irradiation, whereas the presence of the spleen, adrenals, kidneys, and hypophysis is not required.

Animal data, although useful, are by no means directly applicable to man (34). In man, urinary biochemical indicators of radiation injury have proved disappointing. The studies reported after radiation accidents or therapy have not shown good correlations between these urinary constituents and the degree of radiation injury. Wide individual variation in human urinary constituents and the difficulty in quantitatively collecting urine samples further complicate the use of these indicators as measures of radiation injury in the soldier. More extensive study of the changes in urinary products induced by radiation is necessary before meaningful indices can be developed.

(See Section VII, Suggested Areas for Future Research, p 62)
Figure 1

Hourly urinary excretion of taurine after a single dose of whole-body X-irradiation. Minus number on the time scale indicates hours prior to irradiation. Each value is the average of 3 rats (Ref. 33).
E. HEMATOLOGIC CHANGES

The hematologic changes induced by radiation exposure in man have been documented from the experience obtained after industrial and laboratory radiation accidents, the examination of the survivors of Hiroshima and Nagasaki, the study of Marshall islanders exposed to gamma radiation from fallout, and from the studies of patients receiving therapeutic radiation. These have been reviewed recently (4, 35).

Post-irradiation blood studies reveal major changes in the number of leukocytes. For example, the hematologic changes after 200 rad are illustrated in Figure 2. The immediate increase in neutrophils after radiation exposure may be a useful quantitative measure of the degree of irradiation injury. Usually, the complete blood count is considered in an evaluation of the radiation-induced injury of the subject. The lymphocyte level begins to fall immediately and reaches a low level within the first few days after radiation exposure. The magnitude of this decrease is an early index of the degree of radiation injury. The evidence suggests that precursors of different blood cells possess varying degrees of sensitivity to equal amounts of radiation.

Nevertheless, the data from radiation accident victims reveal a similar pattern of change in the cellular elements of blood following exposure to approximately equal, but unevenly distributed, radiation doses. The hematologic changes of 5 individuals exposed to estimated doses of 236 to 365 rad at the Oak Ridge criticality accident are shown in Figure 3. The hematologic changes are greater following larger radiation doses and are reasonably predictable for any one dose. For this reason radiation-induced hematologic changes are the most useful indicators of biological injury.

Lymphocytes are unique among the blood cells because both the precursors and the circulating mature lymphocytes are radiosensitive. Reduction in the number of circulating lymphocytes does not necessarily reflect damage to the precursor cells nor does it require that exposure be uniform over the entire body.

Cell population kinetics of monocytes have shown that these cells are derived from precursors that are radiation sensitive (37, 38, 39) and monocyte counts may thus have some value as an index of radiation injury. However, monocytes are relatively few in number in the blood and are more difficult to count, but these factors would be of secondary consideration. In mice a 400 rad whole-body X-ray dose reduces the absolute monocyte count to one-tenth the pre-irradiation level within 24 hours (Figure 4). The
Typical hematologic changes in man after exposure to 200 rad.
(Courtesy Gould A. Andrews; Ref. 36).
Figure 3

Graph showing average hematologic values of five Y-12 accident victims; bargraph below gives clinical data. (Courtesy Gould A. Andrews; Ref. 36).
The effect of 400 rad whole-body X-radiation on the level of circulating monocytes in mice. The absolute count is represented on the ordinate and the number of post-radiation days along the abscissa. (Courtesy Alvin Volkman; Ref. 38).
striking reduction in numbers is not surprising in view of the observed normal turnover rates of monocytes determined with $^3$H thymidine in rats (Figure 5). The evidence strongly suggests that these cells are part of a population with a rapid turnover and a halfltime in the circulating blood of about 40 hours (40). The fragmentary data on blood monocyte changes in seriously irradiated human subjects should be expanded. For example, a systematic study of these blood elements using $^3$H thymidine in subjects undergoing radiation for therapeutic purposes might be revealing. More experimentation is necessary to explore the potential usefulness of the dynamics of monocyte changes as predictors of radiation injury and in the evaluation of the capacity for recovery of the bone marrow of radiation casualties. For example, in the case of non-homogeneous or poorly penetrating radiation the peripheral lymphocyte counts would surely drop although monocyte counts would be maintained if sufficient bone marrow were spared.

Multiple exposures or exposures to combinations of gamma and neutron radiations may be encountered in military situations. Variables of geometry, type of radiation, and duration of exposure will modify the biologic effects of irradiation. There is a pressing need for more information about the manner in which these variables influence biologic responses, including hematologic changes. Although radiation accidents do not mimic completely the military situation, they provide useful hematologic information on man and suggest directions for future research.

(See Section VII, Suggested Areas for Future Research, p 62)
The increase in the percentage of labeled monocytes in the blood of rats continuously infused with tritiated thymidine. The concomitant diminution of the unlabeled monocyte population is also plotted. The estimated half-time is 40 hours (Courtesy Alvin Volkman; Ref. 40).
F. SERUM IRON LEVELS

Although the mature erythrocyte is generally resistant to radiation, the immature stages of the cell series are highly radiosensitive. The life span of the mature human erythrocyte is about 120 days, so that it is not feasible to utilize a red blood cell (RBC) decrease as an indicator of radiation-induced hematopoietic dysfunction or injury.

Radioactive iron (\(^{59}\text{Fe}\)) has been used to determine the rate of formation and the lifetime of the RBC. The National Academy of Sciences - National Research Council (41) reported that turnover studies with \(^{59}\text{Fe}\) appear to be of little value as indices of radiation injury. To be meaningful, they would have to be done serially and, even then, would add little information beyond that derived from the reticulocyte count. In addition, the labeling of RBC with \(^{59}\text{Fe}\) is more time-consuming than labeling with \(^{51}\text{Cr}\) which is used for the same purpose. Cell turnover studies with \(^{59}\text{Fe}\) or \(^{51}\text{Cr}\) do not appear to offer much promise as indicators of biologic injury from radiation.

Iron is incorporated only into the erythrocyte precursors. The feasibility of using serum iron levels as early indicators of radiation injury rests on the premise that erythropoietic tissue is injured and iron incorporation is depressed. Thus, serum iron levels tend to rise. The normal serum iron value in man is about 100 µg/100 ml. However, there is a wide diurnal fluctuation in the utilization of iron and higher values are present at night (42). The use of serum iron levels as early indicators of radiation damage should be explored.

(See Section VII, Suggested Areas for Future Research, p 62)
G. ETIOCHOLANOLONE IN ESTIMATING BONE MARROW GRANULOCYTE RESERVES

During the past 15 years the kinetics of the granulocyte myelo-
poietic system have been studied intensively. However, as pointed
out by Perry and associates (43, 44), although a great deal is known
regarding the source of cells contributing to leukocytosis, there is
little information concerning the basic mechanisms involved in the
mobilization of granulocytes, their movement, production, rates of
maturation, or survival times. Nevertheless, the study of granulo-
cyte kinetics has assisted in the exploration of methods to combat
the serious "hematopoietic syndrome" of injury following therapeutic
radiation exposure. This information may be of value also in as-
sessing bone marrow injury as reflected in hematologic changes fol-
lowing radiation-induced injury in the soldier.

The study of the myelopoietic system has established the fol-
lowing essential features:

- The bone marrow is the chief area of granulocytopenesis and
cell division in the granulocyte series does not occur after
the myelocyte stage.

- Normally, the granulocyte remains in the marrow for a peri-
od of 1 or 2 days after maturity. In normal individuals
approximately 4 to 5 days are required before new granulo-
cytes are released into the blood as determined by the $^{3}H$
thy midine technique. Labeled granulocytes appear in the
peripheral blood during this interval presumably from the
mass of non-proliferating granulocyte cells that constitute
the bone marrow granulocyte reserve (MGR). The majority of
mature granulocytes are released from the MGR to maintain
the circulating blood granulocytes.

- In infectious diseases, experimental leukapheresis, and
similar conditions that incite leukocytosis, the entry of
granulocytes into the peripheral blood will be accelerated.
Thus, in the therapy of radiation injury the bone marrow is
the principal reservoir for peripheral blood granulocytes.

- After maturation the majority of granulocytes are normally
released into the blood in an orderly fashion and probably
represent only about 2 percent to 5 percent of the total
myeloid cells in the body. The MGR of mature cells is many
times larger (5 to 6 times) than the circulating granulocytes.
The majority of granulocytes that circulate in the peripheral blood come from the marrow population and not from peripheral organs. This fact is significant when determining marrow granulocyte reserves based upon peripheral blood samples.

The granulocytes normally enter the peripheral blood and circulate with a halftime of approximately 6.5 hours. Cells of the myeloid series apparently do not return to the bone marrow in normal individuals and granulocytes circulating in the blood do not exchange freely with those in extravascular areas. Therefore, the circulating granulocytes do not represent the average age of the total granulocyte population. However, granulocytes may be sequestered within capillary beds for variable periods and may re-enter the circulating blood. Failure to take cognizance of this interchange led to the previous belief that granulocytes leave and re-enter the blood stream during their life span. The data do not preclude the re-entry of some granulocytes from certain extravascular areas, but the primary direction of granulocyte movement is from the marrow to circulating blood, to capillary beds, and eventually into the tissues. The essential feature of this process in radiation injury is the dynamic loss of the granulocytes into the tissues in that their function is completed shortly after they move from the vascular system. If the marrow granulocyte pool is seriously damaged within a finite period, the process is delayed. The success of a granulocyte reserve measure will depend upon the early detection of a decreasing bone marrow granulocyte reservoir.

Historically, a bacterial endotoxin was used first to estimate the MGR by the mobilization of granulocytes into the peripheral blood in response to the challenge of an injected bacterial polysaccharide (45, 46). Etiocholanolone, a naturally occurring steroid metabolite, was discovered to cause leukocytosis consisting primarily of mature polymorphonuclear neutrophils when this substance was injected intramuscularly into man (47, 48). Subsequently, this fever-inducing substance has been investigated for its unusual biological properties including its effects on leukocyte kinetics (Figure 6).

Etiocholanolone is a metabolite of testosterone, \( \delta \)-4-androstene-3, 17-dione. It is an isomer of the gonadal hormone androsterone. Etiocholanolone is the 5 \( \beta \)-H (A:B cis) configuration while androsterone has the 5 \( \alpha \)-H (A:B trans) structure as shown in Figure 7. Both steroids are detected in normal urine as 17-ketosteroids usually conjugated with glucuronic acid. Etiochol-
The mean change in circulating granulocytes and mononuclear cells over baseline values following a zero time injection of 0.2 mg./Kg. of etiocholanolone in seven male and seven female normal subjects. The decrease in mononuclears is not statistically significant (Courtesy Seymour Perry; Ref. 11).
Comparison of the configuration of androsterone (5α) with etiocholanolone (5β) when drawn in perspective. (Courtesy Philip K. Bondy; Ref. 49).
anolate has a molecular weight of 290, can be prepared synthetically, is non-antigenic, and is insoluble in water (12).

In hematologically normal patients, etiocholanolone caused a tremendous (98%) increase in the total blood granulocyte pool (TBGP) that was demonstrated later to be due to mobilization of granulocytes from the bone marrow (50). Etiocholanolone did not cause a change in the circulating granulocyte pool (CGP) or the marginal granulocyte pool (MGP). Thus, the usefulness of etiocholanolone as a substance to assess marrow granulocyte reserves in normal subjects was established.

Etiocholanolone was utilized in patients receiving cytotoxic agents to estimate granulocyte reserves, in patients who had normal white blood cell counts, or in those who were leukopenic. In general, etiocholanolone was equivalent or superior to endotoxins as a means of estimating the granulocyte reserves. Patients with normal granulocyte reserves, as indicated by this test, appeared to tolerate the myelosuppressive effects of cytotoxic agents better than patients with abnormal granulocyte reserves (51). It was concluded that etiocholanolone was reliable in the estimation of MGR in subjects with a depressed maturation of the normal bone marrow elements in neoplastic disorders and as a guide to treatment (52).

In radiation injury the time after exposure and the amount of radiation received will be important factors in using etiocholanolone to assess marrow granulocyte reserves. Figure 8 illustrates the granulocyte responses in a patient after depression of the marrow by chemotherapeutic agents in cancer therapy. Etiocholanolone does not affect the level of mononuclear cells, the platelets, or other cellular elements of the blood. The white blood cell (WBC) count may be used as an index of the approximate degree of radiation injury, but the time after exposure as well as the dose of radiation received must be known. A WBC count of 5,000 to 6,000 may present a misleading picture because the granulocyte marrow reserves may be largely exhausted. Such an individual is a suitable candidate for MGR tests with etiocholanolone. By contrast, a subject with a 15,000 WBC count of mainly granulocytes likely has a normal marrow reserve. Clinically, the estimation of the MGR is valuable in determining the safety of a second dose of a bone marrow depressant such as cytotoxic agents or radiation therapy.

In the irradiated subject, the first few days after radiation exposure will be characterized by a leukocytosis that likely represents a mobilization phenomenon. The critical bone marrow depression will be manifested later and will vary with each individual. In bone marrow transplantation in man, the transplant must be made 10 to 12 days before the nadir of the marrow depression. There seems
Granulocyte responses of two patients to etiocholanolone before and after chemotherapy given on days 1 through 5. On the ordinates are shown the peripheral granulocyte and platelet counts. On the abscissa are days of hospitalization. The closed circles represent the base-line pre-injection granulocyte counts and the open circles, the maximum granulocyte responses to etiocholanolone. The enclosed shaded area is considered an estimate of the granulocyte reserves. The broken line shows changes in circulating platelet counts (Courtesy Seymour Perry; Ref. 51).
to have been no clinical use of etiocholanolone in therapy of radiation-induced aplastic anemia in man. However, it appears feasible to explore the use of etiocholanolone in assessing radiation injury for predicting the bone marrow reserves of the individual (13).

(See Section VII, Suggested Areas for Future Research, p 63)
H. TOTAL PROTEIN-BOUND NEUTRAL HEXOSES

Serum glycoproteins have been reported to be increased in a number of infectious, inflammatory, neoplastic, and idiopathic diseases. The significance of these changes is not known, and there is evidence that different disease states produce more or less distinctive alterations in the relative concentration of various carbohydrate species bound to protein (53).

Evans and colleagues studied the effects of ionizing radiations on the concentration and distribution of protein-bound carbohydrates (PBC) in the plasma of mice (54) and beagles (9). The PBC were estimated as neutral hexoses in the plasma during the pre- and post-irradiation period, and the changes observed were related to the survival time of the animals.

The plasma PBC values in C3H mice and beagles were determined before and after whole-body exposures of monoenergetic 14 MeV neutrons or mixed gamma-neutron radiations. The mice were exposed to both sources at a steady state rate of \( \approx 20 \) rad/min. The beagles received pulsed doses of mixed gamma-neutron radiations. In both species, the pre- and post-irradiation PBC appeared to constitute a crude index of radiosensitivity prior to, and prognosis after, irradiation in these otherwise healthy animals.

A striking difference was observed between the mice (Figure 9) and dogs (Figure 10) that died after exposure to mixed gamma-neutron radiations and the more resistant animals that, although exposed to identical doses, survived the experimental period. In the animals that died, the PBC rose to varying degrees and remained elevated until their death. By contrast, the survivors demonstrated little change in PBC, deviating only slightly from their pre-irradiation baseline values. There was an inverse relationship between the magnitude of maximum response and the survival time of the animals. Similar results have been reported for dogs receiving whole-body radiation doses of 600 R (55). Dogs with a low initial level of glycoproteins generally survived 30 days after exposure; animals with high initial levels died.

It was noted that the mean pre-irradiation level of PBC of the 30-day survivors was lower than those that died during the same period (Table 1). The pre-irradiation PBC concentration indicated statistically significant differences in radiosensitivity at the lowest reported dose. This finding together with the inverse relationship between radiation dose and magnitude of difference suggest
Protein-bound carbohydrates as neutral hexoses in the plasma of mice as a function of time relative to exposure to 455 rad of mixed gamma-neutron radiations. Numbers in parentheses are number of mice represented by each curve (Courtesy Adelbert S. Evans; Ref. 54).
Figure 10

Percent increase in concentration of total neutral hexoses in the plasma of dogs at indicated times after exposure to mixed gamma-neutron radiations. Numbers in parentheses are number of animals represented by each curve (Courtesy Adelbert S. Evans).
Table 1

Comparison of Preirradiation Protein-Bound Carbohydrate (PBC) Concentration in Mice Which Died with the 30-Day Survivors after Exposure to 14-Mev Neutrons or Mixed Gamma-Neutron Radiations

<table>
<thead>
<tr>
<th></th>
<th>14-Mev neutrons</th>
<th>Mixed gamma-neutron radiations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>365 rads</td>
<td>455 rads</td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td>Survived</td>
</tr>
<tr>
<td>Number of Mice</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Mean PBC ± S.E.</td>
<td>170.6</td>
<td>151.6</td>
</tr>
<tr>
<td>Δ</td>
<td>± 3.4</td>
<td>± 2.9</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.01</td>
<td>0.08 &lt; p &lt; 0.1</td>
</tr>
</tbody>
</table>

a Milligrams per 100 ml of plasma.
b Δ = died minus survived.

(Courtesy Adelbert S. Evans; Ref. 54).

that this subject is worthy of further investigation. In another series of 27 beagles that received a pulsed dose of 215 rad of mixed gamma-neutron radiations, the mean pre-irradiation PBC concentration of the 13 beagles that survived was lower than that of the 14 that died. Causal relation between the pre-irradiation PBC values and radiosensitivity is not known but it may be a reflection of an inherent metabolic lesion in the sensitive animals that renders them less tolerant to a radiation insult.

Admittedly, the estimation of the structurally and physiologically widely diverse glycoproteins as a single entity cannot be expected to yield clinically applicable criteria. However, Evans (9) has found by preparative fractionation of plasma of irradiated and unirradiated animals, that absolute changes occur in the concentration of transferrin, haptoglobulins, β₂-glycoproteins, and α₂-macroglobulin. These proteins are all rich in bound carbohydrates.
Preliminary analyses of the whole plasma of beagles for radiation-induced alterations in the various carbohydrate species bound to protein - neutral hexoses (as galactose and mannose), hexosamines (as glucosamine), sialic acid (as N-acetylmuraminic acid), and methyl pentose (as fucose) - revealed that the relative alterations of these carbohydrates are distinctive and do not mimic the reported values for hypertensive heart disease, myocardial infarction, or liver disorders. In addition, ultrastructural changes in intracellular organelles thought to be vital in the biosynthesis of complex proteins that parallel the biochemical findings have been shown in the hepatocytes of irradiated mice (9).

Continuation of these studies on protein-bound carbohydrates should give a better understanding of the mechanism of radiation injury. It appears feasible to explore the estimation of particular protein-bound carbohydrates as a prognostic test to aid in following the clinical course of an irradiated individual, and to identify the most radiosensitive individuals prior to possible radiation exposure.

(See Section VII, Suggested Areas for Future Research, p 63)
I. DETOXIFICATION ENZYMES, DRUG METABOLISM, AND BIOCHEMICAL CHANGES

The available information suggests that radiation does not affect the ability of adult animals to detoxify most foreign chemicals (56). The acute toxicity (death within 24 hours) of 10 drugs representing four drug classes (anticonvulsants, hypnotics, hypoglycemics, and psychopharmacologics) was studied in male CF1 mice 2 hours, 1 day, or 6 days after 500, 1000, and 10,000 rad whole-body doses of mixed gamma-neutron radiations (57). The LD₅₀ values for most drugs studied were altered in some of the dose groups of irradiated mice, but no definite pattern of change of drug toxicity was identified, with either radiation dose or post-irradiation time. Alteration of the expected drug response after radiation exposure appears to be the exception rather than the rule for the major drug classes (58). When abnormal pharmacologic responses are obtained from drugs after irradiation of an animal, they can often be attributed to the secondary effects of radiation; e.g. hypotension, rather than to any change in the pharmacodynamics of the drug. If irradiation alters the metabolism of the drug, however, the type of drug response and the magnitude of the action might be changed significantly.

There is evidence that whole-body irradiation in the rat will produce a significant increase in the total liver mitochondria mass. This increase does not appear to be related to a corresponding increase in enzyme activity but the matter has not been fully explored. There are no submicroscopic morphologic alterations of the mitochondria that are characteristic of irradiation injury although they may be found in the future. It is noteworthy that the increase in mitochondrial mass reaches a maximum 6 hours post-irradiation and returns to normal in about 24 hours. This early effect of radiation exposure has not been explained.

In a study of the effects of X-rays on the maturation of the oxidative desulfuration enzyme in young rat liver microsomes, Hietbrink and DuBois (56) observed that doses of 200 or 400 R of whole-body irradiation almost completely inhibited the natural development of the enzyme. This effect was manifest in young, growing rats irradiated at 23 days of age. Radiation had no inhibitory effect on the liver enzyme activity of adult male rats. These findings indicate that the radiation effect was related to some phase of the development of the enzyme system rather than an alteration of the activity of pre-formed enzyme. Other enzymes such as p-nitrobenzoic acid reductase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconic acid dehydrogenase were not appreciably inhibited (56).
In a further exploration of the mechanism of inhibition of the development of the normal oxidative desulfuration enzyme in young rats, it was discovered that radiation of the head, while the remainder of the body was shielded, prevented the development of the enzyme activity to a degree similar to total-body radiation. In addition, the same dose of radiation did not prevent enzyme development if the irradiation was directed only to the regions of the liver and testes (59). It was concluded that there are hormonal factors that normally exert a regulatory influence on the synthesis of these microsomal enzymes in male rats (60). This hormonal control is obliterated by irradiation of the head area, especially the pituitary region (61). These observations on animals should be explored in greater detail.

A provocative finding applies specifically to inhibition of the normal synthesis of enzyme protein in the very young, growing animal. One study has extended the observations on rat hepatic microsomal enzymes to in utero total body, or head region radiation exposure (62). An inhibition was produced but radiation doses above 500 R were required to produce enzyme inhibition in adult rats. It appears that the central nervous system exerts a regulatory role, not only in the development, but also in the maintenance of an optimal level of microsomal enzyme activity in the liver and radiation will modify this central nervous system control.

Radiation of rats will inhibit detoxification of chemical substances that require glucuronide conjugation (63, 64). This effect is not manifest until approximately 3 days post-irradiation. The mechanism of action may be due to the effect of X-rays on coenzyme A or its substrates. This radiation effect has not been explored as a means of detecting the degree of radiation exposure.

These findings illustrate examples of radiation effects that may be quantified and prove to be significant leads to future research. However, because large doses of radiation seem to be required to produce biochemical alterations in adult animals, the feasibility of using these radiation-induced biological changes to indicate the degree of injury may be limited.

(See Section VII, Suggested Areas for Future Research, p 63)
J. CHROMOSOME ABERRATIONS

The study of radiation-induced chromosome aberrations was initiated by the work of Karl Sax in the late 1930's (65). Since the advent of the original report, radiation cytogenetics has been the subject of extensive investigations (66, 67, 68). The various types of chromosome aberrations that have been found in human cells after irradiation are essentially identical with chromosome aberrations following irradiation to animal and plant cells possessing similar monocratic chromosomes. In addition, spontaneously produced chromosome malformations, presumably caused by radiomimetic agents or induced by either corpuscular or electromagnetic wave radiations, are indistinguishable.

The development of relatively simple and reliable methods of culturing mammalian cells in vitro (69, 70, 71, 72) was a significant advance. In 1960, Moorhead and co-workers (73) obtained mitotic cell preparations from in vitro cultured peripheral blood leukocytes. These studies stimulated a rapid development of human cytogenetics. In current practice (74) the culture technique involves the introduction of viable leukocytes, separated from other blood elements or whole blood, into a tissue culture medium that contains a substance to induce transformation and mitosis (75, 76).

The two main types of chromosome structural alterations induced by irradiation are usually classified as: (a) chromosome-type aberrations in which the chromosome is broken as a unit, and (b) chromatid-type aberrations in which only one of the sister chromatids is affected as shown in Figure 11 (77). Chromosome-type aberrations are more useful for dose prediction than chromatid breaks because they are easier to score (fewer types) and less likely to exhibit stage sensitivity and radiation-induced mitotic delay. An example of some chromosome aberrations in a human leukocyte is shown in Figure 12. When scoring the first post-irradiation mitosis, the aberration, whether chromosome- or chromatid-type is present in the cell examined. Following the second post-irradiation division, however, chromatid-type aberrations are transmitted to only one of the daughter cells (78). On the other hand, chromosome-type breaks do indeed proliferate into two cells containing the parent aberration. Furthermore, chromosome-type aberrations are induced in G1 during interphase and prior to DNA synthesis; the stage of most human leukocytes in peripheral blood (79, 80). This is particularly important for considerations of in vivo exposure. Moreover, following irradiation, these cells do not divide in vivo for several weeks (81, 82).
Irradiation of chromosomes at different times during the cell cycle induces different kinds of aberrations. When irradiated in G₁, the chromosomes react as if they were single, that is, not subdivided into two chromatids, and they yield chromosome aberrations. When irradiated in S, G₂, or early prophase, the chromosomes react as if they were longitudinally divided into two chromatids, and the induction of chromatid aberrations is observed. When irradiated in late prophase, subchromatid breaks are induced, and the chromosome reacts as if it were quadripartite even though no new round of DNA synthesis has taken place (Courtesy Prentice-Hall, Inc.; Ref. 77).
Chromosome aberrations in a human leukocyte. The blood sample, taken from a normal female, was irradiated with fission neutrons before culture. The cell has either an incomplete asymmetrical interchange (dicentric) or an asymmetrical interchange plus a deletion (Courtesy Michael A. Bender).
Chromosome breaks produced by radiation are, in general, randomly distributed. The broken pieces can remain apart or rejoin in one of two ways: (a) by restoring the original geometric configuration, or (b) by combining with other broken ends. If the "pieces" remain unattached or if they combine in other than the original geometric configuration an aberration is produced. The best mitotic stage to score chromosome anomalies is usually at metaphase, before anaphase movement obscures the strand relationship (83) (Figure 13).

Radiation-induced chromosome aberrations in man may be useful to indicate radiation exposure from any cause including military exposures or civilian radiation accidents (85), to determine radiation hazards of manned spaceflight (86), and to quantify injury from radiotherapy procedures (87). As expressed by Wald and colleagues (88), "In evaluating the possible usefulness of chromosome studies of a worker population one must consider the uses to which such information might be put. To a lawyer or labor leader such observations of biological events might present a dramatic tool with which to make a forensic point, with an uncertain degree of legitimacy. It should be borne in mind, however, that the immediate use of such information can only be avoided or refuted by good evidence in hand. To the health physicist or industrial hygienist involved in radiation protection, this approach seems to offer the means of determining physical dose when the conventional physical dosimeters fail. It is suggested that such an application is fraught with peril in view of the many variables, including types of radiation, energy spectrum, body parts exposed, time of exposure, and dose rate. To the radiobiologist, cytogenetic observations might seem to offer a means of unravelling the dose response relationship between radiation and cytogenetic aberrations. The same problems in reconstructing the dose which interfere with the use of this technique as a tool in radiation protection work are present here as well. To the epidemiologist, these studies offer a means to relate a possible etiologic agent to diseases whose biologic mechanisms of initiation are as yet not known with certainty. This is done by demonstrating a relationship between chromosome aberrations and the incidence of various late effects which may be associated with radiation exposure, but which are of themselves non-specific in origin. Finally, to the clinician such studies offer, first, a way to detect and evaluate radiation injury already present, and, second, a prognostic tool with which to facilitate prediction of the likelihood of a late effect of exposure and to measure the efficacy of any preventive procedures applied to avert such late effects."
Figure 13

1. INTERPHASE
   Chromosomes not seen as distinct structures. Nucleolus visible.
   Centrioles

2. EARLY PROPHASE
   Centrioles moving apart. Chromosomes appear as long thin threads. Nucleolus becoming less distinct.

3. MIDDLE PROPHASE
   Centrioles further apart, begin to organize spindle. Each chromosome composed of 2 chromatids attached by a common centromere.
   Aster

4. LATE PROPHASE

5. METAPHASE
   Nuclear membrane has disappeared. Centromere of each double-stranded chromosome attached to a spindle fibril at spindle equator.

6. EARLY ANAPHASE
   Centromeres have divided and begun moving toward opposite poles of spindle.

7. LATE ANAPHASE
   The 2 sets of new single-stranded chromosomes nearing respective poles. Cytokinesis beginning.

8. TELOPHASE

9. INTERPHASE
   Nuclear membranes complete. Chromosomes no longer visible. Cytokinesis complete.

Mitosis and cytokinesis of an animal cell
(Courtesy W.W. Norton & Company, Inc.; Ref. 84).
A number of investigators (78, 82, 89, 90) have considered chromosome aberrations as a feasible and accurate estimate of the amount of radiation received. Kelly and Brown (89) summarized the type and extent of \textit{in vivo} radiation damage measured by somatic cytogenetics (Table 2). The use of leukocyte chromosome aberrations as an indicator of the absorbed dose is supported by the following findings: (a) with increasing dose there is a corresponding increase in the number of aberrations in peripheral blood lymphocytes following both partial- and whole-body irradiation, and (b) lymphocytes are fairly widely dispersed throughout most tissues in the body and damage produced in these cells may be a reasonable indicator of the effect on the whole organism. The relative merits of using chromosome aberrations as indices of radiation-induced biological injury are discussed in Part II.

Coefficients of chromosome aberration production can be determined and equations written that accurately describe the aberration in relation to the absorbed dose (85, 89, 90). To obtain meaningful chromosome aberration rate measurements, the following conditions must be satisfied before the coefficients of aberration production can be calculated (90): (a) assurance that the aberrations scored are induced only by the radiation exposure; (b) all cells examined must have received substantially the same radiation dose; (c) the quantity and quality of the radiation must be known; and (d) the cells must be examined in their first post-irradiation division. However, it is a rare human radiation exposure that meets all these criteria. For practical reasons, coefficients of aberration production are generally determined with cells exposed to radiation \textit{in vitro}. These coefficients have been used in the study of accidental human radiation exposures and are described below. Chromosome deletions involve only a single break in a chromosome and exhibit linear dose-effect kinetics:

\[ Y = a + b D \]

Ring and dicentric chromosomes require the interaction of two chromosome breaks and usually manifest dose-square kinetics:

\[ Y = a + c D^2 \]

Where \( Y \) = aberration yield, \( a \) = spontaneous aberration frequency constant (deletions or rings and dicentrics), \( b \) and \( c \) = unknown aberration coefficients, and \( D \) = dose of X- or \( \gamma \)-rays absorbed (85, 90). For neutrons or \( \alpha \)-particles the kinetics for two break chromosome aberrations becomes linear (85). The graphic representation of the dicentric aberrations per cell induced by increasing X-ray doses for human peripheral blood leukocytes is shown in Figure 14.
## Table 2

ABERRATIONS IN HUMAN LEUKOCYTES AFTER IN VIVO IRRADIATION*

<table>
<thead>
<tr>
<th>Dose in Rads (pts)</th>
<th>Type of Radiation</th>
<th>Cause</th>
<th>Abnormalities</th>
<th>Time after Irradiation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (2 pts)</td>
<td></td>
<td></td>
<td>{7-12% aneuploidy</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3% centric, fragm.}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>x-ray diagnostic</td>
<td></td>
<td>dicentric</td>
<td>8 hr</td>
<td>14</td>
</tr>
<tr>
<td>&lt;3</td>
<td>x-ray (7 pts)</td>
<td></td>
<td>diagnostic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ivp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11*</td>
<td>I^131</td>
<td>therapeutic-thyroid</td>
<td>27% aneuploidy</td>
<td>3 days</td>
<td>16</td>
</tr>
<tr>
<td>14*</td>
<td>I^131</td>
<td>therapeutic-thyroid</td>
<td>{22% aneuploidy</td>
<td>5 days</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{4% centric, fragm.}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16*</td>
<td>I^131</td>
<td>therapeutic-thyroid</td>
<td>{26% aneuploidy</td>
<td>1 day</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{7% centric, fragm.}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>n &amp; γ accident</td>
<td></td>
<td>4% aneuploidy</td>
<td>2½ yr</td>
<td>7</td>
</tr>
<tr>
<td>23</td>
<td>n &amp; γ accident</td>
<td></td>
<td>{14% aneuploidy</td>
<td>3½ yr</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{3% centrics, others}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24*</td>
<td>I^131</td>
<td>therapeutic-thyroid</td>
<td>30% aneuploidy</td>
<td>3 days</td>
<td>16</td>
</tr>
<tr>
<td>30</td>
<td>x-ray (4 pts)</td>
<td>diagnostic-gi</td>
<td>2-4% “multihit”</td>
<td>“transient”</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>x-ray (1 pt)</td>
<td>diagnostic-gi</td>
<td>2-4% “multihit”</td>
<td>1 mo</td>
<td>6</td>
</tr>
<tr>
<td>70</td>
<td>n &amp; γ (pt.a)</td>
<td>accident</td>
<td>8% aneuploidy</td>
<td>2½ yr</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{2% minutes, others}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>n &amp; γ (pt.b)</td>
<td>accident</td>
<td>14% aneuploidy</td>
<td>2½ yr</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{5% minutes, others}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>n &amp; γ (pt.a)</td>
<td>accident</td>
<td>16% aneuploidy</td>
<td>3½ yr</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{2% minutes, others}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>220† (extrap.)</td>
<td>I^131</td>
<td>therapeutic-thyroid</td>
<td>30% aneuploidy</td>
<td>7 days</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13% centrics, fragm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>236</td>
<td>n &amp; γ accident</td>
<td></td>
<td>7% aneuploidy</td>
<td>2½ yr</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{1% centrics}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>x-ray therapeutic</td>
<td></td>
<td>30% aneuploidy</td>
<td>4 days</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>spine ankylosing</td>
<td></td>
<td>{10% fragments}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>spondylitis</td>
<td></td>
<td>{8% polyploids}</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>x-ray</td>
<td>therapeutic</td>
<td>normal modal no.</td>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ca of cervix</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>n &amp; γ accident</td>
<td></td>
<td>20% aneuploidy</td>
<td>2½ yr</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{1% centrics}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>270</td>
<td>n &amp; γ accident</td>
<td></td>
<td>16% aneuploidy</td>
<td>3½ yr</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>{8% centrics, others}</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Blood dose—calc. from mc dose a/c Green.†
† From Figure 3 (graph) and Figure 7 (nomogram) of Green.

* Table taken in its entirety from Ref. 89. Individual cited source references can be found in original article.
<table>
<thead>
<tr>
<th>Dose in Rads</th>
<th>Type of Radiation</th>
<th>Cause</th>
<th>Abnormalities</th>
<th>Time after Irradiation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>327</td>
<td>n &amp; γ</td>
<td>accident</td>
<td>14% aneuploidy, 9% dicentrics, minutes, rings, others</td>
<td>2½ yr</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n &amp; γ</td>
<td>accident</td>
<td>12% aneuploidy, 7% dicentrics, minutes, others</td>
<td>3½ yr</td>
<td>8</td>
</tr>
<tr>
<td>339</td>
<td>n &amp; γ</td>
<td>accident</td>
<td>23% aneuploidy, 20% dicentrics, minutes, rings, others</td>
<td>2½ yr</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n &amp; γ</td>
<td>accident</td>
<td>8% aneuploidy, 15% dicentrics, minutes, others</td>
<td>3½ yr</td>
<td>8</td>
</tr>
<tr>
<td>350†</td>
<td>I¹³¹ (extrap.)</td>
<td>therapeutic-thyroid</td>
<td>38% aneuploidy, 6% dicentrics, fragments</td>
<td>3 days</td>
<td>16</td>
</tr>
<tr>
<td>365</td>
<td>n &amp; γ</td>
<td>accident</td>
<td>14% aneuploidy, 7% dicentrics, minutes, others</td>
<td>2½ yr</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>n &amp; γ</td>
<td>accident</td>
<td>14% aneuploidy, 12% dicentrics, minutes, others</td>
<td>3½ yr</td>
<td>8</td>
</tr>
<tr>
<td>600</td>
<td>x-ray</td>
<td>therapeutic (spine) ankylosing spondylitis</td>
<td>none</td>
<td>13 yr</td>
<td>12</td>
</tr>
<tr>
<td>650</td>
<td>x-ray</td>
<td>therapeutic (spine) ankylosing spondylitis</td>
<td>18% aneuploidy</td>
<td>6 days</td>
<td>17</td>
</tr>
<tr>
<td>750</td>
<td>x-ray</td>
<td>therapeutic (spine) ankylosing spondylitis</td>
<td>18% aneuploidy</td>
<td>6 yr</td>
<td>12</td>
</tr>
<tr>
<td>1,200</td>
<td>x-ray (2 pts)</td>
<td>therapeutic (spine) ankylosing spondylitis</td>
<td>none</td>
<td>12-14 yr</td>
<td>12</td>
</tr>
<tr>
<td>1,500</td>
<td>x-ray</td>
<td>therapeutic (spine) ankylosing spondylitis</td>
<td>26% aneuploidy</td>
<td>4 days</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3% polyploidy, 16% rings, dicentrics</td>
<td>20 days</td>
<td>5</td>
</tr>
<tr>
<td>1,800</td>
<td>x-ray (2 pts)</td>
<td>therapeutic (spine) ankylosing spondylitis</td>
<td>none</td>
<td>9-14 yr</td>
<td>12</td>
</tr>
<tr>
<td>1,800</td>
<td>x-ray</td>
<td>therapeutic (spine) ankylosing spondylitis</td>
<td>48% aneuploidy</td>
<td>16 days</td>
<td>17</td>
</tr>
<tr>
<td>1,500-2,500</td>
<td>x-ray</td>
<td>therapeutic (spine) ankylosing spondylitis</td>
<td>aneuploidy</td>
<td>&gt;3 wk-2 yr</td>
<td>13</td>
</tr>
<tr>
<td>(58)</td>
<td></td>
<td></td>
<td>18% polyploidy, chromatid aberrations fragments dicentrics + tri rings</td>
<td>&gt;7 days-5 yr</td>
<td>13</td>
</tr>
</tbody>
</table>

* Blood dose—calc. from me dose s/c Green.†
† From Figure 3 (graph) and Figure 7 (nomogram) of Green.‡
Table 2 - Continued

<table>
<thead>
<tr>
<th>Dose in Rads</th>
<th>Type of Radiation</th>
<th>Cause</th>
<th>Abnormalities</th>
<th>Time after Irradiation</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,020</td>
<td>x-ray</td>
<td>therapeutic ca cervix</td>
<td>45% aneuploid</td>
<td>19 days</td>
<td>17</td>
</tr>
<tr>
<td>2,360</td>
<td>x-ray</td>
<td>therapeutic ca cervix</td>
<td>63% aneuploid</td>
<td>22 days</td>
<td>17</td>
</tr>
<tr>
<td>2,500</td>
<td>x-ray</td>
<td>therapeutic ca cervix</td>
<td>18% aneuploid</td>
<td>6 wk</td>
<td>17</td>
</tr>
</tbody>
</table>
| Not given    | x-ray             | therapeutic ca cervix        | 50%  
|              |                   |                              | chromosome breaks             | 14 days                | 17     |
|              |                   | diagnostic                   | decrease in modal no.            |                        |        |
|              |                   |                              | 28% polyploidy                   |                        | 9      |
|              |                   |                              | 100% Ph' chromosome              |                        |        |
|              |                   |                              | 5% pts. e aneuploidy,            |                        | 18     |
|              |                   |                              | polyploidy, endo-               |                        |        |
|              |                   |                              | reduplication, rings,           |                        |        |
|              |                   |                              | fragm., transloc.                |                        |        |
| Not given    | x-ray (7 pts)     | therapeutic (leukemia)       | 5% pts. e aneuploidy,            | 14 days                |        |
|              |                   |                              | polyploidy, endo-               |                        |        |
|              |                   |                              | reduplication, rings,           |                        |        |
|              |                   |                              | fragm., transloc.                |                        |        |
| Not given    | x-ray             | therapeutic (chronic         | polyploidy                      | 10 days                | 10     |
|              | p32               | myelog., leukemia)           | decrease in Ph'                 |                        |        |
|              |                   |                              | translocation                    |                        |        |
| Not given    | x-ray             | diagnostic                   | aneuploidy, dicentrics,         | 2 mo                   | 15     |
|              |                   |                              | fragments                        |                        |        |

* Blood dose—calc. from me dose a/c Green.19
† From Figure 3 (graph) and Figure 7 (comogram) of Green.19

(Courtesy Sally Kelly; Ref. 89).
The Effects of Increasing X-Ray Doses on the Number of Dicentric Chromosome Aberrations in Human Peripheral Blood Leukocytes.
(Courtesy Peter C. Nowell; Original reports are cited in Ref. 91).
Persistence of chromosome abnormalities for many months and years after irradiation have been documented (92, 93, 94). Thus, the past radiation history becomes important for the soldier in determining the significance of individual aberrations found shortly after an acute exposure.

Because it is a highly specialized, time-consuming procedure, not adapted in its present state as a routine "field" technique, and results are not available for a minimum of 3 days after blood samples are obtained, chromosome aberrations in peripheral leukocytes are not particularly useful to the military as an indicator of radiation-induced injury. On the other hand, the method has practical value in determining whether a moderate to large (e.g. 200-400 rad) dose was received, or only a relatively small dose, e.g. < 25 rad). If exposure is suspected (e.g. an exposed film badge) but questionable, then a chromosome aberration analysis can provide a conclusive answer (85).

(See Section VII, Suggested Areas for Future Research, p 64)
VII. SUGGESTED AREAS FOR FUTURE RESEARCH

PHYSICAL DOSIMETRY

The developments in the field of thermoluminescent dosimetry may have military applications in determining the degree of radiation exposure. There are a number of aspects of this form of physical dosimetry that may be suitable for field use. For example, in military combat a predetermined number of soldiers could wear dog tags with phosphor teflon matrix inserts to provide an estimate of the maximum exposure distribution. Consideration should be given to additional reviews of this subject. Unfortunately, the recorded dose is of limited value in determining the radiation-induced biological injury sustained by the soldier. (See Section VI A, p 21).

RADIATION-INDUCED EMESIS

Post-irradiation vomiting is a clinical manifestation of the radiation syndrome. The onset, frequency, and duration of radiation-induced emesis should be tabulated more accurately and related to the amount of radiation received by the subject. The basic etiologic mechanisms of radiation-induced vomiting should be explored in greater detail in man. A biochemical alteration may be correlated with the incident radiation and subsequent nausea and vomiting. Further research on the etiologic mechanisms, the biochemical alterations, and the clinical correlations of radiation-induced emesis may enhance the diagnostic significance in radiation casualties and lead to improved therapy. (See Section VI B, p 22).

CYTOLOGICAL CHANGES IN THE TESTES

The cells of the testes are highly radiosensitive. However, it is not practical to perform testicular biopsies on suspected radiation battlefield casualties. There is a need to develop methods to utilize the extreme radiosensitivity of the testicular cells to measure the degree of radiation injury. (See Section VI C, p 24).
CHANGES IN URINARY CONSTITUENTS

Alterations in specific urinary constituents following radiation exposure of animals have shown a relationship to the dose. However, the value of these changes as indicators of radiation injury has yet to be proved for man. Additional studies of this type could be made on accident victims and patients receiving radiation therapy to determine if meaningful indices of radiation injury could be developed. (See Section VI D, p 25).

HEMATOLOGIC CHANGES

The recognized hematologic changes in man have been determined usually after single exposures to known sources of radiation for known time periods. These data provide the basis for current understanding and prediction of the degree of injury after radiation. However, multiple exposures of gamma and combinations of gamma-neutron radiation encountered in the military situation, in addition to other variables, make the prediction of the extent of radiation injury difficult. Although radiation accidents do not mimic battlefield exposure, they provide useful hematologic information on man and suggest guidelines for future research.

Preliminary data from irradiated animals, and man after radiation accidents, suggest that cell population kinetics of blood monocytes may be useful as an index of radiation injury. The prompt reduction in the number of these cells is presumably dose-related. A systematic study of these blood elements in subjects undergoing therapeutic radiation should be made. (See Section VI E, p 28).

SERUM IRON LEVELS

Radiation exposure effects are generally reflected in a decreased bone marrow erythropoiesis mirrored by an increased serum iron level. It may be possible to utilize serum iron changes as an indicator of the degree of radiation injury. These measures should be explored in greater detail. (See Section VI F, p 34).
ETIOCHOLANOLONE IN ESTIMATING BONE MARROW GRANULOCYTE RESERVES

The continued study of granulocyte kinetics will be useful in elucidating the dynamics of the myelopoietic system in man and its relation to the general hematopoietic syndrome that develops after radiation exposure. The steroid metabolite, etiocholanolone, has proved to be a useful agent to assess the granulocyte reserve of patients receiving therapy with myelosuppressive agents. Because it is feasible to use etiocholanolone to estimate bone marrow reserves of the radiation-injured man, research on granulocyte kinetics warrants additional emphasis. (See Section VI G, p 35).

TOTAL PROTEIN-BOUND NEUTRAL HEXOSES

Differences in the total protein-bound carbohydrates in the plasma of irradiated mice suggest new opportunities for the detection of radiation injury. These preliminary findings should be extended to additional species including man, and the various individual glycoproteins measured before and after radiation. The newer physicochemical analytical methods may be employed to measure the changes in specific protein-bound carbohydrates induced by radiation exposure. These measures may serve as a prognostic test to aid in following the clinical course of an irradiated man and to identify the most radiosensitive individuals prior to therapeutic radiation exposure. (See Section VI H, p 42).

DETOXIFICATION ENZYMES, DRUG METABOLISM, AND BIOCHEMICAL CHANGES

Radiation-induced alterations in the metabolism of a drug or a chemical test substance may provide an indication of the degree of radiation injury. There is evidence that some oxidative enzymes are inhibited, or prevented from developing, in young rats by radiation exposure to the pituitary region of the brain. There is evidence also that liver microsomal enzymes involved in the metabolism of some chemicals may be modified by irradiation. The plasma clearance of a test chemical might be related to radiation exposure because reduced enzyme activity may be reflected in higher blood plasma drug levels. Because large doses are required to produce changes in animals, additional information is required on the character and causes of these enzymic effects before reliable measures can be developed. (See Section VI I, p 47).
CHROMOSOME ABERRATIONS

The scoring of radiation-induced leukocyte chromosome aberrations is a useful and reliable technique to determine the approximate degree of radiation injury. However, the method is technically difficult and time-consuming. It would be desirable to develop simplified scoring techniques and the use of computers would facilitate the tedious microscopic examinations now necessary. However, computer methodology may not be applicable to battlefield conditions. More extensive use of leukocyte chromosome aberrations to detect and predict radiation-induced injury of the individual warrants additional study. The persistence of chromosome abnormalities for years after irradiation provides a useful radiation history of the soldier to determine the significance of subsequent exposures. (See Section VI J, p 49).
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X. GLOSSARY

Chromatid . . . . . One of the two strands formed by longitudinal duplication of a chromosome that becomes visible during prophase.

Granulocytes . . . Mature granular leukocytes, including neutrophilic, acidophilic, and basophilic types of polymorphonuclear leukocytes, i.e., neutrophils, eosinophils, and basophils.

Hematopoietic Tissue . . . . . The source of the various types of blood cells and other formed elements.

Leukapheresis . . . An experimental removal of white blood cells without the introduction of known toxic factors to incite leukocytosis.

Leukocytopenia . . . A decrease in the number of leukocytes in the blood.

Liver Mitochondria . . . . Organelles of the cell cytoplasm that contain the cytochrome and oxidative phosphorylation enzymes. They are the principal source of energy for the cell.

Lymphocytes . . . . White blood cells formed in lymphoid tissue throughout the body.

Mitosis . . . . . . . The process of division of somatic cells that results in the formation of two daughter cells with exactly the same chromosome number and DNA content as the original cell (see Figure 13, p 53).

Monocytes . . . . . Relatively large mononuclear leukocytes in the circulating blood.

Myeloid reserves . . . Features of the bone marrow that are concerned with myelocyte production and other blood elements.
Phytohemagglutinin. A mucoprotein obtained from the red kidney bean that agglutinates only the erythrocytes in low concentrations. Stimulates blastogenesis and mitotic activity in cultured lymphocytes.

Polycythemia. An increase in the number of red blood cells; erythrocytosis.

Rad. (Acronym for radiation absorbed dose.) The basic unit of absorbed dose of ionizing radiation. A dose of one rad means the absorption of 100 ergs of radiation energy per gram of absorbing material.

Reticuloendothelial System. A cell group of macrophages, collectively the cells in different organs, chiefly concerned with phagocytosis. They occur in lymphatic structures, the liver, spleen, and bone marrow.

Roentgen (R). A unit of exposure to ionizing radiation. It is that amount of gamma or X-rays required to produce ions carrying 1 electrostatic unit of electrical charge (either positive or negative) in 1 cubic centimeter of dry air under standard conditions.

Spermatogonia. Primitive sperm cells that give rise by division to the spermatocyte.

Thrombocytopenia. A condition in which there is an abnormally small number of platelets in the circulating blood.

Total Blood Granulocyte Pool (TBGP). The granulocytes in the calculated amount of total blood including those mobilized from the bone marrow. The marginal granulocyte pool (MGP) is calculated by subtracting the circulating granulocyte pool (GP) value from the TBGP.
PART II

CLINICAL AND LABORATORY OBSERVATIONS USEFUL IN ESTIMATING DEGREE OF RADIATION INJURY

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I. INTRODUCTION

This review of biological changes that indicate the degree of injury following exposure to ionizing radiation is concerned primarily with changes observed in the irradiated human, especially with evaluation of injury under conditions of nuclear warfare, where large numbers of individuals might be irradiated.

The extensive literature related to acute radiation injury and the "acute radiation syndrome" already contains many reports and monographs that describe the signs, symptoms, and laboratory data observed in man following irradiation. This material, of course, is the major source of information for all reviews of the subject, including the present one. Relevant references cited in the text are annotated in the bibliography. In addition, the bibliography lists separately, without annotation, (a) a group of review articles and books selected as being authoritative and, as a group, representative of the literature, and (b) a group of papers that, although not cited specifically in the text, would provide additional highly pertinent documentation. Where possible, references describing observations of human subjects have been selected.
II. RADIATION INJURY IN CONTEXT OF GENERAL MEDICINE

Medical personnel responsible for the management of injured patients must estimate how seriously each patient has been injured. Following exposure to ionizing radiation, the injury manifest by the individual (i.e., the soldier) will depend upon the duration of exposure, uniformity and intensity of the radiation field, the proportion of the total body exposed (or shielded), the physical characteristics of the radiation, the precise conditions of the individual's exposure, and various biological variables such as individual susceptibility, and other types of injuries or stresses applied concomitantly. Since the medical officer will be faced with multiple uncertainties, he must rely upon observations reflecting the state of the patient's intrinsic physiological machinery when estimating how serious the injury is (1). In doing so, he acknowledges that ionizing radiation is only one form of energy capable of causing injury and that patients injured by radiation must be treated according to the current concepts of diagnosis and treatment.

Unfortunately, when there is a question of radiation injury, there is often a preoccupation with the injurious agent rather than with the injury. Although accurate physical dosimetry will of course be of great help, one should, in fact, be extremely cautious in estimating the diagnostic and prognostic significance of biological changes that reflect integral radiation dose. Peripheral blood lymphocytes, for example, which are produced by widely distributed lymphoid tissue, circulate freely and are highly radiosensitive, may show effects of radiation exposure even though only a small part of the body was exposed. Their use as "integrating dosimeters" has been proposed (2).

But consider, for a moment, whether knowing the integral radiation dose would necessarily be helpful in the clinical management of acute radiation injury. Chromosome aberrations in cultures of peripheral blood lymphocytes may accurately reflect total dose to a small part of the upper thorax for example, but not genetic injury to the germinal epithelium where it is especially important. Similarly, the number of morphologically altered or non-viable lymphocytes in peripheral blood after exposure may accurately reflect total dose delivered to one part of the body (or even to the blood extracorporeally) without indicating how sick the patient will be. Clearly it is not dose that the clinician or this report should be concerned with. What the clinician needs is information about the patient and about the probable course of his illness.
Use of the term "biodosimetry" with reference to injury evaluation is a distinctly ill-advised aberration of emphasis. The term implies that the "bio" (e.g. the injured man) is used to measure the physical agent, and that is, unfortunately, the way in which the term is sometimes used by those who become preoccupied with attempting to determine which biological changes can best tell them the radiation dose. Extending this concept, one might expect the surgeon, confronted with a burned patient, to concentrate on determining how many BTU's the casualty had absorbed. In the context of clinical evaluation, the word embodies a false concept and tends to promulgate a distortion in judgement.
III. SELECTING CLINICAL INDICATORS OF DEGREE OF INJURY

Symptoms, signs, and laboratory data indicating the degree of injury in military radiation casualties will be important primarily to medical personnel responsible for clinical management of the injured and to those military personnel responsible for making decisions about deployment of irradiated troops. For such application, clinical indicators of the degree of radiation-induced injury should meet most of the following criteria:

- The magnitude of the change measured should be a function of absorbed dose over the range (approximately) 100-1000 R.

- The necessary determinations should be relatively easy to make and should not require extremely complex, unstable, or exotic equipment. If possible, they should not require the introduction of radioactive material into the exposed persons.

- It should be possible to make the necessary determinations on readily available and serially available biological material, e.g. blood or urine.

- The range of normal values for the parameters selected should be well-defined and should have a relatively small range of variability. Ideally, the range of normal values for the individual in question, rather than the mean for a group, should be the reference value when evaluating post-exposure data.

- Although it is unrealistic to expect any one biological change to be absolutely specific for radiation injury, there should be a high probability that the biological indicators selected will be affected by radiation exposure. In some situations, a measurement that simply indicates that no significant injury has occurred would have great value.

- Changes should be detectable within 24-48 hours after exposure.
IV. CLINICAL AND LABORATORY FINDINGS USEFUL IN EVALUATING RADIATION INJURY

The major sources of information about man's response to acute radiation injury are: (a) the Japanese population exposed to radiation from the atomic bomb, (b) Marshall islanders and Japanese fishermen exposed to gamma radiation from fallout subsequent to testing thermonuclear devices, (c) persons accidentally exposed to large doses of ionizing radiation during the course of their normal activity (criticality accidents, exposure to radiation from radioisotopes, or other sources of radiation), and (d) patients receiving radiation therapy to the entire body.

Although this review is concerned primarily with the irradiated human, it also reflects the large mass of information obtained from laboratory studies of irradiated experimental animals of many species. These laboratory studies have frequently guided clinical studies; in other instances they have provided confirmatory evidence, raised important questions or influenced the clinical work in other ways. Taken together, the available data have shown the following clinical or laboratory findings to be especially helpful in estimating the severity of radiation injury:

A. CLINICAL PICTURE

Patients of greatest concern to the medical officer are those whose radiation injury is not too serious to completely preclude recovery. Various schemes have been proposed for classifying casualties (triage). There is good general agreement that patients who remain asymptomatic during the first few days after exposure probably are not injured seriously enough to require treatment. Absence of nausea and vomiting is especially encouraging during this period (3, 4, 5, 6, 7, 8). These patients would be ranked in category I according to the classification presented in Table 1, which is based on suggestions of several other authors and groups (3, 4, 5, 6, 7, 8, 9, 10).

At the other extreme, patients with immediate onset of extreme excitement, severe gastrointestinal symptoms followed shortly by unconsciousness and intractable hypotension are those whose injury is irreversible (Group IV in Table 1). Therapy other than that required to relieve symptoms would not be indicated, and death may be expected within hours or days.
Table 1

CLINICAL OBSERVATIONS FOLLOWING SINGLE, BRIEF EXPOSURE

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<tr>
<td>Patient Group IV³</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
</tbody>
</table>

1 Observations during first 2 days following exposure
2 No treatment necessary; minimal injury
3 Extremely poor prognosis; symptomatic treatment only
Between the two extremes lie those casualties for whom survival is possible (Groups II and III in Table 1). Some of these casualties may have to return to duty under emergency military conditions. These patients will have sustained more or less serious injury to the hematopoietic system and in Groups II and III to the gastrointestinal epithelium. The signs and symptoms they develop will reflect the site and extent of their injury.

Certain assumptions underlying the following comments about clinical observations in Groups II and III should be noted. It is assumed that the casualties indeed have been in a situation in which radiation exposure was likely, i.e. that the essential history has been taken. In this, as in most other discussions of acute radiation injury, it is also assumed that the whole body has been exposed, essentially uniformly, to a single dose delivered in a short period. These assumptions are, of course, oversimplifications and it is important to recognize them as such when attempting to extrapolate from the hypothetical situation to the real one which may differ from it in many important ways.

Severe, persistent nausea and vomiting during the first two to three days after exposure indicates serious injury and the need for further observation or additional data. If nausea and vomiting are accompanied by diarrhea and fever during this early period, the clinical picture would fit that of the "gastrointestinal syndrome" and a fatal outcome would be predicted in the majority of these patients unless rigorous therapeutic support were possible (3, 4, 5, 6, 9, 11, 12, 15, 16, 17).

In the profile scoring system developed by Thoma and Wald (4), observations are continued and laboratory procedures carried out as indicated by the cumulative scores reflecting severity of injury (Figure 1). This system has proved to be workable and helpful in real exposure situations (14). In most schemes for classifying radiation injury, early erythema, fatigue, and restlessness are considered to be less predictable or difficult to measure, but are, nevertheless, recognized as significant ancillary clinical findings in severely injured casualties (7, 8, 15, 17).

One of the more extensive recent analyses of clinical observations in irradiated human subjects utilizes data contributed by a group of collaborators at several medical centers where the response of patients to therapeutic whole-body irradiation is under study (6). Table 2 and Figures 2 and 3 illustrate the most recent analysis of the pooled data. Some difficulty in estimating the contribution of the patients' underlying diseases to the response is, of course, unavoidable. In spite of this problem, the patterns of response to the various radiation doses conform remarkably well to the patterns observed in healthy persons exposed accidentally.
Figure 1

1. Observe and Record Time of Onset of Clinical Signs and Symptoms

Nausea, Vomiting, Diarrhea Within Minutes and Ataxia, Disorientation, Shock, Coma in Minutes to Hours

Injury Groups I, II, III, IV

Injury Group V

Injury Groups II, III, IV

Injury Group I

Nausea and/or Vomiting and Some Derangement of Blood Count Within 2 Days

Injury Groups II, III, IV

Marked Leucocyte and Lymphocyte Count Derangement in 3 Days

Injury Groups III, IV

Diarrhea Within 4 Days and Marked Platelet Derangement Within 6 to 9 Days

Injury Group IV

PRELIMINARY EVALUATION OF RADIATION INJURY

Schematic plan of profile scoring system of Thoma and Wald (Ref. 4).
Table 2

ESTIMATES FOR EXPOSURE (R) AND ABSORBED (RAD) DOSES REQUIRED TO CAUSE 50 PERCENT OF PATIENTS TO SHOW VARIOUS CLINICAL RESPONSES

<table>
<thead>
<tr>
<th>Clinical Response</th>
<th>Normal Arithmetic Distribution</th>
<th>Log-Normal Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(rads)</td>
<td>(R)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>121+19</td>
<td>97+12</td>
</tr>
<tr>
<td></td>
<td>183+29</td>
<td>147+18</td>
</tr>
<tr>
<td></td>
<td>-11</td>
<td>-16</td>
</tr>
<tr>
<td>Nausea</td>
<td>172+17</td>
<td>139+20</td>
</tr>
<tr>
<td></td>
<td>260+25</td>
<td>210+30</td>
</tr>
<tr>
<td></td>
<td>-17</td>
<td>-26</td>
</tr>
<tr>
<td>Fatigue</td>
<td>181+25</td>
<td>147+35</td>
</tr>
<tr>
<td></td>
<td>274+38</td>
<td>223+53</td>
</tr>
<tr>
<td></td>
<td>-28</td>
<td>-43</td>
</tr>
<tr>
<td>Vomiting</td>
<td>214+22</td>
<td>183+39</td>
</tr>
<tr>
<td></td>
<td>325+33</td>
<td>277+58</td>
</tr>
<tr>
<td></td>
<td>-32</td>
<td>-48</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>239+32</td>
<td>230+235</td>
</tr>
<tr>
<td></td>
<td>361+48</td>
<td>348+357</td>
</tr>
<tr>
<td></td>
<td>-116</td>
<td>-176</td>
</tr>
<tr>
<td>Death</td>
<td>251+28</td>
<td>235+75</td>
</tr>
<tr>
<td></td>
<td>381+43</td>
<td>356+114</td>
</tr>
<tr>
<td></td>
<td>-57</td>
<td>-87</td>
</tr>
</tbody>
</table>

(Ref. 6).
Two examples of probit regression analysis as done in this study of the probability relationships of response to radiation dose, assuming normal and log-normal distributions of population sensitivity. Data used in this analysis represent patients receiving therapeutic irradiation of the entire body. Computed probability that emesis will occur within the first two days following various radiation doses is shown for two types of populations (Ref. 6).
Acute hemopoietic syndrome defined by our estimates of effective single doses for radiation-induced anorexia and lethality in patients. The probit regression lines have shaded fiducial limits. Probability that patients receiving whole-body irradiation will develop prodromal symptoms or will die following various X-ray doses is shown. Note 50% effective dose for producing the "acute hematologic syndrome" (lethality curve) is approximately 250 rad (Ref. 6).
B. PERIPHERAL BLOOD STUDIES

Radiation-induced changes in the number of circulating mature blood cells reflect the high radiosensitivity of the proliferating precursor cells in hematopoietic tissue. Normally, hematopoiesis is in a steady state, the cells that die or are lost per unit time being balanced by the production of new cells. When cell production is interrupted, available cells disappear from circulation at rates determined by their characteristic life spans and by rates of utilization or cell loss (as in hemorrhage). Thus, following exposure to large doses of ionizing radiation, the level of short-lived leukocytes falls rapidly whereas anemia develops more slowly, as one would expect in light of the relatively long life span of erythrocytes (roughly 120 days in man). The concept that the acute radiation syndrome represents a somewhat delayed manifestation of a failure in cellular proliferation is developed and documented in a recent monograph (18). The high susceptibility of circulating lymphocytes to direct injury by radiation is an interesting exception to the general rule that the circulating blood cells are relatively radio-resistant. The diagnostic and prognostic implications of this exception are considered later in this review.

Many authorities have analyzed the effects of various radiation doses on the peripheral blood picture (3, 4, 5, 7, 14, 15, 18, 19, 20). Examples of such analysis, presented graphically, are shown in Figures 4, 5, 6, and 7 (19, 20). It is particularly encouraging that despite considerable variability among circumstances prevailing during the various accidental human exposures that have occurred, hematological changes have been relatively consistent among casualties exposed to comparable large single radiation doses in the lethal range (4, 5, 6, 7, 13, 14, 15, 18, 19, 20, 21, 22).

Leukocyte counts: Leukopenia, long recognized as a cardinal manifestation of acute radiation injury, has proved to be a useful gauge of such injury in both clinical and experimental applications. Salient characteristics of the peripheral blood leukocyte picture in the four injury groups (Table 1) are summarized in Table 3. Although the absolute granulocyte count may fluctuate markedly during the early post-exposure period, causing similar fluctuation in the total leukocyte count, the trend of the absolute lymphocyte count is consistently downward and lymphopenia tends to be proportional to the severity of radiation injury, as indicated schematically in Figures 8 and 9. Indeed, lymphopenia appears to be a bulwark of all major schemes for clinical evaluation of radiation injury (4, 6, 7, 14, 18, 19, 20, 22).
Schematic representation of the relationship between early changes in peripheral blood lymphocyte counts and degree of radiation injury (Ref. 19 and 20).
Some examples demonstrating the relationship between lymphocyte count and the degree of radiation injury in radiation accident casualties (Ref. 19 and 20).
Hematological response expected after whole-body exposure to 100 rad (Ref. 19 and 20).
Hematological responses expected after whole-body exposure to 200 rad (Ref. 19 and 20).
### Table 3

**PERIPHERAL BLOOD LEUKOCYTE COUNTS**  
*(Single short-term exposure)*

<table>
<thead>
<tr>
<th>Degree of Injury</th>
<th>Lymphocytes/mm³ 48 hours post-exposure or later</th>
<th>Total Leukocytes/mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Group I*</td>
<td>Not lower than 800</td>
<td>Minimal change</td>
</tr>
<tr>
<td>Patient Group II</td>
<td>Not lower than 400</td>
<td>Neutrophils variable on days 1 and 2; decreased to approximately 3000 by days 5-10.</td>
</tr>
<tr>
<td>Patient Group III</td>
<td>Not lower than 200</td>
<td>Transient rise in neutrophils and total WBC count within few hours, followed by precipitous drop to approximately 1000 at 10 days.</td>
</tr>
<tr>
<td>Patient Group IV**</td>
<td>&lt; 200</td>
<td>Greater neutrophil and WBC increase, then a fall to 1000 in less than 1 week.</td>
</tr>
</tbody>
</table>

* No treatment necessary; minimal injury.  
** Extremely poor prognosis; symptomatic treatment only.

### Table 4

**GRANULOCYTE HALF REDUCTION TIMES IN X-IRRADIATED PATIENTS**

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. Patients</th>
<th>Half Reduction Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 R</td>
<td>12</td>
<td>18.8 days</td>
</tr>
<tr>
<td>250 R</td>
<td>3</td>
<td>9.8 days</td>
</tr>
<tr>
<td>400 R</td>
<td>1</td>
<td>4.0 days</td>
</tr>
<tr>
<td>?</td>
<td>14</td>
<td>7.8 days</td>
</tr>
</tbody>
</table>

- 105 -
Figure 8

Hematological responses expected after whole-body exposure to 300 rad (Ref. 19 and 20).
Hematological responses expected after whole-body exposure to 450 rad (Ref. 19 and 20).
Depression of the absolute granulocyte and total leukocyte counts is usually apparent as an overall downward trend during the first week after single exposures to radiation doses in the lethal range. A transient "abortive rise" may occur during the second post-exposure week in patients in injury Groups II and III (Table 2, Figures 5 and 6). Although the absolute granulocyte count of man as well as other mammals has usually been found to develop a definite, uninterrupted downward trend only after an interval of several days (4, 6, 7, 14, 17, 19, 23), Adelstein and Dealy observed prompt onset of granulocytopenia and an exponential disappearance rate, for granulocytes, of ten days in a series of patients receiving whole-body exposure to a single X-ray dose of 250 R in preparation for kidney transplantation. The corresponding half reduction times for lymphocytes and platelets were 4 days and 16 days, respectively (24). The observed granulocyte disappearance rate was compared with rates reported by other investigators and results were presented graphically. Results of the comparison are summarized in Table 4. The authors suggest that early assessment of radiation damage on the basis of granulocyte disappearance rates might be feasible.

If the radiation exposure is uniform over the entire body, severe monocytopenia may be anticipated during the first post-exposure day following radiation exposures in the lethal range according to Volkman (25) who has studied the monocyte response in rats (Figure 10). This change is not as well documented as are other leukocyte trends in human radiation accident casualties, possibly because monocyte trends often are not analyzed separately from other leukocyte trends. If a significant proportion of marrow is shielded, marked reduction of the absolute monocyte count is unlikely. Thus, severe monocytopenia during the first post-exposure day can probably be taken as an indication that no appreciable portion of the hematopoietic tissue has been shielded (25). Conversely, if some marrow has been shielded, there may be only a transient monocytopenia or even an absolute monocytosis and an increased percentage of relatively immature monocytes (25, 26). Either of these would be an encouraging finding.

Lymphocyte abnormalities: Several types of morphologically or physiologically abnormal leukocytes have been observed in the peripheral blood of heavily irradiated persons. Some of these cytological aberrations show considerable promise as diagnostic and prognostic aids in the medical management of irradiated casualties. This is particularly true of certain lymphocyte anomalies which reflect the exceptional radiosensitivity of these circulating cells, as mentioned previously (p.100). The major lymphocyte aberrations of interest are: (a) nuclear "homogenization", (b) nuclear pyknosis, (c) chromosome anomalies detectable as micronuclei in blood films.
The monocyte and lymphocyte counts in peripheral blood of mice after exposure to 400 rad.
Note the prompt onset of monocytopenia. Striated bars indicate normal levels ± 2S.D.; ordinates represent cells per cubic millimeter of peripheral blood; abscissae the time in days after exposure to 400 rad (Ref. 25).
(26, 27, 28) or as abnormal karyotypes in lymphocytes induced to transform and undergo mitosis in vitro, and (d) altered reactivity to mitogenic stimuli in vitro. Other less well defined changes have also been observed following irradiation.

The sequence of microscopically observable changes that can be observed in the nuclei of irradiated lymphocytes has been studied extensively (29, 30, 31, 32). Changes may be observed after radiation doses as low as (approximately) 25 R in vivo and 2 R in vitro (30).

Whitfield and colleagues found that the incidence of peripheral blood lymphocytes showing lack of normal nuclear structure ("homogenization") after X-irradiation of rat lymphocytes was a function of radiation dose over the range of 25 R to 200 R delivered in vitro or in vivo (2). A similar dose-effect relationship was demonstrated for human lymphocytes irradiated in vitro. Since damaged cells tend to leave the circulation rapidly [the loss being attributed (2) entirely to the removal of cells by the reticuloendothelial system], blood must be drawn promptly after in vivo irradiation and incubated in tissue culture medium for several hours so that damaged cells do not elude detection and their true cumulative total may be estimated after a period of several hours.

Scaife (31) has suggested that the rate of development of nuclear pyknosis in vitro in peripheral blood lymphocytes obtained from irradiated subjects might be used as a means of estimating severity of radiation injury. In rats and rabbits, the response increased linearly with increasing radiation doses up to 100 R; sensitivity was 5 R and results were available after seven hours of culture. Longer incubation (24 hours) was required for human lymphocytes, however, and there was considerable variability among the individuals studied. Rixon, studying thymocytes of mice, found that radiosensitivity in vitro, as estimated by the development of nuclear pyknosis, varied with cell size and the nuclear/cytoplasmic ratio (32).

Lymphocytes with nuclear fragments (micronuclei representing acentric chromosome fragments) are virtually never seen in the peripheral blood unless there has been some exposure to agents noxious to hematopoiesis. Even small radiation doses may cause an occasional cell of this type to be produced. Rugh found a fairly well defined relationship between radiation dose and the incidence of such cells in rats and suggested that they might be useful in evaluating radiation exposures in man (27). The cells are distinctive but the absolute incidence in man is low, even after large exposures when there is a relative increase (26, 28).
The effect of irradiation \textit{in vitro} on the ability of human lymphocytes to undergo morphological transformation and mitosis when cultured in the presence of phytohemagglutinin has been evaluated by Block and Nachtwey (33). Although their preliminary studies are only semi-quantitative, they indicate that decreasing percentages of lymphocytes undergo morphological transformation and incorporate $^3$H thymidine after irradiation, and that the response to phytohemagglutinin decreases as the radiation dose increases (100, 200, 400, and 800 R). There is relatively little information about the response of lymphocytes to phytohemagglutinin when the cells have been irradiated \textit{in vivo}. Wald, however, was able to obtain preparations satisfactory for karyotype analysis from PHA-stimulated peripheral blood lymphocytes of heavily irradiated casualties in the recent industrial accident in Pittsburgh throughout the post-exposure period (34).

Rat thymocytes irradiated either \textit{in vivo} or \textit{in vitro} have been shown to have a reduced uptake of $^{51}$Cr when incubated with the isotope \textit{in vitro} (35). The effect (% decrease) is maximal approximately four hours after exposure, is greater with greater radiation doses over the range 50-800 rad, and is closely (positively) correlated with the percentage of pyknotic cells. Uptake of radiochrome by cells of other tissues following intraperitoneal injection is also reduced following irradiation (36). When tissue recovery commences, uptake is increased (36). Chromium binding is a property of the intact nucleated cell. Binding by nuclei and mitochondria isolated from irradiated thymocytes before exposure to $^{51}$Cr is not altered (36). The use of chromium binding as an early indication of radiation injury appears to be an interesting possibility. Although binding of Cr by red cells is not altered by radiation, leukocytes isolated from peripheral blood might be used in an \textit{in vitro} test system.

Spangler and Cassen studied electrophoretic mobility of lymphocytes and the frequency distribution histograms of lymphocyte volumes before and after \textit{x}-irradiation of rabbits with 200 R or 400 R (37). The number of larger cells was increased during the second post-exposure week. Four classes of normal lymphocytes were identified on the basis of their electrophoretic mobility. The fraction of lymphocytes with the greatest cataphoretic mobility was increased in blood drawn five minutes after exposure, but the effect was no longer significant in blood drawn 30 minutes after exposure. The authors suggest that the results support the hypothesis that one of the responses of lymphocytes to ionizing radiation is "an increase in cellular metabolism and the transformation of the lymphocyte into a functionally different cell." This concept is not necessarily incompatible with the observations.
of rapid disappearance of pyknotic and otherwise morphologically altered lymphocytes in vivo. The cells with high electrophoretic mobility might be cells that would, in tissue culture, undergo the nuclear changes described by other investigators (29, 30, 31, 38). If such cells were not irreversibly injured, however, they might leave the blood stream and undergo transformation at another site, reappearing subsequently as the larger circulating cells observed during the second post-exposure week. Such a concept is, of course, highly speculative at this stage. Nevertheless, it suggests that some of the morphological changes observed in lymphocytes maintained in vitro after irradiation in vivo deserve more critical study to determine their physiological implications. It would be unfortunate indeed if, in the search for the hypothetical quick and easy prognostic test, important clues to the functions of the lymphocyte were found but ignored.

In comparing various studies, one finds discrepancies in the percentage of lymphocytes found to be affected by a given dose of radiation, and the discrepancies appear to reflect many experimental variables such as the morphological changes or viability tests selected as criteria of effect and the culture conditions. Certain fundamental improvements in experimental methods must be incorporated into the various studies carried out on lymphocytes in vitro before data derived from such studies can be accepted as measure of radiation exposure or injury. Briefly, these are as follows:

With surprisingly few exceptions, in vitro studies of lymphocytes (including estimates of the incidence of chromosome anomalies) have been concerned with relative numbers of cells demonstrating a given effect; for example, the percentage of cells showing altered nuclear structure or the percent undergoing morphological transformation when cultured in the presence of phytohemagglutinin (PHA). Relative numbers in such studies are, at best, of limited significance and at worst may be grossly misleading. The absolute number of viable lymphocytes initially present in the experimental system and the absolute number showing the change in question when the sample is subsequently examined must be known. Obviously, if some of the cells initially present are not viable, the percent subsequently demonstrating one or another phenomenon may be virtually meaningless. Even when the number of viable cells is the experimental variable being measured, quantitative data are essential. Suppose, for example, that at the start of incubation 5% non-viable lymphocytes are found and at the end of incubation the figure has risen to 20%, 40%, and 60% following increasing doses of radiation. The question to be asked is, "20%, 40%, and 60% of what"? If some cells disappear from the culture through lysis, dissolution, adherence to the culture vessel at the time of sampling or through any other mechanism, or if they are added to the culture
through cell division, the results will not indicate the magnitude of effect occurring among the original cell population.

Simple, reliable methods for determining cell counts in tissue cultures are available and should be applied in studies of the type described (39). Appropriate control studies, also frequently omitted, should be included in the experimental protocols, and would go far towards eliminating many discrepancies among results reported. One should know, for example, precisely how many cells of each type are present in the culture, for red cells and leukocytes other than lymphocytes may profoundly affect the fate of lymphocytes in vitro. The numbers of each type of cell present should be maintained constant from culture to culture. Control studies designed to evaluate the effect of irradiated medium and irradiated "other cells" on the cell type of interest are essential in studies of effects of irradiation of lymphocytes in vitro. All of these declarations appear to be stating the obvious, but they refer to requirements that are often widely ignored. For example, there is an optimum concentration of PHA for eliciting lymphocyte transformation in vitro (40). PHA, however, is readily and irreversibly bound by erythrocytes and one might just as well not measure the PHA at all if the "quantitative" estimate of response represents lymphocytes in the presence of unknown or variable numbers of other cell types.

The need for control studies to evaluate the effect of irradiated medium on the lymphocytes (or other cells of interest) in tissue culture systems was alluded to briefly in this discussion of the need for quantitative studies. The question of indirect effects is a fundamental one that appears to be ripe for re-evaluation.

Goh and Sumner recently determined rates of occurrence of chromosome breaks in lymphocytes from normal persons, cultured (with PHA) in the presence of normal plasma and in the presence of plasma from donors who had been accidentally irradiated seven years earlier in a criticality accident (41). They found a normal, low incidence of breaks among cells cultured in the presence of autologous plasma or even plasma from a donor of different sex or differing with respect to at least one blood group antigen. In contrast, there was a distinctly increased frequency of breaks when the plasma was obtained from a previously irradiated, serologically compatible donor. The authors suggest that some substance(s) capable of causing chromosomal aberrations may be produced or activated by irradiation and that the substance(s) may persist or be produced for years after exposure.

If the findings of Goh and Sumner are confirmed, they will have important implications with respect to estimating the severity of radiation injury. The authors emphasize the need to re-evaluate
the methods and interpretation of many experimental cytogenetic studies that have ignored or rejected the possibility of indirect effects of radiation. Identification of the humoral agents responsible for the observed effects would clearly become a major area of research, and once such substances became identifiable and measurable, they could be incorporated into the diagnostic armamentarium of the medical officer responsible for the clinical management of radiation casualties. At present, however, the existence and nature of the agent(s) in question remain to be demonstrated.

Other abnormal leukocytes: Morphologically aberrant peripheral blood leukocytes other than lymphocytes have also been described following exposure to large single doses of radiation, but there are, at present, few quantitative data describing their frequency of occurrence, the degree of correlation with other measures of injury, and their reliability generally as diagnostic and prognostic aids. They are generally considered to be confirmatory evidence of exposure to large radiation doses, although even this limited interpretation of the phenomena must be made cautiously. The "miscellaneous" group includes: (a) giant (probably polyploid) forms of normal cell types (particularly giant, hypersegmented granulocytes), (b) cells with subtle alterations of nuclear structure such as small chromatin appendages, (c) immature forms of granulocytes, and (d) nucleated red cells that are rarely observed in normal peripheral blood (9, 15, 18, 22, 26, 28). As in the case of lymphocytes with nuclear fragments, most of these cells occur rarely, even when their incidence is increased. Many thousands of leukocytes must be examined if a relationship between incidence and radiation dose is to be established. This will not be feasible until rapid automatic methods for identifying and enumerating the cells become available. The feasibility of such instrumentation has been clearly demonstrated, but funds have never been allocated for it (28, 42).

C. HEMATOPOIETIC TISSUE

Although the radiosensitive stages of blood cells normally reside in the hematopoietic tissue, bone marrow studies rank below peripheral blood studies with respect to their usefulness in evaluating radiation injuries, in part, because serial blood samples may be obtained easily and frequently, with little risk to the patient, so that trends -- always important in clinical diagnosis -- can be recognized. Trends of leukocyte counts following irradiation reflect hematopoietic damage promptly because a recognizable deficiency of these short-lived cells in peripheral blood begins to develop soon after the supply is interrupted.
Bone marrow studies that have been performed on irradiated humans have provided important confirmatory evidence of disrupted hematopoiesis and, especially, data that have led to major advances in understanding how radiation affects hematopoiesis. Indeed, these research data are basic to modern concepts of the kinetics of normal blood cell production. Nevertheless, in the context of the practical problem that is the subject of this review, peripheral blood changes remain the most reliable and most sensitive hematological indication of radiation injury.

The mitotic index appears to be one of the most prognostically significant early bone marrow measurements. Radiation doses less than approximately 100 rad did not depress the mitotic index in the marrow of the less seriously injured Y-12 casualties, but doses in the range of 236-365 rad (received in the same accident) resulted in virtual absence of mitotic cells in marrow specimens aspirated four days after exposure (9, 22, 43). It has been suggested that absence of mitotic figures on the 4th post-exposure day might be taken as an indication (confirmatory evidence) that the radiation dose was ~ 236-365 rad.

Marrow cellularity in the Y-12 patients receiving 236-365 rad decreased gradually and was only slightly reduced on the third post-exposure day. The most conspicuous morphologically aberrant cell forms were giant abnormal neutrophil precursors which were present from the 2nd to the 16th post-exposure days.

A recently developed functional test for hematopoiesis (granulocytopenies) may prove useful in evaluating bone marrow reserves by way of changes in the peripheral blood leukocyte count (44, 45, 46). Etocholanolone, a steroid metabolic product, produces a predictable release of granulocytes from bone marrow stores into peripheral blood during a period of approximately 16 hours after administration. It is superior to other agents used to mobilize leukocyte reserves because of its low toxicity and the remarkable reproducibility of the response elicited (44, 45, 46, 47). If the bone marrow stores are being refilled, a surge of granulocytes will be released into the circulation in response to the injection, indicating that the marrow is actively producing granulocytes. This test might be extremely helpful to the medical officer faced with the need to select, among troops recovering from an initial radiation exposure, those who, though still leukopenic, could best return to duty in an emergency. Being able to distinguish between those whose hematopoietic recovery was early but definite and those who were not replenishing granulocyte stores, the medical officer could make the decision with far more confidence than he would otherwise.
Lymphopoietic tissue cannot be sampled readily enough to be considered a "routine" procedure even under normal conditions, and serial biopsies would involve considerably increased risk to the irradiated patient. Hence there is little reason to expect that lymph node or spleen biopsy would augment the diagnostic capabilities of the medical officer.

D. OTHER TISSUES

Although both testicular and intestinal epithelium are highly radiosensitive and a number of dose-related changes in these tissues have been studied in experimental animals, these changes can be observed only if tissue specimens are available or, in the case of the testes, by analysis of semen. Biopsy would not be advisable under the conditions prevailing under the military conditions postulated, and changes observable by analysis of semen would not be detectable during the early post-exposure period (48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58).

E. CHROMOSOME BREAKS

Chromosome breaks produced by ionizing radiation have been of great interest to those concerned with the evaluation of radiation injury as well as to geneticists. Both groups were quick to exploit methods for determining the frequency of chromosome and chromatid aberrations in metaphases of peripheral blood lymphocytes cultured in vitro when those methods became available (59).

The increase in frequency of chromosome aberrations with increasing radiation doses has been extensively documented for both plants and animals. The dose-effect relationship in human cells has been documented by studies of radiation accident casualties and patients receiving radiation therapy (60).

As Bender has pointed out, the basic requirements for human radiation exposures suitable for evaluating the relationships between radiation dose and chromosome aberration frequency are that the subject receive a single, brief exposure of the whole body to a measurable dose of external radiation (60, 61). Determination of aberration frequency should then be made using cells in their first post-irradiation division, for tissues tend to purge themselves of cells bearing chromosome aberrations.
There are several sources of uncertainty or error in estimating chromosome aberration frequencies and in using the estimates to evaluate radiation exposures. Following a given radiation dose to the whole body, for example, the aberration frequency may not be the same in all rapidly dividing tissues (60, 62, 63). As mentioned above, aberration frequency decreases fairly rapidly (through cell division) after exposure, and when cells are cultured in vitro to obtain metaphase spreads for chromosome studies, the frequency may also vary with time in culture (62, 63, 64). There are also many variables in the tissue culture techniques used in various laboratories, and these must be taken into consideration when comparing results. Reliable information about the types of radiation and the dose rate are essential (60, 61). The possible influence of agents other than radiation that are known to produce chromosome breaks must also be taken into account (65, 66, 67, 68). In short, numerical data representing the various types of chromosome lesions, like any other type of biological change in any type of disease, are significant only in the context of all other available information about the afflicted individual. Many of the comments about lymphocyte studies in vitro apply to cytogenetic studies on peripheral blood leukocytes.

F. BIOCHEMICAL STUDIES

Changes in the chemical composition of plasma and urine following irradiation have not been conspicuously helpful to the clinician in estimating the degree of injury following irradiation. Neither have the biochemical studies been fruitless, however, and the following radiation-associated changes may warrant further evaluation in this respect.

Urinary excretion of taurine, a sulfur containing amino acid, has been measured in several of the radiation accident casualties. Increased levels were found in some cases, but there is no clear relationship between clinical condition or estimated radiation doses and urinary taurine levels (69, 70, 71). The patterns of taurine excretion after irradiation are species dependent and urinary taurine levels are greatly influenced by dietary protein intake, as is amino-aciduria in general. The concentration of all free amino acids is higher in leukocytes and platelets than in plasma or red cells, an observation that has led Soupart to suggest that the increased urinary excretion of taurine after irradiation may result from release of intracellular taurine secondary to changes in cell permeability (72).
Creatininuria appears promising as a correlate of the radiation dose per gram of muscle. Creatininuria after irradiation of experimental animals (73, 74) and patients receiving radiation therapy (75) represents failure of muscle to utilize newly synthesized creatine which was synthesized at a normal rate (74). Creatininuria in radiation accident casualties has not reflected the severity of the injury particularly well. It will probably be most useful as confirmatory evidence when there is some question about how much of the body may have been shielded. Heavily irradiated patients in the Lockport accident, who were irradiated most intensively over the head and upper thorax, showed very mild creatininuria (creatine/creatinine ratios) compared to the Y-12 casualties who received much more uniform exposure. Thus, one might pair the persistence of relatively normal peripheral blood monocyte levels with relatively low creatine/creatinine ratios in the urine as indications of non-uniform exposure.

Two metabolites of nucleic acid metabolism have been found in increased amounts in the urine of some of the radiation accident casualties and patients receiving whole-body irradiation as therapy. These are beta-aminoisobutyric acid (BAIBA), a product of thymine metabolism, and deoxycytidine (DOC).

The amount of BAIBA normally excreted varies from individual to individual but is generally in the range of 100-200 micromoles per liter of urine except in "high BAIBA excretors" in whom the high excretion of the compound appears to be a hereditary characteristic not associated with overt disease (76). Urinary levels of BAIBA are markedly increased and variable in patients with malignancies (77, 78). Urinary levels of BAIBA were elevated in all five seriously injured Y-12 casualties during the first post-exposure week. Maximum urine concentrations were observed on the fourth post-exposure day and ranged from 250 to 650 μM/L (78). The relationship of urinary BAIBA concentrations to radiation exposure is much less clear in radiation therapy patients, most of whom have malignant disease (77).

After developing an analytic method for measurement of DOC, Saenger and his colleagues measured the concentration of that nucleoside in the urine of two patients before and after they received whole-body therapeutic irradiation. No DOC could be detected in the pre-exposure 24-hour urine specimens (minimum amount detectable was 0.020 mg/hr) but serial 6-hour urine specimens after irradiation showed excretion rates as high as 7.8 mg/hr in one patient and 3.4 mg/hr in the second. Urine of the patient with the highest concentration contained measurable amounts of DOC for two days; that of the other patient only during the first 24 hours after exposure (77).
If erythropoiesis is interrupted so that iron is not removed from the circulation for incorporation into hemoglobin, serum iron levels tend to rise. This can be demonstrated in experimental animals during a period of several days after irradiation (79, 80, 81). Under ideal experimental conditions, the degree of hyperferremia tends to increase with increasing radiation doses (80, 81). Serum iron levels in man, however, tend to vary considerably from one individual to another and this variability is superimposed on marked diurnal variation (82). Some way would have to be found to cope with this intrinsic variability before serum iron could be applied in the clinical evaluation of radiation injury. The rationale underlying such application makes the possibility sufficiently attractive to justify more extensive evaluation in irradiated patients. Although serum iron concentration was not measured in most of the radiation accident casualties, a rise in the concentration on the second or third days after exposure has been a rather consistent finding in a group of patients receiving therapeutic whole-body irradiation at Oak Ridge (Gould Andrews, personal communication).

The rates at which radioiron is cleared from plasma and incorporated into hemoglobin within erythrocytes also provides information about the state of erythropoiesis in hematopoietic tissues and these rates can be determined with accuracy and precision. The determinations, however, require the administration of radioactive iron which one would like to avoid if possible. Use of the short half-lived isotope, $^{52}\text{Fe}$, would minimize the radiation dose administered and would permit serial studies of iron clearance. Difficulties in obtaining this cyclotron-produced isotope would be formidable, however (83).

In studies using mice and beagle dogs, Evans has observed that the level of protein-bound carbohydrate (PBC) in the plasma has prognostic significance, higher levels of PBC in plasma are associated with higher probability of death following irradiation with monoenergetic 14 MeV neutrons or mixed neutron-$\gamma$-radiation (84). Plasma constituents rich in bound carbohydrate that are present in increased concentration after irradiation include the metal combining proteins, haptoglobins, $\beta$-glycoproteins and $\alpha_2$ macroglobulins. The striking difference with respect to PBC, in all the experiments at all radiation doses studied was between animals that die and animals that survived a given exposure.

Information presently available does not indicate what fractions of the PBC are mainly responsible for the differences described. One wonders whether this observation may be related in some way to the $\alpha$-macroglobulin fraction that has been shown to have a strong radioprotective and therapeutic effect in irradiated rodents (85). The beneficial effects in the experimental studies
were greater with greater doses of the α-globulin. Although, at first glance, the two sets of observations appear to be completely divergent, they are not necessarily so. One might speculate, for example, that the higher α-macroglobulin levels in the sera of radiosensitive animals reflects a physiological requirement for higher levels and that the levels observed after irradiation, although exceeding the levels in animals that survive, are still not adequate to meet the greater demand. Much more information about the biological significance of the various PBC constituents, including α-macroglobulins, will be required before one can do more than speculate about the physiological implications of such observations.

G. BIOPHYSICAL STUDIES

Measurement of radioactivity induced by neutrons has proved helpful in estimating the magnitude of the total radiation dose and the contribution of neutrons to the total dose when exposure is to mixed radiation (86, 87). Radioactivity induced in the exposed individual is primarily 24Na which can be detected and measured in blood, urine, or stool specimens or by whole-body counting. Clothing, metallic objects in pockets, jewelry and the like can also be analyzed for induced radioactivity.

Electron spin resonance techniques applied to bone, tail, hair, teeth, and skin of γ-irradiated rats have shown that measurable signals may be obtained following radiation doses in the lethal to supra-lethal range (88). Only bone, teeth, and skin gave reproducible results, however, and skin proved difficult to prepare for the measurements. Effects of radiation doses ranging from 80-430 rad could be detected in teeth when repetitive scans were averaged by a computer. A readily measurable signal was also obtained in preliminary studies with human fingernail clippings irradiated in vitro with 4 K rad of 60Co γ-rays. Specimens stored at -196°C retained their resonances indefinitely. Presently available data are not adequate to predict whether it will be advantageous to apply this type of analysis in evaluating the severity of radiation injury in human casualties.
V. SOME IMPORTANT EXPOSURE VARIABLES

A. DOSE PROTRACTION AND FRACTIONATION

Careful analysis of clinical and laboratory data representing the various accident casualties and the radiation therapy patients have made it possible to predict, reasonably well, the degree of injury associated with a given radiation dose so long as the radiation is delivered within (approximately) one or two days. Prognosis is less certain when the radiation is administered over longer periods, either continuously at low dose rates or intermittently in several fractions (89, 90).

The case histories of the five persons exposed over a period of several months to γ-radiation from 60Co in Mexico provide dramatic evidence that protraction and fractionation of the radiation dose may profoundly alter the response (91). Four of the five exposed persons died as a consequence of radiation injury, all demonstrating the clinical picture typical of bone marrow hypoplasia. Death was attributable to hemorrhage and infection as in the classical acute radiation syndrome, but it occurred four to six months after the beginning of exposure except in the 10 year old boy who carried the radioactive cobalt source in his pocket for approximately a week, accumulating a large part of his total dose (minimum 2,940 rems, maximum 5,165 rems) during that time. The estimated radiation doses received by the five members of the family and the survival times after the onset of exposure are shown in Table 5.

The Mexican accident illustrates the need to learn more about how the clinical and laboratory findings that precede bone marrow failure following a brief, intense radiation exposure are modified if the total radiation dose is accumulated over a much longer time. The Marshall islanders, who received most of their exposure during a period of approximately two days, reacted very much as one might have expected had the radiation been delivered in a much shorter time (13). How much more extensive must dose protraction be to result in major alteration in the time course and intensity of the anticipated clinical responses? Predictions based on extrapolation from experimental animal studies must be accepted with extreme caution, for species differ widely with respect to the effect of dose fractionation and protraction on the manifestations (including lethality) of radiation injury (90, 91, 92, 93, 94, 95, 96, 97, 98).
<table>
<thead>
<tr>
<th>Patient</th>
<th>Days of Exposure</th>
<th>Age</th>
<th>Minimum Dose Estimate</th>
<th>Maximum Dose Estimate</th>
<th>Time of Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.E.P.</td>
<td>30</td>
<td>10 yrs.</td>
<td>2940 remS</td>
<td>5165 remS</td>
<td>24 days</td>
</tr>
<tr>
<td>M.C.E.</td>
<td>115</td>
<td>27 yrs.</td>
<td>1995 remS</td>
<td>2938 remS</td>
<td>4 months</td>
</tr>
<tr>
<td>M.E.E.C.</td>
<td>99</td>
<td>2½ yrs.</td>
<td>1373 remS</td>
<td>1871 remS</td>
<td>5 months</td>
</tr>
<tr>
<td>A.I.G.</td>
<td>90</td>
<td>57 yrs.</td>
<td>1818 remS</td>
<td>2897 remS</td>
<td>7 months</td>
</tr>
<tr>
<td>J.E.I.</td>
<td>106</td>
<td>---</td>
<td>984 remS</td>
<td>1716 remS</td>
<td>survived</td>
</tr>
</tbody>
</table>
Some examples of the effect of dose rate on the lethal effectiveness of \( x \)- and \( \gamma \)-radiation for several of the larger species of experimental animals are presented in Tables 6 and 7. These tables were compiled by Page, who has used the data in analyzing the kinetics of recovery following acute radiation injury (99). Examples of dose rate and dose fractionation on response of Beagle dogs to irradiation are presented in Tables 8 and 9. Michaelson, et al., have studied the effect of these variables and of partial body exposure on lethality, survival time, and the peripheral blood picture. They found that some fractionation schedules were more effective than single exposures when selected portions of the body were irradiated, "suggesting that cyclic variations in metabolic activity of specific cell types influence the synergism, augmentation, or reduction of the response in relation to the interval between exposures." (100).

The collaborative study of irradiated patients, already mentioned (6), which includes estimates of the effects of dose protraction and fractionation greater than one day but less than eight days, demonstrates that the effective dose for the prodromal symptom complex in the patients is increased about two-fold. Hematopoietic changes appear to be influenced much less by fractionation. The estimated effects of dose protraction and fractionation are shown graphically in Figures 2, 3, 11, and 12.
Table 6

MEDIAN LETHAL DOSE (LD50) VALUES FOR LARGE ANIMALS EXPOSED TO GAMMA OR X-IRRADIATION. a

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>RADIATION SOURCE</th>
<th>DOSE RATE (R/MIN)</th>
<th>METHOD OF EXPOSURE</th>
<th>MEDIAN LETHAL DOSE (LD50)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MID-AIR DOSE (R)</td>
<td>MID-TISSUE DOSE (RADS)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BURRO</td>
<td>1000 Kvp X</td>
<td>7.0</td>
<td>Bilateral</td>
<td>376</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>.85</td>
<td>Multisource</td>
<td>784</td>
<td>280 b</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>.35</td>
<td>Multisource</td>
<td>651</td>
<td>290 b</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>.30</td>
<td>Multisource</td>
<td>585</td>
<td>350 b</td>
</tr>
<tr>
<td>CATTLE</td>
<td>Cobalt-60</td>
<td>6.6</td>
<td>Multisource</td>
<td>200</td>
<td>125 c</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>.9</td>
<td>Multisource</td>
<td>450</td>
<td>150 c</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>.9</td>
<td>Multisource</td>
<td>543</td>
<td>160</td>
</tr>
<tr>
<td>DOG</td>
<td>Cobalt-60</td>
<td>50-65</td>
<td>Bilateral</td>
<td>308</td>
<td>250 c</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>55.0</td>
<td>Bilateral</td>
<td>---</td>
<td>239 c</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>15.0</td>
<td>Bilateral</td>
<td>---</td>
<td>239 c</td>
</tr>
<tr>
<td></td>
<td>2000 Kvp X</td>
<td>15.0</td>
<td>Bilateral</td>
<td>---</td>
<td>266 c</td>
</tr>
<tr>
<td></td>
<td>2000 Kvp X</td>
<td>15.0</td>
<td>Bilateral</td>
<td>315</td>
<td>248 c</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>8-10</td>
<td>Bilateral</td>
<td>319</td>
<td>280</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>6.0</td>
<td>Bilateral</td>
<td>350</td>
<td>335</td>
</tr>
<tr>
<td>GOAT</td>
<td>2500 Kev gamma</td>
<td>32.5</td>
<td>Bilateral</td>
<td>395</td>
<td>240 c</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>7.5</td>
<td>Bilateral</td>
<td>312</td>
<td>200 c</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>1.3</td>
<td>Bilateral</td>
<td>550</td>
<td>350 c</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>800.0</td>
<td>4 Pi</td>
<td>438</td>
<td>380</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>55.0</td>
<td>Rotated</td>
<td>---</td>
<td>644 c</td>
</tr>
<tr>
<td>PRIMATES (Macaca mulatta)</td>
<td>250 Kvp X</td>
<td>22.0</td>
<td>Rotated</td>
<td>530</td>
<td>475 c</td>
</tr>
<tr>
<td></td>
<td>250 Kvp X</td>
<td>22.0</td>
<td>Rotated</td>
<td>---</td>
<td>503 c</td>
</tr>
<tr>
<td></td>
<td>250 Kvp X</td>
<td>13.7</td>
<td>Rotated</td>
<td>600</td>
<td>550 c</td>
</tr>
<tr>
<td></td>
<td>2000 Kvp X</td>
<td>10.7</td>
<td>Rotated</td>
<td>---</td>
<td>670 c</td>
</tr>
<tr>
<td></td>
<td>250 Kvp X</td>
<td>3.0</td>
<td>Rotated</td>
<td>570</td>
<td>510 c</td>
</tr>
</tbody>
</table>

(Ref. 99).
<table>
<thead>
<tr>
<th>SPECIES</th>
<th>RADIATION SOURCE</th>
<th>DOSE RATE (R/MIN)</th>
<th>METHOD OF EXPOSURE</th>
<th>MEDIAN LETHAL DOSE (LD50)</th>
<th>MEDIAN-TISSUE DOSE (RADS)</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MID-AIR DOSE (R)</td>
<td>MID-AIR DOSE (RADS)</td>
<td></td>
</tr>
<tr>
<td>SHEEP</td>
<td>Cobalt-60</td>
<td>11.0</td>
<td>Bilateral</td>
<td>237</td>
<td>145</td>
<td>Hanks (1966)</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>7.5</td>
<td>Bilateral</td>
<td>252</td>
<td>146</td>
<td>Hanks (1966)</td>
</tr>
<tr>
<td></td>
<td>250 Kvp X</td>
<td>7.5</td>
<td>Bilateral</td>
<td>389</td>
<td>245</td>
<td>Mobley (1966)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>4.35</td>
<td>Bilateral</td>
<td>318</td>
<td>194</td>
<td>Page (1968)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>0.5</td>
<td>Bilateral</td>
<td>338</td>
<td>206</td>
<td>Page (1968)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>0.3</td>
<td>Multisource</td>
<td>524</td>
<td>205&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Trum (1955)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>0.06</td>
<td>Free-moving&lt;sup&gt;e&lt;/sup&gt;</td>
<td>495</td>
<td>302</td>
<td>Page (1968)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>0.033</td>
<td>Free-moving&lt;sup&gt;e&lt;/sup&gt;</td>
<td>637</td>
<td>389</td>
<td>Page (1968)</td>
</tr>
<tr>
<td>SWINE</td>
<td>Cobalt-60</td>
<td>50.0</td>
<td>Bilateral</td>
<td>350-400</td>
<td>240&lt;sup&gt;e&lt;/sup&gt;</td>
<td>D. Brown (1968)</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>30.0</td>
<td>Bilateral</td>
<td>510</td>
<td>250&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Tullis (1952)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>18-29</td>
<td>4 Pi</td>
<td>393</td>
<td>228</td>
<td>Chambers (1964)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>18-29</td>
<td>4 Pi</td>
<td>335</td>
<td>218</td>
<td>Chambers (1964)</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>27.0</td>
<td>Bilateral</td>
<td>425</td>
<td>255</td>
<td>Bond (1951)</td>
</tr>
<tr>
<td></td>
<td>2000 Kvp X</td>
<td>15.0</td>
<td>Bilateral</td>
<td>350-400</td>
<td>230&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Tullis (1952)</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>15.0</td>
<td>Bilateral</td>
<td>510</td>
<td>250&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Tullis (1952)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>11.5</td>
<td>Bilateral</td>
<td>375</td>
<td>260</td>
<td>Page (1967)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>10.0</td>
<td>Bilateral</td>
<td>400-450</td>
<td>270</td>
<td>D. Brown (1968)</td>
</tr>
<tr>
<td></td>
<td>1000 Kvp X</td>
<td>9-10</td>
<td>Bilateral</td>
<td>399</td>
<td>270</td>
<td>Nachtway (1967)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>1.0</td>
<td>Bilateral</td>
<td>650-700</td>
<td>425&lt;sup&gt;e&lt;/sup&gt;</td>
<td>D. Brown (1968)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>0.85</td>
<td>Multisource</td>
<td>618</td>
<td>370&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Rust (1954)</td>
</tr>
<tr>
<td></td>
<td>Cobalt-60</td>
<td>0.067</td>
<td>Free-moving&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2000-2500</td>
<td>1350-1700</td>
<td>Taylor (1968)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only studies in which a relatively homogeneous depth-dose distribution was obtained are presented in this table. Those in which unilateral or dorsal-ventral exposures or low-energy radiations were utilized are not included.

<sup>b</sup> Estimated by Trum (1959).

<sup>c</sup> Estimated by Woodward (1967).

<sup>d</sup> Estimated by Bond (1957).

<sup>e</sup> Although exposed from one direction, random-movement of animals resulted in equal exposure to both sides providing an effective bilateral exposure.

<sup>f</sup> Estimated by D. Brown (1968).

<sup>*</sup> Estimate based on data presented in reference.

(Ref. 99).
### Table 7

**SURVIVAL OF DOGS AND GOATS EXPOSED CONTINUOUSLY (22-24 HRS/DAY) TO COBALT-60 RADIATION.**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>DOSE/DAY AND DOSE-RATE</th>
<th>MEAN SURVIVAL TIME (DAYS)</th>
<th>MEAN CUMULATIVE LEthal DOSE (R)</th>
<th>ESTIMATED WASTED RADIATION (DAYS)</th>
<th>ESTIMATED LD$_{50}$ MLA(R)</th>
<th>MLT(RADS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DOGS$^a$</strong></td>
<td>Single-dose @ 13 R/M</td>
<td>19</td>
<td>----</td>
<td>----</td>
<td>258</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72 R @ 0.055 R/M</td>
<td>26</td>
<td>1427*</td>
<td>13-17</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50 R @ 0.035 R/M</td>
<td>38</td>
<td>1438</td>
<td>13-17</td>
<td>900</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35 R @ 0.027 R/M</td>
<td>53</td>
<td>1414</td>
<td>12-17</td>
<td>1050</td>
<td></td>
</tr>
<tr>
<td><strong>GOATS$^b$</strong></td>
<td>Single-dose @ 1.3 R/M</td>
<td>20</td>
<td>----</td>
<td>----</td>
<td>550$^c$</td>
<td>350</td>
</tr>
<tr>
<td></td>
<td>40 R @ 0.033 R/M</td>
<td>42</td>
<td>1696</td>
<td>15-18</td>
<td>1000-1100</td>
<td>650</td>
</tr>
<tr>
<td></td>
<td>20 R @ 0.017 R/M</td>
<td>108</td>
<td>2215</td>
<td>15-18</td>
<td>1850-1915</td>
<td>1100</td>
</tr>
</tbody>
</table>

$^a$Reference - Norris (1967).


$^c$Krise (1968).

*$^*$Rads Midline Tissue Dose

(Ref. 99).
Table 8

MEDIAN LETHAL DOSE FOR 1000 kVp X-RAYS IN BEAGLES

<table>
<thead>
<tr>
<th>Exposure (single session)</th>
<th>Whole-Body</th>
<th>Lower-Body</th>
<th>Upper-Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 R/min</td>
<td>657 R+30 R</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>(RE = 1.0)</td>
<td>1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-12 R/min</td>
<td>502 R+22 R</td>
<td>878 R+9 R</td>
<td>1998 R+48 R</td>
</tr>
<tr>
<td>(RE = 1.3)</td>
<td>(RE = 2.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) mean ± standard deviation

\(^b\) relative effectiveness for lethality compared to 1 R/min

(Ref. 100)
<table>
<thead>
<tr>
<th>R</th>
<th>50-60 R/min</th>
<th>8-12 R/min</th>
<th>1 R/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>260-300</td>
<td>18.25 (11-26)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.8 (14-24)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>22.0 (15-29)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>325-340</td>
<td>14.50 (9-20)&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400-450</td>
<td>16.0 (13-22)</td>
<td></td>
<td>23.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>600</td>
<td></td>
<td>15.2 (11-28)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>29 (23-39)</td>
</tr>
<tr>
<td>750</td>
<td></td>
<td></td>
<td>15.4 (13-17)&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> mean (range)  
<sup>b</sup> 4 fractions at 24 hours  
<sup>c</sup> 4 fractions at 12 hours  
<sup>d</sup> 1 dog  
<sup>e</sup> LD 70-80  

(Ref. 100).
Fractionation of total dose over eight days increases the doses required to produce the same incidence of various prodromal symptoms (Ref. 6).
Figure 12

SINGLE VS FRACTIONATED EXPOSURES

"HEMATOLOGIC RESPONSE LEVEL"

Theoretical
Single dose response

Theoretical
Fractionated dose model
(fr = 2)

○ PROTRACTED DOSES (1.5 R/Hr)

X Single dose

● Dose fractionated within 8 days

<100 <200 <300 <400 <500 >500

DOSAGE (~RADS)

(Ref. 6).
VI. BIBLIOGRAPHY

REFERENCES CITED IN PART II

1. Cronkite, E. P.
   (Discussion) pp. 366, 376 in:
   Physical Factors and Modification of Radiation Injury, L. D. Hamilton, Consulting Editor.

   "My position is straightforward. Radiation injury is a disease due to a physical agent. Its variants can be the results of injury to any and all organ systems. The management will not be influenced or should not be by dosimetric considerations. The patient's course will inform the physician as to the appropriate therapy, and the decisions will be made at the bedside.

   "Dosimetry (depth-dose, energy, LET, etc.) is needed for scientific reasons and is essential for evaluation of late effects, but is not essential for clinical management. In fact, early estimates of air dose without adequate information on dose distribution can be frightening to all concerned." - E. P. Cronkite

2. Whitfield, J. F., Kellerer, S., Brohee, H. and Youdale, T.
   The Feasibility of a New Dosimeter for Biological Dosimetry.

   The incidence of lymphocytes showing lack of the normal nuclear structure ("nuclear homogenisation") after x-irradiation in vitro (rat and human lymphocytes) or in vivo (rats) is shown to be a dose-related phenomenon. After irradiation in vivo, damaged cells are quickly removed by the animal's reticulo-endothelial system. This change is manifest as a drop in lymphocyte count in peripheral blood. If blood is removed promptly after
exposure, however, and maintained in tissue culture medium at body temperature for several hours (so that injured and dead cells are not removed) and then examined, a cumulative total of the number of cells manifesting the nuclear injury during that period can be determined. Tabulated and graphed data are presented to demonstrate: 1. In blood drawn immediately after exposure of rats to radiation doses ranging from 25 R to 200 R and incubated for 6-7 hours, the percentage of lymphocytes with homogeneous nuclei is 70-100 times greater than in blood drawn 6-7 hours post-exposure and examined immediately. 2. The incidence of the abnormal lymphocytes after incubation of cells irradiated in vivo is increased to approximately twice the control value after a dose of 50 R. 3. The relationship between the incidence of abnormal lymphocytes and radiation dose (in vitro) increases linearly with dose between 25 R and 100 R for rat lymphocytes and between 25 R and 75-100 R for human lymphocytes. The authors emphasize that the observations described demonstrate that the lymphocyte can serve as an integrating dosimeter, providing a measure to total dose regardless of how much or what part of the body is exposed.

Atomic Bomb Injury: Radiation.

The authors describe the forms of the acute radiation syndrome observed following total-body exposure to large doses of ionizing radiation and correlate the clinical observations with approximate dose and with prognosis.

4. Thoma, G. E. Jr., and Wald, N.
The Diagnosis and Management of Accidental Radiation Injury.

The authors describe concisely the seven major criticality accidents that had occurred at the time of writing and present a classification of five degrees of radiation injury based on the clinical and laboratory findings in the various
radiation casualties in those accidents, in casualties of atomic bombing and in patients receiving radiation therapy. Hypothetical cases are described to illustrate the various injury groups. A profile scoring method is then developed, based on 1. time of onset and duration of initial clinical symptoms, 2. time of onset and duration of clinical signs and symptoms in the "manifest illness stage", and 3. abnormal hematological changes expressed as increases above and decreases below the "universal mean" (as defined in Standard Values in Blood, Part 1 of ATIBS - NRC Handbook of Biological Data, W. B. Saunders Co., Philadelphia, 1952, E. C. Albritten, Ed.). Daily logging of the integral and cumulative profile scores provides a valuable guide to diagnosis and prognosis in individual cases. Examples of the use of the profile scoring system are presented and discussed with emphasis on the reassuring observation that, "... despite the disturbing physical complexity of nuclear criticality accidents, the key procedures for clinical evaluation of resultant injury are such familiar ones as a detailed medical history, thorough physical examinations and accurate blood counts."

5. Gerstner, H. B. 
Reaction to Short-Term Radiation in Man. 

The author presents a summary and analysis of the literature (published through April 1959) pertinent to the reaction of humans to doses of ionizing radiation just great enough to induce clinical signs and symptoms or to larger doses causing severe acute injury or death. The prodromal reaction is classified as mild, moderate or severe and the classifications are shown to be related to radiation dose, increasing in severity with increasing dose. Absence of prodromal symptoms among an exposed population is considered to indicate an air dose of less than 100 R and presence of prodromal effects is taken as indicating an air dose in excess of 100 R. A graph is presented to summarize the author's estimate of "dose-incidence-time" pattern. This depicts the major characteristics of the
prodromal reaction, namely delay followed by sudden onset of symptoms, maximum incidence between 5 and 8 hours after exposure (regardless of dose) followed by rapid decrease of symptoms. A particularly interesting analysis represents the time of onset of prodromal symptoms in 97 persons who were irradiated either accidentally or therapeutically. The 97 cases include only cases in which the time of onset of symptoms is known with a high degree of accuracy. When plotted as a frequency distribution histogram, the data demonstrate 1. that the time of onset of prodromal symptoms is between 1 and 4 1/2 hours after exposure, 2. time of onset is influenced very little by the percentage of the body exposed, 3. is independent of the type of radiation and 4. is independent of total dose over a wide range of doses. Data are cited to show that the rate of occurrence of prodromal symptoms decreases with increasing age; that the symptoms are higher in women than in men.


Clinical records of 1085 patients from 33 hospitals have been analyzed to determine the relationship between radiation dose and certain clinical and laboratory observations. This summary of the extensive analysis is concerned especially with the effect of dose rate and fractionation of radiation dose on the responses studied. The relationship between the various responses and radiation dose were analyzed by probit regression analysis assuming 1. normal and 2. log-normal distributions of population sensitivity. The analysis shows that man responds like most large animals; his LD_{50} is of the order of 300 rads and he can repair gastrointestinal damage produced at low dose rates even when exposure is fractionated only over a week. No evidence was found for repair of hematopoietic damage during the same period.

This report, compiled by the Scientific Committee on the Effects of Atomic Radiation (U. N.), reviews the knowledge current at the time of publication (1962) about effects of radiation on man and his environment. Descriptions of some major radiation accidents are included in the section on Acute Radiation Injury in Man.

8. Schambra, P. E., Stapleton, G. E. and Barr, N. F., Editors
   Space Radiation Biology.

This supplement contains a group of papers presented at a workshop conference held at the University of California, Berkeley, Sept. 7-10, 1965. It includes sections on cellular effects, radiation effects on mammalian systems and evaluations of radiation effects on man, as well as sections on other aspects of space radiation biology.

   Criticality Accident at the Y-12 Plant.

Clinical and laboratory observations made on five men exposed to mixed neutron-gamma radiation in doses ranging from 236 to 365 rad in a criticality accident at Oak Ridge, Tenn. in 1958 are presented. Three of the five men had nausea and vomiting within a few hours after exposure, a fourth slightly later, and the fifth had mild nausea without vomiting on the second post-exposure day. Initial nausea and vomiting subsided the day after the accident in the casualties who first developed them, but recurred on the second day. The composite hematological picture for the five men and individual leukocyte counts, presented graphically, demonstrate the changes that have come to be considered
typical of the exposures in the LD$_{50}$ range, including an initial leukocytosis and early fluctuation in the leukocyte count, followed by a declining count until the ninth day, an "abortive rise" between the ninth and 16th days, then a further decline which was very rapid after the 22nd day. Minimal leukocyte counts were observed between the 29th and 36th days (minimum values on the 29th and 30th day in the two most seriously injured men). An upwards trend of the leukocyte count was then observed. The lymphocyte count had reached essentially minimal values within 48 hours in all five men, and remained depressed thereafter, showing very gradual recovery after the 30th day. Platelet counts which were somewhat variable during the first 15 days began to decrease thereafter, reaching lowest levels 25-35 days after exposure. During the period when platelet counts were minimal, some signs of hemorrhagic tendency were observed clinically. Lowest platelet values were observed in the two men who received the highest radiation dose. The anticipated gradual decline in red cell and hemoglobin values was observed; minimal values observed between the 35th and 45th days. Reticulocytes were decreased slightly during the first week, showed an abortive rise between the 13th and 25th days and a definite increase between the 30th and 45th days. Morphological changes observed in peripheral blood films as well as observations on bone marrow aspirates obtained serially are described, as are biochemical studies on blood and urine. The various observations are compared with those representing casualties of the Yugoslav criticality accident, particularly the four men who received bone marrow transplants. It is concluded that these four men were probably more seriously injured than were the Y-12 casualties, that bone marrow transplants may have "hastened recovery somewhat" but that the main evidence that the grafts took is the demonstration of donor type erythrocytes in the recipients.

This report summarizes the work of a committee convened to define, in light of present knowledge, the best plan for medical management of large numbers of radiation casualties under austere medical conditions and to evaluate specific courses of research which would improve our ability to cope with civil defense aspects of radiation sickness. Radiation injury is viewed as only one of a number of noxious agents which might be expected to act cumulatively or synergistically under the shelter conditions postulated. The medical significance of pre-existing disease and disability in the shelter population and the medical effects of confining large randomly selected groups of persons under the austere conditions postulated are emphasized throughout. Measures most likely to influence ultimate radiation-related mortality include management of infection and of hemorrhagic manifestations of radiation injury, and management of surgical problems. Presently available chemical "protective agents" are mentioned but their effectiveness in preventing radiation mortality is considered to be doubtful under shelter conditions postulated. Space and water are the most important requirements for survival under shelter conditions, and present allocations should be revised. Recommendations for specific research are made but these recommendations would be subject to revision, depending upon the outcome of the recommendations with respect to operations.
11. Saenger, E. L., Editor
Medical Aspects of Radiation Accidents, A
Handbook for Physicians, Health Physicists
and Industrial Hygienists.

This handbook was prepared as a guide for medical
and health physics personnel in the event of a
peacetime radiation accident. It includes
sections on estimation of doses, clinical features
of the acute radiation syndrome and therapy of
radiation injury.

12. National Committee on Radiation Protection and
Measurements Report No. 29, Exposure to Radiation
in an Emergency (1962).
Available from Section of Nuclear Medicine,
Dept. of Pharmacology, the University of Chicago,
Chicago 37, Illinois.

A committee of internationally recognized
authorities from the United States, under the
chairmanship of Lauriston S. Taylor, reviews the
problem of "possible exposures greatly in excess
of those encountered in normal radiation work
and, in the case of nuclear war, with the cer-
tainty that radioactive fallout will kill many of
those in the areas involved." Various physical
considerations related to estimates of (and con-
trol of) exposure are considered. The clinical
features of radiation injury are described
according to both severity of injury and prognosis.
The dividing line between doses that will and will
not cause sickness that requires medical care is
chosen, arbitrarily, as 200 r. A short section
reviews effects on livestock and agriculture and
three appendices are included, one on definitions
of radiation quantities and units, a second on
equivalent residual dose (which is concerned with
recovery processes that are active during the
period between a first and a subsequent exposure)
and a third appendix on empirical relationships
between contamination and skin dose.
13. Cronkite, E. P., Bond, V. P. and Dunham, C. L., Editors
Some Effects of Ionizing Radiation on Human Beings.
United States Atomic Energy Commission, U. S.

This report describes the accidental exposure of
Marshall Islanders to radiation from fallout from
an experimental thermo-nuclear device exploded at
the U. S. Atomic Energy Commission's Einiwetok
Proving Grounds in the Marshall Islands. Radiation
doses from gamma rays originating externally for
four groups of casualties on separate islands were
260, 100, 120 and 20 R. Clinical and laboratory
observations and treatment are described and dis-
cussed in the context of acute radiation injury
as it has been observed in other clinical and
laboratory studies. This unique accident rep-
resents an important source of information about the
response of man to acute radiation injury and
specifically to γ-radiation delivered at the
changing dose rates that are typical of a radio-
active fallout source. Islanders were transported
and resettled on other islands outside the fallout
area as soon as possible after their exposure
became known. Total period of exposure to external
γ-radiation from fallout was about two days.

14. Wald, N., Thoma, Jr., G. and Broun, Jr., G.
Hematological Manifestations of Radiation Exposure
in Man.
In: Progress in Hematology, Vol. III, pp 1-62,
Leandro M. Tacantins, editor, Grune & Stratton, New

Both prompt and delayed hematological changes
resulting from radiation doses delivered over
short or long periods are reviewed. Effects of
internally administered radioactive isotopes are
also considered briefly. The profile scoring
system for estimating degree of radiation injury
and prognosis of the clinical course in radiation
accident casualties is reviewed. This system
recognizes the importance of evaluating a number of
the most predictable hematological changes plus
the major prodromal symptoms when evaluating
radiation injury.

The major radiation accidents are described, along with the therapeutic use of whole-body irradiation and tissue transplantation in man. Treatment and problems for future study are emphasized. The discussion following the various sections is well and fully reported and is a particularly valuable part of the publication. These accidents all represent radiation injury of civilians and as such are valuable source material to physicians, health physicists and others concerned with the management of accidental acute radiation injury, as well as to those interested in military medicine.


The clinical and laboratory findings in the first two civilian radiation accident casualties (criticality accident) at Los Alamos in 1945 and 1946 are summarized as a basis for discussing the management of such casualties generally. Laboratory findings, particularly creatine excretion, which might be useful in assessing the degree of injury in other accidents are discussed.


A radiation accident is described in which nine civilian employees were exposed accidently to x-rays from an unshielded klystron tube at a military installation in Lockport, N. Y. The exposures were non-uniform, the most seriously injured casualties (four) receiving most of the
x-ray dose to the head and upper thorax. The nature of the injury that caused the various symptoms in the men was not immediately understood and, in fact, it was only on the day after the exposures that the nature of the injury became apparent. Clinical and laboratory findings, especially in the most seriously injured man, are described. The large x-ray dose to the head of this man resulted in a series of manifestations that included signs of central nervous system pathology. All patients survived.


This monograph describes in detail the physiological effects of irradiation of the whole body of mammals. The observations on irradiated humans and the extensive data on animals are analyzed to show that most of the lethal consequences of irradiation of the entire body are mediated through disturbances of cellular proliferation. Methods of treatment that significantly alter the course of radiation injury are also reviewed.


The criticality accident that occurred in Oak Ridge, Tennessee in June 1958 is used as the basis of this discussion of the relationship between the estimated radiation doses received and radiation injury incurred by the casualties. Physical dosimetry was centrally important to the decisions about clinical management of the patients in this accident, as in other radiation accidents, but the clinicians relied most heavily on biological manifestations of injury as early indices of damage actually incurred by the casualties. Typical hematological responses to be expected after uniform, total body exposure to
radiation doses of 100, 200, 300 and 450 rads are presented graphically and data representing not only the Oak Ridge accident (Y-12) but also other major accidents are presented to show that of the various hematological data, the absolute lymphocyte count appears to be most reliable as an early indicator of degree of injury sustained. A set of five graphs showing trends of the absolute lymphocyte counts observed in 17 casualties, grouped according to the severity of injury, is particularly convincing in this respect. A schematic graph, derived from these data, is presented to summarize the relationships between absolute lymphocyte count (during the first two days after exposure) and degree of injury. A bar graph showing approximate levels of radiation causing anorexia, nausea and vomiting following exposures to (approximately) 50 R, 100 R, 300 R, 400 R and 500 R is also included. The authors also stress the importance of determining sodium and sulfur activation (in blood and hair respectively) where there is a significant neutron component to the dose.


This review of accidental acute radiation injury is written primarily for the general physician who may find himself responsible for medical management of casualties in civilian radiation accidents. The major patterns of response to various radiation doses are described (for man) and a diagram is presented to illustrate patterns of survival after various doses. A set of four graphs illustrate the characteristic pattern of hematological changes following single doses of 100, 200, 300 and 450 rads. The authors emphasize the importance of the absolute lymphocyte and neutrophil counts as early indicators of the degree of radiation injury. The diagnosis and clinical management of radiation accident casualties are also discussed.
21. Cronkite, E. P.
The Diagnosis, Prognosis and Treatment of Radiation Injuries Produced by Atomic Bombs.
Radiology 56: 661 (1951).

The author reviews data from studies using experimental animals to observe the effects of lethal total body x-irradiation. Data representing studies of mice, dogs and goats are presented, special consideration being given to hematological changes.

22. Killman, S. A., Cronkite, E. P., Bond, V. P. and Fliedner, T. M.
Acute Radiation Effects in Man Revealed by Unexpected Exposures.

The relative diagnostic and prognostic significance of various changes that have been measured in radiation accident casualties are discussed and illustrated. These include excretion of \( \beta \)-aminoisobutyric acid, mitotic index of bone marrow, the number of DNA synthesizing cells in peripheral blood, and various other changes in the peripheral blood picture. Therapy of the hematopoietic syndrome is reviewed with particular emphasis on the importance of good general medical care and close supervision, large doses of antibiotics if signs of infection are present, and fresh blood in fresh platelet transfusions for hemorrhage secondary to thrombocytopenia.

23. Patt, H. M. and Maloney, Mary A.
A Comparison of Radiation-Induced Granulocytopenia in Several Mammalian Species.

After a radiation dose sufficiently large to bring granulocyte production to a near standstill, (500-600 R), granulocytes were found to disappear from the blood exponentially with a half-time of 10-15 hours in mice, rats, hamsters, rabbits, dogs, and monkeys. A latent period before the onset of
granulocytopenia was observed in all species in contrast to the immediate onset of lymphocytopenia. The length of the latent period varied among the various species and the authors suggest that it may reflect species differences in the non-mitotic differentiating marrow pool.

24. Adelstein, S. J. and Dealy, J. B.
Hematologic Responses to Human Whole Body Irradiation.

Hematologic data from 7 patients exposed to whole body x-irradiation are analyzed and compared with observations reported by other investigators. Three patients (the "index" group) received a first x-ray dose of 250 R followed by a second exposure to 200 R seven days later. Other patients received lower doses. In the index group, lymphocyte, reticulocyte, granulocyte and platelet levels fell progressively, with half reduction times of 4, 6, 10 and 16 days, respectively. The exponential disappearance of granulocytes was prompt in onset after exposure. The half reduction times showed considerable variability among individuals but when the analysis was limited to initial disappearance rates (first 10-12 days), the variability was much less. The authors feel that, "This suggests that the initial disappearance rate is a reflection of the first radiation insult." Response in patients receiving fractionated doses was somewhat unusual in that there was a seven-day lag before the second dose produced deviation from the initial disappearance rate.

25. Volkman, A. and Gowans, J. L.
The Origin of Macrophages from Bone Marrow in The Rat.

As part of a series of studies designed to identify the origin of tissue macrophages the authors studied the emigration of macrophages to glass coverslips on abraded skin (skin windows) or placed subcutaneously. Macrophage labeling by
tritiated precursors of DNA and RNA and their accumulation on coverslips was not abolished by whole body exposure to an x-ray dose of 400 rad (sublethal) even though the peripheral blood lymphocyte count fell to less than 10% of the normal value. After 750 rad of x-ray, a dose in the lethal range, only a few macrophages were found on the coverslips. Shielding the marrow of one limb during irradiation with 750 rad, however, or transplantation of cell suspensions prepared from syngeneic marrow or spleen (but not cells from thoracic duct lymph, lymph nodes or thymus) after such exposure enabled the irradiated rat to produce and mobilize normal numbers of macrophages. Evidence supporting the hypothesis that the circulating monocytes (derived from precursors in the marrow) become the tissue macrophages observed on the coverglasses is summarized.

26. Ingram, M.
Morphologically Aberrant Blood Cells in Hanford Accident Casualties.

The incidence of certain rarely occurring blood cells and of subtle morphological aberrations in more commonly occurring types of cells in three men exposed to mixed gamma and neutron radiation in a critical excursion accident was determined and compared with the incidence of similarly aberrant cells in the Y-12 and Lockport accident casualties. Damage to hematopoietic tissue in the Hanford accident casualties was not severe enough to cause leukopenia. The types of morphological aberrations observed include 1. changes in nuclear structure of the granulocyte series, 2. mild, transient monocytosis and increased numbers of immature monocytes, 3. binucleate lymphocytes and lymphocytes with nuclear fragments, 4. increased numbers of plasmacytoid mononuclear cells, 5. occurrence of nucleated erythrocytes in peripheral blood, and 6. chromatin and chromosome abnormalities in nucleated
erythrocytes of the marrow. The magnitude of the changes observed is seen to correspond most closely with changes observed in the low-dose Lockport casualties.

27. Rugh, R.
An Anomalous Lymphocyte: Possibly Diagnostic for Exposure to Ionizing Radiation or Radiomimetic Agents.

Cytoplasmic inclusions (nuclear satellites) were found in lymphocytes in peripheral blood of mice during a 10-week observation period following whole body exposure to 100-500 R doses of x-rays. The cells were first seen on the first post-exposure day and were most numerous two to four weeks after exposure. The rate of occurrence in peripheral blood lymphocytes ranged from 0.15 to 1.16%. In lymph node lymphocytes the incidence was even higher. The incidence was greater with greater radiation doses over the range studied.

28. Ingram, M. and Preston, Jr., K.
Importance of Automatic Pattern Recognition Techniques in the Early Detection of Altered Hematopoiesis.

There is a vast amount of information about subtly altered hematopoiesis that is present even in routinely prepared films of peripheral blood. Much of this information cannot be utilized at present because of the practical limitations imposed by classical microscopy, namely the need to examine thousands of cells in order to obtain statistically significant data about rates of occurrence of the various unusual cell types. Automatic pattern-recognition techniques suitable for identifying blood cells by rapid topographical analysis may be expected to make such information useful and such techniques have already been shown to be feasible. Methods for evaluating, quantitatively, the occurrence and persistence of unusual cells and fluctuations in their rates of occurrence would make it possible to study large groups of persons.
repeatedly, if necessary over periods of many years after exposure to potentially hematotoxic agents, and to determine whether some abnormal cell lines are premonitory of serious disorders in blood cell proliferation. Such methods would also improve differential leukocyte counting, making it possible to classify routinely large numbers of cells, often according to many morphological criteria not presently exploited.


Fragments of lumbar, sacral and iliac nodes of 4-week-old rats were cultured on semi-solid culture medium or on a fine tantalum wire grid just under the surface of liquid medium. The health of the cultures was assessed by counting the percentage of pyknotic lymphocytes in stained films or sections at the end of the experiments. Various aspects of the culture environment that influence survival of the tissue and lymphopoiesis are mentioned in this report and the process of lymphopoiesis is described in terms of the histological and cytological observations. The radiosensitivity of small lymphocytes is emphasized, particularly observations comparing radiosensitivity in vivo and in vitro. The importance of environmental conditions on radiosensitivity is also stressed. Some of the major observations on radiosensitivity reported may be summarized as follows:

Radiosensitivity of Small Lymphocytes

<table>
<thead>
<tr>
<th>Source of Lymphocytes</th>
<th>Mode of Exposure</th>
<th>LD 50/5 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph nodes</td>
<td>in vivo</td>
<td>150 R</td>
</tr>
<tr>
<td>Lymph node fragments</td>
<td>in vitro</td>
<td>275 R</td>
</tr>
<tr>
<td>Lymph node fragments</td>
<td>in vitro under 0 oxygen tension during irradiation</td>
<td>3400 R</td>
</tr>
<tr>
<td>Whole blood</td>
<td>in vitro</td>
<td>1600 R</td>
</tr>
<tr>
<td>Scattered lymphocytes</td>
<td>in vivo</td>
<td>2250 R</td>
</tr>
<tr>
<td>in intestinal villi</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
30. Schrek, R.
Radiation Effects on Lymphocytes.

The author's opening statement is, "The lymphocyte is a unique cell. It is the only nonmitotic cell that is sensitive to small doses of x-rays."
Morphological changes in lymphocytes irradiated in vitro with 1000 R and observed by means of time-lapse cinematography are described and illustrated. The non-irradiated and irradiated lymphocytes undergo the same series of morphological changes before death but the irradiated cells die earlier. Longevity or 10% survival time of human lymphocytes in vitro was found to be 9.2 days, but after exposure in vitro to 1000 R, was shortened to 1.7 days. A dose as low as 5 R significantly decreased the survival time.

The changes observed in peripheral blood and stained sections of lymphoid tissues examined serially after whole body exposure to various radiation doses are described and illustrated with data representing human patients and experimental animals. Certain species differences with respect to patterns of response are pointed out.

31. Scaife, J. F.
Nuclear Pyknosis and the Development of Radiation Damage in Peripheral Lymphocytes.
EUR-3939e, European Atomic Energy Community, Ispra, Italy, Joint Nuclear Research Center (1968).

An evaluation of a system of "biological dosimetry" based upon the development of nuclear pyknosis in peripheral lymphocytes in vitro, showed the system to be reproducible in rats and rabbits with a linear response up to 100 R, and a sensitivity of 5 R with results available after 7 hours. With human lymphocytes, 24 hours were required to produce an equivalent response which varied from individual to individual and hence can not be established as an overall invariable method of dosimetry. Rabbit lymphocytes labelled with $^{3}H$-cytidine were
found to disappear from the peripheral circulation very rapidly after reinfusion into the same animal. They were essentially all gone after 60 to 90 min. and no difference in the rate of elimination could be discerned for normal or irradiated (200 R) lymphocytes. (Author)

32. Rixon, R. H.
The Effect of Radiation on the Survival In Vitro of Rat Thymocytes of Different Size.

Thymocytes from 4-5 week old male mice were x-irradiated in vitro with single x-ray doses ranging from 10 R to 1500 R. The number of normal and pyknotic cells was determined by examining wet films of the cell suspensions under the phase microscope five hours after irradiation. The relation between cell survival and cell size was linear for x-ray doses from 300 R to 1500 R and the percentage survival (relative to that in the control) was inversely proportional to cell diameter, although the nuclear:cytoplasmic ratio was the same for all cell sizes. The relationship between cell survival and x-ray dose was non-linear for doses less than 300 R. The increased radiosensitivity of larger cells (> 7.9 μ in diameter) is considered to be a reflection of the greater metabolic activity in larger cells, the dose-survival curves being defined by 1. the fraction of cells lethally damaged by increasing doses, and 2. the rate at which lethally irradiated cells lose their nuclear structure.

33. Block, P. C. and Nachtwey, D. S.
Effect of x-irradiation on the Response of Human Peripheral Lymphocytes to Phytohemagglutinin.
U. S. Naval Radiological Defense Laboratory, San Francisco, California

Human peripheral blood lymphocytes (from one donor) were exposed in vitro to an x-ray dose of 100, 200, 400 or 800 R after which phytohemagglutinin (PHA) was added to the irradiated cultures which were then returned to the incubator. Twenty hours after exposure, tracer amounts of 3H thymidine were
added to the cultures. They were incubated again until they were harvested 44, 48, 68 or 72 hours after irradiation. Stained smears were examined to estimate the percentage of cells undergoing morphological transformation and autoradiographs were prepared so that the percentage of cells incorporating the label could be determined. Radiation doses greater than 100 R decreased the percentage of cells responding to PHA and a plot of labelled cells vs. radiation dose at 44 and 48 hours after irradiation was consistent with an exponential survival curve with a "shoulder" extending to 100 R.


In a recent Van de Graaff accelerator accident, three individuals were exposed to 520, 520 and 110 R, respectively, of x-rays ranging in energy from 300-800 KeV. Mean midline doses were estimated at 600 and 300 rads in the first two patients. In view of the gravity of the anticipated injury, advantage was taken of the fact that the first patient had an identical twin. Bone marrow from the latter was transfused into the patient on the eighth post-exposure day. Hematologic improvement began on the 20th day post-exposure in this patient and about the 30th day in the other two men. Cytogenetic observations were made in all three patients on peripheral blood samples daily and then bi-weekly. There was an initial high incidence of cells with chromosome aberrations. This fell somewhat but persisted at a distinctly abnormal level for at least three months post-exposure in the first two patients. The significance of these results will be discussed. (Authors)
Scaife, J. F. and Vittorio, P. V.
The Use of Chromium-51 as a Sensitive Quantitative Criterion of Early Radiation Damage to Rat Thymocytes.

Rat thymocytes were incubated in medium with $^{51}$Cr (as sodium chromate) at 37°C for 30 min. The reaction was stopped by adding sodium ascorbate, chilling the suspension in ice, spinning down, and washing with suspending medium. The packed cells were counted in deep-well type scintillation counter. "The uptake of $^{51}$Cr by thymocytes was found to be directly proportional to the time of incubation up to 30 min., the number of cells, and to the amount of isotope present." It was also observed that various inhibitors and the composition of the medium could influence the uptake of $^{51}$Cr.

Thymocytes from unirradiated animals were used as normal controls. Thymocytes from previously irradiated animals and thymocytes irradiated in vitro were shown to have a reduced $^{51}$Cr uptake 4 hours after exposure. Dose dependency and comparison with development of pyknosis in cells exposed in vivo are shown in the following table:

<table>
<thead>
<tr>
<th>Dose (rads)</th>
<th>50</th>
<th>110</th>
<th>165</th>
<th>400</th>
<th>800</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{51}$Cr uptake (% reduction)</td>
<td>7</td>
<td>11</td>
<td>15.5</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>Pyknosis (% total cells)</td>
<td>5</td>
<td>9</td>
<td>13</td>
<td>24</td>
<td>41</td>
</tr>
</tbody>
</table>

"Doses as low as 25 rads in vitro consistently produced a significant depression of the $^{51}$Cr uptake after 4 hours incubation at 37°C."

The following table shows the development of $^{51}$Cr uptake, viable staining, and pyknosis with time after 800 rads of x-irradiation:
<table>
<thead>
<tr>
<th>In vitro</th>
<th>1 hr.</th>
<th>2 hr.</th>
<th>3 hr.</th>
<th>4 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>(3)</td>
<td>(6)</td>
<td>(10)</td>
<td>(13)</td>
</tr>
<tr>
<td>$^{51}$Cr uptake (% reduction)</td>
<td>10</td>
<td>14</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Staining (% control cells)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In vivo</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{51}$Cr uptake (% reduction)</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>Pyknosis (% total cells)</td>
<td>4</td>
<td>15</td>
<td>25</td>
<td>41</td>
</tr>
<tr>
<td>Staining (% control cells)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figures in () indicate reduction in $^{51}$Cr uptake of non-irradiated cells after 4 hr. incubation. Other figures are changes compared to non-irradiated thymocytes."

The depression in $^{51}$Cr uptake following irradiation is apparently a phenomenon of the intact cell, as neither mitochondria nor nuclei alone showed any difference in $^{51}$Cr uptake following irradiation. Rat red cells and mouse ascites cells showed no change in $^{51}$Cr uptake 8 hrs. after 800 rads, and 4 hrs. after 1000 rads, respectively, a finding that was interpreted as indicating that thymocytes are more radiosensitive than either of the other two cell types.

The protective effects of anoxia (protects up to 150 rads) and various protective agents (no protective effect) were also evaluated by the $^{51}$Cr uptake method.

36. Vittorio, P. V. and Dziubalo, S.
A Study of the Effects of X-irradiation and Protective Agents on Mice Using $^{51}$Cr.

It has been shown previously that radiochromate when injected intraperitoneally is rapidly incorporated into the major organs with a consistent distribution pattern. Furthermore, rapidly proliferating cells appear to incorporate more chromium than do older cells. Increased $^{51}$Cr uptake has been seen following whole body x-irradiation. Therefore in this study, the
$^{51}$Cr content of various organs after intraperitoneal injection of $^{51}$Cr was used as an index of repair in radiation-damaged mice. Groups of male Swiss mice were exposed to 100, 300 or 600 R of x-rays. At various times (from 1 to 29 days) after exposure, the mice were injected with 1 Ci $^{51}$Cr (as sodium chromate) per gram body weight. Twenty-four hours later, they were anesthetized with Nembutal; each abdomen was opened, blood was removed by cardiac puncture, and the liver, spleen, thymus, kidney, testis, lung, heart and duodenum were removed and weighed. Total $^{51}$Cr content of each organ was determined by radioactivity counting in a well-type scintillation detector. In a second experiment, some of the mice received (pre-exposure) intraperitoneal injections of one or both of the radioprotective agents, S-2-aminoethylisothiouradihydrobromide (AET) and serotonin creatinine sulphate (5HT), in order to test the protective effect of those compounds. $^{51}$Cr uptake values are normalized with respect to uptake by unirradiated controls. While $^{51}$Cr uptake of the irradiated mice fluctuated below 100% at times, the maximum changes with respect to the controls were all increases. In the liver, spleen and thymus, peaks were noted on days 1 and/or 3 following exposure to 100 or 300 R. The authors state that these increases "may be due to effects on membrane permeability and disturbances in water balance, and hence may be more of an indication of early damage rather than early repair."


Following exposure to a dose of 200 R of $^{60}$Co gamma radiation, the absolute lymphocyte count of rabbits fell to 25% of its pre-exposure value in 3.6 hours and reached minimal levels (20% of normal) in 24 hours. The frequency distribution histograms of lymphocyte volumes was also altered by the exposure. The relative number of small lymphocytes (7-9 μ diameter) was increased transiently on the second post-exposure day, the relative number of cells with diameters 9 μ-10 μ
was increased between the seventh and ninth days, and the relative number of large lymphocytes (10 \( \mu \) to 14.5 \( \mu \) diameters) was increased on the fifteenth day. Electron micrographic characteristics of the cells at various times after exposure are described and illustrated.

Electrophoretic mobility of lymphocytes (in whole blood diluted with Hank's solution) was estimated for cells obtained five, fifteen and thirty minutes after exposure of rabbits to 200 R. Samples were compared with respect to the number of cells (proportions) obtained in each of four classes that were distinguishable by their relative electrophoretic velocities. The fraction of lymphocytes with greatest electrophoretic mobility (greatest net negative charge) was increased five minutes after exposure. The magnitude of this effect decreased rapidly and was insignificant in the sample of lymphocytes obtained thirty minutes or longer after exposure. In vitro irradiation of blood with 200 R also increased the relative number of lymphocytes with high electrophoretic mobility, and 400 R increased this fraction even more.

The various observations reported are interpreted in light of known and postulated functions of lymphocytes; particularly their transforming capabilities. The authors emphasize that the observations "correlate well with the hypothesis that one of the responses of lymphocytes to low doses of ionizing radiation is an increase in cellular metabolism and the transformation of the lymphocyte into a functionally different cell."

38. Cassen, B. and Gutefreund, W.
Irradiation of Lymphocytes in Freshly Drawn Blood.

Whole blood specimens from normal rabbits were exposed in vitro to 1000 R of 250 kV x-rays and lymphocyte concentrates were prepared immediately thereafter. The lymphocytes were examined in wet preparations by phase microscopy and motility and morphological changes were used to evaluate the viability of the cells. The lymphocytes showed typical ameboid motion as long as six hours
after exposure, and cell counts showed that cells were not lost by lysis or other means during irradiation. The observations "are offered against hypotheses that explain the rapid decrease of lymphocyte count after total body irradiation on the basis of a rapid lethal effect of the radiation on mature lymphocytes. It is not ruled out that they may be sublethally affected."

39. Stewart, C. C. and Ingram, M.
A Method for Counting Phytohemagglutinin-stimulated Lymphocytes.

A technic is described for accurately enumerating the number of viable nucleated cells in phytohemagglutinin-stimulated lymphocyte cultures. The method utilizes the proteolytic enzyme, pronase, to digest nonviable cells and debris in a culture and the cytolytic agent, cetrimide, to strip off cell cytoplasm, leaving a clean suspension of unclumped, individual cell nuclei for counting and sizing. The method was applied to determine changes in the absolute number of cells surviving and the number incorporating tritiated thymidine over a 72 hour culturing interval. Serially determined absolute lymphocyte counts in cultures with phytohemagglutinin demonstrate a net gain in both cellularity and number of cells incorporating labeled thymidine, and the growth appears to be logarithmic throughout the 72 hour observation period.

40. Stewart, C. C., Nakeff, A. and Ingram, M.
Kinetics of Erythrocyte and Lymphocyte Agglutination by PHA.

Using a quantitative agglutination technique, the kinetics of erythrocyte and lymphocyte agglutination by purified Phaseolus vulgaris extracts have been determined. It was found that lymphocytes and erythrocytes are agglutinated in the same manner and that a measurable loss of lymphocyte agglutinating activity occurred when PHA was adsorbed onto erythrocytes.

- 155 -
41. Goh, K. and Sumner, H.  
Breaks in Human Chromosomes: Are They Induced by a Transferable Substance in the Plasma of Persons Exposed to Total Body Irradiation?  

The experiments described were carried out to test the hypothesis that some substance(s) capable of causing chromosomal aberrations is produced or activated by whole body exposure to ionizing radiation, and that it may be produced, or persist for very long periods (years) after exposure. Peripheral blood leukocytes from normal persons were cultured (in the presence of phytohemagglutinin) with 1. autologous plasma, 2. plasma from another individual who had at least one different red cell antigen (ABO system) and, in four instances, was also of opposite sex, or 3. plasma of one of six men surviving seven years after whole body exposure to mixed fast neutrons and gamma rays (criticality accident, see reference #9).

Results of karyotype analysis were as follows:

<table>
<thead>
<tr>
<th>Cells</th>
<th>Plasma</th>
<th>No. Metaphases Scored</th>
<th>% Chromosome Breaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal donor</td>
<td>autologous or homologous</td>
<td>461</td>
<td>1</td>
</tr>
<tr>
<td>&quot;</td>
<td>different ABO blood type and/or sex</td>
<td>643</td>
<td>1</td>
</tr>
<tr>
<td>&quot;</td>
<td>plasma from radiation casualties</td>
<td>1318</td>
<td>8.7</td>
</tr>
</tbody>
</table>

The results appear to support the hypothesis being tested.

The aims of the authors' research on developing a practical instrument for automatic pattern recognition of blood cell images are reviewed, and their CELLSCAN system is described and illustrated. The importance of quantitative information about various cellular morphological characteristics of interest, e.g. number and size of nuclear lobes, vacuoles, presence, size and number of nucleoli, range of variability in cell size and configuration, is emphasized. Examples of the data obtained using the CELLSCAN system are presented to demonstrate how the various morphological characteristics may be recognized and measured.


Data representing bone marrow specimens obtained from the Y-12 accident casualties (see ref. #9) are analyzed to demonstrate the importance of disrupted marrow architecture in the response to radiation injury. Observations from experiments with rats are also presented to show the relationship between radiation dose (550 R, 1000 R or 1500 R) and the percentage of immature myeloid and erythroid cells incorporating H3 thymidine at various times (up to 18 hours) after exposure. After 1000 R, abortive myelocytic regeneration was observed on the fifth and sixth days post-exposure and true recovery began after the seventh to eighth day in the rats. After 550 R, the degenerative and regenerative phases of the marrow response overlapped.
The Effect of Etiocholanolone on Granulocyte Kinetics.

The effect of etiocholanolone on granulocyte kinetics in 12 hematologically normal patients (with neoplastic disease) was investigated using the technique of $^3$H-DFP labeling of autologous blood in vitro. Four hundred to 500 ml whole blood was collected under sterile conditions. Leukocyte labeling was accomplished by incubating whole blood for 45 minutes with 250 to 350 $\mu$Ci $^3$H-DFP. An aliquot (10 ml) of labeled blood was removed for analysis and the remainder was infused into the donor within 10 to 15 minutes. Blood samples were obtained by venepuncture at various times up to 24 hours after the termination of the infusion.

Baseline determinations of the total blood granulocyte pool, TBGP (calculated by isotope dilution method), the circulating pool CGP (calculated by multiplying granulocyte count by blood volume), and the margined pool, MGP (calculated by subtracting CGP from TBGP), were performed. Values for the TBGP (69.3 x 10$^9$ granulocytes/kg and 58.9 x 10$^9$ granulocytes/kg, in two separate studies) were similar to those previously reported.

The administration of etiocholanolone caused an increase in CGP, MGP and TBGP. The CGP/TBGP ratio before and after etiocholanolone changed only slightly (0.34 vs. 0.36). In the etiocholanolone group a 98% mean increase in TBGP was found in contrast to an 8% mean decrease in the control group. The 50% survival time (T 1/2) of circulating granulocytes was similar prior to and following etiocholanolone administration.

The authors conclude that the large increase observed in the TBGP (98%), compared to baseline after etiocholanolone, must be due largely to an influx of cells from the bone marrow, and that etiocholanolone may be a useful agent for the estimation of bone marrow reserve.
The effect of etiocholanolone on the appearance of labeled granulocytes in peripheral blood following administration of $^3$HTdR is described. The study group consisted of seven hematologically normal patients (with malignant disease). Baseline studies showed that the peripheral blood leukocyte specific activity curves for these patients were similar to those previously observed in other hematologically normal individuals. There was an early low level of radioactivity present for 96 to 118 hours (due primarily to labeled large lymphocytes, and to a lesser extent to monocytes and small lymphocytes), followed by a marked rise with peak radioactivity occurring between 120 and 191 hours (due to the appearance of labeled neutrophilic granulocytes). Decline in radioactivity was rapid thereafter. In the current study patients were given etiocholanolone (0.3 mg/kg body weight, I.M.) at varying times before and after $^3$HTdR administration. There was no significant alteration in the peripheral blood leukocyte specific activity patterns when etiocholanolone was given before or up to 50 hours after thymidine administration. When etiocholanolone was administered 74 hours after thymidine administration, an early appearance and an early peak of radioactivity due to early appearance of labeled granulocytes occurred.

The authors concluded that when etiocholanolone stimulus occurred up to 50 hours after $^3$HTdR administration, the mobilization of granulocytes from the mature reserves did not affect the rate of progression of labeled cells through the various myelocytic compartments sufficiently to alter the leukocyte kinetic pattern. Fifty hours after isotope administration, mature labeled granulocytes were not present in the mature marrow reserve in numbers adequate to influence the leukocyte radioactivity curve. It was only after
72 hours (the time interval required for the progression of the myelocyte to the mature labeled neutrophil) that the stimulus of etiocholanolone resulted in release of labeled myeloid cells from the bone marrow. The data provide evidence for an accelerated mobilization of granulocytes from the marrow, and indicate that marrow granulocytes cannot be released by etiocholanolone stimulation until cellular maturation has taken place.

46. Wolff, S., Kimball, H. R., Perry, S., Root, R. and Kappas, A.
The Biological Properties of Etiocholanolone.

The pyrogenic and hematologic responses to the steroid metabolite, etiocholanolone, are reviewed and illustrated with data obtained from studying these responses in patients and in normal volunteers at the Clinical Center, National Institutes of Health. The effect of etiocholanolone on leukocyte kinetics was studied using leukocytes labeled with $^{32}$P or $^{3}$H thymidine. Etiocholanolone was shown to mobilize granulocytes from bone marrow, resulting in a marked increase in the number of granulocytes in peripheral blood. The relationship between granulocyte response to etiocholanolone and subsequent myelosuppression during radio- or chemotherapy is discussed, and the authors point out the merits of this agent when used in estimating granulocyte reserves. Other interesting properties of etiocholanolone, especially its stimulating effect on porphyrin and heme synthesis (liver and bone marrow) are also reviewed.

The Blood and Bone Marrow Neutrophil Response to Graded Doses of Endotoxin in Mice.

Mice were injected intraperitoneally with either 0.01 µg, 0.2 µg, 5.0 µg or 25.0 µg of S. typhosa endotoxin. Absolute granulocyte counts of peripheral blood were determined prior to injection and serially 2, 6, 10 and 16 hours after injection. Bone marrow was collected from one
femur of mice killed 6 or 16 hours after injection and the absolute granulocyte count of this marrow was also determined. The neutrophil response was found to be dose-related, and at least two dose-related kinetic events were found to be operative in instigating the observed changes in neutrophil counts: 1. With small doses of endotoxin, acceleration of release rate from marrow exceeded the rate of loss from peripheral blood. 2. Larger doses of endotoxin were more effective in accelerating the rate of release of neutrophils from marrow, but had an even greater effect on the acceleration of egress of neutrophils from the blood, thus resulting in transient leukopenia.


Rats (120 gm) and mice (20 gm) were placed in separate cages and exposed to continuous gamma radiation from $^{60}$Co. Rats received 50 rads/day for 12, 35 or 105 days; mice for 12 or 35 days. At the end of the exposure period, tracer doses of $^3$H thymidine were injected and some animals were killed at closely spaced intervals from 1/2 to 20 hours thereafter. Similar injections were administered to non-irradiated control animals. Histological studies as well as counts of mitotic and labelled cells in intestinal crypts of each of three sections of each animal's upper small intestine were carried out to evaluate the effects of irradiation. Duration of the various stages of the cell cycle were calculated from the percent labeled mitotic figures found at each sampling interval. The sequence of events observed in the intestinal crypts of continuously irradiated animals consisted of 1. retardation of cell cycle after 1 day of exposure, 2. shortening of the generation cycle by approximately 20%, 3. return to nearly normal values by 35 days in the mouse and 105 days in the rat.
Yuhas, J. and Storer, J. B.
The Effect of Age on Two Modes of Radiation Death and on Hematopoietic Cell Survival in the Mouse.

Female C57BL/6J mice, between the ages of 3 and 24 months, have been tested for three measures of radiosensitivity: "marrow" death [estimated by calculating the LD50(30)], "intestinal" death [estimated by calculating the LD50(7)], and hematopoietic "stem" cell content and survival, as estimated by the ability to form spleen colonies. The two modes of death show dissimilar aging patterns: "marrow" resistance increases to a maximum at 17 to 19 months of age, while "intestinal" resistance declines from 3 months onward. The median lethal dose for 30-day survival is a composite of sensitivity to both modes of death, with "intestinal" sensitivity becoming increasingly important as the age of the mouse is increased.

The resistance to "marrow" death is accounted for by the number of "stem" cells present in the femoral marrow of the aging mouse, and by the surviving number after X-ray exposure. As was the case with resistance to "marrow" death, the number of "stem" cells in the femur increased through 18 months and declined thereafter.

Patt, H. M.
Cell Turnover and Mammalian Radiosensitivity.

The differential response of cell renewal systems to irradiation is influenced by the inherent radiosensitivity of the cell types being considered, the organization and control of tissues and organs of which the cells are a part, and the significance of the cell, tissue or organ with respect to its role in the economy of the organism. In hematopoietic tissue and intestinal epithelium but not in all tissues, maturing cells that are no longer dividing are relatively unaffected by
radiation doses that are lethal for proliferating cells. The importance of the stage of the cell cycle irradiated, the duration of cell cycle and the proliferation rate all profoundly influence radiosensitivity.


The authors describe the clinical and laboratory observations following accidental exposure of a 33 year old graduate student to gamma radiation from a 200 Ci 60Co source. The dose received was estimated to be between 250-300 R, the source being at about the level of the patient's umbilicus during most of the exposure which ended about noon. Malaise, lethargy, nausea and vomiting began about 1 1/2 hours after exposure; diarrhea about 5 hours after exposure. All these symptoms began before the patient knew he had been irradiated and had subsided before he was admitted to the hospital at 11:00 P.M. Changes in leukocyte and platelet counts during the 84 day post-exposure period reported were very similar to those reported by Andrews et al. in their report of the Y-12 casualties (see reference #9), and the doses were also comparable. The patient recovered although his course was complicated by development of a necrotic lesion just above the umbilicus. The lesion which developed in an area of (probably) more intense localized radiation injury, subsequently sloughed and was repaired by plastic surgery. The patient's sperm count was decreased on the 23rd post-exposure day and only 56% of the sperm were motile. The count continued to fall and the percent motile sperm to decrease until 167 days after exposure when no spermatozoa were found. The specimen obtained 15 months after exposure also contained no spermatozoa.
52. Paulsen, C. A.
Progress Report, June 1963-March 1966, Washington University, Seattle, School of Medicine, Contract AT (45-1)-1781 OPAU RL0-1781-6.

The effects of localized irradiation of the scrotums of 31 volunteers (male prisoners) with x-ray doses ranging from 7.5 R to 400 R were evaluated by serial analyses of semen, by testicular biopsies and by measuring the levels of sex hormones in serum. Sperm count was reduced by x-ray doses of 15 R or greater but the effect was not permanent. A dose of 100 R of x-rays caused immediate damage to germ cells, cellular metabolism being measurably altered four hours after exposure. Sperm counts were decreased between 30-135 days after exposure (injury being primarily to proliferating early stages); urinary gonadotropin excretion was increased during this period. Plasma testosterone and estrogen levels were not altered.

53. Nebel, B. R. and Murphy, C. J.
Damage and Recovery of Mouse Testis after 1000 r Acute Localized X-irradiation, with Reference to Restitution Cells, Sertoli Cell Increase, and Type A Spermatogonial Recovery.

After 1000 r of acute localized irradiation many spermatocytes are blocked in first metaphase. Many restitution nuclei similar to those seen after colchicine treatment from between the first and the tenth day after irradiation, the maximum being observed at 6 days. These restituted cells often drift toward the basement membrane. This excess is not explained by cell division as shown by autoradiography, nor by shrinkage of tubules, since the number is abnormally high even after correction for the shrinkage. A major part of the excess number of Sertoli cells in heavily damaged tubules apparently corresponds to the number of restitution cells formed from the blocked first metaphases. The ultimate fate of these indifferent
"Sertoli"-like cells is unknown. Type A spermatogonia were observed between 8 and 10 days after 1000 r with a frequency of 0.007 per tubule. After division, the resulting daughter cells distribute themselves along the basement membrane, soon forming a complete ring of dividing spermatogenic cells, observed at 20 days in recovering tubules. Stage "XII" was observed at 20 days in recovering tubules. Stage "XII" was observed at 5 weeks showing development only through first metaphase spermatocytes.

54. Oakberg, E. F.
Gamma-ray Sensitivity of Spermatogonia of the Mouse.

Hybrid male mice were given a range of doses from 5 to 100 r of 60Co gamma rays, killed 72 hours after irradiation, and all spermatogonia counted in 100 tubule cross sections per mouse. Young primary spermatocytes at stages VII and VIII in the cycle of the seminiferous epithelium also were counted. Late type A, intermediate, and early type B spermatogonia were uniformly sensitive, with an LD50 of approximately 20 to 24 r of 60Co gamma rays.

Scoring of all type A spermatogonia gave survival comparable to intermediate and type B cells at 5 to 23 r, and a significantly higher survival at 30 to 100 r. The curve obtained for type A cells has been interpreted as resulting from a mixed population of cells with at least two different levels of sensitivity. These differences in sensitivity could not be correlated with specific stages in the cycle of the seminiferous epithelium, but appeared to exist even in the dormant period, where uniform radiation response might have been expected.

Earlier work had shown that intermediate spermatogonia can be identified cytologically at stages II and III. On the basis of radiation data of the present investigation, this differentiation can be detected one stage earlier in the cycle of the seminiferous epithelium, namely, at late stage I to early stage II. Differentiation of intermediate spermatogonia from type A cells appears to be unaffected by radiation. (Author)
55. Oakberg, E. F.
Degeneration of Spermatogonia of the Mouse Following Exposure to X-rays and Stages in the Mitotic Cycle at Which Cell Death Occurs.

Results are reported from studies on the effect of low doses of whole-body irradiation and of low dose rates on spermatogonia and oocytes of the mouse exposed to x and γ-irradiation. Neutron exposures at low doses were used for comparisons of relative biological effectiveness. While some significant differences attributable to dose rate were observed, they were not consistent through-out. Both oocytes and spermatogonia gave multi-hit survival curves. The fact that oocyte survival after chronic irradiation was higher in adult than in young females suggests either less effective elimination of radiation damage, or a higher sensitivity of comparable oocyte stages in young females. (Author)

56. Oakberg, E. F. and DiMinno, R. L.
X-ray Sensitivity of Primary Spermatocytes of the Mouse.

The radiation sensitivity of mouse primary spermatocytes to cell-killing was estimated by the number of spermatids produced; sensitivity to chromosome breakage was measured by scoring abnormal divisions atanaphase I and II. There was an inverse rela-
tion between cell-killing and chromosome breakage: pre-leptotene was the most sensitive and dia-
kinesis-metaphase I the most resistant to induction of cell death; whereas pre-leptotene and leptotene were the most resistant and metaphase I the most sensitive to chromosome breakage. The relative sensitivities of primary spermatocyte stages to chromosome breakage are similar to those observed for meiotic cells in other organisms. Stage differ-
ences between leptotene and pachytene in sensitivity to chromosome breakage in the mouse are as large as those sometimes claimed for species differences. The need for accurate information on the duration of spermatogenesis is emphasized in order that
species comparisons should not be confused with stage differences in sensitivity. Determination of the number and morphology of spermatozoa ejaculated at successive intervals after irradiation revealed no changes as spermatozoa and spermatids were utilized, a decrease in number and an increase in abnormal sperm from spermatocytes, a severe oligospermia from destruction of spermatogonia, and a return to normal number after regeneration of the testis. (Author)

57. Oakberg, E. F.

Doses as high as 1000 R had no retarding effect on meiosis or spermiogenesis of cells irradiated as spermatids. Maturation of spermatids arising from cells irradiated as spermatocytes probably is retarded at high doses but not at 100 R. Timing of maturation, according to observations after 100 R, agreed with maturation time observed in experiments where doses were as low as 5 R of γ-rays from 60Co. The author concludes that the estimated duration for spermatogenesis, 34.5 days, is not seriously biased.

58. Bateman, J. L., Johnson, H. A., Bond, V. P. and Rossi, H. H.

Utilizing the decrease in number of resting primary spermatocytes (RPS) in the testes of mice 96 hours after irradiation as a measure of radiation damage, the authors determined the relative biological effectiveness of 250 kVp x-rays 60Co gamma rays and monoenergetic neutrons of 0.43 MeV, 0.62 MeV, 1.00 MeV, 1.80 MeV or 14 MeV. The effect of dose rate was studied in the range of 0.015 to 1.5 rads/min. for 0.62 MeV neutrons, in the range of 0.15 to 15.0 rads/min. for 14 MeV neutrons, and in the range of 1.0 to 100 rads/min. for 250 kVp x-rays. The dose required to reduce
the number of surviving RPS to $50\%$ of that in un-
irradiated mice (ED$_{50}$) was the basis of comparison.

The ED$_{50}$ for $^{60}$Co gamma rays and 250 kVp x-rays (at
all dose rates studied) was approximately 40 rads.
For monoenergetic neutrons, the ED$_{50}$ was 3.7 rads
at 0.43 MeV, 15 rads at 1.4 MeV and between these
two values for neutrons of intermediate energies.
Dose rate did not influence the ED$_{50}$ values for
neutrons. For neutrons of 1.4 MeV, all dose rates
yielded multiple-event curves, but regressions with
neutrons of lower energies appeared to be exponen-
tial.

The gross sensitive volume (gsv: that volume just
large enough to "contain" at least one particle, on
the average, at a dose corresponding to a survival
of 1/e (D$_{37}$)) was calculated and compared with the
volume of the spheroidal nucleus and of the entire
cell. The authors "conclude that it is probable,
but not established, that these cells can be killed
by single particles traversing the cytoplasm only."

59. Moorhead, P. S., Nowell, P. C., Mellman, W. J.,
Battips, D. M. and Hungerford, D. A.
Chromosome Preparations of Leukocytes Cultured
from Peripheral Blood.

The preparation of leukocyte concentrates from
human peripheral blood using phytohemagglutinin as
an erythrocyte agglutinin and a mitogen for leuko-
cytes is described. Details of the technique for
preparation of metaphase spreads are also presented.

60. Bender, M. A.
Effects of Radiation on Chromosomes
In: Symposium on the Pacific Uses of Atomic
ORNL-P-3201 Oak Ridge National Lab.

The author reviews the types of radiation-induced
chromosomal aberrations in man, the kinetics of
their induction, and the effects of such aber-
tations on "germ-line" cells and somatic cells,
emphasizing that in the case of deletions in somatic
cells, "...many, perhaps most, are neither lethal
nor even detrimental." Available data on rates of induction of chromosomal aberrations in germ-line cells and somatic cells of man are reviewed with particular emphasis on the relationship between radiation dose and aberration frequency and on factors influencing that relationship. The most extensive data are those representing aberration frequencies in cultures of human peripheral blood leukocytes after irradiation in vivo or in vitro.

To determine primary aberration frequencies, it is usually assumed that the incidence of aberrations must be determined in the first post-irradiation cell division. The author, however, cites data (from his own and from other laboratories) that aberration yields are as high after the second post-irradiation division as after the first. The simplest explanation for this is considered to be the presence of at least two populations of cells in the culture, one population having a shorter cell cycle and greater radiosensitivity.

There are many uncertainties in the interpretation of the relationship between aberration frequency and radiation dose when considerable time has elapsed between exposure and cytogenetic studies, when exposure has been chronic or fractionated, when only part of the body has been irradiated or when radiation is from internally deposited radioisotopes. Most observations on the incidence of aberrations in man following irradiation are subject to one or more of these sources of uncertainty. A few cases have been studied (some of them by the author) within hours or a few days of nearly uniform, whole body exposure to a single dose, and these have shown "reasonably good" agreement between radiation dose estimates from aberration yields and estimates from physical dosimeters. A case is made for determining aberration rates in irradiated human populations and for automating the process of chromosome scoring.
61. Bender, M. A. and Gooch, P. C.  
Somatic Chromosome Aberrations Induced by Human Whole-body Irradiation: The "Recuplex" Criticality Accident.  

Three men were exposed to mixed neutron-γ-radiation in a criticality accident in Richland, Washington.

Peripheral blood samples obtained during the first two years after exposure were cultured, the aberrations per cell were determined and compared with the expected frequencies that had been calculated using data from previous studies of cells irradiated in vitro. These expected coefficients were as follows:

**Coefficient of Aberration Production in Human Leukocytes**

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>Deletions Per Cell</th>
<th>Rings and Dicentrics Per Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard x-rays</td>
<td>$0.9\pm0.1 \times 10^{-3}/\text{rad}$</td>
<td>$6.0\pm0.4 \times 10^{-6} \text{ rad}^2$</td>
</tr>
<tr>
<td>Uranium-fission neutrons</td>
<td>$4.0\pm0.8 \times 10^{-3}/\text{rad}$</td>
<td>$5.6\pm0.6 \times 10^{-3} \text{ rad}$</td>
</tr>
</tbody>
</table>

Observed frequencies were consistent with expectations based on the physical dose estimates and the coefficients of variation shown above. The aberration frequency dropped after several weeks but some aberrations were found in the sample taken two years after exposure.

62. Evans, H. J.  
Actions of Radiations on Chromosomes.  

The author reviews the subject of radiation-induced changes in chromosome structure and number with particular emphasis on the production of chromosome aberrations in man. The processes of terminal deletion, interstitial deletion, the production of centric and dicentric rings and translocation are described and diagrammed. The
author then presents his own data representing the relationship between dose and yield for the four readily distinguished types of aberrations in human lymphocytes irradiated in vitro with a graded series of x-ray doses ranging from 0 rads to 300 rads. The author found that the kinetics of aberration production as a function of dose is similar for each of the four aberration types. The anticipated relationship between dose and effect for aberrations that require two independently-produced breaks is: \[ y \propto D^2 \]
where \( k \) is a constant and \( D \) is the dose delivered in a very short interval (i.e., minutes). If the dose is delivered over a longer interval (hours), some breaks may heal before others occur, and the dose exponent would then be less than 2. In the author's studies, however, the dose exponent never exceeded 1.3, even when all doses were delivered within two minutes. The data are analysed to show that the bulk of exchange aberrations observed result from the passage of a single electron track through or near the two chromosome arms that are involved. Similar experiments (in vitro irradiation of phytohemagglutinin-stimulated human lymphocytes) in other laboratories have yielded aberration frequencies three or four times lower than those reported by this author. The discrepancy is explained on the basis of differences in technique, the author scoring the aberrations 46-50 hours after the initiation of cultures, before any cells have undergone their second division. The other investigators whose work is cited scored aberrations at 60 to 72 hours when a considerable proportion of surviving cells were undergoing the second mitosis. Since most cells bearing dicentric and ring aberrations are not capable of further division, the second series of cell divisions would result in considerable dilution of the aberrant population by unaffected cells, and aberration yields would appear to be substantially lower. The authors' data indicate that chromosomes in human small lymphocytes are highly radiosensitive and that damage is not appreciably reduced by increasing exposure time.
63. Brooks, A. L. and Lengemann, F. W.  
Comparison of Radiation-induced Chromatid Aberrations in the Testes and Bone Marrow of the Chinese Hamster.  

Chinese hamsters were irradiated with various doses of γ-radiation (50-125 R) from a 60Co source at a rate of 16.6 R/min. At various times after irradiation, certain of the animals were injected with 0.3 ml of 1% colchicine and killed two hours thereafter. The frequency of occurrence of chromatid breaks was determined in the femoral marrow and testes. The number of single-hit breaks per cell per R was calculated to be 0.0015 for bone marrow and 0.0041 for testes. The number of single-hit breaks appeared to decrease as an exponential function with time after exposure (3-24 hours) and values for testes were consistently higher than those for marrow, and at six hours, when the difference was maximal, the testes showed twice as many radiation-induced chromatid breaks as did marrow. The total number of breaks per cell was higher in testes at all times, but the difference decreased markedly with time, the ratios varying from 1.34 to 3.19. Possible explanations for the observed differences are discussed. The authors emphasize that the number of chromatid deletions in a somatic tissue may not reflect the number produced by the same dose in a reproductive tissue.

64. Amos, N., Sasaki, M., Ottoman, R. E. and Veomett, R. C.  
Use of Chromosome Aberrations to Estimate X-ray and γ-ray Dose to Man.  
SAM-TR-67-112. CFSTI, AD-66713, U. of California at Los Angeles School of Medicine, Los Angeles (1967).

The frequency of chromosome aberrations in lymphocytes taken from peripheral blood of five persons accidentally exposed to ionizing radiation was determined, using PHA-stimulated lymphocyte cultures and determining aberration frequencies after 50 hours and 72 hours in culture. Estimation of dose was found to be affected by time in culture, sampling error and sampling time, and by size, rate, distribution and quality of radiation dose.
Nichols, W. W.
Studies on the Role of Viruses in Somatic Mutation.

The present paper reviews a continuing study that is divided into two parts. The first part deals with the chromosome damaging potential of viruses through the study of two model systems, the SR strain of Rous sarcoma virus and measles virus. Chromosome studies were carried out on rat tumor cells exposed to the Rous sarcoma virus in vivo and in vitro, and on diploid (non-tumor) rat and human cells to which the virus was added in vitro. In the second model system, measles, two groups of patients were studied; those who had the clinical disease and those receiving live attenuated measles vaccine. The chromosome effects of measles virus were further studied in vitro in four tissue culture systems. Following this, various fractions of the measles virus were studied in an in vitro system.

At least three types of change involving the chromosomes were recognized: 1. the "single" open break, 2. chromosome pulverization, and 3. cell fusion and spindle abnormalities. The one thought most likely to have mutagenic significance is the "single" break, and the somatic mutation hypothesis of carcinogenesis is discussed from this standpoint.

The mechanism of the single breaks has been approached by a series of comparisons with nucleosides (including deoxycytidine), nucleotides and analogues that inhibit the synthesis of DNA and produce morphologically similar breaks.

The discussion section begins with a review of studies that have demonstrated that a variety of viruses may result in chromosome breaks when cells are exposed in vivo or in vitro. One example cited is the induction of chromosome breaks in vitro in normal human lymphocytes cultured in the serum of patients with infectious hepatitis.
66. Leonard, A. and DeKnudt, G.
Relation Between the X-ray Dose and the Rate of Chromosome Rearrangements in Spermatogonia of Mice.

The relationship between whole-body x-ray dose and the rate of reciprocal translocations in spermatogonia of BALB/c strain mice were studied for doses of x-radiation (300 kV, 20 mA, 100 R/min.) ranging from 100 R to 800 R in increments of 100 R. Testes were studied 10 weeks after irradiation, the major types of examination being 1. determination of testes weights, 2. histological studies of Feulgen-stained sections to determine the percentage of tubules containing the different maturation stages of germ cells, and 3. determining the frequency of chromosome rearrangements on meiotic preparations obtained by means of the air-drying method. Testes weight decreased with increasing dose in agreement with earlier observations by Kohn and Kallman (see reference #)

Following exposure to x-ray doses of 400 R and above, the cell population of the tubules also varied with x-ray dose, fewer of the tubules containing stages more mature than spermatogonia. The percentage of chromosome translocations increased with increasing x-ray dose and the relationship appeared to be linear with a regression coefficient b = 0.017121 ± 0.001048 and a P value of <0.001. The authors point out that there was sometimes "heterogeneity between animals given the same x-ray dose or between the testes of the same animal." The observed dose-effect relationship (for chromosome rearrangements) was found to agree well with data reported by two other groups of investigators.

67. Biagini, C., Brancadoro, P. and Siciliano, A.
Studio delle correlazioni fra le aberrazioni dei chromosomi e la dose nei soggetti irradiati a scopo terapeutico.
(Rapporto annuale luglio 1965-luglio 1966) (In Italian).

The authors determined the frequency of occurrence of chromatid and chromosome interchanges, acentric
fragments, dicentrics, rings and polyploid cells in cultured peripheral blood lymphocytes from a series of patients before and after they received 60Co gamma radiation therapy to a localized area of the body. Initial doses of 10-1000 rads were delivered through a 10 x 10 cm port over the anterior thorax or the anterior iliac crest and blood was drawn for cytogenetic studies 24 or 72 hours thereafter.

Results, which are tabulated and graphed, show that integral dose to marrow (M grads) is the best basis for comparing effects of localized irradiation of different parts of the body. The occurrence of fragments and dicentrics tends to increase linearly with dose per M grad after local exposure and the rate of occurrence of inversions, determined by karyotyping, also tends to increase with increasing integral dose to the marrow (above 1 M grads). The relative frequency of tetraploid cells in culture after 72 hours increased linearly with both skin dose and integral dose to the marrow.


Granulocytic leukemia, which could regularly be induced in RF/Up mice by x-irradiation, and which was also transmissible by cell-free filtrates (from spleen cells), is shown to be associated with two types of abnormalities in karyotype (as determined from spreads of bone marrow cells), namely a significant proportion of cells having 41 instead of the normal 40 chromosomes and a morphologically abnormal (apparently identical) chromosome in many of the cells. The abnormal "marker" chromosome was found most often in cells having 41 chromosomes. The authors discuss the evidence that the leukemia and the chromosome abnormalities are virus-induced and that the virus was present in the mouse in which the leukemia was originally induced by irradiation. They suggest that the virus may have induced the production of the "marker" chromosome initially by altering the
morphology and increasing the stickiness at a specific, highly susceptible region on one of two chromatids of a particular chromosome, resulting in adherence of the affected chromatid to an unaffected chromatid in the next cell division during which the adherent chromatids fail to separate. In subsequent mitoses, stickiness presumably diminishes, chromatids separate normally, although the two altered sister chromatids frequently adhere to each other. Possible parallelisms between the etiology of the radiation induced granulocytic leukemia in the mouse and chronic granulocytic leukemia in man are discussed briefly to emphasize the close relationship between the viral and somatic mutation concepts of leukogenesis.

69. Luzzio, A. J., Kereiakes, J. G. and Sutton, G.
Blood and Urine Tyrosine Levels in Starved X-irradiated Rats.

Starved x-irradiated and starved unirradiated rats were compared with normal control rats by measuring free tyrosine in blood serum and urine, the tyrosine being used as an index of protein degradation. Both starved groups differed significantly from the normal group in that the levels of serum tyrosine increased to a peak at 48 hours, then dropped to subnormal levels by 96 hours. This observation is thought to be analogous to starvation-induced increases in blood lipid and carbohydrate levels. The irradiated rats showed a lower peak than the unirradiated group, which is interpreted as being a result of radiation-induced inhibition of protein catabolism. Urinary tyrosine decreased significantly in both starved groups, but the decrease was greater in the unirradiated group. There was no evidence of direct radiation-induced protein degradation in this study.

70. Angel, C. R. and Noonan, T. R.
Urinary Taurine Excretion and the Partition of Sulfur in Four Species of Mammals After Whole-Body X-irradiation.
Urinary taurine levels and the partition of total urinary sulfur (total sulfur, sulfate and neutral sulfur) were studied in rats, guinea pigs and rabbits before and (daily) after exposure to 800 R (rats, guinea pigs, dogs) or 980 or 1070 R (rabbits). The excretion of taurine was found to be species-dependent. The rat showed an early rise, the guinea pig no rise at all. Rabbits showed no rise or a variable increase during the first three post-exposure days. Dogs excreted either increased amounts of taurine at two time periods after irradiation or a large quantity one day before death. Total sulfur excretion correlated with taurine excretion only in the rat.

71. Mitsuyuki, A., Takahashi, M., Takeuchi, K. and Fukada, M.,
Studies on the Significance of Taurine in Radiation Injury.

Daily excretion of taurine in urine of female mice was measured for one week prior to and fifteen days after whole-body exposure to 690 R of 150 kVp x-rays. Peak taurine excretion was observed on the first post exposure day when urine contained more than three times the amount of taurine excreted daily prior to exposure. The daily excretion then decreased rapidly, minimum values being observed on the fourth post-exposure day. A secondary increase in taurine excretion occurred in parallel with increasing mortality rate after the tenth day. The authors suggest that destruction of lymphocytes, which are very rich in taurine, may be a major cause of taurinuria after irradiation. Observations on administrations of taurine as a therapeutic measure are also reported.

72. Soupart, P.
Free Amino Acids of Blood and Urine in the Human.

This review article also presents some of the author's data on the distribution of free amino
acids in plasma and in erythrocytes, leukocytes and platelets of peripheral blood. In leukocytes, all the amino acids measured, except taurine, were present in concentrations 4-60 times that in plasma. Taurine concentrations in both leukocytes and platelets were several hundred fold higher than the plasma concentrations.

Dose Dependency of Radiation-Induced Creatine Excretion in Rat Urine.

Male rats were exposed to doses of γ-rays ranging from 0 to 1000 R. Sham-irradiation was carried out on controls. Two rats were housed in each cage, and creatine was assayed in 24-hour combined urine collections from each pair. Creatine excretion is expressed as creatine/creatinine ratio, to compensate for non-quantitative urine collection. Plots of creatine/creatinine ratios against days after exposure showed no consistent pattern, but creatinuria was greater after exposure to intermediate doses of radiation than after exposure to low doses, and still greater after exposure to the highest doses. The effect of sham-irradiation was approximately the same as that of 25 R; i.e. a slight creatinuria. The best correlation of creatine excretion with time was obtained by averaging the creatine-creatinine ratios over the first four post-exposure days, and plotting the averages as a function of radiation dose. The plot of the average values per se is not linear, but if standard errors are plotted for each point, a straight line can be drawn within the range of the standard errors (which are about 30%), up to 650 R. At dose levels about 650 R, peak values for creatine excretion were attained more slowly, with the result that the average creatine excretion during the first four days declined as doses exceeded 650 R. Thus the degree of creatinuria after the fourth day would have to be considered in order to distinguish between exposure to high doses and exposure to lower doses.
Gerber, G. B., Gerber, G. and Altman, K. I.  
The Mechanism of Radiation-Induced Creatinuria.  

The authors measured the rate of creatine synthesis  
and rate of efflux of creatine from muscle of rats  
following whole body exposure to x-ray doses of  
650 R, in two groups of control rats (sham irradia-  
ted) and in normal rats not exposed to any of the  
manipulations involved in the irradiation procedure.  
Levels of creatine and creatinine in urine were  
determined for each animal, using a pooled urine  
sample collected over a period of two weeks. Two  
other groups of five rats each were given creatine-  
2-^{14}C parenterally, two weeks prior to x-irradiation  
and the efflux of creatinine from muscle was  
measured by determining specific activity of crea-  
tine and creatinine in urine excreted during a one-  
week and a two-week period after irradiation.

During the two-week post-irradiation period, rats  
excreted approximately the same amount of creatinine  
but about five times as much creatine as did un-  
irradiated rats. The amount of creatine synthesized  
was decreased from approximately 30 mole/100 gm/day  
in both control groups to 17 μ moles/100 gm/day in  
x-irradiated rats. Release of creatine from muscle  
was not increased after irradiation. Taken together,  
the results are interpreted as indicating that  
radiation-induced creatinuria represents failure  
of muscle to utilize newly synthesized creatine  
which was synthesized at a normal rate.

Cavalieri, R. R., Van Metre, M., Chambers, Jr.,  
F. W. and King, E. R.  
Taurine Excretion in Humans Treated by Total-Body  
Radiation.  

Urinary excretion of taurine and creatinine by  
eight patients (with advanced malignant disease)  
was studied before and after total body exposure  
to 60Co gamma radiation. Four of the patients  
received a single dose (one received 900 R, two  
received 450 R, and one received 225 R, measured  
in air; 684, 342 and 171 rads, respectively) and  
four patients received multiple doses of 100 R at
intervals of two or three days, for a total of four, five or six doses. Urinary taurine levels were significantly increased following the single exposures to 450 R and 900 R, but not following 225 R. Increased taurine excretion was also observed (beginning on the fifth day after the first dose) in two of the four patients who received multiple 100 R doses. (Urine of five normal adult males was analyzed to establish normal values for comparison.) The authors comment that, "The source of taurine which is excreted in excess after radiation exposure is not known."

76. Harris, H.  
Family Studies on the Urinary Excretion of \( \beta \)-aminoisobutyric Acid.  

Paper chromatographic methods were applied in estimating the amount of \( \beta \)-aminoisobutyric acid (BAIBA) in 24 hour urine specimens of 345 normal individuals representing a series of family groups. Using criteria established for this study, 33 of the individuals (9.6%) were "high" excretors of BAIBA. "High" values were approximately 70-200 mg BAIBA/g creatinine. Results were consistent on repeated examinations of urine samples collected at intervals (weeks up to two years). This was considered to be a genetically determined characteristic of the individuals. Although the inheritance pattern could not be determined precisely, the hypothesis that the "character" is determined by a common autosomal gene fit reasonably well with the observed familial distribution.

77. Saenger, E. L.  
Metabolic Changes in Humans Following Total Body Irradiation.  

This report describes results obtained during the period 1 Nov. 1961 - 30 April 1963 from a continuing study of the metabolic effects of total and partial body irradiation. The report represents studies on
29 patients with metastatic or incurable neoplasms who were in good nutritional status and had a stable blood picture. The experimental procedures include an initial sham exposure. Doses of 50 to 200 rad of 60Co γ-radiation were delivered during a single exposure of the whole body. Clinical and laboratory studies are described and summarized in tables and graphs. Results and conclusions from the research include:


2. Identification of DOC in the urine following whole body exposure.

3. Xanthurenic acid was found in the urine of some of the patients after exposure.

4. Chromosomal abnormalities were identified in peripheral blood lymphocytes cultured in the presence of phytohemagglutinin.

5. Patients were found to tolerate doses of 200 rad to the whole body relatively well.


Urine of three groups of radiation casualties (five Oak Ridge Y-12 casualties, two Los Alamos casualties of the December 1958 criticality accident and two casualties of the Lockport accident of 1960) was analyzed to determine excretion of creatine, creatinine beta-amino-isobutyric acid (BAIBA), pyrrole-carboxylic acid and "free" hydroxyproline at various times after irradiation. In most of the cases, the concentration of BAIBA in urine and the urinary creatine/creatinine ratios were elevated for two weeks after exposure. (The authors do not indicate what their "normal" value represents.) The authors could not demonstrate a relationship between radiation dose (ranging from approximately
100 to approximately 4500 rads) and urinary levels of UA/UA nor of urinary creatinine/creatinine ratios. Excretion of "free" hydroxyproline was decreased in the three most heavily irradiated patients; pyrrolicarboxylic acid was increased only in the fatally injured man (dose approximately 4500 rads).

79. Hartwig, Q. L., Melville, Jr., G. S., Leffingwell, T. P. and Young, R. J.
Iron-59 Metabolism as an Index of Erythropoietic Damage and Recovery in Monkeys Exposed to Nuclear Radiations.

Three groups of male monkeys were exposed to mixed neutron and gamma radiation from a nuclear device at a test site receiving total doses of 709 REM (3 animals), 629 REM (3 animals) or 544 REM (4 animals). Three additional monkeys traveled to the test site but were not irradiated and six monkeys were not transported to the site but served as laboratory controls. Twenty eight hours after exposure all animals were injected with tracer doses of radio-iron as FeCl₃. In addition to standard hematological studies, iron incorporation into red cells and plasma iron levels were determined 24 hours after injection of ⁵⁹FeCl₃ and then every few days for 50 days or until the animals died. (Seven of the ten irradiated animals died during the third post-exposure week.) Fifty two hours post-exposure, Fe uptake in irradiated animals was 2.1%, compared to 10.7% for controls, and a marked difference in the two groups in this respect persisted for approximately one month. Plasma iron levels were significantly increased in the irradiated monkeys on the fifth post-exposure day although there were no significant differences among the three dosage groups. A second hyperferremic phase was observed in the lowest dosage group. Reticulocytes were absent from peripheral blood by approximately the fifth day after exposure. Only three of 10 irradiated monkeys survived. Hypoferremia was seen in all irradiated monkeys prior to death. Repetition of the studies (using supplemental groups of survivors) 34 days after exposure indicated that erythropoiesis was recovering from the radiation injury.
80. Haley, T. J., Flesher, A. M. and Komesu, N.
Effect of X-irradiation on Bound Iron and
Unsaturated Iron-binding Capacity in Rabbits.

Acute whole body-X-irradiation produced a hyper-
erremia in rabbits. This effect reached its peak
on the 4th day, at which time the iron-binding
globulins were almost completely saturated. The
radiation dose did not affect the iron-binding
mechanism even when the globulins were saturated
prior to irradiation because the saturation re-
sulted in a prolongation of the period of
irradiation hyperferremia. The results support
the theory that irradiation hyperferremia is a
result of decreased iron utilization by the bone
marrow. There does not appear to be any relation-
ship between radiation lethality and hyperferremia.
(Author)

81. Baum, S. J.
A Measure of Nonreparable Injury to Hematopoietic
Stem Cells in Rats Exposed Repeatedly to X-rays.

Groups of polycythemic rats were irradiated, then
injected with erythropoietin of known potency at
various times between 1 hr. and 18 days post-
irradiation. Two days after erythropoietin
administration, the rats received intravenous $^{59}$Fe.
Seven days later $^{59}$Fe incorporation into new
erthrocytes in peripheral blood was measured by
radioactivity counting. Nonirradiated polycythemic
controls were treated in the same way.

The procedure was repeated after a three month
interval, using the same rats. Some groups were
also subjected to a third irradiation after another
three-month rest, then studied for six days post-
irradiation. Results showed recovery to about 75%
normal $^{59}$Fe incorporation by the sixth day in all
cases, but the rate of achieving this recovery was
diminished after the second irradiation, and
diminished still further after the third irradia-
tion. The stimulation of erythropoiesis in
polycythemic animals by erythropoietin is con-
sidered to be a measure of stem cell release, and,
inversely, as a measure of radiation injury to the stem cell population. After the sixth day, however, $^{59}$Fe uptake fluctuated widely, suggesting that other factors were operating to control erythrocyte production. The authors assert that the first 5 to 6 days constitute a "rapid repair phase" in which the red cell renewal system would be fully stimulated and a reduction in stem cell population would be apparent. After six days, however, the recovery process slows down, fewer stem cells are utilized, and the reduction in cell population is marked.


Serum iron binding was studied in 46 hematologically normal adults (mainly hospital personnel): 22 women ranging in age from 21-65 years and 24 men ranging in age from 21-84 years. Serum iron (SI) levels were measured spectrophotometrically using bathophenanthroline to complex with free iron. Latent iron-binding capacity (LIBC) determinations were performed with the $^{59}$Fe saturation excess method. Total iron-binding capacity (TIBC) is the sum of SI and LIBC and reflects the total concentration of transferrin in the serum. The diurnal variation in serum iron described by others was observed and the LIBC also showed diurnal variation but in the direction opposite to that in SI. As a consequence, TIBC remained constant.


Iron-52 is a positron emitter that has been used in the Hammersmith Hospital, London, since 1957 to investigate distribution of erythropoietic tissue in patients. Studies utilize coincidence counting techniques. The short half-life (8.2 hours) have made it possible "to investigate the effect of cytotoxic agents and ionizing radiation on marrow function by serial measurements at various intervals." The isotope is produced by bombarding
high-purity chromium targets (placed at grazing incidence to the beam) with α-particles accelerated in the Medical Research Council cyclotron. The target is placed in the "dee-box" and bombarded for 1 hour. Details of the preparation of the carrier-free isotope are presented. The various aqueous and organic solutions are automatically manipulated by a Technicon proportioning pump. Extraction efficiency is better than 85%. Some long half-lived iron-55 is also present, and to keep the proportion of the long-lived isotope as low as possible, the iron-52 is used as soon as possible after the target has been bombarded. Not more than 3 hours are required for chemical processing.

Evans, A.

C3H mice and beagles were subjected to (whole body) monoenergetic 14 MeV neutrons or mixed gamma-neutron radiations. The mice were exposed to both sources at a steady state rate of ~ 20 rads/min. The dogs received pulsed doses of mixed gamma-neutron radiations. The protein-bound carbohydrate concentration (PBC) in the plasma of the animals was quantified as a function of time relative to irradiation. The pre- and postirradiation PBC appeared to constitute a crude index of radiosensitivity prior to and prognosis after irradiation of these otherwise healthy individuals. Thus, the mean preirradiation PBC of the animals which died was consistently higher than that of the 30-day survivors. After irradiation, a striking difference was observed between those radiosensitive animals which died during the first week after exposure and the more resistant individuals which, although exposed to identical doses, survived the experimental period. Preparative fractionation of the blood plasma by combined molecular sieving and ion exchange chromatography revealed major radiation-induced changes in the concentration of components.
tentatively identified by acrylamide gel electrophoresis as siderophilin, haptoglobins, 
β-glycoproteins, and α2-macroglobulins, all of which are rich in bound carbohydrate. Ultra-
structural changes in organelles thought to be vital in biosynthesis of complex proteins which
parallel the biochemical findings are shown in the hepatocytes of irradiated mouse livers. The impli-
cations of these results as a clue to a portion of the mechanism of radiation damage to physiological
functions as well as their impact on the philosophy of experimental design in radiobiology are dis-
cussed. (Author)


A 19 S α-macroglobulin fraction isolated from the serum of rats and mice increased the survival among
mice exposed to 750 R. The mouse macroglobulin also enhanced radiation recovery of hematopoietic
tissue as measured by colony forming assay and 59Fe incorporation into the erythroid series of
cells. In mice exposed to 400 R and then treated with the macroglobulin fraction, hematopoietic
activity was increased three- to five-fold in the marrow (compared with untreated, irradiated control
mice) and nine- to ten-fold in the spleen. Other serum protein fractions of smaller molecular weight
had no such effect.

86. Harris, P.
Acute Radiation Death Resulting from an Accidental Nuclear Critical Excursion.

The author describes the physical characteristics of the radiation source in the Los Alamos criti-
cality accident of 30 December, 1958, and gives a detailed account of the radiation dosimetry, in-
cluding estimation of the neutron dose. This is a lucid, concise and highly informative presentation
of the various approaches taken to collect data, of the assumptions made in interpreting the data
and of sample calculations.

The accident described in this group of reports occurred at the Los Alamos Scientific Laboratory on 30 December, 1958, and resulted in the death of one man following the largest accidental exposure ever received by a human. The average whole body dose was estimated to be between 3,900 and 4,900 rad. This man died 35 hours after exposure, the clinical picture being that of severe central nervous system injury and circulatory failure. The supplement contains 9 separate reports concerned with a description of the accident, clinical course of the casualty, clinical pathology and biochemistry, gross and microscopic pathology, special studies, dosemetric calculations, health physics studies, report on other personnel exposure and a summary.


Electron spin resonance techniques were applied to various tissues of rats following exposure to 60Co gamma radiation. Of the tissues studied, bone, tail, hair, teeth and skin gave readily measurable signals, but skin and hair were found to be relatively poor tissues to use for estimating dose because skin samples were difficult to prepare for the measurements and there was a large variation between signals obtained when multiple hair samples were compared. Teeth and femurs removed from rats 15 minutes after irradiation showed a linear dose-response relationship for doses of 900-27,000 rads. Teeth gave a greater signal/noise ratio than did bone (femur) and effects of radiation doses ranging from 80-430 rads could be detected when repetitive scans of incisors were averaged in a computer. Preliminary studies with human fingernail clippings irradiated in vitro with 4 krads
demonstrated that this tissue gives a readily measurable signal that has a complex decay pattern including a component easily detected 15 hours after exposure.

The authors conclude that electron spin resonance measurements of teeth offer a potential in vivo dosimetry system for accidental irradiation, over the dose range of 100 to 30,000 rads. Intrinsic variation of the techniques for estimating x- or gamma-ray exposure is said to be of the order of 50-100 rads at doses of 100-1000 rads if multiple samples are examined. Efficiency of resonance production decreases as more densely ionizing radiation is used. Specimens stored at low temperatures (-196°C) retain their resonances indefinitely.

89. Brown, W. M. C. and Abbatt, J. D.
The Relation of X-ray Dose to the Time of Development of Radiation Sickness Following Exposure to a Single Dose of X-rays.

The authors present data, obtained from studies of patients receiving therapeutic x-irradiation, indicating that total-body integral radiation dose cannot be correlated with severity of radiation sickness. They suggest that expression of radiation dose be qualified by the level of dose received in specified organs and tissues of known radiosensitivity.

Dose Rate in Mammalian Radiation Biology
Conf. -68C410 Biol. and Med. (TID-4500)
USAEC Division of Technical Information Extension, Oak Ridge, Tenn. (1968).

The texts of 25 papers presented at a Symposium on Dose Rate in Mammalian Radiation Biology are presented. The effects of both protraction and fractionation of radiation dose on response are considered by the various authors, and both immediate and delayed effects are included. Data
presented include those from studies on cells irradiated in tissue culture, experimental animals irradiated in various ways and radiation therapy patients. Questions asked and answers given during discussion periods following the oral presentation are included.


In March, 1962, a family consisting of a father, a pregnant mother, a 10 year old son and a three year old daughter moved into a house in which the previous occupant had left a 5 curie lead-enclosed $^{60}$Co source. The family had been asked to look after the object but they did not know what it was. Shortly after the family moved into the house, the source, in some way, was removed from the lead capsule. The 10 year old boy carried the unshielded source in the pocket of his trousers for about a week. The mother then placed the source in a drawer in the kitchen. In April the grandmother joined the family in the house. All five persons were exposed to the $\gamma$-radiation over a period of approximately 5 months. One by one the family members became ill and were hospitalized. The boy died on April 29, the mother on July 19, the three year old girl died in mid-August and the grandmother in mid-October. The clinical and laboratory findings are presented. The presenting symptoms were those of infection and hemorrhage; the findings those of severe bone marrow hypoplasia. The cause of the injury was not suspected until after the boy and his mother had died. Precise dosimetry was not possible, but best estimates indicate that the family received $\gamma$-ray doses ranging from (approximately) 1200 R to 4700 R during the protracted period of exposure.
Holloway, R. J., Leong, G. F., Ainsworth, E. J., Albright, M. L. and Baum, S. J. 
Recovery From Radiation Injury in the Hamster as Evaluated by the Split-dose Technique. 

Using the split-dose technique (see ref. #93), the authors determined the LD<sub>50</sub>(30) for hamsters at various times up to 20 days after a "conditioning exposure" to approximately 2/3 LD<sub>50</sub>(30) of 1-mVp X-rays. The recovery pattern was found to be complex. There was only 6% recovery on the third day, but by the fifth day, the "percent remaining injury" was less than 40% and on the seventh day, was only about 25%, the lowest value observed. Thereafter, however, radiosensitivity increased again, so that on the 11th day, 75% of the "initial injury" was observed. When measured once more, eight days later (20 days after the conditioning exposure) the remaining injury had again decreased to 20%. Changes in peripheral blood picture are evaluated in light of the mortality rates at various times after the conditioning exposure. The authors speculate that it would be profitable to study the kinetics of cellular proliferation of hematopoietic precursors in searching for an explanation of the observed "reversion" towards greater remaining injury after the initial phase of recovery.

Nachtwey, D. S., Ainsworth, E. J. and Leong, G. F. 
Recovery from Radiation Injury in Swine as Evaluated by the Split-dose Technique. 

The studies described are part of a series of studies of several species of small and large animals exposed first to a "conditioning" dose of X-rays (approximately 2/3 of the LD<sub>50</sub>), then after periods ranging from 3 days to 107 days, exposed to X-rays a second time ("challenge exposure") to evaluate the recovery from the first dose. Recovery pattern is expressed in terms of percent recovery as a function of time after the conditioning exposure.
Death of the animals was the major criterion of effect. According to this criterion, swine had recovered from 51% of the initial injury within three days. By the seventh day they appeared to have recovered from approximately 65% of the initial injury and by 20 days, the entire population appeared to be radioresistant, the LD$_{50}$ at that time being 16.4% of the LD$_{50}$ of unconditioned animals. The pattern of systemic recovery and the period of radioresistance were not clearly reflected in the peripheral blood picture following a conditioning exposure. The authors emphasize that the phenomenon commonly described as 'recovery' must be considered, in these observations, not as a simple recovery that might be expressed in terms of repair of a certain number of roentgen-equivalents, but as a time-dependent change in radiosensitivity after a sublethal exposure.


The hazard of ionizing radiation in space is described, including the Van Allen belt in which radiation intensities of 100 R per hour may be encountered and solar flares in which solar proton beams may deliver $3 \times 10^6$ R per hour. The importance of radiation dose rate is emphasized as the single most important variable influencing injury sustained by the exposed space traveler. For example, dogs could tolerate radiation doses of 720 R (250 kVp x-rays) when given at the rate of 2 R per minute, but 300 R (1000 kVp) given at a rate of 10 R or 50 R per minute caused 50% mortality. Other examples are also cited. Other data are presented to illustrate the importance of species differences and differences in metabolic rate with respect to the degree of injury incurred as a result of irradiation. A schematic diagram of an experimental approach to the problem of injury and recovery in mammals following radiation exposures at various dose rates is presented and the authors suggest that the experiment be supported by interested agencies.
95. Odland, L. T. and Michaelson, S. M.
Some Observations on Dogs Following Lower Body Exposure to 1000 KVP X-rays.

Studies with dogs given varying doses of 1000 KVP x-rays to the lower body indicated that the 60-day median lethal dose is about 920 R; the limiting factor being the sensitivity of intestinal mucosa cells rather than those of the hematopoietic system. The acute clinical phase of lower body radiation injury is much shorter than with whole or upper body exposures, suggesting the gut and/or other abdominal viscera have a rapid component of total body recovery potential as opposed to primarily hematopoietic damage where recovery is much prolonged. Anorexia, weight loss, vomiting and hypersialosis were the most significant clinical changes, and these appeared immediately post-exposure persisting for 5-10 days. Fractionation of the single doses into four equal components given during brief session over as many consecutive days, decreased morbidity and mortality. Erythropoiesis was relatively unimpaired by the exposure of only the lower body. (Authors)

96. Lamerton, L. F. and Courtenay, V. D.
The Steady State Under Continuous Irradiation.
In: Dose Rate in Mammalian Radiation Biology
TID-4500, Conf. 680410
DTIE, Oak Ridge, Tenn.

Analyses of data from the authors' laboratory and from other laboratories are presented to demonstrate the way in which maintenance of the steady state by renewal tissues under continuous irradiation depends upon 1. the cellular radiosensitivity under continuous exposure at low dose rates and 2. the extent to which tissue can adapt to the continuous irradiation. Responses of intestinal epithelium, bone marrow and testicular epithelium under continuous irradiation in vitro are compared with respect to the nature of the physiological controls over their cell production and the intrinsic radiosensitivity of the proliferating cells.
Male albino rats originally of Wistar strain, were exposed to whole body x-irradiation at doses of 550 to 750 R and/or to thermal trauma over 16% of body surface for 1 or 1.5 min., producing second- to third-degree burns. Those animals that died between 0 and 8 days after treatment, with continual weight loss, were classified as "gastrointestinal-like" deaths. Those that died between 8 and 20 days after treatment or gained weight at any time before death were classified as "bone marrow" type deaths. Results show that "the administration of a sublethal thermal trauma ... caused a significant decrease in survival in rats exposed to x-ray doses between 550 and 750 R. The increase in mortality in the case of the combined injuries was confined to the time period characteristic of injury to the gastrointestinal tract - that is, to 3 to 8 days after x-irradiation." To test the hypothesis that the synergistic effect of thermal trauma on x-irradiation is due to a bacteremia, two groups of rats were given 5 mg of Aureomycin per day for 7 days following treatment. One of these groups had received 700 R of radiation, and the other had received 700 R plus a 1-minute burn. The antibiotic therapy produced no change in mortality. Water consumption and urine volume were recorded daily for the first 8 days after injury; both decreased after x-irradiation, then approached normal again by days 6 to 7. Addition of thermal trauma did not alter this effect significantly. Since other workers using different strains of rats have reported polydipsia and polyuria as effects of x-irradiation, it is suggested that these effects are strain-dependent. Thermal trauma without irradiation produced polydipsia and polyuria.

The authors describe a device they developed so that laboratory animals might be irradiated at dose rates that vary in the same manner as does the dose rate from radioactive fallout; i.e. as a power function of time. Results of preliminary experiments with mice are presented, demonstrating that the LD$_{50}$/30 for a simulated fallout exposure ($^{60}$Co gamma radiation) of 96 hours is significantly greater than the LD$_{50}$/30 for $^{60}$Co gamma radiation delivered at a constant dose rate over the same interval (1109 R and 1008 R, respectively).


Literature relevant to acute lethality studies of irradiated large animals is reviewed with the objective of establishing quantitative relationships between intensity of radiation and lethality. The experiments fall into two main groups, namely those concerned with LD$_{50}$ determinations and those concerned with survival under continuous irradiation. Data used in the analysis are presented in tabular form. The influence of dose rate and exposure time on median lethal dose for various species is presented graphically. There is loss of effectiveness of radiation with decreasing dose rate or protraction of exposure.

Recovery rates, calculated from the quantitative data on loss of effectiveness with protraction of exposure and decreasing dose rate, show that species may be ranked as follows in order of decreasing recovery rates: swine (fastest recovery), mouse, goat, dog, burro, primates. The estimated recovery rates are compared with recovery rates estimated from "paired-dose" experiments in which the first
or "conditioning" dose is 2/3 of the LD₅₀ (in rad). No simple function describes recovery kinetics for all species. Species are ranked according to these estimated recovery rates as follows: mouse, swine and dog as a group show most rapid recovery, goat and sheep are intermediate and burro and primate recover most slowly. Except for swine and mice, there is good agreement between results using the two approaches. The author emphasizes that very little is known about the basic physiological mechanism underlying the recovery phenomenon as studied in such experiments.

Radiation Time-Intensity and Pathophysiologic Correlations in Whole and Partial-Body X-irradiated Beagles.
In: Dose Rate in Mammalian Radiation Biology, Brown, D. G. & T. R. Noonan, editors,
TID-4500 USAEC-DTIE, Oak Ridge, Tenn. (1968).

Two series of Beagles were exposed to 1000 kVp x-rays. The first series consisted of animals given single-session, whole-body irradiation, dorso-ventrally, of 450 R, 600 R or 700 R at 1 R/min, or 400 R and 600 R at 11.4-12.2 R/min. In the second series Beagles were exposed bilaterally to 150 R - 400 R whole-body, 970 R - 2425 R upper-body, or 400 R - 1100 R lower-body in single session at 50-65 R/min or by fractionation at 12, 24 and 48 hour intervals at 50-65 R/min or 8-12 R/min. All were midline air exposure doses (MAD). Sham-irradiated animals were included for comparison. The estimated median lethal midline air exposure dose for the dorso-ventrally irradiated dogs was 657 R ± 30 R (S.D.) at 1 R/min and 502 R ± 22 R at 11.4 - 12.2 R/min. The regression lines were parallel. The estimated relative potency was 1.3 for 11-12 R/min and 2.1 for 50-65 R/min (bilateral) irradiation in comparison to 1 R/min as the standard. Survival time after exposure was inversely related to dose intensity as well as total dose. Shielding influenced lethality in relation to location of radiosensitive
body organs and systems. The 60 day median lethal doses in the second series were 308 R ± 18 R, 878 R ± 9 R and 1998 R ± 48 R with relative shield effectiveness of 1.0, 2.8 and 6.5 for whole-body, lower-body, and upper-body irradiation respectively. In addition to intensity of irradiation, fractionation, or the interval between exposures i.e. 12, 24 or 48 hours, was an important determinant of lethality. For whole-body irradiation at the LD50/60, fractionation at 48 hours was effective in reducing lethality; the relative effectiveness of 12 and 24 hour fractionation was considerably lower. The influence of radiation intensity or the interval between exposures was also reflected by the changes in hematologic indexes. Higher intensity irradiation was more effective in depressing various white blood cell components. The effectiveness ratio for hematological depression for 11-12 R/min vs 1 R/min radiation was 1.3 which is identical with the ratio for acute lethality production in the Beagle. The greater effectiveness of some fractionation schedules over others or over single exposures when selected portions of the body were irradiated suggests that cyclic variations in metabolic activity of specific cell types influence the synergism, augmentation, or diminution of the responses. (Authors)
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A STUDY OF EARLY RADIATION-INDUCED BIOLOGICAL CHANGES AS INDICATORS OF RADIATION INJURY

Final report on this aspect of treatment of radiation injury

Staff Report, Life Sciences Research Office

This document has been approved for public release and sale; its distribution is unlimited

This technical report is the second of three reviews of promising research opportunities to improve the treatment of radiation injury in the soldier and complements the first report on A Study of the Immunologic Aspects of Therapy of Radiation Injury in the Soldier, AD-674262, and a future report, A Study of the Metabolic Aspects of Therapy of Radiation Injury in the Soldier.

This study is a part of a broad review of current research on the biological effects of radiation to explore the possibility of developing improved treatment measures. Early biological changes that reliably indicate the degree of radiation injury a soldier has received would simplify the triage of radiation casualties and facilitate treatment regimens. Prompt diagnosis of the radiation-induced injury is important in prognosis and the military use of these clinical judgements will determine the immediate future role of the soldier.

The scope of the study included: the use of physical dosimetry; early clinical manifestations of radiation-induced emesis; radiation-induced cytological changes in the testes; urinary constituents as predictors of radiation injury; hematologic changes; serum iron levels as indicators of hematopoietic dysfunction or injury; ethiocolanolone in estimating bone marrow granulocyte reserves; total protein-bound neutral hexoses in the plasma as related to radiation sensitivity; effects of radiation on detoxification enzymes, drug metabolism, and biochemical changes; and chromosome aberrations as indices of radiation injury. Suggested areas for future research are emphasized. A critical review entitled Clinical and Laboratory Observations Useful in Estimating Degree of Radiation Injury and an annotated evaluative bibliography constitute Part II of the report.
Bibliographic Survey of Indicators of Radiation Injury

Biological Indicators of Radiation Injury

Radiation-Induced:

- Biochemical Changes
- Changes in Granulocyte Reserves
- Changes in Urinary Constituents
- Chromosome Aberrations
- Cytological Changes in the Testes
- Emesis
- Hematologic Changes
- Serum Iron Level Alterations

Treatment of Radiation Injury