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# BIOLOGICAL EFFECTS OF IONIZING RADIATION

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FOREWORD

No references are given in this chapter because it is intended to be orientative in character and because the number required would greatly increase its length. Some 5000 references, many annotated, have been collected in this field. It is planned to publish them separately.

## BIOLOGICAL EFFECTS OF IONIZING RADIATIONS

### 1. Introduction

The term ionizing radiations is currently used in a loose sense to refer to any type of radiation, either electromagnetic or particulate, which is capable of producing ions in the media through which it passes. The more common radiations which fall into this class are x-rays, gamma rays, alpha and beta particles, and neutrons, and although the discussion in the present chapter draws heavily on the more extensive observations relating to x-rays, an effort has been made to present as broad a picture of the biological effects of ionizing radiation as is warranted by the data presently available.

The physical properties of the radiations under consideration have been reviewed in the preceding chapter. It is worthy of additional note, however, that ionization is not the only process, or for that matter, insofar as chemical and biological effects are concerned, perhaps not even the most important process which occurs when ionizing radiation passes through a given medium. At the outset it should be recognized that ionizing radiation is merely a generic term, now firmly entrenched in our language, which is widely used for convenience. The cause and effect relationship between ionization per se and the chemical and biological effects of ionizing radiation is not nearly as certain now as it seemed to be some 10 or 20 years ago.

Ever since the earliest observation that ionizing radiations are capable of producing biological change, much interest has been evidenced in the manner in which these changes are brought about, and the proposals put forth have have been both many and varied. Most of these may be grouped under two generalizations, known respectively as the direct or target hypothesis, and the indirect or poison hypothesis.

The postulate common to all proposals included in the target hypothesis is that the ionizing radiation acts directly on a very small biologically sensitive structure, i.e., the target, and that the phenomenon under consideration results whenever the corresponding target is hit. Various opinions have been expressed as to the physical interpretation of the target, as well as to what constitutes a hit. Some workers feel a hit is registered when a photon is absorbed within the sensitive volume, others that the production of a pair of ions is the essential process, and still others that the passage of an electron through or near the target is sufficient. In certain instances it is suggested that a target must be hit several times in order to produce the observed effect. Some workers insist that the target hypothesis is strictly a mathematical concept, devoid of unique physical interpretation. This point of view is perhaps not unreasonable since the evidence offered in support of the target hypothesis is mainly of a statistical nature, dealing for the most part either with survival data or the frequency and distribution of genetic changes. On the whole, the target hypothesis has probably been more popular than the poison hypothesis.

The poison hypothesis has as its basic postulate the concept that the radiation produces a certain concentration of some poisonous material which, by one means or another, results in the changes observed. Numerous opinions have been expressed concerning the identity of these hypothetical poisons and the primary effects they produce. Hydrogen peroxide and various forms of "active water" have often been suggested as the specific poison, and are considered to arise by the action of radiation on water which is present in all living materials. Other workers have maintained that the demonstrable effects of ionizing radiation results primarily from a disturbance in cellular physiology secondary to inactivation of enzymes, denaturation of essential proteins, alteration of colloidal properties or changes in protoplasm viscosity, all of which can actually be produced in isolated systems. Other opinions are to the effect that the basic abnormality lies in changes in cell membranes, cellular water balance, etc.

The poison hypothesis finds strong support in observations such as those in which irradiation of nutrient media followed by inoculation with a test organism results in the same lethal effect as is obtained when organisms growing in the media are irradiated directly. Water irradiated with x-rays is said to be only slightly less toxic for paramecia than the same amount of radiation delivered to an aqueous suspension of the organism, and vaccinia virus is apparently inactivated by water previously exposed to alpha radiation. The growth of young plants has repeatedly been shown to be retarded more by blood from animals previously subjected to whole body irradiation than by blood from non-irradiated controls. There are also numerous reports to the effect that irradiated tissue shows more damage from a given exposure when the nutrient medium is also exposed and subsequently left in contact with the tissue. It is readily apparent that observations such as these raise formidable questions whenever one attempts a quantitative evaluation of the biological effects of ionizing radiations.

A further difficulty in interpreting the biological effects of ionizing radiation arises when one begins to consider the time relationships which exist. As is well known, there is usually a lag period between exposure and the appearance of biological effects. Since the primary effects of the radiations are usually considered to be instantaneous, the lag phenomenon has been interpreted as inherent in the biological test systems. This is not unreasonable since delayed responses are commonly obtained when the same systems are subjected to various other noxious agents. Doubt is cast upon the validity of such a view, however, by recent studies on the depolymerization of thymus nucleic acid, in which it was found that changes continued to occur for as much as

10 hours following a single, more or less instantaneous exposure to x-rays.

During the past three decades a great deal has been learned about the properties of atoms and molecules, and the manner in which ionizing radiations interact with them. During this same period, considerable advance has also been made in the general understanding of chemical reactions, and it has become increasingly clear that there is no essential difference between the chemical reactions which follow exposure to ionizing radiations and those induced by heat or exposure to ultraviolet light. With the development of this point of view, it has been recognized that the target and poison hypotheses merely represent a focusing of attention upon different aspects of the complex processes actually occurring in biological materials, and that both hypotheses are far too simple.

## 2. Physical and Chemical Considerations of the Effects of Ionizing Radiations On Biological Systems

Biological materials, in the last analysis, are composed of molecules and for the purpose of orientation it is convenient to begin a consideration of the biological effects of ionizing radiations with a consideration of the changes which occur in isolated chemical systems subjected to these same radiations.

Consider first the general nature of chemical reactions. A stable chemical system is represented by such an arrangement of the constituent atoms that the potential energy of the system as a whole is less than that of any slightly different arrangement. For a given number of atoms there may exist several stable arrangements. For example, in the case of two hydrogen and two iodine atoms there are two stable arrangements, i.e., when like atoms are paired giving $H_2$ and $I_2$ and when the unlike atoms are paired giving two HI molecules. A chemical reaction consists of passing from one stable arrangement to another, and during this transition, the potential energy of the system is greater than that in either stable arrangement. The amount by which the potential energy must be increased before a change to some other stable system is possible reflects the stability of the system under consideration. The larger the required increase, the more stable the system. In a general way it is seen, therefore, that a chemical reaction can occur whenever the system under consideration can exist in more than one stable arrangement, and sufficient energy is available to bring about the transition from one of these arrangements to another.

Another way of stating this general concept is that a stable system, i.e., a molecule or a stable collection of molecules or atoms, is surrounded by an energy barrier and that this barrier must be crossed during the course of a chemical reaction. The essence of reactions induced by ionizing radiations is that the radiation supplies the energy necessary to make passage across these energy barriers possible. A frequently used analogy is that of an explorer in a mountainous region. Picture him in a basin surrounded on every side by mountains with passes extending in several directions. Each of these passes leads to another basin, which in turn is surrounded by more mountains with passes. Provided the explorer has sufficient energy he may ascend through any of these passes and descend again to the basin beyond. So it is in the case of the stable chemical system which, providing it gains the necessary energy, may cross the energy barrier, usually by way of the lowest path, and pass into a new stable arrangement. In terms of the general concept, crossing the energy barrier constitutes a chemical reaction. It is important to recognize that as the topography of the mountainous country in the present analogy is fixed by nature, so the relationships of the energy barriers surrounding a given stable arrangement of any collection of atoms are fixed by the properties of the atoms present. The explorer's only choice is the decision as to which pass to go through. By the same token, the energy made available by exposure to ionizing radiation, or by any other means for that matter, only makes it possible for the system in any one of the possible stable arrangements to cross the restraining energy barrier and reach one of the adjacent stable arrangements. In short, exposure to ionizing radiation can bring about only those reactions inherent in the collection of atoms under consideration.

It should be added that the energy barriers surrounding different stable arrangements of a given group of atoms are not necessarily equal, and that once the system has passed over the first barrier it may find itself surrounded by a low barrier which can easily be crossed in any number of directions, each of which leads to the formation of different products. For this reason, the over-all chain of events may be, and usually is, extremely complex and the end products very numerous.

An increase in the energy associated with a given arrangement of atoms may occur in various ways -- by an increase in translational motion of the arrangement as a whole, by an increase in rotation or internal vibration, by an increase in electronic energy, or by some combination of these. In thermal reactions, molecules gain sufficient energy to cross energy barriers as a result of molecular collisions in which a redistribution of translational, vibrational and rotational energy occurs. In photochemical reactions, the primary energy supplying process is classically considered to be an increase in electronic energy as a result of radiation from an external source. In any medium subjected to ionizing radiation there exist a number of processes whereby incident energy is systematically converted to molecular energy. At various stages in this conversion relatively large amounts of energy are localized in individual molecules, and these energy rich molecules are capable of entering into chemical reactions. When it is recalled that the electronic excitation which occurs with ionizing radiation is not limited to specific atoms and atomic linkages, as in the case of photochemical reactions, and that the increase in thermal energies is not diffuse, as in a system subjected to high temperatures, and that biological materials are multiphase systems in each of which many reactions are possible, it becomes clear that the changes induced in biological materials subjected to ionizing radiation are indeed very complex, and in all probability irreversible. This irreversibility is not so much due to the irreversibility of the individual reactions, but to the extremely small probability of attaining that precise distribution of energy which would be required to force the many chemical systems which are involved back over their respective energy barriers to their initial positions. The effects of ionizing radiations on biological systems often appear to be reversible, however this should probably best be thought of as a repair or recovery phenomenon characteristic of living systems rather than a true reversal. The possibility of exactly reversing the biological effects of radiation by the judicious application of chemical agents is likewise extremely slight because of the complexity of the changes which are involved. It must be admitted, however, that an observed biological effect may possibly be due largely to a very few specific changes, and that in such a case the biological effect might essentially be reversed with the aid of chemical agents possessing the appropriate activity. This is the position taken by E. G. Barron, for example, who postulates that the biological effects of ionizing radiations are largely due to oxidation of -SH containing enzymes, and that specific reactivation can be accomplished with glutathione.

One further point deserves mention in connection with the very broad picture of radiation effects which has been outlined, and this deals with the distribution of energy within an irradiated medium. It is well known that definite differences in energy density and energy localization occur with various radiations, and depend to some extent on the elemental composition of the medium. Since chemical reactions will occur only where energy is available, it follows that the biological effectiveness of various radiations may well be determined, in part at least, by the particular distribution of energy which results. There is as yet, however, little information regarding the exact contribution of energy distribution to the over-all problem of radiosensitivity, and further discussion of this interesting point is deferred.

On the basis of the preceding discussion, one would predict that almost any chemical reaction which can be brought about by heat or exposure to ultraviolet light can also be brought about by any of the ionizing radiations. This is essentially true, however one must add that the quantitative distribution of energy is much different, and that decomposition and recombination play a prominent role in reactions induced by ionizing radiations. As a result, the yield of any one product is usually very slight.

A great deal has been, and is being written, regarding the relative importance of ions, free radicals, hot molecules, activated water, etc., etc., in the detailed mechanism of chemical reactions induced by ionizing radiations. While these questions are all of great importance, their contribution to the over-all problem of the biological effects of ionizing radiation has not yet been very great and consideration of these points may be passed over for the present. In passing it should be emphasized, however, that the purely physical and chemical approach to the biological effects of ionizing radiations is an extremely difficult problem in probability. In principle, it is quite clear that the sole effect of ionizing radiation is to supply energy, which in turn will lead to some or all of the reactions which are possible in the irradiated systems. In attempting to apply this principle to biological materials, however, great difficulties arise because of the tremendous number of ways in which the energy may be parceled out and the astronomical number of reactions which are possible. Add to this the paucity of information regarding the detailed chemistry of living systems, and it seems quite fair to say that progress in unraveling the long chain of events which leads from the absorption of incident radiation to detectable biological change will be extremely slow, unless it actually turns out, as has been suggested, that the biological effects of ionizing radiations are largely mediated through a few key substances, of which water is a promising favorite.

3. Morphological and Physiological Changes Observed in Biological Systems Subjected to Ionizing Radiations

Changes in irradiated cells and tissue are characterized by their detrimental effect and by marked variability both in kind and magnitude. The effects of ionizing radiations on biological systems are not unique for it has been demonstrated repeatedly that diverse chemical and physical stimuli are capable of producing similar cellular responses. Morphological changes for the most part reflect relatively gross damage. Altered cellular physiology, however, results whenever a cell is incapable of compensating perfectly for any modification, large or small, in one or more of its components.

Several attempts have been made to devise some criterion for predicting the radiosensitivity of various cells and tissues, and two hypotheses which have become firmly entrenched in the literature deserve some mention. The first of these is based on the premise that the most actively dividing cells are the most radiosensitive, and that radiosensitivity in general is correlated with high division rates. There are obvious exceptions to this rule, since many tissues and cells characterized by extremely rapid growth, e.g., malignant melanoma, sarcomata, bacteria, and unicellular animals in general, are also characterized by extreme resistance to radiation damage. The second hypothesis devised to explain variable radiosensitivity is that more poorly differentiated cells tend towards greater radiosensitivity. Well known exceptions to this general rule, such as the reticuloendothelial cells which are poorly differentiated but extremely radio-resistant, are also numerous. When it is recalled that the immediate effects of ionizing radiations on cells are changes in individual molecules, which in turn initiate a sequence of events which lead to observable physiological and morphological changes, it is clear that generalities such as those mentioned above hold little promise as an ultimate explanation for differences in radiosensitivity among cells.

The problem of radiosensitivity presents numerous experimental difficulties. Many subtle changes certainly occur before gross results are noted, and other changes may appear after the period of observation has been completed. A dose of radiation which is not lethal and does not produce an immediate gross alteration may, therefore, still have a definite effect on the cell. Furthermore, the ability to survive a given dose reflects recuperative powers as well as susceptibility. In view of these considerations, it seems fair to regard the susceptibility of cells to radiation damage, as determined by usual experimental procedures, as representing the apparent and not necessarily the real radiosensitivity.

3.1 General Considerations of Changes in Cells Exposed to Ionizing Radiation

Many observations of radiation effects are concerned with growth, development, and differentiation of single cells. The observations on growth may be summarized in the generalization that with varying amounts of radiation, growth may be unaffected, temporarily or permanently inhibited, and/or altered in pattern. Examples of the latter are tumor induction, irradiation-induced somatic mutations, and the alterations in growth pattern which may be observed in irradiated tissue cultures. It has been repeatedly demonstrated that the apparent stimulating effect of radiation is a physiological artefact in which the increased mitotic rate after irradiation represents a recovery phase; a purely compensatory response following suppression of mitosis. After doses small enough to permit complete recovery, the increased mitotic rate which follows the initial depression is sufficiently high to allow the irradiated cells to "catch up" with the controls, as regards the number of cell divisions. The compensatory increase becomes progressively less marked as the dose increases, and in severe radiation injury, the compensatory increase in mitosis may be absent.

Increased permeability of cellular membranes as a result of x-irradiation has been stressed by many authors. Although this effect has been produced by irradiation in some instances, its relative importance in the over-all response has yet to be convincingly evaluated. In extreme radiation injury, the cell may become completely permeable with total disruption of its physico-chemical processes and dissolution. With milder degrees of damage, however, permeability may appear to be altered selectively. Altered distribution of Ca and K in irradiated skin for example has been interpreted as an indication that the permeability of the cells and tissues to the respective cations had been altered. Similarly, an increase in cytoplasmic nucleic acid and a decrease in nuclear nucleic acid content have been interpreted by some investigators to be an indication of altered nuclear permeability. The increase in ascitic fluid protein after total body exposure to x-rays has been interpreted as representing increased vascular permeability.

Physico-chemical changes other than altered permeability have also been demonstrated in irradiated cells. Liquefaction and coagulation of cytoplasm have both been described as irradiation effects. L. V. Heilbrunn postulated that cytoplasmic coagulation reflects increased permeability of the injured cell to calcium, and hence is analagous to blood clotting. In view of the fact that mitosis is accompanied by changes in cytoplasmic viscosity, and especially by a marked increase in viscosity during metaphase and early telephase, several investigators have speculated that increased mitotic rates following irradiation may also stem from altered viscosity.

Many attempts have been made to determine the role played by the various cellular components in the response to irradiation. There appears to be good general agreement that nuclei are particularly susceptible to radiation damage, and it has further been suggested that within the nucleus the chromatin material, particularly desoxyribose nucleic acid, is responsible for the radiosensitivity. Delayed, inhibited or otherwise abnormal mitosis following irradiation has been extensively studied and has been correlated in some instances with changes such as "stickiness" and "clumping" of chromosomes. In this respect it is of interest that doses necessary to produce transient depression of mitosis in a tissue may be many times smaller than doses required to produce other visible changes.

The roles of the various cytoplasmic structures in cellular responses to irradiation are not clear and none of the observed changes are pathognomonic of radiation damage. Observations include the presence of multiple centrioles in tissues in which multipolar mitoses have been induced by irradiation; the formation of vacuoles, the disappearance of vacuoles, and degenerative changes in cytoplasmic inclusions.

The lethal effect of x-rays and the effect of x-rays on cell survival have been widely studied from a statistical point of view. In most instances the amount of radiation necessary to kill a cell is extremely high compared to doses which produce barely detectable changes. Likewise doses which produce cell death at some time after irradiation are considerably smaller than those which kill the cell immediately. It is doubtful that the cause of death is the same in the various situations, and it is not entirely clear at present what the nature of the lethal derangement is in any one instance. Disruption of one process on which many other cellular processes are dependent may be "magnified" many times in that the entire cellular metabolism may ultimately be affected. On the other hand, the strong tendency of cells to recover from damage if given a reasonable opportunity probably influences results in all radiation experiments, particularly statistical studies concerned with survival.

3.2 Changes Observed in Lower Animals

Unicellular animals are among the most radio-resistant found, and factors governing their response to irradiation are difficult to evaluate. Six thousand r of soft x-rays proved lethal to Paramecium caudatum if the animals were kept in the medium in which they were irradiated, whereas if they were moved to fresh media shortly after exposure, it was necessary to double this dose in order to produce the same effect. X-rays have been found to sensitize these organisms to heat, the lethal heat exposure being reduced by approximately 88% after exposure of paramecia to 280,000 r. Whether or not such sensitization occurs as regards other noxious agents has not been determined. Changes in mineral metabolism and nitrogen metabolism (as determined by ammonia production) have been observed in x-irradiated paramecia. The influence of cell division on the sensitivity of paramecia to x-rays has not been definitely determined. Some observers have found increased sensitivity during the phase of low division rate, when the paramecium reorganizes after the previous division. Increased sensitivity of non-dividing paramecia has also been demonstrated in an experiment in which colchicine was used to inhibit division. Under conditions of this experiment, the lethal dose was found to be 50% less than for untreated, normally dividing controls.

The simpler multicellular animals show varying degrees of radiosensitivity, but in general have been found to be somewhat more radiosensitive than protozoa. This difference may in part be related to the fact that a single protozoan is relatively independent of other cells. In multicellular organisms, on the other hand, all cells are more or less dependent on each other and adjustments to environmental changes become more complex. There is marked variation in the dosages of x-rays required to kill different individuals of the same species, and further variations in the lethal dosages at various stages of development. The significance of a 100% or 50% lethal dose for the various simpler multi-cellular animals is consequently not entirely clear.

Studies utilizing x-irradiated trichinella larvae fed to healthy rats indicate that after 400 r the number of embryos produced in the intestine of the host is markedly reduced -- after 2000 r embryo formation does not progress beyond late cleavage stages, and after 5000 r the irradiated larvae do not develop into adults in the host. In the worm Rhabditis pellio, a dosage of 5000 r to the nearly adult worms resulted in arrested development of their embryos at an early cleavage stage. The irradiated adults themselves, however, lived significantly longer than non-irradiated controls. This apparent paradox is explained by the fact that the embryos are destructive to the maternal tissues and at the same time are more easily destroyed by irradiation. Prevention of embryonic development thus protects the female from damage, and a lengthened life span results.

### 3.3 Changes Induced in Sperm, Ova and Zygotes

Sperm, ova and zygotes are highly susceptible to radiation damage in almost all forms of living things. It has already been noted, for example, that in certain worms the dose required to prevent the formation of a second generation is considerably smaller than the dose required to kill the adult. Although various explanations have been suggested for the high radiosensitivity of these forms, none of them fits all the facts sufficiently well to be completely satisfactory.

Irradiation of either the sperm or the egg may result in the formation of non-viable zygotes, retardation of development, or the production of anomalous offspring. Rabbit spermatozoa x-rayed in vitro lose the ability to penetrate ova only after extremely high doses (approximately 100,000 roentgens). With doses as low as 500 r, however, the incidence of pregnancy, litter size, and viability of offspring are all decreased.

There is evidence that recovery may occur following irradiation of the ova in some cases. For example, if the unfertilized arabacia egg is exposed to 2600 r, and then fertilized, the first cleavage is delayed. If sufficient time elapses between irradiation and fertilization, this delay does not occur.

3.4 Changes Observed in Embryos

There is considerable variation in the apparent radiosensitivity of the various stages between the zygote and the end of the embryonic development. For example, when grasshopper eggs are irradiated immediately after being laid, 50 r is sufficient to inhibit embryo formation, whereas the lethal dose on the second and third day is 200 r and 500 r, respectively. In drosophila there is likewise a one hundred-fold increase in radio-resistance from egg to imago stage. This general pattern appears also to hold true for amphibia. In the case of higher vertebrates, however, the trend is not so well defined. Although it is generally agreed that sensitivity to radiation damage is greatest in the early stages of development, radioresistance does not develop in direct proportion to the advancing age of the embryo but increases in a more or less erratic fashion with several maxima and minima.

Delay in cleavage has been observed repeatedly and with sufficiently high doses, development may cease at this stage. Multipolar mitoses and the formation of more than two blastomeres has been observed in cases where either gamete of the sea urchin was irradiated before fertilization. The blastomeres resulting from multipolar mitoses have abnormal chromosome complements and may be nonviable or develop anomalously. The possibility of a similar process in somatic cells has been suggested as an explanation of the induction of malignancy by x-rays.

In many species gastrulation appears to be one of the most radiosensitive stages of embryonic development. Great radiosensitivity during gastrulation, however, should be considered in light of the fact that this stage in embryonic development is also remarkably susceptible to noxious agents other than ionizing radiations.

Irradiation of embryos beyond the stage of gastrulation may also prove lethal or result in defective young. In some species, if doses are sufficiently high, embryos die and are resorbed. In species where resorption does not occur, the result of comparable doses is abortion. Various developmental anomalies which result from sub-lethal exposures appear to bear some relationship to the stage of development of the embryo at the time of irradiation.

3.5 Tissue in Vivo

The many published opinions regarding the relative importance of factors which influence the biological response to irradiation indicate that there are few really definite findings on which to base interpretation of changes which occur in the tissues of irradiated multicellular organisms. The following brief account of changes which have been noted in tissues irradiated *in vivo* is therefore limited largely to the presentation of the observations *per se*.

3.6 Vascular System

The effects of irradiation on the vascular system are generally considered to be extremely important, and some radiologists feel that the therapeutic effectiveness of irradiation is due entirely to vascular changes. Presumably disruption of the circulation to an irradiated tissue curtails the nutrient supply, impairs respiratory exchange, hinders the removal of waste products and generally depresses metabolism.

Despite the central significance of vascular changes following irradiation and the extensive literature on the subject, the matter is still controversial. It is, or at least has been fairly well agreed, however, that the initial radiation-induced changes in blood vessels reflect damage incurred by the endothelial cells. Within a few hours or a day after exposure, these cells swell and effectively narrow the capillary lumen. Blood flow in the capillaries is slowed simultaneously. Damaged capillaries then become more permeable, allowing fluid and cells to leave the vessels and enter the tissue spaces. Unless there is co-existing infection in the irradiated tissue, lymphocytes are the first cells to migrate from the capillaries and their appearance in the adjacent tissue spaces in considerable numbers is particularly characteristic of radiation damage. Larger vessels have thicker walls and offer more resistance to the migrating cells. The characteristic cellular accumulations are, therefore, not in adjacent tissues but in the vessel walls where they may contribute to the process of narrowing the lumen. In extreme cases, complete occlusion may result. In general, doses necessary to produce marked direct effects on the arteries and veins are considerably larger than those required to produce equally apparent effects on capillaries.

The erythema which follows exposure to radiation has been formulated as follows: According to J. Borak, the initial capillary narrowing is due to swelling of the rhomboidal endothelial cells in their transverse diameter. With continued swelling, the endothelial cells elongate in their long diameter, and enlarge the capillary lumen. If radiation is sufficiently intense, the dilatation of superficial capillaries becomes apparent as erythema. The first wave of erythema subsides slowly, only to be followed by a second wave at some time after the first post-exposure week. The second wave is regarded as a compensatory capillary dilatation due to local ischemia resulting from narrowing of the arteries and veins, and it is this erythema which is particularly prominent, and which has been used as a method of dosimetry in x-ray therapy.

With sufficiently high doses, a third wave of erythema may be observed approximately one month after exposure. This has been attributed to damage to the veins, which because of their larger lumina require a longer period of endothelial swelling and cellular infiltration for narrowing to become significant. When narrowing has become extensive enough to offer considerable resistance to blood flow, the pre-stenotic portions of the vessels dilate, resulting in erythema.

The increased permeability of damaged capillaries results in edema which in turn exerts pressure on vessels in the edematous area. Veins, with their relatively readily compressible walls and low blood pressure, may be completely occluded by this pressure, particularly if there is already a marked degree of narrowing of the lumen. Extreme narrowing over a period of several months to a year, leads gradually to permanent dilation and tortuosity of pre-stenotic capillaries, a condition which is better known as telangiectasis. The importance of the external pressure in producing telangiectasis is well demonstrated by the fact that telangiectasis is particularly likely to occur in irradiated areas where there is sustained pressure, i.e., from a truss, scar tissue, bone, etc., etc.

In the event that small arteries are occluded (usually under conditions of extremely high dosage, extensive sustained edema and pressure from without) ischemia and tissue necrosis are the end results. If arterial narrowing is not complete, the result may merely be atrophy and depigmentation of the skin.

Changes in circulatory dynamics described above are based on observations of living tissues, usually after exposure to relatively large doses. Fixed tissue sections often fail to demonstrate these changes. In the extensive observations by Bloom et al on blood vessels in routine tissue sections from various irradiated experimental animals the characteristic changes were found to be predominantly in the perivascular connective tissue. Injury of collagen was evidenced by an initial haphazard disorientation of connective tissue fibers, altered staining , and after the initial acute response, hyalin changes and marked shrinkage of the collagen. In instances where collagen damage was extreme, there was a suggestion that vessel walls had actually disappeared, leaving only endothelium. The suggestion is put forth by this group that degenerative changes in the connective tissue might be the primary pathology.

It should be noted that most authors who have written on this subject, especially those who have actually contributed to the experimental work, appear to agree that details of the nature and sequence of vascular changes induced by various intensities of ionizing radiations have not yet been completely and conclusively elucidated.

3.7 Lymphoid Tissue

Lymphoid tissues (lymph nodes, spleen, thymus) rank near the top in the scale of radiosensitivity. Changes in the lymphopoietic tissues have been observed within an hour after a single exposure to x-rays. Changes are first apparent as cessation of mitosis, clumping of nuclear chromatin, pyknosis, and cell and nuclear fragmentation. These changes, which are progressive and result in the production of considerable debris, are soon followed by increasingly active phagocytosis. During the "quiescent period" in which there are no observable signs of further degeneration, phagocytosis may be the only active process apparent in the tissue. Providing the initial damage was not too severe, recovery begins before the debris has been cleared completely. The histological criteria of recovery are resumption of mitotic activity and repopulation of the tissue with normal cells. Recovery of lymph tissues is remarkably rapid after small single doses but is slow and incomplete in animals which survive massive doses. After small doses the only sign of change may be a decreased mitotic index, indications of mild nuclear damage in some cells, and some increase in phagocytosis. The picture may revert to normal in a very short time, e.g., within a few hours after 25 r of x-rays. The source of the parent cells which initiate the recovery phase after severe damage is obscure. They are probably either a few lymphocytes which were spared in the destructive process, or cells which survived in some other site and were carried via the circulation to the observed tissues to initiate repair.

Following large doses of radiation, or following a prolonged series of exposures, diminution in the size and activity of lymphatic tissue generally is a common finding. Inactive, shrunken, hypoplastic nodes overgrown with fibrous tissue of the reticulum are characteristic of gross over-exposure where repair has been incomplete.

3.8 Cellular Elements of Blood

Cellular elements of the blood and blood forming tissues have long been recognized as among the most radiosensitive of mammalian cells. In general, white blood cells are considered to be more radiosensitive than red cells, and of the leukocytes, lymphocytes are ranked first in radiosensitivity.

Following a single exposure to ionizing radiations the number of peripheral blood leukocytes decreases promptly and markedly. In rats, for example, a single whole body dose of 5 r of x-rays has been shown to produce a transient but highly statistically significant reduction of lymphocytes within 15 minutes after exposure. Lymphopenia was definite within 24 hours after exposure in heavily irradiated Japanese A-bomb victims. The lymphocyte picture demonstrates a remarkable capacity for recovery, which characteristically begins promptly and proceeds rapidly relative to the recovery trends of counts of other blood cells. (See Lymphoid Tissue). Depression of the number of granulocytes is characteristically somewhat less marked and less prompt than the lymphocyte depression and recovery tends to occur later and may be less extensive in some cases. Following large doses, the granulocytes characteristically show a transient increase prior to the onset of granulocytopenia.

Mature red cells appear to be relatively radio-resistant. A decrease in the number of mature erythrocytes in the peripheral blood occurs later than the depression of leukocyte count and occurs only after exposures which are many times the minimal effective dose for inducing leukocyte depressions.

It is interesting to note that after relatively large doses of x-rays, the reticulocyte response may be similar in promptness and magnitude to the lymphocyte response. Reticulocyte responses to extremely low doses, however, have not been studied extensively. Recent studies of both peripheral blood and bone marrow in experimental animals indicate that nucleated red cells may equal or surpass the lymphocyte as regards sensitivity to ionizing radiation. In man, however, at least in fatal cases after the Japanese bombing, erythroblastic foci were found to persist in the marrow after all other cell series had been completely depleted. The anemia induced by irradiation is believed to be a manifestation of damage to these precursors. A direct effect of x-rays on mature erythrocytes has been suggested by some investigators. Because of the extremely high doses usually required to produce extensive changes of this nature, however, direct effects of radiation on the erythrocytes are generally considered to have little clinical significance.

Most of the commonly accepted generalities regarding the radiosensitivity of various blood cells are based upon changes following a single exposure to a relatively large dose. Changes observed after a prolonged series of exposures to small doses may be considerably different. Experimental animals have developed persistant lymphocytoses and erythrocytoses following chronic exposure. At the Holt Radium Institute at Manchester, England, the significant hematological findings in the personnel working with radiation for long periods were a decrease in the mean leukocyte count and a relative lymphocytosis. Reticulocytosis and mild anemia have also been observed following chronic exposure, and the appearance of abnormal, immature cells in the peripheral blood of chronically exposed individuals has been noted by several observers. The significance of these observations relative to the occurrence of similar cells during pre-exposure periods has been determined in relatively few cases, however. After large single exposures, and occasionally after chronic exposures, plasma cells may be found in increased numbers in the hematopoietic tissues, and to a lesser extent in other tissues. They are seldom found in the peripheral blood.

The mechanism of radiation-induced alterations of blood clotting remains a controversial subject. In some species there is apparently a heparin-like substance in the blood of irradiated animals during the hemorrhagic diathesis. In other species, however, and under other conditions, the hemorrhagic diathesis exists in the absence of a demonstrable circulating anti-clotting substance. Thrombocytopenia apparently contributes to the abnormality, as do collagen damage and increased capillary permeability. The relative importance of these various contributing factors remains obscure.

3.9 Skin

The skin shows readily observable changes following exposure to relatively small doses of radiation. Almost immediately after exposure, mitosis decreases abruptly. Nuclear condensation and fragmentation, clumping of chromatin, abnormal mitoses and multi-nucleated cells may all be observed even after relatively small doses.

The inhibitory effect of irradiation on mitosis tends to be proportional to the dose and it has been suggested that the time required for mitosis to return to normal might be used as an index of the biological effectiveness of various types and quantities of radiation.

With small doses the effect on the epithelium itself is the most prominent feature, especially with frequent exposures to very low dosages. Larger exposures produce more or less extensive alteration of the small blood and lymph vessels, with migration of leukocytes into the connective tissue, and the production of erythema. (See Vascular System). If severe damage is incurred, the vascularity of the skin may eventually be permanently reduced, resulting in atrophic, shiny contracted skin and the production of altered pigmentation and telangiectasis.

The more marked degrees of damage may be associated with definite changes in various skin appendages. Hair follicles are usually affected by doses which are sufficient to produce "secondary erythema". The cells of the hair follicles show changes similar to those in other irradiated cells and the changes may be reversible or permanent depending on the dosage. In man, x-ray doses above approximately 700 r produce permanent epilation. The errector pilae muscles are relatively radio-resistant and may persist after the cells of the hair follicle have degenerated completely. The sensitivity of the sweat glands and sebaceous glands is of approximately the same order of magnitude as the sensitivity of the hair follicles. Pigmentation of hair is an independent process and may be altered independently of hair production.

Recovery of the skin from severe radiation injury depends to a considerable extent upon the reparative activity of the dermis which, in turn, is directly related to the integrity of the blood vessels. Normal appearing epithelial layers which have grown over eroded areas from surrounding tissues have been observed to slough off if the underlying connective tissue is sufficiently damaged. Total destruction of epithelium with the production of persistent ulcers represents severe skin damage and is particularly likely to result in neoplasia.

3.10 G. I. Tract

The small intestine, particularly the duodenum, is highly radiosensitive and within a few hours after an $LD_{50}$ in common laboratory animals, cytological changes such as nuclear swelling, pyknosis, and fragmentation may be observed. Degenerative nuclear changes in cells of crypts of Lieberkuhn have been described as early as 15 minutes after exposure. These visible signs of damage are not apparent in the cells of the villi until many hours or a few days later. Degenerative changes generally persist for a few days but are soon (usually within a week) followed by regeneration, which may be observed in areas of early damage while degeneration is still progressing elsewhere. Mucus secreting cells are much more resistant to radiation damage than crypt cells. Following extensive degeneration, the mucous cells may be the only intact epithelial cells apparent in the intestine. Even after very large doses these cells may survive, although in such circumstances they may be almost totally devoid of mucus. Following moderate doses of radiation, an absolute increase in the number of mucus secreting cells throughout the intestine has been observed.

The stomach appears to be only slightly less radiosensitive than the upper intestinal tract. Post-irradiation gastritis may occur following moderate doses, and in cases where it is severe may lead to ulceration or eventually to atrophy of the mucosa. Diminished gastric acidity following irradiation has been reported. However, this has not been a constant finding. It is of interest that some of the pigs exposed at Bikini developed achlorhydria and macrocytic anemia.

The large intestine is markedly radio-resistant in comparison to the small intestine. With sufficiently high doses, however, the large bowel too may be severely damaged. Stenosis of the bowel as a result of radiation has been observed in experimental animals, and permanent large bowel sequelae (e.g., strictures, fistulae and atrophic changes) are not uncommon complications in women treated with intensive radiation for pelvic malignancy. A sharp line of demarcation at the ileocecal valve with marked destruction proximally and minimal damage distally has been observed in experimentally radiated animals.

The role of the gastro-intestinal tract in the general picture of radiation damage is believed to be very important, and in this respect, several factors other than direct damage to the epithelium per se must be considered. The likelihood of allowing bacteria to gain entrance to the blood stream as a result of interference with the integrity of the mucosa has long been of considerable interest, and the contribution of G. I. tract damage to the production or enhancement of anorexia, diarrhea, water loss, diminution of food and water absorption, etc., continues to be the subject of considerable study.

Liver - The method of estimating radiosensitivity on the basis of visible changes is particularly poorly suited to the liver. The effect of irradiation on liver function, however, has been adequately studied in relatively few instances. If one accepts the production of visible changes as a criterion of radiation sensitivity, the liver must be considered as extremely radio-resistant. Visible changes, when they do occur, consist of congestion and dilatation of the sinuses, swelling of the parenchymal cells with vacuolization of the cytoplasm and other degenerative cytological changes. In the studies carried out by Bloom et al on the liver of amphiuma, the characteristic nuclear changes in parenchymal cells were described as "clumped" and "vacuous nuclei". Active phagocytosis of circulating debris by Kuppfer cells is a characteristic finding following large doses. In general, recovery from such radiation damage as may occur tends to be rapid and complete.

There are indications that although large doses of radiation are required to produce visible liver damage, smaller doses may be effective in producing other changes. Experimental studies on the production of specific proteases following irradiation with x-rays indicate that detectable increases in liver-specific protease occurs with exposures in the neighborhood of 330 r.

Pancreas - The pancreas appears to be exceptionally resistant to radiation damage. Histologically there are no definite signs of damage even after fairly long periods of exposure to radioactive isotopes deposited in the organ. Although the literature contains a few reports of damage to islet cells, parenchymal cells, etc., after large doses of roentgen irradiation, these effects have not been observed consistently. Deranged physiology following experimental x-irradiation has been reported only after extremely large doses, and in many instances, experimental results of this nature have not been reproducible. In most of the atomic bomb fatalities there were no radiation-induced changes which could be detected by routine histological studies.

3.11 Kidney and Urinary Tract

Results of experiments carried out by numerous investigators utilizing several species of animals and a wide range of radiation intensities and irradiation procedures indicate that doses equivalent to several thousand r of x-rays localized to the kidney, or whole body exposure to doses in excess of the $LD_{50}$, are required to produce extensive renal pathology. As in the case of many other tissues and organs, vascular damage appears to account for much of the observable change, and the damage is usually more marked in the kidney than in the ureters or bladder.

Experimentally, extremely heavy irradiation limited to the kidney may result in severe renal damage and renal insufficiency. In such cases, the characteristic early changes are extreme congestion and edema of the kidney. Degenerative changes in the tubular epithelium progress rapidly, and are usually accompanied by hemorrhage into the tubules. Changes of this nature were observed in some of the histological material from atom bomb fatalities.

3.12 Testes

The effects of irradiation on the gonads has been of considerable interest both to the layman, who tends to associate radiation with sterility, and to physicians and geneticists who have attempted to arrive at an impersonal evaluation of this problem.

Insofar as visible histological damage is concerned, the testes might be ranked among the most radiosensitive tissues. Changes in the seminiferous tubules of men have been described after single doses of as little as 50 r of whole body exposure. There also appears to be a tendency for frequently repeated smaller doses to "accumulate" as regards histological damage in the testes.

There is good general agreement that the precursors (spermatogonia) are the most sensitive of the testicular epithelial cells. Following an $LD_{50}$ dose, spermatogonia may show signs of destruction within a few hours; spermatocytes within a few days. Sertoli cells, interstitial cells, and the reticular cells along the surface of the basement membranes of the tubules appear to be remarkably resistant. The recent study by Bloom et al in experimental animals indicates that cells lining the basement membrane are the source of regenerating epithelium, Sertoli cells apparently contributing little in this respect. The difference in the doses required to produce barely detectable effects of the testes, and the dose required to produce complete and permanent sterility demonstrates the extreme range of sensitivity of the various cell types. Complete and permanent sterility occurs only after doses in excess of the lethal whole body dose for the species under consideration. Recovery to a state of apparently normal reproductivity has been observed following complete depletion of the spermatogenic series (exclusive of the undifferentiated tubule-lining cells). The extensive recuperative powers of the testicles are evidenced by the fact that resumption of apparently normal testicular physiology was the rule in individuals who survived extensive exposure in bombed Japanese cities.

Radiation sufficient to produce at least temporary depletion of one or more maturation stages of testicular epithelium usually results in reduction in size of the testicle. Reduction of testicular size may also occur after frequently repeated small doses of radiation. This is not necessarily - or usually - associated with sterility. Following large single or accumulated doses, an increased incidence of structurally abnormal sperm have been reported in experimental animals. Libido, which is not directly related to germinal epithelium, is not affected by damage localized to the testes.

The genetic effects resulting from irradiation of the testes are discussed in a later section of this chapter.

3.13 Ovaries

The ovaries like the testes, may undergo temporary destruction of epithelium as a result of exposure to radiation. As a rule, however, permanent sterility is produced only by extremely high doses. In fact, when permanent sterility in women is indicated medically, reliance upon x-ray therapy always involves the risk that the ovaries may recover function and require further treatment.

Degenerative changes in primitive ova and follicle cells occur within a few hours after irradiation, and following very large doses, may progress to complete destruction of these elements so that only the relatively radio-resistant interstitial tissue remains. In general, developing follicles are particularly sensitive, and primitive follicles only slightly less so. The ovary of the mouse appears to be singularly sensitive to irradiation as regards both the relatively low doses required to produce visible changes and the marked tendency towards the development of malignancy following exposure. It is interesting that in the mouse, in contrast to other common laboratory animals, externally administered beta radiation appears to have a pronounced carcinogenic effect on the ovary, even when other internal organs show minimal radiation effects.

In general, the ovary possesses great capacity for recovery from radiation damage. In this regard it should be mentioned, however, that decreased fertility, evidenced largely by decreased litter size, has been observed in many species of experimental animals following relatively large doses of radiation.

Radiation has been used to "stimulate" ovarian function in women, and continues to be used for this purpose by some radiologists. The typical case is one in which signs of a mild degree of hypoovarianism or hypopituitarism are associated with sterility. In these cases, three or four small doses (e.g., 50-75 r) of x-ray may be followed by improvement in menstrual function and finally by conception. The rationale for this treatment is not clear. Success has been variously attributed to the hyperemia which is known to occur in irradiated ovaries, "stimulation" of the ovaries or pituitary, rupture of a follicle, destruction of a persistent corpus luteum, and to the possibility of a "general endocrine stimulus".

Although the genetic effects resulting from irradiation of the ovaries and testes are discussed in a later section of this chapter, it should be mentioned here that doses far short of those required to produce immediately apparent effects may result in definite damage to the germ plasm. There is considerable evidence that genetic effects are proportional to the dose regardless of whether the total dose is administered at one time or is cumulative over a long period. For this reason, regardless of the apparent ease of producing functional changes in the ovary by irradiation, this form of therapy should not be, and for the most part is not used for non-malignant gynecological complaints.

3.14 Other Endocrine Glands

The endocrine glands with the exception of the testes and ovaries tend to be relatively radio-resistant.

Adrenal: The adrenal gland appears to rank somewhere between the gonads and the other endocrine glands as regards radiosensitivity. The cortex is generally considered to be considerably more sensitive than the medulla. Temporarily inhibited mitosis has been observed in the adrenal cortex of experimental animals following whole body exposure to doses as low as 50 r. Other cytological changes, however, are demonstrable only after much larger doses. The most characteristic of these changes is a decrease in cortical lipids, a non-specific change which also occurs following stress of many types other than irradiation. Autopsy material from atomic bomb radiation deaths revealed cortical atrophy, degenerative cellular changes, and loss of lipid from the cortex. Hyperemia and hemorrhage into the adrenal have been observed repeatedly following experimental exposure to large doses.

Because of the importance of normal adrenal function in maintaining life, various methods of shielding the adrenal have been investigated as potential protective measures against whole body exposure. Administration of supplements of various adrenal cortical hormone preparations has also been considered in this respect and some experimental results indicate that such measures may be efficacious. On the basis of other experiments, however, it has also been proposed that adrenalectomy protects animals against radiation damage, presumably because of the sparing effect on the lymphocytes. When the various experiments are reviewed, the net results are inconclusive. (See Factors Influencing the Response to Irradiation.)

Pituitary: Depressed blood sugar, decreased growth, and transient effects on gonadotropic function have been described following large doses of radiation delivered to the pituitary glands of experimental animals. The frequency and extent of their occurrence, however, appear to be unpredictable. As determined by the therapeutic application of radiation for pituitary tumors in man, the eosinophile cells appear to be the most sensitive and the basophile cells the least sensitive epithelial components of the gland. A secondary effect on the pituitary was observed in some of the atomic bomb casualties where testicular atrophy was associated with the presence of castration-cells in the pituitary. This observation has also been made in irradiated experimental animals.

Some gynecologists recommend small doses of x-ray directed to the pituitary for women in whom sterility appears to be associated with hypopituitarism. The doses used are small relative to the radiosensitivity of the gland, however, and it is doubtful that such treatment has a direct effect on the pituitary. In experimental animals, a few cases of atrophy of the genitalia have been reported following very high doses; however, these cases are exceptional.

Thyroid: The thyroid is also relatively radio-resistant. The usual changes, even in heavily irradiated thyroids are limited to transient changes in the epithelium and more or less fibrosis, chiefly of the capsule. Radioactive iodine is efficacious in treatment of thyroid disease because it permits sharply localized application of extremely high doses of radiation.

Parathyroid: The parathyroid appears to be even more radio-resistant than the thyroid, and damage even after extremely large doses is usually limited to minimal fibrosis.

Pineal: There is virtually no information relative to the effects of radiation on the pineal gland.

3.15 Lungs

Relatively large doses of radiation are required to produce visible changes in the lung and for the most part, the effects appear to be the results of vascular changes.

Edema and thickening of interstitial tissues are reported to be among the earliest observable changes, and even in Bloom's $LD_{50}$ studies where pulmonary changes were minimal, some peribronchial and interstitial infiltration were noted. The pneumonitis which may develop following exposure to larger doses is characterized by congestion, edema, and degenerative changes in the alveolar cells. Pulmonary fibrosis has been described as an occasional end result of such radiation-induced pulmonary pathology.

Congestion, hemorrhage into alveoli, and areas of necrosis (usually in central portions of areas of hemorrhage) are commonly observed in animals dying from the acute radiation syndrome. Changes of this nature were also observed in autopsy material from atomic bomb fatalities in which the radiation syndrome was a prominent feature. Edema and hemorrhage of the serous surfaces were also noted in these cases.

Inhalation of radioactive materials by experimental animals may result in heavy and prolonged irradiation of the lungs. In these cases, hyalinization and proliferative reaction of the bronchial epithelium suggestive of neoplasia have been observed.

3.16 Muscle

Muscle appears to be remarkably resistant to radiation damage, and most radiation damage in this tissue appears to be related to vascular changes, even following exposure to extremely high doses. This is equally true of smooth muscle, striated muscle, and cardiac muscle. In the case of the skin, the erector pilae muscles may survive doses of radiation sufficient to destroy hair follicles completely, and smooth muscle of the GI tract is usually normal even in the presence of marked destruction of the epithelium. Heavy irradiation may result in hemorrhages into the myocardium. Electrocardiographic changes have been observed following fairly heavy irradiation (i.e., $LD_{50}$).

In the presence of marked hemorrhagic tendencies and increased vascular permeability, however, it is difficult to interpret such changes as representing direct effects on the myocardium.

Under extreme conditions, when radiation has been delivered at remarkably high rates in massive total doses sufficient to produce immediate death, it has been observed that muscle spasticity may occur prior to the onset of rigor mortis.

3.17 Bone

The radiosensitivity of bone varies considerably with the age of the animal under consideration. The radiosensitivity of actively growing bone seems to be considerably greater than that of mature bone. In the former, the relatively abundant and active cartilage cells of the epiphysis undergo denenerative changes fairly readily, and may become completely necrotic. The resulting interference with growth may be severe and irreversible, or minimal and capable of being completely compensated. Retarded or inhibited elongation of long bones has been reported by several investigators, and the cessation of growth of irradiated bones in children, associated with closure of the epiphysis, has been recognized for many years. The altered growth patterns which may result from irradiation of growing bone have generally been attributed to a disordered relationship between resorption of damaged tissue and the formation of new.

In general, heavily irradiated formed bone exhibits decreased vitality, loss of strength, and an increased susceptibility to infection. Formed bone appears to be more resistant in children and young adults than in older individuals. Pathological fractures which occur in irradiated bone usually follow partial decalcification. Healing in such instances is often characterized by delayed and altered repair in which callus forms slowly and in deficient amounts.

Radioactive isotopes deposited in bone generally have a direct destructive effect. Spontaneous fractures are not uncommon in radium poisoning, and characteristically heal imperfectly, usually by fibrous union or pseudarthrosis. In segments of bone from animals containing bone deposited isotopes, the histology has been described as an extremely complex picture, with degeneration and more or less abnormal reparative processes proceeding simultaneously.

The occurrence of malignancies in irradiated bone is included in the discussion of latent effects.

3.18 Nervous System

The nervous system appears to be markedly resistant to radiation damage. In the autopsy material available from populations of bombed Japanese cities, congestion and hemorrhage were the most commonly observed pathological changes, the hemorrhage occasionally being associated with bacterial invasion of affected tissue and damage to ganglion cells.

In experimental animals, extremely large doses of radiation are necessary to produce such visible changes as chromatolysis, swelling, vacuolization and fatty degeneration in ganglion cells. Irradiation by local application of radioactive substances usually results in sharply localized damage. Localized amyloid degeneration has been described as the end result of heavy (3,500-6,000 r) x-ray therapy directed to areas of the human brain; however, this apparently does not occur commonly.

The nerve fibers also are resistant, although demyelinization of nerves and degenerative changes in the nerve cell bodies have been described following very large doses directed to the spinal cord and peripheral nerves of experimental animals.

## 4. Physiological Changes Observed in the Intact Animal

Published reports concerning physiological changes in the intact animal contain a tremendous amount of qualitative data concerning various types of reactions, a very small amount of quantitative data, and very little information concerning the mechanism by which the reactions are produced. It is also common to find divergent opinions concerning almost all types of physiological changes, in almost every species including man.

The material presented in this section is based largely on observations following more or less uniform whole body exposures. This arbitrary restriction is made in an effort to arrive at a picture of the base response to radiation. In instances where radiation is highly localized, the response will obviously be modified in a manner which depends on the magnitude of the exposure, the radio-sensitivity of the exposed tissues, and the role of the irradiated structures in the over-all body economy.

### 4.1 Radiation Syndromes

Following whole body exposure to ionizing radiation a series of physiological events occur in moderate to rapid sequence, the rapidity of onset and severity depending primarily on the total amount of radiation received. The alterations in function usually exhibit a phase of injury followed by a phase of recovery, the latter often being associated with over compensation. Since there is considerable individual variation in the time of onset of injury and recovery reactions, it is often possible to observe a state of depressed activity within a system of one animal of a given species at the same time another animal shows a state of increased activity in the same system. This single phenomenon accounts for many of the apparent inconsistencies which may be observed in the vast literature on this subject.

Making due allowance for individual and species variations, it is possible to sketch the general clinical and physiological pictures which are seen following exposure to ionizing radiation as follows:

Immediate Death: In certain species death occurs during or within a few hours following exposure to very high doses of penetrating radiation (25,000-50,000 r) In these animals there is widespread cellular destruction throughout the entire body. Convulsions have been noted in some cases, and there may be muscle spasticity immediately after death. In other cases there is an abrupt fall in blood pressure, with a state of shock preceding death. In birds, a rapid deposition of urate crystals in the tissues and a marked increase in blood uric acid has been observed during the moribund period.

Initial Death: The initial prostration may proceed to death within approximately 48 hours without a demonstrable period of latency. The clinical picture in such cases is characterized by severe gastro-intestinal dysfunction manifested as nausea, vomiting, anorexia, and diarrhea, as well as signs of circulatory instability, namely vasodilatation and cutaneous erythema. This type of death occurs following exposure to single doses in excess of approximately 3,000 r.

Early Death: Death occurs after four to six days, and is associated with evidence of dehydration, profound lymphopenia and leukopenia and marked gastro-intestinal damage with associated dysfunction. In some cases there may also be marked hemoconcentration. This type of reaction is seen following dosages of approximately 1,000 to 3,000 r.

Acute Death: This group includes almost all deaths from lethal amounts of ionizing radiation. The maximum dosage is approximately twice the $LD_{50}$ for the species. The initial shock reaction persists for approximately two hours followed by a latent period of from two to twenty days during which no specific symptoms are present. Leukopenia commonly begins during the latent period. After this time general malaise, anorexia, vomiting and diarrhea occur followed by hemorrhage, thrombocytopenia and fever. Leukopenia becomes marked and there may be almost absolute lymphopenia. Death occurs approximately 9 to 30 days after exposure. Pathologic findings include extensive breakdown of the more sensitive tissues, particularly bone marrow, lymphoid tissue, gut and testicular epithelium. The bleeding tendency is associated with a prolonged clotting time and a high sedimentation rate. Signs of alteration in the water and electrolyte balance occur with varying severity depending on the species. Other evidence suggests a series of nutritional abnormalities. The entire picture is further complicated by the presence of anemia, depression of the immune processes and increased susceptibility to infection of various types.

Subacute Responses: These include instances where exposures in the lethal or near lethal range do not produce death in a 30 day period. Physiological changes are similar to those which precede acute death and it is often impossible to predict which animals will survive. Subacute responses following lower doses may consist only of transient physiological changes and mild depression of hematopoiesis. Infections and anemia are common. Death rarely occurs except as the result of complicating factors.

Chronic Exposures: Immediate sequelae are usually not observed. The most important finding is the occurrence of latent changes, and these are considered separately in a later section. (See Latent Effects).

## 4.2 Radiation Sickness

This term is commonly used to include the physiological responses which occur in patients undergoing radiation therapy for cancerous lesions in various parts of the body. Symptoms include nausea, vomiting, anorexia, weight loss, fever and intestinal hemorrhage, and are usually most severe following irradiation over the abdomen. The mechanisms leading to these symptoms are in dispute. A great deal of literature deals with the treatment of this symptom complex, however no specific therapy has been developed which is universally successful.

4.3 Body Weight

Although considerable variation exists among species, there is usually an initial loss in weight during the first two to three days. This is associated with prostration, nausea and vomiting, diarrhea, anorexia and reduction in water intake. The severity of the loss and the nature of the symptom complex are directly related to the size of the dose. Following this initial loss there is a stabilization in weight during the latent period when symptoms are not present. With the reappearance of anorexia, fever and other signs already described, a secondary period of weight loss occurs. In some instances, survival is correlated with cessation of such loss and the resumption of more normal food and fluid intake.

In general, the weight loss is similar to that of starvation, however in certain instances the weight loss appears to be greater than would occur in starvation over the same period of time. Nitrogen balance experiments do not indicate excessive breakdown of protein, except during terminal phases when elevated NPN and urea nitrogen may be demonstrated.

4.4 Metabolic Disturbances

Generally speaking, there do not seem to be any metabolic changes which can be considered typical of irradiation damage. A large number of clinical and laboratory studies have been carried out, and these may be summarized briefly as follows:

Protein Constituents: With respect to protein metabolism, balance studies indicate that most of the changes can be accounted for on the basis of starvation, with an added factor related to irradiation, the nature of which is unexplained. There appears to be no gross change in the circulating total protein. In the dog there is a reduction in albumin and a rise in total globulin. A particularly striking observation is the depression of gamma globulin which occurs in near-lethal exposures. Changes in NPN or BUN are not conspicuous until the terminal phase. Creatin values are normal or reduced. Creatinine values are within normal limits. All of these findings may be modified by changes in blood and plasma volume. Alterations in kidney clearances occur only when there are definite lesions in that organ. Certain increases in polypeptidases have been observed. Alterations in nucleic acid metabolism are thought to be associated with excessive cell destruction.

Carbohydrate: Studies indicate that the absorption of glucose during the first 24 hours is delayed. Irradiation is usually followed by a rise in blood sugar, possibly due to glycogen mobilization resulting from adrenal stimulation; however, hyperglycemia appears to be more marked when x-ray is given directly over the liver. Blood lactic acid and blood pyruvate values are reduced.

Fat: Following irradiation there is a rise in total lipid and fatty acids of the blood and definite lipemia has been reported in some instances. Impaired or altered fat absorption has been noted following abdominal x-ray therapy for leukemia. Blood cholesterol values in general are normal or decreased, although elevations occur in some instances. Acetone bodies are increased, perhaps resulting from dehydration, and reduced food intake.

Lipids tend to be decreased in the adrenal cortex. Vitamin A absorption in rats is delayed slightly in the first 24 hours and is normal or increased during the following 6 days, after which a slight decrease is noted. These changes are associated with a decrease in Vitamin A in the liver. Animals surviving a 14-21 day period will generally show almost complete absence of depot fat at autopsy, indicating increased utilization. Mice (which apparently are unable to utilize depot fat during periods of starvation) will die several days post-radiation if they are not fed. Other species will survive without food for extended periods following similar exposures.

### 4.5 Alterations in Fluid and Electrolytes

A number of derangements of fluid and electrolyte balance follow irradiation. As with most other changes, the severity varies from species to species, and depends on the magnitude of exposure. With high dosages, a condition similar in some respects to traumatic shock has been produced. Distention of the intestinal canal, with associated fluid and electrolyte loss, is noted in rats during the first six hours after exposure. In many cases diarrhea, with its associated water loss is a prominent feature of the acute radiation syndrome. In some animals, however, the water intake may be sufficient to compensate for the water lost in diarrhea. After the initial period there may be a rise in serum chloride and a decrease in gastric acidity. Little change is observed in individual chemical analyses during later periods. A gradual increase in plasma volume offsets the decrease in circulating red cell mass and the blood volume remains relatively constant. The interstitial space (thiocyanate space less plasma volume) shows no change or slight increase. Certain findings suggestive of alkalosis with a decrease in circulating total base have been reported. It seems fair to say that marked variation exists in all findings, and that much additional information is necessary.

4.6 Effects on Immunological Phenomena

This aspect of the effect of radiation on the intact animal presents formidable experimental problems, and is the subject of much controversy.

In general, irradiation of blood or serum in vitro has been found to exert a negligible effect on antibodies, although in some experiments where very high doses have been employed, inactivation of opsonins has been reported. The marked susceptibility of heavily irradiated experimental animals to infection has often been assumed to represent an expression of generally diminished antibody formation, but experimental investigation of this hypothesis has raised several questions relative to its validity. On the one hand, it has been shown, in the case of certain specific antibodies, that whole body exposure at any time after antibody production has begun will not seriously depress that function. On the other hand, antibody production will be greatly reduced or completely inhibited if irradiation precedes the onset of antibody formation. The proposal that deficiency in antibody formation results from lymphopenia is without adequate confirmation, since experimental investigations have failed to demonstrate a convincing degree of positive correlation between the amounts of antibody in the serum and in the lymph.

The effectiveness of small therapeutic doses of radiation in infections is variously explained, but poorly documented by experimentally proven facts. Explanations include the attraction of leukocytes to irradiated tissues, various effects on the medium (tissue) on which bacteria grow, increased antibody production in the treated area (presumably from the breakdown of tissue, leukocytes, bacteria, or some combination of these) increased phagocytosis, etc.

Studies concerning the effects of irradiation on the development of anaphylaxis in susceptible animals seem to indicate that irradiation has no significant effect on this particular immune response.

4.7 Organ Specific Proteases

Proteinases specific for tissues which have been irradiated have been identified in the urine of experimental animals and man. These enzymes possess both species and tissue specificity and in many cases appear following doses considerably smaller than those required to produce histological change. For example, doses which have been found to result in the excretion of proteinases specific for liver and brain respectively are 300 r and 247 r in the dog. It has also been found that the increased rate of postmortem autolysis of tissues from irradiated animals is associated with an increased proteolytic activity of the serum.

4.8 Adrenal Responses Following Exposure to Radiation

Certain responses following whole body exposure to large doses of ionizing radiation are suggestive of adrenal stimulation. Elevated blood adrenalin levels following x-irradiation have been described by several investigators, and it has been suggested that this may be responsible for the hyperglycemia previously mentioned.

In many respects the physiological response to whole body irradiation resembles the adaptation syndrome or alarm reaction of Selye. Adrenal hypertrophy and thymolymphatic atrophy have been described as characteristic of the response to irradiation. In one instance, these histological changes were observed in rats receiving doses of 1,980 - 3,680 r localized to the abdomen. Surgical removal of the adrenal prior to irradiation prevented these effects, although mortality was slightly increased. Shielding the adrenal has been found to decrease mortality in some cases, however neither this procedure nor the therapeutic use of desoxycorticosterone or adrenal cortical extracts have been consistently successful in experimentally modifying the acute radiation syndrome. Conflicting reports exist concerning the effect of the administration of cortin, desoxycorticosterone, cortisone, ACTH, and hadacol, on the alleviation of radiation sickness. In some instances, patients receiving therapeutic irradiation localized to the abdomen have been considerably benefited by desoxycorticosterone, however this has not been a consistent finding.

4.9 Influence of Radiation on Reparative Processes

Healing and repair following radiation injury apparently depend on a number of factors; for example the extent and nature of the injury, the tissue under consideration, the general physiological condition of the organism, the integrity of the vascular system, the presence or absence of infection, etc., etc. It is certain that changes in immune processes, hemtopoietic reserves, nutrition and detoxification mechanisms also influence regeneration in the animal receiving whole body exposure.

Retarded healing has been reported following irradiation of wounds with large doses; however in such cases repair merely proceeds at a slower rate and may be complete and normal in all other respects. In rats, the dose required to produce such a delay has been estimated as approximately 1000 r. Delayed repair may be due in part to slowly developing manifestations of radiation damage in the wound area, however this has not been evaluated experimentally. Smaller doses of radiation (i.e. doses less than 700 r) delivered immediately after suturing abdominal skin incisions has been reported to increase the tensile strength of the healing lesions in rats.

## 5. Latent Changes Following Exposure of Biological Systems to Ionizing Radiations

It has already been pointed out that although a considerable proportion of the changes which follow irradiation of biological systems become apparent within a period of hours, days or weeks, certain changes appear much later. In the present discussion these are classed as latent changes. There is, of necessity, considerable leeway in this definition. In some cases, as for example in the induction of cataracts, the damage may have been incurred in a very limited number of exposures followed by a long symptom-free period during which no further exposure occurred. In other cases, however, irradiation may have been continuous over a long period of time. This was true in the case of radium dial workers whose contact with radium ended several years before the appearance of malignancy but who, nonetheless, were being constantly irradiated from the bone deposited material throughout the entire period following the radium ingestion.

Latent effects may, for convenience, be classified into five main types. These are 1) the induction of malignancy (carcinogenesis), 2) the production of heriditable changes in the genes and chromosomes, 3) decreased fertility, 4) premature aging and shortening of the life span, and 5) the induction of cataracts.

### 5.1 Carcinogenesis

The induction of malignancy by irradiation has been recognized since the early days of roentgen ray therapy and has been observed in diverse organisms, including plants, insects, mice, various other experimental animals, and man. The mechanism by which irradiation produces neoplasia is still poorly understood. The evidence in favor of the existence of such a cause and effect relationship, however, is highly convincing.

In some instances at least part of the apparent increase in the incidence of malignancy appears to be related to the fact that tumors appear earlier in irradiated animals, possibly because irradiation enhances an inherent tendency toward the development of tumors. For example, there are strains of mice which are predisposed to develop certain tumors, and following frequently repeated irradiation these animals develop malignancy at a much younger age than the non-irradiated controls. It is felt that acceleration in the appearance of the lesion may merely permit a greater number of predisposed individuals to develop malignancy during their life span, and that many more of the controls would also have developed it had they lived longer. In other instances, irradiation of certain carcinogens has been found to enhance their carcinogenic powers. Irradiation may result in malignant change in previously existing benign conditions which are known to have more or less tendency to undergo spontaneous malignant degeneration. It has also been suggested that the induction of somatic mutations and various related alterations of cell division and growth may be important in the pathogenesis of radiation-induced tumors.

5.1a Skin

During the years before the dangers of ionizing radiations were fully appreciated many physicians and physicists received excessive irradiation of their hands and subsequently developed cancer in these sites. Since that time steady progress has been made in recognizing and reducing the hazards associated with the use of ionizing radiations, and this has been reflected in a sharp curtailment of gross over-exposures. Skin damage of milder degree is still fairly commonly observed among radiologists, however, and in experimental animals, skin tumors may be produced relatively easily either by acute or chronic gross over-exposures. Beta radiation is highly effective in producing skin tumors in experimental animals, and in one study, almost every conceivable type of skin tumor was observed in a group of mice exposed to this radiation.

5.Ib <u>Bone</u>

Bone tumors occur particularly frequently in radium poisoning and have been produced experimentally by poisoning with certain other bone-deposited radioactive isotopes. The characteristic lesion in all these cases is osteogenic sarcoma, which is commonly preceded by radiation osteitis at the same site. There is considerable evidence that radiation osteitis and tumors may also be induced in normal bone which receives heavy irradiation during therapy for malignancy in a nearby part, as for example the ribs in radiation therapy for cancer of the breast.

5.1c <u>Lung</u>

Lung tumors may develop from repeated whole-body exposures to small doses of radiation and from prolonged inhalation of radioactive dusts. In the section on lungs (3.91), abnormal (neoplastic) bronchial epithelium has already been indicated as one result of the latter type of radiation exposure. Radioactive dusts have been incriminated as the etiological agent in the high incidence of lung cancer among certain groups of miners. In most species the tumor which develops originates from the bronchial epithelium, although in others (notably mice and guinea pigs) the tumors appear to originate from the alveolar cells.

5.1d <u>Breast</u>

Cancer of the breast has been noted in several species of experimental animals which receive frequently repeated exposures to relatively low doses of ionizing radiation. In these instances, it is felt that the radiation serves merely as added impetus towards carcinogenesis in a strain of animal in which other requirements for tumor development are already present.

5.1e Ovaries

An increased incidence of ovarian tumors following prolonged chronic irradiation has been observed in many species of experimental animals. In many cases, these tumors follow definite degenerative changes in the ovary. Since non-irradiated mice occasionally develop ovarian tumors following the degenerative ovarian changes which normally occur towards the end of their life span, it has been suggested that irradiation produces ovarian tumors because it initiates the degenerative changes sufficiently early in the life of the exposed animal to allow adequate time for the tumor to develop.

5.1f Leukemia and Related Malignancies

Leukemia and various lymphomata have long been recognized as one of the possible end results of extensive exposure to radiation. Mice and rats appear to be particularly susceptible; however, these malignant diseases have been observed in many species. In this respect it is interesting that the incidence of leukemia among radiologists is unusually high, approximately four times higher than among the population in general, and nine times higher than the incidence among other physicians.

5.1g Other Tumors

Tumors of tissue other than those mentioned above have been observed in various species following irradiation, however their incidence is usually sufficiently low to encourage a conservative interpretation of their relationship to exposure.

5.2 Genetic Effects.

The sociological and medical significance of the genetic effects of irradiation has become a highly controversial subject during recent years.

There are certain pertinent facts in the matter, however, which are based on good experimental evidence, and these may be summarized briefly as follows:

In certain species which have been carefully studied it has been shown that there is a linear relationship between the total dose of radiation received and the incidence of both gene mutations and chromosome aberrations. There is no "threshold" dose for producing an increased mutation frequency. In the case of gene mutations, the dosage rate appears to have no significant influence.

Radiation-induced mutations appear to be of the same general type as naturally occurring mutations. Most of them are recessive, and they are permanent, i.e., there is no healing this type of damage. The great majority of them are also detrimental.

The hereditable effects of irradiation may not be visible or may be difficult to observe. Because most of the mutations are recessive, they will not be fully apparent until two parents, carrying identical mutations transmit the mutation in duplicate to their offspring.

Gene Mutations: All individuals of all species normally carry a relatively large number of mutant genes. This reflects the natural mutation frequency, i.e., the frequency of occurrence of mutations under usual conditions. These mutations are constantly being eliminated from the population because of the death of the individuals who carry them, and after a time the total number of mutant genes in a population represents an equilibrium in which the natural rate of occurrence of the mutations in question equals the rate of their elimination. The mutations are eliminated because they tend to be detrimental. Thus, if a mutation is lethal or severely debilitating it will tend to be eliminated in relatively few generations, whereas if it produces only minimal detrimental changes, its elimination may require many more generations.

Normally, genes are present in pairs, and if, as mentioned above, a mutation is recessive, relatively little effect is noted in individuals with one normal or one mutant gene (i.e., the heterozygous state). For this reason, so long as both parents do not carry an identical mutant gene, their offspring will be relatively little affected, and the mutation may be passed along for generations until the chance mating of two parents, both of whom do carry the same recessive mutation, occurs. In such a case, a certain percentage of the offspring will be doubly endowed with the mutant which will then become apparent. One very important considera-tion in this respect is that recessive genes may posses a certain degree of effectiveness even in the heterozygous state. These effects may be ex-tremely difficult to identify by ordinary means, and are very difficult to study quantitatively; however they may account for many of the minor, vague, poorly understood inadequacies of certain individuals, and may well account for many other more readily apparent common afflictions.

Chromosome Mutations: These are preceded by breaks in the chromo-somes, and result when a chromosome permanently loses a fragment (with its constituent genes), exchanges a fragment with an adjacent chromosome, or when a fragment turns end for end and then rejoins the original chromosome, thereby resulting in a change in the order of the genes within the chromo-some. The various chromosome aberrations occur with a definite frequency following irradiation. The frequency is determined by many factors such as the dose of radiation, the type of radiation, the state of the nucleus at the time of exposure, the nature of the medium in which the irradiation occurs, etc., etc. As in the case of gene mutations, however, the produc-tion of chromosome aberrations by irradiation appears to have no "thres-hold".

In general the principles which govern the transmission of chromosome mutations are the same as those governing the transmission of gene mutations, although the manifestation of the two types of mutations may differ. Chromosome mutations have a greater tendency to result in decreased fertility without apparent effects on the anatomy, whereas gene mutations are likely to produce gross structural defects. This, of course, is only a generality, and it is often impossible to distinguish between gene and chromosome mutations. In general, chromosome aberrations with the most marked effect are those in which a fragment is lost, leaving a gamete deficient in genes. These are particularly likely to result in non-viable zygotes or embryos.

The disagreement which exists among various persons who, for one reason or another, are concerned with the problem of the induction of hereditable effects by irradiation, relates to 1) the statistical consideration of the possibility that sufficient numbers of individuals in a population will be affected by very low exposures to result in a disturbance of the equilibrium frequency, and 2) the probability that individuals carrying a radiation-induced mutation in the heterozygous condition will be significantly harmed, even though the mutation be recessive.

The more conservative opinion, which has been eloquently expressed by H. J. Muller, considers that future generations will not only be saddled with the added radiation-induced changes from the present era, but that large numbers of the future population may receive relatively high exposures almost routinely. In this manner, generation after generation may be expected to contribute at an increasing rate to the number of new mutations, and the rate of occurrence may significantly exceed the rate of elimination. Although this may not be reflected in a sharp increase in death rate in the next few generations, it may seriously interfere with the health and well being of more and more individuals. It is to be expected that extensive effort will continue to be directed towards preserving the life of the afflicted, and although this is a praiseworthy goal, it has genetic significance because it will tend to result in a slower rate of elimination of mutations

From this conservative point of view, no amount of irradiation can safely be considered negligible, even if there are no immediately apparent effects, and every effort should be directed towards reducing exposures to the absolute minimum which is compatible with maintaining a progressive and productive society. This decidedly long-range point of view has been endorsed in essence, by the various atomic energy installations.

The less conservative opinions on this matter point out that slight increases in the frequency of occurrence of mutations are not likely to result in significant alterations of the equilibrium frequency, and that the increase in death rate due to genetic changes among a population of which a relatively small fraction received excessive exposure would probably be entirely negligible. This has been true in many species studied experimentally, and it is predicted that it may also hold true in the case of the populations of bombed Japanese cities.

5.3 Decreased Fertility

Decreased fertility as a result of irradiation includes all conditions which result in fewer viable offspring from an irradiated individual or stock. This concept, which is considerably broader than that embraced by certain groups concerned with only relatively restricted aspects of radiation work is appropriate if one is to appreciate the over-all significance of latent effects.

The specific mechanisms by which radiation results in decreased fertility can only be postulated. In many instances, however, decreased fertility appears to be more or less directly related to the presence of other well recognized radiation effects.

The germinal epithelium is highly radiosensitive, and depending upon the extent of exposure, radiation may result in varying degrees of interference with the production of normal gametes. In man the permanent sterilizing dose is extremely high and greatly exceeds the minimum lethal dose for whole body exposure. Consequently, permanent sterility due solely to interference with the function of germinal epithelium is not of great practical significance. Gametes which have altered genes or chromosomes as a result of exposure, however, may produce non-viable zygotes, or viable offspring with more or less abnormal constitutions. In extreme cases the affected individuals may die before they have reproduced, while milder degrees of affliction may result in generally decreased vigor and the production of fewer viable offspring. This has actually been observed in experimental animals. In cases where hereditary defects influence fertility, the mutations will be passed along from generation to generation, so that over a long period of time, effects which might appear to be insignificant (or which are, perhaps, not immediately apparent at all) may have profound effect. In man, for example, decreased physical or mental stamina may conceivably be sufficiently detrimental to result in failure or inability to provide adequately for the offspring; or might be associated with an increased susceptibility to various illnesses which could be contracted by the children or which might in themselves be responsible for fewer offspring. In other cases the radiation induced changes might directly or indirectly act as contributory factors in failure to marry, etc., etc. Possibilities of this nature, although extremely difficult to test experimentally, figure prominently in the controversy relative to the significance of hereditable effects of irradiation.

## 5.4 Premature Aging and Shortening of the Life Span

There appears to be a high degree of positive correlation between exposure to radiation and the occurrence of both premature aging and shortened life span. This has been observed in several species, particularly in cases of frequently repeated exposures to small doses of radiation over long periods of time. The mechanism responsible for this effect is obscure. In cases where death occurs following the acute radiation syndrome or as a result of radiation-induced malignancy, the life span is shortened without premature aging.

The effects of chronic or low dosage irradiation of many tissues may be indistinguishable from the effects of physiological aging. The problem of recognizing a shortened life span when it is not due to some obvious major pathological change is largely a statistical one and it has been possible to demonstrate this effect only in fairly large groups of experimental animals. The almost imperceptible shortening of a life span would not be of particular concern to persons interested in the preventative medical aspects of radiation work and atomic warfare except for the possibility that premature aging may be associated with other less benign effects such as carcinogenesis and genetic changes.

## 5.5 Induction of Cataracts

Irradiation of the eyes with x-rays, gamma rays or neutrons has been observed to result in the development of lenticular opacities in several species. Some of the earliest observations of this effect were made in animals which had been irradiated in utero, and it has since been shown that the lens in younger animals is relatively highly susceptible to radiation damage. Cataracts have also been observed in adult animals, after both acute and chronic exposure to relatively large doses of ionizing radiation. Recently the occurrence of cataracts following exposure to radiation from cyclotrons has received considerable attention and it has been shown that neutrons are particularly effective in this respect. In the adult human, for example, exposure to x-rays in excess of approximately 2,000 r is required to produce cataracts, while a minimum dose of approximately 50 n units of neutrons (20 MEV or less) appears capable of producing the same effect. Experimentally, chronic exposure to neutrons in doses which are relatively low but considerably above tolerance level may induce cataracts in a large number of exposed animals. The deplorable practice of lining up a cyclotron beam by visual means has apparently been responsible for several of the neutron-induced cataracts which have recently been reported in physicists.

## 6. Factors Influencing the Biological Response to Ionizing Radiation

Many factors have been found capable of influencing the biological response to irradiation. Experiments designed to study these factors are often difficult to interpret because of difficulty in restricting the number of variables, and there are many apparent discrepancies among the results of various investigators. In spite of these differences, however, several major groups of factors are recognized, and these are considered briefly under the following headings.

### 6.1 Species Differences

Species differences constitute a prominent characteristic of radiobiologic reactions. The $LD_{50}$ doses, for example, are 200 r for the guinea pig, 400 r for dogs, 750 r for goldfish, 800 r for rabbits, 2000 to 3000 r for frogs, and 9500 r for adult Drosophila. Another example of species difference is provided by an experiment in which the hematology of two species of laboratory animals was studied over a period of one to two years during which time the animals received daily exposure to small doses of radiation. In this experiment 10 r was the smallest daily dose producing a significant depression of the lymphocyte count in a group of rabbits. The depression appeared to be definite after approximately 8 weeks. In dogs, the smallest daily dose producing a significant depression of the lymphocyte count was 0.5 r, which produced an apparent depression after about six months of daily exposure, and a statistically significant depression after approximately two years of exposure.

It is interesting that the extent of variation from species to species depends upon the variable which has been selected for observation. In rats, mice, guinea pigs and rabbits, for example, 200 r has been found to produce approximately the same degree of histological damage in the lymph nodes and thymus despite the marked differences in $LD_{50}$ doses.

In general the relative sensitivity of various organs is the same in the various mammals which have been studied, and, indeed, the general response to irradiation is more notable for its similarities than for its differences. There are, however, certain species differences in this respect. For example, in the mouse the ovary is particularly vulnerable to radiation damage, as regards the development of both sterility and ovarian tumors. The rabbit differs from other species in that the response following acute exposures is characterized by a drastic depression of blood pressure within a few hours after exposure; a single dose of as little as 50 r produces a distinct fall in blood pressure and after doses in the $LD_{50}$ range the depression may be progressive and lead to death.

6.2 Temperature

Generally speaking, lowering the temperature decreases the magnitude of radiation-induced biological reactions. This has been observed in such test organisms as germinating seeds, chick embryos, tadpoles and the eggs of ascaris and grasshoppers. In some instances a return to normal temperatures after chilling may be followed by the appearance of typical signs of radiation damage, although if chilling is sufficiently prolonged the magnitude of the delayed response may be reduced.

Conversely, the effect of irradiation is often increased by raising the temperature of the irradiated system. For example, exposure of fungus spores, tradescantia and other plants to infra-red radiation before x-irradiation increased the number of mutations produced. Radiation-induced skin damage may be enhanced by exposure to heat either during or immediately before x-irradiation.

In certain instances, the temperature during irradiation has little or no influence on the response, while the temperature during the post-exposure period exerts a profound effect. This has been demonstrated in the case of isolated leukocytes and in experiments utilizing Drosophila eggs, egg albumin and lens extracts. Still other studies indicate that different responses within the same organism may vary widely in their temperature dependence. Chilling irradiated Ascaris eggs, for example, does not alter the radiation-induced delay in cleavage although it increases the percentage of normal embryos which subsequently develop from the eggs.

Chilling as a rule decreases the mitotic rate, and since cells in mitosis tend to be more radiosensitive, some consideration has been given to the possibility that the effects of temperature may be mediated through changes in mitotic activity. This relationship between division rate and radiosensitivity has certain exceptions, however, as for example in the case of paramecia, where the radiosensitivity is reported to be greatest during the non-dividing state. If chromosome breaks are selected as the criterion of radiation damage, chilling may actually increase the effect. This has been shown to be due to failure of restitution of chromosome breaks in the chilled cells; i.e., chilling depresses the degree of recovery following the initial radiation damage.

6.3 Oxygen Tension

The role of oxygen in the response to irradiation is currently the subject of considerable study, as it has been on several occasions in the past. There is as yet, however, no clear understanding of the role of oxygen in radiation-induced reactions. The destruction of bacterial toxins, the inactivation of enzymes, as well as the lethal and mutagenic effects on various organisms have been shown to be considerably greater in the presence of high oxygen tensions. It has also been demonstrated that oxygen acceptors may diminish radiosensitivity in both biological and chemical systems. Tolerance to x-rays is enhanced by hypoxia in mice, rats, bacteria, daphnia, tradescantia and various other organisms.

The possibility that hypoxia and chilling may influence radiosensitivity largely by depressing metabolism has been suggested. In this respect it is interesting that in an investigation utilizing developing grasshopper eggs, "blocking" embryonic development greatly increased the resistance of the eggs to irradiation even when oxygen consumption was markedly increased by the administration of 3.5 dinitro-o-cresol.

6.4 Acidity

The role of intercellular acidity has been studied by adding diffusible acids or bases to cell suspensions. Plant cells and Drosophila eggs are considerably more resistant to irradiation after treatment with weak ammonia. For the most part, the actual pH of the cells has not been determined although studies with diffusible indicators (e.g. neutral red) have been reported. This method suffers from the disadvantage that the presence of an indicator in the cell may well exert some effect of its own on the biological system. In many cases pH has been incriminated as the critical factor largely by inference.

In the case of relatively simple chemical systems the situation is more hopeful. Recent experimental work by one of us* has suggested that changes in pH may influence radiosensitivity by altering the molecular species which are present. Specifically, the decolorization of aqueous methylene blue by x-ray apparently occurs appreciably only when the methylene blue is present in the ionic form. The addition of alkali, which results in the formation of the undissociated base, greatly depresses the rate of decolorization, and the magnitude of the effect is in good agreement with that calculated from the known dissociation constant for the free base. Addition of soluble chlorides and other salts also depresses the rate of decolorization, presumably by a common ion effect.

6.5 Variations in Radiosensitivity with Changes in Physiological State

The response to ionizing radiation is greatly influenced by the physiological state of the test organism, and this subject is presently the object of considerable experimental study. The effects of temperature, oxygen, and acidity have been considered separately and represent factors which are capable of influencing the biological response at all levels of development. For convenience, those factors which may be of especial importance in influencing the biological response in intact adult animals are considered in the following sections.

6.5a Infection

The role of infection is of particular importance in acute radiation injuries, and appears to have a profound effect on morbidity. It

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has been observed that sepsis is more extensive in untreated, acutely irradiated animals which die than in those animals which survive a like exposure. Treatment of animals with antibiotics may afford some protection against the lethal effect of irradiation.

6.5b Hemorrhage

Hemorrhagic phenomena are also particularly important in acute radiation injuries, and have been discussed briefly in earlier sections of this chapter. Hemorrhagic phenomena are commonly related, or associated with, extensive infection. Agents which modify one often modify the other.

6.5c Hypersensitivity

The factor of hypersensitivity has been considered as contributory in the etiology of both radiation sickness and the acute radiation syndrome. A few studies have indicated that antihistaminic drugs may alleviate some symptoms of radiation sickness. On the other hand, other workers have found administration of histamine to alleviate other symptoms of radiation sickness.

6.5d Dietary Deficiencies

Nausea, vomiting, diarrhea and anorexia, commonly occur simultaneously following acute radiation exposure, and are associated with tissue damage, and an increased demand for metabolic building materials. These observations call attention to the possibility that dietary deficiencies may contribute to the over all picture. Although this may be true to a certain extent, deficiencies of this nature do not appear to be determining factors in the development of either the acute radiation syndrome or radiation sickness. The use of various vitamin preparations, particularly the B vitamins in the treatment of radiation sickness has not been consistently successful in alleviating symptoms or decreasing the mortality in groups of acutely radiated experimental animals.

6.5e Stress

The role of stress in determining the response of animals to heavy irradiation is of considerable importance in military medicine, since radiation injury sustained during atomic warfare would almost always be associated with excessive physiological demands. There is evidence that strenuous activity after whole body exposure to large amounts of radiation definitely decreases the chance for survival.

The exact role of the typical "alarm reaction" in the over all picture of acute radiation injury is not clear and experimental studies such as shielding the adrenals, adrenalectomizing or hypophysectomizing animals, administering adrenal cortical extracts or related substances, etc., have not yet been very helpful. In some instances, results of repeated experiments and experiments utilizing various species have not given consistent results. In other studies, the treatment being evaluated was sufficiently drastic to obscure the "normal" response to irradiation.

In considering the problem of stress it is important to recall that irradiation may, in its turn alter the response of an organism to other stimuli. Many observed radiation effects may, in fact, represent altered response to extraneous stimuli rather than specific direct effects of irradiation. For example, rats exposed to approximately $LD_{50}$ doses of neutrons have been shown to develop fatal sepsis following the intravenous administration of viable bacteria much more readily than do control rats. Radiation-induced tumors should perhaps be considered in this same light, inasmuch as they probably represent an altered response to some independent factor or factors already present in the irradiated host or its environment.

6.6 Acquired Radioresistance

Recovery from one or more whole-body exposures to large doses of radiation may result in a decrease in the severity of the radiation syndrome following subsequent exposures to similar doses. The explanation for this phenomenon is obscure. Many of the results of exposure to large doses, e.g. decreased vascularity, fibrosis, and other histological changes which might best be described as characteristic of aging, are also characteristic of radioresistant tissues. Whether or not there is a direct cause and effect relationship between the histological changes and acquired radioresistance, or whether they are merely coincidental has not been determined. In any event, it should be recalled that multicellular animals do not acquire radioresistance to all phases of radiation damage following repeated exposures. In the case of genetic damage, for instance, the effects of repeated exposures are strictly cumulative.

Some attempts have been made to study the specific problem of acquired radioresistance. One of the more recent is that carried out by Dr. Margaret Bloom, who studied the intestinal crypt epithelium of rats which had been exposed repeatedly to doses of 60 r of x-rays. Histological changes were used as criteria of radiation damage, and were found to be progressively less marked following repeated exposures at intervals of about ten days.

A similar phenomenon has been observed repeatedly in tumors subjected to therapeutic doses of ionizing radiation. Not uncommonly a tumor which regresses markedly following an initial course of therapy responds less and less to subsequent courses until finally irradiation has almost no destructive effect. In some instances this change in radiosensitivity is associated with a change in the histological characteristics of the tumor, however, this is not always the case.

In bacteria and certain unicellular animals, acquired radioresistance appears to have a genetic basis. In B coli, for example, there is a spontaneous mutation which results in a marked increase in radioresistance. After irradiation of the cultures with x-rays, the mutation rate is increased and the incidence of this particular mutant may be very high. It is also possible that successive irradiation results in an increase in resistant strains partly because of the principle of survival of the fittest -- i.e., the inherently vulnerable individuals are more easily destroyed and the resistant organisms survive and propagate.

6.7 Selective Sensitization

Consideration of the differences in radiosensitivity among various tissues commonly leads to a consideration of methods of selectively sensitizing tissues, especially neoplastic tissues. Obviously this would be a great boon to radiation therapy. There are three ways in which this end might possibly be achieved, namely, by altering the tissue in question in some way so that it absorbs a considerably larger quantity of radiation; by increasing the response of the tissue to the radiation which it absorbs; or by decreasing the radiosensitivity of normal tissue in some manner. Unfortunately, all of these possibilities have proven difficult to accomplish, largely because they all depend upon selectively altering either the normal or malignant tissue.

6.8 Protective Phenomena

Several factors which modify radiation injury have already been mentioned. On the basis of presently available information it is not possible to identify any one biochemical system, cell constituent, cell type, tissue or organ system as being uniquely important in determining radioresistance or susceptibility. Instances in which a certain degree of protection has been achieved by shielding portions of highly radiosensitive systems (e.g. the bone marrow and spleen) are properly considered as examples of the beneficial results of maintaining adequate physiological reserves. In such cases, protection is achieved without altering the basic character of the response to irradiation.

Studies of a somewhat different nature have indicated that protection against radiation damage can be obtained by avoiding the toxic products formed by irradiation. This represents the "poison hypothesis" and has been most strikingly demonstrated in simpler organisms. Irradiation of bacteria, for example, produces a much more marked effect when the bacteria are left on the medium on which they were exposed. If they are removed to fresh media, the radiation effect is greatly reduced. The irradiated medium apparently contains a "toxic" substance which is formed as a result of irradiation, for when washings from this media are used to make a new media, organisms grown on it manifest the same effects as irradiated organisms of the same strain. Removal of paramecia from media in which they are irradiated to fresh media has been shown to decrease the harmful effects of irradiation. Similarly tissues irradiated <u>in vivo</u> and promptly removed to a new host or to tissue culture show much less radiation damage than similarly irradiated tissues removed to new media after a considerable delay.

A close relative of the poison hypothesis is the postulate that radiation damage is due to the destruction of one or more vitally important substances which are particularly radiosensitive. If this were true, a very basic type of protection agains radiation damage might be achieved by replacement of the vital substance. One of the better known examples of this interesting postulate is that proposed by E. G. Barron, who maintains that enzymes containing the sulfhydryl group are particularly radiosensitive, and the addition of glutathione or other substances which supply sulfhydryl groups has been reported to afford some degree of protection under certain circumstances as was mentioned above. As mentioned in an earlier section of the chapter the possibility of exactly reversing all the biological effects of radiation appears to be very slight indeed.

7. Relative Effects of Various Ionizing Radiations

In general, the various types of ionizing radiation tend to produce closely similar effects on living systems, however certain differences have been observed. Externally applied beta radiation, for example, produces damage which is fairly well localized to the skin, while gamma radiation is highly penetrating and injures both deep and superficial tissues, and ingested radioactive isotopes exert their effects largely on tissues in which they are localized. Differences of this nature are fairly readily explained on the basis of differences in the localization of energy absorption. In progressively smaller organisms, qualitative differences in the effects produced by various types of radiations become increasingly difficult to identify, and if the cell is selected as the biological unit to be examined it becomes virtually impossible to determine by inspection what kind of ionizing radiation produced the observed changes. For that matter, it is equally difficult to determine whether the damage is due to irradiation or to some other noxious agent. These considerations are of central importance in any attempt to contrast the effects of various types of ionizing radiations on living systems.

Although the various types of radiation tend to produce closely similar effects on living systems, they may vary greatly in the efficiency with which they produce these effects. Observed differences of this nature have given rise to the concept of biological effectiveness, which may be defined as the quantity of biological effect produced per unit quantity of energy supplied by the ionizing radiation. Determination of biological effectiveness is thus beset both by problems inherent in making accurate quantitative measurements of biological changes, and by problems involved in making accurate measurements of the amount of energy supplied in the form of the ionizing radiation.

Measurement of biological change is indeed a formidable problem. In many cases the effects are subtle, qualitative in nature, the mere identification of which requires considerable experience and skill, as for example, in the recognition of mutations. Application of the methods of physics and chemistry are attractive, however, even this is difficult for there is almost no manipulative technique which can be applied to living cells without introducing a new variable. Measurement of cell size, viscosity, pH, surface tension, chemical composition, etc., are all subject to limitations in this respect, while statistical studies based on observation of intact organisms (as for example, attempts to determine survival time under certain conditions) fail to differentiate the contributions of many mechanisms which may be important in causing death even in simpler unicellular organisms.

The problems inherent in making accurate measurement of the amount of energy supplied in the form of ionizing radiation are somewhat less difficult, although they have not yet proved capable of an entirely satisfactory solution. The major approaches to a quantitative measurement of ionzing radiations may be classed as biological dosimetry, chemical dosimetry, and physical dosimetry of which only the latter merits detailed consideration.

## 7.1 Biological Dosimetry

Biological dosimetry is largely of historical interest, and is extremely limited in application because of the problems inherent in making quantitative measurements of biological effects. The rationale for biological dosimetry lies in the desire for a biological standard for the measurement of dose in radiation therapy. The effect which found greatest application was radiation induced erythema, and the concept of the erythema dose found wide application until rather recently. The magnitude of certain other radiation induced biological changes varies with exposure, and various attempts have been made to utilize these as radiation dosimeters. The inhibition of growth in seedling plants was one of the earliest to be studied in this respect. Decreased transplantability of irradiated mouse tumors, diminished mitosis in onion root tip cells, inhibition of development of irradiated Drosophila eggs, and altered pigmentation in the nervous system of goldfish have also been proposed as dosimeters.

7.2 Chemical Dosimetry

The possibility of quantitatively measuring ionizing radiations by determining the amount of chemical change produced under specified conditions early attracted attention, and several chemical systems were studied with this in mind. The more popular of these included Eder's reaction (precipitation of mercurous chloride from a solution containing mercuric chloride and ammonium oxalate), decolorization of methylene blue, liberation of iodine from iodides, decomposition of formic acid, decomposition of $Na_2S_2O_8$, and oxidation of ferrous iron. These reactions all shared in the disadvantage that large amounts of radiation produced only slight change. Furthermore, it was soon observed that the production of chemical change by ionizing radiations was very inefficient, in some cases with less than 1% of the absorbed energy being accounted for on the basis of the resulting reaction. Chemical dosimeters were therefore unsuited as primary standards, and had to be calibrated with the aid of an instrument which measured the absorbed energy. Although inherently simple, chemical dosimeters proved impractical and, with improvements in physical dosimetry, soon became obselete.

Renewed interest has recently been focused on chemical dosimeters as an inexpensive means for the semi-quantitative detection of ionizing radiations following such catastrophies as the detonation of atomic explosives.

## 7.3 Physical Dosimetry

The problems implicit in physical dosimetry can best be discussed by considering the requirements of physical measurements and the degree to which these requirements can be met with present techniques.

The complete physical characterization of the dose delivered by radiation entails knowing the following:

1. The energy dissipated per unit mass of the irradiated material.
2. The spatial distribution of absorbed energy.
3. The frequency of various energy events which occur during degredation of absorbed energy to the thermal level.

Consider first the problem of measuring the amount of energy dissipated in a unit mass of irradiated material. The material of interest is, of course, living tissue of one kind or another. A direct measure of the total amount of energy absorbed implies calorimetric techniques. These are out of the question with living tissue because the metabolic heat overshadows the amount of heat produced by even large doses of radiation. Calorimetric measurements of radiation absorption are possible using materials which simulate living tissue, but the techniques are difficult because the temperature increases are small. A dose of 5000 r of high energy x-radiation would raise the temperature of 1 gram of wet tissue about 0.01 C. Since direct measurement of the energy absorption lies beyond the limits of physical techniques in all but a few special cases, various indirect measures must be employed.

A simple relation between the ionization produced in a small volume of gas and the gamma ray energy absorbed per unit volume of the solid walls has been derived by Bragg and Gray. Gray, Rossi and others have extended this relation to radiations other than gamma. Thus the energy absorption at any point in a mass of irradiated tissue can be obtained by measuring the ionization produced in a small gas volume contained in a chamber with walls of the same composition as the tissue. Although this method is not without its critics and its practical limitations, it remains the most direct measurement of energy absorption available. The chief limitations are those of matching the composition of the chamber walls to that of the surrounding tissue, which cannot always be considered homogeneous even in the macroscopic sense, and of maintaining the linear dimensions of the gas cavity small, as compared to the range of the secondary corpuscular radiation. The second limitation can be relaxed if the gas has the same composition as the walls. However, this leads to an explosive mixture when the walls are tissue-equivalent. Within the accuracy to which the various restrictions can be met, it is possible to measure a dose in terms of ergs per gram of tissue. This is the quantity which the International Commission on Radiation Units at its meeting in London July 1950 recommended as the fundamental unit of dose for all ionizing radiations.

The roentgen* unit illustrates a less direct approach to the problem of measuring the energy dissipated per unit mass of irradiated material. Since it is defined in air, the roentgen appears to be a somewhat more easily measured quantity than the erg per gram of tissue, but even this statment

*that quantity of x- or gamma-radiation such that the associated corpuscular emission per 0.001293 gm. of air, produced in air, ions carrying 1 electrostatic unit of quantity of electricity of either sign.

must be qualified. Either the standard free air chamber, or an air-wall Bragg-Gray chamber may be used to measure the roentgen. The standard free air chamber measures the roentgen as it is defined, but at either very low or very high energies such chambers are subject to many practical difficulties. A Bragg-Gray chamber with air equivalent walls measures the roentgen indirectly and subject to the same limitations described above for the tissue-wall chamber, but does so with practical convenience over a wide range of x and gamma-ray energies.

The roentgen, although defined in a manner which permits direct measurement under certain circumstances, possesses two fundamental weaknesses:

> By definition it applies only to x- and gamma-radiation; for a given dose in roentgens, the dose delivered to tissue depends on both the wavelength of the incident radiation and the composition of the tissue.

These weaknesses are particularly unfortunate since the great bulk of knowledge regarding the biological effects of radiation has been obtained with x- and gamma-radiation, and is consequently expressed in roentgens. This situation has given rise to several attempts to introduce units equivalent to the roentgen which are also applicable to other types of radiations. Two such units are the rep* and the gram-roentgen**. Unless used with considerable care such units confuse rather than clarify. To illustrate: 1 roentgen of 100 Kev quantum radiation produces 1 rep in muscle, but 1

*the rep, roentgen-equivalent-physical, is that quantity of any ionizing radiations which produces energy absorption of 93 ergs per gram of tissue.

**the gram-roentgen is the energy dissipated by gamma-rays in 1 gm. of air, i.e., 1 gram-roentgen equals 83.8 ergs in an unspecified amount of tissue, thus 1 rep equals 1 gm-r/gm. of tissue.

roentgen of 12 Kev radiation produces about 0.46 rep in fat and about 9.5 rep in bone. Thus 1 roentgen does not equal 1 rep under any but limited circumstances.

A still different approach to the problem of measuring the energy absorbed from radiation is illustrated by the "n-unit"* which was introduced as a unit for fast neutron dose. Since it is defined in terms of a convenient measurement, this unit can be measured directly, and this is its sole virtue. The exposure conditions must be such that ionization is produced in the thimble chamber by fast neutrons and not by a combination of these with thermal neutrons, gamma rays and radioactivity induced in the walls. If this condition cannot be attained, then suitable corrections must be made. A further difficulty is that two chambers with identical properties for gamma rays may exhibit quite different responses to the same fast neutron exposure. The fundamental objection to the n-unit is the lack of a definite relation between a dose in n-units and the dose in tissue, and that whatever the relation between these two may be, it will be a function of both the neutron energy and the tissue composition. The nature of the definition of the n-unit may contribute appreciably to apparent differences in biological effectiveness of x-rays and neutrons.

It is evident then that the wide variety of physical dose units does little to alleviate the problems of measuring or estimating the total amount of energy dissipated in a unit mass of irradiated tissue. The most direct method available is that of locating a Bragg-Gray chamber at the point of interest. If the chamber walls, cavity dimensions and filling

*the n-unit is that quantity of fast neutron radiation which produces the same ionization in the Victoreen 100-r thimble chamber as would 1 r of gamma radiation.

gas are suitably chosen, reasonably accurate measurements of the dose in ergs per gram of tissue can be obtained. Where the direct approach is not possible, reliance must be placed on conversion factors between more conveniently measureable quantities of radiation and the dose delivered at the point of interest in tissue. The conversion factors between incident flux density and dose rate for gamma radiation as calculated by Marinelli, Quimby and Hine, and for neutrons as calculated by Mitchell, Gamertsfelder, Tait and Biram, are examples.

Measurement of the spatial distribution of the absorbed energy throughout the irradiated material is subject to the same difficulties inherent in the measurement of the energy dissipated per unit mass. As the volume of interest becomes smaller, the practical difficulties multiply rapidly. Sievert has made and used small ionization chambers. Hine has described a crystal probe which automatically traces isotope surfaces in a liquid phantom, and Cassen has described a scintillation probe affixed to the end of a hypodermic needle for use in living tissue, but the extension of such measurements to heterogeneous media, such as a large living organism, and to radiations other than x- and gamma has been slight. Calculations of this sort are so complex as to become manageable only after restrictive simplifications.

Determination of the frequency of the various energy events which occur during degradation of the absorbed energy to the thermal level lies more nearly within the capabilities of available physical techniques than does the measurement of the spatial distribution of this energy. A number of "proportional" devices are capable of making the desired measurements, either in gases, liquids, or solids. When properly arranged, such proportional devices give voltage pulses which bear a linear relation to the size of the ionizing event. Ultimately they will all saturate, i.e., if the size of the ionizing event is increased indefinitely, a point is finally reached at which larger voltage pulses cannot be produced in the sensitive element. At the other end of the scale such devices fail eventually, either because the electronic "noise" inherent in any electrical device obscures the small pulses produced by the radiation, or because the energy steps have become so small that they do not produce ionization, or excitation in the case of the crystal and scintillation counters. Such devices thus have a threshold, and events involving amounts of energy smaller than this will not be recorded.

The threshold limitation of proportional devices is one shared by all radiation instruments which depend on ionization. With very few exceptions all physical devices which measure radiation depend on ionization, and it is worth considering briefly the restriction which this method of measurement imposes.

If the total amount of energy dissipated in a given volume of gas is divided by the number of ion pairs produced by this energy, a figure between 32 and 35 electron volts is usually obtained. On the other hand, if the energies required to produce ion pairs from the various types of molecules in the gas are determined, values between 10 and 15 electron volts will be found. Obviously, then, only about one half of the energy absorbed from the radiation is degraded to thermal energy in steps large enough to produce ion pairs, while the other half slips down the energy scale in steps smaller than 10 or 15 electron volts. This invisible energy loss is of small consequence to the measurement of the total energy dissipated per unit mass of irradiated material as long as the amount of energy required to produce an ion pair remains constant. Fortunately this energy remains constant for a given type of radiation over quite wide energy ranges. However, when one is concerned with the spatial distribution or the frequency of the various events which occur during degradation, the undetected half of the absorbed energy cannot be shrugged off. Energy events in the range of 1 electron volt, and smaller, are of importance in producing chemical changes, and while these events are small, there must be many of them, and one would like to know where they occur in the irradiated material and how many there are of any given size. These determinations lie beyond the limits of present physical techniques.

To summarize: the energy dissipated per unit mass of irradiated tissue can be measured with a tissue-wall Bragg-Gray chamber and expressed with reasonable accuracy in ergs per gram of tissue, as advocated in the recent recommendation of the International Commission on Radiation Units. The spatial distribution of the energy dissipated throughout the irradiated tissue can be determined moderately well on a macroscopic scale using small Bragg-Gray chambers, either gaseous or scintillation, which are properly matched to their environment. Measurement of space distribution on a near microscopic scale is possible only in one dimension with the extrapolation chamber. The frequency of the various events by which the absorbed energy degrades to heat can be measured with reasonable accuracy down to the least energy which will form an ion pair; below this, measurement is not possible with the present physical methods. This shortcoming is to be recognized as a serious limitation because it neglects about half of the absorbed energy and possibly upwards of 95% of the energy-loss events.

## 7.4 Biological Effectiveness

Heated discussion and extensive experimental work have centered on this subject. In the preceding pages, an attempt has been made to sketch briefly the problems inherent in arriving at estimates of biological effectiveness. Clearly the difficulties are very real, and impose serious restrictions on interpretation of experimental work. Unfortunately the matter may be even more complicated than might be expected, because of the apparent dependence of biological effectiveness upon the biological test system selected for study. Neutrons, for example, appear to be more effective than ionizing electromagnetic radiation in producing cataracts and chromosome breaks, but less effective in producing gene mutations. For the present, at least, an honest skepticism regarding the relative biological effectiveness of various radiations seems in order, and current permissible exposure levels which are based on estimates of relative biological effectiveness should be recognized as arbitrary standards, which may be subject to extensive revision as more exact information becomes available.