

MECHANISMS in RADIOBIOLOGY

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VOLUME II

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PREFACE

Active research in radiobiology has been carried on since the beginning of this century. Many fundamental aspects were envisaged by pathologists and physicists in these early days, chiefly in connection with the therapeutic use of X-rays in medicine and the resulting need to study the biological action of these rays. The discovery of H. G. Muller that mutations could be induced by radiation opened a new field for exact, quantitative work. Shortly after the Second World War D. E. Lea made the first, and very successful attempt, to coordinate radiobiological knowledge into a comprehensive picture.

Since then we have witnessed a tremendous expansion of research work in radiation physics, chemistry, and biology, which has been further stimulated by the rapid development of atomic energy. Radiochemical reactions and the mechanism of energy transfer are now far better understood. Precise methods of irradiating biological units with α -particles, neutrons, etc., have afforded more definite notions of the effects on various cell structures, of the mechanism of gene action, cell division, and differentiation.

The development of radiobiology has run parallel with the advancement of biology in general and a wealth of data has thus accumulated, far more than one man can possibly cover. This is why it was felt that a comprehensive treatise, written by experienced scientists, might result in a useful handbook for the student as well as an informative book of reference for the advanced research worker.

Volume I deals with physical and chemical aspects of radiation effects as well as biochemical changes produced *in vivo* and *in vitro*. The treatment of cytological effects has particularly emphasized cellular damage as it appears under the electron microscope. Radiation effects on free living cells and radiation genetics have been presented chiefly with the view in mind to discuss principles and mechanisms of action. In Volume II radiation effects on embryonic and adult organisms are discussed, chiefly in mammals. Special chapters are devoted to immunological processes in irradiated organisms, to the mechanisms of action of protective and sensitizing agents, as well as to the experimental possibilities of enhancing the recovery of irradiated mammals.

The editors are of course aware of the difficulty of covering the whole field of radiobiology and adjacent disciplines. Also, science has made rapid headway between the stage of planning of this book and the final stage of preface writing some two and a half years later. Paramagnetic resonance measurements have been developed as an important tool for determinations of free radicals during the past few years. Some topics, such as bac-

terial genetics and lysogeny have not been included since there are many review articles available. Other, more practical aspects, such as problems related to internal radiation sources have been superficially treated because of their very specialized nature.

The editors wish to acknowledge the pleasant cooperation of all the authors and the Academic Press during the preparation of this book.

Bruxelles and Stockholm

March 5, 1960

M. ERRERA
A. FORSSBERG

ABBREVIATIONS

ACTH — Adrenocorticotropic hormone	ICRP — International Commission for Radiological Protection
AET — Aminoethylisothiuronium bromide hydrobromide	IQ — Intelligence quotient
BAL — British antilewisite = 2,3-dimercapto-1-propanol	LD ₅₀ — Dose lethal to 50 % of animals
CSH — Cysteine	LD ₅₀₍₃₀₎ — Dose lethal to 50 % of animals during the 30 days following irradiation
CSSH — Cystine	MEG — <i>S</i> -mercaptoproethylguanidine hydrobromide
DNA — Deoxyribonucleic acid	MLD — Minimal lethal dose
DNase — Deoxyribonuclease	p.i. — Postirradiation
DOCA — Deoxycorticosterone acetate	PVP — Polyvinyl pyrrolidinone
dopa — 3-(3,4-Dihydroxyphenyl)-alanine	RBE — Relative biological efficiency
DPN ⁺ — Diphosphopyridine nucleotide	RNA — Ribonucleic acid
DPNH — Diphosphopyridine nucleotide (reduced)	RNase (pH6) — Ribonuclease (pH optimum = 6)
EDTA — Ethylenediamine tetraacetate	RNase (pH8) — Ribonuclease (pH optimum = 8)
EEG — Electroencephalogram	STH — Somatotrophic hormone
ERG — Electoretinogram	TEM — Triethylene melamine = 2,4,6-tris(1-aziridinyl)- <i>s</i> -triazine
G.I. — Gastrointestinal tract	TPN ⁺ — Triphosphopyridine nucleotide (oxidized)
GSH — Glutathione (reduced)	TPNH — Triphosphopyridine nucleotide (reduced)
GSSH — Glutathione (oxidized)	

UNITS

ev — Electron volt. The energy of each quantum of electromagnetic radiation is given in ev by $12,400/\lambda$, where λ is the wavelength in angstroms.

rad — Unit of absorbed energy equal to an energy absorption of 100 ergs per gram of irradiated material at the point of interest. It is applicable to any ionizing radiation provided the energy deposited is measured or calculated in the material actually irradiated.

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r — Roentgen, the unit of exposure dose to X- or γ -rays. One r is an exposure dose of X- or γ -radiation such that the associated corpuscular emissions per 0.001293 gm of air produces in air, ions carrying one electrostatic unit of gravity or electricity of either sign.

Produces 2.1×10^9 ion pairs in 1 cc of air.

Absorption of 83 ergs per gram of air or 93 ergs per gram of water.

rep — Roentgen equivalent physical. One rep liberates the same energy in water or tissues as one r of X-rays. (This unit has now generally been replaced by the rad.)

c — Curie, the quantity of radioactive nuclide in which the number of disintegrations per second is 3.7×10^{10} .

n — Neutron unit, the amount of neutron radiations which produces the same discharge of the 100-r Victoreen dosimeter as does one r of X-rays. (The n has now been generally replaced by the rad.)

LET — Linear energy transfer, the linear rate of loss of energy (locally absorbed) by an ionizing particle traversing a material medium. LET can be expressed in kiloelectron volts per micron.

RBE — Relative biological effectiveness is used to compare the effectiveness of absorbed dose of radiation delivered in different ways. *Example:* one rad of α -radiation produces a particular biological response in the same degree as 10 rads of γ -radiation. The RBE depends on many factors which should always be made clear. For X-rays and β -rays, $RBE \approx 1$; for α -rays, $RBE \approx 10$.

rem — Unit of RBE. The dose in rem equals the dose in rads multiplied by RBE.

The definitions of units have been taken from: Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, General Assembly, Official Records of the 13th Session, Suppl. No. 7 (A/3838), New York, 1958; Z. M. Bacq and P. Alexander, "Fundamentals of Radiobiology," Academic Press, New York, 1955.

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CHAPTER 1

General Biology: Gametes, the Developing Embryo, and Cellular Differentiations

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I. Effects of Ionizing Radiations on the Testes and Spermatozoa

A. INTRODUCTION

1. THE GENERAL EPITHELIUM

The first description of *X*-irradiation damage to the testes was made in 1903 (1), and since then there has been a voluminous literature on the subject, probably most of which has appeared during the last two decades. Regaud and Blanc (2) first suggested that changes in sensitivity in the testes accompanied the development of different cell types. It is quite generally agreed now that the various stages from the earliest spermatogonia to the mature spermatozoa exhibit very different sensitivities to the same irradiation. There are type A, intermediate, and type B spermatogonia, which are differentially very radiosensitive. Further, type A spermatogonia are not themselves uniform in sensitivity. Irradiation just prior to the last division has less effect on the cells destined to become dormant type A cells than on those which transform (differentiate) into the intermediate stage and thence to type B spermatogonia. The heterogeneity of sensitivities is indicated also for stages where all type A spermatogonia are in interphase. Thus, markedly different responses exist for cells which may be regarded as cytologically similar (3). It may be possible ultimately to distinguish further between the sensitivities of spermatids and spermatozoa, although at present they are considered to be rather equally radioresistant. Of the nonspermatogenetic elements, the Sertoli and interstitial cells are believed to be very radioresistant (4). In any case, the peculiar radiosensitivity of the testes is due to the spermatogenetic elements derived from the germinal epithelium, and the order of decreasing sensitivity is from the most sensitive spermatogonia, through the spermatocytes and spermatids, to spermatozoa, which are quite radioresistant. Thus, weight reduction in exposed testes is due primarily to actual cell loss, in the order named, the degree depending on the level of exposure.

Short of actual necrosis, there can be damage to germinal cells by irradiation such that there is no evidence of it until mitosis, meiosis, or differentiation is attempted, at which time the cells may go to pieces. This has led to a controversy as to whether the various cells of the germinal epithelium are actually destroyed by irradiation (4-14) or whether meiosis and differentiation cannot occur because spermatogonia stop dividing (15-17). Of course, destruction (necrosis) of any cell would preclude its subsequent mitosis, so that the only way this dispute can be resolved is to determine the minimum level of induced cell necrosis. Even so, this does not eliminate the possibility that a lower level of exposure, sufficient to interfere with

mitosis, meiosis, or differentiation, might not bring about the same end result, namely sterility. It will not be the province of this discussion to include lethal mutations, nor mutational effects of any kind, though the distinction between chromosomal aberrations and dominant lethals (either of which could affect fertility) is not at all clear, even to many cytogeneticists.

This important question of whether necrosis or inhibition of mitosis is the major factor in spermatogonial depletion has been re-examined (10). "The development of spermatocytes from Type A spermatogonia may be summarized as follows: As judged by the number of cells observed per cross section in our material, each dormant Type A spermatogonium undergoes three divisions. Of the resulting 8 cells, one remains as a dormant Type A cell and later starts the multiplicative cycle over; the remaining 7 cells transform to intermediate spermatogonia which divide to form about 14 Type B spermatogonia, the Type B cells then divide to form about 28 primary spermatocytes." This investigator therefore believes that the depletion of spermatogonia after irradiation is due to the actual killing of these cells. Further conclusions of this investigator are worthy of review (10).

"Reduction in number of spermatogonia after irradiation can be explained by killing of the cells, without postulating prolonged mitotic inhibition of Type A spermatogonia. Intermediate and Type B spermatogonia are extremely sensitive to irradiation while Type A spermatogonia are of heterogenous sensitivity, depending upon the mitotic activity and stage of development. Degeneration of spermatogonia occurs primarily as damaged cells reach late interphase or early prophase of their first post-radiation division. A few cells may undergo one or more divisions before degenerating. Irradiation damage to the primary spermatocytes remains latent until the cells enter meiotic metaphase and anaphase. Many abnormal figures typical of chromosomal aberrations then occur, and nuclei of resulting spermatids show an abnormal size variation. Spermatids and spermatozoa show no visible changes with doses up to 1500 r. (mice). However, numerous investigators have established the sensitivity of these cells to genetic damage which is expressed after fertilization" (10). The emphasis on dominant lethals has also been made (18), and through their study it may be possible eventually to prove greater sensitivity differences between the spermatids and spermatozoa. It has also been shown that the intermediate and type B spermatogonia are about seventy times as radiosensitive as are the type A spermatogonia (19).

Although spermatogonia may be of several types, and although they may be resting in mitosis or in active differentiation into primary spermatocytes, it is generally conceded that spermatogonia of any phase are more

radiosensitive than are the later stages in spermatogenesis (maturation). One author (20) suggests that it might be possible for spermatogonia to be mitotically inhibited but nevertheless differentiate. This is improbable, because other studies (21) have shown that any cell in the process of differentiation is in its most radiosensitive phase, much more so than during mitosis. It has even been suggested that there are prespermatogonia which are less sensitive (3, 22). When testes are depopulated of their spermatogonia by irradiation to 1440 r (C57 black mice), a new generation of spermatogonia develops from the prespermatogonia. Even a dose of 138 r depopulated the spermatogonia in 3 to 4 days, and repopulation occurred, but it was delayed after repeated exposures. There is certainly need for clarification in this area.

2. THE RECOVERY OF SPERMATOGENESIS

It is now well known that the testes when subjected to either an acute dose of irradiation or to chronic exposures, followed by sterilization, may "recover" in time and to the extent of being able to reproduce functional spermatozoa. The three suggested sources for "recovery" of the germinal elements are from the Sertoli (nurse) cells, which is very unlikely; from undifferentiated pregerminal cells; or from a few surviving relatively undamaged type A spermatogonia. Whatever the source, this does not mean that the surviving cells and their progeny are without (genetic) damage but simply that they are alive and able to reproduce by mitosis and to produce primary spermatocytes. There is no doubt but that such cells, after exposure to any irradiation, carry more than their prior quota of deleterious, irradiation-induced mutations. It is for this reason that the meaning of the word "recovery" must be clearly understood. It does not mean a return to the preirradiation condition except in so far as the ability to produce functional spermatozoa is concerned. The word is challenged to emphasize the fact that, although fertility is restored, the cells have been altered by irradiation. This phenomenon of "recovery" after high-level irradiation of the testes has no counterpart in the ovary.

This so-called "recovery" or "regeneration" of the germinal epithelium of the testes after irradiation depopulation may be delayed for various lengths of time, depending on the dose and possibly on the rate of irradiation, providing the fractionation is over a long period. At low doses many spermatogonia may be spared sufficient damage so that they can repopulate the testes, but after high levels of exposure regeneration will depend on the survival of a few type A spermatogonia, which then have the task of repopulating a greater volume of the testes, which process naturally takes

longer. Consequently the interval between irradiation, its consequent sterility, and the ultimate regeneration ("recovery") and renewed function of the testes may be a very long period. In some species (e.g., mice) it may be a matter of weeks, and in others (e.g., man) it may be many months. Complete and permanent sterilization of the male can be achieved, but it is more difficult and requires a higher level of exposure than it does in the female, owing to the persistence of a few early spermatogonia. Male mice X-irradiated to the posterior third of the body to 1000 r produced offspring 22 months later, and other males given 600-r whole-body exposures were fertile at 18 months after irradiation (23).

3. THE MEANING OF STERILITY (OR ITS CONVERSE, FERTILITY)

When the testes are exposed to excessively high levels of X-irradiation and are studied cytologically at intervals thereafter, it is quite obvious that X-irradiation causes formed meiotic chromosomes to lose their identity by becoming fluid, sticky, and "less viscous." They tend to flow out along the more rigid spindle fibers, losing their shape to such an extent that their return to normal morphology is impossible. The end point of irradiation changes on the germ cell is the pyknotic nucleus, seen most readily in the spermatocytes. Stages exposed at a time when there are no formed chromosomes develop these abnormal configurations at the time of attempted mitosis or meiosis (24).

Hypoplasia of the spermatogenetic stages will be varied both in time and extent, depending on the degree of irradiation damage inflicted. But the spermatids and spermatozoa, being relatively radioresistant, can be used by the animal for fertilization for a period of days or weeks after exposure, depending (in an inverse relation) on the level of exposure. In an attempt to produce parthenogenetic mammalian embryos in a manner similar to those produced among amphibia (25), C57 black male testes were exposed to 41,000 r and the mice mated with CF1 white females, which produced 8 viable black offspring (26). This was not parthenogenesis, since the offspring carried the paternal coloring, but the fact that the C57 black sperm could tolerate such a high level of exposure and survive through parturition is new. These mice will subsequently be mated to determine effects on fertility and gross genetic factors.* Other males are being exposed to even higher doses of X-rays, in an attempt to eliminate the nuclear (genetic) contributions of the sperm and produce true mammalian partheno-

* These mice, resulting from 41,000-r sperm and normal ova, were viable and fertile and produced F_2 mice. While no gross anomalies appeared in the F_1 , exencephalia and many other anomalies did appear in the F_2 .

genesis. It should be emphasized, however, that these high doses affect neither the motility nor the fertilizing power of mature spermatozoa. Invariably the males die within a few days, owing to radiation scatter to more vital organs. This ability of such highly exposed testes to produce functional sperm is short-lived, even if the animal itself survives. Sterility in the male is therefore never immediate because of the high radioresistance of the spermatozoa, as well as the spermatids. By the time the spermatozoa and spermatids (which are to be transformed to spermatozoa) are used up, the derivatives of the more sensitive spermatocytes, whether or not they can differentiate, are so damaged that they cannot function. In most rodents (rats and mice) there appears to be about a 27- to 28-day cycle from spermatogonia to spermatozoa (20). The peak of hypoplasia of the entire germinal epithelium will occur at about that time. Rather precise intervals for mice have been reported (27) as follows: spermatogonia A—0 days; spermatogonia B—4.7; resting spermatocytes—6.0; first meiotic prophase—9.0; early spermatids—20.0; and spermatozoa—34.5. There is another study, however, in which it is suggested that it may take 6 to 7 weeks to produce spermatozoa in mice (28).

The exact duration of each of the twelve stages in mouse spermatogenesis has now been worked out with great precision (29) as shown in Table I.

Sterility may last for weeks or months, depending on the scarcity of active and functional spermatogonia that are left and their ability to re-

TABLE I
DURATION OF STAGES IN MOUSE SPERMATOGENESIS

Stage	Duration (hours)	Maximum days to release of mature spermatozoa
Spermatogonia		
Type A	Always present	35.5 days
Intermediate	27.3	30.0
Type B	29.4	28.8
Primary spermatocytes		
Resting	31.0	27.6
Leptotene	31.2	26.3
Zygotene	37.5	25.0
Pachytene	175.3	23.4
Diplotene	21.4	16.1
Diakinesis + metaphase 1	10.4	15.2
Secondary spermatocytes	10.4	14.8
Spermatids	229.2	14.4

populate the seminiferous tubules. This has generally been termed "temporary sterility." It must again be emphasized that the repopulation occurs from spermatogonia which survive the irradiation but which must inevitably carry a large quota of deleterious mutations, so that the term "temporary sterilization" must be qualified, since the poststerilization gametes are not (genetically, at least) the same as those present before sterilization was imposed. The word "temporary" implies a return to the previous condition which is not exactly achieved.

Chromosomal mutations give reliable information only on the sensitivity of postmeiotic stages in spermatogenesis of the mouse. These suggest that the spermatids are twice as sensitive as are the sperm, in either testis or epididymis. The sensitivity of type B spermatogonia is relatively so great that it has been impossible to measure their mutagenic sensitivity. The two mechanisms are probably totally unrelated, owing to different processes. It is suggested that the sensitivity of stem cells to chromosome breakage is probably the same as for the mature sperm. X-rays appear to produce an "aging" effect on Sertoli cells (29a).

When regeneration or repopulation occurs, it is thought by some to begin before the peak of hypoplasia (2, 6, 9), and this may indeed be true for low levels of irradiation where the depopulation does not involve cells of every phase of meiosis. Some investigators (30) state that if regeneration does not occur within several months, permanent sterility is inevitable. For instance, "recovery" was slow after 800 r, and rare after 1000 r. Others (17, 30-32) have claimed, however, that repopulation of testes in mice can occur even after 6 or 7 months.* The so-called "recovery" has occurred even later in man. It would be interesting to determine whether there is any correlation between the sensitivity of the germinal epithelium of a particular species and its LD₅₀₍₃₀₎ value, using a wide range of sensitivities such as is found in the LD₅₀₍₃₀₎ values for guinea pigs, rats, and rabbits. It is believed that the order of (relative) sensitivity of the various maturation (spermatogenetic) stages is the same for all species studied.

An estimate of the functional condition of the testes based entirely on an histological examination is difficult (33). Neither the Sertoli cells nor the interstitial tissue is appreciably reduced in adult testes after even high levels of irradiation (16, 34), and there is no decrease in libido and hence no effect on the hormone production. If the testes are irradiated during their embryonic development (i.e., during fetal life) in the mouse, however, and are subsequently tested at 6 months of age, it is found (34) that testes sterility is correlated with the relative number of seminiferous tubules in which germinal epithelium is absent. In those testes where 40% or more

* A recent case became fertile after 2 years of x-irradiation sterility.

of the tubules contained maturation stages, the male was fertile. The completely sterile testes contained 10% or less of their tubules with maturation stages. It was also found that the precursors of the embryonic Sertoli and interstitial cells were radioresistant and that, accompanying sterility, there was a *relative* increase in the amount of interstitial tissue from 15% in the controls to 44% in those that were completely sterilized by X-irradiation. These conditions followed exposure of the fetus to a total of 300 r at 50 r/day during the last 6 days of (mouse) fetal life. *We must therefore conclude that X-irradiation changes in the testes relate primarily to the products of the germinal epithelium.*

4. EFFECT ON LITTER SIZE

Fertility in terms of litter size is not altered immediately after high-level X-irradiation, as litters of 8 or more have been produced from spermatozoa exposed to 41,000 r (26), and the poststerility litters from "recovered" testes approach the normal size (20). When there is reduction in litter size after testes irradiation, it is generally attributed to severe chromosomal aberrations which cause developmental abnormalities, intra-uterine death, or abortions (35, 36). It is very doubtful that spermatozoa, once matured, can be so damaged by ionizing radiations as to be without motility or fertilizing (activating) power, but if the exposure occurs prior to full maturation the damage may be such as to prevent normal pronuclear fusion during fertilization, and this would preclude normal embryonic development.

Further, the sperm nucleus can initiate development and carry it to a critical phase, such as gastrulation, and then prove deleterious to further development, resulting in intra-uterine death. This could be due to genetic factors for some members of a litter, while others survive, thus reducing litter size.

5. SEMISTERILITY

The term "semisterility" has also appeared in the literature. It generally refers to a genetically transmissible condition rather than an immediate and direct effect on available gametes. Fractionated daily or chronic exposures of the spermatozoa are apparently less effective than is a single acute exposure in producing these transmissible reductions in fertility (20). The radioresistant and transient spermatozoa are not so affected by fractionated or chronic irradiation, but the radiosensitive and more persistent spermatogonia may be exposed to a cumulative dose which brings about the more serious condition (so far as development is concerned) of abnormal chromosomes, rather than genic mutations. It is postulated that within any field

exposed to ionizing radiations no two spermatozoa (or any maturation stage) would likely be affected in the same manner (25) and that the mutation or mutations induced in any particular cell would depend on the locus of ionization. Some effects would certainly be more drastic with regard to subsequent development than would others. The "semisterility," transmissible by exposed germ cells, would not therefore be universal among the germ cells exposed.

Although sterility does not necessarily mean testes devoid of germinal elements (34), it has long been known that in most mammals the number of motile sperm per ejaculate must reach a certain numerical value before fertilization seems possible, even though but a single spermatozoon is ultimately used for a single egg. The fact that some seminiferous tubules may appear to be quite normal, and adjacent to them may be tubules all but devoid of germinal epithelium, suggests that the difference reflects the effectiveness of the irradiation on the various stages in spermatogenesis, found in the various tubules. In chronic or in low-level fractionated exposures there may be continual spermatogenesis, but it is at a plateau of spermatozoon production too low to ensure fertilization from an ejaculate. This plateau may be maintained for variable periods, depending on the dose and dose rate of irradiation involved. The plateau may be at the level of *sterility, semisterility, or low fertility*—but not *temporary sterility*, which only follows an acute exposure (37–39).

There is no evidence of an indirect effect of irradiation on the testes. When the testes are shielded, whole-body irradiation is not reflected by any change in the germinal epithelium (40) unless a lethal exposure is involved, killing the animal.

There are, therefore, various criteria of effects of irradiation of the testes: *induced mutation, chromosome aberrations, reduced fertility, semisterility, and permanent sterility*—all involving the germinal epithelium and bypassing the Sertoli and interstitial cells. This has led to some confusion, both in analyzing the effects of various types of irradiation (RBE studies) and in equating fractionation with an acute exposure. There are reports (41) that there is no essential difference between single or fractionated doses, and this may be true as far as genetic sequelae are concerned. There appears to be some evidence, however, that fractionation of the total dose causes a lesser degree of sterilization (30–32, 37–39, 42–44), one explanation being that by fractionating the dose there is a delay in the process of repopulation from undamaged cells. Conversely, then, the so-called "recovery" of the germinal epithelium should follow better the single acute exposure (45). *The maximum chronic exposure which will still allow adequate sperm to mature for successful fertilization has not been determined for any species.* When

determined, this level will be low and yet many times the level for the induction of mutations.

6. RELATIVE BIOLOGICAL EFFECTIVENESS (RBE)

Such a quantitatively radioresponsive organ as the testes should prove of great value in RBE studies (43, 44). Thus far there seems to be no qualitative difference between X-rays, γ -rays, and neutrons, for each can cause sterility. Neutrons, in most biological tests, seem to be about five times as potent as are X-rays, with respect both to postirradiation sterility and to litter size changes (46).

The preceding statements are in the form of a survey, so that it is appropriate that we present below some experimental data from the various vertebrate groups to substantiate them. This discussion will deal only with the testes. It will not be exhaustive but will be quite up-to-date.

B. MARINE ANIMALS

The spermatozoa of the clam *Spisula* are much more radioresistant than are the eggs, and 264,000 r did not alter their fertilizing power, although with increasing exposures there was increasing delay in the initial cleavage time when X-irradiated sperm were used to fertilize normal eggs. The delay never exceeded 15 minutes, which was equivalent to the time between the first and the second cleavages. There was some evidence of parthenogenetic stimulation of the egg if the spermatozoa were exposed to more than 163,000 r of X-rays, but trochophore larvae did develop. Sperm dilution in sea water did increase their radiosensitivity (47).

X-Irradiation of the fish *Fundulus* to 200,000 r did not destroy the motility or the fertilizing power of the spermatozoa. Development of normal eggs fertilized by these sperm reached 12 days, and sperm exposed to 100,000 r and used to fertilize normal eggs gave rise to embryos which were able to develop through the hatching stage (48).

The heads of herring sperm consist entirely of DNA-protamine which disperses after X-irradiation in salt solution and does not give rise to any gel which can be spun down at 20,000 g. The DNA and protein cannot be dissociated by the use of detergents. It is suggested that the bond may be a covalent crosslink. Alpha rays are as effective as X-rays, in contrast to the degradation of pure DNA and protein where sparsely ionizing radiations appear to be more effective (48a).

C. AMPHIBIA

The poikilothermic amphibia are excellent material for laboratory experimentation. Eggs and sperm are available at all times of the year, and

development in the laboratory is normal. It was shown (25) that frog sperm could tolerate as much as 120,000 r and not lose their motility or their fertilizing power. When normal sperm (of *Rana pipiens*) were used, however, to fertilize normal eggs of the bullfrog (*Rana catesbeiana*), the embryos invariably disintegrated at the late blastula stage, owing to the incompatibility of the two genomes. When X-irradiated sperm were used in this same species cross, however, fertilization and development reached 93 % because the irradiated sperm with their genetically damaged nuclei were eliminated after the activation of the egg, and development was parthenogenetic (haploid). This was later further confirmed by a cytological study (49) which showed that 86 % of the developing embryos were haploid and 1 % diploid. In the earlier study (25) it had been shown that when sperm of increasing exposures were used to fertilize normal eggs there was a correlated increase in failure of the resulting embryos to develop, beginning with some at 15 r and continuing to 1000 r when none was normal. By continuing to expose spermatozoa to as much as 120,000 r (and thereby damaging all the chromatin contribution of the sperm), the eggs were activated by the sperm and proceeded to develop under the influence of the egg nucleus alone (gynogenesis). These data relate only to the effects on mature sperm.

D. GUINEA PIGS AND HAMSTERS

The guinea pig is highly radiosensitive ($LD_{50(30)}$ is 225 r), but its testes require a single direct exposure to 4500 r at 75 kvp to cause complete sterilization (50), although lower exposures might well prove effective. At 165 kvp and a lower exposure of 2100 r, fractionated over 6 days, complete sterilization was achieved (51). With continuous chronic whole-body exposure at the rate of 8.8 r/day (8-hour day), the guinea pig appears to be about as sensitive (or resistant) as the mouse (52). When the accumulated exposure reached 326 r there was an appreciable effect on spermatogenesis, and a total of 405 r caused complete hypoplasia of the spermatogenetic elements (6).

For the hamster ($LD_{50(30)}$ is 725 r) a single acute exposure of 5000 r to the testes at 184 kvp caused marked degeneration of the germinal epithelium but did not prevent ultimate (seventeenth week) reconstitution or "recovery" (54). A lower exposure might prove to be equally effective.

E. MICE

This form has provided for the bulk of irradiation studies with regard to fertility and sterility, "recovery," chronic and fractionated exposures, and RBE factors. It must be pointed out that there is a range of several

hundred roentgens between the LD₅₀₍₃₀₎ values for the various strains of mice, and there may well be correlated differences in radiosensitivity of the testes. For instance, effects were described for LAF mice whose testes were exposed to 200 r which gave results similar to those when C3H testes were exposed to 50 r (55). It is therefore important to specify the strain of mouse in all data presented.

A single acute exposure of the mouse testes to 800 r caused temporary sterility (4 months) after an initial period of 2 weeks of normal fertility, and an exposure to 1600 r or more caused permanent sterility. A single exposure of 300 r caused 90% destruction of the germinal elements, 1440 r destroyed 97.5%, and 5050 r destroyed all but the Sertoli cells (56) and resulted in complete sterilization. After 5050 r all the spermatogonia disappeared in 5 days, spermatocytes in 10 days, spermatids in 14 days, and spermatozoa in 21 days (5). The earlier papers did not distinguish between temporary and permanent sterilization, so that doses of 800 r (57), 2000 r (58), or even 3000 r (59) have been reported as the sterilizing dose for the mouse.

Nevertheless, hybrid mice of the 101 and C3H strains given 1000 r to the posterior third of the body (therefore including all testicular material) were able to achieve fertile matings 22 months later; and other hybrids given 600-r whole-body exposures fathered litters after 18 months (23). No doubt the sperm, functional in these matings, were spermatogonia or pre-spermatogonia at the time of X-irradiation. Mice made partially sterile but able to produce some offspring after doses of 500 to 1000 r transmitted to their offspring partial sterility (60).

A single exposure of 1000 r of male C57 black mice shielded above the pelvis caused depopulation of all cells of the mature germ line except the Sertoli syncytium. At 3 weeks no cells were found in the tubules other than the Sertoli lining of the basement membrane. At 4 weeks a few of the tubules showed signs of regeneration of spermatogenesis, and at 10 weeks 30% showed regeneration. The investigators believe that regeneration is brought about by the dedifferentiation of Sertoli nuclei which round up and increase their chromatin content (61). This has yet to be proven.

After chronic exposures (whole body) to 74 r/day and 2500 r/day of γ -irradiation for periods of 30 and 5 days, those mice which received the lower exposure lost their spermatogonial cells by the third day, spermatocytes after the fifteenth day, sperm after the twenty-fifth day, and spermatids after the thirteenth day. At the higher level of exposure, all but the spermatogonial cell types were seen throughout the short 5-day period of observation (62). Chronic exposure of 8.8 r/day (LAF₁) to a total of 1760 r caused temporary sterility, and those receiving upward of 880 r showed

transient decrease in litter size (52, 63, 64). Continuous chronic irradiation of mice at 8.8 r/day caused marked atrophy of the testes in 8 months. This dose produced greater injury within 2 months than did 4.4 r/day in 16 months, suggesting a greater importance of the dose rate over total dose (65).

Mice exposed to the lower dose of 4.4 r/day for 250 days showed no reduction in fertility, so that this may suggest the level of chronic exposure which can be fully tolerated, as far as the fertilizing power of the spermatozoa is concerned. Again, we are not considering the genetic effects. However, C3H mice, similarly exposed but only to an accumulated 800 r, were sterile. Further, fractionation of the daily dose might well establish the threshold for sterilization, since it was the daily chronic level of exposure and not the total cumulative dose that was critical. As an example (66), CBA mice were given 1.64 r, 8 r, or 33.3 r/week. The intermediate dose of 8 r to an aggregate of 200 r produced only 39% sterility, but 33.3 r/week for the same total produced no noticeable effect. At the highest level the onset of sterility occurred at a cumulative exposure of 330 r, and it was 100% at 50 r. The authors believed that either an increase in the total time of fractionation (extension of the chronic exposure) or a decrease in the dose rate was deleterious.

There is but one reference to the possible temporary increase in fertility after chronic irradiation of the testes at 2.5 r/day, and this seemed to occur when the cumulative exposure was between 200 and 400 r (67) and was followed by a rapid decrease in fertility. The number of young was not affected by irradiation. There are varying reports on litter size, particularly from irradiated males during the presterilization period. Doses from 200 to 550 r (58, 68) and 600 r (35) have been reported as causing reduction in litter number, presumably by affecting the mature spermatozoa. This is difficult to accept in light of the fact that exposures of 15,000 to 41,000 r to the testes allowed normal litter size production (26). There is no doubt but that litter size studies should be checked on larger groups of animals for better statistical data.

Regardless of sterility, the testes weight changes have long been recognized as rather accurate radiation dosimeters (32, 69) and have been plotted as a function of time for single organs (13), but it was Lorenz and his co-workers who first used testicular weight as a quantitative measure of radiological damage (16, 70). Kohn and Kallman have exhaustively studied the relationship between radiation exposure and testes weight and have used various types of irradiation as well (41, 71, 72). These investigators offer three major premises: First, testicular weight loss reaches the maximum at about 4 weeks after 1000 r or more (15, 71). Second, weight loss depends

exclusively on the dose to the testes; there is no indirect effect by irradiation of the rest of the body; and it is an exponential function of the dose (71). Third, weight loss is due to actual loss in the germinal cells and is well correlated with the histological changes. Changes in the interstitial tissue and Sertoli cells are trivial (15, 16).

The acute reaction of the testes at 4 weeks was not altered by fractionation of the dose for CAF mice (41). Total doses used ranged from 80 to 240 r, and fractionations were from 2 to 5, given in 1 to 4 days elapsed time. The degree of radiologic injury (i.e., weight loss) was proportional to the total dose and was not affected by fractionation within the limits used. "One effective radiologic event inactivates one biologic unit, most likely the spermatogonium" (41). Extreme protraction or fractionation, however, diminishes injury to the tests. Restitution is inhibited at doses above 600 r, and fractionation of large doses increases damage to the mechanism responsible for restitution.

It has been suggested that "change in weight of the mouse testes be used for the determination of the relative biological effectiveness of different qualities of radiation, or the effects of drugs upon radiation injury in the germinal epithelium" (41, 71, 73). The first suggestion of the use of the testes for such studies was probably made by Storer *et al.* (74), who found RBE values of 1.2 to 2.5 for thermal neutrons, with 1.3 being the best average value obtainable. Studies in Bern on the betatron at 31 Mev showed the n^* value to be somewhat less than 1 (75), and the 22.5-Mev betatron at Sloan-Kettering Institute gave the value of n close to unity (76). With 250- and 1000-kvp X-rays, the RBE was found to be about 0.82 (72). CBA mice exposed to 100 rep of neutrons were permanently sterilized, with 50% weight reduction in the testes (77), but neutrons have been known to be more potent than X- or γ -rays in other biological processes—e.g., cataract development and lethality studies. All seem to agree that the mouse testis is so radiosensitive to various qualities and all quantities of irradiation that it may be a suitable test object for certain RBE studies.

It has also been shown (78) that when male mice are exposed to neutrons (as from the atomic bomb) there is a consequent shortening of the life expectancy of its progeny in a rather direct relationship to the level of exposure. This is probably due to a genetic change in the sperm.

The effects of radioisotopes have not been so extensively investigated. The γ -emitting isotopes, particularly if they concentrate in or near the gonads, might conceivably have an effect on the germinal epithelium, but calculating the exact exposure is a very difficult matter. A study was made

* An " n unit" is that amount of neutron radiation which discharges a 100-r Victoreen Chamber as much as does 1 r of X-rays.

on chronic low-level I^{131} treatment of mice and the effect on fertility as compared with the chronic low-level X-irradiation (79). Doses were up to 4 μ c of I^{131} or 10 r of X-rays per week (single injection or exposure), and at the end of the observation period (44 weeks), when the accumulated exposure was 440 r of X-rays or 176 μ c of I^{131} (i.p.), all males were fertile. This is significant, because females similarly treated were largely sterile, and 10 r of X-rays was found to be more deleterious than 4 μ c of I^{131} . Suckling young mice at 5 days were exposed to I^{131} by injecting the mother with 600 μ c. (i.p.) so that the isotope reached the young through the milk. This resulted in sterilization of the female offspring but only a slight effect on some of the males when tested as adults (80).

F. RATS

The rats, shielded except for the testes, were exposed to doses of 50 to 3000 r and were sacrificed at 7 to 300 days thereafter. After exposures of 130 r there were effects on mitosis and meiosis, and above this level the numbers of spermatogonia were reduced. Above 400 r all cells beyond the dusty spermatogonial stage disappeared but later reappeared (32). Lower doses of 60 r also affected the testes in terms of detectable changes in the germinal epithelium by 4 days with complete recovery by 50 days. The maximum injury after 150 r occurred at 30 days with partial recovery by 50 days; 450 r caused further delay in "recovery" (12, 13).

In a radically different conclusion (81) it has been stated that 500 r directly to the testes caused no morphological degeneration, which the authors attributed to the combined effect of a secondary mediator with the direct effect, neither of which alone could cause damage. This was later described as "a unique type of injury" (82, 83). Such abscopal mechanisms are denied in another study where doses of 60 to 210 r were given directly to the testes with histological consequences, although irradiation of the rest of the body had no appreciable effect (38).

Sterilization doses to the rat testes vary from 1000 r (30) to 3000 r (54), depending probably on differing criteria of sterilization. A dose of 800 r given at one time caused temporary sterility, but the same total, fractionated over 5 days, caused complete sterilization (28, 49). Lower levels of exposure caused varying degrees of germinal hypoplasia, always with "recovery" (6, 11, 14, 17, 32).

An exposure of 500 r directly to the testes showed infrequent occurrence of necrosis or pyknosis in the spermatogonia and other cells, with abundance of mature spermatozoa in the epididymus. The fact that the time required for these phenomena was about 26 days, or the normal duration of the

spermatogenetic cycle in the rat, suggests a specific but temporary inhibition of the somatic mitotic divisions in the dustlike spermatogonia. The dustlike spermatogonia were found to be the most radiosensitive cells with respect to mitotic inhibition, but the most radioresistant (with the exception of the spermatozoa) in their ability to withstand irreparable destruction by X-rays. Regeneration comes from the undestroyed dustlike spermatogonia which are only temporarily suppressed in their maturational activity (84).

With chronic daily exposures of 0.1 to 10.0 r/day for long periods, only the highest dose showed any effect (85). When the daily whole-body exposure was raised to 13.7 r (for 146 days) there was sufficient hypoplasia to produce weight loss of the testes (86). The gonads showed a reduction of some 31% in weight after 105 days.

If 325 r was delivered acutely and chronically (3.25 r/day, 5 days per week) to rat testes and the results compared, it was found that chronic irradiation was about twice as efficient, per roentgen, as was the acute irradiation in reducing the prespermatocyte production in the prerecovery (repletion) period. Acute irradiation was about twice as effective as chronic in terms of the degree to which the "recovery" of spermatocytes was incomplete in the postrecovery period. The inhibition of type A spermatogonia and the normal differentiation of type B spermatogonia were largely responsible for the spermatogonial depletion after either chronic or acute X-irradiation. Spermatogonial death, inhibition of mitosis of type B spermatogonia, and possibly premature or increased rate of spermatogonial differentiation also may have contributed to the depletion which followed acute irradiation (86a).

Young and preweanling rats showed more extensive damage to the germinal epithelium than did the adults with a comparable exposure to X-rays, but there was no real inhibition of spermatogenesis in the young animals (17). When the young rats reached the age of 35 or more days, their testes reacted much in the manner of fully adult testes (82, 83, 87). The spermatogonia of weanlings showed very rapid degeneration after X-irradiation, so that their number was markedly reduced by the second day (in adults this would take longer). There was some effect on the spermatocytes, and on days 3 and 4 the weanlings (but not the adults) showed degeneration of the spermatids (88).

One study made on combined irradiation proposes that small exposures followed, after an interval, by large exposures will have a less damaging effect than those which have not had the preliminary minor exposure. The testes of rats which had received the preliminary exposure of 0.25 r daily for 80 to 100 days before a massive exposure of 600 to 3000 r showed vir-

tually no damage when the massive dose was 600 r, but the controls (without prior chronic exposure) showed extensive testicular damage. The higher levels of X-irradiation were also more damaging in those cases where there had not been any prior low-level exposure (89).

It appears that the germinal epithelium of the rat is more radiosensitive than that of the rabbit and more radioresistant than that of the dog. This differential parallels that of the LD₅₀₍₃₀₎ relationships in general.

G. RABBITS

Rabbit semen has been irradiated by cobalt to levels of 100 to 100,000 r and then used for insemination of normal eggs. At 50,000 r or 100,000 r the eggs were fertilized and cleaved to the two- or four-cell stage; at 10,000 r development was farther, but no blastocyst developed; and at 1000 r about 10 % "normal" blastocysts and fetuses were obtained. At 100 r there was litter size reduction but no other apparent effect (90). Another investigator was unable to get any living embryos or fetuses after irradiating the semen to 1000 r (91).

The offspring of X-irradiated males seem to have poor viability even after as low an exposure as 100 r (92), and those exposed to 300 r and mated to normal females showed 42 % fetal loss, and litter averages reduced from 6.6 to 3.2. Nevertheless, the rabbit testes are regarded as more radioresistant (with respect to sterility) than are the testes of other forms mentioned above. A single sterilizing exposure has been variously reported as 1000 r (93), 1500 r (85), and 2000 r (31), and may actually be higher, depending on what is meant by sterility. When there is whole-body exposure, 800 r causes temporary but complete depletion of the germinal elements (6, 94), with recovery in 4 months. "The rabbit is difficult to sterilize by x-irradiation. Its germinal epithelium is somewhat less sensitive to x-irradiation and more rapidly regenerating than is this tissue of the other laboratory mammals" (20).

When irradiation was fractionated, total exposure above 2000 r was required to cause permanent sterility (32, 95), but when there were six daily exposures per week, ranging from 0.1 to 10.0 r daily, only the highest level of exposure caused sterility and then only after an accumulation of 3100 r (96). With neutrons a comparable result was achieved by 1.7 n/day (97).

H. Dogs

Dogs do not lend themselves satisfactorily to fertility and sterility tests, so that there are relatively few reportable observations. Beagles given daily

whole-body exposures of 3 r for 5 days per week showed a reduction in the sperm count to zero by the twenty-fifth week, at which level the testes remained; hence they were permanently sterilized (20, 98). Even 1.0 r/day produced sterility by 1 year. When this latter dose rate was applied and had reached an accumulated level of 622 r, there was complete atrophy of the germinal epithelium, whereas half of this dose rate (0.5 r/day) and half the total dose caused only moderate atrophy (96). When the still lower level of 0.1 r/day was used for 2 years there were some histopathological changes. Since it has been suggested (20, 38) that the "recovery" of dog testes may be more like that of man, this low level becomes particularly significant. A very low daily whole-body level averaging about 3 r/week or 60 r in 20 weeks caused a decline in the absolute sperm count in semen, reaching its lowest level at 60 weeks with a total of 180 r.

When the dog was exposed to whole-body neutrons at 1.7 n/day to an accumulation of 398 n, there was complete atrophy of the testes. This dose may well have been far above the minimal sterilizing dose.

I. MAN

For obvious reasons data from the human male are very rare and are acquired, in general, through accidents or war. The LD₅₀ for man in whole-body X-ray exposure is estimated at 400 r. A dose of 600 r applied to the testes, will cause permanent sterility (52). It was found that a temporary sterility of 12 months' duration usually followed an exposure of 250 r. It has been estimated, however, that 30 r to the human testes may be detrimental (53). From a genetic point of view it may be much less.

One man, 34 years of age, was accidentally exposed (whole body) to a total of 416 r (γ - and X-rays), suffering severe symptoms but surviving to provide a rather complete testicular history. The sperm counts per milliliter were reduced from over 5 million at 37 days to zero by 7 months. At 10 months a biopsy showed normal Sertoli cells but no spermatogenesis. By 20 months there was still marked atrophy but some evidence of "recovery," so that by 26 months there were almost 2 million sperm and by 50 months over 10 to 18 million sperm per milliliter (99, 100). This is far short of the 60 million generally required for normal fertilization, but nevertheless this individual fathered a child at 58 months after exposure. Those accustomed to working with mice and rats are a bit surprised at this rather long-drawn-out period for the human male to "recover" from sterility, and yet it should be remembered that when we extrapolate from the average life expectancy of the mouse (2.5 years) to that of the human being (75 years) we have a ratio of about 1/30. The reproductive life span of the mouse is approxi-

mately 1 year, extrapolating to about 30 years for man, which is quite average. Although direct extrapolation is very unwise, this disparity between mouse and man is not considered as peculiar in itself but is related to the different life span factors of the two totally unrelated forms.

In one report (94) where orchietomy was indicated because of prostatic carcinoma, controlled X-irradiation of the testes showed that some weeks after 900-r exposure to one individual there was no suppression of spermatogenesis. Although this interval between exposure and histological study was rather short (for man) the supralethal dose used did not have an immediate and drastic effect on the germinal epithelium. Studies of this nature on the human subject are being made currently with highly controlled X-irradiation to one of the testes, with the other (shielded) as the control. The analysis will be cytochemical (25a).

J. SUMMARY: TESTES AND SPERMATOZOA

Of all the cellular components of the vertebrate testes, the spermatogonia appear to be by far the most radiosensitive, and the nongerminal elements, the Sertoli and interstitial cells, are the most radioresistant. In between we find increasing radioresistance among the primary and secondary spermatocytes, spermatids, and finally the spermatozoa, which can tolerate thousands of roentgens without altering their ability to move and to initiate development of the egg by fertilization. However, the level of exposure of the mature sperm which will affect embryonic development is probably near the LD₅₀ dose for the species, whereas the level that causes mutations may well prove to be as low as a single roentgen.

Ionizing radiations affect any cell by direct damage to the chromatin, inhibiting mitosis, meiosis, or differentiation, causing necrosis and pyknosis. The germinal cells are no exception, and the major effect of ionizing radiations on them has to do with nuclear chromatin sensitivity so that higher levels of exposure will demonstrate graphically the irrevocable changes brought about in these nuclear components. Such inhibition of maturation as occurs may not be evident at the time of irradiation but will become evident as any particular cell reaches the stage where mitosis or differentiation is supposed to occur (101).

The above statement should not be construed to mean that all cells are equally radiosensitive, for it is obvious that within the testes there are cells with very wide differences in radiosensitivity. Even among the similar-appearing spermatogonia there are different susceptibilities, and all spermatogonia are hundreds to thousands of times as sensitive as are the mature spermatozoa into which they would normally develop. One might conjecture

ture that the peculiar radioresistance of the mature spermatozoon is due to the fact that it never undergoes mitosis or differentiation. We cannot deny, however, the probability that, even though the spermatozoon is all but impossible to kill by irradiation, it is still subject to mutational changes at very low levels of exposure. Such changes are not reflected in hypoplasia or in sterilization and are more difficult to demonstrate.

Within a wide range, a group of values may be offered to suggest the relative resistance of the several spermatogenetic stages, purely with respect to *destruction* and resultant *sterility* (not to genetic effects). If the radioresistance of the type B spermatogonium has a value of 1, then we may suggest: type B spermatogonia—1; type A spermatogonia—70; spermatids— $300 \pm$; spermatocytes— $400 \pm$; and spermatozoa— $10,000 \pm$. These are only rough estimates and must be regarded as such, but they are probably in the correct relationship. In order that the values not be misconstrued, it is again emphasized that this has to do only with sterility, and it may well be that all above cell stages are equally radiosensitive with respect to (genetic) mutational changes.

Irradiation of the testes with their varied cellular components results in the more sensitive spermatogonia being inhibited in their maturation processes or being destroyed so that, at some time subsequent to the exposure, there is germinal hypoplasia to a degree related to the level of irradiation and damage to these stem cells. The hypoplasia bears in general an inverse relationship in degree to the dose. Although the testes may appear to be histologically devoid of germinal elements, apparently only a very few surviving spermatogonia are necessary eventually to repopulate the gonad, so that there has been evidence of "recovery" of the fertilizing power of the testes in every form studied, providing the exposure has not been too high.

The level of irradiation which brings on sterility varies with different forms, being highest in rabbits and probably lowest in guinea pigs (Table II). It also varies with respect to the meaning of "sterility." Any exposure of the testes will destroy some cells, causing hypoplasia of certain elements, but to effect sterility of temporary or permanent nature a greater number of earlier "stem" cells must be destroyed. It has been suggested that there may be a correlation between the relative sensitivity of the testes and the LD₅₀ values for the various forms, as determined by fertility alone. In any case, the "recovery" which has been seen in all forms after temporary sterility refers to the return of the testes from a depopulated to a repopulated state and does not imply that the germinal cells which reappear are genetically "normal" but rather that they are able to initiate development in a normal egg. Thus far there have been no tests of offspring from poststerility

TABLE II
SUGGESTED X-IRRADIATION LEVELS FOR TESTES OF DIFFERENT
ANIMALS AND PURPOSES^a

Animal	Germ Cell hypoplasia	Arrest of spermatogenesis	Permanent sterility ^b	LD ₅₀₍₃₀₎
Guinea pig	—	325 r	400 r	225 r
Dog	200 r	—	600 r	300 r
Mouse	300 r	500–800 r	800–1500 r	400 r±650 r
Man	300–400 r	600–700 r	900–1500 r	400 r–500 r
Rat	450–500 r	700–800 r	1000–1200 r	700 r±
Rabbit	300–400 r	800 r	1000–2000 r	750–825 r
Fowl	1300 r	—	2500 r	1000 r

^a These figures are only suggestive and are based on consensus of published data.

^b These represent minimum range. The literature describes higher exposures to attain sterility, but often these doses are much higher than necessary.

spermatozoa to determine whether they are qualitatively "normal," a study which is most important.

The sterile period which follows a postirradiation fertile period of varying length may itself vary not only in onset but in duration, depending on the species concerned and the irradiation, both in terms of dose and whether the exposure was single, fractionated, or chronic.

This temporary sterility after high-level irradiation seems to be peculiar to the testes, as it is not found in the ovary, and several decades ago this fact was utilized to render human males sterile for the early period of marriage, without affecting the libido, which was dependent on the radioresistant interstitial cell hormones. This practice is no longer followed because of the probable genetic damage to the surviving cells.

Human data are inadequate and uncontrolled, and extrapolations are fraught with danger and may be inaccurate. For example, it was found that mutations in mice occur some fifteen times as frequently as they do in *Drosophila* after irradiation, so that it is conceivable that they may occur even more readily in the human germinal elements. But this has not been proved or disproved.

The human life span is so much longer than that of any of the other forms studied, and the reproductive life is likewise extended, so that one would expect differences in reactions to ionizing radiations in the human subject as compared with other (vertebrate) testes. For instance, one would expect that the onset of sterility would be longer, sterility would still de-

pend on dose, and "recovery" of fertilizing power would take months. This is especially true, since the number of spermatozoa per milliliter of ejaculate is so high for successful fertilization, even of a single egg. These are, in fact, the findings thus far. Nevertheless, except for the time factor one cannot demonstrate any basic difference in the reaction of the human testes when compared with testes of other vertebrates, so that, until we have more direct data from the human subject, extrapolations can be only suggestive.

K. CONCLUSIONS: TESTES AND SPERMATOZOA

1. As an organ the testes are probably as radiosensitive as any in the vertebrate body, reacting to very low exposures by acquiring mutations in the germ cells and to high exposures by hypoplasia of the germinal epithelium which results in temporary or permanent sterility.
2. Although the sterile testes may later become repopulated with functional cells, capable of motility and fertilizing power, there is every reason to believe that such cells will carry effects altering the development and the expected genome of the progeny.
3. Sterilization is never immediate, regardless of level of exposure, so that litters may result from spermatozoa receiving many roentgens of X-irradiation.
4. High-level irradiation resulting in sterilization does not correspondingly deprecate the secretion of hormones from the interstitial cells, for libido is not lost even though the testes are sterile.
5. For producing sterility the dose rate appears to be more important than the total dose.
6. Short-term fractionation may be more deleterious because different cells in critical phases of development are insulted, but fractionation over long periods requires considerable increase in exposure to attain the same degree of hypoplasia.
7. There is no evidence of indirect effects on the testes, i.e., effects from whole-body irradiation with gonads shielded. To affect the testes, that organ must be directly exposed.
8. The term "recovery," used to describe the revival of testes once sterile, refers to the ability of a few stem cells to repopulate the seminiferous tubules, an ability quite peculiar to the testes. It does not refer to recovery from genetic damage.
9. Type A spermatogonia exhibit heterogenous radiosensitivity among themselves, and those that survive irradiation can repopulate the testes. This may occur within weeks for some mammals and months for others and will depend in part on the degree of irradiation-induced hypoplasia.

10. The matured spermatozoa are exceptionally radioresistant. This may be related to the fact that they no longer undergo mitosis or differentiation, two processes critical for the irradiated cell.

11. There is a suggestion that the differences in sensitivity of the testes of various vertebrate forms may be correlated with the relative LD₅₀ values for the respective forms.

12. The effect of ionizing radiations on the meiotic chromosomes is graphic, but the differentiating germ cell is probably many times as radiosensitive (in terms of survival). The focal point of irradiation damage is the germ cell nucleus, specifically the chromosomes.

13. The vertebrate testes are such a sensitive dosimeter that they may well prove a valuable organ for RBE determinations.

14. Marine and poikilothermic forms have germ cells that can tolerate higher levels of X-irradiation than warm-blooded mammals and still produce viable offspring. This may be due to a combination of low temperature and greater resistance through less complicated (evolutionary) development.

15. The testes of young (immature) animals are more radiosensitive than those of the adult into which it develops.

II. Effects of Ionizing Radiations on the Ovary and Ova

A. INTRODUCTION

The ovary and ova are, in general, more radiosensitive than the testes and their cellular components (except in the embryo). The two organs cannot be directly compared except, possibly, from the point of view of the genetic effects of irradiations. Although it is generally believed that the ovary responds in an all-or-none fashion to irradiation, there are isolated reports of temporary sterility rather than reduced fertility (7, 51, 102). Conversely, low levels of exposure of the human ovary are believed by a few to stimulate ovulation (103), although there is no explanation for this, and the practice is strongly discouraged for genetic reasons. In the female there are interrelationships between the ovary and reproductive accessories, probably through the endocrine system, which makes the situation of the female somewhat more complicated than that of the male. But the ovarian hormones afford the female a degree of whole-body resistance to irradiation not found in the male (104, 105), and such hormones, injected into castrate males, give them comparable protection. Nevertheless, the ovary itself is more radiosensitive than is the testis. Ovarian atrophy brings about changes in the uterus, fallopian tubes, vagina, and mammary glands (20), although

ovarian sterilization in the mouse does not prevent future estrous, as it does by causing amenorrhea in the human female.

The more mature ova and the granulosa cells appear to be the more radiosensitive of the ovarian cellular components, depending in part on their activity. The primary and developing follicles are somewhat less radiosensitive (102, 106). As in the testes, the interstitial tissue, known as the stroma, is very radioresistant. Although there are species differences, these are not so great as among the testes (20). With the exception of the mouse, the order of increasing radiosensitivity might be as follows: (1) corpora lutea, (2) ovarian stroma, (3) primordial follicles, (4) oocytes, (5) developing follicles, (6) mature follicles, and (7) granulosa cells. In the mouse it appears that both the oocytes and the primary or immature follicles are quite radiosensitive (107).

The ovary can be sterilized by low single exposures to ionizing radiations, or to somewhat higher chronic or fractionated doses, but there is no weight loss from hypoplasia comparable to that found in the testes. The failure of the primary follicles to develop leads to stromal invasion rather than to cellular loss. The permanently sterilized ovary may be somewhat smaller than the normal, but this is not a safe dosimeter as it is for the testes. Such an ovary will have no ovarian follicles in any stage of development. But one cannot form generalizations, for there may be changes in the human interstitial tissue without counterpart in the rodent ovary (20). The degenerating mouse ovary peculiarly develops tubular downgrowths of the germinal epithelium, and X-irradiation almost invariably produces ovarian tumors, if enough time is allowed, even after low levels of exposure (52). Such conditions are not generally found in other mammals or the human female, although comparable time relationships have not been determined. In an average of 6.7 years after ovarian irradiation in women (108) there have been no more tumors than in the unirradiated, but since all mice develop tumors in about 12 months a comparable time span for the human would be 30 years (109). Specific data will be presented below.

B. MARINE ANIMALS

1. THE CLAM

The eggs of the clam *Spisula* are considerably more radiosensitive than are its spermatozoa. Some eggs cleaved when fertilized after 189,000 r of X-rays, but none reached the trochophore stage. At 214,000 r there was cytoplasmic and membrane destruction. The normally radioprotective drugs cysteine and cysteamine afforded no appreciable protection to *Spisula* gametes (110).

2. THE SEA URCHIN

In a current study (111) the phenomena of "recovery" and of "protection" at the cellular level have been re-examined, using the egg of the sea urchin *Arbacia*. The prior findings of Henshaw (112) have been confirmed, showing that the X-irradiation of the egg caused a delay in the time of the first cleavage after fertilization, but that this delay was reduced if a considerable time elapsed between irradiation and fertilization. This was called evidence of "recovery" of the egg from irradiation effects. But it was pointed out (111) that although cleavage time might revert toward the normal there was no true "recovery," since the eggs could not develop into embryos (plutei). The delay in fertilization did not bring about a "restoration of the normal state," so that true recovery was not achieved. The cytoplasmic effects of irradiation might have been temporary, but the nuclear (chromatin) effects were permanent and irreparable. In fact, if the eggs were X-irradiated in such a "protective" —SH compound as cysteamine, there was considerably less delay in cleavage and the eggs could develop farther than those without benefit of the "protective" agent. By combining delay in fertilization (after X-irradiation) with the "protective" drug cysteamine, some 30% of the eggs exposed to 125,000 r were able to cleave, while none of the controls divided. Further, it was shown that, even with the —SH compound, the eggs were not "protected" against the normal sequelae of irradiation, even though more eggs would divide through the early stages. Thus, neither "recovery" nor "protection" could be demonstrated, since the nuclear chromatin, so vital to the developmental processes, was irradiation-damaged and no plutei could develop. The fact that the chromatin changes at the cellular level, after irradiation, are irreversible, irrevocable, and irreparable suggests that this may well be true for any living cell subjected to ionizing radiations. The cell membranes are very resistant, showing no changes in permeability even after 100,000 r (113).

3. FISH

X-Irradiation of the eggs of the fish *Fundulus* (48) often caused separation of the outer membrane to form a wide perivitelline space, and such eggs could not be fertilized. Eggs X-irradiated within the body of the female to 100,000 r and fertilized with normal sperm sometimes developed to stage 17 without gross teratologies. Point irradiation of single cells with an α -source at the two-cell stage (20,000 rep) damaged the cell to such an extent that gastrulation was impeded. In general, marine eggs appear to withstand high levels of irradiation better than do mammalian eggs.

C. AMPHIBIA

Ovarian eggs in the frog tolerated 400 r X-rays without any appreciable effect on later fertilization and development. Uterine eggs, which are in suspended metaphase of the last meiotic division, were considerably more radiosensitive, and the fertilized eggs were the most sensitive to X-irradiation. The abnormalities of development caused by X-irradiating the eggs were similar to those after X-irradiation of the spermatozoa; hence it was suggested that the effects were chromosomal. A delay in fertilization after X-irradiation did not allow any of the so-called "recovery" in cleavage time described for *Arbacia* (114).

D. BIRDS

The hen's ovary shows the first signs of X-ray damage at about 400 r (115), and exposures of 50 to 300 r at a rate of 5 r/min had no appreciable effect on total egg production. The fertility and hatchability of such eggs was unimpaired (116).

When the chick embryo was treated with 47.5 μ c of P³² on the fifth day of incubation, all ova of the presumptive ovary were destroyed, and there was moderate atrophy of the ovary by hatching time 16 days later. The primitive ova were the most radiosensitive. Larger doses of P³² brought on the degenerative changes earlier, but if the injection occurred after the fourteenth day there was no effect on the embryonic ovary. There was never any recovery from the damage, and "the primitive sex cells in either gonad were the most sensitive elements" (117).

E. MICE

1. STERILITY

Evidence to date suggests that the mouse ovary reacts somewhat differently from the ovaries of other mammals. Further, it is probably important that in presenting any data the species or strain of mouse be designated, for there are differences in their reactions to ionizing radiations. For example, ovarian tumors invariably follow X-irradiation of female mice even at low exposures if enough time is allowed, and this has not been demonstrated for other forms. This special radiosensitivity may be related to the peculiar sensitivity of its primordial follicles (20). A single exposure of 54 r causes degeneration of some primary follicles by the first day, and 140 r or more causes histological sterility by 43 days (118). Apparently the mature follicles are more resistant, because 150 r does not cause permanent

sterility until after several litters have been produced (119, 120). Again, the figure of 800 r which has been given (121) for permanent sterility is certainly higher than is necessary for most strains of mice, although with such an exposure the onset of sterility might well be earlier. The so-called "recovery" from sterility, seen in the males, has no real counterpart in the ovary, for the usual reaction is "all-or-none." One report (122) states, however, that whole-body exposure of 200 r caused sterility but full recovery within 1 to 3 months. In general, whole-body exposure causes greater damage than does direct X-irradiation of the ovaries (123), although one must consider the difficulty of pinpointing the internal ovary in exclusive X-irradiation.

The destructive effects of X-irradiation of the mouse ovaries probably set in within a few hours after high-level exposure (106, 124). At lower levels the histologic and cytologic changes may be delayed for days or weeks (125, 126). In a study of the onset and duration of sterility in the CF1 mouse (127) it was found that the first changes were in the degeneration of the granulosa cell and the last were in the stroma or interstitial cells. There was some reduction in ovarian size, correlated with failure of germinal elements to develop. There were many atretic follicles, and abundant primary follicles which appeared to be unable to develop. The mass of connective tissue resembled either or both the granular granulosa-type cell and the lutein-type cell, which is large and contains a nucleus devoid of granules. It is possible that these may be precursors of the granulosa or lutein types of ovarian tumors fully described (128-131).

When lower levels of exposure were made at weekly intervals over a long period (6 months), there was evidence of a cumulative effect on the ovaries. This was shown for a dose as low as 1.1 r/day which, at 6 months, caused a decrease in the number of primitive ova and an increase in the number of mast cells (132). A weekly exposure of 4 r was shown to be near the maximum tolerated by the ovary without gross change (127). After 6 months of weekly exposure to 10 r of X-rays CF1 mice had ovaries with only occasional mature follicles, while some females were still fertile at 34 weeks. Above 10 r/week (i.e., 25 or 50 r) complete sterility in all females was achieved by 11 weeks or less. Thus, chronic or weekly exposures allowed the mouse to tolerate a higher accumulated dose than did single exposures, before sterility was achieved. The effects on the ovary were cumulative but not additive.

Since a 30-r single exposure had been shown to be sterilizing to the ovaries of some mice (127), a later study cut the exposure to 4 r/week, as a result of which sterility occurred in some mice by 8 weeks, or an accumulated 32 r of X-rays (109). An exposure of 100 r sterilized all female mice within 8 weeks, and a 60-r single exposure sterilized some mice immediately. If 100

r was given over 30 days complete sterilization was achieved in 30 days. Thus, by fractionation there was a threefold delay in the onset of sterility (112). The exposures are cumulative but not entirely additive, as they are believed to be for genetic sequelae. Thus, some mice could be sterilized at very low levels of exposure, and immediately. There was demonstrated an inverse relationship between dose and sterilization time, more drastic with single than with fractionated exposures (133). In the females irradiation of the ovaries appears to have a cumulative and irreversible effect, whereas in the males the effects were reversible if the total accumulation was not too great, but always greater than in the female.

Since female productivity was lessened by fractionation of the dose of X-rays, a study was made on chronic exposure effects. Mice were exposed to continuous γ -radiation from Cs¹³⁷ for total accumulated doses of 100 to 400 r. For accumulated doses above 100 r, the data appear to show an inverse relationship between dose rate and total young produced. Continuous irradiation at 100 r/week had less effect on female productivity than did fractionated doses of only 50 r/week (five 10-r fractions). Since mitosis does not occur in adult oogenesis, the radiation-induced death of oocytes cannot be due to chromosome imbalance after cell division. The dependence of the radiation effect on dose rate cannot therefore be explained on the basis of chromosomal derangement. The author suggests that the oocyte may be capable of some "repair" (133a).

2. LITTER SIZE

The effect of X-irradiation of the mouse ovary on subsequent litter size appears to be more definite than does irradiation of the testes. When there is a brief or temporary fertile period prior to the onset of sterility, there may be a reduction in litter size. A decline in litter size is more rapid after a higher level of exposure (120). Hybrid mice exposed to 300 r or more of X-rays produced a single litter of decreased size and then became sterile (123), but after an exposure of 50 to 200 r there were multiple litters, but of reduced size.

3. OVARIAN STIMULATION

Some evidence of stimulation of ovulation was shown (132, 133) when mice exposed to 400 r were examined during 2 to 16 days thereafter and found to have 50% more ovulations than expected, based on numbers of corpora lutea. There was still a reduction in litter size of similarly treated mice, however, due to dominant lethals which killed embryos *in utero*. This was confirmed by the finding of nuclear fragments in oocytes.

4. LITTER FREQUENCY

There is another way in which to analyze litter production changes. It was found that from 4 r/week to a 350-r single exposure there was no statistical reduction in litter size that could in any way be correlated with exposure (127). If adequate numbers of females were studied over a period of many months, with full opportunity for mating, it was found that there was a drop in the *total number of litters* produced with increasing exposure, whether the exposures were single or weekly. A single exposure, for instance, of 50 r reduced total litter production to 39 % during the 38-week period of study, and a single exposure of 150 r reduced litter production to 20 %. By fractionating the exposures the females were allowed to tolerate a considerably higher accumulated dose than in a single exposure before sterility set in, but in the meantime the size of the individual litters produced was not reduced.

5. TEMPORARY STERILITY

It has been stated (109) that "in the mouse there appears to be no level of x-irradiation which will render the female only temporarily sterile." An exposure of as little as 20 r, whether given at one time or fractionated to daily exposure of 1 r over 20 days, will reduce the fertile life span and the total number of litters, without reduction in the size of the individual litters produced. There appears to be great variation in the response of females, since 40 r will sterilize some immediately and others not for 26 weeks; and if the dose is fractionated, litters may be produced for 43 weeks. Controls in these experiments would be expected to reproduce for almost a year beyond the sterility period of the experimentals. There was no evidence from litter records that any exposure to X-irradiation stimulated ova production, although uterine resorptions were not investigated.

It has been suggested that ovarian radiosensitivity may not be found to parallel whole-body relative sensitivity of different mammalian species. Guinea pig females, for instance, appeared to be more radioresistant than mouse females as measured by sterility. Even to disturb the estrous cycle of the female guinea pig it required a higher level of exposure than that which caused complete lethality for the mouse. Arranged in order of diminishing radiosensitivity of the ovary there are mouse > man > rat > guinea pig. The effect of irradiation is described as on the germinative epithelium, and the differences between species must therefore be due to differences in the sensitivity of their respective germinative epithelia (133b).

6. RELATIVE BIOLOGICAL EFFECTIVENESS (RBE)

There have been very few RBE studies on the mouse ovary. An exposure to 140 rep of fast neutrons at 8.3 rep/week had no effect on fertility or reproduction, although this same exposure of the males reduced fertility to half. Exposure to 100 rep was completely sterilizing. Thus, sensitivity to fast neutrons appears to be unpredictable by extrapolations from values for X-rays (134).

7. ISOTOPES

Scattered references to isotope effects have appeared. When P^{32} was injected into female mice the ovaries were reduced in size, owing to loss of Graafian follicles, absence of corpora lutea, and condensation of stroma. The dose necessary to achieve absolute sterilization was not demonstrated. This isotope appeared to attack first the granulosa cells (92). Sulphur (S^{35}), even to 60 $\mu c/gm$ body weight, did not sterilize the ovaries (135).

Radioiodine effects have been more energetically studied. A dose of 4 $\mu c/week$ for a total of 176 μc reduced fertility of the female mouse but was not sterilizing, with a wide range of responses among individuals (136). The testes of males were not affected by this weekly dose at 6 months, but the ovaries were degenerate, reduced, with no primary follicles and many anovular follicles, and seldom any corpora lutea. The production of follicle-stimulating hormone was not obliterated (137). The ovaries of young newborn mice are particularly sensitive to I^{131} when it is given to the mother and reaches the suckling young through the milk. The testes of the young are resistant to a 600- μc dose administered to the mother, but the suckling females are affected so as to exhibit reduced fertility by 4 months, and all are sterile by 10 months. Reduced fertility is measured, not by reduction in litter size, but by reduction in total litter production. The 5-day ovary proves to be more sensitive than the 15-day gonad. Sterilization is delayed for 4 months after the single treatment of the mother (138).

8. PROTECTION

Gonadotropic hormones and fresh pituitary implantations after irradiation prevented some mice, exposed to 244 r and 300 r of X-rays, from becoming sterile by 3 months, the ovaries showing "regeneration" and the production of ova and follicles. No follicles matured, however, and no young were produced (139). Ovarian hormones are protective to the adult female (104) and when injected into the castrate male. The female is most radio-resistant during estrous and least during diestrous. Protection of the ovary

by such hormones against X-ray damage so that functional ova are produced has not been investigated.

When BAL was given to female mice repeatedly during internal irradiation by P³², there appeared to be some protection of the ovaries, but protection by BAL against X-irradiation was not achieved (140).

Exposing female mice to 700 r after cysteamine injection did not protect the ovaries (141), and yet either cysteamine or cysteamine injected prior to 50 r of X-irradiation of the female mouse extended its expected functional period of reproduction by 280 % and two- to threefold the number of litters produced, so that there was some degree of "protection" at the lower level of exposure (142). There was no evidence of protection against genetic or developmental consequences of irradiation.

9. OVARIAN TUMORS

It has long been known that the mouse ovary is prone to develop tumors after whole-body X-irradiation (143, 144). Single or fractionated doses of 200 to 400 r produced tumors in 7 to 10 months, consisting of three kinds: granulosa cell tumors, luteomas, and tubular adenomas. Mice exposed to 87 to 350 r all developed tumors if they lived to 17 months after exposure, regardless of the dose. These ovarian growths were readily transplantable autonomous tumors. Since human ovarian tumors appear late in life, the relation of X-irradiation to such neoplasms is not at all certain (128).

Mice exposed to doses of 50 r or more can still have several normal pregnancies and yet develop ovarian tumors in a stock in which tumors are normally nonexistent. Mice at 1 to 3 days X-rayed to 150 r were sterilized (33 %), but neoplasms developed in 76 %. Exposures at 1 to 3 days did not hasten the onset of tumors more than in those mice which had been exposed at 4 to 10 weeks. Tumors developed in the middle-aged or old mice. "It is postulated that a specific delayed effect coupled with a hormonal imbalance provoked by x-rays leads to the development of ovarian tumors" (145).

The mechanism of tumorigenesis challenges the imagination. Certainly it must involve the effects of ionizing radiations on the nuclear components, which are believed to be sixty-six times as radiosensitive as the cytoplasm (146), and yet the cytoplasm cannot be excluded.

F. RATS

The destructive effects of irradiation of the rat ovaries follows within a few hours of high-level exposures (106). An exposure of 750 r had little effect, but 1500 r caused ovarian atresia by 2 to 4 hours, and the post-

irradiation injection of gonadotropic hormones did not produce ovulation but did stimulate ova maturation even when the ovary was in an advanced stage of degeneration. Primitive ova and ova of secondary follicles were the first to show degenerative changes after X-ray exposure. The interphase ova in primary follicles were relatively quite radiosensitive (147).

Sterility has been reported in rats exposed to 800 r or more (54), but this value is far below that reported by others, where local exposures of 3000 r or more seemed necessary (7, 148). The lowest exposure reported was 600 r, which caused marked degeneration in the ovary within 2 days and a disappearance of ova by 4 months (149). Continuous X-irradiation of female rats for 4 months to varying doses caused the offspring of only the highest dose (1410 r) to die shortly before or after birth, presumably by the production of dominant lethals (150).

Daily whole-body exposure of female rats to cyclotron neutrons so as to accumulate 532 n in 1 year produced atretic follicles—absence of growing but persistence of primordial follicles (85). This exposure was probably far in excess of one necessary to sterilize the ovary.

Injection of P³² did not affect the mating responses of female rats, but the number of offspring and of implanted embryos was reduced. The F₁ were not fertile. The estrous cycle of P³²-injected rats became irregular and persisted, sometimes for 30 days after injection. The primordial follicles were not affected by P³², but the ovaries were reduced in size (151). Doses as low as 1.2 μ c/gm body weight caused morphological changes in the ovaries (152).

G. RABBITS

Rabbit ova X-irradiated to 100 r were fertilizable and produced blastocysts (153). But 400 r to one ovary caused it to be reduced in size. It was pale, firm, and without alteration of the succinic dehydrogenase activity but with most ova obliterated by 48 hours. The ova remaining were large and atretic and did not respond to hormonal stimulation of the Friedman test (154).

The same workers (155) found that 400 r to the ovary caused no damage to the stromal cells in pattern or in lipid content but did obliterate many ova, and those remaining were atretic with no stainable lipid and a considerable deposition of periodic acid-Schiff substance (not glycogen). These ovaries would not respond to the Friedman test of hormonal stimulation.

It is reported that 1200 r to the rabbit ovary caused only temporary sterilization (7, 54) but that 2000 r to the ova caused the destruction of ova and the failure of corpora lutea to form; hence the rabbits were ster-

ilized by this dose. During active oogenesis the sterilization dose is reported to be 4500 r or more (156). That there is poor agreement in the rabbit data available is shown by the report that 8.8 r of γ -rays, per 8-hour day, to 11,700 r produced little change in the rabbit ovary (52), whereas 10 r of X-rays per day for 1 year (total 3100 r) caused the disappearance of the ovarian follicles (96).

Also, a 2500-r skin dose to the rabbit sterilized the ovaries, destroying the follicular apparatus completely (157). When the dose was fractionated to five equal consecutive doses, the sterilization threshold was reduced to 2000 r, or for complete destruction was 2500 r. The i.v. injection of β -mercaptoethylamine prior to X-irradiation raised the threshold to 2750 r for sterilization and to 3000 r for destruction. This suggests weak "protection" at the cellular level.

H. Dogs

Although the reaction of dog gonads has been compared to that of human gonads, there are few statistical data regarding the effects of X-irradiation on the dog ovary. A beagle was exposed to 1320 r of whole-body X-rays at 3 r/day and subsequently produced 6 pups, so that this chronic exposure was not in any way sterilizing (98). Another beagle exposed to 403 n (cyclotron neutrons) showed persistent primary follicles, but absence of normal growing follicles in the ovaries (85).

Virgin female beagles 10 to 12 months of age were given whole-body (bilateral) exposures to 250-kvp (Maxitron) rays, and it was found that 300 r was the LD₆₂₍₃₀₎. The reproductive ability of the survivors was not impaired with respect to litter size and puppy mortality, but the reproductive fitness of the large colony of dogs was impaired somewhat by the acute exposure. The study involved several hundred dogs observed for a period of 5 years (161a).

I. HUMAN OVARY

X-Irradiation of the human ovary may result in its sterilization (158, 159) although it was once thought to be very radioresistant (160). In clinical practice irradiation sterilization of the human ovary is sometimes prescribed in cases of fibroids and of breast cancers (161). The former practice of using this method to control premenopausal bleeding has been abandoned.

The range of exposure of the ovary for temporary or for permanent sterilization varies considerably because of (1) variations in individuals, (2) difficulty of coning the radiations to the ovaries, the position of which

varies in different individuals, (3) variations in size or obesity of patients, so that the delivered dose varies, and (4) the margin of insurance desired by the various clinicians. The accompanying tabulation shows, for example, the doses of X-rays to the ovaries recommended by three radiologists in the same department.

	Temporary sterilization		Permanent sterilization	
	Single	Fractionated	Single	Fractionated
G.	200 r	800 r/4 days 1200 r/10 days 2000 r at 200 r/wk for 10 wk	400 r	500 r/2 days 900 r/wk
H.	300 r	400 r/wk		800 r/10 days
M.		400 r/wk		1800 r/5 days

Radium is also being used to achieve complete sterilization, the exposure consisting of 1600 to 1800 mg hr of radium given in 32 hours, accomplished by 50 mg of radium in the fundus of the uterus. This exposure must be increased for younger females. In this way both ovaries are irradiated simultaneously. It may well be that, as rare human data become adequate, the above figures will be revised downward (162).

There have been reports in the literature of permanent sterilization at doses of 320 r (54) to 625 r (159), and single exposures of 125 to 150 r produced amenorrhea in 50% of the women treated.

A single exposure of 625 r caused permanent sterility in an entire group of 72 women (159). Sterilization of the human female brings on symptoms designated as artificial menopause including amenorrhea, flashes, diminished libido, and, in some, depression (163). The practice of irradiation-induced sterilization in the female appears to be disappearing.

Low levels of exposure of the human ovary (and, sometimes, the anterior pituitary) are reported by a few to stimulate ovulation (103, 164-166), but the practice has been vigorously opposed (167, 168). One cannot dispute the statement that ovulation may be stimulated by direct ovarian X-irradiation, but whether it is due to hyperemia or to psychogenic factors has not been investigated. The opposition to the practice is based on the growing conviction that the probable genetic sequelae are too serious and that the practice should be made illegal. Exposures as low as 30 r to the human ovary are believed to be detrimental (53). Fortunately there are only a few physicians subscribing to this practice, but they have accounted for thousands of ovarian irradiations already.

Ovarian tumors in the human female do not follow irradiation within a matter of a decade (108), but, if one extrapolates from the mouse, such tumors would not be expected for some 30 years (162), so that it would not be safe at this time to deny the ultimate tumorigenesis of ovarian irradiation, even in the human female.

J. CONCLUSIONS: OVARY AND OVA

1. Ovarian hormones give the female a degree of radioresistance not found in the male, and yet the ovaries themselves appear to be more radiosensitive than the testes of the same species (except in the embryo, where the reverse situation holds).
2. The ovary may become anovular after X-irradiation, but its weight reduction cannot be taken as a dosimeter of irradiation (as it may be for the testes).
3. The mouse ovary appears to be prone to develop tumors after X-irradiation at almost any level, providing adequate time is allowed. Extrapolation of time for the human development of such tumors would require 30 years, for which there is as yet no postirradiation experience.
4. There is no evidence that eggs damaged by X-irradiation ever "recover" in the sense that normal development will follow fertilization by normal spermatozoa. Neither is there any real "protection" against irradiation sequelae by the so-called protective drugs, even though better cleavage may result. Embryonic development was not "protected."
5. The onset of ovarian sterility may be hastened by the increase of ovarian X-irradiation. There is no evidence of systemic effects, i.e., indirect effects on the ovary when it is shielded.
6. Low levels of exposure are cumulative but not directly additive in their effects. For instance, all mice were sterilized by single exposures of 100 r of X-rays within 8 weeks, but some mice were sterilized by as little as 32 r delivered at 4 r/week in 8 weeks. The responses of individuals differed.
7. There was no evidence of ovarian stimulation by X-irradiation in lower mammals, except possibly for a transient increase in ovulation counter-balanced by greater intra-uterine death.
8. Ovaries appear to be more susceptible to isotope effects than do the testes.
9. The so-called "protective" drugs extended the fertile period of mice who were X-irradiated, but there was no evidence of protection against the deleterious effects of irradiation on the genome or on development.
10. The nucleus of the egg appears to be more than sixty six times as

radiosensitive as is the cytoplasm of the egg, but the egg, having some cytoplasm, is more radiosensitive than is the spermatozoon.

11. There is no evidence of effect of irradiation on the secretion of hormones by the ovary; the stroma is highly radioresistant.

III. Effects of Ionizing Radiations on the Embryo and/or the Fetus

A. INTRODUCTION

The embryo presents entirely different problems with respect to ionizing radiations simply because at no successive intervals of time is it the same. Spermatozoa or ova, or their earlier progenitors, may have a certain uniformity and may be rather inert, though alive, whereas the embryo is a dynamic, ever-changing and ever more complicated mosaic of developing potencies. Since it is agreed that the differentiating cell is more radiosensitive than is the resting or even the mitotic cell, it goes without further proof that the embryo will exhibit radiosensitivities quite different from the gametes which gave rise to it, simply because it is made up largely of differentiating and mitotic cells.

Further, the embryo is more sensitive to ionizing radiations than is any subsequent stage in the ontogeny of the species. Abnormalities, deficiencies, and teratologies can be produced by exposing the embryo or fetus to relatively low levels of ionizing radiation, but such conditions are *never* produced by exposing the adult, no matter what level of irradiation is used. For example, probably the most common embryological effect is microcephaly or deletions in the central nervous system, but these are never produced at any level of exposure of the adult. It is justified, therefore, to consider the embryo and/or the fetus as the most radiosensitive stage in the entire life history of any organism. With such a mosaic of developing centers, each of which is vital to the survival of the embryo, ionizing radiations which prove to be lethal to any center will be lethal to the organism as a whole. The embryo is as radiosensitive, then, as its most sensitive vital organ system under development.

At the same time the embryo possesses certain powers not found at other stages in the life history, namely powers of repair, regeneration, or reconstitution. From an early stage in development it possesses phagocytes which are active and ready to engulf and remove cellular detritus, necrotic cells which have been damaged by ionizing radiation. With these out of the way, the remaining, undifferentiated and undamaged primordial cells are called on by the "organism as a whole" to fill in the deficiencies as best as they can, so that an embryo is formed which is topographically quite

normal but reduced through cellular deletions. This leads to microphthalmia, microcephalia, stunting, and other evidences of loss of formative materials. And yet the embryo and newborn will appear normal, though miniature or reduced. That it is not entirely normal can be demonstrated in cytological or histological analyses, behavior tests, and reduced life span.

Low-level X-irradiation of the gametes may produce mutations, but these may not be evident for generations. Similar exposures of the adult, or of its gonads, may produce sequelae such as tumors, but not for months (mice) or years (man). But the embryo or fetus will exhibit the effects of ionizing radiations almost immediately, and, where repair or reconstitution are at all possible, the results will be evident shortly after hatching or birth. Thus, effects on the embryo usually result in a quite rapid expression of damage and are graphic as compared with effects on its progenitors (the gametes) or its successive stage in ontogeny (the adult).

There is an area of embryonic study which has not been adequately investigated, namely the incidence and onset of the normal late sequelae of ionizing radiations when the embryo is exposed. If it takes from 17 to 35 years for cancer to become macroscopic in man after some causative irritant such as ionizing radiations, would there be the same long latent period when a more radiosensitive organ system of the embryo or fetus is exposed? To what extent may the low-level exposures in pelvimetry be responsible, for instance, for the difference in the incidence of leukemia in children as compared with that in adults? It is important that we evaluate the consequences of embryonic and fetal irradiation, as we have been trying to do for the adult. They may well be quite different.

Specifically, irradiating the gonad primordium in the early embryo may have quite different consequences from irradiation of the adult gonad. The tendency is to forget that the embryo also possesses gonads after a certain stage in development, and that its reproductive cells may have a radiosensitivity different from that of the adult. Further, changes in such cells, which are few in the embryo, will have the greater effect simply because they are the stem cells for thousands of cells of the germinal epithelium of the adult gonad. A primordial germ cell in the 32-day-old human embryo probably gives rise to thousands of cells of the adult gonad, and hence any effect of ionizing radiations on it will be carried to all its surviving progeny.

Exposures of the embryo or fetus do not produce anomalies which are peculiar to ionizing radiations, for there are radiomimetic drugs (triethylene melamine, and nitrogen mustard) and conditions (vitamin A imbalance) which will cause the same types of damage. It is the embryo which reacts to insult. Of all external agents or conditions, however, penetrating ionizing radiations appear to be the most consistently damaging, and those to which

the embryonic anlagen are the most responsive or sensitive. When one realizes that the adult neuron can tolerate something like 10,000 r, but the transforming neuroblast from which it develops may be damaged by 25 to 40 r, we realize that ionizing radiations constitute a most lethal tool for the differentiating cell, which is normally found only in the embryo, where it is abundant.

For fear of misunderstanding, it must be stated here that ionizing radiations have proved and will continue to prove to be one of the most important tools in the advancement of civilization, as demonstrated already in medicine and in nonmedical science and technology. The average human life is longer and happier in part because of the science of radiology. Nevertheless, it is the duty of every radio-embryologist to emphasize that his particular province—the embryo—is extremely radiosensitive, and that its exposure can have far-reaching consequences.

It is impossible to draw a composite picture of "an embryo" or "a fetus," even though there is a basic study of embryology. There is an undercurrent of similarity in development, whether we are studying fish, frog, mouse, rat, or man, but there are real differences even between closely related forms. One might present a composite picture of a spermatozoon, or an ovum, with reasonable accuracy, but not of the embryo. It is therefore even more important that we present data from the various groups of animals, after which an attempt will be made to draw a summary and some conclusions on the effects of ionizing radiations on embryos.

B. MARINE ANIMALS

The squid (*Loligo pealei*) embryo exposed to 25,000 r will be liberated from its jelly capsule, and after 50,000 r 100% of them will hatch. This acceleration of hatching is brought about by a swelling of the jelly and the bursting of the capsule, liberating the motile larva by X-irradiation. Such an embryo can tolerate as much as 200,000 r and survive for at least 4 days. The well-developed vertebrate type of eyes possessed by the squid, which give it a strong phototropistic response, are so affected by 100,000 r, however, that this tropism is immediately nullified in many, and by 200,000 r in 100% of the larvae which continue to move, but without this reaction to light. Growth of these larvae is stopped immediately by exposures of 5000 r or more, but no effect on the smooth muscles and larval motility have been noted before 200,000 r. The chromatophores remain expanded after this exposure. The neural retina, however, shows cytological damage by 3 to 4 days after 800 r (169).

Early embryos of the marine annelid *Chaetopterus* exposed to 255 r or

more were delayed in their cleavages, produced abnormal trochophores which had severe ciliary defects and cytoplasmic blebs, and were very feeble in activity. Exposures to 1020 r or more caused death of most of the larvae before the trochophore stage. Cytologically these early embryos showed multipolar spindles, chromosome fragmentation, and karyokinesis without cytokinesis (170).

The intermitotic stages of the early development of the snail *Helisoma subcrenatum* were from two to four times as radioresistant as those embryos that were in active mitosis. The LD₅₀ for hatching was 300 to 400 r for resting cells and about 100 r for those in mitosis. Later embryonic stages were less sensitive, namely 500 to 1000 r for the trochophore through the early shelled embryo stages. The greatest sensitivity during early cleavages corresponded with the earliest indication of cleavage (i. e., indentation) (171).

C. FISH

Thus far fish embryos have not proved to be very satisfactory experimental material, probably for technical reasons. The salmon embryo in the "eye" stage could survive an exposure of 2500 r for 4 weeks, and then all developed similar degenerative symptoms and died in 30 to 51 days. An exposure of 2500 r of X-rays arrested the development of cutaneous pigment, vascular system, fin rays, and other organs. Exposures of as little as 500 r caused some of these changes in some embryos. It appeared that the gonads and the hematopoietic systems of these embryos were the most radiosensitive, since 250 r affected their normal development (172). Experiments with the fingerling Chinook salmon showed that 250 r increased mortality of embryos, 500 r affected the weight, 750 r the blood development, and 1000 r altered the length (173).

In studies on the trout (*Salmo gairdnerii*) embryo from the fertilized egg to hatching, it was shown that the one-celled stage was by far the most radiosensitive, with an LD₅₀ of 58 r, and as the embryo progressed in development and age it became more resistant, tolerating from 300 to 900 r of X-rays. Exposure during the early "eye" stage, even to 38 r, caused some retardation in growth, but 200 r or more was required to cause the same retardation at later stages of development. Fin ray numbers were reduced by exposures to 75 r or more at the thirty-two-cell stage, late germ ring stage, or early eyed stage (174).

Similar results were found with the marine *Fundulus*, the first cleavage being the most vulnerable to X-rays. A dose of 300 r could be tolerated, however, without causing gross abnormalities. After 1000 r neural differentiation was prevented. The effects of 500 r at the first cleavage were

similar to 1000 r at stage 11 (expanding blastula). It was the anterior neural structures that appeared to suffer most from radiation insult, for headless embryos would develop, having pulsating hearts but without circulating corpuscles (175).

D. AMPHIBIA

The reader should be reminded that the development of the amphibian takes the organism through cleavage, blastula, gastrula, and neurula stages to the larva—also known as the tadpole (frog)—which is an embryo, since it must pass, by metamorphosis, into the salamander (or frog). During all their development the majority of amphibia are aquatic, which renders them the more radiosensitive. (Strictly speaking, all embryos are aquatic, even the mammal.)

The newly fertilized egg and the early blastula stages are the most radiosensitive of all stages in the development of the frog, *Rana pipiens* (176), as measured by the frequency of developmental abnormalities. The ovarian egg, the neurula, and the older tadpole are the more radioresistant. These differences have been explained on the basis of (1) the movement of the gametic nuclei, (2) the relative potency of blastomeres, and (3) the presence of cells differentiating into vital organ primordia. Difficulty arises in studies on these poikilothermic early embryos because the teratologies cannot be quantitated. Exposures of 300 r can be better tolerated at the neurula stage simply because many of the vital differentiations have already been accomplished. The fertilized egg is the more sensitive because it contains chromosome complexes from the two gametes which are migrating toward each other for syngamic fusion. Chromosomes in movement (syngamy or mitosis) appear to be particularly radiosensitive. The use of low-level ionizing radiations on such early embryos will aid, however, in mapping the organ anlagen, since these will be present as developmental potencies highly subject to X-irradiation insult and marked by subsequent anomalies. Such cells or cell areas cannot be identified morphologically, but they may be marked radiocytologically.

Pyknosis or karyorrhexis may be caused immediately in the rapidly dividing cells of the early embryo, but development is never stopped abruptly, nor are developmental abnormalities evident for some time. The most frequent developmental abnormalities produced by exposures of the early amphibian embryo to doses of 180 r or above may be listed as follows: (1) pyknosis and/or karyorrhexis of differentiating cells, (2) exogastrulation, (3) edema, (4) stunting, (5) microcephaly, (6) flexure, (7), aneurogenic development, and (8) amorphous development.

Exposures of the later tadpole (which is still an embryo) to doses of 300 r or more affect the developing central nervous and special sense organ systems so as to destroy the neuroblasts and cause them to be sloughed off into the brain and other cavities (176). This study particularly emphasized that the embryo is especially radiosensitive because of the dynamic nature of the changes rather than because of the presence of any particular morphological entity. Ionizing radiations affect or interfere with the movement of chromosomes, the process of cleavage and gastrulation, the differentiation of cells. Once the organ is formed it achieves much higher radioresistance. This was also suggested in work on European forms (22, 23).

When frog embryos between stages 18 and 25 (early tadpoles of *Rana pipiens*) are exposed to 10,000 r of X-rays, all will die within 6 days; and if the exposure is raised to 25,000 r, death follows in 4 days. The fact that they live as long as they do is of interest, for even 50,000 r did not kill any embryos within 24 hours (177). In fact, once the tadpole stage has been achieved, resistance again declines, probably because of the particular sensitivity of the excretory and respiratory systems which are so vital to survival at these stages. Edema occurs in the belly region, owing to fluid in the coelomic spaces; toward the head, owing to the newly developed lymph spaces; and on the head, owing to the development of the external and internal gills and to the closely adjacent pronephros. Such tadpoles appear to be bloated and covered with blisters due to the failure of the newly formed respiratory and excretory systems to control the flow of fluid through their cells. This is probably an indication of a direct effect on the semipermeable cell membranes. It was not due to any effect of 100,000 r of X-rays on the aqueous medium of the embryos (177).

Amphibian larvae are, nevertheless, quite radioresistant, more so than the adults into which they metamorphose. When 2.2-cm larvae of the salamander *Ambystoma punctatum* are exposed to 15,000 r of X-rays and examined cytologically a week later, they show mitotic arrest and destruction of the widely dispersed nervous elements. Neuroblasts were found to be necrotic and sloughed off into the brain cavities and neurocoele (spinal canal), showing various stages of degeneration (178). Apparently the muscles of these larvae are radioresistant, since they are motile and appear normal at fixation.

These poikilothermic forms (e.g., bullfrog tadpole) may be given some temporary "protection" against irradiation sequelae by refrigeration to 3°C, but the survival of such embryos lasts only so long as they are kept cold (179). When they are returned to the laboratory temperature of 23°C, the normal sequelae of X-irradiation follow without delay. When larval salamanders are X-irradiated at 2° to 9°C and subsequently kept at their

normal temperatures of 28° to 33°C, they appear to show some benefit by developing less severe abnormalities than the controls irradiated at the higher temperature. This had been previously explained as due to the reduced metabolic level of the refrigerated larvae (180).

Although 10,000 r appears to be the LD₅₀ for the tadpole of the bullfrog *R. catesbeiana*, an exposure of 500 r caused extensive destruction of its hematopoietic system (181-183). The postirradiation temperature determined the rate of destruction of the hematopoietic system, and below 13°C the breakdown was very slight. If after several days at this temperature the tadpoles were returned to the normal laboratory temperature, the rate of destruction returned also to that which would be expected at the higher temperature. Both metabolic activity and mitosis were emphasized as the responsible factors for the radiosensitivity of the hematopoietic system. Cells in prophase were destroyed directly by 500 r of X-rays, and cells held in prophase by colchicine were similarly radiosensitive (184). Nevertheless, it was shown that cells in interphase would go to pieces during subsequent prophase if exposed to such X-irradiation, but exposure to 10,000 r caused cell destruction far in excess of those just in mitosis. Doses as low as 25 r showed effects on hematopoietic elements in the anterior kidneys of these tadpoles (185), but exposures to 750 r did not prevent the reappearance of mitotic figures by the fifth day postirradiation. Anoxia reduced cell destruction, or at least delayed its onset (183). The tadpole anterior pituitary gland, when subjected to 20,000 r, did not lose its effect on metamorphosis and the shedding of larval cuticular teeth when implanted in normal tadpoles, which shows that its endocrine function was unimpaired by this very high level of X-irradiation.

When salamander (*Ambystoma punctatum*) larvae at stage 27 were X-irradiated (200 to 600 r) and various embryonic organs were orthotopically transplanted to normal hosts of the same age, such organs tended to develop better than they would have in the originally irradiated donors (186). This is in line with experience previously reported (21) that certain organ anlagen may not be severely damaged, but the host, having some other vital organ systems that are extremely radiosensitive, succumbs and takes along with it these less affected or more radioresistant areas.

The amphibian embryo (tadpole or larva) has been used as a test object for regeneration studies and for investigations involving local and limited ionizing irradiation. Although these studies are important and of real interest, they are not considered to be strictly within the scope of this section on embryonic effects and will be omitted. They rightfully belong in discussions of experimental embryology. For reviews of these studies, see references 187 and 188.

E. CHICK

The chick embryo is warm-blooded and depends so much on its extraembryonic circulation for nutrition and respiration that its radiosensitivity changes from hour to hour. Further, there is evidence that the embryo itself might survive much higher levels of exposure than is the common experience, but it cannot because the extraembryonic blood vessels are so sensitive that their radiation insult results in the death of the embryo. This was graphically shown in a study on the 33-hour and 60-hour embryos (192), where it was found that exposures up to 500 r produced no significant effect on the survival of the 33-hour embryo, whereas the 60-hour embryo could survive only 200 r with the same results. As the exposure of the 33-hour stage was increased to 900 r, all were killed by the fourth day of incubation. But when the embryo was lead-shielded and only the yolk and extraembryonic areas of this early stage were exposed to as much as 5000 r, such lethal effects were not noted. The 60-hour stage failed to survive 1000 r for 24 hours after exposure, but when the embryo was shielded and the extraembryonic areas were exposed similar effects were produced by 1500 r. The difference between the 33-hour and the 60-hour stages was in the degree of development of the extraembryonic vascular bed. Embryonic anlagen exposed to doses that would kill the embryo, when transplanted to normal chorioallantoic membranes, developed as if they had not been irradiated. These included the optic vesicles and hind limb buds, and the results support the thesis that embryos are often carried to death by areas or developing organs that are particularly vital and also particularly sensitive to ionizing radiation. The study also revealed that multinucleate cells appeared in the brain, heart, and head mesenchyme, along with many pyknotic nuclei. Particularly, necrotic neuroblasts were sloughed off into the brain cavities (192) by 21 hours after irradiation, but were cleaned up by phagocytes by 45 hours. Thus, the lethal exposure of the embryo was decreased somewhat below that of the adult chicken, owing to the extraembryonic structures which are not an integral part of the embryo itself. This study was further confirmed to show that disintegration of the extraembryonic vascular plexi rather than the extraembryonic hemorrhage was the prominent feature in 12-day embryos exposed to 800 r of X-rays (193).

Exposures of 600 to 800 r were followed by injury to the capillaries and extraembryonic vascular deterioration and intraembryonic hemorrhage. Blood vessels broke down even before they contained blood (37). It was also shown that the chick mesencephalic wall could be damaged without direct X-irradiation (194). This may be questioned, because the developing mesencephalon is extremely radiosensitive, possibly even to scattered radiations.

The 4-day chick embryo has an extensive vascular bed, and many of the organ systems have been laid down. An exposure of 887 r kills 50% within 6 hours, and 717 r kills within the following 10 days (195). A 9% increase in lethal effectiveness of X-irradiation was found when the dose rate was raised from 36 to 151 r/min. The investigator reports four intervals of death after irradiation, each presumably due to different mechanisms. The *first* death (after 22,000 r or more, at 1500 r/min) was due to scattered hemorrhages, largely between the myotomes. In the *second* (after 1200 to 20,000 r), the hemorrhage occurred suddenly into the extraembryonic coelom. The *third* (after 950 to 1100 r) was caused by intraembryonic hemorrhage, generally in the occipital region. In the *fourth* interval (after 800 to 950 r), the embryos sometimes survived from 3 to 9 days and died for less obvious reasons, although there was usually an accompanying hemorrhage.

In an earlier study (38) it was shown that in acute death studies the LD₅₀ for the 2-day embryo was about 2000 r, for the 8-day embryo was 700 r, and for the 16-day embryo was 900 r. The maximum sensitivity occurred between 8 and 10 days.

In a study with the early stages, from unincubated blastoderm to the 36-hour stage, it was found that exposures of 600 to 800 r showed that the anlagen of the nervous system, the sense organs, the somites, and the notochordal area were the most radiosensitive. The central nervous elements are particularly radiosensitive at the earliest stages, but later the somites become hypersensitive (196). Some embryos developed without any central nervous system or notochord, others without head, and some without caudal structures, as have been found also among amphibian embryos (176).

Attempts have been made to protect the chick embryo with some of the —SH and other compounds found to be effective with adult mice (197). Cysteamine was used prior to irradiation with some protection, but when it was injected into the egg after exposure there was no protection. Protection appeared to be best after the circulatory system was functioning to transport the cysteamine throughout the embryo and its extraembryonic structures. Since then another study reports seven other substances (plus cold) used at tolerable levels, prior to X-irradiation, with only methylamine showing some degree of protection. The investigator therefore felt that protection was not due to the mere presence of the —SH group, to the amine, or to it as a reducing agent. Protection, for both methylamine and cysteamine, was believed to be on the vascular system (198).

Only one RBE study has appeared, probably because the chick embryo

is technically difficult to handle. This showed that the RBE of 1000-kvp X-rays compared with 250-kvcp X-rays, determined on the basis of survival of the 4-day chick embryo at 7 hours after exposure, was 0.897. This value was consistent with findings with other forms (199).

The isotope P³² has been used to study the effect on growing embryonic bone of the chick embryo, but it was found that, in addition to stunting and skeletal defects, the embryonic gonads of both sexes were very radiosensitive with doses of 47.5 to 300 μ c. There was destruction of the germ cells, and none of the male birds developed secondary sexual characters during 60 days posthatching. The ovary appeared to be somewhat less radiosensitive, even as is the case with the fetal mouse (200, 201). The path of injected P³² appeared to be from the yolk to the embryo and thence to its bones, where it concentrated, affecting the nearby developing gonads by irradiation. After the 14-day half-life degradation of the isotope, there appeared to be some reversal of the effects of P³² on the bones and growth, but not on the gonads. The gonads, particularly the ovaries, were affected to a degree related to the total dose. In a later study it was shown that smaller doses of P³² affected the maturation of the immature hematopoietic cells and reduced the mitotic activity of the embryo. Lymphoid tissue and the thymus were very sensitive to β -irradiation and showed an almost immediate reduction in mitotic activity. Peripheral blood counts also reflected damage, the lymphocytes dropping first, with granulocytes next, and then the erythrocytes. All returned toward the normal as the injected isotope became inactive (35).

A study on earlier stages was made (202) by injecting up to 234 μ c of P³² into the yolk sac of 2-day incubated eggs and studying the effects 2 days later. The LD₅₀ dose was established as 100 μ c in 2 days, and 165 μ c was 100% lethal. Effects similar to those after external irradiations were found, such as decrease in mitosis and tissue degeneration involving chromatin clumping, pyknosis, fragmentation of the nucleus, and destruction and fragmentation of the cell. The organ anlagen most affected were the neural tube, optic cup, dense mesenchyme of the gut wall, limb buds, and branchial arches. The least sensitive organ anlagen were those undergoing least developmental activity at the time of injection such as the heart, notochord, lens, and cephalic myotomes. Autoradiographs showed that the P³² concentrated in cells and was found most dense in those organs whose cellular density was highest (44).

Only one study with I¹³¹ has appeared, and the investigators established that the iodine is incorporated in the thyroid as a functioning organ on the eleventh day of the chick embryo when discrete follicles first appear (37).

F. RATS

Although mice and rats have essentially the same gestation period and react in the same manner to intrusions on their embryonic development, it will be more efficient to consider the findings of the two rodent groups separately. References will be specific, but it should be assumed that what is said for the rat could, in all probability, be equally demonstrated for the mouse embryo.

Gross abnormalities of rat skeletons followed fetal X-irradiations, and this constituted the first systematic study of embryonic effects among rodents (205). But it was soon found (206) that the growing central nervous tissues of fetal rats (and mice) could be selectively and extensively destroyed by X-irradiation with virtually no destructive effect on other organ systems. It was also found that this was due to the presence of the highly sensitive differentiating neuroblast, particularly in the brain, cord, some ganglia, and the retina. Doses were reduced to less than 100 r, but scattered areas of damage still were found, so that it was soon evident that the embryonic and fetal nervous system was infinitely more radiosensitive than its adult derivatives. The earliest necrotic cells were found 2 hours after exposure of the embryo or fetus, and by 24 hours whole areas of liquefaction necrosis were found in which floated cellular detritus and polymorphonuclear leukocytes. Phagocytosis of the detritus was described, peculiar to the embryo and occurring within 6 hours. By 48 hours rosette formation was seen in brain, cord, and retina. These were later described by another investigator, probably erroneously, as neoplasias (207). They rarely survive until the time of birth. Despite the resulting rather grotesque brains, the rats were later able to "crawl, then eat, climb, run and bite, but their motor patterns seemed more jerky than normal controls" (206). With fetal doses of only 150 r the rats were smaller than the controls, were without a corpus callosum, with small hippocampus, and striatum incompletely formed. The ventricles were dilated and irregular, and the white matter of the cerebellum was reduced. At higher levels of exposure scattered foci of necrosis were found also in the liver, kidney, adrenal, and bone marrow, and osteoclasts showed pyknosis. This was the initial work of an intensive series which is still incomplete.

It was soon found that X-irradiation effects could be reproduced by certain enzyme inhibitors, radiomimetic drugs (208), so that the reactions of the embryo were not radiation-specific. Nevertheless, ionizing radiations are still the most potent and consistent causative influences of specific developmental abnormalities, and it was learned that an exposure of a fetus to 40 r would necrotize some neuroblasts in the developing nervous

system. The most sensitive region was the anterior striatal periventricular region and the angle of the lateral ventricles where the corpus callosum forms. As the level of exposure was increased, other regions came within the areas of necrotic effects of irradiation. But, since parts of the brain, the cord, and the retina became less radiosensitive (as gestation proceeded), it was evident that ionizing radiations could be used to locate regions of active differentiation in the mosaic of the embryo or fetus. During the first 8 days after fertilization of the egg there appeared to be no irradiation effect on the embryo, but by the ninth day, when the neural tube began to form, the embryo became extremely subject to teratological development even at low exposures of X-rays. Neuroblasts in mitosis were affected, but those which were not dividing appeared to be even more radiosensitive. Differentiation proved to be more radiosensitive than mitosis. The term "neuroblast" is used to designate that intermediate, differentiating, embryonic cell between the highly radioresistant, primitive, and abundant neurectoderm and the even more radioresistant mature neuron.

The primitive, immediate precursor of the neuron is the neuroblast, and that of the neuroglia is the spongioblast, both coming originally from the neurectoderm. The "blast" stage in the differentiation of any somatic cell is its most radiosensitive stage, more so than its precursor or its progeny. It was then evident that both the time of X-irradiation and the dose were related to the type and degree of abnormality produced (208). For example, the cerebellum, which is last to differentiate, is not radiosensitive until the last third of gestation, but the sensitivity persists for several weeks after birth, owing to the presence of neuroblasts. Neuroblasts are present in the rat embryo beginning about the ninth day of gestation. Barron has suggested that the high sensitivity of neuroblasts was due to the inhibition of enzymes with active sulphydryl groups that are particularly concerned with protein synthesis, which process is so important to the differentiating neuroblast. It has been suggested that many enzymes depend on —SH for their activity as electron transfer catalysts (209). Ionizing radiations and radiomimetic drugs (nitrogen mustard and triethylene melamine) may interfere with the very sensitive mechanism for protein synthesis of the neuroblast and hence are thought by some specifically to bring about neurological abnormalities.

"This necrosis of neuroblasts is one of the most specific destructive biologic effects that can be produced experimentally. Forty roentgens selectively destroyed some neuroblasts when a pregnant mouse or rat was given a total body exposure; yet neurectoderm withstood at least 10 times this dose and adult neurons were rarely or not at all destroyed by 1,500 r and were rarely damaged by 20,000 r. This differential effect as the nerve

cell passes from embryonic to adult life is a good example of changing developmental metabolism" (210). Ionizing radiations may indirectly affect the neuroblasts by causing the formation of short-lived oxidizing compounds in the cell water, which would disturb the oxidation-reduction equilibrium of enzymes which have easily oxidizable —SH prosthetic groups (211), or upset the ratio of reduced to oxidized glutathione (212).

Since there is little experience in identifying necrotic or dead neural cells, it is appropriate that descriptions be given here (210). Within 4 hours after a necrotizing exposure the *neuron* goes through maximal change, with marked reduction in cytoplasmic and nuclear volume so that the neuron may be from one-third to one-sixth the diameter of its living neighbor. The cell and nuclear outlines are indistinct; the nucleus becomes homogeneously basophilic, and sometimes beaded and coagulated. A dead *neuroblast* is also reduced in volume (size), but, since it has little original cytoplasm this reduction is not so apparent. What cytoplasm there is becomes eosinophilic or neutral. The nucleus shrinks to one-half to one-fourth its original diameter and becomes coagulated, often a lobulated or beaded mass of densely basophilic material. Neuroblasts can be killed by sulfhydryl agents, radiomimetic drugs, the antisulfhydryl action of steroids, and ionizing radiations, but not by anoxia or hypoglycemia (210).

After a study of the effect of ionizing radiations on over a thousand fetal rats, the statement was again made that "the primitive differentiating

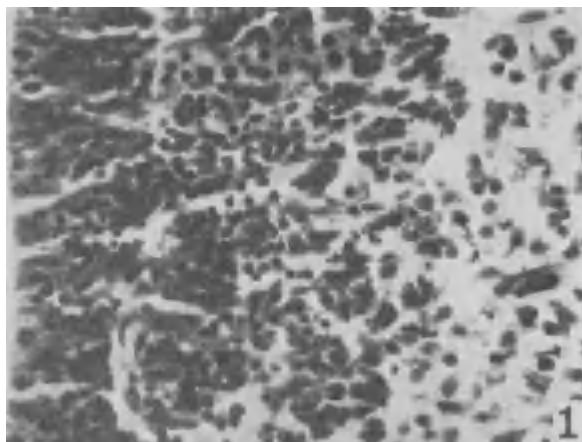


FIG. 1. Widespread necrosis of embryonic cerebral cortex. From S. P. Hicks, *Am. J. Pathol.* **33**, 459 (1957).

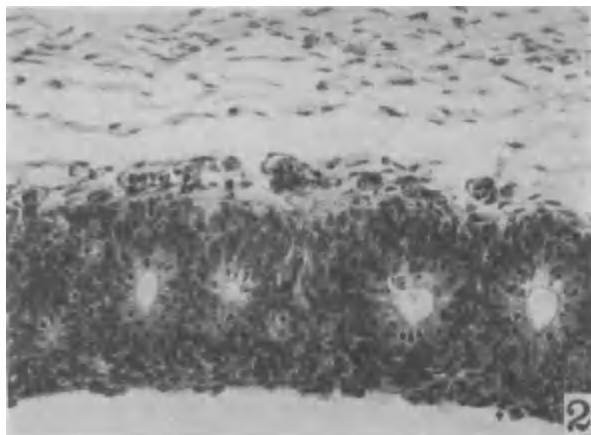


FIG. 2. Cortical rosettes, temporary, due to neuroblast damage. From S. P. Hicks, *Am. J. Pathol.* **33**, 459 (1957).

cells of the nervous system are probably the mammalian cells most easily destroyed by ionizing radiations. In fetal and newborn rats and mice (and other animals) substantial numbers of these cells become morphologically necrotic in less than 4 hours after as little as 40 r is given to the whole body of the mother or the newborn. The effect is a direct one of radiation on the cell, there being no evidence that secondary humoral, maternal, vascular or other factors are responsible" (213). Further study showed that the prior neurectoderm could be destroyed by 600 to 800 r, whereas the matured neurons of the newborn or adult rat could tolerate more than 1500 r, and "relatively few more were destroyed by 15,000–20,000 r" (213). Likewise only a few scattered oligodendroglia were destroyed by 1500 r.

It is obvious that the pattern of malformations resulting from irradiation of the rat fetus will depend on the time of exposure, so that particular areas are insulted when they are most radiosensitive, but the severity of damage is related to the dose. There are times when a small dose will produce a severe effect, whereas at a later time in development to produce a similar effect would require many times the previous exposure.

The presence of rosettes after fetal irradiation has caused considerable speculation, since they are peculiar to the nervous and not to other systems. They are certainly the expression of disorganization rather than organization, although they tend to resemble scattered neural tubes. This may be an expression of an inherent growth tendency of neural tissue, since they have been found in the retina, in sympathetic ganglia, in tumors of the

nervous system, and throughout fetal brains even after exposure to radio-mimetic drugs. They cannot therefore be related to specific effects of ionizing radiations but are a specific reaction of differentiating neural tissue. When first observed they were described as neoplasms (207), but they do not persist and are rarely seen after birth. Another and major effect on the central nervous system is microcephalia, correlated with degree and time of exposure (213). The reductions in the nervous system, whether anencephaly, microcephalia, or microphthalmia, represent neural deletions often obscured by the fact that the developing organism has powers of integrating the remaining plastic and undifferentiated neurectoderm so that there is topographical integrity, though a general reduction in size (volume).

The resistance of rat embryos to 300 to 400 r before 8 days is not explained, but embryos which survive this level of exposure appear to be quite free from teratologies (214). But, beginning a day later (the ninth day) the situation is radically different, owing to the fact that the organ systems (anlagen) have begun to differentiate. Anencephaly and severe brain deficiencies and facial deformities result in exposures at even lower levels (150 r) on the ninth day. On the tenth day abnormalities relate principally to the eyes, with resulting anophthalmia and microphthalmia.

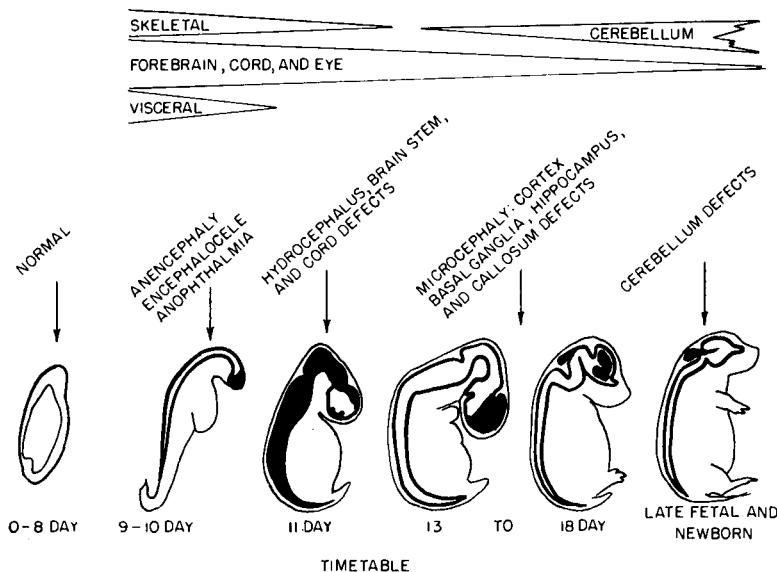


FIG. 3. Schematic representation of specific rat fetal sensitivities at succeeding gestation ages. From S. P. Hicks, *J. Cellular Comp. Physiol.* **43**, 151 (1954). It is now doubtful that rat embryos 0-8 days are truly radioresistant. (author)



FIG. 4. Left, anencephaly in a newborn (Caesarian) rat, resulting from irradiation on the ninth day of gestation. Right, anophthalmia in an adult rat resulting from irradiation on the tenth day of gestation, compared with the normal. The anophthalmic animal is otherwise virtually normal. From S. P. Hicks, *A.M.A. Arch. Pathol.* 57, 1 (1954).

On the eleventh day irradiation causes hydrocephalus and spinal anomalies; on the twelfth day, forebrain abnormalities. In addition, at higher levels of exposure, anomalies may be seen in the heart, vessels, and urinogenital system after exposures on days 9 to 11 (215). But it must be emphasized that even within a single litter, presumably all of which receive the same radiation insult, there may be varying degrees of anencephaly, microcephaly, microphthalmia, etc. This is in part due to the fact that embryonic cells are not uniformly radiosensitive, that the "blast" stage in the production of any cell is particularly radiosensitive, that members of a litter may not all be of the same chronological or developmental age, and that the undifferentiated and radioresistant primitive cells may be redirected in their metamorphoses toward the normal topography of the whole embryo. It is the individual cell that is sensitive to insults of various sorts, but consistently and especially sensitive to ionizing radiations because of the all-pervading nature of such irradiations.

It would be appropriate here to summarize the development and the teratologies which follow ionizing radiations at the early days in development of the rat, about which there is specific information.

First week: Fertilization to implantation. No developmental abnormalities followed irradiation if the embryo survived. This should be further investigated, at low dose levels (see mouse).

Eighth day: Homogeneous mass of blastomeres establishing uterine placental relations, no differentiation. Irradiation retarded growth in survivors, 200 r generally lethal.

Ninth day: No organ development, but germ layers distinguishable. Exposure of 100 r caused malformations in the eyes (90 %), brain (45 %), aortic arches (27 %), heart (27 %), spinal cord (18 %), urinary tract (5 %), and caused situs inversus (18 %) in a single report (52). The tabulated data show the percentages affected with respect to mortality and eye defects (49, 52, 54). Toward the end of the ninth day, somites and neural groove

Dose	Per cent affected	Per cent mortality	Per cent eye defects
25 r	20	0	6—microphthalmia
50 r	59	11.5	72—microphthalmia
100 r	98	26.4	90—anophthalmia
150 r	100	100	100—anencephaly
200 r	100	100 (4 days)	100—entire anterior end

begin to differentiate. Irradiation on the ninth day caused anterior abnormalities largely, but without specific skeletal or visceral deformities. In the brain and mesoderm underlying the notochord there was extensive necrosis, desquamated cells into the amniotic fluid, abnormal anterior pituitary, but no effect on mid- or hindbrain, medulla, or cord, and no phagocytes present (until the fourteenth day).

Tenth day: Rapid and diverse organogenesis, primary nervous and skeletal systems indicated, with eight to twenty somites. Irradiation caused necrosis of neuroblasts and spongioblasts in neural folds, midbrain, medulla and cord, optic pit, mesenchyme, dermatomes, retina, and pituitary. Abnormalities after 100 r occurred in eyes (75 %), urinary tract (25 %), and brain (11 % +). There was an increase in intrauterine death and a reduction in growth. After 200 r microphthalmia and anophthalmia were more frequent, all the above were anomalies accentuated, some encephalo-coele appendages were abnormal, and liver hematopoiesis was reduced. Exposure to 400 r resulted in embryonic death within 24 hours.

Eleventh day: Embryo attains definitive form with major body divisions, central nervous and vascular systems well advanced, and twenty somites. Irradiation caused necrosis throughout the neural axis, somites and mesenchyme, eye and face, with no effect on limb buds, heart, or tongue. Later development showed hydrocephalus and narrow aqueduct, reduced cortex and white matter, hippocampus and corpus callosum unformed, midbrain



FIG. 5. Malformation of cerebellum of rat. It received 200 r at 6 days after birth. From S. P. Hicks, *Physiol. Revs.* **38**, 337 (1958).



FIG. 6. Ventriculocele, hydrocephalus, deficient pallium, and diencephalon, in term fetal rat, the result of 200 r on the eleventh day (about twenty somites). From S. P. Hicks, *Physiol. Revs.* **38**, 337 (1958).

jumbled, striatum and thalamus reduced. There were retinal damage and microphthalmia, skeletal but not visceral abnormalities.

Twelfth day: Rapid and diverse organogenesis, thirty somites. Exposure to 150 r caused brain anomalies with ventricular rosettes, and 300 r caused encephalocele, absence of corpus callosum, dilated ventricles, small skeleton and toes incomplete, almost complete anophthalmia. Skeletal system radiosensitive because of active osteoblasts, but damage seen in midbrain, caudal



FIG. 7. Four hours after 200 r on thirteenth day, with necrotic cells in mantle layer and neurectoderm in disorder. From S. P. Hicks, *Physiol. Revs.* **38**, 337 (1958).



FIG. 8. Rosettes 48 hours later.

FIGS. 7-10. Four steps that characterize the effects of radiation on the thirteenth or fourteenth day of fetal life in the rat. From S. P. Hicks, *Physiol. Revs.* **38**, 337 (1958).

medulla, differentiating layers of cord, otic vesicles, limb and lung bud mesenchyme, caudal somites and nephric mesenchyme (216).

Thirteenth day: All organ systems indicated, thirty-four to forty-one somites. Affected anlagen were cerebral vesicles, reduced hemispheres, striatum and hippocampus, absence of corpus callosum, abnormal retina, caudal medulla, and striatal mass of brain. The liver, heart, and lung buds were unaffected.

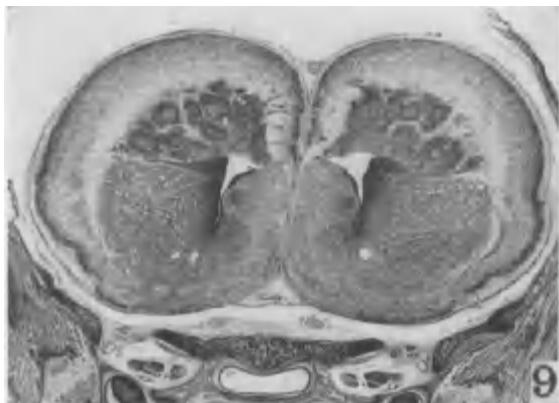


FIG. 9. Rosettes at birth, "subcortical." From S. P. Hicks, *Physiol. Revs.* **38**, 337 (1958).

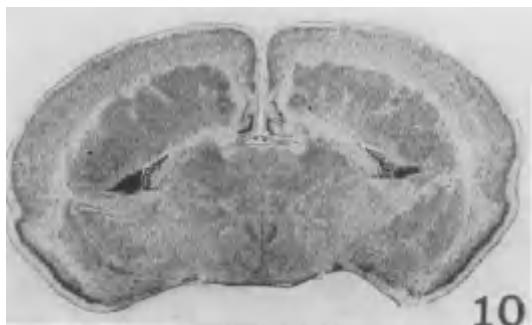


FIG. 10. At 2 weeks, rosettes matured into masses of bizarre cortical tissue. From S. P. Hicks, *Physiol. Revs.* **38**, 337 (1958).

Fourteenth day: Irradiation caused abnormal cerebral hemispheres, loss of corpus callosum, confluence of third and lateral ventricles, rosettes in retina. Phagocytes appear.

Fifteenth day: Irradiation anomalies largely in the striatum, hippocampus, and dorsal cortex, with minimal damage to the anterior neural structures which were already past differentiation. Corpus callosum absent.

Sixteenth to twenty-first day: Corpus callosum always affected, increasing damage to cerebellum with increasing gestational age, architectural damage rather than gross derangement of parts, cortical cells disoriented.

The course of anomaly production by irradiation follows closely the

initiation of developmental (differentiation) activity of various organ primordia, so that anterior neural structures are affected during early development, and skeletal, and visceral effects follow.

The concept of regeneration or repair has been emphasized (216) as being aided by phagocytosis (which begins about day 14) which removes the cellular detritus, and the remaining undamaged primitive cells aid in the replacement of lost cell masses. The result is that the embryo at birth appears to have made almost a complete "recovery," except that there is rather uniform deficiency of cells in organs and in the organism as a whole, with resultant reduction in size. The investigator (216) could find no explanation for the ability of certain organ primordia to regenerate or repair after irradiation insult. Cells which are vulnerable to irradiation damage are at the peak of differential cytology which may be associated with marked sulfhydryl enzyme activity concerned with nucleic acid and protein synthesis (210). The ultimate residual damage is a balance between the severe necrosis shortly after exposure and the ability of the undamaged, remaining primitive cells to repair the loss (215). Some conclusions are worthy of report: "The number and distribution of dead cells is usually far out of proportion to any malformation that might follow . . . the early brain and spinal cord of rats have a capacity to regulate and regenerate comparable to that described in other vertebrates. . . . In a given region destructive damage will be proportional to the number of cells in the radio-sensitive stage . . . Restitution must come from the adjacent neurectodermal proliferating area and recovery may be determined by whether that area is still at peak mitotic activity, or subsiding, or resting prior to producing another crop of migrating cells" (216). This is a different explanation from that previously offered (217, 218).

There have been but few studies on the effect of irradiation from an internal emitter such as injected P^{32} on the rat embryo. Some studies have appeared showing that greater depression of growth is caused by X- than by γ -irradiation of the rat embryo, and that X-rays consistently produce a greater incidence of morphological defects than does the same quantity of γ -irradiation (219, 220). With rather high levels of injection of P^{32} , effects on the skull bones were particularly noted, but other qualitative effects were seen in the ribs, femur, intervertebral discs, and tail. The membranous bones showed excessive porosity and ossification defects (221). The lethal dose of P^{32} varied from 0.46 mc on day 6 to 1.29 mc on day 10 (222), 50% of the embryos dying during expected fetal life, usually by day 14. Within the lethal range the amount of energy absorbed is less with X-irradiation than with β -irradiation from P^{32} , so that X-rays are the more potent, although the investigators agree that RBE statements are difficult to make

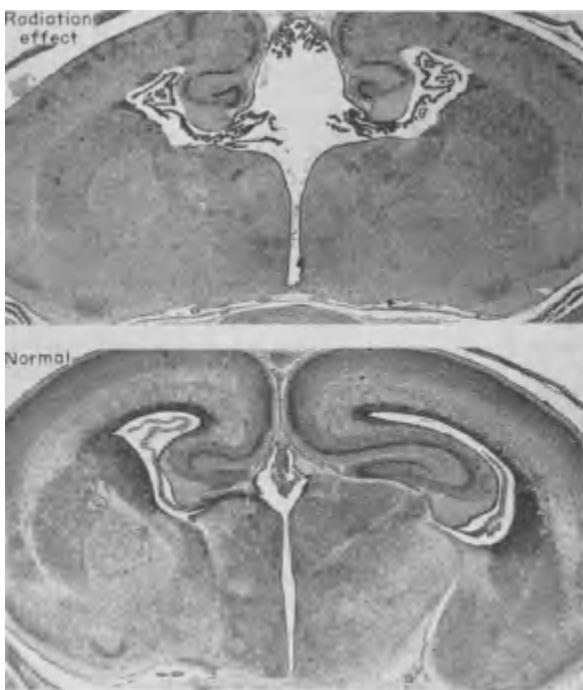


FIG. 11. Malformation of the brain of a young rat irradiated *in utero* on the sixteenth day. The normal brain is that of a comparable young rat irradiated on the eighth day of gestation with 400 r (H & E, low power). From S. P. Hicks, *J. Cellular Comp. Physiol.* **43**, 151 (1954).

here. With increasing fetal age a higher level of injection was necessary to prove lethal, but all doses used had a depressing effect on the postnatal weight (223).

Two studies show biochemical consequences of irradiation of fetal organs (222, 224). X-Irradiation increases the amide-free lipid fraction in the developing brain, and in the liver there is an acute effect which decreases the glycogen and a delayed effect which increases the liver glycogen.

Protective studies on fetal rats have not appeared, but for one exception (225), where it was shown that mercaptoethylamine injected into pregnant rats (days 15 and 18) before X-irradiation to 300 r gave some "protection" to the fetuses. It is obvious that survivors must be followed for some time in order to determine the degree of "protection" afforded. Rats which have been radiation-insulted *in utero* have a better chance of long-term survival

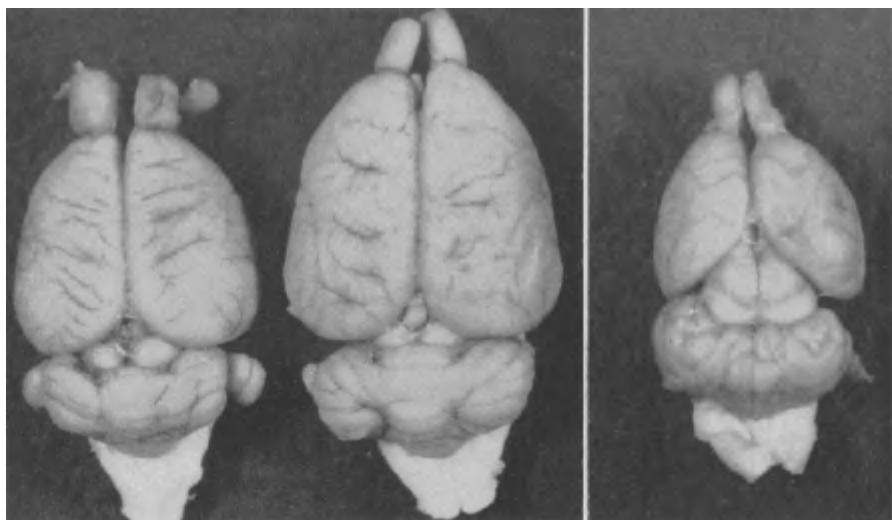


FIG. 12. Radiation microcephaly in adult rats compared with normal (center). *Left*, moderate deficiency of pallium and cerebellum after 150 r on the twentieth day of fetal life. *Right*, severe pallial and cerebellar deformity after 300 r on the seventeenth fetal day. From S. P. Hicks, *Physiol. Revs.* **38**, 337 (1958).

if, after birth, they are given to normal, unirradiated foster mothers (226–228).

Finally, very little has been suggested relative to the possible psychological effects of irradiation on the embryo or fetus as indicated by postnatal behavior (227). Rats exposed at 11 to 19 days of gestation to doses ranging from 300 to 600 r later exhibited more emotion and nervousness in the maze situation than did the controls. There was teeth-chattering, persistent scratching, face washing, defecation, and urination during the last days in the maze, not seen in the controls. Such rats were very difficult to tame. The learning ability of fetally irradiated rats was decreased proportionately to the amount of radiation given (227). There is currently an investigation in progress to determine the minimum level of X-irradiation and the critical fetal age at which effects can be discerned in subsequent neurological behavior, using refined psychological tests. It is believed by many that levels of irradiation of the fetus even below those that elicit histological damage may bring about neurological or psychological sequelae

which could have social consequences in an environment such as exists for man.

G. MICE

The mouse embryo has proved to be extremely valuable in radiobiology not only for determining the chronology of teratologies which follow fetal irradiation but for a better understanding of normal embryology. It is conceivable that in the near future this form will supplant the fetal pig in traditional morphological embryology.

The most exhaustive studies have been made within the last decade, and it has been shown that exposures at $7\frac{1}{2}$ to $8\frac{1}{2}$ days of gestation age to as little as 25 r will cause detectable abnormalities even in relatively small samples (228, 229), but that after the very high levels of exposure of 400 r from day $\frac{1}{2}$ to day $13\frac{1}{2}$ postconception there is no evidence that the embryo is in any way affected by the intermediation of the maternal organism or damage to it (230, 231). It is natural that one would suspect

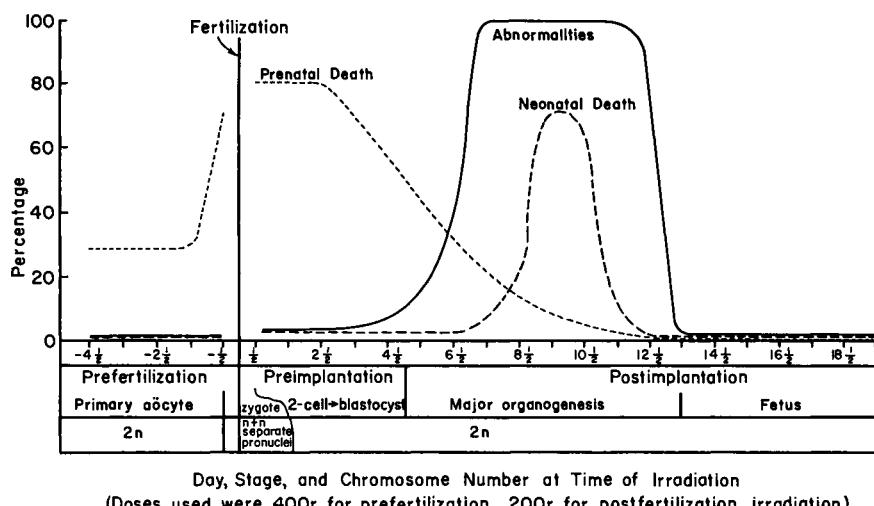


FIG. 13. Incidence of pre- and neonatal death and of abnormal individuals at term after irradiation at various intervals (separated by 24 hours) pre- and post-fertilization. Semidiagrammatic. Doses used were: 400 r prefertilization, 200 r postfertilization. Abnormal individuals may have many more than one abnormality. From L. B. Russell and W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* **19**, 50 (1954).

indirect effects from maternal damage by X-irradiation, but there is apparently no basic difference between the viviparous (mouse) and oviparous (amphibian) forms in this regard; effects on the embryo are direct.

It has been postulated (230) that uniformly distributed ionizations in the embryo may bring about chromosome breaks in randomly hit nuclei (due to initial intracellular damage) which cause chromosomal aberrations sufficient to interrupt or impede mitosis, resulting in cell death. Some damaged but surviving cells might give rise to descendants which affect normal development, or the presence of dead cells plus the failure of complete regulatory power of the remaining cells would independently or together explain the correlation between X-irradiation and teratology. There appears to be a threshold of cell numbers which effect an abnormality, but the degree of that abnormality may be related to the excess number of cells insulted. Some abnormalities are not caused by 200 r but invariably follow 300 r. One supporting fact of this thesis is that hypoxia protects the embryo as well as prevents radiation-induced chromosomal aberrations (230).

The general picture is as follows: Preimplantation embryos (to day 5) are very radiosensitive in the sense that from 43 to 83 % die before 12 days, and the earliest are the most sensitive. Those preimplantation stages which survive the irradiation are generally without gross teratologies. There follows a period between days $6\frac{1}{2}$ and $12\frac{1}{2}$ where irradiations result in the highest incidence of malformations after birth. After day $13\frac{1}{2}$ abnormalities are again difficult to produce by irradiation, so that days $6\frac{1}{2}$ to $12\frac{1}{2}$ must be considered as the most susceptible period for most organ systems and their teratologies. Neonatal death after 200 r has a peak at $9\frac{1}{2}$ to $10\frac{1}{2}$ days but is no different from the controls before day $7\frac{1}{2}$ or after day $11\frac{1}{2}$. Those that are irradiated on day $11\frac{1}{2}$ show the greatest decrease in birth weight, however.

Earlier studies on the external and visceral changes (232) and skeletal effects (233) were exhaustively thorough. They show that exposures to 300 r from day $14\frac{1}{2}$ to birth result in no prenatal death or visible abnormalities at term, although long-term sequelae do follow. If the mouse embryo or fetus is X-irradiated during the most critical phase of $6\frac{1}{2}$ to $13\frac{1}{2}$ days, there may develop one or more of a large variety of abnormalities such as microphthalmia, coloboma, narrow iris, vaulted cranium, snout and nostril abnormality, cranial blister, narrow head, open eyelids, spina bifida, small or imperforate anus, hydronephrosis and/or hydroureter, reduction in tail length, overgrowth of feet, digital reductions, and limb abnormalities. The sensitivity related to a specific abnormality sometimes was correlated with the first evidence of differentiation of that organ, but it could precede that time, indicating that the organ anlage had been set apart prior to any m-

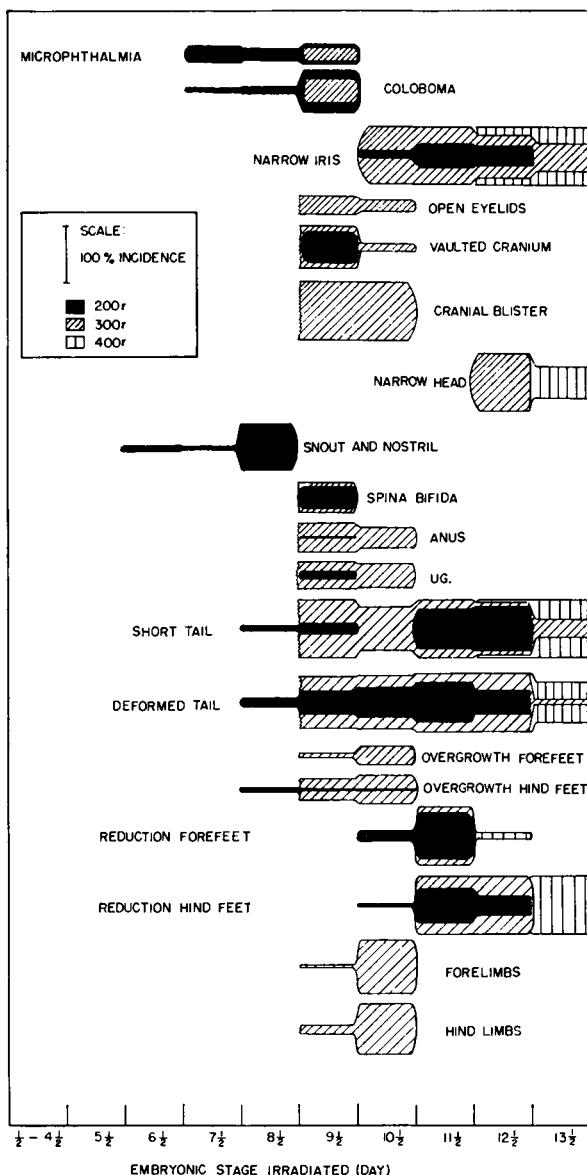


FIG. 14. Critical periods for all the abnormalities described, except those occurring in only one animal in each case (hypospadias and cloaca, situs inversus, pseudencephaly, skin appendage). Representation is (1) by percentage incidence of abnormality and (2) by magnitude of dose required to produce abnormality. Thus, the wider and more heavily shaded a band, the greater is the sensitivity. Serrated end of a band indicates that the dose series was not continued beyond that stage. From L. B. Russell, *J. Exptl. Zool.* **114**, 545 (1950).

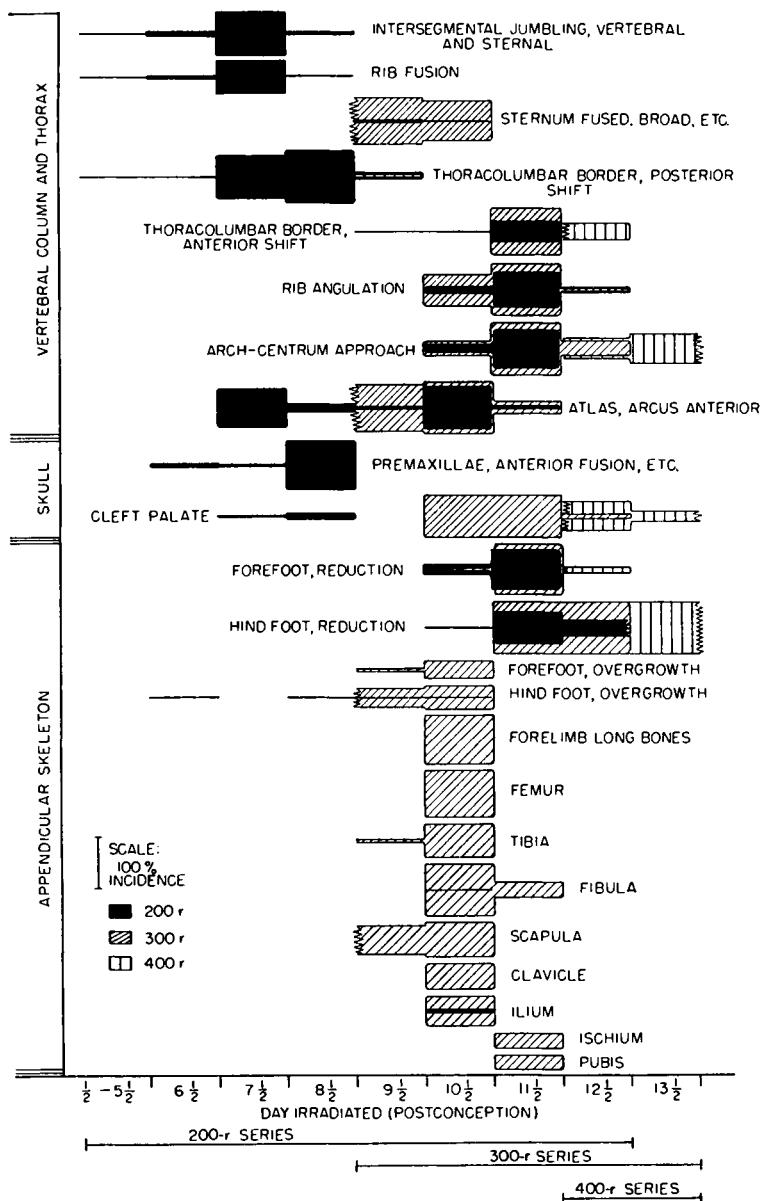


FIG. 15. Skeletal effects of fetal X-irradiation. From L. B. Russell and W. L. Russell, *J. Cellular Comp. Physiol.* **43**, 103 (1954).

phological evidence of its presence. This is a common embryological phenomenon. Although there was a minimum dose which would elicit any particular anomaly, increase of that dose would increase the degree or severity of that abnormality. In a later *skeletal* study (233) the chronology of events was carefully worked out as follows, resulting from irradiation.

Day 7½: Intersegmental jumbling, posterior shift at the thoracolumbar abnormalities of the arcus anterior of the atlas.

Day 8½: Continued and maximum effect shifting to posterior at the thoracolumbar border.

Day 9½: Depression of lumbosacral profile, dorsal ankyloses of lumbar centra, spina bifida, bilateral ossification of thoracic and lumbar centra, arch anomalies, and (after 300 r) reduction in posterior extent of true ribs and rib fusions. There may be severe costal cartilage anomalies and broad fusions of sternebrae.

Day 10½: Bilateral ossification of thoracic and lumbar centra, abnormalities of arcus anterior of the atlas, and continuation of rib fusion effects from day 9½.

Day 11½: Arch-centrum fusions premature, angulation of ribs, reduction or absence of last rib(s), reduction in average number of ossified tail vertebrae, and vertebral ossification ending anterior to caudal margin of the pelvic girdle. Anterior shift of thoracolumbar border (see also day 7½).

By studying the effects of irradiation on early cleavage stages an attempt has been made to attribute the proved effects on the embryo to genetic (at least chromosomal) effects (231). This is broadening the meaning of the term "gene" to include any chromosomal aberration, and, since any such effect would reflect in development, the broad gap between the gene and development has been bridged! This simplifies the situation far beyond that which could be accepted by any embryologist, much as simplification would be desirable. This concept bypasses the regulatory or integrative powers of the "organism as a whole" and would fail to explain the fact that irradiated organ anlagen develop better when transplanted to a nonirradiated host environment. Nevertheless, one cannot deny the relationship of the genome to development—normal development—and that developmental abnormalities can be caused by genic mutations. To draw attention to irradiation-induced developmental abnormalities as phenocopies and to presume thereby that irradiations merely bring about somatic mutations in the embryo may be premature and unwarranted at this time. There is present in the embryo what is referred to as "regulatory powers," which is simply an expression of the ability of the embryo as an integrated

TABLE III
X-IRRADIATION OF THE EARLY MOUSE EMBRYO^a

Embryonic age (days)	50 roentgens				200 roentgens			
	Total number of implants	Per cent "normal" ^b	Per cent resorbed	Per cent exencephaly	Total number of implants	Per cent "normal" ^b	Per cent resorbed	Per cent exencephaly
1½	52	58	42	0	29	33	64	3
1½	90	95	5	0	57	88	10	2
2½	95	73	24	3	68	39	61	0
3½	76	88	9	3	108	46	53	1
4½	53	92	8	0	49	94	4	2
5½	37	77	17	6	78	87	11.5	1.5
6½	77	92	8	0	85	90	5	5
7	23	100	0	0	27	92	0	8
7½	25	96	0	4	29	97	0	3
8	39	92.5	2.5	5	35	51	26	23
8½	26	100	0	0	26	62	0	38
9	20	95	5	0	26	54	38	8
9½	12	90	10	0	20	95	5	0
Totals	625				637			
Average		88%	10%	1.6%		71%	21%	7%

^a From R. Rugh and E. Grupp, *J. Neuropathol. Exptl. Neurol.* **18**, 468 (1959).

^b The term "normal" is used to designate those fetuses which appear to be normal. It is presumed that some of them will show neurological dysfunctions, since they are littermates of exencephalies and resorptions. Also, the term "resorption" includes dead fetuses as well as evidences of complete resorption.

organism to utilize what normal material is left, to develop a topographically whole individual. It is difficult to ascribe this integrative power to genic action if at the same time one ascribes the disintegrative abnormal development also to genic action.

Table III summarizes the data on early embryonic X-irradiation (mouse) at levels of 50 r and 200 r from ½ day (prior to the first cleavage) through day 9½ (after neurogenesis). It shows that intrauterine death and resorption occur most frequently when exposure occurs before cleavage (½ day) and that exencephalies (cerebral hernias) occur most frequently after 50 r on day 5½ and after 200 r on day 8½ (38%). There is either resorption or development of congenital cerebral anomalies after 200 r at any time from fertilization through neurogenesis, and, with few exceptions,

after 50 r also. Other data show that 25 r delivered to the fertilized but uncleaved egg ($\frac{1}{2}$ day) will cause 30% of the embryos to die *in utero*, so that this dose level should be considered as deleterious.*

These data, supported by photographs, have recently been published by Rugh and Grupp (233a). The so-called "critical period" concept will have to be revised in relation to exencephaly, since it can be brought about by X-rays at any time in embryonic development up to 9 days of gestation. Since the so-called "normal" littermates appear normal but are all stunted, with respect to controls, it must be assumed that the early embryo is not so tolerant of X-irradiation as was once thought. Extrapolating to the human embryo, we must now suggest that there is danger of neurological damage at any time through 40+ days and that pelvic X-irradiation of the female should be discouraged except during the 9 days after the onset of regular menstruation, when pregnancy could not occur.

The statement has been made that X-irradiation of the mouse embryo prior to about $8\frac{1}{2}$ days will not elicit surviving teratologies, and that all fetuses that are delivered appear to be normal. The reason for this sort of statement is that the mouse mother generally eats her abnormal young at birth, and gross anomalies must be found by Caesarian section prior to the time of expected delivery. As a result it has been demonstrated that exposures of 200 r will cause exencephalies in mice at any time from day $\frac{1}{2}$ to day $9\frac{1}{2}$, and that even exposures of as little as 50 r will cause such severe teratologies at $2\frac{1}{2}$ or $8\frac{1}{2}$ days.* Although 50 r on either of these days caused some exencephaly (see Figs. 16 to 21), exposures to 25 r had no such drastic effect. Doses of 50 r fractionated as 25 r on day $2\frac{1}{2}$ and day $8\frac{1}{2}$ did produce severe cerebral anomalies, however, including exencephaly. The phenomena described are not peculiar to insult of ionizing radiations but rather are an expected embryonic reaction when the insult occurs at such times. In the earlier instance it is probably due to interference with the mechanism of gastrulation, and in the later instance to direct damage to neuroblasts of the developing brain and head mesenchyme which would normally enclose the brain as cranial roof (233b).

Although the above projects (228-233) cover the bulk of studies on embryonic effects in mice, there have been other studies which tend to fill in some of the gaps which were unavoidable. Females $15\frac{1}{2}$ days pregnant were exposed to a single dose of 300 r of X-rays, and the embryos were studied from postpartum days 1 to 240 for skeletal effects. This time of exposure is after the major bones have been laid down, but there is continued growth, so that the study was to determine any effect on growth after initial differentiation. It was found that the dimensions of the femoral,

* Exencephaly has been produced by 15 r at 1.5 days, at the 2-cell stage.



FIG. 16. Two mice from a single litter whose grandfather was a C57 black male receiving 40,125 r of X-rays to the testes before mating with normal CF1 female. The F₁ were apparently normal and mated to normal CF1 mice to produce a litter containing an exencephalic embryo, the anomaly probably due to translocation the effect of which one would expect to skip a generation. (257)



FIG. 17. Two members from a single litter which received 150 r of X-rays *in utero* at 8½ days, showing exencephaly without evidence of cerebral hyperplasia. This was due to deletion of neuroblasts and mesoblasts after X-ray insult at the most critical phase of development. (26)

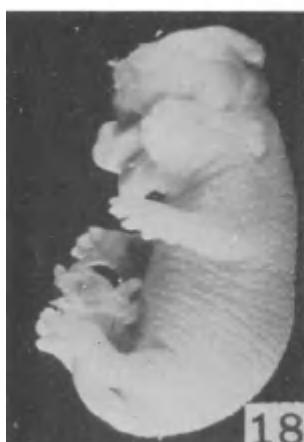


FIG. 18. Mouse fetus which received 150 r of X-rays at 9 days of gestation, showing gross anomalies of the entire head, but rather normal structures posterior to the head. Cephalic teratologies due to insult at time they were differentiating. (26)



FIG. 19. An entire litter at 18 days after *in utero* exposure to 200 r of X-rays at 8½ days of gestation, showing 5 out of 11 individuals with cerebral hernias (exencephalia) in both sides of uterus, and different positions. (26)

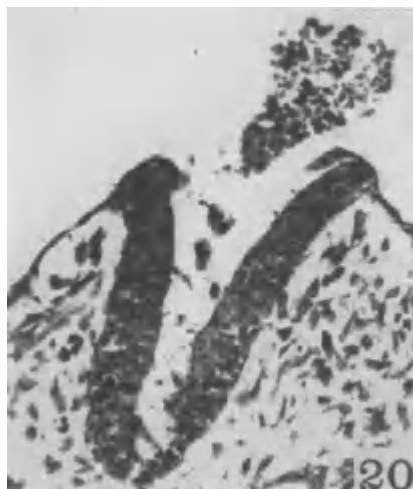


FIG. 20. Neural groove of 8½-day mouse embryo 4 hours after being exposed to 200 r of X-rays, showing sloughing off of neuroblasts into developing neurocèle, and disruption of neural ectoderm. (26)



FIG. 21. Section of head of mouse at birth, showing extent of exencephalia after 50 r of X-rays at 8½ days of gestation. Note occluded telocoeles, everted brain through unformed cranial roof. (26)

parietal, and mandibular bones (selected as representative of different types of bone) were unchanged, and no evidences of abnormalities appeared. The embryos as a whole were stunted, and the retardation in growth was statistically demonstrated, but it was proportional, in contrast with the effects of irradiation during the period of skeletal differentiation (234).

Day 13½ was selected as one which could be studied for neurohistological effects related to neonatal death in the mouse embryo. It was found that exposures to 100 r at this time did not cause sufficient damage to be detected by histological examination, but that such mice at weaning or early maturity would exhibit behavior reactions (hyperexcitability) which indicated functional damage at least (235, 236). In this connection it has been said (237) about the human embryo that "from the social viewpoint it is probable that the production of an individual with a subnormal brain may be a worse catastrophe than one with almost no brain." Mouse embryos at 15½ days can tolerate 300 r and survive until birth, however, but will die shortly thereafter. The macrophages are unaffected by this irradiation and are abundant and active in attempts to clean up the cellular detritus from such an exposure. When the dead cells are removed, however, there are not sufficient neurectodermal cells to continue normal morphogenesis of the brain, so that this irradiation on developing neural tissue alone could account for the lethal effects. The study shows the value of X-rays in mapping out the areas of concentration of neuroblasts at different stages in embryonic development (235).

The embryonic eye of the mouse begins to form about day 12, and for several days it is extremely vulnerable to ionizing radiations. This is evident if embryos are exposed at day 12½ or 13½ to X-rays of 50 to 300 r and studied within 4 hours. A study at this time is misleading, for it shows a retina so disorganized that it is difficult to imagine it could be reconstituted. By 24 hours, however, there appear abundant phagocytes in and



FIG. 22. Normal mouse at 2 months of age, showing normal eyes (238-240)



FIG. 23. Mouse at same age (2 months), but one which received 250 r at 12½ days of fetal age, showing microcephaly (50%). (238-240)

around the retina, cleaning away the dead cells, so that by 72 hours the eye appears to be quite normal in topography (238-240). Upon closer examination at 2 months of age, it is evident that there is an ill-defined rod layer, slightly reduced outer and inner nuclear layers, and interrupted

Day and Dose	Average diameter (mm)	Relative volumes (%)
Controls	3.355	100
150 r at 12½ days	2.970	69.6
250 r at 12½ days	2.670	50.6

ganglion cell layer of nuclei. These mice appear to have considerable vision, are alert, and will avoid objects, but they are definitely microphthalmic. Measurements at 6 weeks of age are shown in the tabulation. Thus, a sublethal exposure of 250 r reduces the eye volume by 50 %. Thus far it has not been possible to determine whether there is any reduction in visual acuity.

A preliminary study (241) on fetal irradiation and subsequent fertility of mice showed that, if 300 r was given over 6 days (days 14 to 19 at 50 r/day) and the mice were tested for fertility at 6 months of age, there was no uniformity in reproductive possibilities among littermates. This suggests that litter members might be of sufficiently different developmental age as

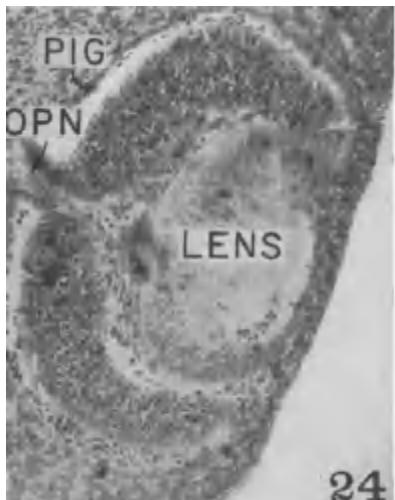


FIG. 24. Eye of $12\frac{1}{2}$ -day mouse embryo 4 hours after X-irradiation to 250 r, showing widespread pyknosis. (238-240)

Fig. 25. Enlarged view of Fig. 24 (238-240)

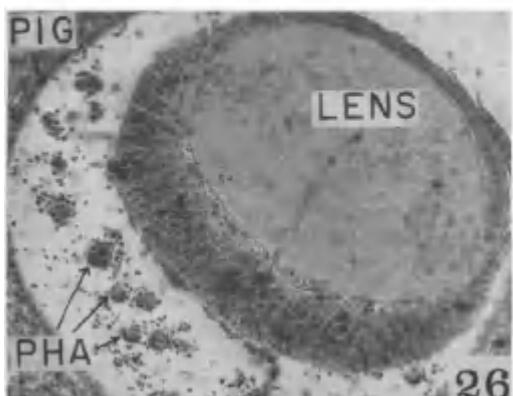
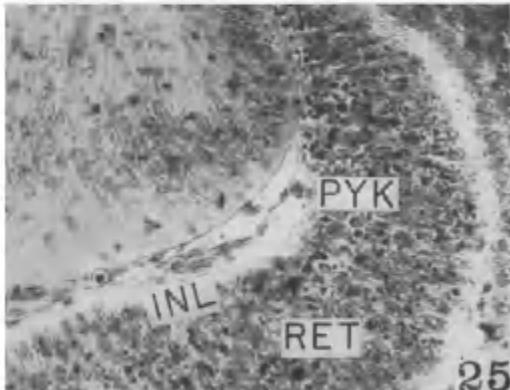


FIG. 26. Eye of $13\frac{1}{2}$ -day mouse embryo 24 hours after being X-irradiated to 250 r at $12\frac{1}{2}$ days, showing active phagocytosis of dead cells and detritus. Phagocytes seen even among retinal cells. (238-240)

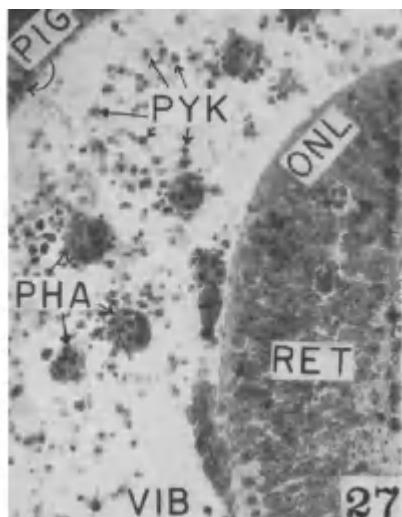


Fig. 27. Enlarged view of Fig. 26 (238-240)

Key to labels: PIG—pigmented layer; OPN—optic nerve; INL—inner nuclear layer; PYC—pyknotic nuclei; RET—retina; PHA—phagocytes; ONL—outer nuclear layer; MIT—mitosis; VIB—vitreous body; and COR—cornea.

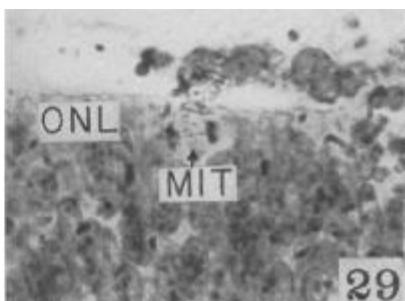
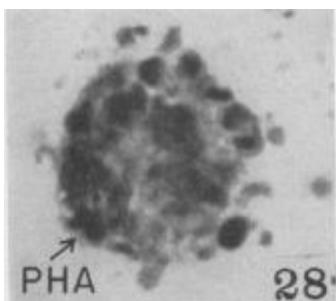


FIG. 28. Single phagocyte containing fourteen or more dead neuroblasts in vicinity of retina at 24 hours after X-irradiation. (238-240)

FIG. 29. Mitotic figure in outer nuclear layer of retina 24 hours after 250 r at 12½ days of fetal age. (238-240)

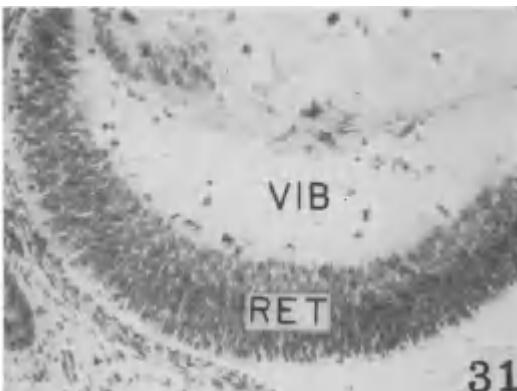
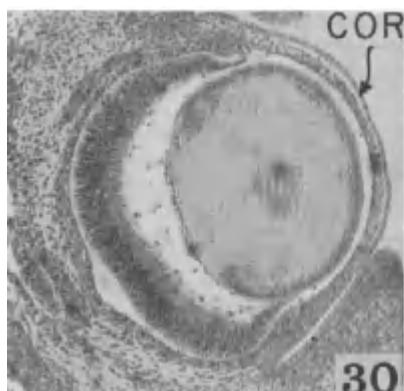


FIG. 30. Reconstituted retina of fetal mouse 72 hours after X-irradiation to 250 r at age of 12½ days. (238-240)

FIG. 31. Enlarged section from Fig. 30 showing apparently normal retina. (238-240)

to be differentially susceptible to X-irradiation sterilization. Certainly the exposures were as uniform as can be achieved. Roughly two-thirds of all offspring were sterile, but more males than females were completely sterile. It was found that those males in which 40% or more of the seminiferous tubules contained spermatozoa were fertile and those with less than 10% were invariably sterile. The Sertoli and interstitial cells of the testes and stroma of the ovary were undamaged, indicating that even though they were in a developmental stage when exposed (in the embryo) these cells

were radioresistant. There was a relative increase in the interstitial tissue of the testes from 15% in the controls to 44% in the irradiated males at 6 months of age. Thus, testes primordia were more radiosensitive than the adult testes or than the adult ovary, but the embryonic ovary was the more resistant (even than the adult ovary). This is a reversal of the expected results, in view of the suspicion that the embryo, in whole or in part, is more radiosensitive than is the adult into which it develops (241). This study has been further refined, since it has been shown (242, 243) that fractionation of exposures of the mouse fetus has statistically greater damaging effects than single exposures. This further study was made with single exposures of 50 to 200 r at days 15½ to 18½ of fetal age, and each of 500 mice was tested by continuous matings at 2 to 9 months of age for reproductive activity. It was found that no exposure caused sterility of the females, but that males exposed on days 15.5 and 16.5 were seriously reduced in fertility, and a considerable number were completely sterile. In direct contrast with the adults the testis primordium is more radiosensitive than the adult testis, and the ovarian primordium is more radioresistant. There was no fluctuation in litter production or litter size, indicating that the degree of reproductive potential was constant throughout the period of study. There was no evidence of temporary sterility or any change in the fertility level during the entire reproductive life. This is in contrast with the fact that an exposure of as little as 20 to 30 r to the adult ovary will reduce its fertility, and 100 r will sterilize all female mice within 8 weeks (244).

Cysteamine, injected into the pregnant mouse on day 14½, prior to fetal exposure of 300 r, will increase the expected survival from 40 to 55%; in addition, the mice will show an average increase in weight at 1 month from 6.83 gm for the unprotected mice to 11.44 gm for those protected by cysteamine. The results are even more graphic if the 17½-day fetus is exposed to 700 r of X-rays, with and without this protective drug. There were no survivors when the drug was not in the body of the pregnant female at the time of X-irradiation, but there were 79% survivors at 1 month of those protected by cysteamine (245, 246). Cysteamine and cystamine were also tested for their protective value toward fetuses at 15½ to 17½ days exposed to 600 r of X-rays. No controls at 15½ days survived, but the majority of drug-protected fetuses survived to at least 1 month of age. Exposures of 700 r at 17½ days reduced controls to 19% at 30 days, cysteamine-protected fetuses to 50%, and cystamine-protected fetuses to 41%. There was no suggestion that treatment of the fetuses in this manner in any way "protected" them against the normal sequelae of X-irradiation,

but rather that it simply allowed them to survive the irradiation better (247).*

It has been shown that low exposures of the adult mice gives them some protection at a later date against higher levels of X-ray exposure (248). Since the embryo is, in general, quite radiosensitive, it was thought that it too might be conditioned against subsequent postnatal exposures at the lethal level. Mice pregnant from $13\frac{1}{2}$ to $16\frac{1}{2}$ days were given exposures ranging from 10 to 300 r. It was found that exposures at 25 r or greater were deleterious with respect to the ability of the mouse subsequently to survive X-irradiation exposure. Exposures of 10 r, however, showed benefit at each gestational age, for both sexes, although not all values were statistically significant. The best values were obtained for $15\frac{1}{2}$ days at 10 r for either male or female, with increases in survival of 17.4% and 24.4% above the controls, all of which died when tested at 4 months after whole-body exposures of 525 r. The significance of this finding is open to conjecture (250, 251).

There are but few isotope studies on fetal mice. It was shown (250) that iodine concentrated in the fetal thyroid first on day 16, which time corresponds with the first appearance of follicles. The I^{131} uptake was increased with gestational age. Neonatal growth was retarded by fetal exposure to radioiodine, and the reproductive activity of fetally exposed females was reduced. Males were unaffected. The thyroids of offspring were fibrotic, with later compensatory hyperplasia and adenoma formation, and ultimate colloid goiters. In one-third of the surviving offspring at 9 to 12 months there developed chromophobe adenomas of the pituitary gland, so that the use of radioiodine in the treatment of pregnant human patients after

* Exencephalia, or herniated brain, which is closely allied to anencephaly (known to occur in the human) has been produced in the mouse either by X-irradiating the testes of the grandfather, or by irradiation of the embryo at any time prior to the completion of neurogenesis (9.5 days). Exposures of 50 r, either singly or fractionated, have caused this anomaly in embryos. Exposures of 50-r X-rays, if given to the embryo within 0.5 day of conception (prior to the first cleavage), will increase from 5.7% to 42% the number of uterine deaths and resorptions, indicating that this is the most sensitive period of all. Further, exposures of 5 r at this time will increase resorptions from 5.7% to 15%, and 15 r at 1.5 days caused exencephalia.

Some 15 so-called "protective agents" have been used in an attempt to prevent the development of X-irradiation congenital anomalies but only cysteamine, cystamine, and anoxia have been at all effective, reducing the incidence of anomalies and embryonic or fetal deaths, and increasing the percentage of "apparently normal" fetuses (247a). It is believed that exposure of the embryo results in a deficient individual, particularly with respect to the central nervous system, so that normality is probably only "apparent" in exposed litters (247b).

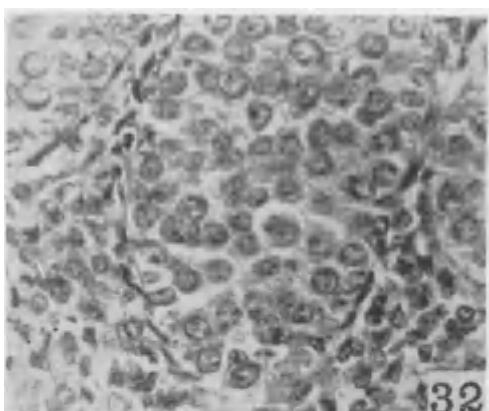


FIG. 32. Section of normal embryonic testis at $15\frac{1}{2}$ days of fetal age of mouse, at time of irradiation.

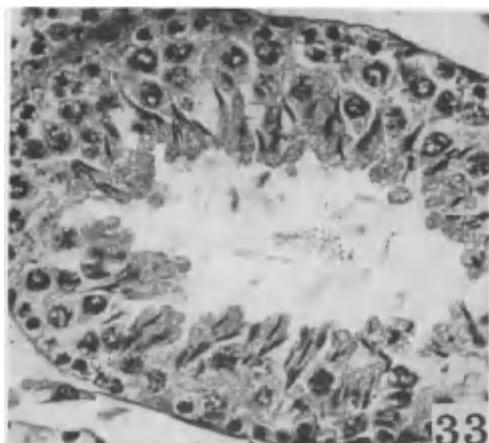


Fig. 33. Section of normal testis, showing tubule with abundant germinal epithelium at 9 months of age.



FIG. 34. Pairs of testes from mice at 9 months of age after fetal X-irradiation to 200 r at $15\frac{1}{2}$ days. Note great variation in size response, correlated with variations in fertility (see text).

FIG. 35. Section of sterile testis at 9 months after fetal irradiation at $15\frac{1}{2}$ days to 200 r. Note abundant interstitial tissue, but seminiferous tubules devoid of germinal epithelium.

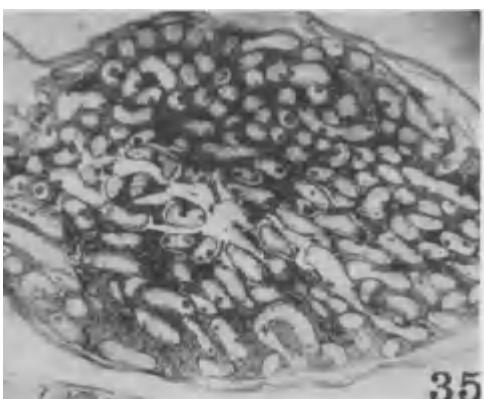


FIG. 36. Section of sterile testis at 9 months after fetal X-irradiation to 200 r at $15\frac{1}{2}$ days.

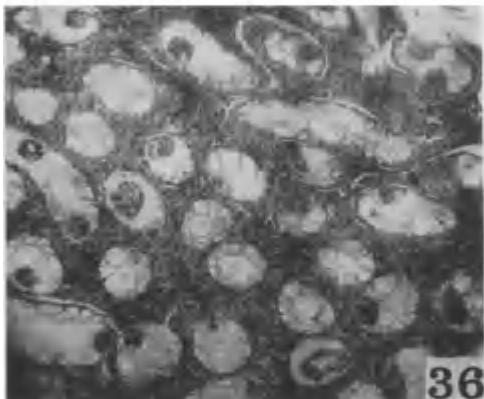
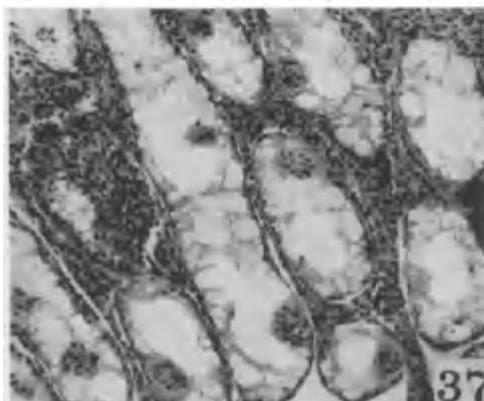


FIG. 37. High - powered view of sterile testis showing complete destruction of the germinal epithelium at 9 months after fetal X-irradiation to 200 r at $16\frac{1}{2}$ days.



Figures 32-37 from R. Rugh and S. Jackson, *J. Exptl. Zool.* **138**, 209-221, 1958.

the first trimester has been discouraged. In another study (251) it was shown that if pregnant mice are treated with thiocyanate (KSCN) the concentration of available I¹³¹ in the fetuses is considerably reduced, as it was also in the mother. It was postulated that the drug passes through the placenta to enter the fetal thyroid, before the iodine has been bound organically, and there acts in a manner similar to that in the adult thyroid gland. It had been shown previously (252) that thiouracil, methimazole, and thyrotropic hormone all modified the damage to the fetal thyroid when the pregnant mouse was treated with any of these drugs prior to treatment with I¹³¹. Pregnant mice, by virtue of the active thyroids of the contained fetuses, always retain more radioiodine than does the nonpregnant or the male mouse, but so much of the I¹³¹ concentrates in the fetuses that the mother's thyroid is somewhat protected by a reduction in the expected concentration (253). The avidity of the fetal thyroid (after day 17) for radioiodine, however, makes the fetus subject to pituitary adenomas in later life, against which KSCN afforded some protection.

A purely embryological study has been made correlating the equivalent ages of the mouse and the human embryos, which should be mentioned here, even though it in no way involved ionizing radiations. The curve was made by matching stages of embryonic structures in both man and mouse with the two gestation ages. The rate of development of the mouse with respect to man increases with increasing age, particularly after day 14. There are strain differences among mice, but the curve is of such value that, even with minor errors for some particular strain, it is offered here for reference (254).

H. MAN

Every irradiation-produced anomaly in the human fetus has been experimentally produced in the mouse or rat embryo by X-irradiation at a comparable stage of development so that it is justifiable to extrapolate from effects on the mouse or rat to the human fetus in regard to specific organ susceptibilities with the proviso that any statement is prefaced with the word "probable" (255). There is a time differential of about 13 between mouse and man, so that the period of greatest radiosensitivity in the human embryo is greatly extended. Table IV above gives the correlations in development of mouse and man, particularly during the critical radiosensitive period.

Implantation of the human ovum takes place at about 10 or 11 days after insemination (256). Radiation tolerance during this preimplantation

TABLE IV
CORRELATIONS IN DEVELOPMENT: MOUSE AND MAN DEVELOPMENTAL
STAGE: ORGAN PRIMORDIA

Mouse	Man	Age (days)	Embryo (mm.)	Development
5	11			Implantation
	14	0.15		Germ layers, extra embryonic membranes
	16	0.40		Primitive streak
8	20½	1.5		Neural groove, blood islands, notochord
9	25½	2.4		Cephalization, extensive vascularization, neural folds meet, primordia of sense organs, thyroid, limbs, muscles, pronephros, branchial arches, somites
10.5	28½	4.2		Primitive brain with vesicles, complete circulation, GI tract and derivatives, meso-metanephros, vertebrae, 31 somites, yolk hemopoiesis
11.5	33½	7.0		Genital ridge, heart, liver, mesonephros protuberant, limb and lung buds, 5 brain vesicles, all sense organs, cardiac septa, and 38 somites
12.5	36½	9.0		Heart chambered, nerves and ganglia differentiating, thyroid anlage bilobed
13.5	38	12.0		Sexless gonad primordia, liver hemopoiesis, brain flexures, limbs, thymus, GI tract actively differentiating
14.5	47	17.0		Cerebral hemispheres, corpora striatum, thalamus, blood vessels all actively differentiating, endocrine glands, peripheral and sympathetic nerves, eyes well formed
15.5	65	40.0		Cerebral cortex, intestinal villi, thyroid follicles, first ossifications, sex differentiation with sex cords and germinal epithelium

Note: The highly radiosensitive neuroblasts are present throughout the embryo and fetus from about day 25 in the human embryo (day 9 in the mouse) until some time after birth.

period is not known. The most radiosensitive period in the entire human prenatal life probably occurs from conception through about day 38, immediately after implantation. After day 38 higher levels of irradiation are necessary to produce anomalies, and generally not the same anomalies occur. This is because during the earlier period there is a great abundance of embryonic cells in their most radiosensitive stage. It is now believed that an exposure of as little as 40 r to the human embryo before day 28 might conceivably produce serious abnormalities (257). It has already been

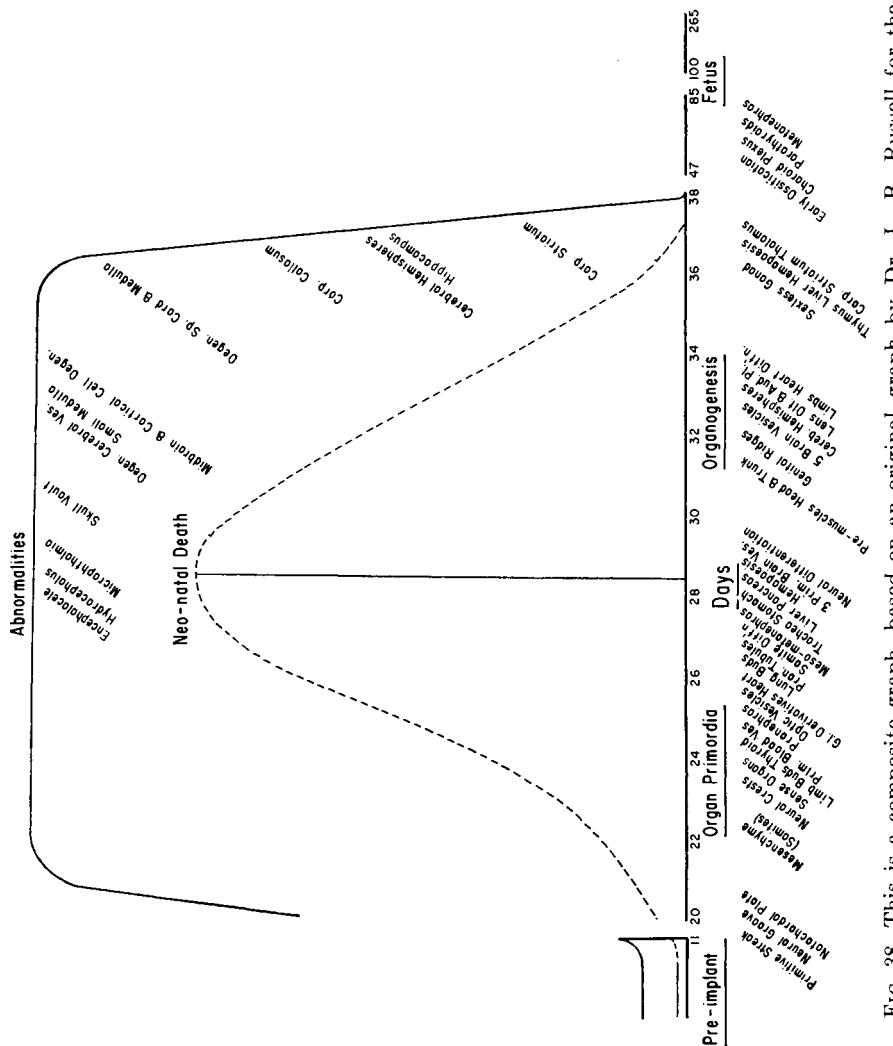


FIG. 38. This is a composite graph, based on an original graph by Dr. L. B. Russell for the mouse, but here extrapolated for man and based on both irradiation data and known embryological facts. From R. Rugh, *J. Pediat.* **52**, 531 (1958). (Note: Unpublished data from Rugh and Grup indicate that this graph will have to be revised with respect to the preimplantation period, since 50 r at $1\frac{1}{2}$, $11\frac{1}{2}$, and $21\frac{1}{2}$ days also caused exencephalia, or "herniated brains.")

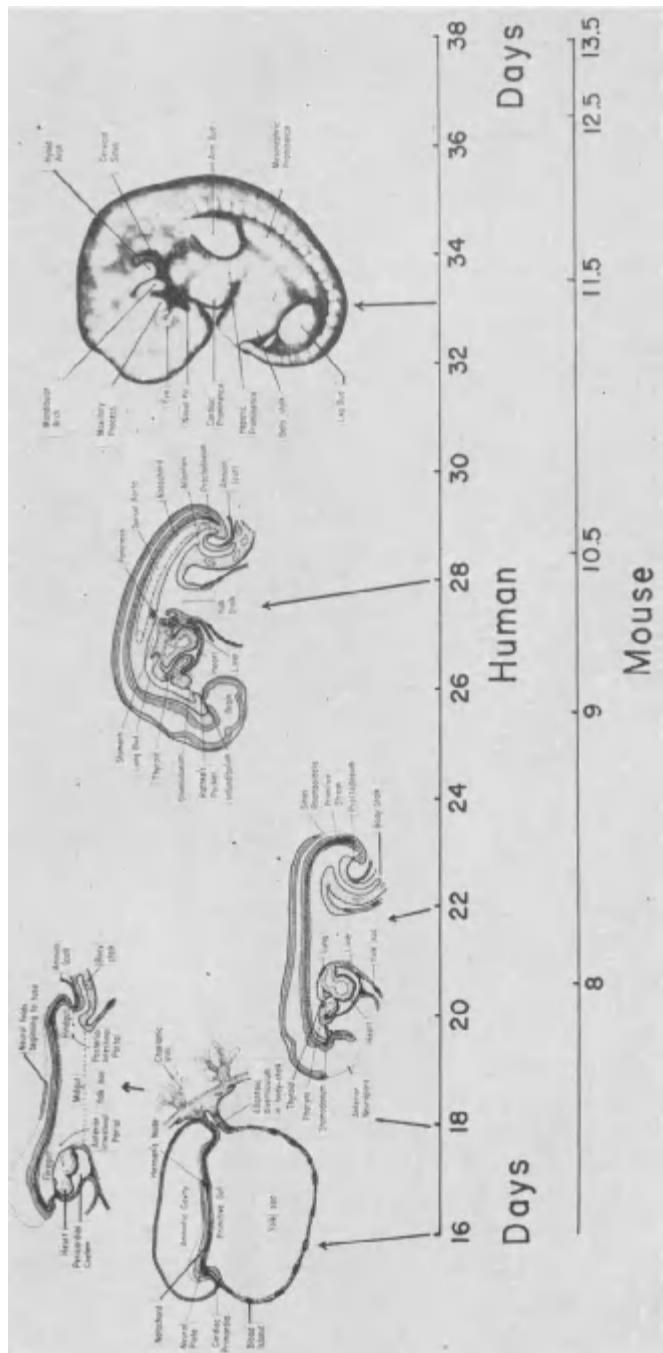


FIG. 39. This graph shows the normal development of the human embryo at times comparable to those for the mouse when the most critical anomalies can be produced by X-irradiation insult. The human figures are taken, with permission, from Dr. B. M. Patten's "Human Embryology," 2nd ed., McGraw-Hill, New York, 1953. Figure reprinted from *J. Pediat.* 52, 531 (1958). (Note: This graph shows the period of maximum neuroblast concentration, hence most susceptible neurological damage from X-irradiation. Recent data indicate, however, that similar damage (e.g., exencephaly) can be produced by X-irradiation at any time after conception, even before implantation.)

shown that X-irradiation of the rat embryo at a comparable stage has caused anencephaly, or the total absence of the brain (49). It is during this period of the human development that all the organ primordia are laid down by rapid differentiation from primitive cell types. The most radiosensitive period in the etiology of a cell is during its transformation from the embryonic state to the adult state, whether it be a neuroblast, myoblast, osteoblast, or erythroblast. During this period of about days 18 to 38 in the human embryo such transformations are occurring in almost every one of the tissue varieties. The greater severity of effects after fractionation of exposure is due to the fact that a greater variety and different distribution of formative cells are exposed, so that more organ primordia are radiation-insulted during their respective critical stages of development.

High-energy X-irradiations are uniformly distributed throughout the embryo and fetus which are at all times mosaics of actively differentiating cells and thereby of varying sensitivities. Irradiation destruction is proportional to the number of radiosensitive cells, but the specific anomaly produced is related to the differentiating organ system(s) possessing such cells. During the period of most active differentiation one would expect to produce the maximum possible damage, even with low-level exposures, and to produce anomalies at a later period in embryonic development would require a higher level of exposure. There are levels of exposure below which detectable anomalies are rare. Irradiation effects are qualitative rather than quantitative, as in most biological reactions. A knowledge of the chronology of human development would allow one, however, to predict with reasonable certainty the types of anomalies that might follow therapeutic levels of irradiation which include the embryo.

Modern experimental embryology includes an analysis of the formative processes which antedate the morphological evidence of their presence. Interruption or interference with these formative processes results in teratologies which the embryo and fetus can rectify to a certain extent under major topographical influences. In general these formative processes precede the morphological evidence of active differentiation in any specific organ or tissue. After about 40 days gross teratologies are difficult, and after birth they are impossible, to produce. The clinician must be reminded, however, that at all times in the human embryo and fetus there are some sensitive neuroblasts which are very radiosensitive, and also some primitive germ cells which can accumulate the effects of X-irradiation.

Aside from the classical review of human fetal irradiation consequences (258), human data are now appearing in the literature, much of which is coming out of studies in Hiroshima and Nagasaki. It is becoming apparent

that low levels of exposure may elicit functional changes even in a single cell which cannot be measured by known laboratory means (259), and somatic cell changes may occur, even in the embryo, which may contribute to disease processes many years after the exposure to X-rays (260). When an embryo or fetus is exposed, the whole organism is exposed, it being relatively so small, and "a young recipient of X-rays is more apt to develop late sequelae than is the adult" (259).

During the decade after the exposure of embryos and fetuses at Hiroshima and Nagasaki it has been shown that there is a reduction in head dimensions and definite mental retardation. The closer the pregnant Japanese woman was to ground zero, and the least protected, the more apt she was to have a microcephalic child, often with other evidences of stunting (88). Thirty-three children exposed *in utero* at Hiroshima had head circumferences two or more standard deviations below normal, and twenty-four of these were between 7 and 15 weeks of gestational age at the time of exposure. Mental retardation occurred in 15, or 45 %, of these children (255).

In the girls exposed *in utero* to the bomb during the first half of the gestational period, the mean height, weight, and head circumference were definitely smaller than in the unirradiated controls. In the boys such a reduction was found only in the mean head circumference. In another study both boys and girls showed stunting effects (261). Exposure during the second half of gestation had no such effects, but the boys exposed during this period scored less in Koga intelligence tests (262). With regard to skeletal maturation, the exposed girls scored higher than the exposed boys. Neurological abnormalities were found more frequently in children exposed *in utero* when less than 2000 meters from ground zero, but severe neurological anomalies, such as have been described for rats and mice, were not found (262). This may be due to poor survival of children so affected, because the above statements were made from a study of only the surviving children of 6 to 7 years of age. Also, all such reports as the above may be modified in time, because tests at 6 to 8 years will not reveal irradiation effects which may not develop until adolescence or later (263).

Among 205 children exposed *in utero* at Hiroshima, microcephaly and mental retardation occurred in greater frequency, with congenital dislocation of hips, mongolism, congenital heart disease, and hydrocele and other scattered effects following (264). Seven of 11 mothers who were within 1200 meters of the hypocenter had children with microcephaly and mental retardation; the controls (beyond 1200 meters) had no such effects. In another study of 30 mothers at Nagasaki who showed major signs of ex-

posure, and who had been within 2000 meters of the hypocenter, there were 23.4% fetal deaths and 26% neonatal or infant deaths, and among 16 surviving children there were 25% who showed mental retardation. The over-all morbidity and mortality was approximately 60% in this group of exposed fetuses, in contrast with 10% beyond 2000 meters and 6% for the general population. When the exposure occurred during the second or third trimester there was greater fetal, neonatal, and infant mortality than in the control groups. Although the effects of trauma, burns, and infection were considered, it was believed that the above percentages relate quite specifically to the effects of ionizing radiations (265).

Microcephalic idiocy, attributed to the effect of ionizing radiations on the human embryo or fetus, was reported many years ago (258, 266, 267). At the same time there have been isolated reports of fetal irradiation without obvious sequelae (268-271), even though exposures were 900 r at the fifth month or 4790 r during the period of 90 to 112 days, and the mothers were sterilized as a result. There has been evidence that in one of these instances a later examination of the child indicated neurological symptoms that were not apparent at 18 months of age. Attention must be called to the fact that there cannot be controls for such observations, and I.Q. tests and disease susceptibility data will have to await a considerable period.

In direct opposition to the above observations, there are specific reports of deleterious effects. Diagnostic X-rays examination of a pregnant woman at 4, 5, and 6 months was followed by delivery of a child showing microphthalmas, microcornea, syndactyly, brachydactyly, strabismus, hypermetropia, amelogenesis, and odontogenesis imperfecta (272). In a case of radiotherapy for castration in the fourth month of an unrecognized pregnancy, the fetus received upward of 900 r and at 2 years showed atrophy of the lower extremities and areas of depigmentation alternating with those of hyperpigmentation; an encephalogram indicated external hydrocephalus, porencephaly, and microcephaly, and psychometric tests revealed complete idiocy. The report includes references to 168 similar cases in the literature, indicating that exposure during 4 to 5 months had effects equivalent to that in the mouse at 10.5 days of gestation. A further statement is made to the effect that during the first 2 months irradiation of the embryo resulted in 100% damage, from 3 to 5 months it caused 64% damage, and from 6 to 10 months some 23% damage. Therapeutic abortion was recommended, since 34% of all cases of embryonic or fetal irradiation resulted in severe abnormalities without incurring spontaneous abortion (273). It will help in properly assessing such data if radiologists will report the irradiation

factors and probable delivered dose in greatest detail and accuracy so that eventually adequate and reliable human data will be available.

Statistics are being studied on the incidence of leukemia and cancer in offspring of mothers X-irradiated during pregnancies. It seems probable that leukemia incidence is doubled, and the authors believe that, besides producing genetic damage, roentgen pelvimetry may occasionally cause leukemia or cancer in the unborn child (272). A later report (274) indicates that among the 515 leukemia cases, after fetal irradiation, there appeared 15 mongolian idiots, and there were neoplasms of brain, spinal cord, kidneys, suprarenals, lymph nodes, and other sites.

The question arises as to fetal death and congenital malformations in the offspring of radiologists. Owing to the increasing awareness of the irradiation hazards and better protection of the clinician in fluoroscopy (particularly), it is likely that there may not be any statistically valid effect on fetal and infant death rates among offspring of radiologists (275, 276), although after 35 years there appeared to be a decline in the fertility pattern of exposed persons. Whether this was irradiation-induced or voluntary cannot be determined. At the same time it must be emphasized that the radiologist is giving service in a hazardous profession, and that overcaution is recommended, particularly until we know more about specific human data.

Radioiodine has been used to determine the onset of function in the human fetal thyroid as occurring after the fourteenth week (277). Two cases have been reported to show not only that pregnant women receiving large doses of I¹³¹ develop hypothyroidism but also that congenital hypothyroidism occurs in the offspring as a result (278). There has not yet been sufficient time to determine whether such children will develop pituitary adenomas such as occur about 9 months after fetal mice are so exposed. These and other studies indicate, however, that there is no placental barrier to the transfer of isotopes from mother to embryo or fetus. In fact, radio-sodium has been used to test placental function, particularly at about the time of parturition when delivery may be delayed (279).

I. GENERAL SUMMARY: EFFECTS OF IONIZING RADIATIONS ON THE EMBRYO AND FETUS

1. The damaging effects of ionizing radiations are primarily on the nucleus and particularly on the chromosomes.
2. The fertilized egg is more radiosensitive than is either gamete, owing probably to interference with the syngamic process.
3. Because of the dynamic nature of the embryo, irradiation studies

TABLE V

MAJOR ABNORMALITIES FOUND IN THE MAMMAL (HUMAN, MOUSE, RAT) AFTER FETAL X-IRRADIATION^a

<i>Brain</i>	<i>Skeleton</i>
Anencephaly	Uniform reduction: stunting
Porencephaly	Reduced skull dimensions
Microcephaly	Vaulted cranium
Encephalocele (herniated brain)	Narrow head
Mongolism	Cranial blisters
Reduced medulla	Funnel chest
Cerebral atrophy	Congenital dislocation of hips
Mental retardation	Reduced and deformed tail
Idiocy	Overgrown and deformed feet
Neuroblastoma	Digital reductions
Deformities;	Calcaneo valgus
Narrow aqueduct	Abnormal limbs
Hydrocephalus	Syndactyly
Rosettes in neural tissue	Brachydactyly
Dilation of third and lateral ventricles	Odontogenesis imperfecta
Spinal cord anomalies	Exostosis on proximal tibia
Reduction or absence of some cranial nerves	Metaphysis
<i>Eyes</i>	Amelogenesis
Complete absence-anophthalmia	Scleratomal necrosis
Microphthalmia	<i>Miscellaneous</i>
Microcornia	Situs inversus
Coloboma	Hydronephrosis
Deformed iris	Hydroureter
Absence of lens and/or retina	Hydrocele
Open eyelids	Absence of kidney
Strabismus	Degenerate gonad
Retinoblastoma	Atrophy of lower extremities
Hypermetropia	Cutaneous depigmentation and hyperpigmentation
Congenital glaucoma	Motorial disturbance of extremities
Partial albinism	Increased probability of leukemia
	Congenital heart disease
	Deformed ear
	Facial deformities
	Pituitary irregularity
	Dermatomal and myotomal necrosis

^a The above irradiation-induced anomalies have been reported in the works of Russell, Hicks, Miller, and Rugh for various mammalian fetuses, including the human.

cannot be quantitated or reproduced exactly. Nevertheless, there is a stage in early development when the embryo is more radiosensitive than is the organism at any other time as measured either by survival or by the production of anomalies. The peculiar hypersensitivity of the embryo may be related to its relatively high level of hydration, compared with any subsequent stage in development.

4. Teratologies such as can be produced by ionizing irradiations of the embryo and fetus are never produced in the postnatal organism.

5. There is no evidence of recovery of embryonic cells from the deleterious effects of ionizing irradiations. If damage cannot be tolerated by the organism, the cells are phagocytized.

6. The differentiating (formative) cell is the most radiosensitive, more so even than the same cell subsequently differentiated and in mitosis. Thus there are particularly sensitive stages in the derivation of every tissue and organ.

7. The neuroblast has been the focal point of study because its destruction may be least tolerable to the developing organism, and since its derivatives (neurons) permeate all other organs and tissues damage to neuroblasts has widespread effects. There may be functional neurological effects at levels of irradiation too low to have histological or cytological corollaries.

8. In general, maximum organ damage occurs if irradiation insult is imposed at the time of organ differentiation. It is known in experimental embryology, however, that organ differentiation may precede the visible appearance of the organ anlage. For this reason ionizing radiations may prove of unique value in mapping the presumptive areas of an early embryo in a manner more accurate than by extirpations.

9. As has long been known, the embryo is unique in having many undifferentiated, primitive cells which can be redirected to compensate for cellular damage and loss so that the organism develops as a topographical unit. Irradiation such as to damage cells of the embryo will result in deficiencies (deletions) which can be tolerated to the extent that residual, undifferentiated cells can be directed toward their replacement. Nevertheless, any irradiation-damaged embryo or fetus will be deficient to a degree somewhat related to the level of insult, and the ultimate damage will be a balance between the initial effect of irradiation and the ability of the "organism as a whole" to repair the damage by redirection of the undamaged primitive cells, and the regeneration of otherwise lost parts.

10. Polydactyly is not an expression of hyperactivity but of a splitting

of potencies and may result from the interposition of dead (irradiation-killed) cells. Hence it, too, is an expression of a deficiency.

11. The major differences in irradiation sensitivity of embryos of the various phyla represented are possibly due to the temperature differences of the environment during development, so that poikilothermic forms appear to be the more resistant. Nevertheless the same anomalies may be produced in any vertebrate embryos studied, by ionizing radiations.

12. There is some evidence that some of the so-called "protective" drugs may provide the embryo or fetus with better survival value when X-irradiated, but there is no evidence that these drugs protect any embryo against either the genetic or long-time sequelae of irradiation exposure.

13. Embryos which do not survive X-irradiation are generally killed by effects on one or more vital organ systems, which take along to death other systems which could survive in an otherwise normal environment. This has been demonstrated by transplantation of organs from moribund embryos to a normal host environment where such organs survive and develop normally.

14. It is now believed that even diagnostic exposures (e.g., 0.1 to 20 r) of the early embryo may result in severe teratologies, particularly if the insult occurs during the early development of the central nervous system (before 38 days in man). The threshold dose which will produce an anomaly has not been determined, although 5 r increases resorption of the very early precleavage stage in the mouse.*

15. Anomalies are not peculiar to ionizing radiations, but, owing to the all-pervasive nature of such irradiations, a greater variety of anomalies can be produced than by any other externally derived irritant. It must be emphasized that the embryo is a mosaic of innumerable developmental potencies, each of which has a period of extreme radiosensitivity.

16. The consequences of embryonic or fetal X-irradiation are such as to force the suggestion that such exposures be avoided at all cost.

IV. References

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CHAPTER 2

General Biology: The Adult Organism

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I. Introduction

On the tissue and organ level the damage caused by ionizing radiation is of an extremely complex nature. This is a consequence of the diversity of changes occurring on the cellular level, consisting in morphological changes as well as alterations of the biochemistry and physical chemistry of the cellular components. The end result of all these changes is the clinical manifestation of radiation injury.

In addition to the primary effects of the irradiation on the various organs, secondary effects also must be taken into consideration. Morphological damage to a tissue or functional disorder of an organ after irradiation may be a secondary phenomenon due to an impaired circulation. The irradiation may also cause a variety of hormonal adaptation and defense reactions, elicited and controlled by nervous centers as well as by humoral factors (see, e.g., ref. 1). Selye (2), for example, assumes such secondary effects of irradiation to be evoked mainly in the pituitary and adrenal glands.

Early studies within the field of medical radiology and radiobiology had revealed the highly variable response of different tissues and organs to irradiation as far as both the time of appearance and the severity of the changes are concerned. Thus, the blood-forming organs, the skin, the mucosa of the gastrointestinal tract, and the gonads were found to react earlier and more extensively than, e.g., the nervous system, the muscles, and the connective tissues. These observations led to the introduction of the concept of radiosensitivity.

It has been shown earlier in this volume (see Chapter 5) that different kinds of cell in the adult animal body display different types of radio-

sensitivity. According to decreasing sensitivity, the body cells can be ranged in approximately the following order: lymphocytes, erythroblasts, myeloblasts, megakaryocytes, spermatogonia, egg cells, cells of jejunal and iliac crypts, cells of germinative stratum, cells of sebaceous glands, hair matrix cells, cells of sweat glands, eye lens, cartilage cells, osteoblasts, epithelial cells of blood vessels, glandular epithelium, liver cells, epithelial cells of renal tubuli, glia cells, nerve cells, alveolar epithelium of lungs, muscle cells, connective tissue cells, and osteocytes.

As a consequence of the difference in radiosensitivity depending on the cellular level, the organs in the body will also display a varying degree of sensitivity. In general, the radiosensitivity of a tissue is proportional to its ability to reproduce and inversely proportional to its degree of differentiation (3). In accordance with this, the organs can be appropriately ranged in the following order of diminishing radiosensitivity:

1. *Differentiating tissues*: lymphatic nodes, spleen, thymus, lymphatic tissue in other organs, bone marrow.
2. *Rapidly dividing tissues*: testes, ovaries, mucous membranes of intestinal tract, skin, hair follicles.
3. *Slowly dividing tissues*: cartilage, growing bone tissue, liver, adrenals, kidney, pancreas, lungs, nervous system, muscles, connective tissues, and bone tissue.

The factors determining the radiosensitivity are very complex, and the classic concept of radiosensitivity is by now considered not to be fully adequate. Thus, it will depend not only on the animal species and its metabolic state but also on the methods used for determining the sensitivity. It is necessary to consider not only the morphological changes but also the functional ones. The fact remains, however, that different species as well as different individuals within the same species react differently on a given dose, and the lethal dose varies with the species. This is shown in Table I, which gives the lethal doses ($LD_{50(30)}$) for some mammalian species.

The principal factors influencing the response of an animal organism to ionizing radiation will be cursorily mentioned here. The main physical factor is the radiation dose. The dose-response relationship has been extensively studied. It is generally agreed that for most somatic effects a threshold dose exists below which no detectable effect occurs. Above this dose the severity of the response increases with increasing dose. As a general rule the effect of a certain dose is greater, the larger the part of the body irradiated, a total-body exposure producing the greatest effect. In partial-body exposure, certain parts, as for example the abdomen, are more sensitive than others, e.g., the extremities or the head.

TABLE I
LETHAL DOSES ($LD_{50(30)}$) IN VARIOUS MAMMALS

Animal species	Lethal doses (r)	Ref.
Dog	315	(4)
Guinea pig	400	(5)
Pig	400	(6-8)
Man	400	(9, 10)
Goat	500	(11)
Monkey (rhesus)	550	(12, 14)
Mouse	550	(15)
Rat	600	(16)
Donkey	650	(6)
Rabbit	800	(8, 17)
Hamster	800	(15)
Bat	15000	(18, 19)

The distribution in time of the radiation exposure is also of importance (see, e.g., ref. 20). A short-time (acute) exposure to a certain dose always has a greater effect than if the same dose is given as a chronic exposure over a long time or is administered fractionally. In these latter cases the dose-effect relationship will be more complex, as biological factors such as recovery and sensitization may be effective and alter the response.

In addition to the physical factors mentioned, the response will also be subject to biological variations depending on factors such as constitutional differences and age. Young individuals are usually more radiosensitive than adults. During senescence the sensitivity increases again.

The response of a mammalian organism to irradiation may be regarded as an integration of the effects on its various organs and on the interrelationship between the organs as far as functional effects are concerned. In this chapter dealing with the effects of ionizing radiation on the adult organism, the main emphasis will be placed on the effects on the various organs or groups of organs in the body. Because data of the detailed effects on complex systems are incomplete, it will not always be possible to give an integrated picture and to treat in detail the interrelationship of the effects on various organs. As far as possible the effects produced will be related to the dose received. This is easy as far as the blood and blood-forming organs are concerned but has not been consistently accomplished for certain other organs owing to lack of data regarding certain dose levels. External irradiation and internal contamination with radioactive isotopes have been treated separately and, when possible, in the first case the effects of acute, chronic, and fractionated irradiation are dealt with. Both morphological and functional effects of irradiation are discussed.

II. Blood-Forming Organs, Blood, and Lymphatic and Reticuloendothelial Systems

A. BLOOD-FORMING ORGANS AND CIRCULATING BLOOD

The marked radiosensitivity of the hematopoietic organs was early emphasized by Heineke in his classic work of 1903 (21). Since then, and especially during the last fifteen years, an enormous literature has accumulated on that topic, and a number of good reviews have been published (see, e.g., refs. 22-29). Owing to the enormous amount of data available concerning changes in the hematopoietic system and blood after irradiation, the following treatment will be only fragmentary.

Heineke (21, 30, 31) pointed out that the changes found in the circulating blood are primarily due to effects on the hematopoietic tissues. It had early been realized that even feeble radiation to a blood-forming organ causes an arrest of the hematopoiesis with changes in the peripheral blood as a consequence. Such changes, therefore, have become an important indication of exposure to ionizing radiation. According to Heineke (31), the lymphocytes decompose almost immediately after the exposure, and the reaction always starts regularly at the same time, independent of the dose.

It is generally agreed that the changes in the formed elements of the circulating blood are due both to primary lesions in the hematopoietic tissue caused by the irradiation and to a secondary action through some kind of neurohormonal mechanism (32, 33). The blood-forming organs have a very large functional reserve capacity, and exposure to small doses of irradiation, therefore, may not always be reflected as changes in the circulating blood.

1. BONE MARROW AND BLOOD

The bone marrow is highly radiosensitive, although not as sensitive as the lymphatic tissues. After large doses (several hundred roentgens) the reaction is very rapid, and cessation of mitosis and degeneration of hematopoietic cells may be seen by 30 minutes or less (34) after the exposure (26). Heineke (30) found an apparent destruction of bone marrow cells within 3 hours after irradiation, with maximum destruction after about 11 hours, and early regeneration in 5 to 6 days. Complete recovery was seen after 3 to 4 weeks. After large doses there is progressive damage and hypoplasia. The early changes in irradiated animals include a relative and absolute decrease in the numbers of normoblasts and other premature blood cells and an increase in the segmented granulocytes.

For a detailed account of the effect of irradiation on bone marrow the reader is referred to the reviews of Töppner (35), Dunlap (25), M. A.

Bloom (26), and Jacobson (29). In the following discussion only a short account of the main morphological and physiological changes will be given.

The various kinds of hematopoietic cells show different sensitivity. Erythroblasts are more sensitive than myelocytes, and myelocytes are more sensitive than megakaryocytes (26, 36), irrespective of the kind of radiation that produces the damage. This newer view about the sensitivity of the various blood cells and precursors is in contradiction to that of Heineke, who considered the erythropoietic cells to be radioresistant, and also to the results of many other earlier authors (cf. refs. 26 and 37). According to Bauer (27), the erythroblasts and hemocytoblasts are the most sensitive, followed by myelocytes, myeloblasts, and finally megakaryocytes. The most sensitive of all blood cells are the lymphocytes and their precursors. The macrophages and especially the reticular and fat cells of the bone marrow are very resistant. It has been found (26) that the less sensitive cells which are not destroyed by the irradiation may be transformed into each other or into spindle cells or blood-forming cells. Dilated sinuses and fatty and gelatinous marrow replace the hematopoietic marrow cells. The marrow may be edematous and hemorrhagic. The reticulum cells are often increased in number.

There is some evidence for the existence of species differences in the radiosensitivity of bone marrow, but the degenerative changes found in the bone marrow after irradiation are qualitatively of the same type. In spite of the fact that the LD₅₀₍₃₀₎ for guinea pigs is much lower than for the rat, the rat bone marrow seems to be more sensitive than that of the guinea pig. A peculiar fact is that the marrow close to the epiphyseal cartilage plate reacts earlier and more severely than the cells in the metaphysis and the shaft on the long bones. This may be due to some differences in physiological activity which are not yet known (26). Similar effects have been described and are more easily understood after irradiation from incorporated radium. After treatment of rats with radium, Thomas and Bruner (38) found an aplastic marrow in the ends of the long bones, and a hyperplastic marrow in the middle two-thirds of the shaft.

The differences in the reactions on irradiation between the erythropoietic and myelopoietic systems are especially evident after fractionated irradiation. They can be easily studied in the chicken, as the two systems are separated in this species. The erythropoiesis is located intravascularly in the bone marrow, the myelopoiesis extravascularly.

The depletion of the bone marrow caused by internal radiation is more gradual than after external exposure. In this case much less cell debris is found, as phagocytosis has had time to remove it (26). Hyperplastic and atypical cell forms occur.

Whole-body irradiation causes a strong reduction of the content of

ribonucleic acid and deoxyribonucleic acid in the bone marrow (39-41). The decrease in cytoplasmic RNA content of morphologically unaffected cells is an early finding (41). Later, after cell destruction, there is complete absence of RNA. During that phase there is, however, still some DNA left.

The synthesis of saturated and unsaturated fatty acids is increased two- to threefold immediately after irradiation (42).

The bone marrow has a large capacity for regeneration, which starts early after irradiation. Even after doses near the LD₅₀ range, regeneration is evident after 1 or 2 weeks. In rats and rabbits, areas of erythropoiesis begin to regenerate earlier than the myelopoietic system (26, 28). In mice the two types of cells start regeneration about simultaneously, or the myelocytes first (26).

The effects of ionizing radiation on the hematopoietic cells in the bone marrow are also, although somewhat later, reflected in the peripheral blood. The circulating blood corpuscles are generally relatively resistant to irradiation, possibly with the exception of the lymphocytes. The reduction of the formed elements in the blood, therefore, are due more to a reduced supply from the damaged bone marrow and the life span of the different blood cells than to a direct injury to the mature circulating corpuscles. The mean lifetime of the circulating lymphocytes has been determined to be from some hours to 1 to 2 days, that of the leukocytes about 4 to 7 days, and that of the erythrocytes about 3 to 4 months. For erythrocytes much lower values have, however, also been reported (43).

With regard to lymphocytes, there are no prominent species differences involved (44). The lymphocyte count in the peripheral blood is supposed to be the most sensitive index of somatic radiation injury (45).

A reduction of the numbers of lymphocytes occurs even during the first hours after the exposure. The degree and duration of the lymphopenia is strongly related to the dose received. Doses as low as 250 mrem of X-irradiation in man can cause a temporary reduction (46), and a significant decrease is evident after doses of less than 100 r. After 300 r or more there is a strong decrease, and it may take 2 months or more for regeneration to be completed. After repeated doses of a few roentgens, morphological alteration of the lymphocytes may appear. There is an increase of binuclear cells (47), and the occurrence of bilobed lymphocytes has been reported to be more readily detected than a mere reduction in number (46). The changes in the blood lymphocytes correspond to the alteration of these cells in other organs (lymphocytes in spleen and lymph nodes, thymocytes).

The change in the numbers of granulocytes follows a somewhat different pattern. During the first 24 hours after irradiation with moderate to high doses, there is an increase in number, which has been interpreted as a

stimulation effect. Then, the number of granulocytes decreases, and after about 3 to 5 days a minimum value is reached. There are also morphological changes in the cells, probably due to mitotic disturbances with formation of giant granulocytes. The specific activity of granulocyte hemin iron decreases after X-ray exposure (48), even with an unaltered number of cells, which is also an indication of an inhibition of myelopoiesis. After the first week the number of granulocytes sometimes increases again, possibly after an abortive rise. The regeneration curve of the granulocytes usually shows an undulatory character, and the numbers may sometime during this phase exceed the pre-exposure value. Eosinophile granulocytes have been reported (49, 50) to increase after whole-body X-irradiation in guinea pigs.

The erythrocytes and proerythrocytes react most slowly, owing, at least partly, to the relatively long life span of the red blood cells. It is, however, evident that these cells are reduced more rapidly than what should be expected only from their life span (51), indicating a destruction of circulating cells. The proerythrocyte curve has a course similar to that of the granulocytes. The anemia will reach its maximum after about 2 weeks. Irradiation at or below the median lethal dose does not hemolyze erythrocytes directly, but some change caused by massive irradiation results in increased destruction of erythrocytes. It has been postulated that this change is a damage to the capillary wall, causing diversion of erythrocytes into tissue spaces and lymphatics, resulting in injury of some erythrocytes (52).

The number of thrombocytes in the circulating blood drops, the rate of decrease following essentially the same pattern as that of the leukocytes. This and the reduction of thrombokinase in the thrombocytes have been made responsible for the increased clotting time (53) and defects in hemostasis after irradiation (54). Alterations in plasma volume have also been attributed to the changed permeability of the capillary walls (55).

Belcher *et al.* (56) and Baxter *et al.* (57) have studied the rate of formation of new erythrocytes in X-irradiated rats by means of the Fe⁵⁹ technique. Even after a dose of 50 r they found a considerable transient reduction in the incorporation of Fe⁵⁹. Minimum values were found after 2 days. After 2 more days the rate of incorporation was normal again. Richmond *et al.* (58) have studied the inhibition of the hemoglobin synthesis in rats after whole-body irradiation. One day after the exposure to 600 r the inhibition of the globin synthesis is three times as strong as that of the hemin synthesis. After a week, on the contrary, the synthesis of hemin is almost nil, whereas there is still some synthesis of globin. Other investigators also (59-62) have observed an impaired hemoglobin synthesis after irradiation. The carbonic anhydrase activity may be depressed (63).

In addition to the reduction of the number of white and red blood cells, other morphological and also a number of biochemical changes are present in the blood after irradiation. These changes reflect the effects of radiation on the tissues and the functions of various organs in the body.

The concentration of total plasma proteins is relatively little changed. A slight reduction is found during the first postirradiation period. The various protein fractions, however, show significant alterations (64-71). The albumin fraction is reduced. The reduction may amount to about 50 %. The α_1 , α_2 , and β -globulin fractions are increased. Thus, the globulin-albumin ratio is higher than normal. The serum lipoproteins may also be subjected to changes after irradiation (72, 73).

Caster and Armstrong (74) and many others have reported changes in the electrolyte balance in the blood. The irradiation often induces a negative sodium and potassium balance, whereas the chloride balance is positive. The serum iron is increased (75). This, in addition to an increased concentration of bile products, is indicative of an increased destruction of erythrocytes. Plasma copper levels are elevated, but the plasma magnesium is decreased after irradiation (76). The increase in the concentrations of nonprotein nitrogen, amino acid nitrogen, and urea likewise is an indication of an increased destruction of hematopoietic and other cells. For further details of the biochemical changes in blood after irradiation, reference is made to Patt and Brues (77).

Some of the enzyme systems of the blood may be subjected to changes after irradiation, although the purified enzymes, when exposed *in vitro*, are often remarkably resistant (78-80). The cholinesterases are among the enzyme systems which have been studied. Despite the great interest in this enzyme system at the present time, there are relatively few reports on the effects of irradiation *in vivo* on cholinesterase in the literature.

Lüthy (79) found in roentgen-irradiated rats a decrease in the activity of cholinesterase in the serum after 500 r and 1000 r, although after 250 r the values usually lay within the normal limits. After 500 r—that is, a little less than the LD₅₀ for rats—there was often a decrease in enzyme activity which was manifested as early as a few hours after the irradiation. The results were inconsistent, however, some animals showing an increased activity, the lower the dose given. The same variable results were obtained in guinea pigs by Ord and Stocken (81). After 1000 r—that is, two to three times the LD₅₀—they generally observed a drop in the unspecific cholinesterase activity 6 hours after irradiation, with a tendency to recovery in 48 hours.

In mice exposed to 25 to 300 r of total-body roentgen irradiation Sabine (82) noticed an increase in the cholinesterase activity of the erythrocytes 4 days after the exposure. High values were observed after 300 r on the

third to fifth days. After that there was a sharp decrease, and values far below the normal were usual at the end of the first week; recovery was complete after 3 weeks.

Lundin *et al.* (83) found a reduction of about 30% during the first 2 days of cholinesterase activity in plasma after whole-body irradiation of male guinea pigs. No changes in the activity of acetylcholinesterase in erythrocytes could be demonstrated as a result of irradiation.

2. LYMPHATIC TISSUE

The lymphatic tissue in lymph nodes, tonsils, Peyer's patches, intestines, etc., are exceedingly sensitive to irradiation. There is a rapid destruction of lymphocytes which is reflected in the peripheral blood by a reduced number of circulating lymphocytes. The lymphatic nodes or organs are reduced in size (84), and atrophy of lymphoid tissue, often with ulceration of the tonsils, is a common finding in animals dying early after exposure to high doses of ionizing radiation (85).

In rabbits, 50 r of whole-body X-irradiation is the lowest dose that may be expected to produce detectable morphological changes (84). After 100 r there may be only minimal damage, and even after 400 r the majority of the nodes are only partially destroyed. Doses of 600 to 800 r completely destroy the lymphatic nodules. After 3 weeks, formation of new nodules begins.

In rats doses of 400 r and 600 r produce the same changes as in rabbits. A dose of 175 r to guinea pigs produces essentially the same degree of damage to the lymph nodules as after about 400 r in rats and rabbits. Fast and slow neutrons produce changes of the same kind as X-rays. Internally administered radioactive isotopes cause the same kind of histological damage to the lymph nodes as is seen after external irradiation.

The activity of β -glucuronidase is increased during the first period after irradiation when the lymphatic tissues undergo a rapid decrease in weight (86). During the reconstitution phase the findings are more variable. The activity change of the enzyme is a secondary effect.

For further details about the effects of ionizing irradiation on the lymphatic tissues, the reader is referred to the extensive study made by De Bruyn (84).

3. SPLEEN

It is generally agreed that the lymphocytes of the spleen are as sensitive as other lymphocytes. This had been noted by Heineke (87), who observed destruction of the lymphoid follicles, disappearance of cells of the marrow, and increase of pigments after irradiation. As in other similar organs, the reticular cells are the most resistant. An excellent account of the effects

of irradiation on the morphology of the spleen has been given by Murray (88), and for details reference is made to this publication.

After an exposure to 400 to 800 r of X-rays, mitosis is inhibited almost immediately, and some lymphocytes die (88). During the following hours most lymphocytes disappear. The most resistant lymphocytes generally are the large ones, the medium-sized being the most sensitive. The erythroblasts in the red pulp are destroyed. The myelocytes degenerate at a later time than the lymphocytes and erythroblasts. The debris of white and red cells are rapidly metabolized by the reticular cells.

Because of the rapid and extensive destruction of cells, the spleen reduces in size and weight. The reticular part, however, undergoes a relative and possibly also an absolute increase in size. Some attempts at regeneration are evident, essentially by transformation of reticular cells. The recovery is slow after high doses. After lower doses (100 to 200 r) the destruction is much less than after higher doses.

In summary, according to Murray (88), the effects of a single $LD_{50(30)}$ of X-rays on the spleen of the rabbit can be divided into four stages. The first stage, lasting 1 to 3 hours, is a phase of destruction with disintegration of almost all lymphocytes. During the next stage, the phase of phagocytosis and elimination of debris, which generally lasts from 3 to about 17 hours, the dead cells are removed. The reticular cells undergo a relative increase. The organ is reduced in size. During the week after the first postirradiation day, there is a phase of relative inactivation with further dominance of the reticulum and sporadic attempts at regeneration. The stage of regeneration through active mitotic proliferation of lymphocytes usually starts after the first week and lasts for about 3 to 4 weeks.

A number of investigations have been devoted to the effects of irradiation on various enzyme systems in the spleen and other tissues, especially the phosphatases and phosphorylases (89-93), as well as the deoxyribonucleases (94). There is usually an increase in the phosphatase activity in the spleen, but the findings are inconsistent and the increase is interpreted as due to the reduced organ weight (91, 93). An increase of adenosine triphosphatase and 5-nucleotidase in the rat spleen has been observed after 200 to 500 r (95) and 800 r (96) of whole-body irradiation. Maximum activity occurs 24 to 48 hours after the exposure.

A marked reduction of deoxyribonucleic acid accompanies the involution of the spleen after irradiation (96-99). The loss is probably due to its being released as a soluble polynucleotide (96). The —SH content of the spleen of mice exposed to 20 to 800 r of whole-body X-irradiation is lower than normal during the first 3 postirradiation days, but thereafter it increases and reaches supernormal values (100). Cytochemically, a decrease of protein-bound sulphydryl groups has been found to occur as

early as 2 hours after irradiation (101). A significantly reduced incorporation of C^{14} -formate into serine, cysteine, nucleoproteins, and lipids has been observed after whole-body exposure of rats to high doses (2000 to 5000 r) of X-rays (102).

Internal Irradiation. Internally administered P^{32} (2.5 $\mu\text{c}/\text{gm}$) or Sr^{89} (3.6 $\mu\text{c}/\text{gm}$) has no or only slight effect on the spleen (88). After Sr^{89} injection, hyperplasia of ectopic myelopoiesis has been found. $\text{Ba}^{140}\text{-La}^{140}$ (5.6 $\mu\text{c}/\text{gm}$) caused rapid moderate depletion of the white pulp but hyperplasia of ectopic myelopoiesis in the red splenic pulp. Y^{91} (2.0 $\mu\text{c}/\text{gm}$) and $\text{Zr}^{93}\text{-Nb}^{93}$ (3.0 $\mu\text{c}/\text{gm}$) have severe depleting effect on the organ. Small doses of Pu^{239} (0.08 $\mu\text{c}/\text{gm}$) exert only a moderate effect on mice spleen, but more severe damage is caused by the same dose in rats. Radium causes a somewhat less severe damage than plutonium.

For the dose dependence of the changes in the spleen after acute and chronic external and internal irradiation, see Section II. A.5.

4. THYMUS

The main change observed in the thymus after exposure to ionizing radiation is a reduction in size, mainly due to a destruction of thymocytes. It should be kept in mind, however, that reduction of thymic weight can be due to other factors than irradiation. Kallman and Kohn (103), studying the effect of irradiation on the thymus of CAF_1 mice, found that the weight of the organ was reduced about 25 % by the mere handling of the animal preparatory to irradiation. Decreased food consumption per se can possibly account for half the thymic weight loss after a dose of 400 r in mice. Minimum postirradiation thymic weight is a curvilinear function of the radiation dose. Kallman and Kohn (103) assume that the thymus is composed of two independently reacting cell populations with different sensitivities. The exposure of the head during a massive whole-body irradiation markedly influences the cellular response in the thymus.

It has long been known that the thymocytes are exceedingly radiosensitive. Pyknosis and disintegration of these cells is pronounced within 24 hours after irradiation (104). The more resistant cells which remain after irradiation include epithelial and connective tissue cells and macrophages. All these kinds of cell contain much more cytoplasm than the thymocytes.

In rabbits, rats, mice, guinea pigs, and chickens exposed to a single whole-body $\text{LD}_{50(30)}$ of X-irradiation, the following results have been found (105). There is an initial destructive phase during which most of the lymphocytes die. A phase of cleaning up of the organ follows when debris is removed by macrophages and probably also in part through lymphatic channels. Sparse thymocytes are found in the medulla. The

next phase is characterized by inactivity with continuous shrinkage and proliferation of connective tissue. During the last phase, that of regeneration, there is again an increase of thymocytes proceeding from the medulla outward to the periphery. The length of the periods and the degree of the changes are rather closely correlated to the dose received. After lower doses a smaller percentage of the cells die. Below 175 r there is usually no depletion of the cortex. The reticular cells are radioresistant and are not damaged by 800 r. The Hassall's corpuscles, which are small, concentrically striated masses of epithelial cells found in early stages of development of the gland, are not affected.

The preservation of lymphocytes after irradiation with large doses is more evident in the cortex than in the medulla. Generally, a close correlation exists between the degree of cellular destruction, the width of the cortical layer, and the weight of the organ. The nuclei of many of the surviving lymphocytes may be pyknotic, but the cellular membrane is usually intact. In animals in which karyorrhexis and phagocytosis are prominent in the spleens, these changes are usually absent in the thymus.

The reactions of the thymus to fast and slow neutrons are qualitatively the same as after X-ray exposure. Quantitatively, the relative biological effectiveness (RBE) ratio of X-rays to fast neutrons is about 5, and of X-rays to slow neutrons about 1 (105).

Local irradiation of the thymic region or irradiation of the body with the head shielded with large doses (10,000 to 30,000 r) has been found regularly to be followed by significantly greater cellular damage and weight loss of the thymus than after a whole-body exposure to the same dose.

According to Murray (105), external whole-body β -irradiation from P^{32} in mice with a dose of 5000 rep, which will kill about 20 % of the animals, has no direct effect on the thymus.

Whole-body re-exposure to a massive dose of radiation within an hour after an initial whole-body exposure to a small dose will regularly be followed by less weight reduction and cellular damage in the thymus than after a whole-body exposure to the small dose alone.

Chronic whole-body exposure to γ -rays gradually causes depletion of lymphocytes. The effect is dependent on the dose, and changes were seen after 6 months when 8.8 r was given daily and after 8 months with 4.4 r daily (106). A dose of 1.1 r daily was effective first after 10 months. There was a strong increase in mast cells in these animals, but plasma cells were not prominent.

The effects of irradiation on the function of the organ are almost unknown. Some changes in the nucleic acid content of the thymic cells after irradiation have, however, been found in recent years. The DNA level of

the thymic cell was not significantly changed after 4 doses of 168 r each, given every 8 days to young mice (104). The RNA content, on the other hand, displayed a roughly threefold increase 1 to 5 days after irradiation. This increased content of RNA during the first few days after irradiation is supposed to be a histochemical expression of the transient change in the cell population of the irradiated thymus.

Single whole-body irradiation of mice has also been found to increase reversibly the three nucleodepolymerases, DNase, RNase (pH 6), and RNase (pH 8), in the thymus of the mouse (107). Twenty-four hours after administration of 160 r the DNase activity was six times the normal activity. The corresponding values for RNase 6 and RNase 8 were two and ten times as high as normal, respectively. An increased RNase 8 activity was observed as soon as 20 minutes after irradiation. Even a dose as low as 40 r increased the activity of the depolymerases. Changes in the cell population are considered not to be the only explanation for the increase.

During the early stages of radiation injury an increased lipid content is found in the thymocytes in mice (108). Large lipid-laden epithelial reticular cells are conspicuous in the inner zone of the cortex and in the medulla. The changes are most evident in young animals. In old thymuses, the accumulation of lipids is less marked, and they are more variable in their distribution.

Internal Irradiation. The damage of the thymus is more gradual and more prolonged after internal irradiation than after an external one (109). In the case of α -emitting isotopes, the depletion of the thymocytes is usually nonuniform.

Intraperitoneal administration of 1.1 μ c of Ra per gram of body weight in mice rapidly depletes the thymus of mice (105). This is also the case with Na²⁴ (50 to 80 μ c/gm). On the other hand, P³² (2.5 μ c/gm) had no depletory effect on the thymus.

Zr⁹³-Nb⁹³ (3 μ c/gm) and Ba¹⁴⁰-La¹⁴⁰ (5 μ c/gm) were effective in rat thymus within 2 weeks. Sr⁹⁰ and Y⁹⁰ located in the sternum and Y⁹¹, and Ce¹⁴⁴-Pr¹⁴⁴ in the lungs may cause damage to the adjacent thymus. In the case of inhalation the effect on the thymus was more pronounced than in the lungs proper.

5. DOSE-RESPONSE RELATIONSHIP FOR CHANGES IN BLOOD AND HEMATOPOIETIC AND LYMPHATIC TISSUES

The dose-response relationships for observed radiation-induced changes in blood and in hematopoietic and lymphatic tissues are here tabulated according to increasing doses of acute, fractionated, and chronic whole-body exposure and for radioactive material.

a. Single (Acute) Whole-Body Irradiation
Less than 25 r

Blood:

Lymphocytes: Reduction shown statistically in rats (110).

Leukocytes: Fall in number as soon as 1 hour (Stundenreaktion) in rabbits. Increase within 3 hours. No reaction below 3 r and within $\frac{1}{2}$ hour p.i. (postirradiation) (111). In lambs no significant decrease in lymphocytes after 15 r (112).

Spleen: At 3 hours p.i. nuclear structure of lymphoblasts looks mellow after 2 r in rats (113). After 6 hours there is still karyorrhexis and pyknosis of the lymphoid cells after local irradiation of the spleen of rats (114).

25 to 100 r

Blood:

Lymphocytes: Lymphocyte values fall below control in 24 hours and return to normal at 24 to 28 hours in various species. In rabbits, Cronkite (115) found a maximum depression of 25% in 48 hours, with a return to normal before 16 days.

Leukocytes: Within 2 to 4 hours leukocytosis, followed within 8 to 12 hours by leukopenia, consisting mainly in lymphopenia in mice. In rabbits, due as much to leukopenia as to lymphopenia (116). In lambs, significant decrease of leukocytes after 30 r (112).

Reticulocytes: Significant increase 24 hours to 7 days (117) in rabbits.

Bone marrow: Earliest changes after 8 hours in mice (118). On third day p.i. increase in promyelocytes and normoblasts. Shift to the right (35).

Spleen: Range between 25 and 50 r lower limit in rats, at which cell damage is directly notable after 24 hours. At 30 minutes p.i. fine-grained protoplasm of lymphoblasts with dusty appearance. No destruction (113). After 3 hours increased ability to hydrolyze adenosine triphosphatase and 5'-adenylic acid in rats and mice; maximum within 72 hours (98).

Lymphatic tissue: Lowest dose range giving histological changes in rabbits (84). At 1 hour p.i. in mice, reticular cells in lymph node detectable. At 4 hours p.i. necrosis in germinal follicles. At 8 to 12 hours recovery started.

100 to 200 r

Blood:

Lymphocytes: Maximum depression (50%) after 24 to 48 hours with return to normal before 36 days in several (110, 115, 118a).

Leukocytes: Leukocytosis in mice within few hours p.i., followed by leukopenia involving neutrocytes as well as lymphocytes. Recovery from second week to 4 to 6 weeks (118). Neutrophiles: No change until dosages greater than 100 r in rats (110).

Reticulocytes: After 24 hours increase within 4 to 5 days, then sudden fall to low levels. Normal after 2 weeks in rabbits (117).

Bone marrow: Mitosis stopped almost completely. Resumed by 3 hours p.i. At 14 hours normal picture (36). At 12 hours loss of marrow cells in mice. At 48 hours recovery starts. At 1 week normal appearance (118).

Megakaryocytes markedly reduced on the fifth day in rats (119).

Spleen: Slight changes. Karyorrhexis and single large phagocytes (120). Within a few hours destruction of small lymphocytes in mice. At 24 hours increase in reticular cells. Recovery starts at 48 hours. At 4 to 6 weeks essentially normal (118).

Lymphatic tissues: Marked changes only in some nodules in rabbits (84).

200 to 300 r

Blood:

Lymphocytes, reticulocytes, and heterophiles: Between 2 and 11 days p.i. (rabbits) abnormal forms of myeloid series seen (118a).

Leukocytes: Slight leukocytosis 2 to 4 hours p.i. (mice) followed by leukopenia, mainly lymphopenia, minimum the fourth day. Recovery after 3 to 4 weeks (118).

Reticulocytes: At 1 hours lower limit of normal in rabbits. At 2 days increase over normal, followed by a decrease with minimum at 12 days. Normal after 4 weeks (117). Marked reduction beginning at 78 hours and persisting for 120 hours in rats (110).

Bone marrow: At 12 hours apparent cell loss in mice, extensive at 24 hours. At 48 hours only a few cells, at 4 days active degeneration. At 1 week recovery approaching (118). Abnormal forms of myeloid series in several species, suggesting effects produced in precursors, which after a series of divisions no longer survive (118a). Reduction (80%) of megakaryocytes after 48 hours in rats (119).

Spleen: During 24 hours p.i. destruction of cells, mainly lymphoblasts of germinative centers in rats. At 48 hours lymphoblasts practically disappeared. After 1 week follicles consist of a large germinative center surrounded by a thin ring of mature lymphocytes (120).

300 to 400 r

Blood:

Lymphocytes: Maximum reduction (74%) in 24 hours, return to normal before 50 days in rabbits (115), later in other species (118a).

Leukocytes: Immediate depression and rapid recovery in monkeys (121). Maximum depression at third day in rats. At 10 days recovery begins, completed at 30 days (122).

Erythrocytes: At 168 to 448 hours substantial reduction in rats (110). In other species occasionally slight anemia (118a). Maximum anemia in rats after 14 to 16 days. Macrocytic anemia (122).

Reticulocytes: At 72 hours successive reduction with increase in dose up to 500 r in rats. Beyond this dose no further reduction (110). After fourth day decrease in rabbits, normal after 2 weeks (117).

Bone marrow: At third day increase in promyelocytes in rats. At doses <300 r, increase in normoblasts; at >300 r, reduction in red cell series but increase in erythrocytes (35). In mice, changes similar to those in the rabbit at 400 r. Recovery indicated at 5 days p.i. (36).

400 to 500 r

Blood:

Lymphocytes: Lymphopenia immediately in mice (118).

Leukocytes: Marked leukopenia in 4 to 5 days in guinea pigs (118). Initial rise in absolute heterophile values at 400 r or more in rabbits (118a). Minimum at 4 days in mice. Recovery at 3 to 4 weeks (118). Granulocytopenia severe for a short period only. Lymphopenia of longer duration in mice (123).

Erythrocytes: Little change in erythrocyte levels in guinea pigs (118).

Bone marrow: Erythropoiesis affected earlier than granulocytopoiesis in rabbits. Megamyelocytes appear (36). Tissue damage greater in guinea pigs than in mice (118). Within 24 hours almost complete disappearance of nucleated red cells in mice. Regeneration of erythroid series commences around the seventh day. Diminution of myeloid marrow during first week (123). At 4 hours cell loss. Necrosis at 1 week. At 2 weeks considerable recovery, but marrow abnormal in mice (118). Majority of nodules are only partially destroyed. Rapid degeneration. At 5 days normal appearance in rats and rabbits (84).

500 to 600 r

Blood:

Lymphocytes: At 15 minutes reduction in rats. Almost zero at 15 hours (110, 111). In rabbits maximum depression (90 %) in 48 hours; return to normal before 50 days (115). Increase in neutral red granules after 600 r in rabbits (115, 118a).

Leukocytes: Transient early leukocytosis followed by depression at 24 to 36 hours in rats (110). After a few hours, shift to the left, fully de-

veloped after 24 hours. After local irradiation of left tibia shift to the left on third day (124). At 72 hours p.i. neutrophiles almost totally absent. Regeneration at 12 days. Normal values at 25 days (110, 111). Heterophiles show two initial peaks at 12 and 24 hours followed by reduction after 72 to 96 hours, recovery within 23 days in rabbits (118a).

Erythrocytes: Moderate decrease, lowest on fourth day in rats (111). Progressive large fraction of rabbits showed anemia after 500 to 800 r. At 800 r normochromic anemia, maximum at 14 days, recovery by twenty-third day (118a). In rats exposed to 500 to 800 r significant drop at 168 hours followed by rapid decline (110, 111).

Reticulocytes: Regeneration at 168 to 280 hours depending on dosage (rats).

With higher doses animals die before regeneration occurs (110).

Platelets: Similar to granular series, reduction later, minimum on eighth day, recovery within 1 to 2 weeks in rabbits (118a). In rats reduction by fifth day. Increasing reduction with dosage up to 400 to 500 r. No further reduction at 600 to 1000 r (110). At 4 to 6 days before death significant decrease of plasma and blood volume in dogs (55).

Bone marrow: Maximum destruction at 2½ to 5 hours in rats. At 8 days severe hypoplasia but never complete aplasia. At 12 days regeneration, completed on fortieth day. Maximum reduction of megakaryocytes at 4 to 12 days, when regeneration started. Completed at approximately 41 days (110, 111). In mice degeneration after 6 hours. RNA content in cytoplasma of morphologically unaffected cells decreases. Later cell destruction with complete absence of RNA. Similar with DNA, but some DNA remains even during complete cell destruction. At 4 days complete aplasia; at 4 to 8 days regeneration. Almost normal on twelfth day (41).

Spleen: Corresponding to lymphocyte findings in the blood, maximum necrosis in follicles after 6 to 12 hours in rats (110, 111). "Acid-active" DNase markedly decreased. Maximum activity by 24 to 48 hours p.i. in rats (95).

Lymph Follicles: At 1 hour reduction, maximum reduction at 6 hours in rats. At 24 hours regeneration, completed at 20 days. At this time the lymphocytes in peripheral blood below normal (110).

600 to 700 r

Blood:

Lymphocytes: Maximum depression (90 %) at 48 hours, return to normal before 50 days in rabbits (115); in monkeys, in agreement with other species (125).

Leukocytes: At third day maximum depression in rats. Recovery at 10 days, completed at 30 days in rabbits (122). Maximum depression of heterophiles (75%) at 96 hours. Return to normal in 9 days (125).

Erythrocytes: Maximum anemia in rats after 14 to 16 days, back to normal first at 94 days (122). In monkeys depression at 10 to 20 days (125).

Reticulocytes: Decrease at fourth day in rabbits. At twelfth day regeneration followed by increase to normal during following week (117).

Platelets: Depression at 7 to 18 days in monkeys. The findings are essentially in agreement with those in rabbits, rats, and dogs (125).

Bone Marrow: Hematopoiesis almost completely eliminated by 2 days in rats. Erythroblasts markedly reduced at 3 hours and completely gone at 14 hours. Myelocytogenesis practically absent at 2 days. Aplastic condition for 7 to 9 days, but by 14 days hyperplastic condition. Regeneration observed at 9 days (36, 122). On third day great decrease in young forms of granulocyte series, but increase in segmented, relative increase in reticular cells, plasma cells, and monocytes in rats. Increase in erythrocytes (35). After 24 hours decrease of megakaryocyte content (119). In mice during the first 30 hours only minimum destruction. At 40 to 80 hours low plateau in cellularity. From 85 to 99 hours regeneration with polymorphonuclear leukocyte series predominant (126). In the bone marrow of swine slower reaction than in lymphoid tissues. At 50 minutes slight decrease in erythroblasts and normoblasts. At 9 hours immature erythroid elements decreasing. At 13 hours most destroyed, first signs of injury to myeloid series in myeloblasts, beginning necrosis. Granulocytes disappear gradually, rare at 90 hours. Megakaryocytes react in the same manner but more slowly (127).

Spleen: After 1 hour destruction of small lymphocytes and erythroid series in mice. Maximum destruction at 7 to 12 hours. At 30 hours lymphocyte values at low plateau. At 60 hours regeneration, not completed at 99 hours. Polymorphonuclear leukocyte series more resistant. Regeneration evident at 80 hours. Slight decrease in megakaryocyte numbers after 30 hours (126).

Lymphatic Tissue: Same as in spleen, but later and slower. After 50 hours occasional extensive infiltration with eosinophilic polymorphonuclear cells in mice (126). According to Stearner *et al.* (122), after $\frac{1}{2}$ -hour damage, at 2 days aplastic condition in rats persisting until about ninth day, when regeneration starts, completed at 35 days (122). Great majority of nodules completely destroyed, with a "nodule-free" period for about 3 weeks in rats and rabbits (84). The intensity of cytochemical reactions for pentosenucleoprotein, protein-bound SH groups, and alkaline phosphatase decreased within 2 hours p.i. in spleen and lym-

phatic nodes in rats (101). In swine after 50 minutes, injury to small lymphocytes. At 13 hours central parts of lymph nodes depleted of lymphocytes, which reappear at 36 to 45 hours up to 164 hours; slow repopulation (127).

700 to 800 r

Blood:

Lymphocytes: Maximum depression (90 %) at 48 to 72 hours in rabbits. Return to normal before fiftieth day (128, 129).

Granulocytes: Immediate rise from 3000 to 16,000 mm³ followed by a decrease reaching maximum after 72 hours in rats. After this, slow increase (128). In rabbits maximum depression (90 %) at 96 hours. Return to normal before 23 days (128).

Bone Marrow: Decrease in granulocytes corresponding to rise in peripheral blood followed by slow increase in rats (128). In swine hematopoietic cells begin to diminish at 8 hours. No regeneration before death. Erythroblasts more sensitive than megakaryocytes. Terminally marrow depletion of nearly all hematopoietic cells except primary reticular cells (129).

Lymphatic Tissue: Necrosis well established by 4 hours in swine, more extensive at 8 hours. At third day repopulation of lymphoid cells noted (129).

1000 to 6000 r

Blood:

Erythrocytes: Large drop in erythrocyte volume in rabbits after 1000 r as measured by P³²-tagged cells, beginning on third day and reaching a depth at about 17 days followed by a slow recovery (130).

Reticulocytes: At 2 days a decrease; at 6 days no reticulocytes in rabbits after 3000 r. After 6000 r a decrease after 24 hours (117).

Bone Marrow: Only scattered islands of hematopoietic tissue remain, and cell reproduction in these islands stagnant after 1000 r in swine (131). After 1200 to 3600 r in rats strong devastation; only a few granulocytes seen on third day (35).

Lymph nodes: Lymphoid cells particularly small, lymphocytes depleted after 1000 r in swine (131).

b. Fractionated Whole-Body Irradiation

0.25 r/day during 1 year (total 80 r) (rats)

Spleen: Increased number of lymphocytes, in some cases hyperplastic, irritated follicles, increase in reticuloendothelial and nondifferentiated epitheloid elements.

Thymus: Increase in size (111).

7.7 r/day for 8 months (rats)

Blood:

Lymphocytes: Reduced after 1 month, remained at a low level.

Heterophiles: Not decreased.

Lymphatic tissue: With the possible exception of a slight hyperplasia of lymphatic tissue, no evidence of histological damage (122).

5 to 25 r/day (mice)

Blood:

Leukocytes: Decrease mainly due to lymphocyte loss, varies progressively as irradiation proceeds and directly with size of daily dose (132).

Reticulocytes: No change in the curve observed before 120 days. Maximum at 154 days. After 155 days rapid decrease (133).

Bone Marrow: At 5 r hyperplastic, at 25 r extremely hypocellular. At 15 to 20 r similar to 25 r showing extensive aplasia. At 10 r both aplasia and hyperplasia (132).

Spleen: Similar but not so severe as in lymphatic nodes.

Lymph nodes: From abnormal hyperplasia in 5-r group to extensive aplasia in 25-r group. Many small lymphocytes in 5-r group but only swollen reticular cells in 25-r group. In 10-r, 15-r, and 20-r groups intermediate states (132).

1 r/day for 1 to 2 years (rats)

Not possible to demonstrate unequivocally hematopoietic changes (110).

10 r/day for 1 to 2 years (several species)

Detectable changes in some of the hematopoietic elements within a year. Mean survival time affected (110).

Daily irradiation (rats)

Granulocytes: After 5 r \times 4 daily increase, 5 r \times 16 daily fall, 5 r \times 33 daily significant fall, 20 r \times 11, given twice a week for 5 weeks = 220 r, initial decrease (111).

50 r every second day (mice)

Blood:

Reticulocytes: Decrease after first irradiation for 2 weeks, after this an increase.

$20\text{ r} \times 10$ daily (rats)

Spleen: By 24 hours after end of irradiation most of lymphoid tissue missing.

At 8 days regenerative processes started. After $4\frac{1}{2}$ weeks structure of follicles apparent (120).

$50\text{ r} \times 10$ daily or $100\text{ r} \times 5$ daily (rats)

Spleen: Similar to single 500-r dose (120).

$100\text{ r} \times 10$ daily (rats)

Spleen: At 4 weeks after beginning of irradiation follicles extremely reduced. Lymphoid tissue completely disappears (120).

$33.5\text{ r/day up to 3 months (maximum survival time)}$ (rats)

Blood:

Lymphocytes: Fall rapidly; after 1 week 35 % of controls.

Leukocytes: Reduction after 1 week, followed by continuous fall; at 66 days 28 % of controls.

Erythrocytes and hemoglobin: Only slightly decreased after 50 days, if at all. Rapidly developing terminal anemia (in a week; in guinea pigs > 1 month) (122).

$50\text{ r} \times 5$ twice weekly (mice)

Blood:

Leukocytes: After first three irradiations quick fall in lymphocytes, slight decrease in granulocytes. After four to five irradiations further slight decrease in lymphocytes; granulocytes fluctuate around a slightly lowered level (134).

$75\text{ r/week given during 8 hours 1 day a week; single and repeated}$ (rats)

Blood:

Leukocytes: At day 1 p.i. decrease; by day 5 recovery continuing to day 34. Decline mainly due to lymphocyte count (59).

Erythrocytes: At 14 days decrease.

$100\text{ r} \times 10$ and $200\text{ r} \times 10$ every second week (monkey)

Blood:

Leukocytes: Depression throughout the irradiation program after which recovery is very slow. Still below normal $1\frac{1}{2}$ years after first irradiation (121).

c. Chronic Whole-Body Irradiation

0.11 to 8.8 r during 8 or 24 hours per day 7 days a week for 3 years
(Guinea pigs, mice, rabbits)

Blood: At 0.11 r only in guinea pigs equivocal reduction in total leukocyte count within a year. At 1.1 r definite reduction chiefly in lymphocytes within first year. No comparable effects in mice and rabbits. At 2.2, 4.4, and 8.8 r significant effects in all three species. Reduction in lymphocytes. Acute onset of severe anemia a common cause of death in guinea pigs. A macrocytic anemia of obscure etiology (118a).

1.1 to 8.8 r/day continuously (mice)

Blood: At 4.4 r lymphocytes depressed below controls, not clear whether significant. At 8.8 r total leukocyte and lymphocyte counts significantly depressed. Red cell count and hemoglobin values reduced only after 8.8 r daily for about a year.

Bone Marrow: Replacement of cellular marrow, progressive increase in number of mast cells at 8.8 and 4.4 r. No changes at 1.1 r.

Spleen: No changes at 1.1 r, only at 4.4 and 8.8 r. Increase in number of mast cells in 4.4 r at 8 months; maximum (three times that in controls) at 16 months, in 8.8 r beginning at 4 months, *maximum* (four and one-half times controls) at 16 months.

Thymus: Increase in number of mast cells, decrease in lymphocytes. At 1.1 r/day after 10 months, at 4.4 r/day after 8 months, at 8.8 r/day after 4 to 6 months.

d. Whole-Body Irradiation, Radioactive Elements

12 μ c: No effect in mice.

23 μ c: Moderate to severe leukopenia.

48 μ c: Moderate to severe leukopenia, lymphopenia, death before 8 days (118a).



20 μ c/gm (mice): *Bone marrow:* Destruction and subsequent replacement by abnormal reticular cells containing greater amounts than normal of glycoprotein, glycogen, and alkaline phosphatase, and associated with a glycoprotein-containing matrix which calcifies (135, 136).



4.5 μ c/gm (rat): A single dose causes changes in the blood picture at intervals from 1 to 15 days after injection (137). Strong decrease in DNA

phosphorus concentration in spleen and thymus due to inhibition of DNA synthesis or of cell regeneration (138).

0.015 $\mu\text{c}/\text{gm}$: No effect in rabbits, rats, and mice (118a).

0.068 $\mu\text{c}/\text{gm}$: Moderate reduction in heterophile values (118a).

0.034 $\mu\text{c}/\text{gm}$: No anemia (118a).

2 $\mu\text{c}/\text{gm}$: Moderate anemia (118a).

0.10 $\mu\text{c}/\text{gm}$: At 4 days p.i. leukocytes reduced to approximately 60 % in dogs. All survived (118a).

Sr⁹⁰

0.5 $\mu\text{c}/\text{gm}$ (rabbits): *Blood*: After 48 hours no abnormalities; after 6 to 8 weeks anemia and leukopenia. Reticulocytes 2 to 6.5 %. Reduced number of platelets; after 1 year no significant change apart from slight fall in hemoglobin.

Bone marrow: After 48 hours no abnormalities; after 6 to 8 weeks replaced by a loose spindle cell connective tissue. Very few hematopoietic cells. After 1 year in tibia and skull aplastic marrow but in femur hyperplastic (139).

1 $\mu\text{c}/\text{gm}$ (rabbits after 1 year): *Blood*: Anemia and leukopenia; reticulocytes 1.7 to 4.8 %.

Bone marrow: Replaced by a loose connective tissue consisting of spindle-shaped and stellate cells with abundant mucoid intracellular material (139).

0.1–0.5 $\mu\text{c}/\text{gm}$ (rats): *Bone marrow (femur)*: After 1 week little effect but decrease of marrow cellularity noted. After 3 months still some fatty, acellular marrow in metaphysis but rather active hematopoiesis in the central portion of the shaft. Later on gradual recovery of hematopoiesis (140).

2.5–3.5 $\mu\text{c}/\text{gm}$ (rats): *Bone marrow (femur)*: Loss of hematopoietic activity, but in center of shaft some evidence of slight activity (140).

5.0 $\mu\text{c}/\text{gm}$ (rats): *Bone marrow (femur)*: Loss of hematopoietic activity, almost entirely composed of hemorrhagic fat tissue (140).

0.2 $\mu\text{c}/\text{kg}$ (monkeys): Changes in peripheral blood usually only short time before death. Changes in bone marrow earlier (141, 142).

Y⁹¹

Y⁹¹ (single dose per os in rats)

<10 $\mu\text{c}/\text{gm}$: No hematological effect.

10 $\mu\text{c}/\text{gm}$: Temporary lymphopenia.

20 $\mu\text{c}/\text{gm}$: Initial elevation in erythrocyte and hemoglobin followed by severe anemia reaching *maximum* at 20 days, 75 % reduction in lymphocyte within 3 days, normal by 35 days (118a).

Y⁹¹ (daily dose in rats)

0.3–2 $\mu\text{c}/\text{gm}$: No anemia, sustained heterophilia, moderate lymphopenia with maximum at 60 days. Recovery to normal by 90 days (118a).

I¹³¹

10–90 $\mu\text{c}/\text{gm}$ (rats): Atrophy of lymphopoietic tissues.

Ba¹⁴⁰-La¹⁴⁰ (intraperitoneal administration in mice)

0.2 $\mu\text{c}/\text{gm}$: Slight decrease in leukocytes, lymphocytes, and heterophiles during 29 days.

1.9 $\mu\text{c}/\text{gm}$: Significant and rapid reduction in all these elements for 60 days.

17 $\mu\text{c}/\text{gm}$: Rapid reduction and death within 15 days (118a).

Au¹⁹⁸

4 mc (rats): Destruction of cells in the bone marrow with edema, hemorrhages, and fatty degeneration (143).

Ra

0.01 $\mu\text{c}/\text{gm}$: No hematological changes in mice, rabbits, and rats.

0.03 $\mu\text{c}/\text{gm}$: Anemia and leukocyte reduction in rats and mice.

0.1 $\mu\text{c}/\text{gm}$: Leukocyte reduction in rabbits but no anemia.

0.94 $\mu\text{c}/\text{gm}$: Reticulocyte and platelet reduction in rats (118a).

Pu

0.0003 and 0.0006 $\mu\text{c}/\text{gm}$: No changes in 300 days in mice, rabbits, and rats (118a).

0.0025 $\mu\text{c}/\text{gm}$: Significant decrease of lymphocytes in blood in dogs (144).

0.003 $\mu\text{c}/\text{gm}$: Minimum to moderate depression of leukocytes and platelets within 100 days in mice, rabbits, and rats (118a).

0.0063 $\mu\text{c}/\text{gm}$: Moderate to severe leukopenia in all three species. Anemia and early death (135 days) in rats and mice. Platelet reduction. Macrocytosis in rats, less significant in rabbits and mice.

6. LEUKEMIA INDUCED BY IONIZING RADIATION

The leukemogenic effect of ionizing radiation has been known for a long time. All types of ionizing radiation are leukemogenic in mice. The induction rate varies with the total dose, the dose rate, and the interval at fractionation. The leukemogenic action is markedly influenced by partial-body shielding, genetic, and physiologic factors (145).

In mice thymic lymphomas are common, but myeloid leukemia has also been induced. The thymus is under control of adrenal hormones, and thy-

mectomy prevents the spontaneous development or inducibility of lymphomas originating in the thymus. Studies on the induction of lymphoid tumors of mice by ionizing irradiation have been reviewed by Kaplan (146). The minimal effective dose for induction of lymphoid tumors after a single dose of X-ray is about 300 r. The incidence of lymphoma increases with dose (147).

Irradiation in a thermal column indicates that in mice the threshold dose for thymic lymphoma induction is between 128 and 518 r (148).

In mice exposed to ionizing radiation from a nuclear detonation, the incidence of thymic lymphomas was greatly elevated in proportion to dose. In groups exposed to more than 700 r, the frequency of thymic lymphoma was greater in males, and the threshold dose was about 500 r. In females the threshold dose appeared lower; even the 192-r group showed a distinct increase (149).

The incidence of lymphoma for a given dose is not affected by daily fractionation, but intervals of 4 to 8 days give a higher incidence, shorter latent period, and significantly steeper probit regression line. If the interval is increased to 16 days, the response is decreased (147).

Leukemia developed earlier and in a higher percentage (60 to 70 %) of mice exposed to 8.8 r daily of γ -radiation than in nonirradiated controls (40 to 50 %). A daily dose of 4.4 r hastened the onset of leukemia but did not materially increase its total incidence (150).

Local irradiation is ineffective in the induction of mouse lymphoma, but fractionated alternate irradiation of the upper and lower halves of the body yielded lymphoid tumors at a rate almost identical with that of equivalent whole-body irradiation (151).

After a massive whole-body exposure to ionizing radiation from a nuclear detonation the induction of lymphomas within 16 months was high, dropping rapidly after doses above LD₅₀, with smaller doses being negligible below 400 rep. The earliest cases appeared in the fourth month postirradiation, and the peak incidence occurred at 7 to 12 months (145, 149).

The lymphoma incidence in mice is strikingly reduced by shielding during irradiation of thigh, lower extremity, or lower abdomen. There is a relationship between the volume of tissue shielded and the degree of protection (152).

In spite of high accumulated doses no increase in leukemia incidence has been observed in guinea pigs and rabbits (150).

With regard to internal contamination, Watanabe (153) showed that 0.3 to 0.5 μ c of P³² per gram of body weight given twice a week ten times was the optimum level to induce leukemia. He also induced leukemia at a level of 0.1 μ c/gm twice a week seven to sixteen times, but at 1 μ c/gm only osteosarcomas but no leukemia appeared. A level of 0.3 μ c of Ce¹⁴⁴

per gram twice a week ten times also induced leukemia. With Sr⁸⁹ administered in the same way, leukemia was observed at a level of 0.1 $\mu\text{c}/\text{gm}$, but at a level of 0.3 $\mu\text{c}/\text{gm}$ only osteosarcomas were observed (153).

B. LYMPH

The effects of ionizing radiation on the lymph vessels (see below) and lymph are relatively little known. The lymph flow in the thoracic duct of cats is unchanged during the first hours after a lethal dose of whole-body X-irradiation (154). Some time after the irradiation an increased lymph flow from the thoracic duct of dogs has been reported (155). A decrease of lymphocytes and an increase of the erythrocyte counts in the lymph after large doses (650 to 750 r) of X-irradiation of rats and dogs have been reported (156).

The glucose concentration of the lymph is not altered during the early postirradiation period after X-ray exposure (157). Nonprotein nitrogen and the concentration of uric acid is increased during the period of maximum lymphocyte destruction.

C. RETICULOENDOTHELIAL SYSTEM

The effect of ionizing radiation on the reticuloendothelial system will be dealt with in the discussions of various other organs. In this paragraph only some complementary data will be given.

Morphologically, the reticuloendothelial system is generally considered to be radioresistant. Concerning the functional capacity of this system after irradiation, the results are by far more diverging. Some authors (158, 159) report a decreased function, whereas others (123, 160, 161) have found normal activity or hyperfunction (hyperphagocytosis). Mostly, a normal activity is said to prevail after irradiation (123, 162, 163). Such changes after whole-body irradiation as decreased phagocytotic activity, bacteremia, and reduced spleen size may, at least partly, be interpreted as a decreased function of the reticuloendothelial system.

III. Gonads

The gonads and their products, especially the spermatogonia and the egg cells, are sensitive to ionizing irradiation. Fractionated irradiation is stated to be more effective in causing sterility than is a single larger dose (164-167).

A. MALE GONADS

The effects of irradiation on the male gonads were first reported by Albers-Schönberg in 1903 (168). He sterilized the testes of rabbits and guinea pigs by daily exposures of 15 to 30 minutes, sterility being obtained

after 135 to 377 minutes. In the same year Frieben (169) noted a marked diminution in size of testes after X-irradiation. Also a number of other authors (170-174) have found that all types of external and internal irradiation that cause cellular depletion are effective in reducing the size of the testes and in causing a gradual cessation of the spermatogenesis. A review of the effects of external ionizing radiations on gametogenesis and fertility in mammals has been published by Casarett (175).

Bergonié and Tribondeau (176) regarded the primary spermatocyte to be the most susceptible to X-irradiation. According to Wigoder (164) and Metz (177) the spermatogonia should be the most resistant. Later experiments have changed this view, and now it is generally agreed that the spermatogonia are the first to be affected (174, 178, and many others). All spermatogenic cells may disappear without visible necrosis of testicular tissue. The mature elements of the seminiferous tubules continue their development after irradiation, but there are no longer any spermatogonia formed (179). The mature sperm is fairly radioresistant.

Heller (174) has made an extensive study of the effects of ionizing radiation. A summary account of her findings is given here. For details the reader is referred to her original publication.

Rabbits exposed to 50 r of X-irradiation showed no changes in the germinal epithelium. After 100 r there were dead spermatogonia 8 hours after the exposure. At 14 hours after the irradiation the degeneration had decreased, and at 24 hours the picture was the same as in nonirradiated animals. After 400 r some spermatogonia were dead at 8 hours postirradiation and still more at 48 hours.

After 600 and 800 r spermatogenesis was completely absent in many of the testes at 9 and 11 days. On the first day dead spermatogonia and a few dead spermatocytes were observed close to the basement membrane. After 9 days most of the tubuli showed only Sertoli cells and a varying number of primary spermatocytes. In some animals the intertubular connective tissue was edematous. The changes in the epididymis were insignificant, consisting only in slight inflammation of the connective tissue and occasional dead epithelial cells.

The damage to the mouse testis after exposure to 350 r is similar to that observed in rabbits after 600 r with the exception that regeneration begins earlier in the mouse testis. The quantity of outstretched basophilic spermatogonia along the basement membrane is striking. This finding suggests that regeneration of germinal cells originates from spindle cells on the basement membrane. The epididymis is probably undamaged by 350 r.

After 600 r to rats, spermatogenesis is almost absent at 21 and 31 days after exposure. Signs of beginning recovery are usually apparent by a month after irradiation. The Sertoli cells and the epithelium of the epidid-

ymis are not significantly altered. In guinea pigs 175 r causes early death of spermatogonia and a few spermatocytes followed by a virtual elimination of all spermatogenesis. The Sertoli cells are apparently unaffected.

In mice exposed to 600 r of X-rays, the type A spermatogonia degenerate to a degree sufficient to explain depopulation of the germinal epithelium (180). Intermediate and type B spermatogonia are completely destroyed between 3 and 24 hours after irradiation, and after 48 hours they have completely disappeared. The damaged spermatogonia degenerate either in interphase or on entrance into prophase of their first postirradiation division. Late type A, intermediate, and early type B spermatogonia were found to be uniformly sensitive after irradiation with 20 to 24 r of γ -irradiation from a Co⁶⁰ source (181). After 30 to 100 r the type A cells showed a significantly higher survival than the intermediate and type B cells. According to Spalding *et al.* (182), who exposed rats to external γ -irradiation in doses of 2500 to 100,000 r (dose rate > 1000 r/min), the type A spermatogonia appears to be relatively radioresistant. After 2000 r no recovery is seen in mice. The interstitial cells remain intact even after doses as high as 5000 r (183).

If repeated small doses are given with short time intervals, a cumulative effect is noticed which is more striking than for any other organ (184). In mice, for example, daily doses of 80 r of X-rays cause progressive damage. After only four doses dead spermatogonia are found, and after nine doses the spermatogenesis has almost completely ceased. There is a strong decrease in spermatocytes. After fourteen to thirty-five daily doses spermatogenesis gradually ceases, and only Sertoli cells are left. Fractionated as well as acute (185) irradiation reduces the weight of the testis. In CAF₁ mice two to five irradiations given in 1 to 4 days elapse time and with a total time dose of 80 to 240 r caused a reduction of testicular weight, the maximum reduction occurring about 4 weeks after the exposure (186).

Even doses as small as 0.25 r administered daily for 80 to 100 days have been shown (187) to cause biological effects on the gonad epithelium. If these "pretreated" animals were exposed to massive doses (600 to 3000 r), the damage to the gonad epithelium was less marked than in the control animals exposed to the same massive dose.

A dose rate of 0.3 r/week or 0.6 r/week did not produce any other definite damage than certain sperm abnormalities in dogs (188). After 3.0 r/week, the sperm counts declined progressively from the twentieth to the thirtieth week of exposure (total dose 60 to 90 r) with concomitant increases in abnormal, immature, and dead sperms. Chronic irradiation is reported (189) to be about twice as efficient, per roentgen, as acute irradiation in reducing the prespermatocyte production in the prerecovery (depletion) period. On the other hand, acute irradiation is about twice as effective as

chronic irradiation in terms of the degree to which the recovery of prespermatocytes is incomplete in the postrecovery period.

After 8.8 r daily of γ -irradiation from radium, there is an early destruction of some spermatogonia in guinea pigs. A total dose of 326 r is sufficient to abolish the spermatogenesis completely (174).

Exposure of rabbits or mice to fast neutrons in comparable doses and times results in an even more severe testicular damage than after X-irradiation. In addition, there are also inflammatory changes to be seen in the epididymis (174, 190).

External β -irradiation (2500 rep) from a P³² source is followed by extensive testicular damage near the source. This type of irradiation caused development of giant cells and bizarre-shaped cells in addition to the dead cells in the injured tubules (174).

In addition to temporary or permanent sterility, there may be a diminished number of sperms and an impaired viability, and chromosome translocations in the sperm cells (191, 192). The interruption of spermatogenesis has been ascribed by Bryan and Gowen (193) to be due to inhibition of mitosis of spermatogonia probably as a result of curtailment of deoxyribonucleic acid synthesis. There may also be certain endocrine response to irradiation. If the irradiation causes sterility, "castration cells" appear in the pituitary gland (194).

Internal Irradiation. Some radioisotopes, when introduced into the body, may cause severe damage to the germinal cells. This applies to, e.g., Ra, Pu, Zr⁹³-Nb⁹³, Sr⁸⁹, P³², Ba¹⁴⁰-La¹⁴⁰, and Y⁹¹. Ra, Pu, and Zr⁹³-Nb⁹³ are stated (174) to be more destructive than the others. Qualitatively, the changes are mainly the same as after external irradiation.

Carcinogenesis. It is not certain whether tolerable doses of whole-body irradiation or massive local doses will induce specific tumors in the testis.

The male accessory organs and passages all seem to be relatively radio-resistant, but the statements in the literature on the effects of ionizing irradiation on these organs are scanty (184).

B. FEMALE GONADS

Since the investigations of Halberstädter (195) and of Bergonié *et al.* 1905 (196), it has been known that the ovary may be severely injured by ionizing radiation, and during the last forty-five years numerous studies have confirmed these early findings. For reviews of the literature the reader is referred to Lacassagne and Gricouloff (179), Warren (197, 198), Bloom (199), and Bloom and Bloom (184).

After irradiation with sufficiently large doses the ovary may atrophy, and a temporary or permanent sterility may result. As a sequela of the

changes in the ovary the secondary sex characters are changed (200), owing to the fact that the tubes, uterus, vagina, and mammary glands will also show signs of atrophy.

The egg cell is the most sensitive component of the ovary. The corpora lutea and the intestinal cells are stated to be much more resistant. Furthermore, the radiosensitivity of the ovum and of the follicular cells is dependent on the functional state (201), and it is also subject to species dependence.

Irradiation of beagle dogs with 100 or 300 r does not impair the reproductive ability of survivors with respect to litter size and puppy mortality (202).

If mice are irradiated with 350 r total-body exposure (199), the ova and follicles are degenerating 3 days after the exposure, and after 2 weeks no ova are left in the shrunken ovaries. A dose of 700 r of external γ -irradiation causes about the same damage to the mouse ovary as 350 r of X-irradiation. According to Schugt (203) and Murray (204) the primitive ovum of the mouse is very radiosensitive. After only 54 r many follicles are destroyed, and 140 to 150 r causes histological sterilization.

After 600 r in rats, pyknotic and some vacuolated ova are found by 1 day after the exposure. After 3 weeks there is an obvious reduction in size of the ovary which consists mostly of interstitial cells, a few degenerating follicles, and some corpora lutea. Some months after the exposure, the interstitial cells have disappeared; fibrous degeneration is seen, and also some cysts. After 400 r almost all primitive ova and the follicles have degenerated 16 hours after the exposure. The damage is more severe after 600 r, but even this dose seems not to sterilize the ovary completely. Lacassagne and Gricouloff (179) state that 600 r to rats causes only minimal changes in the rat ovary, but that 1600 to 1800 r destroys most ova. For complete sterilization about 3000 r is required.

In rabbits 100 r of X-rays cause no morphological changes in the ovary (199). After 400 r there is clumping of the chromatin of the primitive ova at 24 hours after the exposure. After 600 to 800 r disintegration of a large number of primitive ova is seen. After 1200 r there is pyknosis, chromatolysis, and fragmentation of the primitive ovum cell. The follicular cells are damaged, and the whole follicle may disappear in 3 or 4 days. After some months there may be some signs of regeneration which may follow an exponential law (205). Hemorrhages sometimes appear in the follicles. For complete destruction of all ova, 2000 to 2500 r are probably necessary. The interstitial cells show little effect, but new cells do not develop. Interstitial cells may also form chords and "glands" (184). A dose that is enough to destroy all developing follicles will inhibit the development of corpora lutea.

According to Bloom (199), 350 r of X-rays causes more damage to the ovary of the mouse than 400 r to that of the rat, and 400 r is more dangerous to the rat ovary than 600 r to that of the rabbit.

A dose of 700 r of pile γ -irradiation causes extensive ovarian damage to mice and probably also sterilization (199). Slow and fast neutrons have about the same effects as X-rays. The LD₅₀₍₃₀₎ for fast neutrons is stated (199) to be for mice about 90 n and for rabbits about 140 n. The effect of 117 n of fast neutrons on the rabbit ovary is about the same as of 400 r of X-irradiation (199).

Bloom (199) exposed mice ovaries to external β -irradiation from a P³² source and found that this kind of irradiation is very destructive. Two months after the administration of 1000 rep (the LD₅₀₍₃₀₎ is about 4800 rep), the ovaries were shrunken and had no intact ova. Many follicles contained no ovum. After exposure to 2500 rep the signs of atrophy and degeneration were even more pronounced. After 4 months no primitive ova or follicles were left, and after some time fibrous degeneration of the ovary was apparent.

Using fractionated irradiation with γ -rays for a period of 8 hours daily Eschenbrenner (206) and Spargo *et al.* (106) found that 1.1 r/day for 16 months did not completely destroy the ovary, as there were still some growing follicles left. After 4.4 r daily, sterilization was produced in 8 months, and with 8.8 r daily in 4 months. Continuous γ -radiation (from Cs¹³⁷) even at a dose rate of 100 r/week has considerably less effect on female reproductivity than have fractionated X-ray doses of only 50 r/week (207).

A reduction of the frequency of estrus has been described (208) in mice after 200 r and temporary abolition after 400 r, and the effect on the estrus cycle has been stated to serve as an adequate index for determination of degree of radiation injury (209).

Internal Irradiation. Bloom (199) has studied the effect of irradiation after administration of P³², Sr⁸⁹, Y⁹¹, Ba¹⁴⁰-La¹⁴⁰, Po, Ra, and Pu. Some of these isotopes exert no visible or only a slight effect on the ovary, even in relatively large doses. This applies especially to Sr⁸⁹ and Y⁹¹ injected into rats or mice. P³² (2.5 μ c/gm) and Ba¹⁴⁰-La¹⁴⁰ (14 μ c/gm) affect the organ only slightly.

Radium and plutonium, on the other hand, have a strong effect on the ovary and cause severe degenerative changes in the ova and follicles, Pu being more effective than Ra in destroying the ovary. Thus, plutonium seemed to be about ten times as effective as radium after intraperitoneal injection (199).

Carcinogenesis. In spite of the high sensitivity of the ovary to ionizing radiation it is still not known whether radiation will produce ovarian

tumors in any other species than mice. Rabbits are refractory to ovarian tumor development (150).

The available evidence indicates that the induction of ovarian tumors depends on the action of gonadotropic hormones to which granulosa and lutein cells of the irradiated ovary of the mouse retain sensitivity (150).

In the first study (210) describing the induction of ovarian tumors by X-ray, mice were exposed to single or repeated large doses of X-radiation. Irradiation caused a fifteenfold increase in incidence of ovarian tumors over that of controls.

After irradiation by ionizing radiation from a nuclear detonation the incidence of ovarian neoplasms was very high at all dose levels, but very low among the controls. Another study has indicated that, if a threshold dose of about 30 r is exceeded, ovarian tumors will develop in most female mice. At high doses the incidence of ovarian tumors is lower. This fact is related to reduced longevity, since the latency period is relatively long—12 to 15 months (149).

After γ -irradiation of 0.4 to 8.8 r daily, 60 to 100% of mice, observed until natural death, developed ovarian tumors, whereas the incidence in the controls was 12% (150).

The induced ovarian tumors include luteomas, granulosa cell tumors, tubular adenomas of germinal epithelium, cystadenomas, hemangiomas, and, most frequently, mixtures of these types (149).

It is suggested that both a direct and an indirect mechanism are involved in the development of tumors in irradiated mice and that intact ovarian endocrine function inhibits the development of tumors.

Kaplan (211) implanted irradiated ovaries intramuscularly into irradiated and nonirradiated mice. This gave rise to many of the above-mentioned ovarian tumors. No such tumors occurred, however, when irradiated ovaries were implanted into nonirradiated, nonovariectomized mice (211).

Another study has also shown the endocrine influence. Gardner (212) gave mice that had been irradiated with 280 to 380 r prolonged injections of estradiol benzoate. They did not develop ovarian tumors, as did mice given testosterone propionate.

C. UTERUS

The only uterine damage observed after irradiation is an increase in the incidence of uterine carcinomas in rabbits. In eleven out of twelve rabbits exposed daily to doses of 1.1 r or more of γ -radiation and in two of six nonirradiated controls uterine carcinoma developed. The neoplasms metastasized more widely and appeared earlier (33 to 50 months) in the ir-

radiated animals than in the controls (57 months). The sensitivity of the rabbits' uterus to ionizing irradiation is unique, and it is possible that this tumor results from a hormonal imbalance initiated by the irradiation (150).

IV. Endocrine Organs

The endocrine glands are generally considered to be rather radioresistant, although the statements in the literature differ. The difficulty of separating effects of radiation direct on the endocrine organs from systemic effects in whole-body radiation may at least partly account for these discrepancies. The endocrine glands are the organs that reflect most quick, possibly with the exception of the lymphatic system, changes in other parts of the body (213). The endocrine imbalances that occur after whole-body irradiation, especially as far as the thyroid, adrenals, and gonads are concerned, may be assumed (214) to be referable to the reaction of the pituitary. The close interplay between the thyrotropic, adrenotropic, and gonadotropic factors of the pituitary supports this assumption.

A. THYROID GLAND

The normal thyroid gland is fairly radioresistant (213, 215), and it is generally agreed that several thousand roentgens is required to induce morphological changes in the thyroid gland of the guinea pig, rat, rabbit, or dog (216, 217). The thyroid plays an insignificant role in the response to whole-body irradiation in mammals. The reaction is not significantly altered by thyroidectomy (218-220).

Local exposure of the thyroid area of young or mature rats to 5000 r of X-irradiation does not cause any morphological changes or modify the basal oxygen consumption or body weight (217). The hyperplastic thyroid gland, on the other hand, is rather sensitive to ionizing irradiation, and a diminution of the gland has been reported, e.g., in irradiated opossums (215).

After larger doses of local irradiation the gland may be hyperemic and show a slight secretory hyperactivity. Cytoplasmic damage and damage to cell nuclei are only occasional. Hemorrhages, manifested by the presence of red cells within the colloid, may occur. In some species, e.g., white rats, more severe morphological changes have been described (221). After 600 r of X-irradiation there were nuclear pyknosis and epithelial desquamation, and hyaline appearance due to the opening of some vesicles, pseudo-inflammatory changes, and congestion with the presence of lipophage cells. Mitochondrial swelling and vacuolization may occur, and droplets and granules appear, suggesting an increase in lipid material (222).

Activity changes after irradiation have been described by a number of authors. After sublethal X-ray doses (500 r), the thyroid gland of rats shows

hyperactivity; after lethal doses (800 r), hypoactivity (223). An initial increase in activity during the first postirradiation day, succeeded by a progressive decline during the following days (224) or normal values (225), has also been observed.

Irradiation of the whole body or abdomen of rats causes an increased uptake of I^{131} (226), indicating an increased activity. It is not seen in the newborn animal. Closon and Betz (227), on the other hand, found that as early as 48 hours after a total-body irradiation of rats with 800 r the uptake of iodine was markedly decreased. The total amount of I^{131} present in the gland 5 days after irradiation was only 17% of the injected iodine as compared with 40% in nonirradiated animals. The rate of uptake was also lower than normal. Even after very high local doses (up to 15,000 r) there is an initial hyperactivity (228, 229).

The activity changes described are not constant, and this, as well as the fact that there is no significant increase in the basal oxygen consumption of irradiated animals, has led a number of investigators (230-232) to the conclusion that there is no significant alteration of thyroid activity after external irradiation.

Internal Irradiation. The most important radioactive isotope causing internal irradiation of the thyroid gland is I^{131} . This isotope, which constitutes about 50% of the fission products from a reactor, will concentrate in the thyroid. Also the rhenium and astatine isotopes will, when administered to the body, accumulate in the thyroid gland. Radium implants have been found to cause hemorrhage and necrosis of the gland (233).

Relatively large doses of radioiodine are required to disturb thyroid function (234-238). Partial destruction of the organ with colloid degeneration occurs in rats 6 days after injection of about 70 μ c of I^{131} . The accumulated dose to the gland from this dosage is calculated to be about 20,000 rep (234). Thyroid function was not disturbed after injection of 30 μ c of I^{131} in rats (236). Administration of 10 μ c of I^{131} per gram of body weight to rats induces slow decrease to a final constant subnormal value (239). No gross differences have been found (240) in the sensitivity of the thyroid of weanling and adult rats after administration of 0.6 to 5.7 μ c of I^{131} .

Chronic administration of 240 to 1800 μ c of I^{131} per day to sheep causes chronic inflammation, necrosis, and fibrosis in the gland with eventual replacement of the glandular tissues by scar tissue (241).

In contrast to the changes brought about by iodine, astatine has a more pronounced effect (242). The metabolic rate of rats decreased abruptly to 74% of the control value between 6 and 8 days after the administration of 0.8 μ c of At^{211} per gram of body weight (239). The difference in action between I^{131} and At^{211} has been suggested to be due to the different nuclear properties of the two isotopes.

Carcinogenesis. Administration of 300 to 400 μc of I^{131} to mice may cause a complete destruction of the thyroid. Somewhat lower doses (200 to 300 μc) destroy the gland, leaving a few atypical acini. Some of these show the morphological appearance of a precancerous lesion.

After a single injection of 400 μc of I^{131} two rats out of ten sacrificed after 18 months had anaplastic, nonencapsulated neoplasms, one of which was invasive (243).

In six of seven sheep fed with 5 μc of I^{131} per day for their lifetime, tumors were observed (244).

After a single dose of 400 μc of I^{131} in a series of rats maintained on low-iodine diet, Frantz *et al.* (245) found only one thyroid neoplasm, a benign follicular adenoma. After X-irradiation of similar rats, 900 r in 300-r doses delivered in 5 days, they observed a large number of benign adenomas and tumors thought to be carcinomas, designated as the "solid" type. Papillary and follicular carcinomas were found in the X-irradiated rats but not in the controls.

The dual role of radiation and goitrogens in the development of experimental thyroid cancer in the rat has been investigated by Doniach (246). After a dose of 30 μc of I^{131} adenomas developed in ten out of sixteen rats. With larger doses of I^{131} the tumor incidence declined sharply. When the 30- μc dose of I^{131} was followed by methylthiouracil therapy, five thyroid cancers developed in twenty rats so treated.

When 1100 rads of X-ray was administered to rat thyroid followed by methylthiouracil therapy, thyroid cancer developed in seven out of twenty-one rats but only in one of thirteen animals given only X-rays.

B. PARATHYROID GLANDS

The parathyroid gland is considered to be the most resistant of all endocrine organs, but the reports in the literature on the effects of ionizing radiation on this gland are exceedingly few.

After large doses, hyperemia and an increase in the connective tissue of the gland may be seen (213, 215). In the parathyroids of hamsters irradiated with 1500 r (lethal dose) there was no evidence of pyknosis, karyorrhexis, nuclear swelling, vacuolization of cytoplasm, or cell destruction of any kind (213). No tumors have ever been described in this organ after whole-body or local irradiation.

C. PITUITARY GLAND AND EPIPHYSIS

1. PITUITARY GLAND

It is sometimes stated that the normal hypophysis is rather radioresistant (216). The diseased gland may be more sensitive.

The most radiosensitive part of the organ is the anterior lobe. This has been evidenced in the ground squirrel (247, 248) and in the hamster (213). An air dose of 1000 r or more to the pituitary region reduces the growth of young rats (248). Degenerative changes in other parts of the gland have been described only after very heavy irradiation. In rats cathode-ray and X-irradiations distinctly changed the hormonal pattern of the pituitary, and 8000 r was found to be lethal (248a).

Irradiation of the pituitary of young animals seems to depress the growth and development of the animals (184). A slight increase in the weight of the gland 3 months after irradiation with low doses has been observed in infantile animals, however, not accompanied by any signs of an increased function (249).

The morphological changes in the pituitary gland are rather indefinite. Usually there is swelling and increase or decrease of acidophile cells (250). With increasing X-ray dose, Denniston (247, 248) found the acidophiles to decrease in number. After 2000 to 2500 r to rats a reduction of the acidophiles of 50 to 75% is found and also numerous pyknotic nuclei in the chromophile cells (251). The protoplasm becomes cloudy and shrinks. The nucleoli are enlarged. The blood sinuses are sclerotic and become progressively engorged with erythrocytes.

In many investigations no morphological changes (179, 252, and others) were found. Single exposures of adult female rats to 200 to 500 r over the pituitary region give no degenerative changes in the gland (253). The low sensitivity to irradiation of the pars intermedia may be due to its low blood supply (213).

Tobias *et al.* (254) used deuterons for selective irradiation of the pituitary gland in rats. The doses were 3150 to 25,000 rep. The degree of destruction of the organ was found to be a function of time as well as of dose. High doses (18,900 rep or more) were necessary to cause rapid destruction of the organ. With lower doses (6300 rep) the destruction was not complete, even many months after the irradiation. The different cells of the anterior pituitary show about equal sensitivity to this kind of irradiation. Body growth and thyroid growth were not impaired, but the testes showed degenerative changes late after irradiation. There was no evidence of stimulation of any functions of the anterior pituitary, nor any recovery after these doses of deuteron irradiation.

Whole-head irradiation with large doses, but which do not cause any morphological changes of the anterior lobe, may induce an increase of thyrotropic and gonadotropic hormones (255). It is, however, not known whether the hypophyseal discharge occurring several hours after exposure is a direct consequence of irradiation or of neural or humoral stimuli such as epinephrine or tissue breakdown products (77). The early transient physi-

ological changes after hypophyseal irradiation may also be secondary to an increased vascularity or altered permeability (256).

After hypophysectomy, adrenal changes are not found in irradiated animals. Pituitary ablation may increase the sensitivity to whole-body irradiation (257).

Carcinogenesis. In mice exposed to ionizing radiation from a nuclear explosion an unanticipated and perhaps the most significant finding was the induction of pituitary tumors. These tumors were more common in females. Of all radiation-induced neoplasms pituitary tumors have the longest latency period. The incidence rate increases with length of survival. Thus, absence in the highest dose groups is explained by reduced longevity. If correction is made for survival, a correlation is found between dose and pituitary tumor induction. All pituitary tumors studied were of chromophobe type. Atrophy of adrenals, being a late effect of irradiation, was probably the inciting cause of the pituitary tumors (149).

In mice, destruction of the thyroid by I^{131} (200 to 300 μ c) is regularly followed by the development of pituitary tumors (258). Radiation thyroidectomy, however, also occurs with smaller amounts of I^{131} ($\sim 30 \mu$ c) in mice on a low-iodine diet, but it is not followed by pituitary tumor development. This raises the question whether pituitary neoplasia is attributable, in part at least, to a primary effect of ionizing radiation on the pituitary. Thyroid implants or adequate thyroxine treatment prevents induction of tumors but not implants of pituitary, ovarian, or adrenal tissues (259, 260).

Pituitary tumors will also develop after 30 μ c of I^{131} in mice on a low-iodine diet if this dose is followed by whole body irradiation (545 r). Therefore it is postulated that ionizing radiation induces these growths in pituitaries preconditioned by radiothyroidectomy (261). It is also conceivable, however, that the pituitary changes are secondary to irradiation of the ovary or adrenals. Depression of ovarian function is likewise followed by stimulation of the basophile cells of the pituitary (262).

In rats and dogs radiothyroidectomy is not followed by development of pituitary tumors (263).

The effects of irradiation on the epiphyseal gland is essentially unknown.

D. ADRENALS

The highly varying and contradictory effects of irradiation on the adrenals may, at least in part, be due to the extreme variations in the structure of this gland from species to species, in animals of the same species, and even in the same animal under different conditions. Owing to the fact that the adrenals are very sensitive to and will reflect body stresses of various kinds, it is difficult to distinguish between such effects and changes attributable exclusively to irradiation. The adrenals themselves appear to be

rather radioresistant. In general, the medulla seems to be more resistant than the cortex.

Degenerative changes in the adrenals have been observed after heavy local irradiation (more than 5000 r), but after 1000 r there was only minimal morphological alteration in the cortex and medulla (264, 265). Fisher *et al.* (266) were able to produce signs of adrenal insufficiency only with large doses and after a latent period of 3 months.

No detectable morphological alteration was produced in the adrenals of rabbits after an acute irradiation with 1500 r, but after higher doses degenerative changes developed (265). Rabbits that had been exposed to 2200 to 2500 r of X-rays locally showed marked degeneration in the cortex, especially in the zona fasciculata and zona reticularis. No effect was produced in the medulla. An initial transitory hyperemia was followed after 6 days by degeneration. Often there was a slight lymphatic infiltration. The changes were most pronounced at about 3 months after the exposure and were manifest for a long time. The glomerular zone, which is assumed to be the main source of regeneration of the cortex, was not damaged. Birkner *et al.* (267) found in white rats exposed to X-ray doses of 250 to 3000 r a continuous increase in the size but a decrease of the number of nuclei in the cortex. These changes were observed 4 hours after irradiation.

A progressive increase in adrenal activity as measured by an increase in the weight of the organ has been reported (268). Structural and biochemical changes in the adrenal cortex cannot always be interpreted in terms of adrenal function, however. Nor has the role of the reactions of the adrenals in radiation sickness been clearly established. Adrenal shielding affords, however, some protection, and adrenalectomy, e.g., in mice, seems to decrease the tolerance to irradiation. Martin *et al.* (269), on the other hand, found no functional changes in dogs in which one adrenal had been removed and the other was irradiated with a dose large enough to cause fibrotic changes and to cause cachexia and death if given to an isolated loop of small intestine.

After irradiation, as after various kinds of other stresses, there is an early increase in potassium tolerance, suggesting a hyperactive phase. Such a transitory increase in potassium and also in histamine tolerance has been observed in mice subjected to sublethal (425 r) as well as to lethal (700 to 800 r) whole-body irradiation (270).

Total-body irradiation is followed by a marked depletion in adrenal ascorbic acid content (271-274, and many others). That is, however, not a specific change after irradiation but occurs also after other kinds of stress; the degree of adrenal ascorbic acid depletion is considered to be an index of the severity of the stress (2), and it may also indicate the amount of ACTH released (275). The pre-exposure value of ascorbic acid is re-established

after a few days. Wexler *et al.* (272) found that half-body irradiation was much more active in depletion and repletion of adrenal ascorbic acid in rats than was total-body irradiation.

Also the lipids of the gland are depleted after irradiation (272, 276). After two days the glands are replete with lipid, filling the glands from the zona glomerulosa to the juxta-medullary border of the zona reticularis. This lipid has a completely altered physical appearance compared with that before the exposure. This filling of the cortex with an "exhaustion" type of lipid has been interpreted (257) as an attempt toward a possible adaptation.

X-irradiation in rats causes an initial reduction in adrenal cholesterol followed several days later by a normal or elevated cholesterol concentration. This rise several days later after a median lethal dose may be interpreted as an overstimulation in excess of cortical hormone demand (268). It is often associated with hypertrophy of the gland and followed by a marked terminal depression.

There is some evidence of an increased excretion of 17-ketosteroids in the urine some days after irradiation (268, 276-279). The intense depletion of lipids and the dilatation of capillaries in the adrenal cortex seen early after irradiation has been interpreted as an indication of an intense discharge of corticoids, and radiation seems to give rise to a state of hypercorticism for a duration which is related to the radiation dose received. Deoxycorticosterone, 11-dehydrocorticosterone, whole adrenal extract, cortisone, and adrenocorticotrophic hormone have been used experimentally to prevent radiation sickness but without success (280-284). This, however, is also the case in other kinds of stress.

Some protection has been obtained in rats by shielding the adrenals. This may point to the possibility that primary injury of the gland decreases its capacity to respond to the stress of irradiation (285, 286). The susceptibility of adrenalectomized animals, on the other hand, to the lethal action of irradiation is especially striking after lower doses. The toxic or otherwise injurious reactions after irradiation of larger parts of the body seem to be potentiated in the absence of the adrenals or pituitary (257, 286-289).

Internal Irradiation. Some radioisotopes, e.g., Ra, Pu, Sr, Zr⁹³, and Nb⁹³, may accumulate in the adrenals. No clear-cut changes have been reported after injection of these radioisotopes (184), but more or less complete medullar degeneration and pyknosis of cortical cells have occurred in very severe intoxications (290). Local application of radium needles can cause necrosis in the organ.

Carcinogenesis. Casarett reported that adenomas of the adrenal medulla appeared to be more numerous in rats surviving longer than 1 year after intravenous injection of polonium (1 μ c/kg or more) than in controls (291).

V. Skin and Epidermal Derivatives

Skin lesions were the first injurious effect reported early after the discovery of radioactivity (292). Even before the turn of the century a number of publications appeared describing skin damage in human subjects due to ionizing radiation (see ref. 293).

Skin reaction, as observed microscopically, is, in addition to the changes in the bone marrow, the first observable indication of an exposure to irradiation. Even after small doses, cellular changes in the skin may be apparent within an hour after the exposure. The first macroscopically visible effect in the skin is the erythema. The skin has a great capacity for local repair, but after large doses of acute or chronic exposure the irradiation of the skin may result in chronic changes and neoplastic transformation.

A number of excellent reviews have been published on the effects of radiation on the skin (293-304), and therefore the subject will only be summarily treated. Most data available in the literature concern the effects of irradiation on human skin. This presentation will, however, be confined to the effects in mammals other than man.

A. SKIN

The degree of skin lesion after irradiation depends on the dose received and also on the species of animal. Individual differences as to sensitivity are also fairly large. Furthermore, the various structures of the skin display relatively large differences in sensitivity.

Epithelial changes in the skin of the mouse ear have been described after a dose as low as 35 r (305). The mitoses in the epidermis undergo a substantial reduction.

After 600 to 800 r of total-body irradiation some inflammatory reactions with slight hyperemia and some edema are seen (179, 293, 306). There may also be some migration of heterophile leukocytes and lymphocytes, and some destruction of mast cells. After higher doses the epithelial cells become swollen and vacuolated. Vascular changes are presumed to play a great role. The smaller vessels may be more or less completely closed by endothelial proliferation or thrombi. After severe irradiation the inflammatory changes may be followed by progressive sclerosis with loss of elastic fibers and hyalinization of the collagen. The skin reactions generally are more severe after exposure to lower ranges of kilovolts (307).

Devik (308) has studied the effects of acute X-irradiation on the skin of a strain of hairless mice. After a dose of 2700 r the first sign of injury was a sudden occurrence of a great number of abnormal mitoses in the epithelial cells. Destruction of epithelial and connective tissue cells caused the inflammatory reaction. The vascular factor was supposed to be of secondary

importance in the pathogenesis of the acute skin reaction. The vascular changes, however, are most conspicuous in the vicinity of the most severely injured epithelial cells and least in the radioresistant muscle tissue. Regeneration is supposed to be due to the existence of a few radioresistant cells which survive.

X-Rays (100 kv) have a superficial effect on rabbit skin (309). Doses in excess of 2700 r are necessary to produce erythema. Doses of 7000 and 14,000 r cause atrophy of the epidermis, hair follicles, and glands, the atrophic changes extending to the muscle coat of the cutis. Healing occurs with fibrosis and disappearance of rete pegs. Induction of hyperplasia in the epidermis has been observed after roentgen irradiation (310).

Extensive studies have been made on the effects of locally applied radioisotopes on the skin, and it has been found that exposure of the skin to external β -irradiation may induce severe skin lesions.

According to Moritz and Henriques (311), irradiation of the pig skin superficially with the radiation from S³⁵, Co⁶⁰, Sr⁹⁰, Y⁹⁰, Y⁹¹, or Cs¹³⁷ produced epidermal atrophy, appearing 1 to 2 weeks after the exposure and lasting for 2 to 3 weeks. Usually, there were also erythema and exfoliative dyskeratosis followed by epilation and chronic radiation dermatitis. Often ulcerations and transepidermal necrosis were seen. Healing was slow, and often a chronic radiodermatitis persisted.

Exposure of mouse skin to 2500 to 5000 rep of β -rays causes an intense skin reaction after some time (293). Almost immediately an invasion of heterophile leukocytes is observed. After 10 days the basal layer begins to break down, and after 3 weeks an intense sloughing of the epidermis appears with the formation of very slowly healing wounds. An inhibition of the appearance of fibroblasts occurs after heavy β -irradiation of the rat skin (312). Skin burns in mice after partial-body β -irradiation from Sr⁹⁰ have been described by, among others, Crook *et al.* (313).

A dose of 4000 r of β -rays from P³² plaques coating the entire surface of the mouse causes denudation of the epidermis (moist dermatitis). New epidermis is continually formed under the sloughs (314). According to Snider and Raper (315) a single surface dose of 5000 rep of β -irradiation from an external P³² source causes the same changes as occur after X-irradiation.

Jacobsen *et al.* (316, 317) have exposed female rats to β -irradiation from plaques of Sr⁹⁰-Y⁹⁰ with a dose rate of 14.6 r/sec. After 200 or 400 r daily to a total dose of 6000 r, a dry desquamation was observed; and after 12,000 r, epilation occurred. A single dose of 3000 r produced wet desquamation in 14 days. The threshold dose for wet desquamation to occur at 600 r/day was 6000 r. Similar changes have been reported after exposure of rats, guinea pigs, dogs, and pigs to 450 to 100,000 rads of β -irradiation (318). A dose of 15,000 rads produces edema of the irradiated site by the first

day in rats and occasional edema in guinea pigs and dogs. Higher doses reduce the latent time before ulceration and increase the intensity of the erythema.

Exposure of rabbits to 2500 and 5000 rep causes the same skin changes as in rats and mice (293). A much more severe injury was inflicted by 12,000 rep. After 4 to 6 weeks the epidermis had almost completely disappeared. Beginning regeneration was evident after 8 to 10 weeks, but the skin had a clearly pathologic appearance. Also after 28,800 rep there was eventually healing, the new epithelium probably originating, at least partly, from the hair follicles. The defect in the superficial tissues after β -irradiation is replaced by tissues from the middle and deep corium which is moved to the surface by fibroplasia (319).

It has been observed (320) that some cells in the epidermis of hairless mice will survive a dose of the order of 100,000 rep of α -rays from polonium. This kind of exposure will cause a suppression of mitosis for a very short period. After 6 hours, swelling and pyknosis of nuclei occur, at 2 days necrosis starts, and after 20 days the epidermis has atrophied.

The threshold dose for inhibition of mitosis in guinea pig skin with X-rays or β - or γ -irradiation is stated (321) to be 400 r.

A γ -irradiation dose of 8.8 r daily given to guinea pigs caused a thickening of the epidermis and edema after about 3 months' exposure (293). According to Storer (322) thermal neutrons are about 1.7 times as effective as X-rays in inhibiting mitoses in the skin of mice.

It has been found after exposure of mouse skin to X-rays or γ -irradiation that there is a hyperpigmentation of the skin due to an increased amount of epidermal melanin. This is associated with an increased melanogenic action of melanocytes in the basal layer of the epidermis (323).

It is generally believed that the late deleterious changes in irradiated skin are directly the result of the radiation damage to the epithelial cells, and indirectly the result of starvation and anoxia of these cells due to vascular radiation trauma. Radiosodium clearance measurements have, however, led to the startling observations that the effective blood flow in these densely fibrotic, scarred, and atrophied tissues is functionally unimpaired at any time up to years after irradiation (324). This supports the assumption that the vascular damage observed plays no role in the late deleterious changes after skin irradiation. Some of the changes in the skin after irradiation may be secondary to infection in radiation-induced ulcerations.

Exposure of the skin to ionizing radiation causes an inhibition of the respiratory system and the anaerobic glycolytic enzyme system (325). After local exposure of the skin of rats to high doses (14,000 and 28,000 rep) of β -rays from radiostrontium, there was an immediate transitory depression of oxygen uptake and glycolysis followed by a marked stimulation (326).

When necrosis developed, the values fell below normal. Healing was accompanied by a rise in the glycolytic rate.

Beta-irradiation of the skin of rats depressed the local reaction to certain inflammatory stimuli (327). The histamine content of skin is not changed after β -irradiation. It is assumed that β -rays act by interfering with a tissue protease system which plays an important role in the mechanism of inflammation. Contrary to these observations of Ungar and Damgaard (327), Eisen and Wilson (328) found that the histamine content of rat skin decreased to about half the normal value within 5 days after exposure and remained low until the tenth day. The lowered content of skin histamine may be attributable to the concurrent degeneration of subcutaneous mast cells. This reduction in mast cell numbers observed after larger doses may conceivably be related to an enhanced adrenal cortical activity.

During some days after the irradiation there may be a considerable reduction in energy-rich phosphate compounds in the skin. Thus, it has been found (329) after 700 r of X-irradiation of rat skin that there is an initial elevation of DNA during the first 2 days followed by a decrease until the twenty-fifth day. There was also during the first 24 hours a small increase in the total acid-soluble and inorganic phosphorus of the skin followed by a 20 to 30 % decrease in the total acid-soluble phosphorus. Phosphagen phosphorus and ATP decreased for a few days and then rose to about 50 % above normal values (330, 331).

A substantial reduction of the hexokinase activity of skin is observed after whole-body irradiation. Even 3 weeks after 700 r of X-rays, the enzyme activity was still 25 to 50 % below normal. A brief increase occurs in phosphatase activity followed by a decrease to a minimum at 4 days after the exposure (332), the reduction being less after local irradiation.

In rabbits total-body X-irradiation with 600 and 1200 r causes a decrease in skin RNA and DNA (333). After large doses (20,000 r), an immediate formation of small amounts of secondary peroxides has been observed in the skin (334).

Internal Irradiation. Data concerning internal irradiation of the skin are sparse, and many administered radioisotopes seem to exert no or only minor effects in the skin. Thus, the α -irradiation from 0.1 to 0.3 μ c of Pu²³⁹ per gram of body weight given in intramuscular injections to mice gave no changes in the skin (293). The same was the case after intraperitoneal injections of 1.0 μ c of radium per gram. Nor was any damage caused by the β -irradiation from 0.8 μ c of Sr⁹⁰ per gram, administered intraperitoneally to rats, or from 2.0 μ c of Y⁹¹ per gram, given intravenously in the same species.

After subcutaneous implantation of polonium-impregnated nickel plates, hyperplasia of the epidermis occurs (335). Vascular changes are prominent.

Local injection of Y^{91} or Pu^{239} , which have an affinity to collagen, produce long-lasting inflammatory changes (184), and, according to Rhoades (336), the epithelial cells and the walls of smaller vessels may calcify after injection of radium.

B. HAIR

Irradiation of the skin causes destruction of the hair follicles and loss of the hair. There are marked species differences as far as epilation is concerned, and generally man is more susceptible to this kind of skin damage than most other mammals. Although temporary epilation in man usually results after 400 to 500 r of hard X-rays and permanent baldness after 700 r (197, 198), doses in excess of 2000 r are generally required to produce epilation in the common laboratory animals (337). In the C57BL mouse strain, however, Chase (338) produced permanent epilation with a single dose of 1000 to 1500 r of 100-kv whole-body X-irradiation.

Geary (339) has studied the sensitivity of the hair follicle of the rat to soft (44-kv) X-rays during the various phases of the hair cycle. The medium effective dose for complete epilation was between 1030 and 1750 r at various periods of the cycle. The matrix cells of the growing follicle were found to be among the most radiosensitive cells in the skin.

If the irradiation is sufficient to produce permanent epilation, the hair follicles are severely damaged, with cessation of mitosis, vacuolation, and focal necrosis (184). There may be some polymorphonuclear and mononuclear infiltration but usually no strong leukocytic infiltration. Recovery from the other skin reactions often occurs before maximum epilation is seen (340).

The regrown hairs after a temporary epilation often are not accompanied by sebaceous glands, and the hair is coarse (198). The new hair may be white or gray, owing to lack of pigmentation caused by a destruction of melanoblasts. Graying of hair in mice and hamsters may be seen even after about 200 r (341). The white hairs result from the destruction of dendritic cells. In albino mice, after local exposure of the skin to 1200 r of X-irradiation, the numbers of clear cells which are thought to be homologous to the melanocytes decrease substantially (342).

Except for the well-known nail changes in man after chronic exposure, nails and claws of mammals seem to be resistant to irradiation.

C. SKIN GLANDS

The sebaceous glands are very radiosensitive (337, 343). After irradiation their secretion is diminished. The epilation dose will also destroy most sebaceous glands.

The sweat glands are also sensitive (300, 343). The sweat secretion is

decreased or completely inhibited after an epilation dose. After heavy irradiation, degenerative changes are seen, and the glands may disappear completely.

D. MAMMARY GLANDS

The duct system of the mammary gland is stated to be very radioreistant (184). According to Turner and Gomez (344), a dose of 2800 r to 3600 r is necessary to inhibit the development of secretory acini in the rabbits. In pregnancy, the secretory epithelium seems to be more susceptible.

E. CARCINOGENESIS

Skin. The skin is more resistant to tumor induction by irradiation than most internal organs. Cutaneous tumors, however, were the first noted in man, owing to the relatively soft X-radiation used earlier and lack of adequate filtration.

In animals, tumors of the skin have been induced mainly by β -irradiation, but induction by X-irradiation has also been reported.

Rats surviving a single dose of 660 r of whole-body irradiation showed a high incidence of neoplasms. The animals developed a wide variety of tumors, both benign and malignant, especially in the skin and subcutaneous tissues. Of 69 tumor-bearing animals, 43 had one or more malignant neoplasms. There were 14 rats with fibrosarcoma, and 8 with carcinomas of the skin (345).

Stutz and Blüthgen (346) showed that the rat tail proved to be a particularly suitable object for generating cancer by fractionated X-rays. The size and distribution in time of the individual doses were more important than the total dose for the induction of cancer. The carcinomas arose from hyperkeratoses or more frequently in the closest neighborhood of necrotic and ulcerating tissue. The carcinogenic dose is a sublethal dose.

Single massive or repeated small doses of β -radiation from P^{32} to rats induced tumors of practically every type that can arise from skin and connective tissue. They appeared at 10 months to 1 year after irradiation. The optimal single dose for tumor induction was in the range of 4000 to 6000 rep. In the case of fractionation, the doses of 50 rep/day were most effective. Epithelial tumors were present in large numbers in certain experimental groups but almost absent in controls. The incidence of subcutaneous tumors increased only by a few per cent in the irradiated females, but in males the increase was manyfold. The tumors in many instances showed a high degree of differentiation with a tendency for the epithelium to form squamous cell and basal cell carcinomas, as well as adenomas, and for connective tissue to form tendon, cartilage, and even bone (347).

Schubert *et al.* (348) investigated the correlation between tumor incidence and latent period on the one hand and the intensity of the source of radiation used in continuous application of pure β -rays from Sr⁹⁰-Y⁹⁰ on the other. Glass pellets with Sr⁹⁰CO₃ were implanted subcutaneously in 80 rats and intraperitoneally in 134 rats. The pellets differed in activity (15, 30, 45, and 85 μ c). Malignant tumors developed in 91 animals. The tumor rate seemed to be proportionate to the logarithm of the activity, whereas the latent period showed a linear relationship with activity. In the subcutaneous series, tumor formation was preceded by local epilation. In animals given pellets with an activity of only 1.5 μ c, no tumors developed throughout life.

The appearance of skin tumors in rats exposed to electrons from a Van de Graaff accelerator has been investigated by Davis and Alpen (349).

Epilating doses of 1200 rads cause more tumors than either a non-epilating dose of 600 rads or a dose of 2000 rads or more. The majority of the tumors are epitheliomas of benign type or very low malignancy and are first seen at 5 to 6 months. The most striking finding is the number of tumors in the 1200-rad group where five or six tumors per rat are not unusual.

After subcutaneous injection of Y⁹⁰ in mice the incidence of malignant tumors was about 25 % after doses of 10 and 30 μ c per mouse. At higher doses the incidence was lower (350).

After large internal doses of P³², squamous cell carcinomas of the face developed in 3 of 19 rats after a latent period of 254 to 354 days (351).

Mammary Glands. The carcinogenic effect of ionizing radiation on the mammary gland and the endocrine factors involved in the induction process have been the subject of a few publications.

According to Lorenz *et al.* (352) the acute massive whole-body irradiation of mice reduces the over-all incidence of mammary tumors in comparison with that of the controls, but the incidence of these tumors was high in those irradiated mice which developed granulosa cell tumors of the ovary. These findings were attributed to the decrease in estrogen production after irradiation of the ovaries and excessive estrogen production by granulosa cell tumors.

Guinea pigs have also been shown to be sensitive to the induction of breast tumors by ionizing radiation, although in this species the carcinogenesis is unrelated to granulosa tumors (150).

Rats given a single exposure of whole-body irradiation were observed for 11 months postirradiation for the occurrence of mammary tumors. It was found that 56 % of the rats exposed to 400 r of γ -irradiation, 43 % exposed to 400 r of X-rays, and 31 % exposed to 200 r of γ -radiation developed one or more mammary tumors. Seventy-six tumors were observed in 48

rats exposed to whole-body irradiation: 43 adenofibromas, 20 adenocarcinomas, 5 fibroadenomas, 3 fibrosarcomas, and 5 not classified; 12 of the 20 adenosarcomas developed during the first 4 months postirradiation (353).

According to Bond *et al.* (354), a dependence of the radiation-induced neoplasia on ovarian function was indicated by the fact that ovariectomy greatly reduced the incidence of mammary neoplasia. Dose dependency studies indicated that the incidence of mammary gland tumors was linear with dose from 25 r up to 400 r and that a threshold, if present, is small. They state that radiation changes may not manifest themselves maximally as neoplasia unless the endocrine status of the animal is favorable.

The incidence of mammary tumors in female mice after an exposure to ionizing radiation from a nuclear explosion increased in the dose groups 192 to 687 r. Failure to demonstrate an increase at higher dose levels was due to reduced longevity. The neoplasms which occurred early were predominantly adenomas and adenocarcinomas, whereas those developing later were sarcomas (149).

Also after chronic γ -irradiation an increased incidence of mammary gland tumors has been observed in female mice. Of the female mice free of the milk agent, 47% developed tumors after 8.8 r daily as compared with 4% of the controls. The incidence of carcinomas was approximately 20% and of sarcomas 30%. Most of these tumors were associated with ovarian tumors, usually of the granulosa cell type (352).

Chronic fast neutron irradiation in rats also increases the incidence of malignant tumors, most of them in mammary glands (355).

After continuous irradiation of mice, the mammary tumors and malignant tumors of the nasopharynx were increased in incidence by 6.4 rads weekly of fast neutrons, but the incidence of other malignant tumors was unchanged or decreased (356).

Exposure of rats to sublethal amounts of astatine (At^{211}) resulted in the early appearance of large numbers of mammary tumors, many of them malignant, and in the production of an altered functional state simulating menopause (357).

VI. Nervous System

It is generally agreed that the nervous tissues, especially of adult animals, show a remarkable radioresistance (197, 198, 358, 359). This is true at least as far as morphological changes are concerned, but transient functional changes have been reported at comparatively low doses.

The developing nervous tissue, on the other hand, is much more sensitive, and in fetuses and newborn animals radiation damage may be en-

countered also in the nervous system (360). Hicks (361) found that, if mice and rats were irradiated with doses as small as 150 to 200 r during the later two-thirds of pregnancy, the developing neuroblasts were severely damaged and severe malformations of the brain resulted. Irradiation of rats and mice (3 to 12 months old) caused necrosis of scattered oligodendroglia and subependymal cells, retinal rod cells, and occasional neurons in the pyramidal lobe and olfactory brain.

Earlier it was generally believed that total-body irradiation with median lethal doses usually would produce no functional disturbance in the nervous system (362). Later investigations have, however, shown that the nervous system, as far as function is concerned, is rather radiosensitive and that functional disorders can be produced by rather small doses. Changes in the electroencephalogram (EEG) have been reported after very low doses. Transitory changes can be produced after whole-body irradiation with less than 100 r. After 300 to 400 r the EEG may be altered for about 1 week, and 800 to 900 r gives rise to immediate modifications that will last until death. During irradiation with high dose rates, the EEG changes occur very early during the exposure (363). The effects are elicited also from the cerebellar cortex (364). Other changes observed after rather low doses are reduced excitability, changes in the conditioned reflexes, and disturbed balance between excitation and inhibition.

A. BRAIN

Whole-body irradiation with large doses (more than 6000 r) has been found to cause in some instances an increased motor excitability (365).

After local irradiation of the brain with large doses, delayed necrosis of brain cells has been observed in rabbits and monkeys (366, 367), and hemorrhages in the brain have been reported in mice (368) and in dogs (369). Foci of radionecrosis can cause various neurological disturbances, notably epilepsy of the so-called Bravais-Jackson type (370). Changes in the specific gravity (371, 372) and composition (373) of blood have been described after irradiation of the brain. Relatively large unilateral doses (2000 to 4000 r) given to the cerebrum of monkeys have been found to produce contralateral limb paralysis (366).

The chief neurological signs in dogs which survived for 14 to 28 hours after X-irradiation of their heads with 23,000 r were disturbance of equilibrium and tensor rigidity (374). Arterial pressure, heart rate, and respiratory rate did not change significantly until about 1 hour before death, and the carotid sinus reflex was unaltered until 30 to 60 minutes before exitus but then deteriorated rapidly. At the terminal stage the pressor response of the medullary vasomotor center to electrical stimulation decreased in

parallel with that of the carotid sinus reflex. The vital centers in the medulla oblongata may be directly damaged by large doses of X-rays. The failure of respiration is the cause of death in such cases.

If female albino rats are irradiated with X-rays in doses of 500 to 800 r in nonpregnant animals and 150 to 400 r in pregnant animals, the amide-free lipid fraction decreases in the adult but increases in the developing brain (375). The increase of the amide-free lipid fraction in the fetus after maternal irradiation has been proposed to be due to a chemical destruction of embryonic cell elements, especially in the germinal structures in the forebrain.

After sublethal X-ray doses there is an increased ability of the brain to synthesize acetylcholine (376, cf. also 377). The cholinesterase activity of the mouse brain is not influenced by even very high doses (80, 82). After exposure to 700 r of whole-body irradiation the brain content of deoxyribonucleic acid was found to be lowered during the first day and during the 4- to 10-day period postirradiation (378). During the same time the low-frequency brain activity is also reduced. In dogs, local exposure of the head to 5000 r of γ -rays from Co^{60} causes a decrease in the potassium content of the cerebral cortex. The content of sodium, chloride, and water is unaffected (379).

The effects of irradiation of the brain on the behavior of the animal have been extensively studied in monkeys (380-384) and in other animals (385-389).

Internal Irradiation. Internal irradiation by Sr^{89} or radium in doses below the $\text{LD}_{50(30)}$ has no effect on the brain or spinal chord (360).

Carcinogenesis. No brain tumors of experimental animals attributable to exposure to ionizing radiation have been reported.

B. PERIPHERAL NERVES

Peripheral nerves are still more resistant to ionizing irradiation than the brain and spinal chord. In the literature there is an almost complete lack of statement of morphological changes in the peripheral nerves and autonomic nervous system of adult animals (360, 390-392).

The sciatic nerve of the rat shows no influence of doses between 4000 and 10,000 r (393). Complete degeneration of the nerve results only after doses as high as 75,000 r or more.

Changes in ganglion cells in rabbits have been observed only after irradiation with several thousand roentgens (394). The main changes are chromatolysis and vacuolization. According to Novick (395) local irradiation of the head of rabbits with 3000 r causes some histological changes in the trigeminal ganglia. Immediately after the exposure there was an extensive chromatolysis followed after some hours by a certain recovery.

After 3 days there was a new phase of chromatolysis, lasting for several weeks. The animals were dyspneic and displayed exophthalmus, somnolence, and orientation disturbances with postural abnormalities immediately after the exposure. During the second phase of chromatolysis they suffered from emaciation and excessive salivation.

After very large doses of X-rays there may be an effect on peripheral nerve activity. Thus, Bachofer (396) found in rats that during X-irradiation with 6000 r/min the amplitude of the peak of the stimulated nerve increased by about 60% during 80 minutes. The velocity of conduction was increased during the first 10 minutes but thereafter decreased. It is supposed that the increased spike amplitude of the irradiated nerve is due to an increased permeability of the nerve membrane. Similar results have been described also by other investigators (397). Irradiation with very large doses (200,000 to 300,000 r) may produce a decrease in amplitude of the action potential and a retardation of the conduction velocity (398).

Sciatic nerves of rabbits are more sensitive to irradiation than are, e.g., frog nerves (399). Doses in excess of 45,000 r cause complete nerve block within an hour after irradiation.

VII. Sense Organs

The only sense organ that is of any interest as far as radiation damage is concerned is the eye. It has been observed among the survivors after the atomic explosions in Japan (400, 401) and also in accidentally exposed cyclotron workers (402) that opacities of the eye lens, cataracts, develop as a sequela of the exposure.

A. EYE

It has long been known that ionizing irradiation can cause severe inflammatory processes in the conjunctiva and sclera. The doses necessary to bring about these changes are of the same order of magnitude as those effective in producing skin damage.

Some of the ocular lesions occurring after total-body irradiation are certainly due to systemic alteration (403). This is generally true for the retinal hemorrhages, retinitis, choroiditis, and iridocyclitis developing days to weeks after the exposure.

Cibis *et al.* (404) studied the effect of high-intensity (260-kvp) X-irradiation on the eyes of guinea pigs, rabbits, dogs, and rhesus monkeys. Doses between 1700 and 35,000 r caused vascular and cytological response in all ocular tissues with the exception of the sclera. The most significant change in the eye of adult animals is the death of the rod cell population. This becomes functionally noticeable within 10 minutes after exposure by changes in the electroretinogram (ERG), loss of the pupillary reflex to light, night

blindness in monkeys and dogs, and complete blindness in rabbits and guinea pigs.

The changes produced in the rabbit's eyes by very high-energetic radiation in a 20 Mv betatron (16 to 19 Mev electrons and 23 Mev X-rays) are about the same as those caused by lower-voltage radiation (405). Doses of 1650 r caused an early transient uveitis followed by a delayed keratitis. Granulomatous changes occurred in the vitreous body after doses of 1800 r or more. No alterations were found in the sclera or optic nerve.

Cataract. The occurrence of cataract after exposure of the lens to ionizing radiation has been demonstrated in both man and experimental animals. The first report of such postirradiation lens opacities was presented to the Ophthalmologic Society in Heidelberg as early as 1905, and in 1908 Birch-Hirschfeld (406) stated that irradiation probably could cause lens opacities of cataract type. Most observers consider the injury to take its origin primarily in the lens as a direct damage either to the epithelium or to the lens fibers and their proteins. The nature of the lens changes has been supposed to be an alteration in the physical, chemical, enzymatic, or cellular processes taking place in the lens. It may, however, also be secondary or indirect, caused by impaired nutrition resulting from vascular injury in the ciliary body. Also changes in the permeability of certain phase boundaries produced by the irradiation may be responsible. Irradiation of the ciliary body, but with the lens protected, will, however, give no cataractous changes, which indicates that the first, the primary, action on the lens is the predominant (407). According to this theory the primary lesion is located in the cell nuclei of the germinative zone. The radiation cataract apparently is not a nutritional cataract.

The first signs of morphological damage to the lens are punctate vacuole formation, located toward the periphery of the posterior part of the lens (408), the beginning of displacement of nuclei in the lens bow, clamping of chromatin, and nuclear fragmentation in the epithelium. Extrusion of chromatin material from the cell nucleus often leads to death of the cell. Even minimal quantities of X-rays can cause an arrest in the growth of the lens (409). These early morphological changes precede the development of clinically visible changes for several weeks.

Irradiation of part of the eye shows that exposure of the ciliary body alone does not produce cataract. It is necessary to irradiate the periphery of the lens (410). The histopathologic changes in the ciliary body are transient. No appreciable changes in the permeability of the ciliary body are found.

Alter and Leinfelder (407) also showed that the more actively growing parts of the lens, e.g., the lens epithelium and fibers of the equatorial region, are the most sensitive. Exposure of these parts causes the same changes

as do exposure of the entire lens. The central part of the lens, on the other hand, is more resistant, and twice the dose that produces cataract on exposure of the peripheral portion is ineffective centrally. Exposure of one quadrant of the lens only to X-rays produces opacities only in this quadrant.

Poppe (411) found that as small a dose as 250 r of X-rays produces permanent damage to the lens. The latent period for the development of cataract, however, was more than 5 months. The latent period generally is inversely proportional to the roentgen dose (412). The threshold dose of X-rays for the production of serious cataract seem to be about the LD₅₀. Even smaller doses may, however, produce lens opacities.

In mice Upton *et al.* (413) found that opacities were readily induced by X-irradiation locally to the eyes, the threshold dose being about 15 r. The changes increased in severity with dose and time after irradiation. The incidence of cataract approached 100% at 33 weeks after exposure to 45 r or more. These results are in striking contrast to the much higher doses necessary to produce cataract in the human eye, where about 600 r of 100 kv X-rays is needed to produce lens opacities (414). Young animals are more susceptible to cataract formation than adults. The lens opacities caused by whole-body irradiation are identical with those caused by local irradiation of the eye (415).

The effect of other densely ionizing radiations (α -particles, deuterons) on the cell nuclei greatly exceeds that of X-rays. According to von Sallman *et al.* (416) the RBE for lens damage for α -particles is about 4.1, and for deuterons about 3.9.

Changes seen after local β -irradiation of the eyes are telangiectasis of the conjunctiva, keratinization of the conjunctiva epithelium, atrophy of the sclera, superficial punctate keratitis, corneal vascularization, corneal scarring, iritis, iris atrophy, and radiation cataract. Surface doses of more than 5000 rep are necessary to produce such changes (417-419). According to McDonald *et al.* (420) the minimum cataractogenic dose in the rabbit receiving limbal irradiation is 500 to 1000 rep. The latent period is directly related to the dose.

Neutrons have a higher ability to produce cataracts than has X-irradiation (421). The RBE for cyclotron-produced neutrons for cataract production is three to nine times that for 200-kv X-rays (422).

Exposure to 60 to 150 n in a collimated beam of 6- to 8-Mev neutrons failed to produce clinically evident lens cataracts in dogs within 2 years after irradiation. In puppies and young dogs some vacuolization of the lens occurred but no cataract. A dose of 800 to 900 n caused cataract in 60 to 75% of the eyes of adult dogs. Complete necrosis of the globe, orbit, and surrounding tissues occurred in 3 to 4 months after exposure to 1500 n (423).

A single dose of 33 to 100 n of fast neutrons produced definite cataracts in 2 to 5 months in rabbits. A total neutron whole-body irradiation of 52.7 to 83.7 n administered in 16 rabbits at weekly intervals in doses of 3.1 n failed to produce cataract in 4 to 12 months after cessation of exposure (423). Ham (424) supposes 10 to 20 rep of fast neutrons to be sufficient to induce lens opacities that may interfere with eyesight.

Electroretinographic changes have been described (425) in rabbits after irradiation of their eyes with γ -radiation from Co^{60} . Doses of less than 3000 r gave an initial increase in activity (mainly of *b* and *c* waves) followed by a decrease and an irreversible disappearance. After large doses (over 7000 r) a complete disappearance of activity was found early after irradiation. Irradiation of the occipital region with γ -rays had no effect on the ERG. For a constant stimulus and adaptation, the attenuation of the ERG components (*a* and *b* waves) is related to the visual cells affected (426).

Glyceraldehyde phosphate dehydrogenase, glyoxalase, and acetaldehyde oxidase activity have been found (427) to decrease during the development of lens opacities after irradiation of rabbits. The aldolase activity is unchanged except in the opaque lens where it is diminished. The content of coenzyme A and nicotinic acid undergoes a gradual fall during the development of the cataract.

No changes have been found within the first days after irradiation of very young rabbits in the content of the lenses with regard to the high-energy phosphate groups of adenosine triphosphate and adenosine diphosphate, or in hexose monophosphate, hexose diphosphate, phosphoglycerate, or inorganic phosphate. There is no demonstrable decrease in the turnover of deoxyribonucleic acid within the first 2 weeks after irradiation (428).

Harderian Gland Tumors. An unexpected neoplasm was observed by Furth *et al.* (449) in mice exposed to ionizing radiation from a nuclear explosion. Adenocarcinoma of the harderian gland of the orbit developed. This is a locally invasive neoplasm, often obliterating the orbit and invading the skull and adjacent soft tissues. The induction rate of these tumors is very low and apparently not dose-dependent. No tumors of the harderian gland have been observed in controls. Their induction mechanism is unknown.

VIII. Respiratory Tract

The question of the radiosensitivity of the respiratory tract is somewhat under dispute. This may at least partly be due to the fact that the air passages and lungs are built up of cellular structures of different radiosensitivity. The richly ramified vascular system of the lungs is susceptible to irradiation. The same applies to the lymphatic tissue of the lungs, the bronchiolar epithelium, and the cells lining the alveoli and atria. The carti-

lage of the air passages and the pleura, on the other hand, are radioresistant. Pulmonary infections may render the tissue more sensitive to irradiation (429). Pulmonary changes after irradiation (radiation pneumonitis), however, have mainly been described only after fairly large doses and especially as a consequence of repeated large doses. In severe radiation pneumonitis and fibrosis the respiratory function may be affected by impaired diffusion across the alveolar-capillary membrane (430). The most common symptoms after pulmonary irradiation are dyspnea, cyanosis, decreased chest expansion, and alteration of breath sound (431).

Engelstad (432) has studied the effects of large doses of X-rays (1500 to 9000 r) on the lungs of rabbits. As soon as 2 hours after the exposure, degenerative changes were seen in the lymphatic tissue. There were also increased mucus secretion, hyperemia, slight edema, and leukocyte infiltration in the stroma. Swelling of the cells lining the alveoli and atria is also an early sign of pulmonary radiation damage (429). The columnar cells often become cuboidal and lose their cilia. Anaplasia of alveolar and bronchial epithelial cells, and rupture and reduplication of the elastic and hyalin membrane lining the alveoli are also typical changes. After some weeks degenerative changes in the bronchial epithelium, acute bronchopneumonic changes, and increase in alveolar macrophages are seen.

High doses of X-irradiation are supposed (433) to induce a process consisting in capillary damage, extravasation of liquid blood constituents and soaking of the tissues, and collagen swelling. This process is then followed by fibrous interstitial induration of the pulmonary parenchyma.

Ivanov (434) irradiated dogs with 500 r of total-body X-rays (dose rate 16.8 r/min). Pulmonary changes occurred, and the course of events could be divided into four phases. Until the third day there is marked hyperemia with erythrodiapedesis and edema in the alveoli, and subpleural emphysema. The second phase is a symptom-free "occult stage." On the seventh day the third stage begins in which an increased permeability of the vessels is the main phenomenon. The wall is imbibed with plasma. From the tenth day on there are massive perivascular blood effusions. Necrosis may develop in the hemorrhagic areas. A neutropenic bronchopneumonia may develop owing to bacterial infection.

Eventually, the fourth phase, which is a resorptive and regenerative one, starts in the surviving animals, with proliferation of connective tissue, sclerosis, sometimes bone formation, and slight proliferation of bronchial epithelium. Large doses cause extensive sclerosis, usually with bone formation. Smaller doses with no severe effect on the epithelium cause only slight fibrosis. The most important factors for the occurrence of radiation fibrosis of the lungs are supposed (430) to be the total dose delivered to both lungs and the brevity of the period in which a significant volume of

TABLE II
RADIOACTIVE ISOTOPES WHICH CONCENTRATE IN THE LUNGS

Radioisotope	Physical half-life (days)	Effective half-life (days)	Maximum permissible body burden (μc) (ICRP)	Maximum permissible concentration in air ($\mu\text{c}/\text{cc}$) (ICRP)
Ni ⁶³				
Po ²¹⁰	138.3	31	0.02	10^{-7}
Ra ²²⁰ + daughters	6.3×10^{-4}			10^{-7}
Ra ²²² + daughters	3.83			10^{-7}
Th-natural (insoluble)	5×10^{12}	4.3×10^4	2×10^{-3}	3×10^{-11}
U-natural (insoluble)	1.64×10^{12}	120	0.01	3×10^{-11}
U ²³³	5.9×10^7	120	0.016	3×10^{-11}
Pu ²³⁹	8.8×10^6	360	0.02	2×10^{-12}

lung tissue is irradiated. Fractionated doses or spreading out of the dosage over a longer time is less effective.

Internal Irradiation. The greatest radiation hazard to the lungs emanates from inhaled radioactive dust or gas. Several hazardous radioisotopes concentrate in the lungs. The most important are listed in Table II.

The radioactive particles, when inhaled, will locate at various levels of the respiratory tract, depending on the particle size. Particles of about 0.3 to 6 μ are trapped in the alveoli, those about 6 to 20 μ in the bronchiolae, those 20 to 60 μ in the bronchi, and particles larger than 60 μ in the upper parts of the respiratory passages. Particles smaller than 0.3 μ are to a considerable degree exhaled again. For further details of the effects of inhaled radioactive isotopes the reader is referred to ref. 435.

Carcinogenesis. In mice exposed to total-body irradiation within the tolerance range no acute changes occur in the lungs, but many irradiated animals develop pulmonary adenomas late in life (436), since the tumor development time is long and the incidence rate very low. Furth and Furth (437) gave evidence that massive doses of γ -radiation can induce lung tumors in mice. The increase in lung tumor incidence observed in females was slight but statistically significant.

Lorenz *et al.* (150, 438) exposed mice to chronic γ -irradiation, giving 8.8 r daily and a total of 2400 to 2500 r over a period of 10 months. The mice were killed at 11 months of age. The incidence of adenomas of the lung was 75%, in controls 45%.

The incidence in RF mice irradiated in a thermal column and by X-rays was markedly elevated after high doses of radiation. The effect was somewhat obscured by the long latency of the pulmonary neoplasm. Thus,

tumors occurred in 33 % of the females surviving 18 months, whereas only 15 % of the nonirradiated controls had developed lung tumors after 18 months of age (148).

In recent years lung cancer has been observed in animals experimentally given radioactive materials.

Lisco and Finkel (439) induced bronchiogenic carcinomas in rats by inhalation of an unknown quantity of radioactive cerium oxide. This compound covers the bronchi tenaciously. The induction time was 9 months after the end of exposure.

Further evidence of the carcinogenicity of inhaled radioactive particulate matter was gathered by intratracheal injection of $\text{Ce}^{144}\text{F}_3$ into rats at dose levels of 50, 25, 15, and 5 μc . Of 93 treated rats, 13 developed squamous cell carcinomas in the lung. The tumor induction time was short. The earliest carcinoma was found in a rat that died 48 days after exposure to 15 μc of Ce^{144} , and three other tumors were found in rats that died within 100 days. The smallest carcinogenic dose was found in the case of a rat that had been sacrificed 178 days after exposure to 50 μc . The calculated dose was 2400 rads (440).

Sulfur-35 has also been used in an inhalation study. Sixteen rats were subject to ten weekly intratracheal insufflations of 375 μc of S^{35} in the form of BaSO_4 particles with a diameter of 1.45 μ . Two rats which died 312 and 319 days after the exposure showed extensive squamous cell carcinomas in the lungs, and 1 rat died with a carcinoma *in situ* at 118 days (441).

Bair *et al.* (442) exposed mice to aerosols of several radioactive compounds of varying tissue solubility in order to evaluate the hazards of inhaled radioactive particles. The fate of the inhaled radioactive particles and the biological effects of them were determined. Mice inhaling "insoluble" particles, either $\text{Ru}^{106}\text{O}_2$ or $\text{Pu}^{239}\text{O}_2$, have been shown to return the radioisotope in the lung with "biological half-lives" of 30 to more than 200 days. Although $\text{Sr}^{90}\text{SO}_3$ showed a long biological half-life after inhalation, it was found to be rapidly dissolved in the lung and translocated to the skeleton. It was found that mice exposed to radioactive particles showed two types of lesions: tumors and a sterile nondetermined pneumonitis. Four kinds of tumors were revealed: nonmalignant papillary cystadenoma, malignant squamous cell carcinoma, malignant fibrosarcoma, and a malignant nondifferentiated tumor. The latent period was at least 200 days. As little as 0.003 μc of $\text{Pu}^{239}\text{O}_2$ was found to cause malignant growth. Larger quantities of $\text{Ru}^{106}\text{O}_2$, 4.5 μc , were required to cause lung cancer.

IX. Circulatory System

Changes in the circulatory system, namely in the blood vessels, have been observed almost as long as ionizing radiation has been used for therapeutic and experimental purposes. Gassmann (443) seems to have been the

first to describe radiation-induced vascular lesions. A large number of papers have appeared since then (for reviews, see, e.g., refs. 77 and 444). The best known vascular effect is that in the skin causing the radiation erythema.

A. HEART

Radiation effects on the heart are much less known, and in contrast to the blood vessels the heart has generally been assumed to be radioresistant (77, 444-449). This assumption has been based essentially on the negative histological findings (450). Biochemical observations by Caster and Armstrong (451), however, indicate that myocardial lesions occur which resemble those in other radiosensitive tissues. As a plausible explanation for the earlier overlooking of these changes they propose that the typical lesions after irradiation occur at the molecular level and, therefore, are more easily revealed by biochemical than by histological methods (450).

Marked histological changes after exposure of rabbits to 1000 and 8000 r of 75-kv X-rays and 31-Mev betatron X-rays have been found in the heart muscle (452). Myocardial hemorrhages have been reported to occur in dogs dying after acute or chronic X-ray exposure (453, 454). Ross and Ely (455) found myocardial lesions in the terminal stages in dogs which had been exposed to cyclotron neutron irradiation. Caster *et al.* (450) point to the presence of marked functional changes in the heart in animals after irradiation with LD₅₀ doses, and they suggest that a cooperative factor in radiation death may be some form of heart failure.

The heart rate was found to be unaltered in the dog but increased in the rabbit during the first hours after irradiation (454, 456). This change may be compensatory to an initial hypotension. During the terminal stage the pulse rate is usually increased in the dog but not always in the rabbit.

Electrocardiographic alterations after irradiation have been reported by a number of authors (446, 449, 452, 457-459). Often they have been interpreted as secondary changes. Fulton and Sudak (458) observed ECG disturbances in male hamsters after whole-body X-irradiation with 1000 r but not after 600 r. In the terminal stages of the radiation injury there were depression of the amplitude of the T and P waves, bradycardia, depression of the S-T segment, and in some cases prolonged Q-T intervals and reversed T waves. A strong vasoconstriction was seen in these animals.

X-Irradiation causes a release of potassium from the isolated rabbit heart and also from the heart irradiated *in situ* (460). Peak values of 12% above normal were obtained after irradiation with 2500 r, the dose rate being 500 r/min. Even a dose of 500 r is effective in causing liberation of potassium. After 1000 r the amount of potassium released was more than four times as high during 1 hour in the irradiated as in the normal hearts. It has been suggested (458) that the changes in the electrical activity of

the heart after whole-body irradiation may be due to an altered plasma potassium level resulting from reduced adrenal activity.

Internal Irradiation. In mice injected intraperitoneally with 1.0 $\mu\text{c}/\text{gm}$ of radium, swelling of the myocardium and of the cardiac muscle fibers around pulmonary vessels has been observed (444).

B. BLOOD VESSELS

Radiation within the total-body exposure tolerance causes only minor damage to blood vessels, but after larger doses, and especially after larger doses to a localized area, extensive vascular damage may occur. In the earlier literature the emphasis was laid mainly on alterations in the endothelium (461-464). Efskind (465), however, states that endothelial cells of larger vessels are radioresistant. According to later views, the most constant and obvious changes are in the collagen (444). For a review of the earlier literature the reader is referred to Rhoades (444).

The blood vessels of irradiated animals show changes in all layers of the walls (444). The tunica adventitia is the most reactive, owing to its relatively high collagen content, and the degree of damage is related to the content of collagen. With very high doses hyalinization of collagen fibers occurs. Edema is often present around the fibers. It has also been observed (466) that irradiated rabbit ears have a larger ability for edema formation than nonirradiated ears.

In animals killed 4 to 5 months after exposure some vessels lacked any tunica adventitia, whereas in others the tunica was composed of densely packed thick collagen fibers. After high dosages also the tunica media may disappear. The elastic membranes are very resistant. The endothelial cells become swollen only after very large doses, e.g., from internal β -irradiation.

The endothelium in irradiated areas has a decreased capacity to form new capillaries which may play a role in certain postirradiation changes. This has been shown in mouse skin by Mervin and Hill (467) after irradiation with single doses of 400 to 1500 r. The revascularization after burns was decreased.

Whole-body X-irradiation of young mice has been found (468) to produce the changes of normal aging in the arteries with increased amount of ground substance in the vascular media, increased numbers of interlamellar fibers, and fraying and raggedness of the elastic fibers in the media. These changes are not seen in old animals.

After local irradiation of the legs of rabbits with high doses (2000 to 3000 r), some vascular reactions of inflammatory character have been noted (444). The walls of the vessels were often edematous. The endothelium of such edematous vessels looked swollen. By 24 hours after administration of 2000 r only the endothelium remained in the vessels within

the field of irradiation. All other elements of the vessel walls had broken away. No clear changes were observed after doses lower than 800 r.

The effects on the circulation after whole-body irradiation and after local irradiation of a portion of the wing have been studied in the bat (469). The hibernating bat is very radioresistant. After total-body irradiation with 10,000 to 60,000 r, there were marked adherence of leukocytes to the vessel walls, clumping of erythrocytes, stagnation of blood, and some increase in capillary permeability. The threshold for circulatory disturbances after local irradiation with 50-kv X-rays was about 50,000 r.

In mice exposed to 5000 rep of external β -irradiation from a P^{32} source (444) the blood vessels in the skin 24 hours after the exposure had become thinner owing to loss of collagen. The total number of small vessels seemed to have decreased. An increase in the number of patent blood vessels and capillary dilatation have been observed in irradiated skin (456, 470). Attempts at repair were evident after 2 months. The vessels were distended and often thinner than normal. Vessels close to ulcers were completely degenerated. Telangiectasis may result after heavy irradiation. After 2500 rep about the same picture was seen, but fewer vessels degenerated completely.

Internal Irradiation. In rats administered 0.5 μ c/gm of radium in intraperitoneal injection, severe vascular damage resulted (444). Two months after the injection there was such extensive damage to the tunica adventitia that in many vessels only the endothelium was left. Injection of radium chloride caused highly radioactive material to be deposited in the walls of the vessels during the weeks after the administration. Histologically, these deposits had the same appearance as the calcium deposits in arteriosclerosis. In the mouse, which is considerably less sensitive to radium than the rat, injection of 1.0 μ c/gm of radium caused essentially the same vascular changes as 0.5 μ c/gm in the rats.

The administration of ThO_2 (Thorotrast) causes changes similar to arteriosclerosis and thromboangiitis obliterans, and the formation of granulomas (471).

It is by now generally believed that the vascular damage caused by external as well as internal irradiation is due to a primary injury to the vascular structures and not to damage to the nerves supplying the vessels as proposed by David and Gabriel (472).

The views on the influence of radiation on vascular permeability are diverging. There is some evidence for the statement that massive doses are necessary to affect the cell permeability (77). A number of papers suggest that there is an increased vascular permeability often combined with endothelial damage after whole-body irradiation (e.g., refs. 155, 473-477). Various substances such as labeled plasma, erythrocytes, Evans blue, and colloidal radiogold disappear more rapidly from the circulation of mice and

rabbits after X-irradiation, the disappearance rate reaching a maximum in the second week after irradiation. The disappearance of the tagged substances is probably not due to phagocytosis, as there is no significant alteration of macrophage function (476, 478). Other authors have, however, found a decreased vascular permeability after irradiation (479). The effects of irradiation on the pulmonary capillary bed damaged before or after the irradiation by a high-pressure blast exposure are contradictory (480-482).

The increased disappearance rate for some labeled substances after irradiation may be due to a radiation-induced vascular fragility (155, 476, 483, 484). An increased fragility of capillary walls in the rat peritoneum has been demonstrated by Griffith *et al.* (485) after exposure to implanted radon. The increased capillary permeability after roentgen exposure has been supposed by Rieser (486) to be due to liberation of heparin into the circulation as a consequence of the irradiation.

There may also be a marked vasodilatation. The temperature rise in skin concomitant with the erythema speaks in favor of that assumption (487). Bleeding due to increased vascular permeability has been found by some investigators (483, 484, 488, 489) to be reduced by certain flavonoids, e.g., rutin.

Early circulatory disturbances have been observed in some species after irradiation. Thus, in rats and rabbits the blood pressure drops during the first 24 hours after the exposure (454, 456, 490-492). In the rabbits even a dose as low as 50 r of whole-body exposure will reduce the blood pressure (456). Blood-pressure changes have also sometimes been observed after local irradiation of the hind legs. After the initial drop, the blood pressure recovers and remains somewhat below normal for some days and then gradually declines again. In the terminal stage the arterial pressure often is very low (456). During the fall in blood pressure the plasma histamine level is increased in the rat and the rabbit (456, 491). Irradiation causes an immediate but transitory reduction of the rate of blood flow through the ear of the rabbit (493).

An initial hypotension has not been observed in the dog. The arterial pressure remains essentially constant until the acute terminal period (see, e.g., ref. 494). After exposure to massive doses of X-irradiation over the abdomen or over the whole body a shocklike state has been observed in dogs (495, 496).

A slight increase in blood pressure has been recorded during irradiation of the cardiac area and of the midbrain. Irradiation of the whole head, on the other hand, causes a decrease which can be prevented by vagotomy (77).

Shielding of the kidneys reduces the incidence of increased vascular sensitivity to irradiation in the second postirradiation week in rats (497). The shielded animals are in better general condition than the nonshielded

animals. The vascular hypersensitivity is probably due to the general debility rather than to a renal vasoexcitor material, as local irradiation of the kidneys fails to increase vascular sensitivity.

C. LYMPHATIC SYSTEM

The effects of ionizing radiation on the lymphatic system are little known. A dilatation of lymphatics has been observed in the skin after irradiation, and some signs of an accelerated cutaneous lymph flow in irradiated rabbit skin during the first hours after exposure have been presented (456). No significant changes occur in the output of lymph from the thoracic duct of the cat (154). Several days postirradiation an increased lymph flow has been noted in the dog (155).

X. Alimentary Organs

Under this heading will be discussed the gastrointestinal tract, the salivary glands, and the liver and pancreas. The various parts of the alimentary system show rather great differences in radiosensitivity. The stratified squamous epithelia lining the gastrointestinal tract are of the same sensitivity as the same kind of epithelium in the skin (179). The intestinal mucosa has been found to be much more sensitive than the gastric mucosa. Furthermore, it was early noted (390, 498) that the small bowel is more radiosensitive than the large one. Lesions have been observed even during the first hour after exposure (499-501). The most impressive changes are located in the epithelial cells, although the effects are not confined only to them.

Early extensive gastrointestinal damage followed by death within several days is common after a massive dose of radiation to the abdomen or to the whole body (496, 502, 503). The intestinal injury is generally considered to be responsible for the toxicity resulting from such exposure. It has been found that the mean survival time of mice after exposure to doses within the range of 1000 to 12,000 r is 3 to 4 days and that early death occurs only if a large portion of the intestine has been irradiated (503). After lower doses several competing mechanisms contribute to the lethal outcome. In the median lethal dose range mortality is related in time to a bacteremia of intestinal origin (504).

Nausea, vomiting, and anorexia in radiation sickness were first described by Walsh in 1897 (505).

A. GASTROINTESTINAL TRACT

Krause and Ziegler (506) seem to have been the first to report changes in the gastrointestinal tract after irradiation. They observed degenerative alterations in the intestinal mucosa. The most significant early observa-

tions, however, were those of Regaud *et al.* (498) and of Hall and Whipple (502) and Warren and Whipple (507). After irradiation of the abdomen of dogs, differences in the radiosensitivity of the epithelial elements and in the various parts of the system were observed by Regaud *et al.* (498). In the stomach the zymogenic cells were found to be more sensitive than the parietal cells, and in the intestines the basal cells of the crypts of Lieberkühn were more vulnerable than the villous epithelium. A number of later investigations (499, 501, 502, 508-510, and others) have essentially confirmed these results. Warren and Whipple (507) and Warren (359) found that the dog, the cat, and the rabbit are more sensitive than the guinea pig and the rat as far as the gastrointestinal tract is concerned.

1. UPPER PART OF DIGESTIVE TRACT

Reports about the effects of irradiation of the upper part of the digestive tract are relatively few. The stratified squamous epithelium of the mouth and esophagus shows changes similar to those in the skin. Differences in sensitivity in the various parts of the mouth (tongue, inner surface of the cheeks, nasopharynx) have been described by Coutard (511). Large doses may cause necrosis of the epithelium and underlying tissues.

2. STOMACH

The changes occurring after irradiation of the stomach are of two main types, namely: (1) functional and degenerative morphological changes and subsequent repair beginning shortly after the exposure, and (2) development of gastric ulcers some weeks later (109).

Destructive changes in the stomach and intestines may be seen as early as 30 minutes after exposure to moderate doses (501). They are most pronounced after 8 hours. After a single exposure to 500 to 800 r similar effects are found in mice, rats, rabbits, and chickens. Nuclear swelling, pyknosis, and fragmentation is marked as early as at 3 hours in the gastric pits and intestinal crypts. Also Warren and Whipple (507) and Tsuzuki (499) reported nuclear changes and destruction very early after irradiation.

The first few days after irradiation are characterized by hyperplastic regenerative activity with continued degeneration of many cells. At 21 days after 500 to 800 r all the mucosae, possibly with the exception of the duodenal crypts, are normal. The damage is greatest in duodenum, least in colon and rectum.

A dose of 50 r has no visible effect on the gastrointestinal canal of rabbits, but after 100 r a slight damage occurs. A dose of 800 r of 200-kv whole-body irradiation in rabbits causes early degeneration and temporary absence of mitoses in the necks of the fundi glands. The surface epithelium and the cardiac and pyloric glands are more resistant. Some parietal cells of the

fundí glands may show wrinkling of the nuclear membrane or pyknosis and vacuolization of the cytoplasma during the early postirradiation stage.

The production of gastric ulcers by irradiation has been reported by some authors (512-515). The ulcers develop after a latent period of several weeks. They often appear singly and are located almost entirely in the corpus ventriculi along the lesser curvature. Ulcers often appear in the stomach and also in the duodenum after intensive irradiation of the epigastrum.

Irradiation of the stomach reduces the gastric acidity. After large doses extensive atrophy of the gastric glands develops (516-521). According to Desjardins (522), irradiation causes a marked decrease in gastric secretion. A marked decrease is also observed in the pepsin content, but its composition is only little altered. In dogs, the parietal cells, which are presumably the source of hydrochloric acid precursors, are only slightly damaged by irradiation, in contrast to the greater loss of zymogen granules in the chief cells (508). The secretory depression which has been observed after exposure to X-rays, β -rays, and neutrons is probably a local effect. The peptic activity of the gastric juice is diminished but generally not to the same extent as the acidity (517, 518). Uspenskif (523) noted an early acid secretion after irradiation. One to three days later, a phase of secretory inhibition occurs. This in turn is followed by a phase of spontaneous gastric secretion with lower acidity.

The mechanism of functional disturbances in the gastrointestinal tract after irradiation is relatively little known in spite of a number of investigations during recent years. The results are, however, diverging. It has been found that irradiation is followed by a delayed gastric emptying (see, e.g., refs. 518, 524-526). Direct exposure of the stomach itself is not necessary to produce delayed emptying. Irradiation of the upper or lower half of the body, however, causes less retardation of gastric motility (525). Gastric retention rather than altered intestinal motility is supposed (524) to be responsible for the marked reduction in amount of material available for intestinal absorption after exposure to minimal lethal doses of radiation. Lethal irradiation results in progressive distension of the stomach with fluid (527).

Kimeldorf (528) observed in rats exposed to 85-kv or 250-kv X-irradiation, after an initial latency, a depression in motility which was manifested in the amplitude rather than in the frequency of the contractions. The change in motility was evident within 5 to 9 minutes after the start of the exposure and lasted for a duration which was related to the dose. The minimum effective dose was 1000 r. According to Kimeldorf, the depression of gastric motility during irradiation exposure appears to be considerably less radiosensitive than other gastrointestinal responses such as increased food retention.

Anorexia and weight loss have been observed in a number of species after exposure to ionizing radiation. These changes are fairly good indicators of the severity of irradiation (454, 529). Anorexia, vomiting, and diarrhea are, however, generally not observed in animals after exposure of parts of the body remote from the abdomen, and they are, therefore, supposed to be due to a primary injury to the gastrointestinal tract. One exception is exposure of the head as demonstrated in rats by Smith *et al.* (530). They advance the assumption that the early anorexia may be of a neurogenic or humoral origin. Also the delayed gastric emptying as a result of the increased pylorus sphincter tonus may be responsible for the anorexia.

A transient diminution in food intake in the rat has been noted after a dose as low as only 50 r of whole-body irradiation (530). After 250 to 1000 r the food intake decreases to about 10% of normal. After administration of a median lethal dose the food intake may return to the normal level after a few days, but it decreases again several days before death.

3. INTESTINE

The duodenum is the most sensitive part of the intestinal tract (531). In rabbits exposed to 800 r of total-body X-irradiation, marked degenerative changes in the duodenal epithelium are seen by 30 minutes after the exposure, and they persist for 3 weeks or more (501). They consist in varying degrees of nuclear swelling and clumping of chromatin in the cells of the villous, crypt, and Brunnerian epithelium. The most extreme damage is located to the basal cells of the crypts of Lieberkühn. They are the site of nuclear fragmentation and karyolysis for some days. The Paneth cells lose their staining properties within 2 hours after irradiation and display irregular granulation. The epithelium of the Brunner's glands is more resistant than the villous or crypt epithelium.

A whole-body exposure to 800 r causes less severe injury to the ileum than to the duodenum. Qualitatively the changes are similar, but recovery is more rapid in the ileum. The degenerative changes are most marked in the basal crypt cells. Early effects seen in the lamina propria are edema and absence of inflammatory cellular reaction. The effects on the lymphatic follicles of the ileum and the appendix are similar to those encountered in other lymphatic tissue. Even if the crypt epithelium is the most sensitive, destruction of the entire intestinal lining can occur after lethal doses, leaving fragmented crypt cells, denuded villi, edema, hemorrhage, and ulcers. In young animals, the extent of radiation damage to the small intestine is greater, and the injury occurs earlier than in older animals (532).

Doses of 50 r or less have no effects on rabbit intestine. After 100 r there is only a depression of mitosis, and occasional dead crypt cells. A dose of 400 r causes less severe damage than 800 r, with fewer cells affected and a

more rapid recovery. Large doses (2000 r) to the abdomen of dogs lead to extensive intestinal damage with severe hemorrhages and ulceration and death of the animal (390, 498). Intestinal crypt abscesses are the earliest recognized sign of ulcerative colitis.

The colon is believed to be as radiosensitive as the stomach, and the rectum is said to have about the same sensitivity as the mouth.

External whole-body exposure to fast neutrons of mice (96 n) and rabbits (117 n) and of mice to slow neutrons gave the same qualitative alterations in the intestinal mucosa as whole-body X-irradiation (501).

No changes were observed in the intestines of mice which were exposed daily to 8.8 r of γ -rays during an examination period of 2 to 16 months (501). Daily total-body exposures to 80 r of X-irradiation caused mild damage after the first few exposures, but after twenty to thirty-five exposures the mucosa was normal. Recovery thus was complete even after a total dose of more than 2800 r. After fractionated doses some radioresistance may be acquired by the crypt epithelium (533).

Intestinal motility and tonus are increased by irradiation (502, 534-537). After small X-ray doses, tonus returns to the pre-exposure state a few minutes after the exposure. Increased contraction and hyperactivity are largely prevented by parasympatholytic drugs and ganglionic blocking agents (537). Body shielding or vagotomy affords only a slight reduction of the intestinal response to irradiation. It is supposed that this response is due to a direct action of the radiation on cholinergic elements in the intestinal tract. Radiation damage in the small intestine is often characterized by intestinal occlusion.

A decreased intestinal absorption has been observed by several authors after irradiation (534, 538, 539), whereas others (e.g., ref. 540) found no basic disturbances.

Respiration of the small intestine is reported (539) to be inhibited several hours after exposure of rats to 700 r. Oxygen consumption is normal initially but later on depressed. Nucleic acid, protein, and ash content of the crypt epithelium is decreased soon after X- and neutron irradiation (540-542). The rate of synthesis of DNA and RNA in the intestine of rats and rabbits is substantially reduced after X-irradiation, but the protein synthesis is relatively unaffected (543).

Inhibition or decrease of glucose, fructose, and xylose absorption (544) and a diminished phosphorylation of fructose have been demonstrated as early as 4 hours after X-irradiation (539). Detrick (545) found that the glucose absorption by the perfused surviving rat intestine was greatly inhibited during the period 3 to 6 days postirradiation, although the gut appeared histologically normal.

An increase in fecal fat content after irradiation has been observed by

Mead *et al.* (546). This was stated to be due to intestinal desquamation as a consequence of the irradiation. An increase was also evidenced by Morehouse and Searcy (547), whose observations are indicative of a real stimulation in fecal fat synthesis. In contrast to these reports, Coniglio *et al.* (548) have noted a decrease in fecal fat after radiation exposure. This was correlated to the lowered food intake during the postirradiation period. A reduction in the rate of intestinal glucose absorption which could not be attributed to retarded gastric emptying or to a diminished intestinal hexokinase content was found by Dickson (549).

X-Ray doses of 650 to 2000 r increase the rate of flow of water and sodium from the blood into the intestine. The flow in the reverse direction is decreased (550). The net flow in irradiated animals is from blood to intestine, whereas the opposite is the case in nonirradiated animals.

Ileal and jejunal nonspecific cholinesterase activity levels are reduced after irradiation. At 48 hours' irradiation with 650 r, the activity is lowered in rats (551), and activity values 50% of normal have been measured (552). It is also reduced in guinea pigs after 250 r, but not in rhesus monkeys at 48 hours and 7 to 9 days after 800 r (551).

A retention of certain bacteria in the irradiated gut has been demonstrated (553). The normally predominant lactobacilli decrease in number, but the coliforms and other strains increase after total-body irradiation of rats (554). The irradiation itself is responsible, at least in part, for these effects. Often there is a marked accumulation of bacteria on the surface of the mucosa.

Internal Irradiation. The alimentary tract is one of the main routes for the internal administration of radioactive isotopes to the body. All radioisotopes administered *per os* may exert radioactive effects in the gastrointestinal tract. Generally, they do not accumulate there but are absorbed to be located in other critical organs, or excreted again. Internally administered radioactive isotopes affect the gastrointestinal tract epithelium in essentially the same way as do X-rays and other kinds of external radiation (501). There are, however, some differences. Thus, with most of the parenterally administered isotopes, there is no massive destruction of the epithelium as after a single dose of 800 r of X-rays.

Alpha emitters such as radium and plutonium seem to cause more nuclear swelling than do β - and γ -emitting isotopes. The changes in the gastrointestinal tract after administration of α -emitters occur at later intervals and with lower doses than with β -radiating isotopes (501).

Intracardial injection of Zr⁹³-Nb⁹³ causes substantial destructive lesions in the small intestine, with extensive areas of sloughed epithelium. Also parenterally administered Na²⁴ and Ba¹⁴⁰-La¹⁴⁰ cause severe damage to the gastrointestinal mucosa. P³² (2.5 μ c/gm), on the other hand, produces only

moderate damage. After administration of Y^{91} (20 $\mu\text{c}/\text{gm}$) less damage is found, and after Sr^{89} (3.6 $\mu\text{c}/\text{gm}$) still less (501). After intragastric administration of Ru^{106} chloride the largest radiation dose is delivered to the lower part of the large intestine (555). The doses to other parts of the gastrointestinal tract are lower by as much as a factor of 5.

Large doses of mixed fission products (23 to 33 $\mu\text{c}/\text{gm}$) were found to cause extreme necrosis of the mucosa in the rat, most marked in the lower ileum and colon (501). Smaller single doses of mixed fission products or of Y^{91} , or of daily doses of Y^{91} as high as 2 $\mu\text{c}/\text{gm}$ for 3 months, produced only minor changes.

B. SALIVARY GLANDS

According to Desjardins (556) the salivary glands are exceptionally sensitive to irradiation. The serous cells are more radiosensitive than the mucous ones, and both are more sensitive than the duct epithelium (557). Severe irradiation damage may end in fibrotic degeneration of the gland.

The reports in the literature of the effects of irradiation on the salivary glands are scant. The most complete studies have been performed by English and his collaborators.

Submaxillary glands of rats examined at 16 hours to 100 days after exposure to 1000 to 1500 r of X-rays showed changes consisting primarily in alteration in size, shape, and staining characteristics of nuclei of the acinar cells. Changes were also observed in other salivary glands, but they were not so characteristic as in the submaxillary (558). Within 2 to 3 days after exposure to 3000 to 5000 r a significant increase was observed in the glucose-6-phosphate dehydrogenase and isocitric dehydrogenase in rats when the activities were related to the total protein content in the submaxillary and sublingual glands (559). The total activities for the whole salivary gland did not increase, however, because of a weight loss of the gland after the exposure. The total activity of glucose-6-phosphate dehydrogenase remained normal, whereas isocitric dehydrogenase was significantly decreased between the fourth and fourteenth days but tended to rise toward the end of the 20-day observation period.

Rats have also been irradiated with the body shielded with the exception of the salivary glands (560). After 2000 r the values of glucose-6-phosphate dehydrogenase were higher in the irradiated animals. No significant alteration of isocitric dehydrogenase was found after irradiation of the exteriorized salivary gland.

Local irradiation of the head of dogs with 1750 r of X-rays caused considerable morphological changes in the salivary glands (561). The parotid, submaxillary, and sublingual glands were atrophied, fibrosed, and altered in morphological details. A dog sacrificed 68 weeks after the exposure to 1000 r had, however, relatively normal-appearing salivary glands.

C. LIVER

As judged from morphological studies, the liver is remarkably radio-resistant (529, 562-565), although minor cytological changes have been observed. This may be attributed, in part, to the exceedingly large regenerative capacity of the liver, and partly also to the low oxygen tension of liver tissue.

Pohle and Bunting (564), for example, exposed the liver of adult rats to doses of 600 to 2500 r of X-irradiation. This gave no incidence of liver necrosis. Minute cytoplasmic changes have been reported. Temporary atrophy of liver cells may occur. Studies by Rhoades (565) in mice, rats, and guinea pigs which had been exposed to total-body irradiation with 25 to 1200 r confirm these findings. The changes that occur may not be due to the primary effect of the irradiation on the liver cells but are rather secondary to general toxicity. Liver necrosis has been observed after exposure of the liver of dogs to 1880 to 5250 r of X-irradiation (566).

With a dose of 50 r daily to rats, the animals survived more than 40 days in good conditions. Finally, their liver showed fatty degeneration (567). White rats exposed to daily partial doses of 38.4 r of γ -irradiation displayed a reduction of body weight, increase of liver weight supposedly due to an increased metabolism of proteins in the liver, and a great increase in fat metabolism. A significant increase in liver mass has been found (568) in rats as early as 6 hours after exposure to as small a dose as 200 r of X-rays. Contributing factors to this increase were water, lipids, and glycogen, the most striking change being in the glycogen content. After a total-body dose of 1200 r the increase in glycogen accounted for two-thirds of the total increase in the solid constituents of the liver. Also North and Nims (569) noted an increase in liver glycogen during the first 2 days after whole-body irradiation, although there was a decrease in liver cholesterol. These changes have been supposed to be indicative of adrenal cortical activity.

Rats kept on a low-protein diet which survived 450 to 500 r of whole-body irradiation (53 % survival) developed cirrhosis of the liver (570). No cirrhosis was found in irradiated animals fed an adequate diet.

The increase in liver glycogen is possibly bound to fasting (571), as other experimenters have instead found a reduction in liver glycogen after irradiation (572-574). Levy and Rugh (575) exposed hamsters and mice to severe and continuous radiation until death. In the hamsters this meant a dose of about 110,000 r received in about 3.5 hours. Liver glycogen was drastically depleted in these animals.

X-Irradiation of the liver region of mice with up to 12,000 r (576) reduced the liver weight relative to the body weight. The size of the liver cells decreased principally to a decrease in the content of lipids. The fat was found principally in cells at the center of the liver lobules, in con-

trast to the nonirradiated animals in which the fat is equally distributed. Peripheral cells, nearer the portal areas, are more sensitive to irradiation than centrally located cells.

The conversion of acetate-C¹⁴ to lipid-C¹⁴ in liver slices prepared 24 hours after whole-body irradiation was increased as much as twentyfold in rats and twofold in guinea pigs (577, 578). The changes were roughly correlated with the liver glycogen levels. No changes were observed in mice. It is supposed that the increase in blood lipid levels after X-irradiation reflects these changes in liver lipids. A nonspecific fatty infiltration is sometimes seen. Sudanophil fat thus has been found in the mouse liver after irradiation (579). It has been attributed to the release of histaminelike tissue breakdown products.

Doses of 1000 r cause a significant decrease in the oxidative phosphorylation in rats but have no effects on citrulline synthesis (580).

Changes in the liver mitochondria have been found in mice after exposure to 500 to 1200 r. Their structural stability decreased and they become vesiculated, globulated, and fragmented and also decreased in numbers (581). Local as well as whole-body irradiation affects the enzymatic activity of isolated liver mitochondria from adult rats (582). Although the succinodehydrogenase activity remains unaltered, the oxidation of pyruvate is reduced, and this entails the decrease in the oxidative phosphorylation.

Small doses of roentgen rays given during the early stages of liver regeneration show an immediate effect in arresting mitosis, and later on an inhibition of the DNA synthesis (583). This has also been found after exposure of regenerating rat liver to γ -irradiation (584). On the other hand, there was an increased rate of incorporation of P³² into cytoplasmic RNA. The changes in DNA and RNA synthesis are, however, inconsistent, and Gershbein and Krotoszynski (585) found no significant changes in the amounts of DNA and RNA per unit weight of fresh tissue after irradiation of the exteriorized rat liver with 6500 r of X-rays. The ribonuclease activity of whole liver displays no significant changes in whole liver homogenates of rats which have been exposed to 600 r of whole-body X-rays (586). In the separated liver mitochondria there is a significant decrease in RNAase activity.

Total-body exposure of rats to 500 r of X-irradiation has no effect on nonmercapto enzymes such as catalase, alkaline phosphatase, esterase, arginase, and rhodanese in liver (587). Most sulphydryl enzymes in the liver are not affected even by large doses. A dose of 700 r has only little effect on the concentrations of oxidized and reduced DPN, but a dose of 9800 r has been found to lower the level of reduced DPN by 15 to 30 %. The amount of oxidized coenzyme is not significantly changed even after

this dose (588). The results of irradiation on liver enzymes are, however, not consistent. Thus, in a study of the effects of a whole-body exposure of mice with 600 r, Hori (589) found a strong decrease in the activity of catalase and oxidative phosphorylation and an increase in alkaline phosphatase, succinodehydrogenase, choline oxidase, cytochrome oxidase, and deoxyribonuclease. No changes were found in ribonuclease, arginase, esterase, and tyrosinase.

After exposure of rats to thermal neutrons from a nuclear reactor, the level of coenzyme I in liver was temporarily depressed. Pre-exposure levels were re-established by the fourth day postirradiation (590). Exposure to fast neutrons, on the other hand, induced a rise in coenzyme I. The increase appeared to be related to the intensity of the higher-energy flux.

Whole-body irradiation with 500 r inhibits the sulfanilamide acetylation in rat liver by 50% (591). Pretreatment with cysteamine and pantothenic acid increased the survival rate but did not influence the rate of acetylation.

An approximately twofold increase in total plasma amino acid concentration is seen at 1 hour after irradiation with large doses (592), although there is a decrease in the total plasma protein content. Some hours after irradiation the amino acid level in plasma may be even higher. This increase may be attributed to the decrease of the utilization of amino acids in the liver (593). The alterations observed in serum proteins after irradiation generally are, however, not associated with significant changes in liver function (594).

An abnormal liver function after irradiation may be indicated by the terminal rise of kynurenic acid excretion, which suggests some alteration of the tryptophan metabolism, and further by an augmented urinary excretion of coproporphyrin and urobilinogen. Some decrease in the output of bile salts has been observed after large doses administered to the liver of dogs (595).

Internal Irradiation. A number of radioactive isotopes accumulate in the liver. The most important of these isotopes are tabulated in Table III. On the whole, the effects of internal irradiation on the liver are relatively little known.

The distribution of P^{32} , Sr^{89} , Y^{91} , Ba^{140} - La^{140} , and Ce^{144} - Pr^{144} , when internally administered, is diffuse and homogeneous (565). There were only slight morphological changes in the livers studied by Rhoades (565), despite the deposition of the radioactive isotopes within the liver tissue. Radium and especially plutonium, on the other hand, tended to be more unevenly distributed and to collect in clumps. Plutonium and yttrium may cause the occurrence of enormous liver cells with budding nuclei some months after the administration of the isotope. Many of the dividing nu-

TABLE III
RADIOACTIVE ISOTOPES THAT ACCUMULATE IN THE LIVER

Radioisotope	Physical half-life (days)	Effective half-life (days)	Maximum permissible body burden (μc) (ICRP)	Maximum permissible concentration in (ICRP)	
				Water ($\mu\text{c}/\text{cc}$)	Air ($\mu\text{c}/\text{cc}$)
Sc ⁴⁶	85	13	5	0.3	5×10^{-8}
Sc ⁴⁷	3.43	2.8	11	3	6×10^{-7}
Sc ⁴⁸	1.83	1.6	3	1	3×10^{-7}
Mn ⁵⁶	0.108	0.106	8	0.4	4×10^{-6}
Co ⁶⁰	1.9×10^3	8.4	3	2×10^{-2}	10^{-6}
Ni ⁵⁹	9.1×10^7	8	42	0.3	2×10^{-5}
Cu ⁶⁴	0.54	0.53	120	6×10^{-2}	5×10^{-6}
Ag ¹⁰⁵	45	2.8	19	2	10^{-5}
Ag ¹¹¹	7.5	2.1	39	5	3×10^{-5}
Cd ¹⁰⁹ -Ag ¹⁰⁹	330	77	45	7×10^{-2}	7×10^{-8}
Ta ¹⁸²	111	60	6	10^{-1}	2×10^{-8}
Au ¹⁹⁶	5.55	5	8	5×10^{-2}	2×10^{-7}
Au ¹⁹⁸	2.69	2.6	3	4×10^{-2}	2×10^{-7}
Au ¹⁹⁹	3.3	3.1	9	9×10^{-2}	4×10^{-7}

clei show polypliody. After intravenous administration of ThO₂ (Thorotrast) which lodges in the Kupffer cells, liver necrosis may result.

D. PANCREAS

Both the exocrine and endocrine parts of the pancreas are markedly radioresistant. Various kinds of irradiation ranging in doses from 10 r to more than 1000 r of external or internal sources produce no morphological alteration (109, 565). Seino (596) reported degeneration of the pancreas in rabbits after exposure to 750 r, and scarring after 1000 r. This is, however, not in agreement with the findings of other authors.

There seem to be no radioactive isotopes which have a selective accumulation in the pancreas. After injection some isotopes (Y⁹¹, Ra, Pu²³⁹) have, however, appeared in the pancreas (565). After dosages of less than 1.0 $\mu\text{c}/\text{gm}$ there was no deposition of any of these isotopes in the pancreas. Plutonium is deposited in the organ after intravenous injection but not after intramuscular.

The volume of pancreatic secretion has been found to decrease temporarily after irradiation in dogs (597). There was no definite alteration in

the alkalinity of the pancreatic juice after doses of 400 to 1000 r. Exposure to 400 to 600 r, however, markedly depressed the output of amylase, lipase, and trypsin as observed at 12 to 36 hours post-irradiation. These functional alterations were not correlated to any observable histological changes.

Doses of 500 to 2000 r of whole-body X-irradiation in mice do not destructure the mechanism responsible for the synthesis of the specific protein amylase (598). Amylase levels and synthesis were considerably increased at 24 hours after 2000 r. The increases are not related to the nutritional state of the animal. At least partly they may be due to an initially increased cholinergic stimulation of the pancreas after irradiation.

No changes in the RNA concentration or in the *in vitro* incorporation of P³² into RNA occurred after irradiation. The hyperglycemia seen after irradiation of the pancreatic region is probably not due to a primary effect of irradiation on the pancreas.

E. CARCINOGENESIS

1. GASTROINTESTINAL TRACT

Tumors of the intestinal tract in rats and mice are rare. Even after whole-body exposure to ionizing radiations, including X-rays, γ -rays, and fast and slow neutrons, the incidence of intestinal tumors in mice is less than 1% (149, 599).

Significant numbers of intestinal neoplasms, however, have been reported in rats after very high doses of radiation to the bowel mucosa produced either by feeding radioactive yttrium (600), by localized irradiation with 190 Mev deuterons (601), or by lethal doses of whole-body X-irradiation followed by parabiosis postprotection (602).

Nowell *et al.* (603) have shown that exposure of mice to sublethal doses of fast neutrons results, as a late effect, in a high incidence of intestinal cancer. In contrast, mice exposed to even higher doses of X-rays developed no intestinal cancer. This marked difference has been extended to a study involving the production of cancer in the stomach by these radiations. Mice were subjected to neutron irradiation (270 to 520 rep); 36% developed precancerous lesions of the stomach lining, whereas only 9% of X-irradiated mice (800 r) developed such lesions. The finding that these lesions in the X-irradiated group were associated with severe degeneration of stomach blood vessels in two-thirds of the cases is of greatest interest. Such lesions were, however, noted in only one of the neutron-irradiated mice. The result suggests that fast neutrons exert a direct cancer-producing effect on the lining of the mouse stomach, whereas with X-rays any gastric tumors formed are produced, at least in part, by indirect local blood vessel damage (604).

2. LIVER

Induction of malignant liver-cell tumors has not been described in animals, but there is some evidence that massive doses of ionizing radiation may cause benign hepatomas in mice.

Lorenz (150) noted an increased incidence of hepatomas in mice after chronic γ -irradiation. Also after irradiation in thermal column and by X-rays the frequency of hepatomas in RF mice was slightly increased after whole-body irradiation (148).

In the investigation by Neary *et al.* (356) on the effect of chronic neutrons the histological examination of the hepatomas suggested an invasive habit of growth, but in only one mouse in the highest dose level group, 6.4 rads weekly, were multiple metastases certainly identified in the lungs.

After intravenous injections of 40 to 160 μ c of colloidal Au¹⁴⁸ into mice no early death occurred, but cirrhosis was produced in 85% of the C57 BL mice and 90% of the RF mice. The cirrhosis was accompanied by bizarre nuclear abnormalities and cytoplasmic degeneration of liver cord cells with nodular regeneration and occasionally cholangiomatoid hyperplasia. Hepatomas occurred in 15% of the C57BL mice and 32% of the RF mice (605).

XI. Urinary Tract

A. KIDNEYS

The kidneys are probably rather resistant to ionizing radiation, but the statements in the literature are not in accord (for reviews see, e.g., refs. 606-610). Total-body irradiation of rabbits and rats with 600 to 800 r or of mice with 300 to 400 r of X-rays does not cause any visible morphological renal changes (610). Usually doses of several thousand roentgens are necessary to cause renal damage (609), and kidney damage is generally not seen in total-body-irradiated animals (502, 529, 610, 611). Impiombato (612) could not produce any renal damage in rabbits with doses lower than 2000 r, and Warren (608) assumes the threshold dose for effects on the kidney to be 3000 to 5000 r. After doses of 3000 r or more, secondary renal effects are encountered, owing to vascular damage. The least radioresistant part of the kidney is the proximal convolution (184).

Neutron irradiation with 117 n of fast neutrons or 400 arbitrary units of slow neutrons has no effect on the kidneys (610).

Daily exposures in a few cases of mice to 80 r of X-irradiation produced no kidney abnormalities even after thirty-five daily doses of 80 r (610).

Some renal changes have been described by Antopol and Glaubach (613) in C57BL mice that had received 600 r of total-body irradiation. They had large pyknotic nuclei in their glomeruli, and there was a progres-

sive increase in intercapillary material. Animals that died had arteriosclerotic and necrotic arteries in the kidneys and progressive glomerulosclerosis. After irradiation with large doses (4700 r) a renal lesion consisting in tubular degeneration, vascular injury, and interstitial scarring with slight glomerular hyalinization has been described (614). The picture was similar to that of an early hydronephrosis.

Kidney shielding was found to reduce the incidence of increased vascular sensitivity in irradiated rats which occurs in the second week after irradiation (497). This hypersensitivity is probably due to a general debility, as it does not occur after local irradiation of the kidney.

Local irradiation of the exteriorized kidneys with rather high doses (2500 r) of X-rays increased the urine flow 4 weeks after the exposure (614a). After 4000 r the filtration rate was 43% below normal 4 weeks after the irradiation. The renal plasma flow was unchanged after 2500 to 4000 r during the first week, but 4 weeks after 4000 r it was 51% below normal. It is concluded that both increase and decrease of renal function are results of a primary action of the irradiation on the kidneys.

The question of functional renal disturbances after irradiation are, however, somewhat controversial. Kidney function is generally believed not to be seriously disturbed after whole-body irradiation except in the last, agonal period (544). The most sensitive index of acute irradiation effect on the kidneys is the tubular function. The urinary excretion of water and nitrogen and the specific gravity of urine are not significantly changed after irradiation in dogs (77).

Polyuria has, however, been observed early after irradiation in rats and rabbits. In rats an increased excretion of sodium, potassium, and chloride has been reported to occur during the first days after exposure (615). In the terminal stage of radiation injury in dogs an increased content of nonprotein nitrogen and urea nitrogen of blood is an indication of impaired renal function. This is probably due to circulatory disturbances and renal hemorrhage.

The changes in the diuresis in total-body irradiation may also be due to an effect on the pituitary gland. Edelmann (616) observed a marked polyuria the first day after irradiation in intact but not in hypophysectomized rats. Kay and Entenman (617), on the other hand, found that increased diuresis was produced in head-shielded body-irradiated rats but not in head-irradiated rats. The adrenals, spleen, head, legs, and gut were found to be of no importance for the X-ray-induced diuresis. Shielding of the exteriorized kidney prevented diuresis, but the kidneys had to be irradiated before diuresis occurred. Pancreatectomy prevented diuresis.

Blood in the urine after irradiation is usually a sign of renal damage located in the glomerular and tubular epithelium. A less severe injury to

the kidney is often manifested by an increased amino acid content in the urine. Thus, amino aciduria has been observed in man (618) after 25 to 100 rep of γ -irradiation or neutron irradiation. Some evidence of an inhibition of kidney respiration and of oxidative processes requiring the presence of sulfhydryl enzymes has been presented by Barron (619).

Mendelson and Caceres (620) exposed one kidney after surgical removal of the other to 2010, 2750, and 3780 r of X-rays in dogs. After the larger doses the inulin clearance and renal blood flow temporarily increased and thereafter decreased to a significant degree. They suppose that the ultimate damaging effect of large doses of X-rays to the kidney probably is vascular in nature and affects whole nephrons.

In mice (LAF) exposed to ionizing radiation from a nuclear explosion fatal degeneration of the kidneys frequently occurred at dose levels above 500 r but was rare at lower levels (149). The degenerative process is always bilateral, and often it is associated with generalized edema. After exposure to higher dose levels nephrosclerosis is often a significant sequela (621-623).

Internal Irradiation. A number of radioisotopes may concentrate in the kidneys. In Table IV are given the data of the most important of these isotopes.

For some of the pertinent isotopes, e.g., uranium, the rate of excretion

TABLE IV
RADIOISOTOPES THAT CONCENTRATE IN THE KIDNEYS

Radioisotope	Physical half-life (days)	Effective half-life (days)	Maximum permissible body burden (μc) (ICRP)	Maximum permissible concentration in: ICRP	
				Water ($\mu\text{c}/\text{cc}$)	Air ($\mu\text{c}/\text{cc}$)
Cr ⁶¹	26.5	22	600	0.7	10^{-6}
Mn ⁵⁶	0.108	0.104	25	0.15	4×10^{-6}
Ge ⁷¹	11.4	3.9	72	10	4×10^{-6}
As ⁷⁶	1.12	1.09	11	0.2	2×10^{-6}
Tc ⁹⁹	4.3	2.1	5	3×10^{-2}	3×10^{-6}
Rh ¹⁰⁵	1.52	1.5	9	0.4	2×10^{-6}
Ru ¹⁰⁶ -Rh ¹⁰⁶	365	19	4	0.1	3×10^{-8}
Tc ¹⁰⁷					
Te ¹²⁷	90.4	13	4	3×10^{-2}	10^{-7}
Te ¹²⁹	32	10	1.4	10^{-2}	4×10^{-8}
Ir ¹⁹⁰	10.7	7.3	23	10^{-2}	8×10^{-7}
Ir ¹⁹²	70	17	3	9×10^{-4}	5×10^{-8}
Pt ¹⁹¹	3	2.9	2	6×10^{-3}	2×10^{-7}
Pt ¹⁹³	4.3	4.0	3	5×10^{-3}	2×10^{-7}
Au ¹⁹⁶	5.5	5	32	5×10^{-3}	2×10^{-7}
Au ¹⁹⁸	2.69	2.6	10	3×10^{-3}	$\times 10^{-7}$
U-natural (soluble)	1.64×10^{12}	30	0.04	10^{-4}	3×10^{-11}

is so slow that it could be anticipated that severe damage should be inflicted to the renal cells. It is therefore surprising to find only relatively few reports of renal damage after local internal irradiation. Thus, Bloom (610) was unable to find any clear evidence of damage due to irradiation after administration of Ra²²⁶, Pu, Zr⁹³-Nb⁹³, Po²¹⁰, P³², Sr⁸⁹, Ba¹⁴⁰-La¹⁴⁰, Ce¹⁴⁴-Pr¹⁴⁴, or Y⁹¹ to rats and mice. Thomas and Bruner (38) have reported that the kidneys of rats injected with radium showed changes of an acute parenchymatous nephritis. There were also numerous areas of calcification. Rats given 0.06 and 0.125 μ c of plutonium citrate per gram of body weight, and which died 2 to 4 weeks after the exposure, showed extensive acute necrosis of the proximal convoluted tubules (Lisco, cited in ref. 610).

Some of the radioisotopes that accumulate in the kidneys can cause damage to these organs not only through radiation but also through a pure toxic action on the glomeruli or the tubular epithelium (cf. uranium nephrosis).

B. URETERS, BLADDER, AND URETHRA

Statements in the literature of injury to the ureters, bladder, and urethra through irradiation are very sparse. The epithelium is resistant, but ulceration of the mucosa may be seen. If large numbers of cells have been expelled, the passages may be blocked by detritus masses. In the connective tissue an early transient erythema and a secondary erythema after 3 to 4 weeks may occur (109). In case of very severe exposure, the control of the bladder may be abolished.

C. CARCINOGENESIS

The information on tumors induced by radiation in the urinary tract are very scarce. Spargo *et al.* (106) report an adenoma of the kidney which appeared 14 months after 8.8 r daily of γ -irradiation of mice. Also Berdjus *et al.* (624) have observed, among late effects of whole-body irradiation on mice, adenomas in kidneys ($\sim 8\%$) whose pathogenesis could be traced through a number of well-defined stages.

After intravenous injection of 10 μ c/kg of polonium, neoplasms have occurred including benign and malignant nephromas and a fibroscarcoma (625).

XII. BONE AND CARTILAGE

A. BONE TISSUE

Bone tissue is generally believed to be relatively radioresistant (300, 626). There exists, however, a number of observations which indicate that bone tissue damage may occur even after a rather low dosage. This is es-

pecially the case in young individuals. The fetal skeleton has been found to be highly radiosensitive (627).

Especially the structures responsible for bone growth may be severely damaged by radiation. The growth of the long bones may be inhibited. This is seen, e.g., in the rat after the LD₅₀₍₃₀₎ of 600 r (628). The epiphyseal cartilage of the long bones is often separated from the spongiosa, and the growth in length of the bone is temporarily stunted. After completion of growth, the bone is more resistant. The characteristic feature of radiation damage to bone is the maintenance of the integrity of structure with eburnation and devitalization (629), but without leukocytic reaction (304, 630). This maintenance of structure even after heavy irradiation exposure is due partly to the special structure of bone tissue, partly to a slowing down of its metabolic processes (630). Massive bone necrosis even after moderate doses, has, however, been reported (626). Necrosis is especially likely to occur in the jaw bone (631) after administration of radioactive isotopes. Spontaneous fractures after irradiation have been reported by a number of authors (e.g., 38, 632-634). There are considerable species variations in the sensitivity of bone to ionizing radiation (635-637).

External Irradiation. A single dose of 400 r of X-irradiation administered to the whole body of rats reduces the number of osteoblasts. After 600 r, i.e., the LD₅₀₍₃₀₎, the continuity of the epiphyseal cartilages of femur and tibia with the spongiosa was often interrupted (628). An initial excessive hypertrophy of cartilage cells in the ossification zone occurred at 3 days after the exposure. A week later there was a complete separation between cartilage and spongy bone. Some dead osteocytes appeared in the lamellae of the spongiosa but not in the dense cortical bone. Osteoblasts disappeared but reappeared after some weeks. Recovery was generally complete after about 2 months.

A dose of 175 r of X-rays given to guinea pigs does not significantly affect the skeleton. In mice given 350 r of X-rays, there was no evidence of dead osteocytes or osteoblasts. Growth of bone length was unaffected (628).

After repeated doses of 80 r/day administered to mice, bone cells and bone-forming cells were undamaged, and active growth in length continued when the total dose was 720 r (9 days) (628). At 24 and 35 days (1920 and 2800 r) the bone growth had ceased, which, however, was not solely attributed to the irradiation, as the normal bone growth was finished at this time. A daily dose of 8.8 r of γ -irradiation from a radium source had no visible histological effect on the skeleton of guinea pigs when administered repeatedly from eleven to eighty-two times (628).

Local irradiation of rat leg bone with 2000 r of X-rays does not alter the bone ash percentage, indicating that there is no decalcification (638).

The growth of bone per unit body weight is disturbed. The retardation in osteogenesis after this dose is permanent and irreversible. A single dose of 5000 r of X-rays given to the femurs of guinea pigs was found to cause a necrotic process involving the osteocytes (639). It was followed by complete osteonecrosis. No degenerative signs in the blood vessels were observed at that time. After about 2 weeks there was a reaction in the periosteum and in the periosteal osteoblasts. The main cause of the osteonecrosis is supposed to be a primary effect of radiation on the osteocytes.

A dose of 5000 rep of β -rays from an external P^{32} source to a section through the paw of a mouse resulted in breaking away of osteoblasts from the endosteum which were seen as isolated cells in the outer layer of the marrow cavity (628).

The histopathologic changes in bone after exposure of various kinds of ionizing radiation have been summarized by Copeland (630) and Warren (304). The characteristic result is the disappearance of the osteoblasts and the osteocytes, giving the lacunae in which they were located the appearance of empty spaces. After very high doses (more than 100,000 r) the osteoblasts disappear within the first few hours after the irradiation. Abnormal spaces may be seen about the lamellae, with enlargement or obliteration of the canaliculi. The osteoclasts are somewhat more sensitive than the osteoblasts. A damage to the osteoclasts may at least partly account for the eburnation due to a reduction in the resorption of the bone. Sometimes, although more rarely, the damage is instead manifested as a rarefaction as a consequence of excessive resorption. Halisteresis rather than osteoclastic hyperactivity may be the cause in such cases.

The cells of the epiphyseal line are much more sensitive than are the osteocytes. The normal columnar arrangement becomes disorganized, and some cells are damaged and disappear. The changes in the osteocytes themselves are not very striking and consist in pyknosis and vacuolation before the cells disappear.

A characteristic feature after irradiation of bone tissue is the absence of any zone of demarcation between normal and irradiated parts. Owing to its poor circulation and low cellularity, irradiated bone is more easily infected than normal bone, especially if necrotic spots are present. There is no sequestration in the usual sense of this word, but necrotic bone, acting as an infected foreign body, may be sequestered. The periosteum is often edematous and fibrotic, and the vessels disappear. The osteoblastic layer is frequently obliterated. Healing of fractures is not significantly altered after moderate doses.

After local exposure of rat bone to relatively high doses (2000 r) of X-rays there is a reduced uptake of Ca^{45} in the irradiated leg (640). The alkaline phosphatase activity is sharply depressed, the depression being

closely parallel to the impairment of bone growth. This depression is probably a result of the destruction of the osteoblasts, as these cells presumably are the precursors of the enzyme in bone. The decreased uptake of P^{32} in bone after large single doses (2000 r) of irradiation (641) is probably also a consequence of this depression of the activity of the alkaline phosphatase. This effect is maintained 8 to 10 weeks after the exposure, when a slow recovery begins. The changes developed more slowly in the tibia than in the knee joint.

Internal Irradiation. The most important kind of irradiation with regard to bone damage is the internal one. This is due to the fact that a large number of the existing radioactive isotopes are "bone seekers" and thus concentrate in the skeleton when introduced into the body. In Table V the most important of the bone-seeking isotopes are tabulated. When long-lived radioactive elements, whether natural or artificial, have been incorporated into the skeleton, they are eliminated again only slowly. Meanwhile, they continuously irradiate the bone and the sensitive bone marrow cells, with danger of marrow destruction and leukemia, bone necrosis, and tumor formation.

A number of the bone-seeking isotopes are concentrated in the skeleton, primarily because of their metabolic similarity with calcium which is an essential constituent of the bone, as the bone salt is made up of hydroxyapatite. The deposition of other radioactive elements is due to the special physicochemical state of the bone salt. To the first group belong elements such as phosphorus, molybdenum, strontium, radium, uranium, and others. They are concentrated in the skeleton especially through an ion exchange process; i.e., calcium in the hydroxyapatite is exchanged for the radioactive element. As a consequence of this, the accumulation is larger in young individuals than in adults, and it is also dependent on the calcium uptake and calcium metabolism of the individual. An isotope concentrated in the skeleton by this mechanism is not firmly fixed but can be replaced and partly excreted again. The rate and amount of excretion depend on the growth status of the bone.

Elements belonging to the other group are, e.g., plutonium, yttrium, actinium, thorium, polonium, and zirconium. They are concentrated in the skeleton by means of colloidal adsorption. Thus the uptake of these elements in the skeleton is essentially independent of the age of the individual and of its calcium metabolism. When once they are fixed in the skeleton, there is no significant elimination of these isotopes.

The microstructure of bone and its ability to act as an ion exchanger has been the subject of extensive study in recent years (see reviews in refs. 642-644). Only a brief account of the mechanism of uptake of isotopes will be given here. For a more detailed treatise the reader is referred to the above-mentioned reviews.

TABLE V
RADIOACTIVE ISOTOPES WHICH CONCENTRATE IN THE SKELETON

Radioisotope	Physical half-life	Biological half-life (days)	Effective half-life (days)	Maximum permissible total body burden (μ c) (ICRP)
Be ⁷	53.61 \pm 0.17d	400 ^a	48	725
F ¹⁸	1.87h	140 ^a	0.078	5
P ³²	14.30d	1,200	14	10
Ca ⁴⁵	163.5 \pm 4.0d	18,000	151	14
V ⁴⁸	16.1d	50 ^a	12	10
Zn ⁶⁵	245.0 \pm 0.8d	23 ^a	21	400
Ga ⁷²	14.2h	3,000 ^a	0.59	3
Sr ⁸⁹	51d	4,000 ^a	52	2
Sr ⁹⁰	27.7 \pm 0.49y	4,000 ^a	2700	1
Y ⁹⁰	64.24 \pm 0.30h	>500 ^a	2700	1
Y ⁹¹	58.0d \pm 50m	<500 ^a	51	3
Zr ⁹⁵	63.3d	180 ^a	48	10
Nb ⁹⁵	35d	50 ^a	21	44
Mo ⁹⁹	2.85d	150 ^a	2.8	17
Sn ¹¹³	112d	149 ^a	44	84
Ba ¹⁴⁰	12.8d	120	12	1
La ¹⁴⁰	1.67d	35 ^a	1.6	7
Pr ¹⁴³	13.7 \pm 0.1d	50 ^a	11	6
Ce ¹⁴⁴	290d	350(500 ^a)	180	1
Pr ¹⁴⁴	17.5m	50 ^a	180	1
Pm ¹⁴⁷	2.52y	100 ^a	140	25
Nd ¹⁴⁷	11.3d	35 ^a	8.4	
Sm ¹⁵¹	98.5y	40,000 ^a	3.9 \times 10 ⁴	90
Eu ¹⁵⁴	16y	1,400 ^a	820	7
Ho ¹⁶⁶	27h	37 ^a	1.1	4
Tm ¹⁷⁰	127d	110 ^a	59	4
Lu ¹⁷⁷	6.7d	6	3.2	18
W ¹⁸¹	140d	5 ^a	4.8	24
Pb ²⁰³	2.16d	<730 ^a	2.16	61
Pb ²¹⁰	19.4y	<730 ^a	675	0.2
Ra ²²⁶ + 55% daughter products	1622y	20,000 ^a	1.6 \times 10 ⁴	0.1
Ac ²²⁷	21.8y	12,000 ^a	10 ³	0.01
U ²³³	1.63 \times 10 ⁵ y	300 ^a	300	0.04
Th ²³⁴ -Pa ²³⁴	24.1d	40,000 ^a	24.1	2
			24.1	2
Th-natural	1.4 \times 10 ¹⁰ y	40,000 ^a	4.3 \times 10 ⁴	0.01
Pu ²³⁹	2.44 \times 10 ⁴ y	43,000 ^a	4.3 \times 10 ⁴	0.04
Am ²⁴¹	462y	890 ^a	890	0.06
Cm ²⁴²	162.5 \pm 0.3d	600 ^a	120	0.06

^a In critical organ; other values stated refer to total body.

The explanation of the great ability of the bone mineral salt to concentrate various ions very rapidly is the fact that the hydroxyapatite is present as very small crystallites. They have the shape of rods or filaments with the dimensions of about 250×50 Å units and are oriented along the collagen fibers. Because of the small size of the crystallite, the surface of the total skeletal mineral salt is very large, about 130 m^2 per gram of bone salt. Furthermore, a large number of the chemical groups such as Ca and PO_4 have surface positions. Therefore, the crystallites may have varying exchange properties, and this is the basis for the surface adsorption properties of the bone salt. The amount of radioisotopes incorporated per unit volume of bone tissue will depend on the number of reactive sites per unit volume. The number is greater in the spongy bones—e.g., pelvic and jaw bones, ribs, vertebrae, and skull bones—than in the compact ones.

X-Ray microscopic investigations have shown that the mineral salts are unevenly distributed. This nonuniform distribution seems to be a result of the continuous rebuilding which is going on in the bone. The radioactive isotopes incorporated into the bone become localized to these poorly mineralized structures and will there create so called "hot spots." Thus, they become fixed in those parts which are undergoing calcification at the time when the isotope is taken up by the skeleton. The higher the rate of metabolism in the tissue, the greater is the amount of isotope that becomes fixed in it.

The earlier studies on the effects on bone of internally administered radioactive isotopes were essentially concerned with radium (38, 631, 634, 645, 646). During the last ten years, however, a large number of publications on the effects of other isotopes has been issued. In recent years the greatest interest has centered around some of the bone-seeking fission products, especially the radioactive strontium isotopes Sr^{89} and Sr^{90} with its daughter product Y^{90} .

The incorporation of radiostrontium into bone has been dealt with in many publications (636, 644, 647–664). For a review of the uptake, retention, and elimination of radiostrontium in the animal organism the reader is referred to Engström *et al.* (644).

The high toxicity of Sr^{89} has been demonstrated in works from the Argonne National Laboratories (665). It was found that a single intraperitoneal injection of slightly less than $5 \mu\text{c}$ of Sr^{89} per gram of body weight to adult rats produced 50% mortality in 30 days. Heller (628) found $2.9 \mu\text{c}$ of Sr^{89} per gram of body weight to be lethal in 2 weeks in 95% of 50-gm rats. The $\text{LD}_{50(30)}$ for Sr^{89} in young mice is reported by Brues (665a) to be $8 \mu\text{c}/\text{gm}$ body weight. Forty-nine per cent of the animals which had received $5 \mu\text{c}/\text{gm}$ and which had survived for 200 days died before 250 days. Fourteen per cent of these animals had developed bone tumors.

Concerning the toxicity of Sr⁹⁰, Finkel and Scribner (666) found the LD₅₀₍₃₀₎ for mice to be 6 $\mu\text{c}/\text{gm}$ body weight, and for dogs an LD₅₀₍₃₀₎ of 0.15 mc/kg was reported by Finkel and Brues (667). In monkeys a single injection of 0.2 mc/kg has been proved fatal (637). Ray *et al.* (664), studying the acute toxicity of Sr⁹⁰ after a single intraperitoneal injection in 4- to 6-week-old male Long-Evans rats, obtained an LD₅₀₍₅₀₎ of 2.5 to 3.0 $\mu\text{c}/\text{gm}$ body weight. Anderson *et al.* (668) finally, for another rat strain (Sprague-Dawley), got an LD₅₀₍₃₀₎ of slightly less than 2.5 $\mu\text{c}/\text{gm}$.

In young animals whose skeleton is growing, the largest concentration of radiostrontium is found in the regions of active bone growth—that is, in new bone and in cartilage undergoing calcification. In old animals the isotope is deposited preferably in the spongy bone of the metaphyses of the long bones where the metabolism is more intense than in the compact bone. In the latter there is also an uneven distribution, as the isotope is concentrated in the haversian systems and in the endosteum and periosteum. An increased deposition of strontium is also seen in the developing calluses in the vicinity of healing fractures.

The damage to bone by radioactive strontium consists in early death of a number of osteocytes, the appearance of a fibrous marrow, disappearance of almost all osteoblasts, and the gradual "severance" of the epiphyseal cartilage plate from the spongiosa (628). There is abnormal hypertrophy and vacuolization of cartilage cells and finally the appearance of an atypical, almost acellular fibrous bone.

Ba¹⁴⁰-La¹⁴⁰ cause essentially the same damage to bone as radiostrontium, but whereas after radiostrontium extensive bone damage persists for many months, there is considerable repair after only a few weeks in the case of Ba¹⁴⁰-La¹⁴⁰. This was observed by Heller (628) after administration of 5.6 $\mu\text{c}/\text{gm}$ to mice. Both P³² and Na²⁴ have a considerably less damaging effect than radiostrontium.

The deposition of radioactive yttrium in bone has been studied by a number of authors (636, 669, 670, and others), and it has been found that the deposition of this element in the skeleton differs both in mechanism and localization from that of strontium and phosphorus. The retention of yttrium is greater than for strontium and is less affected by age than that of strontium. It is concentrated in the sites of active bone growth beneath the epiphysis but not beneath the endosteum or periosteum (670). The damage done to the bone by radioactive yttrium is of the same degree as that caused by radiostrontium. Zr⁹³-Nb⁹³ and Ce¹⁴⁴-Pr¹⁴⁴ cause less severe damage than strontium. W¹⁸⁵ is retained by osseous tissue probably as insoluble calcium tungstate (671).

The α -emitting isotopes radium and plutonium resemble each other closely in their effects on bone tissue. Martland (631) observed a softening

of the bone as an effect of radium poisoning due to a partial decalcification. Both radium and plutonium have a more severe effect than strontium. They cause cessation of normal growth, early overgrowth of bone, and extensive death of osteocytes (628). The elimination of radium and plutonium from the body is slower than that of strontium (650).

B. TEETH

A single dose of 1500 r of localized head X-irradiation to young rats has a clear effect on the developing incisor teeth (672). Some time after irradiation (about 6 weeks) there is a visible break in the incisor formation, located at the level of the tooth that was under formation at the time of exposure. The incisors of sacrificed animals are separated into two segments. English *et al.* (672) found that in the maxilla the first segment was frequently lost at 100 days, leaving a stumplike tooth. In the mandible, the second formed segment frequently grows lateral to the original tooth, thus in fact producing an extra incisor.

An increased tendency for dental caries has been observed after irradiation. Dietary factors seem to play a part in the occurrence of this change (673). Morphologically, extreme damage is done to the tooth-forming elements, and the growth of the tooth is inhibited. The tooth germ was found also to be damaged but to a lesser degree. In general, there is a recovery of the tooth-forming tissues after irradiation (672).

Bone-seeking isotopes are also accumulated in the growing teeth and antlers (674). This is especially evident in the very rapidly growing incisor teeth of rats in which the uptake, e.g., of radiostrontium is often more pronounced than in other bone tissue (644, 675, 676).

The effect of P³² on the fetal tooth primordia of mice has been studied by Burstone (677). Two to six days before expected parturition 5 to 17 μ c of P³² per gram of body weight was injected into the mothers. It was found that the teeth of the fetus are more radiosensitive than are the teeth of newborn animals. Newly differentiated cells are more radiosensitive. The development of the molars were retarded and, as far as the third one is concerned, completely inhibited.

C. CARTILAGE

The sensibility of cartilage to irradiation is different for different locations of this tissue, and it also depends on the age of the animal and the stage of development of the cartilage. It is generally agreed that the epiphyseal cartilage of young animals shows a marked radiosensitivity. In adult animals it is generally assumed (626, and others) to be rather resistant. After heavy irradiation, which may cause necrosis of the cartilage, recovery is poor (678).

Whole-body as well as local irradiation may cause the cartilage cells to be abnormally swollen. This has been observed in rats 3 days after a single exposure to 600 r of X-rays as the first sign of degeneration in the zone of provisional calcification (628). Many of the cells, after some days, become hypertrophic, vacuolated, and get pyknotic nuclei. Local exposure of an epiphysis of the long bones to rather high doses (1500 r or less) damages the cartilage, with a stunting of the bone growth as a consequence (679-682). The columns of cells and the intercellular bridges are deranged, the epiphysis becomes thinner, and the growth mechanism is disturbed after about 2 weeks.

Most of the bone-seeking isotopes are also highly damaging to cartilage, especially to the epiphyseal cartilage. Swelling and death of cartilage cells are early signs of such cartilage damage. Later on the cartilage may be invaded by vascular connective tissue, and it also becomes irregular and may be broken up into fragments. In mice, Sr⁸⁹ causes an abnormal hypertrophy and vacuolization of cartilage cells. Radium has an even more severe effect (628).

D. CARCINOGENESIS

The induction of bone tumors by ionizing radiation has been observed both in man and in animal experiments. A number of reports on bone tumors after administration of various bone-seeking radioactive isotopes and a small amount of data on bone tumor production by external radiation are available.

External Irradiation. After single whole-body irradiation bone tumors have been reported in rats after 600 r (683) and 700 r (684). At other dose levels of whole body irradiation no data are available.

Cater *et al.* (685) have published a preliminary report of the production of bone tumors in rats by a single dose of 3000 r of γ -radiation given to the knee joint. The effect of courses of treatment with growth hormone and thyroxine after the irradiation has been investigated, and there is some evidence that both these agents increase the incidence of bone tumors in the animals.

In the chronic irradiation experiments by Lorenz *et al.* (686) with γ -radiation and with daily dose rates from 0.11 to 8.88 r on mice, guinea pigs and rats, no bone tumors were reported, although other types of neoplasms were found.

Internal Irradiation. The carcinogenic effect on the bone tissue of bone-seeking radioactive materials has been evidenced in a number of experiments during the last 10 years. The most comprehensive study is that of Brues *et al.* at the Argonne National Laboratory. The relationship between incidence of injected dose of isotope in mice has been reported for Pu and

Ra (687) and for Sr⁹⁰ and Ca⁴⁵ (688). Preliminary data by Finkel (689) indicate a relative effectiveness in inducing malignant bone tumors. Thus, from the appearance of 10% tumor-bearing mice in 25 days, Pu²³⁹ was found to be twenty times as effective as Ra²²⁶, and Ra²²⁶ was five times as effective as Sr⁹⁰, ten times as effective as Sr⁸⁹, and fifty times as effective as Ca⁴⁵.

The dose of isotope necessary to induce carcinogenic transformation of bone tissue in mice and rats is less for Sr⁹⁰ (0.009 to 0.4 μ c/gm) than for Sr⁸⁹ (0.7 to 1.6 μ c/gm). Vaughan and Jowsey (690) report an osteogenic sarcoma in the tibia of a young rabbit which died 180 days after a single intravenous injection of 0.5 μ c of Sr⁹⁰ per gram.

In a Russian investigation, 103 rats were observed by roentgenography at intervals from 192 to 549 days after administration of 0.4 μ c of Sr⁹⁰ per gram of body weight. In 85% osteosarcomas developed. The most common location was the distal metaphysis of the femur (43%) and the proximal end of the tibia (40%) (691).

Injections of Sr⁸⁹ and Ca⁴⁵ produced malignant bone tumors in rats after repeated intraperitoneal administration (ten daily or ten monthly injections). Of the two isotopes, Sr⁸⁹ proved to be the more effective. The majority of the tumors was found in the distal end of the femur and the proximal end of the tibia as in the Russian study. Tumors of the spine and pelvis were produced only by Ca⁴⁵ (692).

If mice were given frequent small doses of Sr⁸⁹, 22 to 30 μ c/gm, intraperitoneally or intravenously, divided into ten or eleven doses during a period of 35 to 40 days, osteogenic sarcomas were induced in 46% of the animals. Most of the tumors were localized in the long bones. The highest radioactivity in the femur shifted in the course of time from the distal part of the bone to the shaft. According to Watanabe (693) the osteogenic sarcoma does not develop from newly formed bone but from mesenchymal cells which are capable of differentiating from long-term irradiation.

In an experiment described by Casarett *et al.*, rats of different ages ingested a water solution of Sr⁹⁰ in 10 or 30 days. The total doses were sufficient to result in skeletal retentions of about 1 to 50 μ c after 2 months. If these were 1 and 2 μ c, no osteosarcomas appeared, but radiation injury of bone marrow and zones of endochondral bone formation was observed. A skeletal retention of 15 μ c gave a high incidence of osteosarcomas of various types, some cases of leukemia, and considerable life shortening. At 50 μ c, marked life shortening, severe pathology of bone marrow and bone tissue, and some bone tumors occurred (694).

Also P³² induces osteogenic sarcomas after internal administration in single or repeated doses. The incidence of tumors ranged from 22 to 50% after 1 μ c to 12 μ c/gm body weight. Amounts of P³² less than 1 μ c/gm failed

to produce neoplasms. The osteosarcomas occurred in different bones, most frequently in the jaws, and metastasized to the lungs. The latent period was 160 to 314 days (695, 696).

Induction of osteogenic sarcomas after very low doses is observed after administration of Th and Pu. After intravenous or intraperitoneal administration of 0.0001 μc of Th per gram of body weight, osteosarcomas were found which originated from the pelvis, scapular bones, the thorax, and thoracic vertebrae. In dogs the tumors were observed after 3 years, and in rats after 8 to 16 months. After a dose of 0.0005 $\mu\text{c}/\text{gm}$ the animals died earlier, with no signs of malignant growth (697).

The following doses of Pu were given by intraperitoneal injections to rats: 6.3×10^{-3} , 1.89×10^{-3} , 0.63×10^{-3} , and $0.315 \times 10^{-3} \mu\text{c}/\text{gm}$ body weight. The two highest doses considerably shortened the life span, but the two lowest doses gave the same life span as the controls. Osteosarcomas and various tumors of the soft tissues were produced. The number of animals with tumors decreased with diminution of the dose. The greatest number (54%) was found in the group of rats receiving $1.89 \times 10^{-3} \mu\text{c}/\text{gm}$. The latent time was shortest after high doses (6.3×10^{-3} and $1.89 \times 10^{-3} \mu\text{c}/\text{gm}$) and increased with diminishing doses (698).

XIII. MUSCULAR AND CONNECTIVE TISSUES

A. MUSCLE

Muscle tissue is among the most radioresistant tissues of the body (699, 700).

In rats, when the lower extremities were exposed to 6000 to 72,000 r of X-rays, it was found that 6000 r caused only slight muscular atrophy, whereas 72,000 r caused total necrosis (701). The intermediate doses gave a gradual increase from atrophy to necrosis. Destructive lesions of scattered muscular fibers were observed as early as 3 hours after 72,000 r. After a week most of the leg tissues were necrotic, with no evidence of fibroblastic proliferation to replace the gangrenous muscle. Exposure to 12,000 to 26,000 r caused coagulation necrosis of smaller areas and atrophy of nonnecrotic parts.

Gross and histological changes were observed by Gerstner *et al.* (702) in rabbit muscle after exposure to 50,000 and 100,000 r of X-rays. The alterations include early edema, small hemorrhages after 24 hours, and necrosis after 72 hours.

The inhibition of contraction of striated muscle by radiation may require many thousands of roentgens (77). After doses between 36,000 and 72,000 r of X-rays given to the forelimb of rats, the muscular function was unchanged during the observation period of 22 hours, but there was a red-

dening of the skin and a gelatinous swelling of the skin and muscles (703). Rabbit muscles exposed to 72,000 r showed a gradually progressive impairment of function (702). Complete abolishment occurred within 24 hours after exposure and was accompanied by severe histological alterations. These results may be compared with those obtained in frog muscle with doses above 50,000 r. This gives early fatigue, contracture, prolongation of relaxation time, and decreased contraction amplitude for heavy loads.

An increase in extracellular water (edema) measured by inulin and Na²⁴ space determinations is seen after irradiation with large doses (703). It is related to the dose, with a threshold for its occurrence between 10,000 and 40,000 r. The muscle fibers lose no potassium per unit dry weight but may possibly gain a minimal amount of sodium.

Internal Irradiation. There are only relatively few radioisotopes that may concentrate especially in the musculature. Among these isotopes are K⁴², Rb⁸⁶, Cs¹³⁷-Ba¹³⁷, and the Tl isotopes. Very little is known about the effects of internal irradiation on muscular tissue.

B. CONNECTIVE TISSUE

The connective tissue is generally stated to be very radioresistant. The effect of X-rays on connective tissue can be an inhibition of formation of connective tissue ground substance possibly induced by effects on the fibroblasts that are supposed to produce the mucopolysaccharides of the ground substance or an increase in the rate with which it is broken down.

It has been found that the absorption of intracutaneous wheals of normal saline (704) or glucose (705) is much more rapid in irradiated than in nonirradiated tissue. A dose of 200 to 500 r is sufficient to be effective in this respect.

Factors of importance for the absorption of subcutaneous injected material are: the blood flow in the area, capillary permeability, the osmotic pressure of the blood, and, finally, a factor that has been termed the penetrability of the connective tissue. It is believed that the increased rate of intracutaneous absorption after irradiation is mainly due to the last of these factors. Also dyes, e.g., Evans blue, injected intradermally spread more rapidly in irradiated animals, e.g., after 750 r in rats (706).

The irradiation-induced permeability of dermal connective tissue appears 4 to 7 days after the exposure and may last for 2 to 3 weeks. It coincides in time fairly well with the period of increased vascular permeability and purpura. The higher permeability is associated with an increase in stainable acid polysaccharide in the connective ground substance and with a reduction of the number of mast cells in the dermis.

Ionizing radiation may exert a direct action on the ground substance. Schoenberg *et al.* (707) thus have shown that X-rays are able to depoly-

merize hyaluronic acid. Brinkman (707a) has found a pronounced decrease in the viscosity of mucopolysaccharides from dermis connective tissue fibrils.

XIV. Shortening of Life Span by Irradiation

After exposure to ionizing irradiation the animal may recover again, but if the dose has been large enough the animal will die after some time, the time before death being shorter, within some limits, the higher the dose. If the animal recovers, it will, however, be subjected to a shortening of its life expectancy. This reduction is partly due to the cancerogenic effect of the irradiation, partly to a number of radiation-induced processes which are not known in detail but which cause an accelerated aging. Degenerative changes occurring after irradiation, manifested as nephrosclerosis, anemia, osteitis, dermatitis, and sterility, may be contributing factors.

Although the effect of life shortening has not yet been clearly established in man, an increasing number of observations during recent years (708-735) has proved that it exists in animals. The degree of life shortening may be subjected to species differences.

Brues and Sacher (736) and Blair (715) have presented theories of the life-shortening effects of radiation. Blair's theory is based on the assumption that the injury is proportional to the dose, that it is composed of two parts, one reparable and one irreparable, proportional to the total dose, and finally that the lethal threshold of injury decreases directly as the life expectancy.

Generally, the survival time after the exposure to a given dose rate is inversely proportional to the amount of radiation absorbed. It is, however, not yet known whether there exists a threshold dose or intensity below which there is no reduction of the life span (726).

If the radiation dose is received as a chronic irradiation over a long time, the reduction of the life span per total dose is, generally, less than after a short-lasting irradiation (714). Furthermore, some observations (737, 738) of the life span after small chronic doses really indicate the existence of a threshold, as mice exposed to 0.1 r/day throughout their whole life had a slightly longer mean survival time than the nonirradiated controls in spite of the fact that they displayed a significantly higher incidence of neoplasms. Carlson *et al.* (738a) irradiated male rats from 4 to 16 months of age at a rate of 0.1 r/hr for 8 hours each day and at two temperatures, 25°C and 5°C. They found about 20% increased life expectancy and higher oxygen consumption in the irradiated animals.

The repair rate for a large number of small irradiations is lower than for a small number of larger doses. It has been suggested (739) that this is because the small doses do not induce enough damage to stimulate maximum repair. The shape of the slope of the survival curve of whole-body-

irradiated animals shows that the mortality is distributed in the course of time (729). In partial-body-irradiated animals the shortening of the life span occurs at different times after the exposure, depending on the area irradiated.

According to Failla and McClement (720) no statistically significant life shortening can be determined for doses less than 0.5 r because of the small number of animals used for the determinations.

The rate of recovery from neutron-induced damage is slower than the recovery from X- or γ -radiation damage (732).

Boche (740) has suggested that the shortening of life span is proportional to the total accumulated dose according to the formula

$$t - t_0 = k dt$$

where t and t_0 are the life spans of irradiated and control animals, respectively, d is the daily dose, and k is a constant which is -0.04 for γ -rays.

The assumption of a linear relationship between the reduction of the life span and the dose in the case of a single exposure to X-rays and neutrons has been supported by the work of Storer and Sanders (733), who obtained a value of about 5% shortening of the life span of mice per 100 r of whole-body irradiation with thermal neutrons. In animals exposed to neutrons and γ -rays from a nuclear detonation it was found (734) that these neutrons shortened the mean life span by 6.7% per 100 rads and the γ -rays by 2.6%. According to Hursh (721), a single sublethal dose of X-rays has a life-shortening effect in rats and mice of 25 to 50% per 1000 r, the proportional amount of shortening increasing with dose size. For chronic irradiation the shortening is estimated to be approximately 10% per 1000 r. These values are in good agreement with those of Blair, who gives about 23% and 10% for acute and chronic irradiation, respectively.

For the explanation of the accelerated aging and life-shortening processes, theoretical considerations have been applied (735, 741). The distribution in time of death after radiation has been supposed to be explained by a distribution in genetic information content in the organism rather than by the distribution of sensitivity or the random fluctuation of bits. Death should occur when the accumulation of genetic noise in the somatic cells reaches a critical value which is dependent on environmental factors. Also Failla (719) has considered the mechanism in terms of gene mutations in the somatic cells of the body. The observations of other investigators (728, 742), however, argue strongly against the somatic mutation theory of aging.

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CHAPTER 3

Immunology

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I. Introduction

This chapter concerns the effects of ionizing radiation on immunity in the broad sense of the word. Normal immunity mechanisms are of a complex character and have not yet been satisfactorily clarified. Explanations of the effects of radiation on immunity are therefore frequently a matter of speculation. On the other hand, some observations of the effects of ionizing radiation on the defense mechanisms of the organism throw light on their basis. They include the finding that changes in the defense capacity of an irradiated organism are closely correlated to certain pathophysiological changes evoked by radiation. Radiation can therefore serve as an indirect means of revealing anatomical or physiological factors which participate in immunity mechanisms.

Soon after the discovery of X-rays, a number of workers turned their attention to the question of the way in which this new physical agent acted on the defense mechanisms of living organisms. The studies of Heineke (1, 2), which demonstrated the marked radiosensitivity of lymphoid and myeloid tissues, laid the first concrete foundations to this problem. Another

important landmark was the finding of Benjamin and Sluka (3) that rabbits irradiated with X-rays prior to the injection of bovine serum eliminated the antigen more slowly and formed precipitins in lower titers than unirradiated controls or animals irradiated 4 days after the injection of antigen. These experiments not only demonstrated the inhibitory effect of radiation on antibody formation but also revealed one of the conditions on which this effect largely depends—the time relationship between irradiation and the administration of antigen. The third type of basic observation concerns the general increase in the susceptibility of irradiated animals to infections to which they are normally resistant, probably on the basis of innate immunity. This applies in particular to infections produced by normal intestinal flora. The chief work in this field are the experiments of Warren and Whipple (4) and the histological demonstration by Chrom (5) of invasion of the blood stream, spleen, liver, and lymph nodes of irradiated mice, a week after exposure, by bacteria normally inhabiting the intestines.

These basic findings on the effects of irradiation of the organism on its immunity mechanisms—changes in antibody formation, radiosensitivity of the reticuloendothelial system, inhibition of natural immunity to autoinfections—formed the starting point of a whole series of further experiments. Some authors made a detailed experimental analysis of individual manifestations of the effects of radiation on immunity and studied their mechanisms; others investigated the possibility of the direct practical application of this form of the biological effects of radiation.

Two different trends developed in the practical utilization of the effects of radiation on immune reactivity. Experiments on the treatment of certain infectious diseases by low radiation doses, usually applied locally, were based primarily on the concept that radiation doses too low to be harmful might nonspecifically stimulate the reticuloendothelial system and thus raise the resistance of the organism. In transplantation problems, the main question is the utilization of the inhibitory effect of irradiation on the immune reactivity of the recipient to homo- and heterografts of normal or neoplastic tissue.

The starting point of the largest number of experimental studies, however, was the marked increase in the susceptibility of irradiated animals to endogenous and exogenous infections, and the demonstration that bacteremia, evoked by normal intestinal flora and other types of infection to which animals are normally resistant, plays an important part in postirradiation death. These findings, which clearly demonstrate that ionizing radiation affects the defense of the organism, have been studied from every possible aspect and verified in different species of animals and different types of infection—parasitic, bacterial, viral—in relation to the experimental conditions—the route of infection, the radiation dose and its time distribution,

the time relationship to irradiation, the administration of the infective agent, etc. This experimental approach to the question of the relationship between radiation and immunity has not, however, proved altogether satisfactory. Different mechanisms of innate and acquired immunity, often difficult to distinguish accurately from one another, participate in the defense of the organism against an infective agent capable of multiplication. They include the barrier properties of tissues, different cellular defense reactions, the natural bactericidal properties of blood, and nonspecific and specific antibody reactions. Probably none of these mechanisms is based on a simple one-stage reaction, or on the function of a single type of cell. It can therefore be expected that the ultimate effect of radiation on each of them will be the function of the relative radiosensitivity of all its components. As a rule, however, it is not possible to study the effect of radiation on each of these mechanisms separately. In most cases we subject the whole organism to radiation and evaluate its reaction by some characteristic feature of its immune response; this is then the outcome of the whole complex of subsidiary changes in the individual types of defense reactions and their components. Radiation can probably also act on all these defense mechanisms indirectly, by influencing other functions of the organism. Anemia, leukopenia, disturbances in the balance of the plasma proteins and electrolytes, injury to the gastrointestinal tract, and changes in adrenal function can also develop as secondary manifestations in the changed defense of the irradiated organism.

The complexity of the effect of radiation on the defense of the organism is manifestly the main reason why a large proportion of the experimental material has failed to give an unequivocal reply to the question of the mechanism of this effect. Only the main experimental findings are reviewed below (for details, see refs. 6-8), and attention is paid chiefly to the latest attempts to analyze subsidiary questions from which certain general conclusions can be drawn.

II. Enhancement of Immunity by Radiation

Before attempting to analyze the effects of radiation on individual immune mechanisms, it should be borne in mind that radiation does not always have an inhibitory effect. The favorable effects of irradiation of the organism on the course of different types of infection are reviewed by Bass and Jarošchka (9) and by Taliaferro and Taliaferro (6). The latter authors evaluate the findings on the beneficial effects of radiation roughly as follows:

Doses with a beneficial effect were all lower than inhibitory doses and were usually applied to a small area of the body. It cannot be assumed that these doses had a direct germicidal effect, as they were too low to cause injury to the majority of parasites *in vitro*, with the exception of certain developmental stages of some metazoan parasites. In principle, they con-

sider the following four possible mechanisms of an increase in immunity due to radiation:

1. Production of antibodies, nonspecific germicidal substances, and enzymes, or their liberation from cells destroyed by radiation. Whether the amounts involved are sufficient to be responsible for the given effects is still a moot question.
2. An increase in the number of some of the cellular elements which participate in immune mechanisms. Bloom (10), however, on the basis of extensive observations, throws doubt on the "stimulating effect" of radiation and ascribes some of the findings explained in this way to temporary reparative overcompensation after mild initial radiation injury.
3. Stimulation of the reticuloendothelial system. It has been presumed, though not proved, that macrophages in the reticular organs may be stimulated by the ingestion of small quantities of lymphocytes destroyed by radiation.
4. Vascular changes leading to hyperemia, an increase in the supply of blood, and of substances contained in the blood, to irradiated sites, with a consequent increase in antibody efficiency.

Conclusions on the beneficial effects of radiation on immunity would be necessarily premature, despite the abundance of experimental material. The findings of earlier authors, made when dosimetry was still undeveloped, should be taken with the greatest reserve.

III. Inhibitory Effects of Radiation

The beneficial effects of radiation on the formation of specific antibodies in correlation to time relationships between the administration of antigen and irradiation will be discussed in the relevant section.

The beneficial effects of radiation on immunity are known rather as the exception, whereas findings on the inhibitory effects of radiation are extremely numerous. There is no point in reviewing here work in which this fact has been repeatedly confirmed, on the basis of an abnormal incidence of autoinfections or increased sensitivity to experimental infection in irradiated animals. The only communications discussed are those which throw light on the mechanism of this increased sensitivity caused by radiation.

A. INHIBITORY EFFECTS ON INNATE IMMUNITY

1. MECHANICAL BARRIERS

It has been demonstrated that acute irradiation can impair the barrier function of certain tissues which normally prevent the penetration of an infective agent into the blood stream. This applies to the skin, the lungs,

and the intestinal mucosa. The gastrointestinal tract of widely differing species of experimental animals displays marked sensitivity to different types of ionizing radiation. Within an hour after exposure to different radiation doses, varying degrees of injury occur to the crypt epithelium, which is more vulnerable than the villous epithelium; the mucosa of the small intestine is considerably more sensitive than that of the colon. On increasing the radiation dose, numerous cells are injured, the villous epithelium is denuded, the lamina propria is less cellular than normal, and the follicular lymphocytes are considerably damaged. Larger radiation doses injure the glandular epithelium, and pathological changes in cellular metabolism appear to be associated with the development of gastric ulcers, which sometimes perforate into the abdominal cavity only 9 days after exposure. In severe cases tumors of the gastrointestinal tract develop. Pierce (11) reviews histological changes in the gastrointestinal tract which lead to interruption of the continuity of the wall of the intestine and to a breakdown of the mechanical barrier against the entry of intestinal flora into parts of the organism to which they normally have no access. Many of these changes are reversible, and the possible extent of regeneration depends primarily on the radiation dose. In the gastrointestinal mucosa it is precisely the cells responsible for regeneration which are the most radiosensitive (10).

2. CELLULAR DEFENSE MECHANISMS

The barrier properties of tissues evidently do not consist only in their function as a mechanical barrier against infection but also include the active participation of cellular defense mechanisms. Troitsky *et al.* (12) found that the wall of the appendix of an adult rabbit is normally capable of blockading and destroying vast quantities of bacteria which would otherwise be capable of migrating from the intestine, even if the crypt epithelium were undamaged. These authors used histochemical reactions for polysaccharides, which are present in large quantities in the appendix of the normal rabbit and the presence of which is evidently an outcome of intracellular digestion of the bacteria. The concentration of these polysaccharides in the appendix of irradiated rabbits is far lower, and histological preparations show the presence of free, unaltered bacteria, which are never found in unirradiated animals. A study of the details of phagocytosis showed that it is not complete. Macrophages containing phagocytosed bacteria are often destroyed, and the bacteria are thus liberated again. Silverman *et al.* (13) also demonstrated that, 60 hours after irradiation of mice with 1200 r, normal intestinal microorganisms are found only in spleen and liver cultures, but later they reappear in the blood. This also shows the inability of the relevant cellular elements in the spleen and liver to engulf and destroy the microorganisms.

The decrease in the resistance of irradiated organisms to infection is thought by many authors to be due to impairment of the phagocytic mechanism. Since macrophages are markedly resistant even to large doses of ionizing radiation (10), the suppression of their phagocytic activity after irradiation is explained by indirect effect, i.e., by a blockade caused by the ingestion of large quantities of destroyed lymphocytes (6), which are highly radiosensitive. Functional tests of the reticuloendothelial system of irradiated animals also show reduced ability to adsorb trypan blue. The decrease in cellular infiltration observed in inflammatory processes after massive doses of total-body irradiation is possibly associated with leukopenia of the circulating blood (14).

3. CLEARANCE MECHANISMS

The natural resistance of organisms to certain infections obviously depends on their ability to eliminate the infective agent from their bodies within a short time. Troitsky *et al.* (12) found that rabbits normally resistant to dysentery per os, which even after large doses (5×10^6 Flexner's bacillus) do not become carriers or display any clinical signs of the disease, usually die after the same dose if irradiated prior to infection with a dose of 800 r. The survivors long remain carriers, thus demonstrating that they are unable to clear the bacteria completely from their bodies. Gordon and Miller (15) studied a similar phenomenon in rabbits to which *Klebsiella pneumoniae* was administered intravenously. In normal animals the micro-organisms were irreversibly eliminated from the organism within a few hours, whereas in irradiated animals the bacteria, after first disappearing, reappeared in the blood, and their numbers then increased until the animal died.

4. BACTERICIDAL EFFICIENCY OF THE BLOOD

Marcus and Donaldson (16) found that exposure to given doses of radiation destroyed the normal bactericidal activity of rabbit serum. They further showed that this was not the outcome of bacteraemia, as the same radiation effect is found in animals without bacteraemia. The cause of this phenomenon is evidently not some chemical radiation product in the blood, since the addition of serum from irradiated rabbits to normal serum does not reduce its bactericidal efficiency (17). These authors considered the possibility that suppression of the bactericidal properties of serum might be due to heparin released from mast cells destroyed by radiation. Heparin produces the same effect, which can be abolished by a suitable dose of protamine sulfate. This hypothesis could not be proved, however. The hypothesis of the anticomplementary effect of heparin in concentra-

tions inhibiting the bactericidal effect of serum (18) was likewise not confirmed. This question is therefore still open.

5. THE PROPERDIN SYSTEM

In some animals, the production of properdin, a serum protein of low specificity, but analogous to some extent to specific antibodies, takes place without previous antigenic stimulation. Properdin appears to participate in the destruction of bacteria, virus neutralization, etc. (19). The properdin level in dogs shows a definite decrease during the postirradiation period; the maximum decrease corresponds to maximum lethal effect, and an increase occurs in survivors. Zymosan, which alters the properdin level *in vivo*, provokes a double increase in the leukocytes of irradiated dogs at the time of maximum leukopenia (20). In irradiated rats, properdin provides partial protection after LD₉₀ (19).

6. OTHER NATURAL DEFENSE MECHANISMS

Apart from the natural defense mechanisms cited above, which can be influenced by radiation, it is possible that others, the nature of which is unknown, and which may in some way be connected with the rest, also exist. Adler and Shechmeister (21) described a marked increase in the sensitivity of irradiated mice after doses of 250 to 300 r to the toxin of *Clostridium septicum*. This was not due to suppression of antitoxin formation, since irradiated animals are not adequately protected by passive transfer of a dose of antitoxin sufficient for protection of the controls but require considerably larger quantities. Perkins and Marcus (22) also found that, despite the fact that radiation doses above LD₁₀₀ do not reduce the titer of preformed antibodies in mice, these are not alone able to protect the irradiated animals against experimental infection with the corresponding infective agent. Allen *et al.* (23) assume that immune antibodies normally form a complex together with some anticoagulant of the heparin type which, after they are consumed, is liberated and reduces blood coagulability. This could be a possible cause of postirradiation hemorrhage which, together with other factors, would facilitate bacterial invasion of the blood stream by intestinal flora.

7. MODIFICATIONS OF THE EFFECTS OF RADIATION ON INNATE IMMUNITY

The inhibitory effect of radiation on innate immunity can be favorably influenced by a number of processes. One of the relatively most successful is the administration of antibiotics to irradiated animals; this does not directly stimulate defense mechanisms, however, but only helps the irradiated organism over the critical period of radiation sickness, until the animal recovers and is again able to resist bacterial invasion. This measure does

not therefore contribute to a solution of the question of the mechanism of the inhibitory effect of radiation on immunity. Greiff *et al.* (24) found that, as the radiation dose increased, the rickettsiostatic property of streptomycin decreased, until it disappeared completely. This radiation change lasted for at least 4 days. The cause of the phenomenon is somewhat obscure. It may be similar to the low efficiency of passively transferred antitoxin in irradiated animals.

Bogomolets *et al.* (25) took Metchnikoff's concept and applied it in different species of irradiated animals, to which they administered antireticular cytotoxic sera in small doses, which stimulate the function of the reticuloendothelial system. Where the damage is reversible, this method gives positive results. Its effect is manifested in several different indices of stimulation of the reticuloendothelial system, however, and it is impossible to differentiate its influence on separate mechanisms. Many questions still remain unresolved and require further research.

B. INHIBITORY EFFECTS ON ANTIBODY FORMATION

Actively acquired immunity is another important immune mechanism. Synthesis of specific antibodies is induced by the penetration of antigen. Well-known antigens include proteins and polysaccharides, and whole cells, such as bacteria and foreign cells, can play the same role. Antibodies agglutinate bacteria (agglutinins) or dissolve them (lysins). Since the effect of radiation on antibody response obviously also depends on whether the organism has previously been in contact with antigen which provokes the reaction in question, primary and secondary reactions will be discussed separately.

1. PRIMARY REACTIONS

As already stated in the Introduction, the discovery of the inhibitory effect of irradiation on antibody formation was made at the same time as the observation that, if total-body irradiation is carried out just before administering antigen, the effect is much more marked than if it is carried out after. The findings of Benjamin and Sluka (3) on changes in precipitin formation were confirmed by the detailed experiments of Hektoen (26, 27) for hemolysins. A review of later experimental findings was submitted by Taliaferro and Taliaferro (6) and by Talmage (7). The question of the effect of radiation on antibody formation in correlation with the time interval between exposure and administration of the antigen has been studied in great detail and is an example of the way in which the study of the effect of radiation on antibody responses has provided a basis for the formulation of certain hypotheses on their mechanisms.

Experiments using nonliving antigen have produced the most definite

results. This has greatly simplified the experimental conditions and has excluded a number of subsidiary factors which must be taken into account when using antigen capable of multiplication.

The results obtained by earlier authors in this field caused them to conclude that the degree of sensitivity of the antibody response is not the same throughout its course. It was assumed, for example, that a radioresistant tissue reserve exists which is able to maintain antibody production which has already commenced, i.e., when irradiation is carried out after the administration of antigen. Kohn (28) was of the opinion that the event most sensitive to irradiation is cellular fixation of the antigen, and that the full development of radiation disturbances responsible for inhibition of antibody formation can take some time, e.g., a day or more. This time would then determine the interval between irradiation and subsequent administration of antigen required for inhibition of the antibody response.

Dixon *et al.* (29) expressed the hypothesis that antibody reactions have two phases. According to these authors, the first is a radiosensitive adaptation phase and the second a radioresistant production phase. Their experimental results with the formation of precipitins against bovine γ -globulin were in good agreement with this concept, although, as shown by further work, it was greatly simplified. Taliaferro *et al.* (30) and Taliaferro and Taliaferro (31) made a detailed examination of the relationship between the effect of radiation on hemolysin formation (against Forssmann's sheep red cell antigen) and the time correlation between exposure and administration of the antigen. Their results are given in Fig. 1. This makes it clear that:

1. The maximum damage is caused by irradiation 12 to 24 hours prior to administration of the antigen, evaluated by the maximum titer, the length of the induction period, and the relative antibody formation rate.
2. If the antigen is administered just after irradiation, so that the development of the process impairing the antibody mechanism has not progressed, the amount of antibody is almost equal to normal values, but production is retarded and its relative rate is less.
3. On administering antigen shortly before irradiation, the results are similar, but antibody production is higher than normal.
4. If antigen is administered 4 days before exposure, antibody production attains values approximately equal to the maximum normal titer but is retarded and develops at a slower rate. This period, 4 days, is longer than the normal induction period, and it is therefore evident that in this case antibody production is not impaired by radiation.

Similar conditions were found by other authors, using other antigens and species of animals. Certain quantitative differences appear to be ex-

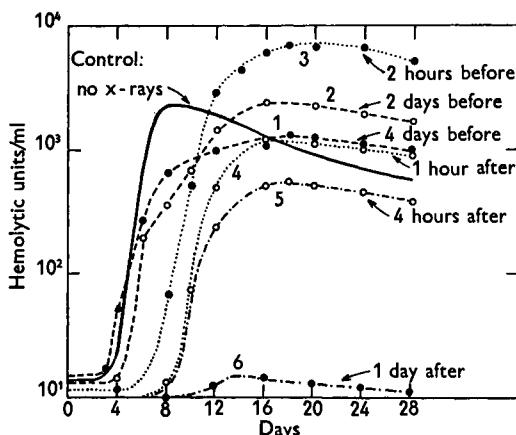


FIG. 1. Mean hemolysin curves from six groups of rabbits for 28 days after one intravenous injection of sheep red cells (0.125 ml of 1% per kilogram) given from 4 days before to 1 day after total-body irradiation with 500 r. A control mean hemolysin curve from nonirradiated rabbits is included. The time of injection of antigen in relation to X-rays is given at the right of each curve. Redrawn from Taliaferro and Taliaferro (31).

plainable on the basis of specific differences in the radiosensitivity of subsidiary mechanisms in the antibody process, or of the different half-life of different antigens, the digestion of which can also be variously affected by radiation. The different molecular weight of the given antibodies—hemolysins, precipitins—and the rate of their metabolism may likewise be responsible for some of the differences observed. In general, however, there is good agreement between the results of the different authors. Figure 2 compares the production curves of antibodies against antigens administered at different times before or after irradiation. The first is taken from the work of Taliaferro and Taliaferro (31) and illustrates the mean values of antisheep hemolysin titers in irradiated rabbits. The second is from experiments carried out in inbred mice by Gengozian and Makinodan (32). These authors expressed immune reactivity by an empirically introduced factor, which they termed "relative immune status." This is the sum of relative changes in four different indices of heteroagglutinin formation: induction period, rate of appearance of circulating antibody, mean peak titer, and mean total titer. In view of the differences in species, antibodies, evaluation criteria, and radiation doses, the results are surprisingly in agreement.

On the basis of his experimental results on the effect of radiation on antibody formation, Taliaferro (8) concludes that this process consists in three successive phases. His hypothesis is also in agreement with the time course of titers after the administration of antigen and with the way in which labeled radioactive amino acids are incorporated (33).

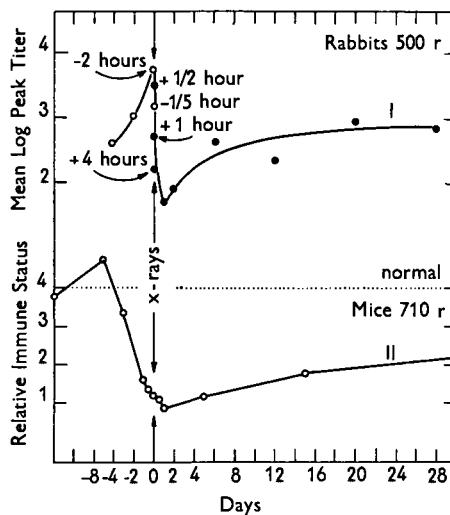


FIG. 2. Antibody production in total-body irradiated animals when antigen is given before (−) or after (+) X-rays. *Curve I*, redrawn from Taliaferro and Taliaferro (31); MPT = mean log peak hemolysin titer from fourteen groups of rabbits after one intravenous injection of sheep red cells given from 4 days before to 4 weeks after total-body irradiation with 500 r. *Curve II*, redrawn from Gengozian and Makinodan (32); RIS = relative immune status of mice forming agglutinins against sheep red cells administered from 10 days before to 30 days after total-body irradiation with 710 r.

a. Preinduction Period. Taliaferro terms the first phase the "preinduction period." He assumes that it covers a brief event essential for initiating the development of the antibody-synthesizing mechanism. This phase is highly radiosensitive, and it is by this characteristic that its duration can best be determined. It appears to last for 1 to 4 hours. Damage to this phase requires a given threshold dose, which—at any rate, for hemolysin—is sharply defined.

The character of the changes which occur during the preinduction period is still a matter of speculation. The original concept of Kohn (28) that it involves fixation of antigen is rejected by Fitch *et al.* (34) on the basis of a histological study of binding of the antigen in the reticular cells of recipients irradiated prior to hemolysin production. They assume that it rather involves suppression of proliferation of antibody-forming cells or inhibition of their proteosynthesis. Interesting results in this respect were obtained by Stevens *et al.* (35). These authors assumed that, if plasma cells participate in antibody synthesis, postirradiation inhibition of antibody formation ought to be a reflection of some functional change in these cells. DNA synthesis is a particularly radiosensitive cell function. They therefore deter-

mined the correlation between the effect of radiation on antibody formation and DNA synthesis. They based this study on their own observation that irradiation has little effect on mature plasma cells but directly inhibits mitosis of preplasmacytes. In that case, only undamaged, healthy cells would participate in postirradiation antibody formation; these, however, decrease in number from day to day and are not regenerated. The decrease in antibody formation on a given day after exposure ought therefore to be proportionate to the decrease in the number of mature plasma cells. On the basis of this hypothesis, Stevens *et al.* calculated theoretical values for postirradiation inhibition of antibody formation and compared them with the experimental findings. The agreement between the two set of values was remarkably good.

Dixon *et al.* (29) propounded the concept that the radiosensitive phase in antibody production was modification of the γ -globulin-synthesizing generator to antigen. Spiers (36) found a correlation between radiosensitivity of the preinduction period and the postirradiation decrease in eosinophile concentration. He therefore assumed that eosinophiles are part of the mechanism of antibody formation, possibly by providing an enzymatic template, which is phagocytosed and utilized by mononuclears for antibody formation.

The curve obtained by Taliaferro and Taliaferro (37) for the relationship between inhibition of the immune response (Fig. 3) and the radiation dose is a typical sigmoid curve, like that which characterizes the lethal effect of radiation at cell level. This relationship might reflect the fact that inhibi-

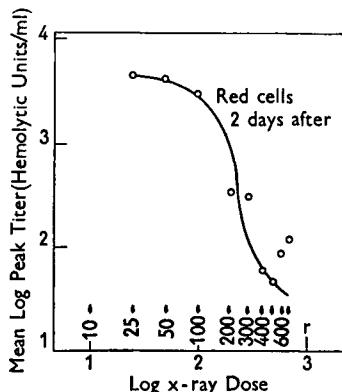


FIG. 3. Two dose-response curves relating mean log peak hemolysin titer to log dose of X-rays (a) from ten groups of rabbits after one intravenous injection of sheep red cells given 1 to 4 hours before total-body irradiation with 10 to 700 r, and (b) from nine groups of rabbits injected with the same amount of antigen 2 days after administration of the same amounts of X-rays. Redrawn from Taliaferro and Taliaferro (37).

tion of antibody formation by radiation is the function of the percentage of injury or of destruction of certain radiosensitive cells. If we consider the role of lymphocytes in this association, it corresponds to the rapid depletion of lymphocytes in the peripheral blood which commences immediately after exposure and reaches minimum values within about 24 hours. The time required for recovery of normal antibody production after irradiation is also in good agreement with the time required for the number of lymphocytes to return to normal. The finding of Dixon *et al.* (29) that antibody production, once begun, is practically uninhibited even by large radiation doses (800 r in rabbits 3 days after the administration of antigen) likewise indicates that lymphocytes participate in the early phase of antibody formation rather than in the actual production period. Taliaferro also outlined other possible explanations of the changes caused by the effects of radiation in the preinduction period of antibody formation. He is of the opinion that the possibility of the participation of several types of cells in this phase cannot be excluded, or that several generations of the same type of cell may participate. Irradiation could destroy or severely injure some of them—metabolic changes, inhibition of mitosis, etc.; if some such event occupied a key position in the process, it might be the cause of inhibition of the whole antibody reaction. The character of the initial process of the antibody reaction can be evaluated indirectly from experiments investigating the modification of the effect of radiation on antibody formation by certain experimental procedures.

b. Induction Period. After the preinduction period, sublethal or median lethal irradiation of the recipient does not affect the total quantity of antibodies produced, but their appearance is considerably retarded. In some cases the maximum titers are actually higher than in the unirradiated controls. The effect of radiation during the induction period could be explained on the basis of retardation of the development of the apparatus which synthesizes antibodies.

c. Production Period. According to present-day concepts, the actual antibodies are not produced from preformed γ -globulins but from a pool of free amino acids. This process is relatively resistant to direct irradiation of the body of the producer. This relative resistance does not, however, attain the same degree in different species of laboratory animals and is confined only to a given range of radiation doses. Maurer *et al.* (38) found that antibodies produced by irradiated rabbits do not differ immunochemically from those of normal rabbits.

2. SECONDARY REACTIONS

The findings of different authors on the inhibitory effects of radiation on antibody formation are in relatively good agreement as regards sensitivity

of the primary response. Data on radiosensitivity of secondary response are markedly at variance, however.

Dixon *et al.* (29) found that secondary response to bovine γ -globulin is relatively radioresistant. Taliaferro *et al.* (30, 31) found that the secondary reaction of hemolysins is radiosensitive. Taliaferro (8) describes three possible causes, which are supported by experimental evidence, of radiosensitivity of secondary response in some cases observed:

1. Radiosensitivity of secondary response depends indirectly on the effectiveness of antigenic stimulation; a secondary reaction to antigen with a short half-life may therefore be more radiosensitive than a secondary reaction to antigen which is eliminated more slowly from the blood stream.

2. More effective antigenic stimulation can also be achieved with larger amounts of antigen. Talmage *et al.* (39) successfully reduced radiosensitivity of secondary response by using large doses of antigen. These authors assume that an immunized organism injured by irradiation is less sensitive to a weak antigenic stimulus, and that small doses of antigen are blockaded in a secondary reaction by antibodies already present.

3. Crosland-Taylor (40) found that the "radioresistance" of a secondary reaction may be due—at least in the case of certain antigens, such as tetanus toxoid—to the fact that the change in the irradiated organism responsible for inhibition of the secondary reaction does not develop until long after irradiation. It is therefore not observed if antigen is administered soon after irradiation. If this "latent period" in inhibition of the secondary antibody reaction to radiation is particularly long, recovery may meantime occur, in which case radioresistance of secondary response could appear to be absolute.

The question of the radiosensitivity of the secondary reaction has also, in substance, been studied by authors who investigated the significance of preirradiation immunization with bacterial antigens for the resistance of animals infected with the appropriate infective agent after exposure. Smith *et al.* (41) found that immunization prior to irradiation increased resistance to infections induced after exposure, but only in the range of sublethal radiation doses. Perkins and Marcus (22) obtained similar results with the administration of a single radiation dose. When they administered considerably larger, fractionated doses, however, previous immunization retained its effectiveness. From this they deduced that the secondary antibody reaction of the organism is capable of recovery, and that the administration of sufficiently small part doses of radiation at sufficiently long intervals did not result in summation.

3. INDIRECT EFFECTS

The effects of radiation on acquired immunity which have been discussed above can all be ascribed to more or less direct influences on the individual phases of the antibody-forming mechanism. It is impossible, however, to exclude a priori the contingency that radiation may also affect the ultimate degree of the antibody reaction indirectly.

Dixon *et al.* (42) considered whether total-body irradiation in any way altered the ability of the organism to metabolize antigen. Using radioactive labeled proteins, they found, however, that the course of the non-immune phase was exactly the same in irradiated and unirradiated rabbits and that the inhibitory effect of irradiation was not manifested until the immune phase of elimination. In the case of cell-bound antigens, however, conditions may be different, as evidenced by the finding of Donaldson *et al.* (43) that radiation inhibits intracellular digestion of phagocytosed erythrocytes. This could indirectly affect antibody formation by reducing the efficiency of antigenic stimulation.

Ingraham (44), who determined the way in which radioactive labeled erythrocyte stromata were distributed in the irradiated organism, found no significant differences as compared with the normal. Werder *et al.* (45) studied the effect of radiation on the serum proteins and found that the greatest damage occurred to the γ -globulin fraction.

Another question to be considered is whether abnormal destruction of preformed antibodies occurs in an irradiated recipient, as this might resemble a decrease in the degree of immune response. Hollingsworth (46) showed that irradiation of rabbits with a dose of 300 r does not affect the destruction rate in antibodies transferred passively 24 hours after exposure. The rate of disappearance of antibodies and the postirradiation recovery rate vary markedly, however, in different species of animals and for different types of antibodies. Marcus and Donaldson (47) found a more than tenfold decrease in bacteriolysins in irradiated rabbits over a period of 9 days. This finding may be of importance in postirradiation infections; animals with inhibited antibody production could rapidly be deprived of preformed antibodies. In an experiment in which irradiation was carried out only a few days after the administration of antigen, radiosensitivity of the newly synthesized antibodies might affect the results. Perkins and Marcus (22), however, showed that even doses above LD₁₀₀ did not reduce the titer of previously synthesized antibodies.

4. MODIFICATION OF THE EFFECTS OF RADIATION ON ANTIBODY SYNTHESIS

It is known that certain experimental procedures can reduce the pathophysiological effects of radiation to such a degree that the resultant injury corresponds to the effects of much lower radiation doses administered with-

out protective measures. Some authors have also studied the question of the influence of similar procedures on antibody formation.

Jacobson and Robson (48) found that rabbits irradiated with 500 or 800 r were capable of forming anti-sheep hemolysins if their spleen was shielded during exposure. Splenectomy of the shielded spleen 24 hours after exposure and 24 hours before administering antigen did not prevent at least partial recovery of antibody synthesis. These authors therefore assumed that this favorable effect was mediated by a humoral factor from the shielded spleen, which stimulated nonsplenic antibody formation. Süssdorf and Draper (49) found that shielding the appendix in rabbits completely protected antibody synthesis, but shielding the spleen, liver, or bone marrow provided only partial protection. Results with spleen or bone marrow homogenates from unirradiated animals, which effectively reduce mortality in lethally irradiated individuals, have not been completely in agreement as regards their influence on antibody formation.

La Via *et al.* (50) found that the injection of spleen cells led to a certain degree of recovery of antibody-forming capacity in rats, but Smith and Ruth (51) had negative results in mice. Jaroslow and Taliaferro (52) achieved varying degrees of recovery of hemolysin-forming capacity in irradiated rabbits with HeLa cell preparations, yeast autolyzates, and cell suspensions or extracts of certain tissues from unirradiated animals. Harris *et al.* (53, 54) found that radiation-inhibited antibody formation can be renewed in rabbits if antigen is administered together with spleen, lymph node, or blood leukocyte cells from unirradiated donors.

C. INHIBITORY EFFECTS ON TRANSPLANTATION IMMUNITY

Evidence of the basic possibility of inhibiting immunological reactivity by means of radiation gave some authors the idea of utilizing radiation in transplantation problems. This possibility was studied by Murphy (55), who regarded lymphocytes as the only source of resistance of the organism to foreign tissues. He therefore used radiation as a means of effectively eliminating lymphoid tissue and successfully grafted several homo- or heterologous tumors in irradiated recipients. Clemmesen (56, 57) made similar observations, which he supplemented by the important finding that tumor heterografts are successful in irradiated recipients only in the case of a first graft. If immunity has already developed in the recipient, and if the first graft implanted without irradiation regresses, irradiation prior to repeated grafting of the tumor does not favorably affect the fate of the graft.

Dempster *et al.* (58) used irradiation of the recipient with a dose of 250 r in skin homografts in rabbits and obtained maximally double the time of survival as compared with survival in unirradiated animals. The favorable

effects of irradiation were again manifested only in the first series of grafts; irradiation of the recipients prior to repeating the graft—i.e., when transplantation immunity had already developed—produced no changes as compared with the unirradiated controls.

Harris *et al.* (59) and Dixon (60) use sublethally irradiated rabbits as recipients of homologous spleen cells. These go on producing antibodies longer than in unirradiated recipients, evidently as a result of weakening of the transplantation immune response which develops against them. Toolan (61) uses sublethal radiation doses, sometimes in combination with cortisone, for conditioning the recipient prior to heterotransplantation of human tumors in animals.

It is seen, therefore, that sublethal irradiation of animals delays the development of transplantation immunity for some time. The fact that X-irradiation does not suppress a pre-existent immune state is in agreement with differences in the radiosensitivity of primary and secondary antibody response.

IV. Long-Term Changes in the Immunological Reactivity of the Irradiated Organism

A. THE DEVELOPMENT OF RADIATION CHIMERAS

Convincing evidence has been produced in recent years demonstrating that irradiation of the organism with massive total-body doses of radiation can completely or almost completely destroy its capacity for immune response. The cause is evidently associated with the destruction of lymphoid tissues. Since this form of irradiation also severely injures the other hematopoietic tissues, the organism would normally die. It can be induced to recover, however, by transfusion of a suspension of homologous or heterologous cells from the spleen, bone marrow, or embryonic tissues of an unirradiated donor (62, 63). During the past few years, various laboratories have provided indirect (e.g., 64) and direct (e.g., 65, 66) evidence that the recovery of lethally irradiated animals treated in this way is due to recolonization of their tissues by the healthy donor cells, which thus constitute successful homo- or heterografts of myeloid cells and also, obviously (67, 68), of lymphoid cells. These individuals are thus actually chimeras, i.e., organisms producing cells of more than one genotype.

The state of chimerism is per se evidence that the immune reactivity of the irradiated organism has been completely suppressed. The fact that radiation chimeras can survive is proof, on the other hand, that they are capable of defense reactions, in particular against infections, to which they are constantly exposed. What is the explanation of this apparent discrepancy? Leaving aside cases in which the cause of immune reactivity is re-

generation of the recipient's own lymphoid tissues and recovery of their function, it is seen to be due to greater resistance of certain individuals to the radiation, so that the final effect represents only temporary depression of immune reactivity, like that produced by sublethal radiation doses in individuals of average sensitivity. Radiation chimerism in such cases is therefore only a temporary state.

B. IMMUNOLOGICAL MANIFESTATIONS OF RADIATION CHIMERAS

The immunological activity of radiation chimeras can be evaluated by the following signs:

1. BEHAVIOR OF RADIATION CHIMERAS TO GRAFTS

The most important experimental results show that lethally irradiated animals which are protected with homo- or heterologous hematopoietic cells after exposure tolerate skin (64, 69) or neoplastic (70) homo- or heterografts of the same origin as the therapeutically administered hematopoietic tissue (from the same donor or from another individual of the donor's inbred strain). This is not surprising if it is borne in mind that survival of tolerant recipients is due to successful grafts of hematopoietic donor tissues. In that case there is no reason why they should display greater resistance to other tissues, whether skin or tumor. On the basis of the cytological evidence provided by Ford (71) and Mitchison's immunological test (72), that the regenerating lymphoid tissues of radiation chimeras are likewise of the donor type, successful skin or tumor homo- or heterografts in radiation chimeras can therefore be regarded as auto- or isografts in relation to transplanted cells capable of immune response.

2. ANTIBODY SYNTHESIS IN RADIATION CHIMERAS

The formation of antibodies against different antigens was studied in lethally irradiated animals which survived after the administration of homologous or heterologous hematopoietic cells from unirradiated donors (70, 73). The aim of these studies was to determine whether the recipient or the donor is responsible for their formation. The fact that this question arose shows that the conditions of the experiments, and primarily of the radiation dose, did not exclude the possibility of regeneration of the lymphoid tissues of the recipient and recovery of their immune activity. The study of antibody formation in radiation chimeras can therefore be an index of their immune activity only if chimerism has actually been proved. Present concepts on the correlation between chimerism, transplantation immunity, antibody synthesis, and the incidence of delayed death are based mainly on investigations in different animals or in different laboratories. The results may therefore be loaded with considerable error. Brocades

Zaalberg *et al.* (69) found three different types of survivors in a single group of lethally irradiated mice, after the administration of rat bone marrow cells: (1) heterologous chimeras, in which the peripheral blood contained only rat erythrocytes and granulocytes, and on which rat skin grafts survived permanently; (2) individuals with a mosaic of rat and mouse erythrocytes and granulocytes, in which rat skin grafts showed some deterioration; and (3) mice in which regeneration of the animals' own hematopoietic tissues occurred and rat skin grafts were sloughed.

This raises the question of whether regeneration or substitution of individual lymphoid and myeloid cell elements of donor and recipient can take place independently, or whether the process is parallel. The findings of some authors differ as regards the formation of serum proteins in radiation chimeras; in some cases the serum proteins appear to be of the donor type, and in others of the recipient type (74-76). It is possible that conditions in heterologous radiation chimeras are even more complex than in homologous chimeras and that the phylogenetic distance of recipient and donor may be an important factor.

The experimental results obtained to date with antibody synthesis in radiation chimeras do not yet therefore permit the formulation of unequivocal conclusions.

3. THE DELAYED DEATH PHENOMENON IN RADIATION CHIMERAS

Cases of delayed death are frequent among homologous and heterologous chimeras. Various evidence exists that this is not a late reaction of the organism to irradiation but an *in vivo* immune reaction of the transplanted donor lymphoid tissue against the tolerant host. Delayed death is evidently due to the same cause as "runt disease," or the death of young animals in which immunological tolerance is induced by an intraembryonal injection of spleen cells from adult donors (77, 78). The explanation of delayed death on the principle of the immune reaction of the graft against the tolerant host is supported by the results of experiments in which (a) delayed death does not occur if the genetic combination of the donor of the hematopoietic cells and the irradiated recipient does not fulfill the conditions for reaction of the graft against the host, and (b) delayed death occurs if the genetic combination of recipient and donor fulfills these conditions.

Delayed death does not occur in the following cases: (i) In combinations in which the donors of the protective material are F_1 hybrids of two inbred strains and the recipients are individuals of either of the parent strains (79). (ii) In a homologous combination of embryonic donor and adult irradiated recipient—in this case delayed death need not occur if immunological tolerance to the tolerant host can be induced in the embryonic lymphoid elements. The experiments of Lengerová (80) demonstrate that this is

feasible, since delayed death does not occur in irradiated mice to which homologous embryonic liver cells are administered. (iii) On using F₁ hybrids of two inbred strains homozygotic at the H-2 locus (the strongest histocompatible locus known) as recipients, and either of the parent strains as donors (suitable strains are BALB/c and DBA/2, which are identical at the H-2 locus, H-2^d) (81). In this combination, in which incompatibility is confined only to weak loci, runt disease also fails to develop after the induction of immunological tolerance.

Delayed death occurs in the following cases: (i) In the usual homologous and heterologous chimeras, in which conditions are also suitable, however, for a reaction of the recipient against the graft, if regeneration of lymphoid tissue has occurred. Model experiments with sublethal radiation doses showed that death can also be provoked on this principle (79, 82). (ii) In a combination of F₁ hybrids of two inbred strains heterozygotic at the H-2 locus as recipients, and of either of the parent strains as donors (83). (iii) In a combination in which the donors are previously immunized against the recipients (84).

4. OPEN QUESTIONS IN THE PROBLEM OF RADIATION CHIMERAS

One important question is why delayed death does not always occur in experimental situations in which the necessary conditions are fulfilled, and why some homo- or heterologous chimeras survive. These cases can only be explained by the development of a state of immunological neutrality between graft and host. In the host it is characterized by nonspecific, complete elimination of immune reactivity, and in the graft by specific tolerance to the host. It is not a case of nonspecific tolerance of the graft, like that caused by exhaustion of the lymphoid tissues, since in homologous chimeras skin or tumor grafts of the host strain take successfully (84, 85), but not those of other strains (86). The mechanism of the development of this tolerance is not yet clear. Among known mechanisms—actively acquired tolerance, enhancement, immunological paralysis—the most acceptable appears to be immunological paralysis evoked by constant contact of the donor's lymphoid tissues with the excess of the host's antigens (85). Other experimental models of immunological paralysis against graft antigens have not yet been satisfactorily elaborated, however.

The reason why a state of immunological neutrality develops in some chimeras, whereas in others delayed death occurs on the immune reaction principle, is manifestly associated with the individual variability of a series of factors. These include, in the first place, differences in the radiosensitivity of the lymphoid and myeloid tissues of irradiated animals of the same strain to the same radiation dose.

The number of questions which this line of research has raised is obviously

much greater than those which it has solved. It seems, however, that the only correct interpretation will be one capable of explaining all the apparently conflicting results and of determining the conditions under which one of the various possible mechanisms will predominate. Today it can already be stated that in the range of lethal or sublethal radiation doses new conditions develop for the long-term, or even the permanent, modification of the immune reaction of irradiated organisms. It cannot be asserted that this is associated with qualitative differences in the effect of sublethal and lethal radiation doses, just as it would be simplifying matters too much to insist that one phase of antibody formation is radiosensitive and that the other is radioresistant. The differences are all quantitative, and if the radiation dose is raised it will finally reach a limit at which even the most resistant elements are injured. Evidence that the difference is only quantitative is furnished by findings that in a small percentage of cases transplantation of erythropoietic homologous elements can occur even in sublethally irradiated rats, in a combination of a genetically very closely related host and donor, differing only in one or a few weak histocompatible genes (87), and by the observation of van Bekkum and Vos (88) that even in the supralethal range of radiation doses some recovery of the defense capacity of the organism against foreign tissues is not essentially impossible. The range of absolute lethal radiation doses, however, obviously exceeds the upper limit of radioresistance of the majority of lymphoid tissues with average resistance, and elimination of the immunological reactivity of most irradiated organisms is therefore relatively uniform within this range.

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CHAPTER 4

Mechanisms of Protective and Sensitizing Action

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I. Introduction

A. HISTORICAL BACKGROUND

The early phase of radiobiology was largely concerned with studies of the differential radiosensitivity of tissues. The prevailing ideas concerning the mode of action of ionizing radiation corresponded by and large to the present-day concepts of direct action, a view offering little hope of influencing radiosensitivity by external means.

The first attempts to modify the radiation response were reported by Schwarz (1), who demonstrated the effect of ischemia on the radiosensitivity of human skin. In the years 1920 to 1930 the fundamental phenomena with regard to the effect of oxygen, temperature, and hydration on radiosensitivity were studied in a variety of systems (2-6). These earliest attempts to modify radiation effects on living systems were of a purely academic nature.

The concept of chemical protection emerged from radiochemical studies of pure chemical systems. This pioneer work on the indirect action of ionizing radiation (7-10) revealed that in solutions containing two solutes the presence of one solute influences the radiochemical transformation of the second solute. These findings were correctly interpreted as the result of a competition between the two solutes for the "activated water" (10). The terms protection and protective agents were not introduced, however, until Dale (11), Friedewald and Anderson (12), and Evans *et al.* (13) in the years 1940 to 1942 were able to reduce by specific chemical substances the radiation damage to biological systems such as crystalline enzymes, virus, and suspensions of *Arabacia punctulata* sperm.

The studies of Fricke *et al.* (10) on two-component systems revealed that different substances possess different ability to react with "activated water." Considerable advances along this line were made by Dale *et al.* (14, 15), who measured the "protective power per molecule" of a series of substances. Dale *et al.* (15) discovered the unique ability of various sulfur-containing compounds to inactivate the free radicals from water. Later, Barron *et al.* (16-18) observed the great sensitivity of thiols to the indirect action of ionizing radiation.

In view of the above-mentioned facts it may seem surprising that chemical protection *in vivo* was not discovered until 1949. In that year Patt *et al.*

(19), Chapman *et al.* (20), and Herve and Bacq (21) observed the protective action of cysteine, glutathione, and sodium cyanide, respectively.

In the subsequent years a large number of substances were screened for protective action, particularly with regard to the lethal effects of ionizing radiation in mammals. From a practical point of view the discovery of the protective action of cysteamine (22) and of its *S*-guanido derivative aminoethylisothiuronium bromide hydrobromide (AET) (23) probably represent the most significant advances.

B. DEFINITION OF THE TERMS PROTECTION AND SENSITIZATION

It is firmly established that the radiation response may be altered by procedures operating either before and during or after the exposure. The effects brought about by pre- and posttreatment seem to be different in nature as well as in degree. It therefore seems appropriate to restrict the terms chemical protection and sensitization to the reduction and the enhancement in the radiation response accomplished by procedures acting before or during the radiation exposure (24). Formally, these definitions are purely descriptive. Most workers, however, seem to assume more or less tacitly that the terms imply an alteration in the immediate radiochemical lesions. In agreement with Latarjet and Gray (24) the term restoration will be used to denote reductions in radiation lesions accomplished by procedures brought to act after the radiation exposure.

C. THE PROTECTIVE AND SENSITIZING SUBSTANCES

A considerable number of substances with widely different chemical and physical properties have been found to possess protective or sensitizing action. In order to facilitate the subsequent discussion of their mode of action, a tentative classification of the compounds will be given (Table I).

The data have been selected on the basis of activity *in vivo*. The classification, which corresponds fairly closely to certain specific chemical, physiological, and pharmacological characteristics, is believed to reflect different mechanisms of action. The substances represent typical examples of each group. Numerous other active compounds, as well as inactive analogs, may be found in the references given.

D. THE SCOPE OF THE ARTICLE

The main experimental facts concerning protection and sensitization phenomena have been covered in a number of review articles (55-60). Therefore no attempt will be made to summarize the vast number of experiments on protective and sensitizing action in chemical and biological systems. Only selected experiments which serve to illuminate basic mechanisms of action will be discussed.

TABLE I
REPRESENTATIVE EXAMPLES OF PROTECTIVE AND SENSITIZING SUBSTANCES ACTIVE IN MAMMALS

	Group	Compound	Dose (mg/kg)	Animal	Effect	Ref.
Sulfur-containing compounds	The cysteine-cysteamine group	Cysteine Cysteamine Aminoethylisothiuronium bromide, HBr (AET) Glutathione	950-1200 i.p. 75-250 i.p. 200-480 i.p. 800-1000 i.p.	Mice, rats Mice, rats Mice, monkeys Mice, rats	Protection	(19, 25, 26) (22, 26-28) (23, 29) (20, 30-32)
	β -Homocysteine Isocysteine Thioethanol		445 i.p. 400 i.p. 150 i.p.	Mice Mice Mice	Sensitization	(33) (33) (26)
	Other sulfur-containing compounds	Dithiocarbamates Thiourea	500-600 i.p. 2500 i.p.	Mice Mice	Protection	(30, 34, 35) (26, 36)
	Specific enzyme inhibitors	Sodium cyanide Sodium azide Carbon monoxide	5 i.p. 6.5 i.p. —	Mice Mice Mice	Protection	(30) (30) (37)
	Hormones	Estradiolbenzoate Deoxycorticosterone acetate ACTH	12.5 i.m. 15 hr before irradiation 50 i.m. for 10 days 25 i.m. for 7 days	Mice Mice Mice	Protection	(38) (38) (38)
		Thyroxine	Desiccated thyroid for 13 days.	Rats	Sensitization	(39)

	Methylandrostenediol	10-25 mg per animal	Rats, mice	(40)
Stimulants and depressants of the central nervous system	Reserpine Chlorpromazine Amphetamine Serotonin	4 s.c. 24 hr before irradiation 20 i.p. 1 i.p. 95 i.p.	Mice Mice Mice	Protection (41) (42) (41, 43) (30, 43, 44)
Other pharmacologically active compounds	<i>p</i> -Aminopropiophenone Pitressin	32 i.p. 23 units i.p.	Rats Rats	Protection (45) (46)
	Synkayvite and related compounds Porphyrins Aneurine diphosphate Lactoflavin-5-phosphate 3,4-Benzopyrene Monooiodoacetic acid	10 mg per animal for 15 days — 77 i.p. 77 i.p. 83.5 s.c. for 3 days 35 i.p.	Rats Rats Rats Rats Rats Mice	Sensitization (47) (48) (49) (49) (50) (51)
Metabolites and inert compounds	Fructose Pyruvic acid Formic acid	13500 i.p. 700 i.p. 92 i.p.	Mice Mice Mice	Protection (30) (30, 52, 53) (30)
Inert compounds	Ethylenediaminetetraacetic acid Propylene glycol Ethanol	150-580 i.p. 3000 i.p. 5920 orally	Mice Mice Mice	Protection (3, 35) (30) (54)

When the available data on chemical protection and sensitization are considered, previous attempts to explain all protection phenomena on the basis of a single mechanism seem unwarranted. On the contrary, the conclusion seems inescapable that the radiation response in a particular system can be influenced in a number of ways. It becomes increasingly clear that several mechanisms may contribute to the protective effect of one particular group of compounds and that the different types of protective action may exert their main effect by different mechanisms.

In the present discussion an attempt is made to call to the reader's attention the numerous possibilities existing for protective and sensitizing action, many of which have been largely ignored. Our understanding of the mechanism of action of protective and sensitizing agents is very incomplete, and on many important aspects experimental data are lacking. Many conclusions here presented will therefore be tentative. At this stage of the development it appears important to attempt to present a logical framework into which new data can be fitted.

In the opinion of the present authors the phenomena of protection and sensitization are two closely linked aspects of radiosensitivity. It appears that in cells and tissues there exists a physiological level of chemical protection which can be modified in either direction. We have, therefore, chosen to discuss the phenomena of protection and sensitization together.

E. REMARKS ON THE MODE OF ACTION OF IONIZING RADIATION

Current views on the mechanism of the direct and indirect action of ionizing radiation are discussed elsewhere in this treatise. The sequence of events from the initial impact of the ionizing particle to the observable effects can be represented diagrammatically as in Fig. 1.

Phase I represents the excitation of water molecules and the forward and radical reactions leading to the formation of the molecular products hydrogen and hydrogen peroxide, as well as a number of free radicals.

Phase II consists in the ionization or excitation of nonessential substances present. This may be brought about either by direct hits or by interaction with the active agents of phase I. An intervention of phase II complicates considerably the concept of indirect action. The intervening substances may bring about either a reduction or an enhancement of the biological effect, depending on their chemical nature. Thus, the energy may be dissipated with the termination of the chain of events. On the other hand, semistable radicals with long diffusion paths and with specific reactivity toward sensitive sites may possibly be formed.

Phase III represents the transfer of radiation energy to the actual target molecules. This may occur either by direct hits or by interaction with the products of phase II or phase I. The ionized or activated target molecule

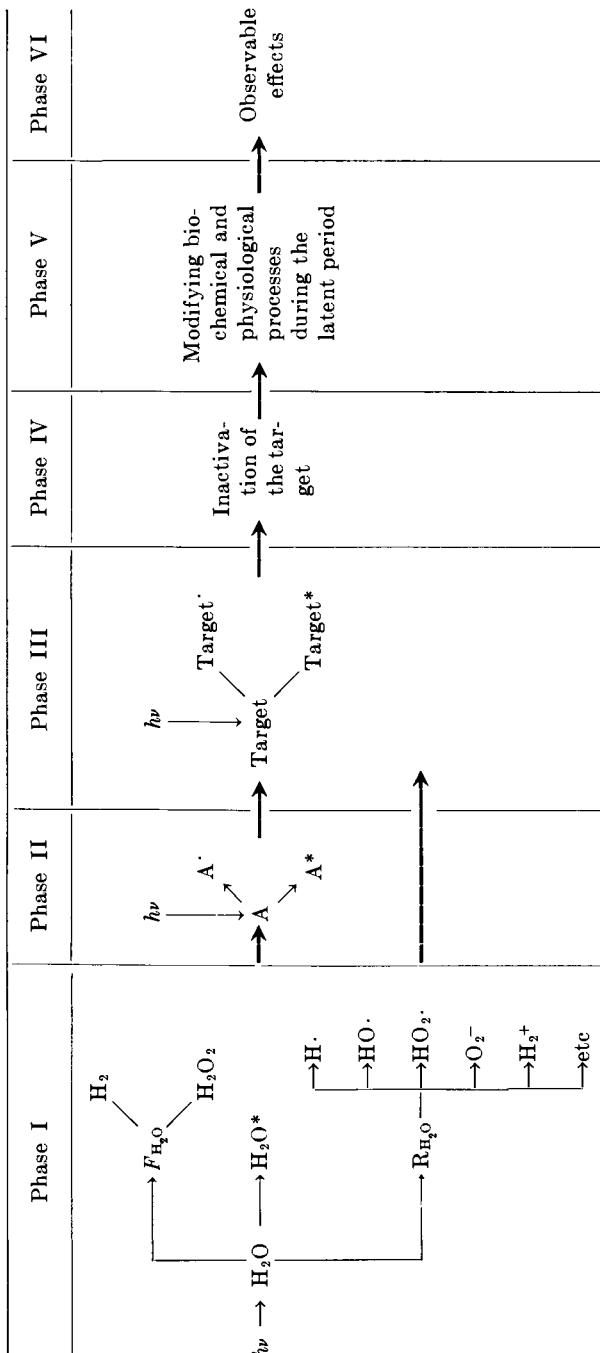


FIG. 1. Diagrammatic representation of the events following the absorption of radiation energy. * = activated molecules.

may, depending on its chemical nature, exist as such for a shorter or longer period of time.

Phase IV represents the irreversible chemical changes leading to the loss of the specific properties of the target.

Phases V and VI. In simple *in vitro* systems the result of phase IV can usually be measured in some way or other. In complex biological systems, however, there will always be interposed a latent period during which physiological and biochemical factors may modify the observable end result. The factors modifying the radiation damage during phase V are covered elsewhere in this book.

II. General Aspects of Protective and Sensitizing Action

A. THE PROTECTIVE AND SENSITIZING EFFECTS

In Table II the most important radiation lesions against which protection has been observed are listed. Also included are various radiation effects against which no protection has been obtained. In Table III examples of sensitizing action are presented. Owing to the paucity of data, this table, in contrast to Table II, has been arranged according to the compound and procedure used.

Whereas in chemical systems the radiation lesions and the protective effects frequently can be observed directly and immediately, it seems to be a general phenomenon that in living systems radiobiological damage can be observed only after a certain period of metabolic and physiological activity. Consequently, the actual biological unit or process being protected need not be identical with the biological end point chosen. Thus, a protection—e.g., against bone marrow damage—may well be due to the protection of a regeneration factor (99).

The specific functional units, the destruction of which is responsible for the observable radiation damage, are usually referred to as the “targets.” In this discussion we shall be concerned mainly with the processes whereby the radiosensitivity of target molecules can be modified, whereas their nature and physiological functions will only briefly be considered.

B. RADIOSENSITIVITY, A CELLULAR PROPERTY

It is generally considered that the main and typical radiobiological effects are brought about by cellular lesions (see ref. 58). Similarly, it appears that the factors which modify radiosensitivity exert their effects on the cells proper. A number of experiments can be enumerated in support of this thesis:

In order to afford protection, the agents must be administered prior to the radiation exposure. Even when administered shortly after the irradiation they are in general inactive (56).

TABLE II
THE MAIN RADIATION EFFECTS AGAINST WHICH PROTECTION
HAS BEEN DEMONSTRATED

Radiation response	Protective effect	Ref.
WHOLE ANIMALS		
Radiation syndrome		
Weight loss	++	(61)
Hematopoietic response	+++	(25, 61)
Radiation death (single dose)		
Bone marrow death	+++	(25, 61, 62)
Intestinal death	++	(63-65)
Central nervous system death	(+)	(66)
Esophageal death	+	(67)
Radiation death (fractionated dose)	(+)	(68)
Regression of tumor growth	+	(69)
Histologic and cytologic effects		
Chromosome aberrations	+	(70, 71)
Capillary damage	+	(72)
Early degenerations in spleen, liver, and the intestines	+	(63, 73, 74)
Late effects		
Genetic effects	0?	(75)
Premature aging	0	(76)
Neoplasia	0	(76-79)
Cataract formation	+++	(80, 81)
Graying of hair	+	(82)
SIMPLE BIOLOGICAL SYSTEMS		
Reduced viability		
Fertility of sperms (<i>Arabacia punctulata</i>)	++	(13)
Multiplication of viruses and bacteria in suspension	+++	(12, 83-85)
Colony formation in bacteria	++	(86)
Growth of germinating seeds	++	(87)
Growth of <i>Allium cepa</i> roots	++	(88)
Inactivation of transforming principle	+++	(89)
Lethal action in infusoria	++	(90, 91)
Genetic effects		
Back-mutation in <i>E. coli</i>	+	(92)
Specific suppressor system in <i>Drosophila melanogaster</i>	++	(93)
Morphological and physiological effects		
Chromosome aberrations		
<i>Tradescantia paludosa</i> cuttings	++	(94, 95)
<i>Allium cepa</i> roots	++	(88)
Thymocyte survival (vital staining test)	++	(96)
Inhibition of reticulocyte maturation	++	(97)
Block to DNA synthesis in <i>E. coli</i> strain B/r	++	(98)

TABLE III
EXAMPLES OF RADIOSENSITIZING ACTION

Compound	Radiation response	Sensitizing effect	Ref.
Isocysteine	Lethal action, rats	+	(33, 40)
β -Homocysteine	Lethal action, rats	+	(33, 40)
Penicillamine	Lethal action, rats	+	(40)
Thioethanol	Lethal action, rats	+	(26, 40)
Synkayvite	Tumor regression, rats	+	(100)
	Response of bronchial carcinoma	+	(100)
	Colony formation, <i>E. coli</i> and yeast	0	(101)
	Lethal action, rabbits	Some Protection	(102)
	Lethal action, rats	+	(47)
	Lethal action, rats	0	(49)
	Lethal action, mice	+ (?)	(48)
	Lethal action, <i>Paramecium caudatum</i>	+++	(103)
Megaphen	Growth inhibition of ascites tumor in mice	+	(104)
Monoiodoacetic acid	Lethal action, mice	+	(51)
Methylandrostenediol 3,4-Benzopyrene	Lethal action, rats, mice	+	(40)
	Lethal action, rats	+	(50)
Malonic acid	Reduced survival of <i>Saccharomyces ellipsoideus</i>	+	(105)
Maleic acid		+	(105)

In the case of cysteamine and cystamine, it has been shown that the period of protection coincides fairly well with the interval during which the compounds are present in the organism (106).

Local infiltration of cysteine into the skin of guinea pigs protects the infiltrated area against X-ray-induced epilation (83). In principle, the same results have been obtained by application of the protective agent in an ointment or by ionophoresis (107, 108).

Significant protection of *E. coli* is obtained when the cells are centrifuged out from a cysteamine solution and washed immediately before irradiation (109) indicating that the cysteamine resides within the cells.

It is generally felt that the radiosensitizers as well must be present in the cells in order to be active, although little experimental evidence is available on this point. Mitchell and co-workers have observed that tetrasodium 2-methyl-1,4-naphthohydroquinone diphosphate (synkayvite) and related compounds tend to concentrate in those tissues where sensitizing effects can be observed (100).

C. THE RELATIONSHIPS BETWEEN THE PROTECTOR DOSE, THE RADIATION DOSE, AND THE EFFECT

An adequate description of a radiation effect requires that the response be measured over a certain dose range. In some pure chemical systems a straight-line relationship is found. In biological systems more complex dose-effect relationships are usually observed (110, 111).

In principle, the effect of protective or sensitizing agents can be evaluated with regard to any radiation lesion. The most satisfactory measure of the effect is the "dose reduction factor" or the "dose amplification factor," i.e., the ratio between equieffective doses in the presence and in the absence of the substance tested. If the dose-effect curves in the presence and in the absence of the protective agent are parallel, the choice of reference point is not decisive. Otherwise, the results must be referred to a certain standard effect (e.g., the LD₅₀).

With increasing ionization density (linear energy transfer) the relative biological efficiency increases and concurrently the effect of protective agents will decrease (112, 113). The bulk of the data reported on protective and sensitizing action refers to experiments with conventional X-rays in the energy range 100 to 200 kev.

Important information concerning the mode of action of a protective or sensitizing compound can be obtained by measuring the relationship between the protective or sensitizing effect and the amount of substance administered. For most protective compounds the protective action will increase with the dose. In the case of cysteine, the degree of protection against lethality in mice was found to increase linearly as the log of the cysteine dose (25). Such relationships are consistent with the view that the compounds themselves participate in the dissipation of the radiation energy. Usually, the protective power is expressed as the dose reduction factor on a molar basis, relative to that of a reference compound, e.g., cysteamine.

In some cases, dose-effect relationships are observed which constitute evidence against the view that the compounds participate directly in the radiochemical events. In the protection of mice with amphetamine, optimal

effect was obtained with 1 $\mu\text{g}/\text{gm}$ of mouse, whereas an increase in the dose to 3 $\mu\text{g}/\text{gm}$ actually resulted in a sensitization (43). In the protection of *E. coli* by sodium dithionite, the effect occurred in the concentration range from about $5 \times 10^{-4} M$ to $5 \times 10^{-3} M$, but an additional twentyfold increase in the protector concentration did not further raise the radioresistance (86). This protector dose-effect curve could be ascribed to a mechanism of action by way of oxygen removal, as the oxygen was completely removed by $5 \times 10^{-3} M$ sodium dithionite.

Many protective substances are toxic in high doses. The optimal protective effect which can be obtained will therefore be a compromise between the toxicity and the protective action on a molar basis. In animals, the highest degree of protection obtained against the lethal action corresponds to a dose reduction factor of about 2 (56). In bacterial systems, dose reduction factors up to 4 may be obtained (109). By combining pre- and post-treatment, factors of about 4 have been obtained in mammals, and up to 12 in bacteria (109).

III. The Interaction of Protective and Sensitizing Agents with Radiation-Induced Free Radicals

A. THE "RADICAL SCAVENGER" HYPOTHESIS

As pointed out above, the concept of chemical protection was developed on the basis of radiochemical studies on dilute aqueous solutions, where the indirect action is dominating. The protection observed under these circumstances was shown to be due to a competition between the protector and the indicator molecules for the active agents formed from water (10, 11). This mechanism, which was thoroughly studied and well corroborated in pure chemical systems, has been widely believed to be the main mechanism of action also *in vivo* (30, 56, 114).

In its original version the radical scavenger hypothesis was concerned only with the inactivation of the radicals formed from water. It is clear, however, that in biological systems free radicals formed from other cellular constituents (phase II, Fig. 1) may intervene, and the interaction of the protective agents with these secondary free radicals must be taken into consideration.

In all reactions between a free radical and a saturated (nonradical) scavenger molecule the product of the interaction will also be a free radical. Clearly, if a substance is to be able to protect target molecules by this mechanism, the new radical formed must be less reactive toward target molecules than the original free radicals themselves. The possibility also exists that the new radical formed may possess enhanced reactivity toward specific target molecules. In this event a radiosensitization will be observed.

In addition to the requirements mentioned above, several other conditions must presumably be fulfilled for a substance to act as an efficient radical scavenger. The compound must possess a high reactivity toward the free radicals, and obviously it must be present in the solution immediately surrounding the target molecules. This implies that, in the case of living organisms, the agents must be able to penetrate cellular and subcellular membranes.

In the last analysis, protection by way of a radical scavenger mechanism must be dependent on a specific radiochemical property of the protector molecule. Admittedly, in living systems several other properties of the protector molecule may also influence the radical scavenger effect, but a high ability to inactivate radicals is obviously a necessary requirement and probably the decisive factor.

In the discussion of the mode of action of the protective agents the radical scavenger mechanism has played a dominating role. In view of this fact it is deplorable that exact data on the radical-capturing ability of the numerous protective compounds studied in biological systems are largely lacking. The pronounced chemical specificity exhibited by the compounds active *in vivo* is one of the most striking phenomena in chemical protection. This is particularly true with regard to the protective thiols. Almost no quantitative data are available, however, on the relative ability of these thiols to inactivate free radicals.

It is felt that an appraisal of the significance of the radical scavenger mechanism must be based on a comparison between chemical structure, radical-capturing ability, and protective action in various systems. An attempt will first be made to examine the available data with the purpose of deducing the principles governing the relationship between chemical structure and radical-capturing ability.

B. CHEMICAL ASPECTS OF RADICAL INACTIVATION

1. EXPERIMENTAL PROCEDURES

The ability of compounds to inactivate free radicals can be assessed from different types of data.

a. *The "Limiting Concentration."* In dilute solution the yield of radiochemical reactions will, within a certain concentration range, be independent of the solute concentration (the "dilution effect") (10, 115). In this range the radicals will all react with solute molecules. Below the "limiting concentration" radical recombination becomes important as a competing reaction. It follows that the "limiting concentration" can be used as a measure of the specific ability of the solute to capture the free radicals formed (110).

b. Competition Experiments in Two-Solute Systems. In two-solute systems the radical-capturing ability of the one solute can be calculated from its effect on the radiochemical transformations of the second solute. Strict radiochemical studies along these lines were carried out in the pioneer investigations of Fricke *et al.* (10), Dale *et al.* (11, 15), and Lea (110). It has also been demonstrated that in a two-component system the second solute may bring about a sensitizing effect (116, 117). In this case the radicals formed from the second solute are apparently more deleterious to the target than the original free radicals.

It is to be regretted that since the discovery of *in vivo* protection only limited attention has been devoted to this type of study.

c. Protection Experiments in Polymer Systems. The indirect action of ionizing radiation may bring about pronounced physicochemical changes (polymerization, depolymerization, or crosslinking) in certain polymer systems. These phenomena have been extensively used in experiments where the radical-capturing ability of a great number of compounds has been determined (30, 118, 119).

In his studies on the degradation of polymethacrylic acid, Alexander concluded that the HO₂· radical was primarily responsible for the degradation (120). The protective effects observed in this system were therefore believed to be due to a specific inactivation of HO₂· radicals. No doubt this polymer system may give relative values for the radical scavenger ability of various compounds. The validity of the conclusions with regard to the specific radicals involved have been seriously doubted, however (121-123).

d. Biological Test Systems. A great variety of biological systems have been used for measuring protective activity under conditions where the indirect action most likely is predominating. From such experiments conclusions with regard to radical-capturing ability can be drawn only with caution. Interfering processes, such as oxygen deprival and possible influence on cell metabolism, should be borne in mind.

e. Studies on the Radiochemistry of Protective and Sensitizing Agents. When protective or sensitizing agents inactivate free radicals, they will become chemically changed. Obviously, information on the nature and the yield of these chemical changes undergone by the compounds may throw light on their mode of action. Comparatively little work has been done along these lines. A limited number of studies, particularly on the sulfur-containing protective agents, have been carried out. The powerful electron spin resonance method which permits the detection and in certain instances the analysis of the electron distribution of free radicals has recently been introduced into the field of radiochemistry. Important information on the relationship between chemical structure and radiochemical properties has been secured by this approach.

A few contributions are available in which the radical scavenger ability

2. RESULTS

of a limited number of compounds has been measured and the fundamental physicochemical parameters have been calculated. Some of the results have been tabulated in Table IV.

Lea (110) calculated on the basis of data for the "limiting concentration"

TABLE IV
THE RELATIVE ABILITY OF VARIOUS COMPOUNDS TO COMPETE FOR THE
FREE RADICALS FORMED FROM WATER

Compound	Zp (protector) ^a p_1 (dinucleotide)	Protec- tion of polymeth- acrylic acid ^b (%) ^c	p (protector) ^d p_1 (carboxy- peptidase)
Glycine	4.6×10^7	18	
Sodium oxalate	4.6×10^7	0	2×10^{-3}
Sodium nitrate	4.6×10^7	0	
Leucylglycine	4.6×10^8		
Sodium hippurate	4.6×10^9		
Glucose	1.5×10^{10}	21	0.79
Sucrose	1.5×10^{10}		
Potassium thiocyanate	1.5×10^{10}	42	
Sodium formate	1.5×10^{10}	37	3.1
Fruuctose	4.6×10^{10}	40	
Sodium nitrite	4.6×10^{10}	62	
Sodium nucleate	4.6×10^{10}	54	
p (absolute value)			
Methyl alcohol	0.25	27	
Oxalic acid	0.037	0	
Tyrosine	0.037	43	
Glutathione	0.018		
Thiourea		69	4.7
Dimethylthiourea			1.9
Dimethylurea		31	0.4
Alloxan		0	2.1×10^{-2}
Urea		0	7.5×10^{-4}

^a The affinities of substances (1 gm. mole per liter) for activated water, relative to that of alloxazine adenine dinucleotide. For definitions of Z and p , see the text. The data are taken from Lea (110).

^b Protection against radiation-induced degradation of polymethacrylic acid (Alexander *et al.*, 30).

^c Per cent protection: $\frac{C - T}{C} \times 100$, where C and T are the decrease in the viscosity of the polymer solution irradiated with 1000 r in the absence and in the presence, respectively, of the protector.

^d Data taken from Dale *et al.* (15).

the specific ability of certain compounds to inactivate free radicals. The results were expressed in terms of the parameter, p , which is equal to the fraction of the collisions between the protector molecule and the radicals, resulting in inactivation of the latter. Although the absolute p values given in Table IV are subject to considerable numerical uncertainties, they demonstrate clearly the enormous reactivity of free radicals, since they indicate that from one out of four to one out of a hundred collisions results in the inactivation of the radical. For comparison it may be noted that in an ordinary chemical reaction having an activation energy of about 24,000 cal/mole only one out of 10^{18} collisions will result in reaction. From the table the significant fact emerges that the inherent ability of methyl alcohol to inactivate free radicals is more than ten times that of glutathione.

On the basis of data from Dale (14), Lea further calculated the relative ability of a number of compounds to compete for the free radicals formed from water (110). The affinities of the substances for activated water were expressed relative to that of alloxazine adenine dinucleotide as the quotient Zp (protector)/ p_1 (dinucleotide). In this expression Z is equal to the number of collisions per second undergone by a single radical with the compound in question (present in a concentration of 1 M). The data (Table IV) reveal a striking difference in the reactivity of different compounds. Thus, fructose appears to be about one thousand times as active as glycine.

The measurements of relative protective ability were extended by Dale *et al.* (15) using carboxypeptidase and alloxazine adenine dinucleotide as indicator molecules (Table IV). They discovered the unique protective ability of various sulfur-containing substances (thiourea, dimethylthiourea, sodium thiosulfate, and colloidal sulfur). In these experiments it was observed that for some substances (glucose, thiourea, sodium formate, dimethylthiourea) the protective power (with carboxypeptidase as indicator) decreases with increasing solute concentration. This phenomenon of the "changing quotient" was interpreted to mean that, although the protector molecules were able to remove the free radicals, they remained in an active state and could in turn inactivate indicator molecules.

In the table some of the data of Alexander *et al.* (30) on the ability of a series of compounds to protect polymethacrylic acid against radiation-induced degradation are included for comparison. Although their results could not be expressed by the same fundamental parameters as the data of Dale *et al.* (14, 15) and of Lea (110), they reveal the same order of relative protective ability. Admittedly, a few exceptions (oxalic acid and glycine) are apparent. In the subsequent discussion extensive use is made of the data of Alexander *et al.* (30), which we consider a measure of the unspecific ability of the compounds to inactivate free radicals from water.

Competition experiments giving quantitative information on the rela-

tive radical-capturing ability of thiols have not been published. Information concerning this point must therefore be sought from experiments where thiols have been tested in various biological systems under conditions where the indirect action most probably predominated.

Evidence is available from studies of bacterial systems (124) that different thiols (cysteine, mercaptosuccinic acid, mercaptopyruvic acid, 2-(2-mercaptoethoxy)ethanol, and BAL) offer protection in proportion to the sulfhydryl concentration in solution. These compounds were effective in the presence of oxygen and also gave some protection in oxygen-free suspensions. Hirsch (125) found that cysteine, phenylthiourea, BAL, and sodium thioglycolate were about equally effective in protecting DOPA against X-ray-induced oxidation to melanin. In a number of instances, low concentrations of thiols have been shown to protect efficiently against radiation damage (84, 88, 94, 97, 126-128), but the data do not permit quantitative interpretation regarding the relative radical-capturing ability.

Apparently, thiols in general possess a pronounced ability to inactivate free radicals from water. This ability seems in part to be shared by compounds containing other forms of divalent sulfur. Several *in vitro* competition experiments have demonstrated a moderate protective ability of methionine, S-allyl mercaptoethylamine, thiosemicarbazide, cystine, cystamine, and S-di(ethylamine) (30, 128, 129). Additional evidence on this point has been obtained from radiochemical studies of various sulfur-containing agents. From an analysis of the degradation products on irradiation of the compounds in solution it is apparent that the main radiochemical reactions involve the sulfur atoms. Thus, irradiation of methionine leads to the formation of homocysteine, methioninesulfoxide, methioninesulfone, and homocysteic acid (130, 131). Thiols are converted to the corresponding disulfides (18, 132, 133), as well as to the further oxidation and reduction products, sulfenic acid, sulfonic acid, and H_2S (133-135). In aqueous solution neither cysteine nor reduced glutathione gives rise to liberation of ammonia, in contrast to the results obtained on irradiation of most amino acids (136, 137).

Independent evidence indicating that stable sulfur radicals are readily formed has been obtained in microwave paramagnetic resonance studies (138, 139). Irradiated methyl mercaptan, ethyl mercaptan, and thioacetamide did not reveal recognizable amounts of the hydrocarbon radicals formed by irradiation of the analogous oxygen compounds (methyl alcohol, ethyl alcohol, and acetamide). Strong indications were obtained that the odd electron eventually became localized on the sulfur atoms. The exact nature of the radicals could not be established, owing to the fact that S^{32} does not possess nuclear spin.

Altogether, it is clear from the evidence cited above that thiols in general

are very efficient radical scavengers. Although quantitative data are lacking, the conclusion seems warranted that different thiols are approximately equally effective in this regard. This fact stands in striking contrast to the results from protection experiments *in vivo*, where a pronounced structural specificity is observed. In animals only the thiols belonging to the cysteine-cysteamine group are active, whereas other thiols are without protective effect. As a striking example may be mentioned allylthiourea, which in the polymer experiments of Alexander *et al.* (30) was found to be one of the most active radical scavengers. This substance has been found by other authors (140) to be devoid of protective action *in vivo*.

Interesting results with regard to the relation between chemical structure and radical-capturing ability have been obtained by Bachofer and Hartwig (141) using T1 bacteriophage as indicator. The relative protective activity of various substituted benzoic acid derivatives was measured under carefully controlled conditions of oxygen supply and pH. Whereas unsubstituted benzoic acid exhibited slight protective activity, substitutions in the ring by hydroxyl or amino groups considerably increased the effect. The amines were more active than the corresponding phenols. The position of the substituting group turned out to be of importance. In both series the protective effect decreased in the order ortho > para > meta. The protective effect increased with the pH in the interval from 5 to 9. The conclusion was drawn that the resonance of the benzene ring as such is not sufficient to confer significant protection, but that certain activating groups must be present either to act as points of entrance of the energy or to activate certain positions of the molecules (141).

Evidence was quoted above that dithiols are twice as active as monothiols in capturing free radicals from water (124). No analogous relationship seems to hold true with regard to alcohols. Thus, there is no evidence that polyvalent alcohols are more efficient radical scavengers than monovalent alcohols (30). Burnett *et al.* (124) found that a number of alcohols (methanol, ethanol, 2-propanol, 1-butanol, 2-methyl-1-propanol, 2-methyl-2-propanol, 1,2,3-propanetriol, 1,2-propandiol, 1,2-ethyl-1-dioxydiol) were about equally active on a molar basis in protecting *E. coli*. It thus appears that in the case of alcohols the structure of the molecule as a whole is more important than the number of alcohol groups.

Only a limited number of experiments are known in which the presence of a second solute has clearly *increased* the indirect action of ionizing radiation on an indicator molecule. Various aliphatic alcohols have been shown to exert a pronounced potentiating effect on the oxidation of ferrous sulfate (116) and on the radiochemical reduction of DPN⁺ in the absence of oxygen (117). The presence of these alcohols also alters the radiochemical reduction of cytochromes to give a product not identical with that obtained

from enzymatic reduction (142). These data were explained by assuming that the active alcohol radicals formed by interaction with the water radicals in turn reacted with the indicator molecules. Hematoporphyrin has been found to enhance strongly the radiation effect on *Paramecium caudatum* (103). The data suggest that this effect is due to a deleterious action of an activated protoporphyrin molecule. Attempts to demonstrate a potentiating effect of synkayvite in bacterial systems have failed (143), indicating that synkayvite does not enhance the indirect effect of ionizing radiation.

3. CONCLUSIONS REGARDING CHEMICAL STRUCTURE AND ABILITY TO INACTIVATE FREE RADICALS

Generalizations regarding the relationships between chemical structure and radical-capturing ability have been advanced by previous authors. The importance of a free amine group in the molecule has been stressed by Bacq and Herve (144). Particularly β -substituted ethylamines were thought to possess an outstanding radical-capturing ability. It is true that this structure is found in many compounds active both *in vitro* and *in vivo* (e.g., cysteamine, thyramine, β -phenylethylamine, etc.). In our opinion no special significance can be attributed to the amine group with regard to radical-capturing ability. In the case of the aromatic amines it seems more likely that the amine group influences the radical-capturing ability of the benzene ring. With regard to cysteamine and its derivatives, there is no radiochemical evidence that the amine group enhances the radical-capturing ability of the thiol. On the contrary, the data quoted above indicate that different thiols are equally effective in this regard.

Alexander *et al.* (30) conclude from their studies on protection against the degradation of polymethacrylic acid that the only characteristic shared by the many protectors such as amines, sulfur compounds, nitrite, and cyanide is the presence of a lone pair of electrons. It appears that this generalization is of little use in distinguishing between active and inactive radical scavengers, as the above characteristic is shared by almost all oxygen-, sulfur-, and nitrogen-containing compounds. Moreover, the authors demonstrate themselves that in their test system aniline is equally efficient in the $-\text{NH}_2$ and in the $-\text{NH}_3^+$ form.

Doherty *et al.* (114), on the basis of extensive studies of the protective ability of AET derivatives *in vivo*, suggest that these compounds may be effective radical scavengers owing to an ability to form a free radical stabilized by resonance. This resonance is visualized to be dependent on the formation of a five- or six-membered ring structure held together by hydrogen bonds between the sulfur and the nitrogen functions. No experimental evidence is presented in support of this hypothesis.

The laws governing radical-capturing ability are at present poorly understood. The data are still insufficient on many important points to permit definite conclusions. Nevertheless, it appears that certain generalizations can be made with some degree of confidence. No attempt will be made to draw conclusions regarding the ability to inactivate specific free radicals.

In general, compounds containing divalent sulfur atoms possess appreciable radical-capturing ability. The thiols are outstanding in this respect. Various aliphatic mercaptans seem to be about equally effective. Similarly, a number of thiols in which the ionized form participates in a resonance system (dithiocarbamates, thiocyanide, thiouracil, thiosemicarbazide) seem to be very efficient radical scavengers. A number of other substances containing divalent sulfur such as disulfides, thiourea derivatives, thio ethers, and the inorganic compound thiosulfate possess only moderate scavenger activity.

The particular ability of divalent sulfur compounds to capture radicals is probably related to the low electronegativity of these sulfur groups. Presumably, either the sulfur radicals resulting from the interaction are unreactive or they are readily stabilized by interaction with water molecules or with oxygen (145).

In general, functional groups such as amines, acids, alcohols, aldehydes, ketones, and amides seem to confer only modest radical scavenger ability on a compound. Numerous examples in support of this generalization can be found (14, 15, 30, 56). Apparent exceptions to this statement can be explained by the presence in the molecules of either sulfur groups or special structures, such as branched carbon chains, resonating systems, or cyclic structures, features which will be dealt with below. The exceptional scavenger activity of formic acid may be explained by its special radiochemical properties (146). Hydrocarbons with straight chains (*n*-hexane) appear to be inactive, whereas the alicyclic structure (e.g., cyclohexane and piperidine) appears to confer some scavenger activity on a molecule.

Nonresonating carbon-carbon double bonds (e.g., oleic acid, cyclohexene) do not seem to bring about radical scavenger ability. The resonating double bonds of aromatic structures (e.g., benzene, dibenzanthracene, pyridine, and indole) do give rise to some activity. The activity of compounds with double bonds seems to be decreased by substituent groups which tend to pull electrons out of the double bond. Such an effect is brought about by carboxylic acid groups, as is apparent from comparisons of benzene with benzoic acid and of phenol with salicylic acid (30). In contrast, minus electromeric substituents, which will increase the electron density in double bonds, appear to enhance the activity strongly. As examples of this effect in aliphatic compounds may be mentioned allylamine, allyl alcohol, and allylthiourea (30).

In the aromatic series, minus electromeric substituents seem to create excellent ability to inactivate radicals. Evidence is available that in the case of aromatic amines and phenols the effect increases with increasing pH (141), indicating that a minus electromeric effect is of significance. In this event, positional isomerism would be expected to exert a pronounced effect. That this is indeed the case is evident from the work of Bachofer *et al.* (141). The role of resonating structures in the stabilization of free radicals has been confirmed in electron spin resonance studies (147-149). Such studies have established the significance of a particular one-electron form of resonance which seems to be analogous to the phenomenon of hyperconjugation (150). Thus, in the case of certain branched-chain compounds containing the isopropyl group the hyperfine structure of the radicals is suggestive of an equal coupling of the lone electron to six protons, indicating that the lone electron moves in an extended orbital covering the three carbon atoms.

C. THE SIGNIFICANCE OF THE RADICAL SCAVENGER MECHANISM IN BIOLOGICAL SYSTEMS

For many years it was widely believed that ionizing radiations exert their effect *in vivo* mainly by indirect action, and the fact that a number of radiation effects could be reduced by protective agents was taken as evidence in support of this view. The belief in the radical scavenger mechanism has been most explicitly stated by Alexander *et al.*, who concluded: "We suggest that $\text{HO}_2\cdot$ radicals play an important role in the lethality produced by X-rays, and that the protectors studied in this paper function by removing this radical" (30).

The above view has since been seriously challenged, and today the balance of evidence seems to favor a modified target theory, in which the target is extended to include a certain sphere of surrounding water (151). In cells only free radicals formed within this sphere of water are believed to interact with target molecules. Such a view was suggested by Gray (see ref. 110) in 1946. Data on the effective diffusion length of free radicals within yeast cells have been presented by Hutchinson (152), who arrived at a value of about 30 Å. The modified target theory leaves only little room for indirect action and for protection by inactivation of free radicals *in vivo*.

The role of the radical scavenger mechanism in protection of living systems by the different groups of protective agents may be summarized as follows.

1. SIMPLE LIVING SYSTEMS

The majority of protection experiments involving suspensions of cells, such as bacteria, phage particles, sperm, and eggs, have been carried out

under conditions where the indirect action most probably is dominating. The limited degree of chemical specificity observed for most groups of protective compounds seems to correlate well with the data on their radical scavenger ability in chemical systems. It is therefore believed that under these conditions the various groups of protective agents act mainly by a radical scavenger mechanism. Whereas the most efficient protection is observed with compounds probably penetrating into the cells, a certain degree of protection can also be obtained merely by inactivation of radicals in the surrounding solution. This is made likely, e.g., from the studies of Evans *et al.* (13), who observed protection of *Arabacia punctulata* sperm by gum arabic, gelatin, and egg albumin, substances which most probably do not penetrate into the cells.

Evidence exists that in simple living systems other mechanisms of protection may also be operating. The possibility of protection by way of an oxygen effect will be discussed below. In the case of the compounds of the cysteine-cysteamine group, indications exist that protection by mixed disulfide formation may also occur. Electron spin resonance studies of cysteamine-protected yeast irradiated in the frozen state suggested that the protection under these circumstances was due to interaction of the cysteamine with the yeast proteins (B. Smaller, personal communication). Also the phenomenon of "superprotection" observed in bacteriophage irradiated in broth (153), as well as the fact that cysteamine-protected bacteria remain protected even after washing of the cell (109), suggests that the protective agents may in part act by being fixed to cellular structures.

2. WHOLE ANIMALS

In whole animals the radical scavenger mechanism seems to play a different role in the protection offered by the various groups of protective compounds.

a. The Cysteine-Cysteamine Group. Probably the most striking fact about protection *in vivo* by thiol compounds is the pronounced specificity with regard to the chemical structure (26, 29, 33, 114), a fact which will be discussed later. The *in vivo* specificity stands in contrast to the above conclusion that different thiols are about equally effective in their ability to inactivate free radicals from water. In our opinion, these findings constitute decisive evidence against the view that the thiols act *in vivo* by a radical scavenger mechanism. The mixed disulfide mechanism discussed below provides an explanation of the chemical requirements for protection by thiols.

b. The Dithiocarbamates. In studies *in vivo* these compounds, which are effective radical scavengers (30), show only slight chemical specificity (34).

The dithiocarbamates do not form mixed disulfides with the biological thiol compounds so far tested (154), and studies of their behavior *in vivo* have not given any clue to their mode of action (155-157).

No definite evidence seems to be available for the mode of action of these compounds *in vivo*. Their high protective potency in mice compared with that of other effective radical scavengers (e.g., thioethanol, thioglycolic acid) suggests that, if the dithiocarbamates act by a radical scavenger mechanism, they must somehow become concentrated within the volume of the "extended target."

c. *The Pharmacologically Active Compounds.* Strong evidence is available that the compounds belonging to this group do not exert their protective action *in vivo* by a radical scavenger mechanism but that their action must be ascribed to other specific mechanisms.

Alexander *et al.* have maintained that this group of compounds also acts by a radical scavenger mechanism *in vivo*. Admittedly, several of these compounds are effective radical scavengers (e.g., tryptamine, histamine, thyramine) (30). It follows from their data, however, that the corresponding amino acids (tryptophan, histidine, and tyrosine) are equally effective radical scavengers but possess no protective action *in vivo*. The argument advanced in attempts to explain this discrepancy (30)—that the amino acids penetrate cell walls at a slow rate—is not supported by the available experimental evidence (158, 159).

Many of the pharmacologically active substances are active *in vivo* in extremely low doses [e.g., cyanide (30), benzedrine, serotonin (43)]. Furthermore, some of these compounds (epinephrine, β -phenylethylamine) failed to protect thymocytes *in vitro* under conditions where the indirect mechanism of action most probably was operating (127). Convincing evidence that entirely different mechanisms must be considered has been obtained in the case of reserpine, which exerts strong protective action in mice 24 hours after the administration of as little as 0.08 mg (41).

d. *Metabolites and Inert Compounds.* These nontoxic compounds in general afford protection in doses which are ten to one hundred times as great as those of the cysteine-cysteamine group. In view of the high doses used, and since they possess a moderate radical scavenger ability *in vitro*, it appears likely that their modest protective effect *in vivo* should be ascribed to a radical scavenger mechanism.

IV. Modification of Radiosensitivity by Interference with Radiolysis

In this section a number of factors which have been thought to influence radiosensitivity by modifying the radiolysis of water or by interacting specifically with a particular product of the radiolysis will be discussed.

A. PROTECTIVE AND SENSITIZING ACTION BY WAY OF THE OXYGEN EFFECT

1. REMARKS ON THE OXYGEN EFFECT

The fact that radiation effects in pure chemical systems as well as in biological systems are influenced by the oxygen concentration is well established, and this "oxygen effect" is extensively treated elsewhere in this book.

The relationship between radiosensitivity and the oxygen tension of the immediate surrounding medium seems to be nearly the same for a variety of biological systems, such as mammalian cells, thymocytes, Ehrlich mouse ascites tumor cells, *Tradescantia* pollen grains, *Vicia faba* roots, and chick fibroblast cultures (see ref. 160). With increasing oxygen tension the radiosensitivity increases sharply and then levels off at a partial pressure of approximately 70 mm Hg. This partial pressure corresponds to an oxygen concentration in water of about $1.5 \times 10^{-4} M$.

The interpretation usually offered for the oxygen effect is that oxygen will react with the reducing $H\cdot$ radicals to form $HO_2\cdot$ radicals. Such a modification of the radiolysis was assumed to increase the "equivalent oxidation-reduction potential" of irradiated water (161, 162). The special significance attributed to the $HO_2\cdot$ radical in the mechanism of action of ionizing radiation (30) has been subject to considerable doubt. The short mean diffusion path (30 Å) found for radiation-induced free radicals in yeast cells led Hutchinson (152) to doubt that $HO_2\cdot$ radicals can be formed at all in cells, since the oxygen molecules are spaced with a distance of 180 Å or more. Furthermore, the dissociation constant of $HO_2\cdot$ has been reported to be of the order of 10^{-2} , implying that at pH 7 only the mildly reducing radical O_2^- will be present (163). Moreover, in certain systems (164, 165) the presence of oxygen reduces the radiation effects. The present controversial situation is reflected in the following statement by Weiss (123): "There seems to be no justification to explain almost everything in radiobiology by the action of $HO_2\cdot$. . . It is unfortunate that so much time still has to be wasted now to point out that these views were really of very little consequence."

Alternative explanations for the oxygen effect have been advanced, views which will be considered later.

2. THE SIGNIFICANCE OF THE OXYGEN EFFECT

Whatever the mechanism of the oxygen effect, it is quite clear that the oxygen tension strongly influences the radiation response. In any system the actual oxygen concentration will be determined by two factors, the oxygen consumption and the oxygen supply. The necessity of taking both these factors into consideration has been stressed by Gray (166). The fail-

ure of many previous authors to do so complicates the interpretation of numerous data in the literature.

In *in vitro* systems in equilibrium with air, the oxygen tension in solution can be reduced by about 50% (to 70 mm Hg) before the radiosensitivity is affected. In animals the oxygen tension of resting skeletal muscle was found to be approximately 20 mm Hg (167). Probably about the same tension exists in several other tissues. On this basis an increase as well as a decrease in the oxygen tension might be expected to influence the radiosensitivity.

a. *Factors Influencing the Oxygen Consumption. Spontaneous oxidation.* Many substances readily undergo spontaneous oxidation with the consumption of oxygen. Therefore, in *in vitro* systems, under conditions where the supply of oxygen is limited, such compounds may undoubtedly reduce the radiation effects by reducing the oxygen tension. Such a mechanism was shown by direct measurements of the actual oxygen concentration to account for the protective effect afforded by NaSH, NaHSO₃, Na₂S₂O₄, and Na₂S₂O₅ in *E. coli* suspensions (168). *In vivo*, however, several autoxidizable substances (hydrosulfite, ascorbic acid, and borotetrahydride) have been tested for protective effect without success (see ref. 55).

The removal of oxygen has repeatedly been suggested to be the cause of the protection afforded by thiols, since these compounds are known to be rapidly oxidized to the corresponding disulfides. Gray (166) has carefully reproduced the experimental conditions used in various previous protection studies on plant material and single cells in suspension. In the pH interval 6.5 to 7.5, cysteine in a concentration of $5 \times 10^{-2} M$ removed the oxygen from the solution in the course of a few minutes. Cysteamine has been found to be still more efficient in this regard (169, 170). Gray concluded that in several previous *in vitro* experiments involving cysteine (88, 96, 171) the actual oxygen concentration in solution at the time of irradiation was probably reduced to the extent that the protective effect could be ascribed to anoxia. It should be clear that such a mechanism can explain only some protective effects achieved by thiols *in vitro*. In the experiments of van Bekkum and DeGroot (127) protection of thymocytes was obtained by cysteamine in concentrations which were too low to influence significantly the oxygen tension. Furthermore, Hollaender and Stapleton (109) observed dose reduction factors as high as 12 for cysteamine protection of *E. coli*, whereas oxygen removal gave a dose reduction factor of 3. Also, *E. coli* suspensions which had been kept in cysteamine for 30 minutes and then were washed and resuspended remained highly resistant to the lethal effect of radiation.

Conclusive evidence is available that oxygen removal by spontaneous oxidation of thiols cannot explain their protective effect *in vivo*. Firstly,

such a mechanism cannot account for the high structural specificity of the thiols protective *in vivo*. The widely different rates of autoxidation of different thiols show no correlation with their protective action *in vivo* (170). Secondly, certain thiols (cysteamine, AET, and cysteine ethyl ester) are equally protective when given in the form of the disulfides, which are unable to consume oxygen by spontaneous oxidation. Finally, in rats Cater and Philpot (172) could not observe any change in the oxygen concentration of mammary tissue as the result of cysteine administration.

Influence on the metabolic rate. It is well established that the metabolic rate of isolated cells *in vitro* can be greatly influenced by the concentration of substrates. Therefore, in protection experiments involving cellular suspensions the possibility exists that the protector may have influenced the oxygen tension by an effect on the rate of metabolism. Hollaender and Stapleton (86) have suggested that such a mechanism may account for the protection of *E. coli* by various metabolites. The finding that α -alanine offered protection, whereas β -alanine did not protect, and the finding that cyanide abolished the protection afforded by formate, succinate, pyruvate, and serine were taken as evidence in support of this view. Goucher *et al.* (see ref. 58) found protection of *E. coli* against X-irradiation on preincubation of dense bacterial suspensions with succinic acid or ethanol. When the suspensions were shaken immediately before the irradiation, the protection was abolished, a finding providing strong support for the above mechanism. The alternative possibility that variations in the metabolic rate per se might influence the radiosensitivity has been excluded by Howard-Flanders and Alper (173) and by Trowell (174). These authors have demonstrated that under constant oxygen concentration in the medium differences in the rate of metabolism did not influence the radiosensitivity.

Conversely, it might be expected that under *in vitro* conditions the oxygen tension and the radiosensitivity of cells will be increased by the use of inhibitors blocking the respiratory chain of enzymes. A sensitizing effect has indeed been observed with cyanide in the case of tumor cells (6) and *Vicia faba* roots (175), and for carbon monoxide in the case of *Tradescantia* microspores (176). In a number of other experiments, however, particularly in whole animals, the respiratory inhibitors exert a protective effect (30, 170, 171).

Also, in the case of protection *in vivo* a mechanism involving the removal of oxygen by an influence on the rate of metabolism has been considered. This explanation has been invoked for certain metabolites (fructose, glucose, amino acids, pyruvic acid, etc.) which afford a modest protection when given in large doses. The implicit assumption that an increased supply of metabolites will increase the cellular respiration rate *in vivo* can be seriously questioned. The metabolic rate and the oxygen consumption of animals

seem to be admirably controlled (158). The levels are determined by species, sex, age, body weight, surface area, nutritional status, temperature, and muscular activity. Variations in the supply of metabolites do not influence the metabolic rate except for the small variations due to the specific dynamic action. This latter effect is limited exclusively to the liver (177). Salerno *et al.* (178) have suggested that thiols may bring about their protective effect *in vivo* by way of an increased rate of metabolism. This view was in part based on the finding that tissue slices from the organs of cysteine-treated rats exhibited an increased oxygen consumption. The respiration rate under *in vitro* conditions, however, does not necessarily reflect the respiration rate *in vivo*. Furthermore, De Schryver was unable to reproduce the above results (179).

The oxygen consumption *in vivo* can, however, be significantly increased by pharmacological means (e.g., by thyroxine, dinitrophenol). Contrary to what might be expected, hyperthyroidism brings about an increase in the radiosensitivity of animals. Dinitrophenol alone did not influence significantly the radiosensitivity of mice (180) but appeared to potentiate slightly the protective action of cysteine.

In view of the above facts it seems unlikely that a significant chemical protection can be obtained in whole animals by an influence on the metabolic rate.

b. Factors Influencing the Oxygen Supply. In whole animals the oxygen supply to tissues will be determined by a number of parameters, such as the rate of lung ventilation, the oxygen transport across the alveolar membranes, the cardiac output, the oxygen capacity of the blood, the dissociation curve of oxyhemoglobin, the arteriovenous coefficient of oxygen utilization, and the status of the capillary bed. Since no method is available for the direct measurement of the intracellular oxygen tension, attempts have been made to draw conclusions on the basis of measurements of the above parameters. In many studies, however, the data presented appear to be insufficient to permit reliable conclusions.

Under physiological conditions, the supply of oxygen to the tissues is controlled by very efficient regulatory mechanisms. In general, mammals are capable of meeting a tenfold increase in the demand for oxygen without altering markedly the unloading pressure of oxygen in the capillaries. Under conditions where the unloading pressure tends to drop, the intracellular oxygen tension may be maintained by an opening up of capillaries with a consequent shortening of the diffusion distance. Also it should be kept in mind that the oxygen tension in specific organs may be decisive. Thus, in the case of the lethal action of ionizing radiation, primarily the oxygen tension in the bone marrow will be of interest.

An interference with the oxygenation of blood has been suggested to be

the underlying cause of the protection obtained by thiourea (181), as an interstitial lung inflammation was demonstrated one hour after the administration of protective doses. No data on the actual oxygen content of blood were reported, however. The above interpretation seems unlikely in view of the fact that protection by thiourea has been observed a few minutes after its administration (36), at which time lung edema presumably could not have been developed.

The protective effects observed in rats and mice by the substances sodium nitrite, *p*-aminopropiophenone (45), and carbon monoxide (37) have been ascribed to an interference with the ability of hemoglobin to transport oxygen. It is well established that carbon monoxide poisoning results in a very pronounced tissue anoxia. Carbon monoxide combines effectively with hemoglobin to form carboxyhemoglobin, which is unable to transport oxygen. Furthermore, carboxyhemoglobin displaces the dissociation curve of the remaining oxyhemoglobin so as to reduce the unloading pressure of oxygen (see ref. 182). These effects are not counteracted by an increase in the cardiac output (183). Methemoglobin formation has qualitatively the same but quantitatively less effect on the dissociation curve of oxyhemoglobin (see ref. 182). With sodium nitrite and *p*-aminopropiophenone efficient protection was observed with doses giving 50 to 70% methemoglobinemia during the period of irradiation (45). This degree of methemoglobinemia may or may not bring about a tissue anoxia, depending on the efficiency of the compensatory mechanisms. Patt (55) has pointed out that the maximum methemoglobinemia does not coincide with the time of optimal protection. In conclusion, it seems likely that tissue anoxia may be a contributory factor to the protection afforded by sodium nitrite and *p*-aminopropiophenone, but it has not been established that this mechanism is the main cause of the protection.

A possible mode of action of protective agents is that they may interfere with the oxygen supply to the tissues by way of effects on the cardiovascular system. Frequently, the doses used in protection experiments are sufficiently toxic to induce shocklike conditions, which conceivably may impair the oxygen supply (27). A number of protective compounds exert specific vascular effects (e.g., epinephrine, Benzedrine, serotonin, Pitressin). There appears to be no consistent relationship, however, between the effect of these compounds on the circulatory system and their protective properties, a fact which has been stressed by Alexander *et al.*, (30), particularly with regard to the sympathicomimetic amines. It is felt that such effects can only in part explain their protective action.

In the case of cysteine and cysteamine, the pharmacological effects on the cardiac output, blood pressure, and arteriovenous coefficient of oxygen utilization have been measured in dogs and rats (184, 185). Cysteine in

doses of about 300 mg/kg produced a transient decrease in the cardiac output and in the stroke volume, whereas the arteriovenous coefficient of oxygen utilization was slightly increased. Cysteamine in equiprotective doses appeared to be without significant effect. The data were taken to indicate that tissue anoxia can in part explain the protection afforded by cysteine, whereas no such effect is operative in the case of cysteamine.

Although it seems well established that the lethal action of X-rays on animals is not increased when the oxygen content of the inspired air is raised (55, 186), the possibility exists that the radiosensitivity of certain tissues with poor oxygen supply can be increased by such measures. This principle has been used (187, 188) to increase the radiosensitivity of tumors, which frequently are poorly vascularized. Since hemoglobin is saturated with oxygen at a partial pressure of about 100 mm Hg, only the amount of oxygen physically dissolved in the aqueous phase of blood can be increased. In order to obtain a significant effect, the partial pressure of oxygen must therefore be raised by several atmospheres.

3. CONCLUDING REMARKS

With reference to the preceding discussion it can be concluded that in *in vitro* systems an effect on the oxygen concentration may be a contributory or even a decisive mechanism of action of protective and sensitizing measures. It seems clear, however, that in many cases (e.g., compounds of the cysteine-cysteamine group) other mechanisms are also operative.

The significance of cellular oxygen concentration in the mechanism of action of the protective agents *in vivo* is by no means clear. The experimental results frequently cited in support of such a view only provide circumstantial evidence. Clearly, data on the intracellular and tissue oxygen concentration are strongly needed. The measurements of Cater *et al.* (189) by means of microelectrodes introduced into the tissue under investigation are of considerable significance, although the electrodes used (diameter 60 μ) were too large to give values for the intracellular oxygen concentration. These authors were unable to demonstrate any significant effect of cysteine administration. On the other hand, breathing of oxygen resulted in a rapid rise of the oxygen concentration, whereas breathing of nitrogen had the opposite effect. After epinephrine injection, the oxygen concentration fell to zero, but it recovered within 2 minutes. Vasopressin caused a slower fall and recovery. The radiosensitizers 2-methyl-1,4-naphthohydroquinone diphosphate and tetrasodium trimethylhydroquinone diphosphate did not influence the oxygen tension.

Although the preceding discussion indicates that removal of oxygen plays only a subordinate role in the mode of action of the protective agents *in vivo*, the conclusion seems unavoidable that the oxygen effect and the ac-

tion of protective agents are somehow causally related. A number of facts can be enumerated in support of this view.

The protective effect in rats of cysteine and *p*-aminopropiophenone can be abolished by allowing the animals to breathe pure oxygen under pressure during the irradiation (185). Conversely, oxygen poisoning can be effectively counteracted by cysteamine administration (190). In most biological systems the optimal dose reduction factors obtained are about the same for oxygen deprival and protective agents. Although exceptions may be found (70, 109), most investigators have failed to observe an additive effect of protective agents and oxygen deprival, and it seems clear that most of the protective effect disappears in the absence of oxygen. On the other hand, in certain sensitization experiments, a synergistic effect of the sensitizing agents and oxygen is observed. The marked enhancement of the radiation lesion to *Paramecium caudatum* observed in the presence of extremely low concentrations of hematoporphyrin was apparent only when oxygen was admitted (103).

The explanation that the protective agents act by removing oxygen is not the only possible interpretation of the above-mentioned relationships. It would appear that the available facts can be more satisfactorily explained by assuming (191) that at some stage in the development of the radiation damage a competition takes place between oxygen and the protective agents. If oxygen renders irreversible the radiochemical damage to the targets, whereas the protective agents in a competing process tend to repair the radiochemical damage, the data can be understood.

B. OTHER FACTORS INFLUENCING RADIOSENSITIVITY BY MODIFYING THE RADIOLYSIS OF WATER

1. CATALASE

No doubt, radiation-induced formation of hydrogen peroxide may play a role in radiochemical and radiobiological lesions. The quantitative significance of H_2O_2 in radiobiological damage has been subject to considerable controversy. The ubiquitous occurrence in living cells of the enzyme catalase, which specifically decomposes hydrogen peroxide, renders it unlikely that hydrogen peroxide production is of major significance.

In certain pure chemical systems a protection effect can be obtained by adding catalase to the solutions. The degree of protection obtained has been taken as a measure of the radiochemical effect of hydrogen peroxide (17).

In addition to its main effect, catalase also possesses peroxidatic activity in the presence of certain substrates. It is known that catalase activity is greatly enhanced by the presence of nitrate, ethanol, and formate (192),

and an influence on catalase activity has been suggested as the mode of action of sodium nitrate (193). This hypothesis has been tested by Bachofer (194), using T1 phage suspensions. In this system the presence of nitrate impaired the protective action of catalase, an effect which was ascribed to an increased peroxidatic action of catalase on the phage. Altogether, there seems to be no convincing evidence that protective or sensitizing procedures act by way of catalase *in vivo*.

2. INERT GASES

The pronounced effect of oxygen on radiation damage is generally attributed to the fact that molecular oxygen is a biradical participating readily in radical reactions. *A priori* the inert gases might be expected not to influence radiosensitivity. Ebert and Howard (195) observed, however, that when *Vicia faba* roots were irradiated in air, plus increasing pressures of hydrogen, nitrogen, helium, crypton, or argon, protective effects were observed of the same order as those obtained in the anoxic state. The different protective effects observed for the different gases correlated fairly well with their partition coefficients in lipid-water mixtures. The tentative explanation was offered that the gases acted by displacing oxygen from lipid-water interphases. Since hydrogen offered protection also in the absence of oxygen, its effect was in part ascribed to its known interaction with OH radicals.

3. THE pH

It is well known that pH has a pronounced effect on the radiolysis of water. In general, the oxidizing power of irradiated water increases with decreasing pH (161, 162). The effect of pH is particularly well studied with regard to the formation of H_2O_2 , the yield of which is fairly constant between pH 3 and 8, and increases strongly on the acid side and decreases on the alkaline side (see ref. 196). The important effect of pH on the dissociation of HO_2 was mentioned above.

In many instances changes in pH may also affect the state of target molecules. If a radiation response is pH-dependent, it may therefore be difficult to distinguish between these two mechanisms. In living systems the pH is regulated within narrow limits, giving only slight possibility of altering the course of radiolysis.

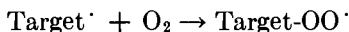
V. Modification of Radiosensitivity by Interaction with Activated Target Molecules

As the result of absorption of radiation energy, whether by a direct or an indirect mechanism, the target molecules will be left in an activated state (Fig. 1). These molecules, which either are electronically excited or have become free radicals (charged or uncharged), will become stabilized

in the course of a certain period of time. The excited molecules are inherently unstable and will dissipate the excess energy within a very short time. Usually the polyatomic excited molecules will return to the ground state. The free radicals, in contrast to the excited molecules, may be more or less stable, depending on the chemical structure, and their subsequent fate will largely depend on their environment. Thus, there is a definite time interval before the target radicals become stabilized by intramolecular reactions or by interactions with the environment, and it is highly probable that during this period protective and sensitizing agents may interact and alter the course of the chemical events. These views have been clearly stated by Alper (191), who has suggested the term methionic reactions to describe the reactions whereby an activated target molecule becomes chemically stabilized.

A number of experiments can be cited which demonstrate that under certain circumstances the time involved in methionic reactions may be quite considerable. Zimmer *et al.* (197) have demonstrated by electron spin resonance measurements that free radicals formed on irradiation of barley embryos persist for hours and days after the radiation exposure, in the presence as well as in the absence of oxygen. Patt *et al.* (96) observed that the protection of thymocytes *in vitro* by cysteine was equally effective whether the cysteine was added one minute before or one minute after the exposure. Similarly, it has been found that in grasshopper neuroblasts the radiation-induced inhibition of mitosis could be abolished when the material was placed in hypertonic salt solution within 50 seconds after the radiation exposure (see ref. 198). Perhaps the most striking demonstration of the existence of a time interval during which the radiation damage is still reversible has been provided by Künkel *et al.* (199). These authors observed a convincing protective effect when the hibernating loir (*Glis glis*) was irradiated in the hibernating state with an otherwise lethal dose, then kept at low temperature for 21 days, and injected with cysteine immediately after having been returned to room temperature. Also, the "aftereffect" observed on irradiation of DNA solutions *in vitro* (200) can be interpreted as a methionic reaction.

Although the physical and chemical processes involved in the methionic reactions are as yet inadequately understood, important data have been accumulated on the reactions of ionized organic molecules in solution. Of particular interest is the interaction of oxygen with radiation-induced free radicals to form hydroperoxides:



More or less stable hydroperoxides have been detected on irradiation of RNA or DNA in aqueous systems in the presence of oxygen (200, 201).

The formation and subsequent decomposition of such hydroperoxides may possibly account for the aftereffect. Obviously, the radiochemical formation of organic hydroperoxides in the presence of oxygen provides one possible mechanism for the biological oxygen effect in general (191).

A second important reaction after ionization of an organic molecule is the process of charge transfer. As an example may be mentioned the study of Manion and Burton (202), who found that the hydrogen production observed on irradiation of cyclohexane is strongly reduced by the addition of benzene. Presumably, the ionized cyclohexane transfers its charge to benzene, which has a lower ionization potential and is more stable in the radical form, owing to its resonating system.

The methionic reactions obviously offer numerous possibilities whereby changes in the chemical composition of a system can either protect or sensitize target molecules. Evidence that sulphhydryl compounds can protect target molecules by acting as transfer agents which convert the free radicals back to their original form has been presented by Prévot-Bernas (203) and by Charlesby and Alexander (204). The latter authors showed that cross-linking with gel formation of polyvinyl pyrrolidone in aqueous solutions could be completely prevented by the addition of cysteamine or 8-hydroxy-quinoline. The protective agents were thought to act by stabilizing active centers on the polymer molecules, which had been produced by either direct or indirect action of the radiation.

At the present time it is hardly possible to decide to what extent protective and sensitizing phenomena *in vivo* can be ascribed to an interference with methionic reactions of target molecules. Bacq and Alexander (56) have enumerated several facts which, in their opinion, constitute strong evidence against the view that such reactions play an important role in protection *in vivo*. On the other hand, Alper (191) believes such reactions to be of considerable importance in protection mechanisms. It would appear that the restorative effect obtained by administration of protective agents immediately after radiation exposure may find a logical explanation by such a mechanism.

A major objection against the view that the above reactions contribute significantly to protective action *in vivo* is that it fails to account for the pronounced chemical specificity observed within most groups of protective agents. The specificity of the protective agents might possibly be explained in terms of the above reaction mechanism if the active agents were capable of being concentrated at the surface of the target molecules. Bacq *et al.* (35) have demonstrated a correlation between protective ability and chelating properties for a number of compounds (dithiocarbamates, ethylenediaminetetraacetic acid, certain phenols). Furthermore, the protective effect of these compounds *in vivo* appears to be reduced by cupric ions with which

they complex effectively. Possibly, the chelating agents in the free form have a particular ability to become adsorbed onto the target molecules.

In the case of the cysteine-cysteamine group of compounds, the mixed disulfide formation with target molecules can be looked on as a special mechanism whereby a limited number of thiols and disulfides can bring about, in a specific manner, a salutary modification of the methionic reactions of target molecules.

VI. Modification of Radiosensitivity by Pharmacological Action

A. SPECIFIC SYSTEMIC EFFECTS

An inspection of Table I immediately reveals that a number of the protective and sensitizing substances possess pronounced and typical pharmacological effects. The question to what extent these well-known properties can explain the effects on radiosensitivity has in part been covered above. Thus, it was discussed at length to what extent the radiobiological effects of a number of compounds can be related to their effects on the metabolic rate and the oxygen supply.

In general, the pharmacological properties of the compounds in question can only to a limited degree account for the protective and sensitizing actions observed. This view is based on the finding that among a group of compounds possessing a specific pharmacological effect only a few substances influence radiosensitivity. Moreover, structurally related compounds with no or opposite pharmacological action may be found which do influence the radiosensitivity. Thus, Alexander *et al.* (30) were unable to demonstrate any consistent relationship between protective action and the vascular effects of a number of substances.

The study of the cysteine-cysteamine group of protective and sensitizing agents has confronted pharmacology with a number of new compounds, the pharmacological effects of which were previously unknown. In the case of some of these substances, their actions on the cardiovascular system (205-208), on carbohydrate metabolism (205, 209), and on hormonal balance (38, 210, 211) have been studied. Also for this group of compounds the pharmacological properties do not offer any clue as to their mode of protective and sensitizing action.

A few of these agents (cysteine, glutathione, cysteamine, and homocysteine) occur in cells as part of essential metabolic systems. The possibility has been considered by various authors (51, 144) that protective agents may function by replacing cellular constituents inactivated by the irradiation. Most compounds of this group cannot be envisaged to function in this way, however. Moreover, definite evidence is available that cysteamine given as such is not incorporated into the coenzyme A molecules in mammals *in vivo* (212).

The firm belief in the role of SH groups in radiation damage prevailing in the postwar years led several investigators to attempt to increase the radiosensitivity of animals by pretreatment with agents specifically inactivating SH groups. Patt *et al.* (213) observed no radiosensitizing effect with *p*-chloromercuribenzoate. Langendorff and Koch (51) found monoiodoacetic acid to possess a slight but definite radiosensitizing effect in mice, whereas alloxan, diphenylthiocarbazone, 1-naphthylthiourea, and sodium tetrathionate were without effect. Mitchell and Simon-Reuss (214) concluded that the radiosensitizers of the synkayrite type usually exhibit reactivity toward sulfhydryl groups *in vitro*. Exceptions were found, however, indicating that the interactions of these compounds with cellular sulfhydryl groups cannot explain their radiosensitizing action.

B. CONDITIONING OF CELLULAR METABOLISM

A striking phenomenon in radiobiology is the pronounced differences in radiosensitivity found among different organisms. The LD₅₀ varies from a few hundred roentgens in mammals up to several hundred thousand roentgens in Protozoa (see ref. 56). The underlying cause of these large differences undoubtedly must be sought in inherent differences in the cellular biochemistry and physiology of the organisms. Also within the same species variations in the physiological state influence the radiosensitivity (215, 216). These latter variations are moderate and of about the same order of magnitude as those which can be achieved by chemical agents.

The logical possibility that the chemical agents may modify radiosensitivity of animals by conditioning the metabolism has been explored only partially. A priori, it might be anticipated that changes in the radiosensitivity brought about by such a mechanism will exhibit a characteristic time-effect relationship. Presumably, a certain time is needed to condition the metabolism in the desired way. On the other hand, an altered metabolism with changed radiosensitivity might be expected to persist for a certain period of time.

Various metabolic disorders are known to be associated with an altered radiosensitivity (see refs. 52, 217). A notable increase is observed in hyperthyreosis, a fact which is particularly evident with regard to the hyperthyroid gland proper. In diabetes the sensitivity is decreased. Attempts have been made to influence the radiosensitivity of animals by altering their basal metabolic rate. Thyroxine medication increased the radiosensitivity, whereas induced myxedema was without effect. The mechanism of the above action of thyroxine is completely unknown.

The higher radioresistance of female rats as compared to males has prompted several investigators to study the role of the sex hormones in radiosensitivity. Langendorff and Koch (218) observed that castration increased strongly the radioresistance of male mice, whereas an opposite

effect occurred in female mice. Certain estrogenic hormones have been reported to afford a slight radioprotective effect (38). The increased radioresistance of castrated male mice could not be further raised, however, by administration of estradiol (218). In the case of methylandrostenediol a radiosensitization has been observed after daily administration for 10 days (219). An attempt has been made to obtain information as to the underlying cause of the sex difference in radiosensitivity by analyzing dose-lethality curves (220). It was possible to resolve the dose-lethality curves in three components, only one of which is influenced by the sex hormones. The first and second components reflect bone marrow death and intestinal death, respectively. The third component, which is the one being influenced by the sex hormones, is mainly of importance in the low dose range. No information is yet available on the type of radiation damage expressed in this component, nor is it known by which mechanism the sex hormones influence this factor.

The role of the adrenals in the radiosensitivity of animals has been extensively discussed (see ref. 38). It seems clear that the adrenal function influences radiosensitivity to a slight but definite extent, inasmuch as procedures which modify the adrenal response will also alter the radiosensitivity. Adrenalectomy (or hypophysectomy) decreases to a considerable extent the radioresistance toward the lethal action of whole-body irradiation (31, 221, 222). Administration of cortical hormones to adrenalectomized animals restores the radioresistance to normal levels. The effect of these hormones on normal animals is disputed, however. Prolonged stimulation of the adrenal cortical activity for about a week prior to irradiation by injections of salicylate, bacterial toxins, ACTH, or DOCA, or by exposure to cold or to a sublethal dose of X-rays, will increase slightly the radioresistance of animals to whole-body irradiation (38). On the other hand, when these procedures are applied after irradiation, the radioresistance is decreased. Cortisone appears to have a deleterious effect when administered before as well as after irradiation. On the basis of these findings, Betz concluded (38) that all procedures which reduce the adrenal response to the stress of irradiation have a beneficial effect on radiosensitivity. The impression is gained that the adrenals modify the radiation response by altering restorative processes. Bacq *et al.* (223) have observed that in cysteamine-protected animals the initial adrenal response to radiation is unchanged, as measured by the fall in the cholesterol content, the ascorbic acid content, and the weight of the adrenals, whereas the second response, about the fourth day, is reduced. It appears likely that this latter effect is a consequence and not the cause of protection.

Numerous attempts have been made to influence the radiosensitivity of animals by prolonged medication of substances believed to stimulate the

bone marrow or other radiosensitive tissues. No definite effect has been obtained by administration of vitamin B₁₂, folic acid, or citrovorum factor, substances known to be of importance in erythropoiesis (224, 225). Because of the probable role of the capillaries in the pathogenesis of the radiation injury, the bioflavonoids extracted from citrus fruits ("vitamin P"), which are believed to act on the intracellular cement of the capillary walls, have been tested for protective action. Although these substances failed to protect animals against total-body irradiation, definite protective effect against radiation erythema of the skin was reported (226). In human subjects treated with bioflavonoids for 10 days before irradiation of the nail bed, a striking effect on the capillaries was observed (72).

As mentioned above, it has been discovered that several substances, usually classified as stimulants or depressants of the central nervous system, possess protective properties. These compounds include chlorpromazine (42), tryptamine, serotonin, amphetamine, methamphetamine, and reserpine (41, 43, 44). These substances are active in extremely low doses. In the case of amphetamine, the optimal protective dose in mice is about one-sixth of the usual pharmacotherapeutic dose, and the larger doses exhibit a radiosensitizing effect (43). In the case of reserpine, the degree of protection, as judged from dose-lethality curves, is of the same order as that obtained with cysteamine or AET. Optimal effect is obtained when the drug is administered 12 to 24 hours before the exposure (41). It is well known that reserpine causes a release of serotonin, epinephrine, and norepinephrine from certain tissues. The significance of this fact with regard to the protective effect of reserpine is not obvious.

The protective effects obtained by the above substances may be unrelated to their effects on the central nervous system, since a number of other substances with similar stimulating or depressing action (cardiazole, ephedrine, caffeine, eventine, mescaline) fail to show protective activity *in vivo* (41). The data suggest that these substances exert their effect on radiosensitivity by an effect on the cellular metabolism.

The well-known law of Bergonié and Tribondeau relating radiosensitivity to mitotic activity obviously offers a possibility of modifying radiosensitivity by procedures influencing the rate of mitosis. In animals, however, the induction of erythroblastic hyperplasia by treatment with phenylhydrazine hydrochloride or by repeated bleeding prior to irradiation (227), as well as by exposure to reduced oxygen tension (228), gave the unexpected result that the radiation-induced bone marrow atrophy was reduced by these procedures. The authors concluded that the effects were probably due to some metabolic change within the cells, rendering them less susceptible to radiation.

It is well established that different stages of mitosis possess different

radiosensitivity. It would therefore seem possible that protective effects might be obtained by compounds capable of arresting temporarily mitosis in a radioresistant phase. No systematic study appears to have been carried out on the radioprotective action of mitotic inhibitors. Therkelsen (229) has demonstrated that in chick heart fibroblast tissue culture certain sulfur-containing radioprotective substances (cysteamine and dimethylcysteamine) arrest mitosis in the metaphase, whereas the nonprotective compounds *S*-methylcysteamine and methyl-bis(β -mercaptoethyl)amine failed to influence the mitotic process. Glutathione, on the other hand, did not possess any effect on mitosis. In view of the short time interval between the administration and the appearance of the protective effect, it appears unlikely that the above mechanism is of significance in explaining the protective action of the compounds of the cysteine-cysteamine group.

VII. Modification of Radiosensitivity by Physicochemical and Chemical Alterations of Target Molecules

A. MODIFICATION OF RADIOSENSITIVITY BY CHANGES IN THE PHYSICOCHEMICAL ENVIRONMENT

Living cells consist of highly complex mixtures of molecules, in part organized in functional units such as nuclei, mitochondria, and microsomes. This organization entails that cells contain numerous phase boundaries. The possibility that radiation effects on phase boundaries may be of particular significance should therefore be considered. In experiments *in vitro*, pronounced effects of small radiation doses on the zeta potential of colloid particles (230) and on the inactivation of enzyme films (231) have been demonstrated. Also, in the case of chemical protection, an adsorption of the agents on surfaces has been invoked to explain the existence of saturation phenomena (232).

It may be useful to recall briefly the main properties of a macromolecule (e.g., a protein molecule) which can be modified by changes in the chemical environment. Proteins carry on their surfaces dissociable groups, the state of which will determine their electrical charge (the zeta potential), the degree of association between like molecules, as well as the degree of hydration. Small molecular ions will tend to be concentrated in the surface layer of bound water, or they may become specifically bound to protein surface groups. The state of certain chemical groupings (SH, SS, prosthetic groups) may be governed by the oxidation-reduction potential of the surrounding solution. It should thus be realized that under different conditions a single molecule may exist as different molecular species, and a priori it would therefore be expected that variations in the physicochemical environment may modify the radiosensitivity of target molecules.

In complex biological systems and in mammalian tissues the environmental changes compatible with cellular function and life are limited. In many simple biological systems, however, such as suspensions of micro-organisms, the composition of the medium, and probably that of the interior of the cells, may be varied within wide limits.

Relatively few experiments have been carried out with the specific aim of revealing the effects of physicochemical variations in the environment on the radiosensitivity of target molecules. In the opinion of the present authors such effects have not been given sufficient consideration, and in many experiments they have been entirely overlooked.

1. THE EFFECT OF pH

The yield of many radiochemical reactions is dependent on the pH of the solution. This pH dependency has been carefully studied in a large number of cases.

It appears that pH exerts no consistent effect on the yield of the reactions assembled in Table V. The data do not easily lend themselves to interpretation, since variations in the pH also influence the nature and relative amounts of the free radicals formed in solution. Table V contains results which cannot be explained solely on the basis of an effect of pH on the radiolysis, however. Thus, the H₂S formation from cysteine shows a maximum value at about pH 6, whereas the H₂S formation from glutathione exhibits a minimum value at this pH value. As the reactions probably proceed by similar mechanisms, it is reasonable to assume that this difference resides in different pH effects on the radiosensitivity of the targets. The radiochemical oxidation of cystamine to the sulfinic and sulfonic acids (133) shows definite minimum yields in the low pH range, in spite of the fact that the equivalent oxidation-reduction potential is increasing with decreasing pH.

The data on the inactivation of enzymes are obviously difficult to interpret, since the underlying radiochemical reactions are unknown. Okada (237) concluded that the pH effect on the radiosensitivity of ribonuclease is due to an influence on the deactivation reactions of the free radicals from water, as the effect was found to be dependent on the enzyme concentration. On the other hand, McDonald (238) and Scholes and Weiss (233) conclude from their radiochemical studies on trypsin and nucleic acids, respectively, that the ionic nature of the target molecules is an important factor influencing their radiosensitivity.

Effects of pH on the radiation response of biological systems have been definitely established in the case of germinating fern spores and in *Paramecia*, which on exposure to increasing concentrations of carbon dioxide or ammonia exhibited varying radiosensitivity with definite maximum and

TABLE V
THE EFFECT OF pH ON THE YIELDS OF VARIOUS RADIOCHEMICAL
REACTIONS IN DILUTE SOLUTION

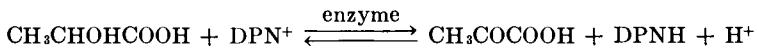
Substance	Oxygen	Effect measured	Maximum ionic yield at pH	Minimum ionic yield at pH	Ref.
Formic acid	Absent	H ₂ formation	2 to 3	8 to 12	(10)
Formic acid	Absent	CO ₂ formation	2 to 3	8 to 12 (Zero)	(10)
Adenine	Absent	NH : formation	1 to 2	6 to 12 (plateau)	(233)
Yeast adenylic acid	1 atm. O ₂	NH ₃ formation	1 and 9	5 and 12	(233)
Pentosenucleic acid	1 atm. O ₂	NH ₃ formation	2 and 9	5 and 10	(233)
Alanine	Absent	NH ₃ formation	3 and 9	1 and 6 and 11	(234)
Pentosenucleic acid	1 atm. O ₂	H ₃ PO ₄ formation	2 and 7.5	5 and 11	(233)
Thiourea	Air	S _x formation	2 to 3	8 to 11 (almost zero)	(235)
Cysteine	With and without O ₂	H ₂ S formation	6	2 and 10	(135)
Glutathione	With and without O ₂	H ₂ S formation	4 and 7	2 and 5	(135)
Cystamine	Air	RSO ₂ H formation	5 to 8	3 and 9	(133)
Cystamine	Air	RSO ₃ H formation	5 to 8	3 and 9	(133)
Chymotrypsin	?	Inactivation	2	7	(236)
Deoxyribonuclease	?	Inactivation	3	9	(237)

minimum values (239, 240). It is unlikely that in higher animals pH effects are important in protective and sensitizing procedures, inasmuch as pH is very efficiently regulated in mammals. This view is supported by the *in vivo* measurements of Cater *et al.* (189), demonstrating that a variety of physiological procedures as well as the administration of protective and sensitizing agents influence the pH only to a slight degree.

2. THE EFFECT OF THE OXIDATION-REDUCTION POTENTIAL

The oxidation-reduction potential of a given system at equilibrium is a well-defined parameter, which significantly influences the radiosensitivity of many systems. This fact is readily apparent from a consideration of the system pyruvic acid-lactic acid. In the presence of the enzyme lactic de-

hydrogenase and DPN the following equilibrium is rapidly established:



The ratio pyruvic acid/lactic acid is determined by the ratio DPN^+/DPNH , and vice versa. The radiochemical fates of pyruvic acid and lactic acid turn out to be widely different (241, 242). Whereas lactic acid at pH 7.4 and in the presence of oxygen is radiochemically oxidized to pyruvic acid, pyruvic acid itself will undergo profound radiochemical degradations to carbon dioxide, acetaldehyde, and possibly acetic acid. Also, DPN^+ and DPNH exhibit pronounced differences in radiosensitivity (243).

There is growing evidence that a number of other biologically important compounds—e.g., cytochrome c (244), myoglobin, and hemoglobin (245)—may exhibit different radiosensitivity in their reduced and oxidized forms. Of special significance is the influence of the oxidation-reduction potential on the radiosensitivity of the thiol-disulfide system. This effect is clearly brought out by comparing the radiochemical reactions of the partners of the couples cystine-cysteine and oxidized glutathione-reduced glutathione (*vide supra*).

In metabolizing cells it is not possible to assign a particular oxidation-reduction potential to the cell as a whole, since the many oxidation-reduction systems are not in equilibrium. The metabolic reactions involve a step-wise change in the oxidation-reduction potential along the chain of enzymes and coenzymes involved. To the extent that oxidation-reduction systems are coupled, changes in the one couple will influence the state of the second couple. Changes in the glucose-6-phosphate dehydrogenase system may thus influence the state of a number of cellular thiols and disulfides by way of the TPN^+/TPNH ratio and the glutathione reductase system (246, 247).

The possibility has been considered that protective or sensitizing agents might bring about their effect by causing a shift in the oxidation-reduction potential at a specific level of the metabolic chain (53, 248). The available experimental evidence does not support such a view. Clearly, cystamine and cysteamine will influence the oxidation-reduction potential of cells and tissues in opposite directions, although both substances offer protection. Langendorff *et al.* (53) failed to observe any correlation between the protective effect of various metabolites and their standard oxidation-reduction potentials. The measurements of Cater *et al.* (189) of the “oxidation-reduction potential” in mammary tissue of the lactating rat after administration of the protective agents cysteine, cysteamine, and glutathione, as well as of the radiosensitizing agent synkayvite, in all cases demonstrated a fall in the potential. The interpretation of the latter results is difficult, since it is not known which system or systems are responsible for the potentials created at the electrodes.

Laser (249) has presented data linking the mechanism of the oxygen effect to the oxidation state of the respiratory enzymes. He observed that the optimal protective effect obtained in various microorganisms by the removal of oxygen was approximately the same as that accomplished by irradiating the organisms in the presence of both oxygen and various respiratory poisons (carbon monoxide, potassium cyanide, hydroxylamine, sodium azide). On the basis of the known interaction of these poisons with cytochrome a_3 , he concluded that oxygen removal and the respiratory poisons probably both act by stabilizing the oxidation-reduction couples of the respiratory chain in their reduced form, which was supposed to be the more radioresistant. The previous finding of Bacq *et al.* (90) that cyanide offers only slight protective effect in cyanide-resistant Protozoa is consistent with the above interpretation.

3. THE EFFECT OF IONIC STRENGTH

Observations may be found in the literature indicating that the ionic yields of radiochemical reactions may be influenced by the ionic strength and the nature of the buffer. Such effects have been investigated in considerable detail by Bachofer and Pottinger (85) in studies on the inactivation of T1 phage. A pronounced decrease in the radiosensitivity of the phage was observed when the salt concentration in the medium was increased in the interval 10^{-4} to $10^{-2} M$. In this particular test system the ion effect was considerable, giving dose-reduction factors of 100 to 1000. Some specificity with regard to the action of particular ions could be observed in so far as the ammonium ion, the nitrate ion, and the nitrite ion possessed considerably greater effect than a series of other ions. Although it cannot be excluded that the effect of these ions on radiosensitivity was in part due to an interference with radiolysis, the pronounced effects by salts were probably largely due to physicochemical alterations of the surface of the phage particles. It may be significant that different cations were effective in an order corresponding with that of the Hofmeister series for the salting out of proteins.

The significance of variations in the salt concentration and the ionic strength with regard to the radiosensitivity of mammalian cells and tissues is doubtful. It is relevant, however, to recall the effect previously mentioned of varying salt concentrations on the radiation-induced inhibition of mitosis in grasshopper neuroblast (see ref. 198).

4. THE EFFECT OF TEMPERATURE

There seems to be no reason to assume that the primary absorption of radiation energy is affected by the temperature. The pathways of the energy dissipation and the consequent chemical reactions (the methionine reactions) would be expected to be temperature-dependent, however.

In general, fairly large temperature changes are necessary to demonstrate a significant effect on the radiosensitivity of target molecules. This is evident from numerous studies of proteins in the dry state. The radiosensitivity decreases with decreasing temperature, although the effect is moderate even down to temperatures of liquid nitrogen (see ref. 55). A pronounced temperature effect has been observed in the interval 40° to 100°C.

The temperature effect on the radiosensitivity of proteins has been demonstrated in a most elegant way by Setlow (250), who determined the cross section for deuterons with respect to inactivation of catalase in the dry state. When the temperature was raised from 100° to 375° K, a stepwise increase in the inactivation was observed, corresponding to the inactivation of one-fourth, one-half, and the whole molecule. Since catalase is known to contain four heme groups, the data indicate a stepwise increase in the probability of transferring radiation energy from one unit of the molecule to the other ones. At higher temperatures the cross section increased sharply, indicating that in this temperature range also excitation energy contributed to the inactivation process.

The effect of temperature on the radiosensitivity of living systems is obviously complex, and a diversity of results has been obtained (see refs. 55 and 56). As previously mentioned, irradiation of animals in the hibernating state does not appear to reduce the lethality but to delay the effect (199, 251). In the cooled state the radiation damage seems in part to be reversible. This interpretation is consistent with the view that the delay in the radiation effects observed after cooling of the animal may be due to a retardation of the methionine reactions.

B. MODIFICATION OF RADIOSENSITIVITY BY CHEMICAL COMBINATION OF TARGET MOLECULES WITH ORGANIC COMPOUNDS

It is now well established that when an organic molecule is ionized by direct hits the charge can migrate within the molecule. Although, for statistical reasons, the initial ionizations will be randomly distributed, an analysis of the reaction products demonstrates that certain bonds are preferentially broken. This has been clearly demonstrated in experiments on organic vapors (252) as well as on dry material (253). It appears that charge migration may be of particular significance in proteins where the polypeptide chains exist in a semiresonating state.

Numerous examples are known (see refs. 110 and 253) in which a single direct hit abolishes the biological function of a macromolecule (e.g., enzymes, virus particles). This fact is most easily explained by the assumption that the ionization can migrate within the molecule to the structures which are essential for the biological function. Gordy (148) has suggested the possibility that similar effects may be brought about also by indirect action.

When free radicals from the surrounding medium remove an electron from target molecules, a similar result is conceivably produced as when the molecule is struck by a direct hit.

When a chemical combination is formed between a target molecule and a second organic compound, the possibility exists that an ionization can be transferred from the one moiety to the second one and thereby increase the effective volume and the sensitive surface of the target. Such a situation may increase the probability of damage by direct or indirect action. On the other hand, the possibility also exists that the combination may bring about a protective effect by offering additional possibilities for dissipation of energy from the ionized target molecule.

Several cases are known in which the chemical combination between an enzyme and a second large molecule leads to an increased radiosensitivity. Thus, it was observed (254) that a combination of hyaluronidase with hyaluronic acid resulted in an enhanced radiosensitivity of the enzyme, as evidenced by an increased cross section for deuterons. The calculated target size corresponded to the combined enzyme and substrate, indicating that ionizations in the latter compound were transferred to the enzyme part of the complex. On the other hand, Doherty (255) found that α -chymotrypsin could be protected against γ -rays by the addition of its substrate. Also, the inactivation of catalase by X-rays was found to be reduced when its ion centers were complexed with cyanide (256). The protection by hydrogen peroxide could similarly be ascribed to a combination with the enzyme.

The suggestion has also been made that small-molecular protective compounds (cyanide, cysteamine) might temporarily combine with target molecules to form more radioresistant complexes (144). No experimental evidence was presented in support of this hypothesis, however, which later was abandoned (56) when it was found that cyanide is an effective protector for polymers.

Experimental evidence that protective agents may exert their action by combining with target molecules was first obtained in our laboratory (169, 257). It was shown that on administration of S³⁵-labeled cystamine or cysteamine to mice, a substantial fraction of the compound was recovered in the form of mixed disulfides with thiol and disulfide groups of body constituents. Radiochemical studies of S³⁵-labeled cystamine provided direct evidence for the exceptional radiosensitivity of the SS bond (133, 134). On this basis a mechanism was formulated whereby the compounds of the cysteine-cysteamine group could be envisaged to protect sulfur-containing target molecules against the direct as well as the indirect action of ionizing radiation.

C. THE MIXED DISULFIDE MECHANISM FOR THE ACTION OF THE COMPOUNDS OF THE CYSTEINE-CYSTEAMINE GROUP

1. GENERAL ASPECTS OF PROTECTION BY THE CYSTEINE-CYSTEAMINE GROUP

The thiols and disulfides which are chemically related to cysteine (i.e., the so-called cysteine-cysteamine group) constitute the group of protective agents which has been most extensively studied *in vivo* and which seems to offer greatest promise. Numerous attempts have been made to explain the protective action of these substances. The radical scavenger hypothesis and the mechanism involving the oxygen effect are the most important theories advanced. These mechanisms, which have been dealt with above, can by and large explain the protective effects observed *in vitro*, but they can account for only a fraction of the protection offered *in vivo*.

The *in vivo* protection offered by the cysteine-cysteamine group exhibits certain characteristics, which should be explained by any theory attempting to account for their mechanism of action. These characteristics, which have been extensively discussed (26, 56, 60), will be summarized briefly here.

The compounds afford protective action a few minutes after the administration, and the effect lasts for about 30 to 60 minutes.

With increasing protector dose, an increasing degree of protection is obtained.

No uniform protection is obtained against the various forms of radiation damage. Catsch (62) observed with cysteamine and AET different dose-reduction factors for the different modes of radiation death. Thus, the dose-reduction factor was 1.4 for intestinal death and 2.13 for bone marrow death. In mammals, the dose-reduction factors do not exceed 2.

The compounds exhibit pronounced and specific chemical requirements for protective or sensitizing action *in vivo*. On the basis of the systematic and comprehensive studies carried out by Langendorff *et al.* (26, 33), by Bacq and Herve (144), and by Doherty *et al.* (29, 114), the following generalizations can now be made:

The sulfur atoms must be present either as a free *sulphydryl* group or as a *disulfide* group. The protective S-substituted compounds have been shown to be capable of undergoing spontaneous chemical rearrangement under physiological conditions, with liberation of the SH group. As examples may be mentioned the isothiuronium compounds, which rearrange to the corresponding guanidoalkyl thiols (258), as well as α -homocysteine-thiolactone (259) and *S*-acetylcysteamine. The ring compound *o*-amino-thiazoline, which has protective activity, is also capable of liberating free SH groups (29). Conversely, those isothiuronium derivatives which are unable to rearrange to the free thiol are devoid of protective activity (29).

Only a limited number of disulfides have been tested for protective action. Cystamine (see ref. 56), di(guanidoethyl)-disulfide (29), and *N,N'*-tetramethylcystamine (33) seem to be as protective as the parent compounds. On the other hand, cystine (32) and cystine diethyl ester (114) were found to be inactive. Oxidized glutathione has also been reported to be inactive (see ref. 56). The failure of cystine to afford protection can reasonably be explained by its low solubility.

The active thiols all contain an *amine group* which seems to be essential. Alkylations with aliphatic or alicyclic residues appear to increase the protective ability on a molar basis (33, 114), although the concomitant increase in the toxicity reduces the protection attainable *in vivo*. The conversion of the amine to a guanido group seems to enhance substantially the protective activity. The only quaternary alkylated amine which seems to have been tested, viz., thiocholine, was devoid of protective action (71). *N*-Acyl derivatives (pantetheine, acetoacetyl cysteamine, aletheine, and *N*-acetylmethylcysteamine) are generally inactive. *N*-Acetylcysteamine and glutathione, which have a moderate protective activity on a molar basis, are exceptions.

The structure of the *carbon chain* also influences the protective effect *in vivo*. Maximal protective effect is obtained in compounds where the thiol and the amine functions are separated by two or three carbon atoms (29, 33). An increase in the chain length or a branching of the chain reduces the activity. The introduction into the molecule of a carboxyl group, in addition to the amine, leads to a decrease or an abolishment of the protective ability or even to a sensitizing effect, depending on the position of the carboxyl group. Thus, the α -amino acids (cysteine and homocysteine) are relatively weak protectors on a molar basis, whereas β -homocysteine and isocysteine possess a sensitizing action (40). Since *d*- and *l*-cysteine are equally protective (260), the stereoisomeric configuration of the protective molecule seems to be without significance.

2. THE MIXED DISULFIDE MECHANISM

The mixed disulfide mechanism proposed by Eldjarn and Pihl (169, 257, 261) to account for the protective and sensitizing action of the compounds of the cysteine-cysteamine group may be stated as follows:

1. During the period when the compounds of the cysteine-cysteamine group exert protection, they exist in the body largely in the form of mixed disulfides with the SS and SH groups of tissue constituents. This binding is temporary, as the mixed disulfide will be reduced by the disulfide-reducing systems of the body (e.g., glutathione reductase). The protective residues thus liberated will be subject to metabolism and excretion.

2. The conversion of a target SH or SS group to a mixed disulfide with the protective agent is assumed to bring about a partial protection of the target sulfur atom against the *indirect action* of ionizing radiation in the following way:

When the mixed disulfide bond is attacked by free radicals (Fig. 2), one of the sulfur atoms becomes oxidized to a sulfenic or sulfonic acid, whereas the other one will be reduced to an SH group. Clearly, the target sulfur atom is being protected to the extent that it becomes reconstituted as a functionally active SH group.

3. The mixed disulfide bond is assumed to serve as the ultimate source of the electrons repairing ionizations created by *direct hits* (Fig. 3). On interaction with water, the ionized mixed disulfide bond is believed to be split, with the formation of one SH group. In this way the formation of a mixed disulfide between the target molecule and the protector is envisaged to offer a partial protection against direct hits, as it provides additional possibilities for dissipation of the radiation energy in a nondeleterious way.

4. The appreciable amounts of protective thiols normally present in the tissues (glutathione, cysteine, homocysteine) are assumed to exist partly in the form of mixed disulfides with target molecules. It is believed that the radiosensitizers of the cysteine-cysteamine group strip the target mole-

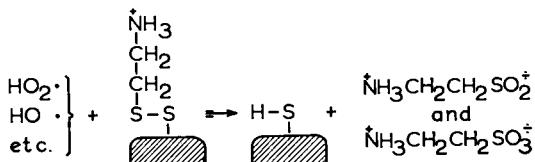


FIG. 2. The mixed disulfide mechanism for protection against the indirect action of radiation.

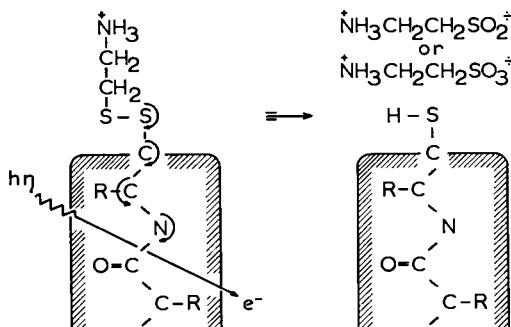


FIG. 3. The mixed disulfide mechanism for protection of target molecules against direct hits.

cules of their protective residues, owing to a stronger tendency to form mixed disulfides with the naturally occurring "protectors" than with the target molecules.

The mixed disulfide mechanism is now supported by a considerable body of evidence. In the following paragraphs the available radiochemical, chemical, and biochemical data on mixed disulfides will be discussed. The purpose is to examine to what extent these data are consistent with the established biological facts concerning protection *in vivo* by thiols and disulfides.

a. *The Radiochemistry of the Disulfide Bond.* The mixed disulfide mechanism involves the assumption that the disulfide bond exhibits characteristic radiochemical properties versus the direct as well as the indirect action of ionizing radiation. A body of evidence is available in support of this view.

In contrast to the assumption of Barron *et al.* (16), disulfides are not radiochemically stable but have been found to be highly susceptible to the indirect action of ionizing radiation. The radiochemical disappearance of cystine has been studied by several authors (132, 262), but the special properties of the disulfide linkage were not revealed until the decomposition products were studied in detail. Radiochemical studies of S³⁵-labeled cystamine in solution demonstrated the formation of 2-aminoethanesulfinic acid (hypotaurine) and taurine (133, 134). Also, the formation of cysteamine was detected. The degradation products indicated that the radiochemical reactions all took place at the sulfur atoms. Experiments in this laboratory (263) have confirmed and extended these studies. Irradiation of cystine solutions similarly gives rise to the corresponding sulfinic and sulfonic acids, as well as to cysteine. In both instances the yields of the oxidation reactions were about 1. When the spontaneous oxidation of the radiochemically formed cysteamine and cysteine was suppressed by the presence in the solution of ethylenediaminetetraacetic acid (EDTA), the yields for the formation of these reduction products as well approach 1.

Evidence for the unique radiosensitivity of the disulfide bond has been obtained also in the case of proteins. Drake *et al.* (264) demonstrated that, when insulin was irradiated in solution at pH 3, among the fourteen amino acids analyzed in the hydrolyzate cystine was by far the one most susceptible to destruction. Also, the decomposition product cysteic acid was identified. The liberation of thiol groups on irradiation of serum albumin with X-rays was demonstrated by Goldblum *et al.* (265). These data indicate that also in the case of protein disulfides the main result of the indirect action is an oxidation of part of the sulfur, while a significant fraction is concurrently reduced to thiols.

Strong evidence has been presented that the disulfide groups are of

special significance also when molecules are struck by direct hits. Although as previously pointed out the initial ionizations of organic molecules will be evenly distributed, the disulfide bonds are selectively affected under such conditions. This is clearly brought out by the studies of Engelhard *et al.* (266) on the chemical changes of serum albumin resulting from irradiation with ultraviolet light. After irradiation with light of a wavelength preferentially absorbed by aromatic structures, a liberation of thiol groups could be demonstrated. Similar conclusions have been reached by Gordy *et al.* (138) on the basis of paramagnetic resonance studies of various peptides and cystine-containing proteins in the dry state. Characteristic resonance patterns were obtained, indicating that the majority of the lone electrons resulting from the irradiation resided on the SS groups of cystine moieties. The conclusion was drawn that "The evidence is strong that whenever radiation knocks out an electron to create a hole or vacancy at any given point in the protein, this hole or vacancy is quickly filled by an electron borrowed from a cystine group" (138). Gordy and Shields (145, 149) have summarized their latest work on a number of proteins: "Most, though not all, proteins investigated give either a doublet resonance characteristic of glycyl-glycine, a field-dependent resonance characteristic of cystine, or a combination of the two. This is a surprising observation when one considers that the individual proteins are generally composed of some twenty different amino acids, almost all of which give characteristically different resonance patterns."

Gordy *et al.* (138) assume that in the SS radicals a three-electron SS bond exists which will strengthen rather than weaken the bond. This may well be true in the dry state. It is likely that in the presence of water the SS radical will undergo chemical reactions analogous to those described above for indirect action. Evidence that the SS radical is highly reactive is found in the fact that, on admission of oxygen, the cystine-like resonance was killed without the formation of an observable secondary radical (145).

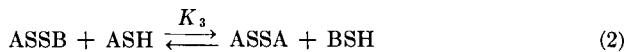
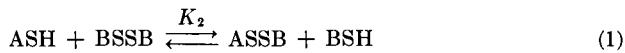
No data are as yet available on the radiochemistry of mixed disulfides. In these cases the possibility exists that the asymmetric distribution of the electrons in the sigma bond of the SS linkage may cause a preferential reduction of one of the sulfur atoms. In this event the electronegativity of the sulfur atom of the protector moiety (reflected in the pK value of the thiol) might be expected to be an important factor in determining the protective ability.

b. The Chemistry of Mixed Disulfide Formation. A crucial question from the point of view of the mixed disulfide mechanism is to what extent a correlation exists between the ability of compounds to form mixed disulfides with cellular constituents and their protective action *in vivo*. Since the protective compounds are active when injected immediately prior to the ir-

radiation, the mixed disulfide mechanism requires that the active compounds react rapidly and extensively with target molecules.

No information is available as to the chemical nature of the cellular target molecules. In the studies carried out in this laboratory on the ability of various protective and nonprotective thiols and disulfides to enter into mixed disulfide formation, cysteine (CSH), cystine (CSSC), and oxidized and reduced glutathione (GSSG and GSH) were arbitrarily chosen as prototypes of target molecules. These compounds were selected, as the bulk of the cellular thiol and disulfide groups are parts of cysteine and cystine molecules of peptides and proteins.

The rate of mixed disulfide formation. In thiol-disulfide interactions the two consecutive reactions 1 and 2 occur:



In such nucleophilic exchange reactions the mercaptide ions are the active molecular species entering into the reactions (246, 267). For this reason it might be expected that thiols with high pK_{SH} values would react too slowly at pH 7.4 to be able to function as effective protective agents.

In an investigation of the role of the pK_{SH} value the unexpected observation was made that the rate of interaction of a series of thiols with cystine (CSSC) at pH 7.4 and 37°C was, with relatively few exceptions, approximately the same. The reaction rate of these thiols was found to obey the Brönsted equation with the following parameters (268):

$$\log k_{\text{S}^-} = 0.73 \times pK_{\text{SH}} - 1.88$$

(k_{S^-} given in liter \times mole $^{-1}$ \times minute $^{-1}$ referring to the interaction of the thiol with the one of the sulfur atoms of CSSC).

For all substances following this equation (cysteine, glutathione, homocysteine, cysteamine, monomethylcysteamine, dimethylcysteamine, diethylcysteamine, morpholylcysteamine) a fairly rapid reaction rate was observed, leading to equilibrium values in the course of 1 to 3 minutes. Apparently, the decrease in the concentration of the ionized thiol at pH 7.4 with increasing pK_{SH} value is effectively counteracted by a concurrent increase in the nucleophilic reactivity of the ionized thiol.

Several thiols did not obey the above equation but reacted at distinctly slower rates. This was the case with certain *N*-acyl derivatives (*N*-acetyl-cysteamine, aletheine, and coenzyme A), as well as thioethanol and thioglycolic acid. Compounds in which the ionized thiol is part of a resonating system (thiocyanide, ergothioneine, thiolhistidine, etc.) did not interact at all with cystine and oxidized glutathione.

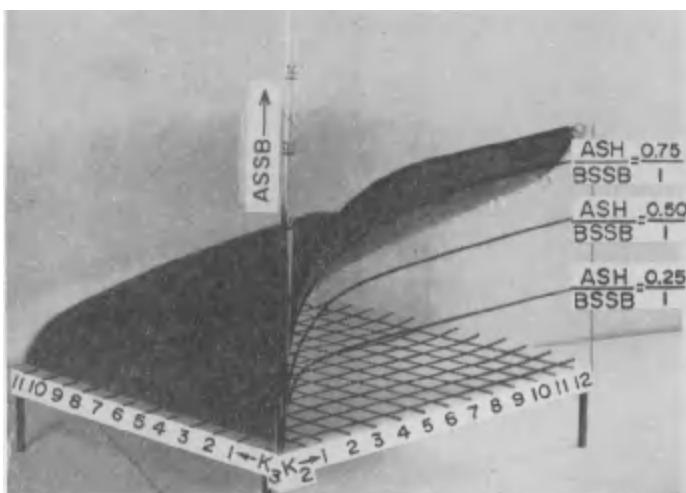


FIG. 4. The equilibrium concentration of mixed disulfide ASSB as a function of the equilibrium constants K_2 and K_3 . The surface depicts the final ASSB concentration when both reactants were initially present in a concentration of 1.

The above reaction rates support the mixed disulfide mechanism in so far as all thiols with strong protective activity react rapidly with cystine, whereas those with little or no protective activity in general react slowly or not at all with cystine to form mixed disulfides (see Table VI).

The extent of mixed disulfide formation. In a given thiol-disulfide system the amount of mixed disulfide present at equilibrium will be determined by the initial concentrations of the reactants and by the equilibrium constants K_2 and K_3 of equations 1 and 2.

The equilibrium constants K_2 and K_3 have been measured for a series of systems in which thiols were reacted with cystine (CSSC) or oxidized glutathione (GSSG) (154, 269, 270). In order to facilitate the comparison between different thiol-disulfide systems, the mixed disulfide content is presented graphically as a surface in three dimensions (Fig. 4) as a function of K_2 and K_3 . This model, which represents the general solution of consecutive reactions of this type, has been constructed on the assumption that the initial concentration of both reactants is equal to 1. It appears that the mixed disulfide concentration may vary from zero to almost unity, depending on the numerical value of the equilibrium constants.

In Fig. 5 the equilibrium constants of systems of radiobiological interest have been plotted in diagrams which represent the bottom plane of Fig. 4. When the two figures are considered together, the practical significance of the equilibrium constants is readily apparent. It is obvious from the diagram that all the protective compounds exhibit a pronounced ability to

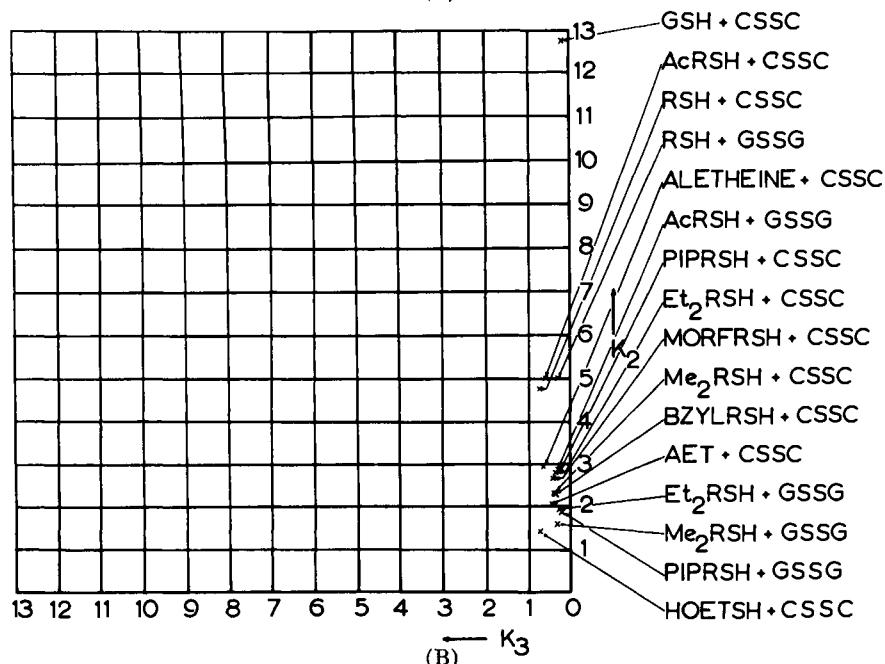
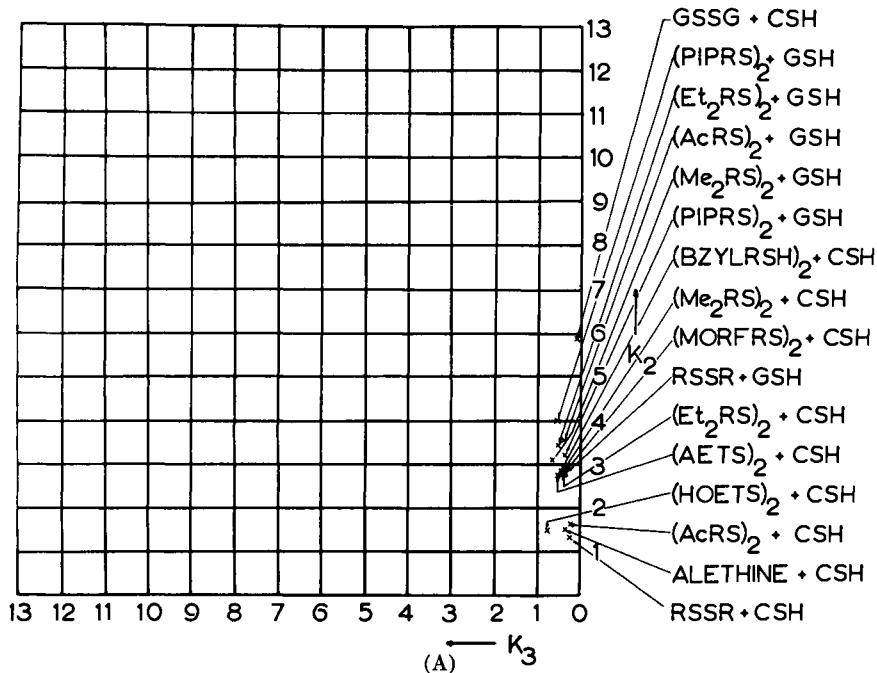


FIG. 5. The equilibrium constants K_2 and K_3 for a number of thiol-disulfide systems. The diagram corresponds to the bottom plane of Fig. 4. A, the interaction of various thiols with cystine (CSSC) and oxidized glutathione (GSSG). B, the interaction of various disulfides with cysteine (CSH) and reduced glutathione (GSH). Abbreviations: RSH-cysteamine; AcRSH-acetylcysteamine; $\text{Me}_2\text{RSH-N-dimethylcysteamine}$; $\text{Me}_3\text{RSH-thiocholine}$; $\text{Et}_2\text{RSH-N-diethylcysteamine}$; PIPRSH-*N*-piperyldycysteamine; MORFRSH-*N*-morpholylcysteamine; BZYLRSH-*N*-benzylcysteamine; AET-aminoethylisothiuroniumbromide HBr; HOETSH-thioethanol.

form mixed disulfide with glutathione and cystine. This is true whether the protective agents are present in the SH or in the SS form. The K values refer to conditions where oxygen is absent. In the presence of oxygen an oxidation of thiols to disulfides will take place with a consequent increase in the equilibrium concentration of mixed disulfide.

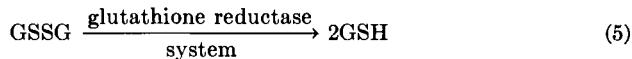
In addition to the systems presented in Figs. 4 and 5, a number of other thiol-disulfide systems have been studied. In several instances the results permit definite conclusions with regard to the ability to form mixed disulfides, although equilibrium constants could not be obtained. As mentioned above, a series of thiols did not interact with cystine and oxidized glutathione to form mixed disulfides. This applies to a number of nonprotective aromatic thiols (thiophenol, *o*-aminothiophenol, 4,6-dimethyl-2-mercaptopyrimidine, 2-mercaptopthiazoline, thiolhistidine, 2,4-dinitrothiophenol, and ergothioneine). Similarly, several aliphatic sulfur compounds, such as thioacetamide, thiocyanide, and diethyldithiocarbamate, did not enter into exchange reactions with aliphatic disulfides. The common feature of these thiols is that the ionized thiol groups are stabilized by resonance. In some cases the interaction of the corresponding disulfides with cysteine and reduced glutathione, respectively, was studied. In all instances a rapid reduction of the disulfide was observed with a concomitant appearance of CSSC and GSSG. Only a transient formation of small amounts of mixed disulfides was detected. Apparently, in mixtures of the above thiols with cystine and oxidized glutathione the equilibria are displaced completely to the left, owing to a large difference in the oxidation-reduction potentials.

In the case of several dithiols (2,3-dimercaptopropanol, dithiopentaerythrit, and thioctic acid) the dithiols in the reduced form rapidly reduced CSSC, with no appreciable formation of mixed disulfides.

By and large, the data summarized above on the amount of mixed disulfide formed between thiols and CSSC and GSSG support the mixed disulfide mechanism (see Table VI). The few apparent exceptions (benzylcysteamine, *N*-dibutylcysteamine, and piperidylcysteamine) will be discussed later.

c. The Biochemistry of Mixed Disulfides. The optimal protective effect of the agents in the cysteine-cysteamine group is obtained when the compounds are administered immediately before the radiation exposure. As pointed out above, the effect is of limited duration. These facts imply that a binding of the protective agents to target molecules must be of a temporary nature and that mechanisms must exist for the release of the protective residues. Direct evidence that cysteamine residues become released from the blood proteins was obtained in experiments on mice (106). The observations may be explained by the finding that, provided small amounts of reduced glutathione are present, a variety of disulfides can be reduced

by glutathione reductase (247), an enzyme which is widely distributed in mammalian tissues. It was demonstrated that this reduction involves a two-step spontaneous thiol-disulfide exchange reaction between the disulfide and the reduced glutathione (equations 3 to 5) followed by the enzymatic reduction of the oxidized glutathione:



The preferential protection of the bone marrow by cysteamine and AET (62) suggests the possibility that this might be due to a selective concentration of the compounds in this tissue. Studies on the organ distribution of cystamine and cysteamine by means of a method which determines both the free and bound compounds demonstrated a moderate degree of selective pickup in thyroid, kidney, spleen, and bone marrow (271). In the case of cysteine and glutathione no specific distribution pattern has been found (55).

Attempts to study the intracellular distribution of S^{35} -labeled cysteine in liver, kidney, and spleen 15 minutes after administration indicate that the radioactivity is limited mainly to the nucleus and the mitochondria near the nucleus (272). Bradford *et al.* (273) have observed with S^{35} -labeled *S*-mercaptopethylguanidine HBr (MEG) a preferential localization of the radioactivity to spleen, bone marrow, and liver. The major activity of all the particulate fractions was tightly bound and could not be removed by dialysis against cyanide, a finding indicating that the binding was probably not due to mixed disulfide formation. Other forms of binding of sulfur-containing protective agents should therefore be considered. In this connection it may be relevant to recall that thiols may interact with aldehydes and ketones to form semimercaptals and thiazolidines. Thus, a slow but extensive interaction has been demonstrated between labeled cysteamine and a series of carbonyl compounds, particularly those containing adjacent activating groups (274). Evidence that such a thiazolidine formation may not be without radiobiological significance has been provided by Littman *et al.* (275).

d. Concluding Remarks. From the radiochemical and physicochemical data summarized above, the conclusion seems inescapable that disulfide groups occupy a central position in the mechanism of dissipation of radiation energy. This applies to both the direct and the indirect mechanism of action. Thus, the thesis that the formation of additional disulfide bonds by mixed disulfide formation with protective residues confers increased radioresistance on a molecule seems well substantiated.

In general, the data on the rate and extent of mixed disulfide formation of a series of protective and nonprotective thiols and disulfides (Table VI) provide strong support for the mixed disulfide mechanism. Also, in the case of protein SH groups, a rapid interaction has been observed with cystamine and cystine (276). On the basis of this evidence the previously inexplicable finding that a number of thiols which are protective *in vitro* lack protective action *in vivo* is logically explained by assuming that these compounds are unable to form mixed disulfides with the target molecules, as they fail to interact with cystine and oxidized glutathione.

In view of the arbitrary choice of cystine and glutathione as prototypes of target molecules, the consistency of the chemical data with the biological data must be considered remarkable. Benzylcysteamine, *N*-dibutylcysteamine, and piperidylcysteamine, which lack protective activity *in vivo* (33), appear to be the only exceptions. Owing to their high toxicity (278), however, these substances were tested with much lower doses than those used with cysteamine and the other protective thiols.

Whereas the above data on the ability of thiols to form mixed disulfides are in excellent qualitative agreement with their effect on radiosensitivity, they do not adequately reflect the direction of the effect (protection or sensitization) and the relative activities. The finding that the sensitizing thiols form mixed disulfides with cystine and glutathione is obviously in agreement with the mechanism proposed.

The fact that it is possible either to raise or to lower the radiosensitivity of organisms by chemical, biochemical, or physicochemical means shows that in tissues there exists a "physiological level of chemical protection" which will be determined by the biochemical make-up of the cells. This point of view emphasizes the close relationship between pathophysiology, radiosensitivity, and the mechanism of chemical protection and sensitization. In view of the mixed disulfide mechanism, special significance should be ascribed to glutathione, since this compound is present in tissues in concentrations of about 1 gm/kg, which is of the same order as the dose given to obtain optimal protection.

To the extent that the mixed disulfide mechanism holds true, a deeper understanding of the factors influencing the rate and extent of mixed disulfide formation might give hints as to the chemical nature of the target and also serve as a guide in efforts to develop more efficient protective agents. It therefore seems of interest to attempt to analyze the structural factors determining the stability of mixed disulfides.

From the high equilibrium concentrations found in the systems studied, it can be inferred that the mixed disulfides between the protective thiols and cysteine and glutathione are somehow thermodynamically stabilized. It appears unlikely that the increased stability can be attributed to prop-

TABLE VI
PROTECTIVE OR SENSITIZING ACTION *in vivo* OF VARIOUS THIOLS AND
THEIR ABILITY TO FORM MIXED DISULFIDES WITH
GLUTATHIONE AND CYSTINE

Compound	Extent of mixed disulfide formation with CSSC and GSSG	Rate of mixed disulfide formation with CSSC and GSSG ^a	Ref. ^b
<i>Protective thiols</i>			
Cysteine	Extensive	Fast (2-3 min)	(19, 25, 26, 114)
Homocysteine	Extensive	Fast (3-5 min)	(26, 30)
Cysteamine	Extensive	Fast (1-2 min)	(22, 26, 30, 33, 114)
Aminoethylisothiuronium bromide HBr (AET)	Extensive	Fast (2-3 min)	(23, 29)
N-Methylcysteamine	Extensive	Fast (2-3 min)	(26, 33)
N-Dimethylcysteamine	Extensive	Fast (2-3 min)	(26, 33, 114)
N-Diethylcysteamine	Extensive	Fast (2-3 min)	(33, 114)
N-Morpholylcysteamine	Extensive	Fast (2-3 min)	(33)
N-Acetyl cysteamine ^c	Extensive	Slow (10-15 min)	(26, 114)
Glutathione	Extensive	Slow (10-15 min)	(20, 30)
Aletheine ^c	Extensive	Slow (15-20 min)	(26, 114)
Cysteine ethyl ester	Extensive	Fast (1-2 min)	(114)
2,3-Dimercaptopropanol ^c	Moderate	Slow (15-20 min)	(26, 114)
<i>Sensitizing thiols</i>			
Thioethanol	Extensive	Slow (10-15 min)	(26)
Penicillamine	Extensive	Slow (12-17 min)	(40)
<i>Inactive thiols</i>			
Di-N-butylcysteamine ^d	Extensive	Fast (3-5 min)	(33)
Thioglycolic acid	Extensive	Slow (15-20 min)	(26)
Thiocholine	Slight	Fast ($\frac{1}{2}$ -1 min)	(71)
N-Piperidylcysteamine ^d	Extensive	Moderate (4-6 min)	(33)
N-Benzylcysteamine ^d	Extensive	Moderate (4-6 min)	(26, 33)
1-Amino-7-mercaptoheptane	Moderate	Slow (40-60 min)	(33)
Thiocetic acid	Traces	Slow (15-30 min)	
Dithiopentaerythrit	Traces	Slow (10-15 min)	(277)
<i>o</i> -Aminothiophenol	None	No interaction	(114, 277)
Thiophenol	None	No interaction	
2,4-Dinitrothiophenol	None	No interaction	
4,6-Dimethyl-2-mercaptopypyrimidine	None	No interaction	(277)
2-Mercaptothiazoline	None	No interaction	(23)
Thiolhistidine	None	No interaction	(277)
Ergothioneine	None	No interaction	(30)
Thioacetamide	None	No interaction	(30, 277)
Thiocyanide	None	No interaction	(30)

^a The figures in parentheses give the time needed to reach equilibrium.

^b The references refer to the protective or sensitizing action of the compounds.

^c Doubtful protective effect.

^d Protective action tested only with very low doses on account of the toxicity.

erties of the SS bond, since the electronic asymmetry would be expected to reduce the bond strength. Therefore, some type of extra stabilizing force probably exists between the two moieties of the molecules. One possible explanation is that hydrogen bonding occurs between the amine of the protector moiety and the carboxylate ion of the "target." Evidence in support of this view is found in the exceptional stability of the mixed disulfide between glutathione and cysteine (279). A molecular model of this compound shows that the configuration permits the formation of *two* sets of hydrogen bonds, viz., between the amine and the carboxylic acid groups of the γ -glutamyl and cysteinyl residues, respectively. Conversely, the nonprotective compound thiocholine forms only slight amounts of mixed disulfides with CSSC and GSSG. This finding is consistent with the well-known fact that quaternary amino groups do not form hydrogen bonds.

From these considerations it may be surmised that the actual target molecules of mammalian cells probably possess in proximity to thiol and disulfide groups a negatively charged group (e.g., carboxylate or phenolate ions) capable of forming hydrogen bonds with the amines of the protector.

The above view that a two-point attachment takes place between the protector and the target molecule is consistent with the bulk of the previously mentioned structural requirements for protective activity. On this basis, a number of facts can be given a logical explanation. Thus, this theory may explain the obligatory requirement for an amine group in the protector molecule, the fact that optimal protection is obtained with a carbon chain length of 2 or 3, the reduction of protective ability obtained by acylation of the amine, the lack of protective ability of quaternary amines, and the fact that enantiomeric compounds are equally protective.

VIII. Summary of Protective and Sensitizing Mechanisms

The most probable mechanism of action *in vivo* of the various groups of protective and sensitizing agents is summarized in Table VII. Many of the conclusions are tentative. This summary and the preceding presentation serve to emphasize the complexity of the protective and sensitizing mechanisms in biological systems. It appears that the frequent attempts to attribute all protection phenomena to a single mechanism are not warranted.

It must be admitted that, despite considerable efforts, the procedures developed with the purpose of reducing or increasing the radiosensitivity of mammals are so far of limited practical value. These endeavors have contributed materially, however, to our understanding of basic mechanisms in radiobiology. The systematic search for procedures influencing the radiation response has considerably broadened our understanding of the processes underlying radiosensitivity. This phenomenon, which for generations has been correlated with the cellular morphology, may in the not too distant

TABLE VII
PROBABLE MECHANISM OF ACTION OF PROTECTIVE AND
SENSITIZING AGENTS ACTIVE *in vivo*

Group	Substance	Action	Main mechanism of action
Sulfur-containing compounds	Cysteine-cysteamine group Dithiocarbamates } Colloidal sulfur } Thiourea }	Protection or sensitization Protection	Mixed disulfide formation Inactivation of free radicals
Pharmacologically active compounds	Enzyme inhibitors (NaCN, NaN ₂ , etc.) Hormones Stimulants and depressants of the central nervous system Carbon monoxide } Sodium nitrite } <i>p</i> -Aminopropiophenone }	Protection Protection or sensitization Protection	Temporary influence on target radio-sensitivity Alterations of cellular metabolism Alterations of cellular metabolism
Metabolites and inert compounds	Fructose Glucose } Pyruvic acid etc.)	Protection	Reduction of cellular oxygen tension Inactivation of free radicals
Substances with specific resonating structures	Synkayvite and related compounds } Porphyrins }	Sensitization or protection	Chemical modification of target molecules

future be understood in terms of biochemical and radiochemical mechanisms.

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CHAPTER 5

Recovery and Therapy of the Irradiated Organism

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I. Introduction—General Survey of Factors Influencing Recovery

The irradiation of living organisms leads to damage and death of many different cell types, and the resulting clinical picture is a complicated one. At present the knowledge of the physiopathology of radiation sickness is still far from complete.

The reactions of living organisms after a damaging dose of ionizing radiation are determined by a variety of physical and biological factors which can be briefly summarized as follows: the physical factors, including the radiation quality, the radiation dose, and the dose rate; and the bio-

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logical factors, including the nature of the irradiated organism and the status of the organism, during and after irradiation.

In the evaluation of experimental results all these factors should be kept in mind. The radiation quality obviously determines the penetration of the radiation, especially in the larger organisms. Consequently the distribution of the dose throughout the body is dependent on this factor. For instance the effects of external β -radiation are limited to the skin; soft X-rays do not penetrate further than the superficial tissues; and very hard X-rays and γ -rays cause a fairly homogeneous dose distribution throughout the entire body, provided a suitable distance between the source of radiation and the irradiated animal is employed, which is particularly important with larger animals.

The latter conditions, which lead to a uniform total body irradiation, have proved to be most useful for the student of radiation sickness. This chapter will be concerned mainly with recovery and therapy after whole-body irradiation of various mammalian species.

Internal irradiation, which results from the introduction of radioactive isotopes into the body, will not be discussed at any length, because in many cases a very specific localized irradiation occurs according to the distribution of the various isotopes, in particular with the nuclides which emit particles of a limited penetrating power.

The relation between total body dose and effect has been studied in many mammalian species. Even within a species considerable differences have been noted, as is illustrated in Fig. 1, which shows the mortality of three different strains of mice after various doses of total-body irradiation. Of course, it is quite possible that other factors (e.g., differences in dosimetry and differences in the care of the animals) are involved as well, but similar differences of radiosensitivity between strains in the same laboratory have been observed, not only with regard to acute mortality but with regard to several other radiation effects as well (3, 4).

In the study of factors influencing recovery of the irradiated animal, death has been used as an end point most frequently. In the present state of radiobiological knowledge in which we are still trying to identify these factors, this seems to be not only fully justified but also the most efficient approach. Admittedly it is a very crude method, but most other indices show a much larger variation after a fixed dose of irradiation.

As in the case of other toxic agents the value of therapeutic measures after total-body irradiation has been estimated at the midlethal dose as well as after radiation doses slightly exceeding the minimum LD_{100} . In general the latter procedure yields more dependable results; because of the steep rise in mortality between the LD_{10} and the LD_{90} in most laboratory animals, minor variations in experimental conditions may easily cause a

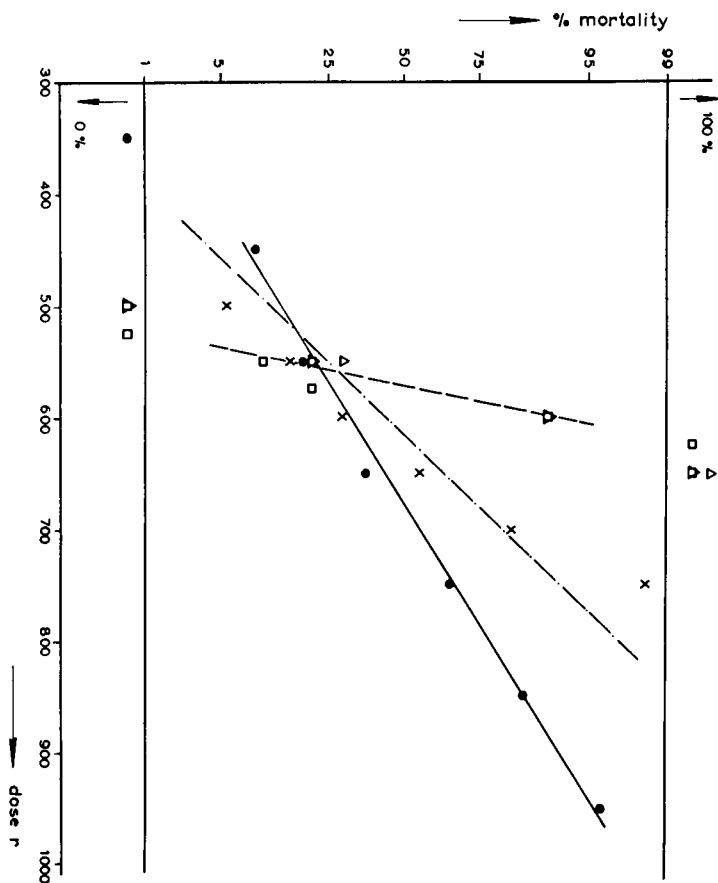


FIG. 1. Relation between X-ray dose and 30-day mortality in three mouse strains. Δ ♂ and \square ♀ CBA mice (author's observations); ● ♂ Swiss mice (1); \times ♀ CF₁ mice (2).

change of mortality which in case of a decrease simulates an action of the therapeutic agent under investigation. On the other hand, at supralethal radiation doses minor therapeutic effects may not become evident. In view of the relatively recent development of the research into the treatment of radiation injury it is not surprising that in general these investigations have been limited to a dose region not exceeding about twice the minimal LD₁₀₀. It is also understandable that these efforts have concentrated on the acute radiation syndrome rather than on the chronic and late effects of irradiation.

After acute irradiation in the dose region mentioned, two major syndromes have been recognized. After doses up to 500 to 1000 r (depending

on the species) the degree of injury to the hematopoietic tissues determines the chances of survival. In lethal cases the pancytopenia with the resulting hemorrhagic diathesis and septicemia constitute the main symptoms of the typical bone marrow syndrome, from which the animals die between the sixth and the twentieth days after irradiation.

After larger doses (1000 to 10,000 r in most species) the damage to the intestinal tract is the determining factor. The survival time is shorter, usually 3 to 5 days. The animals probably die from a disturbance of the fluid and mineral balance which results from diarrhea.

The bacteremia which accompanies the final stages of both syndromes has been attributed rather indiscriminately to the damage of the intestinal epithelium which would permit a penetration of intestinal organisms into the blood stream. In the case of bacteremias accompanying the bone marrow syndrome this explanation is clearly inadequate, since the intestinal lesions have healed completely several days before microorganisms can be detected in the blood. Wensinck *et al.* (5, 5a) have shown that bacteremias in irradiated mice suffering from the bone marrow syndrome are usually not caused by intestinal microorganisms, but instead by bacteria which normally inhabit the upper respiratory tract. Furthermore infected epithelial lesions were observed on the base of the tongue and sometimes in the larynx by Vos *et al.* (6). These lesions may well prove to be the ports of entry of the invading microorganisms.

On the other hand, it has been found that the blood of animals dying from the intestinal syndrome is sterile in many cases which show an excessive damage of the intestinal epithelium, amounting to a complete denudation of the villi. This would indicate that bacteremia is not the direct cause of death in the intestinal syndrome.

In some species (e.g., the mouse) the lethal dose region in which death is due to bone marrow failure and that in which the intestinal injury induces death are clearly separated. In other species (e.g., the rat) it is usually difficult to induce a lethal bone marrow syndrome in 100% of the animals because the intestinal syndrome develops after relatively small doses of radiation. Since the treatment of these two syndromes is essentially different, the latter species is not particularly suited for the study of the therapy of the bone marrow syndrome.

The radiosensitivity of the numerous mammalian species that have been studied does not vary greatly. The LD₅₀ of acute irradiation with penetrating X- or γ -rays lies between 300 and 800 r for most species. The lowest values have been recorded with guinea pigs and dogs. It is not clear whether this is due to a greater radiosensitivity of the animals as such or to the fact that under laboratory conditions these species usually suffer from a relatively large number of latent infections.

A modification of the radiosensitivity of the individual animals may be brought about by a variety of pre- and postirradiation treatments. The majority of the effective preirradiation treatments change the susceptibility of one or more tissues with regard to the damaging effect of ionizing radiation. For all practical purposes this may be considered as a localized or general decrease of the effective radiation dose. The various methods of chemical protection fall into this category.

Postirradiation treatment, on the other hand, does not influence the acute damage to the individual cells but acts obviously by promoting the processes of recovery. It seems theoretically possible that some preirradiation procedures may favorably influence the recovery processes apart from decreasing the initial damage, although no proved example of such a mechanism is known to the author. A unique way to promote recovery—and at present the most promising treatment of the bone marrow syndrome—is provided by the transplantation of viable bone marrow cells. In this case the failing hematopoietic system is replaced by the daughter cells of the donor bone marrow, one of the most beautiful examples of therapy by substitution.

II. Some Physiologic Factors Influencing Recovery

A. METABOLIC RATE, ENVIRONMENTAL TEMPERATURE, HIBERNATION, ANESTHESIA

The literature contains numerous instances of a decrease in radiosensitivity by the above factors or related ones when present during the irradiation. In most cases there is sufficient reason to attribute the protective effect to localized or generalized anoxia. The reader is referred to the exhaustive reviews by Patt (7) and by Patt and Brues (8) and to Chapter 4 of this volume for more information on this subject.

It is our purpose to examine the effect of factors which have been applied after the completion of the irradiation. Several reports are available which show that recovery can be promoted in microorganisms by a temporary inhibition of metabolism when initiated soon after irradiation. Latarjet (9) described an increased survival of X-irradiated *Saccharomyces ellipsoideus* when the cells were kept at 5°C for a few days after the irradiation. Extensive studies on the response of irradiated *Escherichia coli* cells to storage in the cold were made by Hollaender (10). A significantly increased recovery was obtained when the cells were stored at temperatures below the optimal growth temperature. Storage at 4° to 6°C had no beneficial effect, which is contradictory to the findings of Pratt *et al.* (11).

Kimball (12) has shown that exposure to streptomycin after X-irradiation decreases the number of recessive lethal mutations induced in *Para-*

mecium. Kimball postulated that the mutational process is not completed immediately and that streptomycin treatment allows more time for spontaneous recovery by delaying the cell before the stage at which mutation becomes irreversible. Although certain differences have been noted, these phenomena may be related to the increased recovery which has been observed in microorganisms after incubation in nitrogen-deficient media after ultraviolet irradiation (13). This has been tentatively explained by assuming that any slowing down of the synthesis of essential compounds as a result of the irradiation will be lethal to cells that are engaged in division but not to resting cells. If the cells are permitted to recover these synthetic processes before the next cell division, death may be prevented.

As early as 1939 Cook (14) showed that *Ascaris* eggs which were allowed to develop at 25°C after exposure to a dose of 5000 r yielded only 1 to 2% of normal embryos, whereas eggs receiving the same dose and placed at 5°C for 8 weeks developed 45% of normal embryos after being returned to 25°C.

In amphibia and mammals a lowering of the body temperature after irradiation may delay the onset of symptoms, but no evidence of an increased recovery due to this treatment has so far become available. The survival time of frogs after irradiation with doses of 1000 r and 3000 r was greatly increased by keeping the animals at 5° to 6°C continuously after exposure (15). On removal of the animals from the cold after 60 to 130 days, however, there was no difference in the time course of mortality nor in absolute survival from that observed in frogs maintained at 23°C. This suggests that the radiation damage is not repaired in the metabolically depressed frogs. It was shown by Allen *et al.* (16) that the rate of destruction of hematopoietic cells in irradiated tadpoles is greatly influenced by the post-irradiation environmental temperature. At temperatures below 12°C the destruction is greatly delayed. Similarly Duryee (17) described a delay of more than 20 days in the development of lesions in the ovarian ova of amphibians which were kept at 5°C after the irradiation.

When hibernating mammals such as marmots (18) and squirrels (19) are irradiated and maintained in the hibernating state, mortality is greatly reduced. When the surviving hibernators are awakened several weeks later, symptoms of radiation sickness appear, and the animals die after the same period of sickness as nonhibernating controls. Smith and Grenan (18) reported that their marmots given 650 r during the hibernating phase showed only the blood changes characteristic of hibernation. After their return to room temperature both erythrocyte and leukocyte concentrations fell abruptly. When the irradiated squirrels studied by Doull and DuBois (19) were maintained indefinitely in the poikilothermic state, mortality eventually occurred, beginning on the fifty-fourth day after 800 r, on the forty-

second day after 1000 r, and on the thirty-eighth day after 1200 r of X-rays. In the nonhibernating animals nearly all animals died before the tenth postirradiation day. This also shows that the development of radiation injury is merely drawn out and by no means prevented by hibernation. Some histological observations have been made by Brace (20) on the tissues of irradiated hibernating marmots. In the blood-forming organs the only evidence of irradiation was the disappearance of eosinophilic cells, which seem to be the only dividing cells in these tissues during hibernation. After return to room temperature there was no reappearance of hematopoietic activity and the marrow became aplastic. Unfortunately there are no observations on immediate cytological irradiation effects in these hibernating animals, the shortest interval after irradiation being 3 weeks. Brace postulates that the delay in the appearance of radiation changes is closely related to the rate of cell division and that interference with cell division is an important factor in the lethal process. The possibility remains, however, that hibernation also inhibits the development of other modes of radiation-induced cell death which are not dependent on division.

The experiments performed by Künkel *et al.* (21) on hibernating loirs (*Glis glis*) deserve some comment. These investigators irradiated hibernating animals at 4°C with a total-body dose of 700 r. After 21 days—an interval during which 83 % of the animals irradiated with the same dose in the nonhibernating state at 20°C had died—the animals were returned to room temperature, which resulted in awakening. A few days afterward the animals began to die, and at 51 days 67 % of the animals had succumbed. Another group of 15 animals was injected intraperitoneally with 500 mg of cysteine per kilogram immediately after the return to room temperature, that is, 21 days after the irradiation took place. This treatment resulted in 100 % survival at day 51. These results would indicate that cysteine is able to give full protection even when administered 21 days after irradiation. It is clearly impossible to explain these data in the light of the existing opinions on the protective mechanism of sulfhydryl compounds. If the claims of Künkel *et al.* could be confirmed, a radical revision of our current thinking on chemical protection would be necessary. Several years ago Maisin *et al.* (22) also reported a beneficial effect of cysteamine and of glutathione when injected after the irradiation. In these experiments the liver region of the rats was shielded during the irradiation; in nonshielded rats post-treatment with cysteamine had no effect. These experiments were immediately repeated by Straube and Patt (23) and by the present author (unpublished); in both cases the results were essentially negative. Langendorff *et al.* (24) have claimed confirmation of Maisin's results by employing median lethal doses of X-rays, but any work in this dose region is liable to criticism because of the great variability observed in the untreated con-

trol animals. Maisin himself could only partly reproduce this phenomenon in subsequent experiments (25).

Few of the drugs which have been used to induce postirradiation anesthesia have had any appreciable effect on survival (see review article by Langendorff and Koch, 26). Smith and Smith (27) employed intermittent sedation by phenobarbital for 11 days after irradiation in mice, which resulted in a slight increase in mortality. A rather striking beneficial effect of chemical hibernation (*anaesthésie potentialisée*) by Laborit's method has been reported by Gros and Comsa (28). When the treatment was started 5 or 10 hours after irradiation with a dose of 800 r the 20-day mortality of their guinea pigs was reduced substantially. These findings, however, require additional confirmation, preferably in other animal species.

The finding that an increase in these parameters enhances radiation mortality is consistent with the above observations on the effect of a decrease of temperature and metabolic activity. It should be pointed out that this need by no means represent a specific effect, since a similar relation is known to exist in the case of many other types of injury. Among the treatments that increase the metabolic rate as well as the mortality of irradiated animals are the administration of thyroid hormone and dinitrophenol (29), moderate (27) as well as exhaustive exercise (30), and exposure of nonacclimatized mammals to a cold environment (31, 32). Surprisingly the continued administration of thiouracil and propylthiouracil beginning 22 days prior to irradiation did not decrease the mortality (29).

B. NUTRITIONAL FACTORS

Relatively little information is available on the effect of nutrition on recovery after irradiation. Certain vitamins with a known or presumed role in hematopoiesis have been administered to irradiated animals; these experiments will be dealt with in other sections.

The eating patterns of various animal species after irradiation differ widely. Guinea pigs continue to eat and gain weight during the first days, even after lethal doses of irradiation, whereas rats refuse food during the same period, at which time they suffer from a stasis of gastric contents and from gastric dilatation. In monkeys a lethal dose of X-rays evokes emesis within a few hours, but usually the appetite recovers within a day or two. When rats were force-fed during the first 3 days after irradiation, both the survival time and the percentage survival were decreased (33, 34). It is possible that the anorexia in irradiated rats is actually beneficial because it safeguards the damaged intestinal epithelium from additional injury and stress. Fasting for a few days after irradiation did not alter the mortality in rats, mice, or guinea pigs significantly (33).

The influence of the protein level of the diet in the postirradiation period

was studied by Smith *et al.* (35). An increase of the casein content caused a slight reduction in the mortality; the addition of methionine and cystine to low-casein diets did not influence the death rate.

The effect of dietary protein and fat on mortality after administration of P³² to mice has been studied by Cornatzer *et al.* (36). A high-protein, high-fat diet had a detrimental effect. Optimal survival was obtained with a diet containing 10% protein and 5% fat.

In a series of investigations Cheng and co-workers (37) have demonstrated an increased susceptibility to radiation injury in rats which were kept on a fat-free diet. This applies to both single and repeated whole-body irradiations. Supplementation with ethylinoleate increases resistance to normal levels. Whether essential fatty acids have a specific function in recovery from radiation injury remains subject to speculation.

No systematic investigation into the optimal levels of proteins, carbohydrates, and fats in the diet of irradiated animals is known to the author. The influence of various vitamins on radiation mortality has been studied by comparing animals on a vitamin-deficient regime with animals that received vitamin supplements. Generally the deficient animals were more radiosensitive, and no indication of a specific role of any vitamin has come to light.

With regard to the proper alimentation of patients suffering from radiation sickness, the observed species differences in the sensitivity of the intestinal tract warrant considerable caution in extrapolating the results of animal experimentation to man. Observations on whole-body-irradiated patients indicate a surprisingly fast return of appetite. It remains the general opinion—which seems based on common sense—that victims of radiation should receive an easily digestible diet of a balanced composition with ample amounts of proteins and vitamins.

III. Specific Therapy of the Bone Marrow Syndrome

A. SYMPTOMATIC TREATMENT

As has been mentioned before, the main manifestations of the bone marrow syndrome in mammals are septicemia, hemorrhagic diathesis, and anemia. Before the introduction of bone marrow transplantation numerous attempts had been made to alleviate these pathological conditions in irradiated animals by symptomatic treatment involving blood transfusions, injections of isolated peripheral cell types, and the administration of various antibiotics. Since the control of the disease depends principally on marrow restoration, long-term survival as a result of the above-mentioned types of treatment can be expected to occur only after irradiation doses which permit a relatively rapid regeneration of the bone marrow. Because

of the complexity of the experiments most investigators have studied the effects of treating one of the major symptoms only. Little success with regard to the prevention of radiation death has been reported from these attempts, the reason being that the animals died from the defects that were not corrected. If septicemia could be prevented, death ensued a few days later from hemorrhage, and vice versa.

It is of considerable significance that much better results may be obtained by a more integrated treatment, providing what is essentially good clinical care. Bond and his collaborators (38) treated dogs by the transfusion of fresh blood to substitute for the loss of platelets, by the administration of potent antibiotics as soon as fever occurred, and by correcting deviations of the fluid and mineral balance. Although the number of dogs in this study is as yet small (10 untreated, 9 deaths; 10 treated, 1 death), their results support the current concept that adequate symptomatic treatment will be of some benefit to human victims of radiation.

Any detailed discussion of the considerable amount of experimental work relating to the effects of blood transfusions and antibiotic treatment falls outside the scope of this chapter, but a few remarks seem nevertheless appropriate.

The use of frequent whole-blood transfusions to combat the anemia which accompanies the later stages of the bone marrow syndrome had little effect on mortality of irradiated dogs (39). The platelet and leukocyte levels were not restored by this treatment, nor was there any difference in the incidence of febrile reactions and the occurrence of gross hemorrhages as compared with the untreated control group (40). The hemorrhagic state involves a continuous loss of erythrocytes and in some instances massive bleeding. Effective replacement of these losses is usually impossible. The continued administration of platelets, either freshly isolated or with fresh blood, has a much better effect because the bleeding can be stopped temporarily. Allen *et al.* (39) were able to maintain the platelet count of lethally irradiated dogs at about 100,000 per cubic millimeter by repeated transfusions of platelet concentrates. At autopsy the extent of hemorrhage was distinctly less than in the controls, and no evidence of severe bleeding into any organ was observed. Nevertheless the treatment did not influence survival rate or survival time.

The effects of early exchange transfusion of irradiated dogs with the obvious objective to replace damaged peripheral cells and to remove postulated toxic factors have been studied in a limited number of dogs. In the experiments of Salisbury *et al.* (41) and of Swisher and Furth (42) there was some benefit from this treatment, but others (43) failed to confirm this.

The results obtained with antibiotics in irradiated animals are variable

(44), most likely because the choice of drugs, dose, and administration schedule has not received the consideration which is customary in the clinical treatment of human patients. It seems that most of the favorable results have been obtained in mice with streptomycin (45, 46), and in dogs with the tetracycline group of antibiotics (44). The mortality in rats was little influenced by parenteral or oral treatment with antibiotics (45, 47), although oral administration of aureomycin was reported to decrease radiation mortality by one group of investigators (48). Such differences between the species are to be anticipated because of differences in bacterial flora as well as differences in the pattern of radiation damage. Since the favorable results—amounting to 50% reduction of mortality—were obtained without any other selection of antibiotics than trial, it seems certainly worth while to investigate the effects of an antibiotic treatment specifically directed at the causative microorganisms involved in the development of the septicemia. There is of course the possibility that the eradication of one strain of invading bacteria will be immediately followed by the appearance of another, which would restrict the usefulness of antibiotics very severely. It should be stressed that the indication for the antibiotics in addition to other therapeutic measures is not disputed in any way by these remarks.

B. TRANSPLANTATION OF HEMATOPOIETIC CELLS

1. INVESTIGATIONS LEADING TO BONE MARROW TRANSPLANTATION

From a consideration of the pathology of the bone marrow syndrome it will be evident that this treatment is essentially a form of substitution therapy and at present the most successful one—at least under certain specific experimental conditions. The story of the discovery of the beneficial effects of bone marrow in acute radiation disease and of the elucidation of the mechanism by which this effect is achieved is a fascinating one. The advances made during the past ten years have evoked a tremendous interest, not only from radiobiologists but even more from workers in the fields of immunology, hematology, and tissue transplantation.

Work along these lines was initiated by Jacobson and Lorenz. In 1949 Jacobson *et al.* (49) discovered that shielding of the spleen during irradiation caused a considerable reduction of mortality in mice, which finding emphasized the key position of the hematopoietic system in radiation sickness (the mouse spleen is essentially a hematopoietic organ). The shielding was effected by exteriorizing the spleen, and every attempt was made to keep the blood supply of the organ intact. In some cases, however, a total infarction of the spleen could not be prevented, but these animals were found to be equally well protected. Jacobson correctly interpreted this observation as indicative of a beneficial effect of a spleen autograft. In 1951

his group (50) reported the therapeutic action of implanted autologous and isologous spleen in irradiated mice, and soon afterward similar effects were obtained with intraperitoneal injections of isologous spleen cells. In the meantime Lorenz (51) and his associates had obtained an impressive protection by the administration of isologous bone marrow to mice and guinea pigs immediately after irradiation. In the following years these two groups of investigators greatly extended their studies, Jacobson's by concentrating on isologous cell preparations, Lorenz's by investigating a number of homologous and heterologous host-donor combinations. Other workers joined in this approach, and, although a number of failures were reported, confirmation of the main findings could be achieved. Table I summarizes the results obtained with bone marrow suspensions until the end of 1955.

By that time interest in the mechanism of the therapeutic action of spleen and bone marrow preparations had gradually increased. Histological studies of the spleen-protected animals had revealed the same massive destruction of the hematopoietic cells of the bone marrow during the first few days as in the nonshielded mice, but an intensive regeneration of the bone marrow as well as of the lymphoid tissues was observed to begin on the fourth day, leading to a complete restoration on the eighth day. The regeneration of the thymus occurred somewhat more slowly, being complete between the twelfth and the fifteenth days. In the liver and the thy-

TABLE I
RESULTS OF BONE MARROW TRANSPLANTATION IN IRRADIATED ANIMALS
(Data available at the end of 1955)

Recipient	Donor	Effect on survival	Reference
Mouse	Isologous	Beneficial	(52, 53)
Mouse	Homologous	Beneficial	(54)
Mouse	Rat	Beneficial	(55)
Mouse	Guinea pig	Beneficial	(54)
Mouse	Rat	None	(56)
Mouse	Dog	None	(55)
Mouse	Rabbit	None	(55)
Mouse	Guinea pig	None	(57)
Mouse	Rabbit	None	(57)
Rat	Isologous	Beneficial	(58)
Rat (non-inbred)	Isologous	Beneficial	(59)
Rabbit	Rabbit	Beneficial	(60)
Guinea pig	Isologous	Beneficial	(52)
Guinea pig	Guinea pig	Beneficial	(57)
Guinea pig	Rabbit	None	(55)
Dog	Dog	Dubious	(61)
Hamster	Hamster	Beneficial	(62)

mus foci of erythropoiesis and myelopoiesis were found. In the nonshielded mice regeneration was absent or consisted merely of some isolated nests of erythropoietic cells by the tenth day (63).

From his observations Jacobson tentatively concluded that the mouse spleen contained a humoral factor capable of stimulating the regeneration of blood-forming tissues. This concept will be referred to as the *humoral hypothesis*. The beneficial effects of bone marrow injections and postirradiation parabiosis were also attributed to this humoral factor by many investigators, because these procedures resulted in an acceleration of hematopoietic recovery which was comparable to that observed after spleen shielding and spleen implantation (54, 64). The possibility of a proliferation of injected hematopoietic cells with subsequent colonization of the host tissues was also recognized, but this *cellular hypothesis* was not much favored because a number of observations seemed to support the humoral hypothesis:

1. Jacobson reported that the removal of the shielded spleen 1 hour after the end of the irradiation did not alter the degree of protection afforded by the shielding procedure (65). A small but significant protection was retained even when the spleen was removed as soon as 5 minutes after the irradiation. At that time it could not be imagined that the number of hematopoietic cells that would be ejected from the spleen into the circulation during this short period would suffice to repopulate eventually all the blood-forming tissues. The idea of a humoral factor's being liberated by the protected spleen seemed much more attractive.

2. In a number of mice that survived the irradiation as a result of the implantation of donor spleens, no spleen graft could be recovered (66). This observation, although in itself providing strong support for the humoral hypothesis, was not confirmed by other investigators and it was actually contradictory to earlier findings reported by Jacobson. The present author observed a correlation between the survival of the irradiated mice and the take of the spleen transplants. This and similar observations in the case of intraperitoneal bone marrow transplantation caused a number of workers to intensify their attempts to obtain more insight into a possible cellular mechanism.

3. It was claimed by Cole's group that cell-free extracts of the hematopoietic tissues were therapeutically effective (67, 68). These authors even suggested that the humoral factor might be identical to a nucleoprotein (69, 70). The results of Cole *et al.* have not been confirmed by other workers (53, 57, 71), and it seems now to be certain that their positive findings were entirely due to the presence of large numbers of intact living cells in the "extracts." Significantly, Cole *et al.* were among the first to provide experimental proof for the transplantability of foreign bone marrow in irradiated animals.

4. The most convincing argument in favor of the humoral hypothesis and one that for many years seemed to be irrefutable was provided by the observations of Lorenz *et al.* (51, 52, 54) that homologous and heterologous tissues are therapeutically effective. The degree of breakdown of the immunological defense system in irradiated animals was apparently not fully realized at that time. There were probably very few who were willing to consider the possibility of foreign cells repopulating the hosts tissues. In 1954 Barnes and Loutit (57), who were among the first supporters of a cellular mechanism, wrote: "We cannot presume that the heterospecific cellular material would survive in the host; even homospecific cells are unlikely to survive unless genetically and antigenically very similar to those of the recipient."

Although the results of Lorenz and co-workers with heterologous bone marrow could not be confirmed by others for a number of years, additional evidence was accumulated by the same group (55). The high quality of their work made it necessary to take their observations into account for any hypothesis on the mechanism of bone marrow therapy.

Nevertheless by 1955 an increasing number of authors expressed their doubt as to the correctness of the humoral factor hypothesis. Some did not accept the value of the arguments presented above under 1 and 2. In addition the following observations could be explained more satisfactorily by the cellular mechanism.

Various investigators agreed on the superiority of the intravenous administration of spleen or bone marrow preparations as compared with the intraperitoneal injection (52, 53, 72, 73, 74). This would be expected in case of a transplantation of hematopoietic cells.

The marked sensitivity of the therapeutic factor to heat (57, 70) and toward ionizing radiation *in vivo* and *in vitro* (57, 65, 69) was similar to that of living cells of the hematopoietic system. Furthermore, storage of the factor in the frozen state could be achieved only when methods were used that had been proved successful in the preservation of living cells (75).

In 1955 Barnes and Loutit (76) drew attention to an important difference between the effects of isologous and homologous hematopoietic tissues, which was not noted by Lorenz's group previously because of the relatively short observation period (20 to 30 days). Although 30-day survival rates were similar, the mice treated with homologous tissue began to die soon after the thirtieth day, and by day 100 all animals were dead. No such secondary mortality occurred after treatment with isologous tissue. No therapeutic effect whatsoever was obtained with homologous spleen in mice which had been immunized against the donor strain prior to the irradiation (57). In 1956 Mitchison (77) demonstrated the presence of A strain antigens in the spleen, the lymph nodes, and the peritoneal exudate

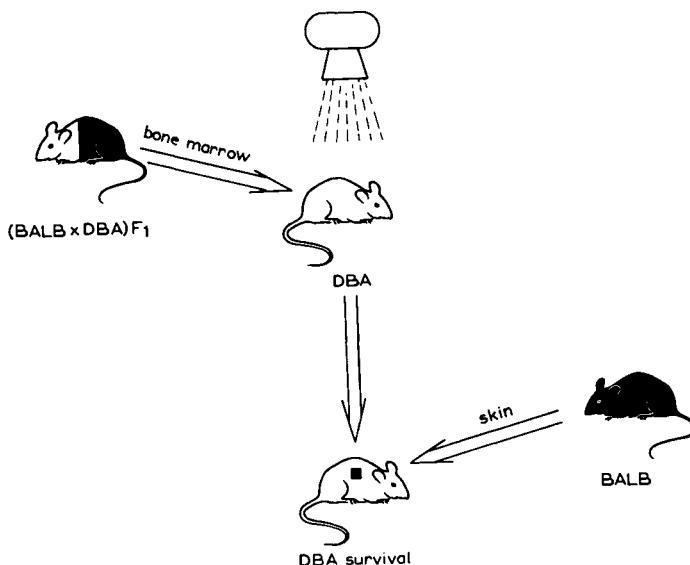


FIG. 2. Schematic representation of the design of the experiments of Main and Prehn (79).

cells of CBA mice that survived a lethal dose of irradiation as a result of the administration of bone marrow of A strain mice. Using a different method Merwin and Congdon (78) demonstrated donor strain antigens in the hematopoietic tissues of irradiated mice several weeks after the injection of homologous bone marrow.

Since a humoral factor might well possess antigenic properties, these observations could not be considered to prove the existence of a cellular mechanism. On the other hand, the persistence and the concentration of the donor-type antigens were exceptionally high, which seemed more difficult to reconcile with the concept of a humoral factor. In this period the experiments performed by Main and Prehn (79) evoked considerable interest. These authors injected $(\text{BALB} \times \text{DBA})\text{F}_1$ bone marrow cells into lethally irradiated DNA mice. The survivors received BALB skin grafts which took in most cases (Fig. 2). These results could be more easily explained by assuming that the bone marrow of the host had been replaced by donor tissue than by the action of a humoral factor.

A few months later Lindsley *et al.* (80) supplied convincing evidence that a transplantation of homologous hematopoietic tissue can be achieved under certain conditions. In irradiated rats which had been treated with homologous bone marrow, donor-type erythrocytes were identified with a serological technique several months after the irradiation. In a few animals the donor-type erythrocytes nearly completely replaced the host's cells.

In these experiments the donor and recipient strains were probably closely related, and it remains doubtful whether this combination can be regarded as strictly homologous.

As Lindsley *et al.* were not able to show a correlation between the survival of the irradiated rats and the persistence of donor-type erythropoietic activity, their results were insufficient to prove the validity of the cellular mechanism in bone marrow therapy. It should be obvious, however, from the reports discussed above that a profound change of ideas with regard to the mechanism of bone marrow treatment was taking place.

Soon afterward, in 1956, definite proof of the cellular mechanism was provided simultaneously by three different groups (81-83). A number of other publications containing confirmation as well as additional evidence have subsequently appeared, so that it can now be stated with certainty that the host's hematopoietic system is replaced by isologous, homologous, and heterologous cells according to the nature of the bone marrow donor. (The animals carrying a foreign bone marrow have been called chimeras.) The implications of this discovery reach far beyond the problem of the treatment of radiation sickness. Its main interest actually lies in the fields of transplantation biology and immunology. The homologous and even the heterologous transplantation of a complex tissue, with localizations throughout the entire host organism and preservation of its various functions, has been shown to be achievable by the simple procedure of intravenous injection of a limited number of cells.

It is now known that the foreign hematopoietic system may under certain conditions continue to function for the remaining life span of the host. This surprising violation of immunological incompatibility barriers seems to provide great possibilities for future experimentation.

2. IDENTIFICATION OF HOST AND DONOR CELLS

A variety of techniques has become available that permit a differentiation between host and donor cells in radiation chimeras. A list of these is presented in Table II.

A most elegant method was introduced by Ford *et al.* (81). It is based on the identification of the chromosomes in metaphase cells. Rat cells contain 42 chromosome pairs, many of which have a characteristic metacentric appearance. They can be easily distinguished from mouse cells which contain only 40 chromosome pairs, all of which are telocentric. The method has also been applied to the study of homologous chimeras by using donor mice carrying a morphologically recognizable marker chromosome. Beginning on the fifth day after transplantation the majority of the identifiable cells in the bone marrow, the spleen, the thymus, and the lymph nodes were found to possess the characteristic chromosomal makeup of the donor.

TABLE II

DIFFERENTIATION BETWEEN HOST CELLS AND DONOR CELLS IN HOMOLOGOUS AND HETEROLOGOUS RADIATION CHIMERAS

Cell type	Host-donor combination	Method	Authors
Hematopoietic cells	Mouse-mouse	Identification of chromosomes	Ford <i>et al.</i> , 1957 (81)
	Mouse-rat		
Bone marrow	Mouse-mouse	Cross-trans-plantation	Vos <i>et al.</i> , 1956 (83)
	Mouse-rat		
Erythrocytes	Rat-rat	Agglutination	Lindsley <i>et al.</i> , 1955 (80)
	Mouse-mouse	Electrophoresis hemoglobins	Welling and van Bekkum, 1958 (84); Rosa <i>et al.</i> , 1958 (85)
	Mouse-rat	Agglutination	Vos <i>et al.</i> , 1956 (83); Makinodan, 1956 (86)
Granulocytes	Mouse-rat	Alkaline phosphatase	Nowell <i>et al.</i> , 1956 (82); Vos <i>et al.</i> , 1956 (83)
	Male-female rabbits	Sex chromatin	Porter, 1957 (87)
Thrombocytes	Mouse-rat	Agglutination	Smith <i>et al.</i> , 1957 (88)
Thymocytes	Mouse-rat	Agglutination	Urso <i>et al.</i> , 1957 (89)
Lymph gland cells	Mouse-rat	Cytotoxic action of antisera	Brocades Zaalberg and van Bekkum, 1958 (90)

As a transformation of host cell chromosomes through the influence of the injected foreign material is quite inconceivable, these findings supplied indisputable proof of a cellular repopulation.

Unfortunately the applicability of this technique is rather limited because the animals have to be either sacrificed or operated upon. Furthermore only a small proportion of the cells in the squash preparations presents its chromosomes in a way permitting identification, and a cytological classification of the hematopoietic cells in metaphase is virtually impossible.

The test devised by Vos *et al.* (83) for the identification of bone marrow cells also requires the sacrifice of the chimera. It makes use of the observation that the number of homologous or heterologous cells required to protect irradiated mice is about twenty times the number of isologous cells that is needed to achieve a similar protection. The results obtained at 14 and 30 days after bone marrow transplantation were in complete agreement with those reported by Ford. The method is depicted schematically in Fig. 3.

Agglutination with specific antisera has been employed to differentiate between rat and mouse erythrocytes (83, 86), thymocytes (89), and blood platelets (88). Some quantitation of the proportion of host and donor cells

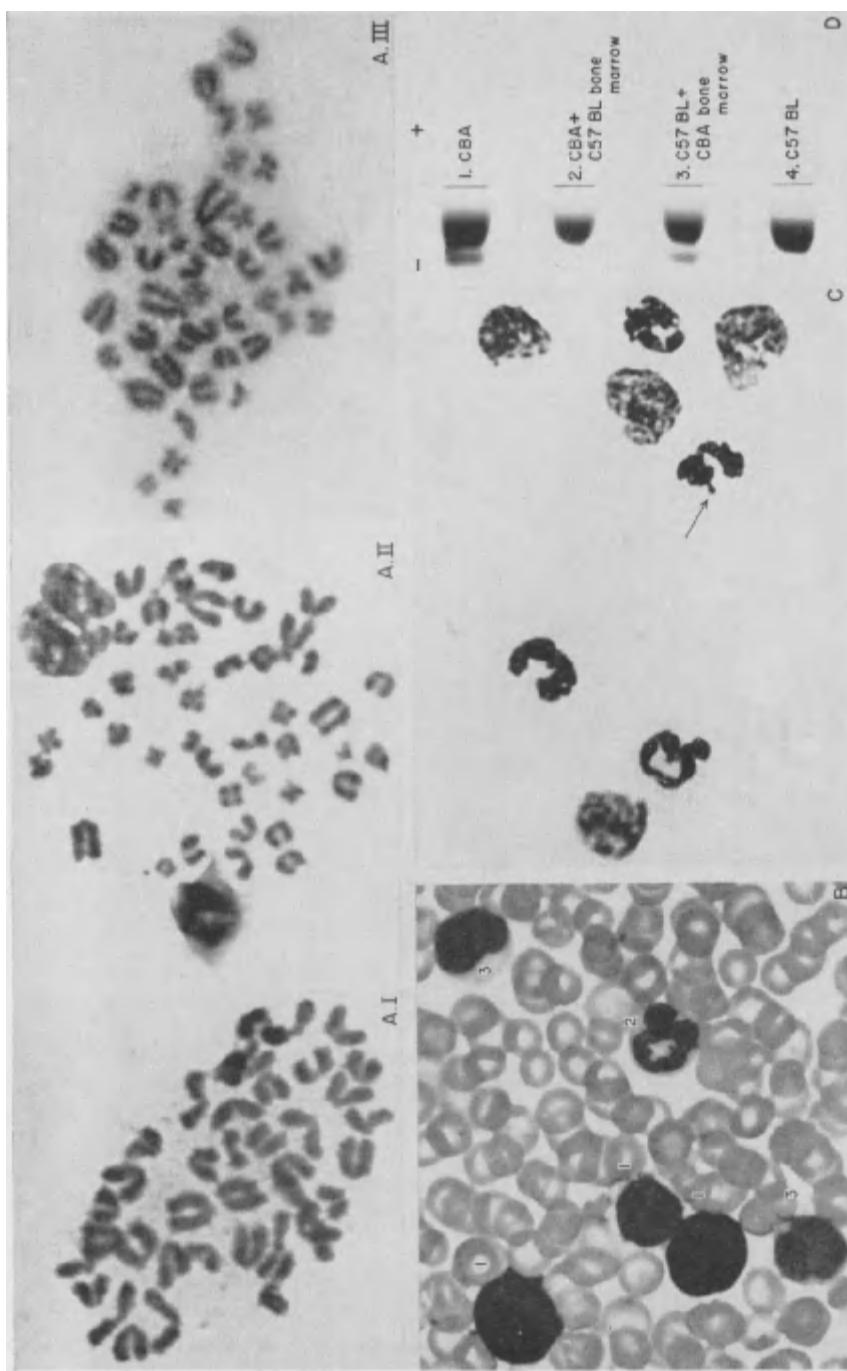


FIG. 3

can be obtained with these procedures. The cytotoxic action of antiserum has been used for the identification of cells from the lymph nodes of mice that were protected with rat bone marrow (90).

Nowell *et al.* (82) and Vos *et al.* (83) independently applied the alkaline phosphatase reaction in blood smears to distinguish positively reacting rat granulocytes from mouse granulocytes, which do not show the presence of the enzyme (Fig. 4). Both this technique and the serological method of erythrocyte typing require only very small samples of blood, so that repeated determinations in one animal can be performed without harm. The replacement of granulocytes and erythrocytes in an irradiated mouse after treatment with rat bone marrow is shown in Fig. 5. The time required for a complete replacement is likely to be related to the life span of the host's cells.

The differentiation between host and donor-type cells is more difficult to perform in homologous combinations. Ford's chromosome identification as well as the cross-transplantation technique of Vos *et al.* may be used in some combinations, but both these methods are laborious, and repeated determinations on the same animal cannot easily be performed.

Two groups of investigators (84, 85) have introduced the use of differences in the electrophoretic behavior of hemoglobins between some mouse strains to demonstrate the presence of host or donor erythrocytes in homologous radiation chimeras. The replacement of the host's erythrocytes appears to be completed somewhat earlier than in the case of heterologous bone marrow transplantation, but this is probably due to the fact that the hemoglobin differentiation is not sensitive enough to detect a small proportion of either one of the hemoglobins in a mixture of the two types (91).

In rabbits the sex chromatin of the granulocytes was used as a marker by Porter (87), but this technique does not permit a quantitative determination of the proportion of host and donor cells.

FIG. 3. Various methods used to distinguish between host and donor cell types. A, chromosomes in bone marrow cells, prepared according to the method of Ford *et al.* (81). Courtesy Dr. L. M. van Putten, Rijswijk. I, mouse bone marrow cell. II, rat bone marrow cell. III, cell from the bone marrow of a mouse, 5 months after irradiation and rat bone marrow transplantation. B, blood smear of a mouse, 319 days after irradiation and rat bone marrow transplantation. Alkaline phosphatase reaction. The picture shows three positive granulocytes (1), one negative granulocyte (2), and two mononuclear cells (3). Courtesy Dr. B. G. Crouch, Rijswijk. C, blood smear of a male rabbit, 60 days after irradiation and transplantation of female rabbit bone marrow. Arrow points to sex chromatin in a granulocyte. Courtesy Dr. B. G. Crouch, Rijswijk. D, Agar electrophoretograms of mouse hemoglobins. Courtesy Mr. W. Welling, Rijswijk. 1. Normal CBA mouse. 2, CBA mouse, 150 days after irradiation and transplantation of C57BL bone marrow. 3, C57BL mouse, 280 days after irradiation and transplantation of CBA bone marrow. 4, normal C57BL mouse.

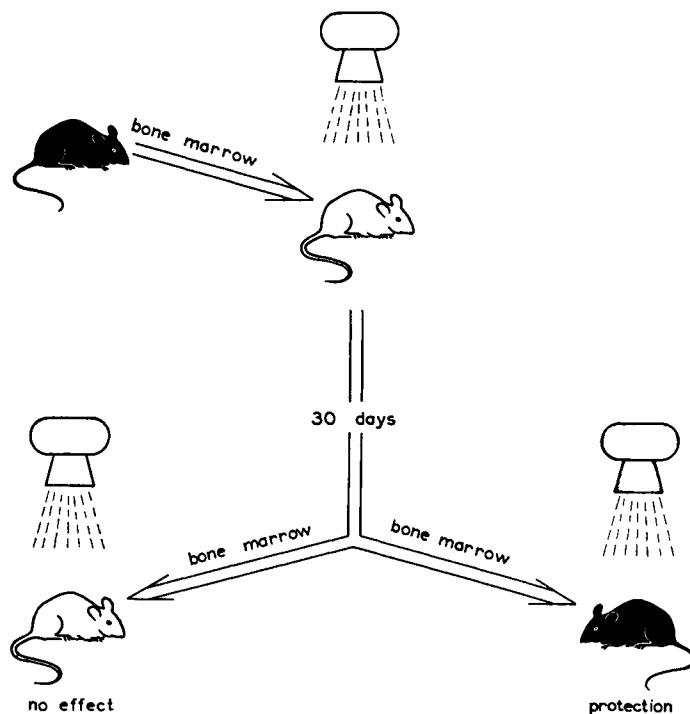


FIG. 4. Schematic representation of the cross-transplantation of bone marrow as employed by Vos *et al.* (83) to identify the bone marrow in radiation chimeras.

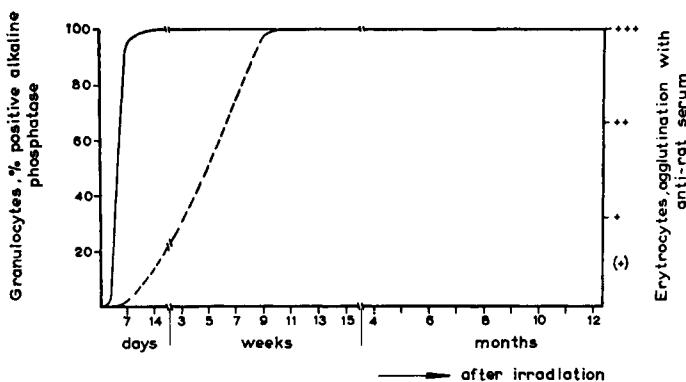


FIG. 5. Replacement of granulocytes (—) and of erythrocytes (----) of the host by those of the graft in mice after irradiation and rat bone marrow transplantation. Schematic representation of results obtained by van Bekkum and Vos (101).

In man the transplantation of bone marrow from donors whose erythrocytes differ in one of the minor blood groups from that of the host has permitted an evaluation of the functional state of the erythropoietic activity of the graft.

The results obtained with the methods discussed above have shown that a complete replacement of the erythropoietic, the myelopoietic, and the thrombopoietic components of the blood-forming system is the rule when bone marrow or spleen is transplanted in lethally irradiated animals. The identity of the lymphoid cells in radiation chimeras has been uncertain for some time, the atrophic condition of the lymphoid system in radiation chimeras being a pitfall for many attempts at differentiation. Ford *et al.* (81) recognized cells bearing donor-type chromosome configurations in the lymph nodes and the thymus, but those cells have not been identified cytologically as belonging to the lymphoid series. The presence of donor-specific antigens in the lymphoid organs of the radiation chimeras was demonstrated by Merwin and Congdon (78). Urso *et al.* (89) reported the presence of cells that could be agglutinated by antirat serum in the thymus of mice treated with rat bone marrow, and the results obtained by Brocades Zaalberg *et al.* (90) with similar radiation chimeras suggest a complete repopulation of the lymph nodes by cells of the rat type.

Although the methods used to identify the lymphoid cells leave much to be desired, the accumulated evidence strongly indicates that a replacement of the host's lymphoid system by donor-type cells takes place. Since the lymphoid system is generally supposed to be the site of immunological reactivity, it would follow that in radiation chimeras the immunological system could be of the donor type as well. This would imply that in the case of homologous and heterologous chimeras the host's own tissues might provide a (continuous) antigenic stimulus to the foreign immunological system which has invaded the same organism. The possible consequences of this peculiar state of affairs have been the subject of intensive research during the past few years and will be discussed more thoroughly in one of the following sections.

3. THE REQUIREMENTS FOR A SUCCESSFUL TRANSPLANTATION OF HEMATOPOIETIC TISSUE

a. Transplantation Techniques. Therapeutic effects have been reported after a variety of transplantation procedures, e.g., the implantation of donor tissue (spleen) in the peritoneal cavity, the intraperitoneal and intravenous injection of cell suspensions, the injection of cells directly into the spleen.

The most effective method, being the one that requires the smallest number of cells to afford a certain degree of protection, has been found to

be the intravenous injection. Under identical conditions about seventy-five times as many cells were needed to obtain a comparable protection when the intraperitoneal route was used (92). These experiments were done with isologous bone marrow. It is noteworthy that the injection of donor cells directly into the spleen is about equally effective as the intravenous injection. This would suggest that a large portion of the intravenously injected cells reach the hematopoietic tissues. Immediately after the intravenous injection of rat bone marrow suspensions into irradiated mice, alkaline phosphatase-positive cells were detected in the lungs. After 24 hours and 48 hours these cells had disappeared (93).

The optimal interval between the irradiation and the transplantation of hematopoietic cells has not yet been investigated in a systematic way. A 24-hour interval has currently been favored by many investigators on the basis of the following essentially theoretical considerations: Firstly, most of the cellular debris has been removed from the hematopoietic tissues after 24 hours, which might provide better conditions for the arriving donor cells. Secondly, both Taliaferro and Taliaferro (94) and Williams *et al.* (95) have shown that the intensity of the primary response to soluble antigens in irradiated animals is dependent on the interval between irradiation and challenge, the minimal response being obtained with a 24-hour interval. In the case of foreign bone marrow transplantation the host's immunological reactivity may determine the fate of the transplanted cells, but it should be stressed that there is no direct evidence to show that the irradiated animal's response against cellular antigens follows the same pattern as against soluble antigens.

Some indications as to the maximal interval after which transplantation can modify the radiation mortality are available. Jacobson *et al.* (50) found 20% survivals among irradiated mice which received spleen implants after an interval of 2 days compared with 38% survivals after immediate transplantation. Cole *et al.* (96) reported some therapeutic effect of treatment with isologous spleen suspensions 45 hours after the irradiation, and Congdon *et al.* (52) obtained a beneficial effect when the interval was 3 days. An increased survival time but no increase of 30-day survival rate was obtained by treating mice with isologous bone marrow 5 days after the irradiation (97).

Little information is as yet available on the optimal conditions for bone marrow therapy after multiple and prolonged irradiations. A limited number of human patients have received bone marrow after consecutive total-body irradiations; no evidence of a proliferation of donor tissue was obtained (98).

A beneficial effect of bone marrow treatment after 1500 r administered over 25 hours was reported in mice by Barnes *et al.* (99). In guinea pigs

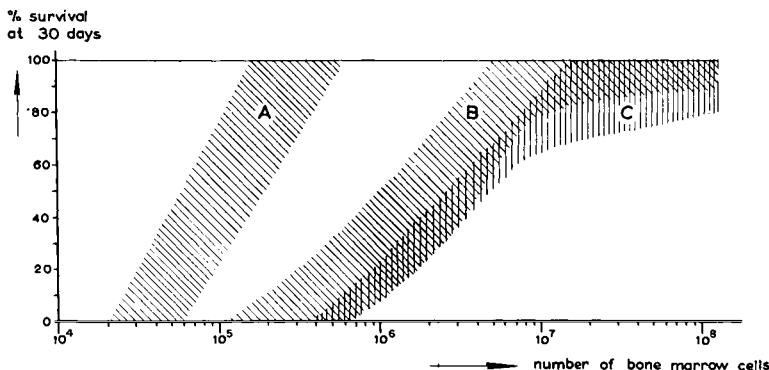


FIG. 6. Relation between number of bone marrow cells administered to irradiated mice and 30-day survival. Data derived from van Bekkum and Vos (101). A, isologous and parent to F_1 hybrid combinations. B, homologous and F_1 hybrid to parent combinations. C, heterologous combination: rat bone marrow.

subjected to a long-term irradiation, transplantation of bone marrow was performed several months after the completion of the irradiation. It seemed that the treatment had some favorable effect (100).

b. *Antigenic Differences between Host and Donor.* This relation is certainly one of the most important factors in bone marrow transplantation. Its significance in regard to the initial (30 days) recovery from the effects of a lethal X-ray dose may be illustrated by the fact that in mice roughly twenty times as many cells are required in the case of treatment with homologous bone marrow as with isologous marrow (83). Even more cells are needed for a successful heterologous transplantation (101) (Fig. 6). Many failures have been reported with homologous and heterologous cells under conditions which permitted excellent recovery with similarly prepared isologous cells.

As has been illustrated in Fig. 6, the number of parent strain cells required to protect irradiated F_1 mice is equal to the number of isologous cells. In the reverse combination many more cells are needed, as is the case in homologous bone marrow transplantation. It will be noted that these results bear resemblance to those obtained with skin transplantation between parent strain mice and their F_1 hybrids. Parent strain skin uniformly* takes in F_1 recipients, whereas F_1 skin is rejected by the parent strain mice. This remarkable analogy warrants the assumption that the immunological

* Except in strains which show the Eichwald-Silmser effect (a rejection of male skin by females from the same strain (102)) F_1 hybrid females may reject skin of the male parent in combinations of these strains (103). The bone marrow transplantsations referred to are not complicated by this phenomenon, since males were used as recipients and females as donors throughout the experiments (101).

reactivity of lethally irradiated mice is not completely suppressed. At present there is little if any reason to attribute the failure of small numbers of F₁ cells to protect parent strain mice to a biochemical incompatibility between the grafted cells and the host.

After irradiation of mice with doses in the midlethal region homologous and heterologous cells induce considerably less recovery than after supralethal doses of radiation. Under the former conditions the administration of foreign cells may even result in increased mortality (104). Another serious disadvantage of the use of foreign hematopoietic tissue is the so-called secondary disease which develops about 30 days after the irradiation in mice that have been protected from the initial lethal effects. These complications will be discussed in more detail in subsequent sections.

Certain host-donor combinations are difficult to classify as either homologous or isologous. The F₁ hybrid-parent strain combinations have been discussed above from the point of view of initial transplantability. With regard to the occurrence of secondary disease the reverse relationship has been found: hybrids carrying parent strain marrow may develop the disease, at least in some laboratories, but parent strain mice carrying hybrid marrow remain unaffected.

It seems likely that the antigenic differences between the host and donor rats which were used by Lindsley *et al.* (80) were less than those which exist between various inbred strains of mice, firstly, because transplantations of bone marrow succeeded after sublethal doses of irradiation, and secondly, because no symptoms of secondary disease were observed, although the donor marrow persisted for many months in some of the treated animals.

It is impossible to predict whether the exchange of bone marrow transplants between randomly bred animals will be comparable to strictly homologous transplantations between inbred strains. Additional information on this point is urgently required because of its significance with regard to the applicability of bone marrow transplantation in human patients. So far some therapeutic successes have been reported with host-donor combinations derived from randomly bred populations, namely in guinea pigs, rats, dogs, and monkeys (see also Table I). Although it is most likely that the survival of the irradiated animals in these experiments was due to a proliferation of donor cells, none of the authors has provided any proof that transplantation actually occurred.

Thomas *et al.* (98) observed a definite though temporary proliferation of donor hematopoietic tissue in a leukemic patient who was treated by intermittent whole-body irradiation (total dose 450 r) and administration of homologous bone marrow. A proliferation of erythropoietic donor cells lasting 90 days has probably occurred in a patient who received injections

of pooled bone marrow to combat a bone marrow aplasia (105) induced by tetramine.

Proof of a successful transplantation of heterologous bone marrow has so far been obtained only in irradiated mice treated with rat bone marrow. Surprisingly, the reverse combination has consistently failed. Some therapeutic effects with other heterologous combinations have been reported. Lorenz *et al.* (54) obtained an increased 20-day survival of irradiated mice by treatment with guinea pig bone marrow. A significant increase of the survival time of irradiated mice was noted after treatment with bone marrow from guinea pigs and hamsters by van Bekkum and Vos (101). In none of these instances did the authors determine the identity of the hematopoietic cells in the survivors. Jacobson *et al.* (106) obtained a definite and lasting therapeutic effect in irradiated rabbits which received fetal mouse liver and spleen. There was no evidence, however, of the presence of mouse hematopoietic cells in the survivors. Therefore the therapeutic effect has not been explained satisfactorily in this case. Failure of fetal mouse liver to protect irradiated rabbits has been reported by Porter and Moseley (107).

c. *The Nature of the Graft and Its Preparation.* In irradiated mice therapeutic effects have been obtained with a variety of tissue preparations, among them spleen, bone marrow, bone brei (108), fetal liver (53), and leukemoid blood (109). Negative results were obtained in therapeutic trials involving thymocyte suspensions and lymph node cells (110).

Several attempts have been made to identify the cell type which is responsible for the therapeutic activity of hematopoietic tissue grafts. So far it has not been possible to obtain satisfactory separation of the various stem cells by the use of differential centrifugation. An indirect approach was made by Cole *et al.* (111) as well as by Vos (110), who compared the efficacy of bone marrow suspensions from normal mice and from mice in which the erythropoiesis or the myelopoiesis had been stimulated. Erythropoietic stimulation was obtained by treatment with phenylhydrazine, and myelopoietic hyperactivity was obtained in animals bearing a transplantable carcinoma. In every experiment normal bone marrow and spleen were found to be superior on the basis of the number of cells required to produce a certain degree of protection, which led both groups of investigators to the conclusion that precursors of all hematopoietic cell series are required for a successful transplantation and that the optimal proportions of the various stem cells are apparently provided by bone marrow from normal animals. This problem has some interesting aspects with regard to current ideas concerning the origin of blood cells. At present it seems likely that after the initial embryonic period each type of blood cell has its own particular stem cell (polyphylytism) instead of being derived from multi-

potent mesenchymal cells (unitarism). The results described above are in essential agreement with this point of view, and additional support has been obtained from long-term observations of heterologous radiation chimeras. Among these, some rare cases were encountered in which the erythrocytes were all of the mouse type, whereas the granulocytes were all of rat origin, this peculiar state being maintained for many months (101).

The *in vitro* culture of hematopoietic cells and the subsequent study of their therapeutic action have served a dual purpose. Firstly, an evaluation has been made of the possibilities of this technique in the production and the preservation of bone marrow. In the second place, it has been possible to investigate the therapeutic effect of primitive mesenchymal cells, since a rapid dedifferentiation usually occurs in bone marrow and spleen cultures. The results indicate that dedifferentiation is accompanied by loss of therapeutic activity (112, 113), which is consistent with the observations discussed earlier. The most favorable results from the point of view of the preservation of protective activity were reported by Billen (114), who maintained therapeutically active cells for 24 days *in vitro*. Under the conditions of the latter experiments no cell proliferation was observed in the cultures, so that the procedure merely allowed a number of cells to survive.

The problem of storage of hematopoietic tissue with preservation of its protective activity has become of practical importance since bone marrow transplantation is being attempted in human patients. In 1955 Barnes and Loutit (75) succeeded in keeping spleen at -70°C for 80 days without appreciable loss of activity, by freezing the tissue in glycerol according to the method of Polge, Smith, and Parkes.

Thomas *et al.* (98) have applied the same method to human bone marrow, but their results were difficult to evaluate. Mouse bone marrow which was stored in a similar way was found to be therapeutically active after storage for 6 weeks. At a temperature between 2° and 4°C mouse bone marrow could be preserved in Tyrode solution (115).

The quantity of hematopoietic tissue required for the successful treatment of irradiated animals varies with the type of tissue, with the immunogenetic relation between the recipient and the donor and with the method of transplantation. Unfortunately, quantitative work involving viable cell counts has been limited. Moreover, a comparison of the data published by the various groups of investigators is rather difficult because of differences in irradiation techniques, in the preparations of cell suspensions, etc. In some cases the degree of genetic homogeneity of the animals has not been defined, leaving the reader at a loss whether to classify the combinations as isologous or as homologous ones.

Van Bekkum *et al.* (92, 101) have published detailed quantitative in-

formation which permits a comparison of the therapeutic activity of various hematopoietic tissues in a number of host-donor combinations. These authors have counted the numbers of nucleated eosin-resistant cells that were injected. Jacobson *et al.* (53) performed nucleated cell counts in 2% acetic acid solution, but their data involve only one host-donor combination. Other authors express the size of the graft in spleen or femur equivalents, which permit a rough evaluation only of the number of cells administered.

The majority of the investigators used 30-day survival rates to evaluate the therapeutic effect of the grafted tissues. Since under the experimental conditions employed in most investigations 30-day survival is dependent on a proliferation of the transplanted cells, this method of screening can be considered to be adequate.

d. Spleen Implantation. After a uniformly lethal dose of whole-body X-irradiation (1025 r) Jacobson (65) obtained 38% survival in CF₁ mice by the intraperitoneal implantation of four spleens of CF₁ donors 1 to 22 days old. No protection was observed when two spleens only were implanted. With donors 4 to 5 weeks old, two spleens caused 45% of the treated mice to survive. The CF₁ mice are presumably fairly homogeneous, but the exact degree of inbreeding has not been stated.

Isologous spleen was found to be effective on intraperitoneal implantation into irradiated inbred CBA mice (J. A. G. Davids, O. Vos, and D. W. Bekkum, unpublished data). Negative results were obtained by Rudali *et al.* (116) with spleen transplants in two inbred strains of mice, but the degree of inbreeding was again not specified. In four incompletely inbred mouse strains van Bekkum failed to observe any therapeutic effect from intrastrain spleen transplants (117).

These results indicate that the therapeutic implantation of intact spleen tissue is moderately protective only in the case of isologous host-donor combinations.

Jacobson *et al.* (106) have published some preliminary results which seem to be at variance with this conclusion. These authors implanted mouse spleen into the intraperitoneal cavity of irradiated rabbits which resulted in an increased survival. Some of the spleen grafts were found to be vascularized and to show cell proliferation as well. The report does not contain information concerning the origin of the regenerated hematopoietic tissues of the surviving rabbits.

e. Suspensions of Spleen Cells. The relation between the number of intravenously injected isologous spleen cells and the survival of lethally irradiated CF₁ mice has been investigated by Jacobson *et al.* (53). The results seem to indicate that the protective activity decreases with the age of the spleen donors, but no distinct relation between the number of cells

and the therapeutic effect emerged. The protection did not exceed 80% in any of the experiments, and the variation of the results was considerable. The fact that a mortality peak occurred on the sixth postirradiation day is suspicious of the presence of a complication of the bone marrow syndrome (e.g., early death due to an infection) in these experiments, which may have influenced the results.

About 100% protection has been obtained by Barnes and Loutit (57) by the intravenous administration of $\frac{1}{10}$ equivalent of isologous spleen (about 5×10^6 cells) in irradiated CBA mice.

Between 1 and 4×10^6 isologous spleen cells were found to protect 80 to 100% of irradiated C57BL mice when the intravenous route of administration was employed (110). After receiving 50×10^6 homologous spleen cells intravenously, 9 out of 16 lethally irradiated CBA mice survived for 30 days postirradiation, but all died soon afterward (118). This secondary mortality is the usual complication of homologous and heterologous bone marrow transplantation. The spleens were derived from donors a few days old, which may be of significance in view of observations that foreign lymphoid cells from adult donors may kill irradiated mice within a short period, even when foreign bone marrow is administered at the same time (119, 120). There have been no other reports in the literature of protection obtained with homologous spleen cells.

A striking reduction of mortality from 95% in untreated rabbits to 25% in treated animals was observed by Jacobson *et al.* (106) as a result of the intravenous injection of 25 to 30×10^6 spleen cells from newborn mice. It is noteworthy that the white blood counts did not return to normal any faster in the treated animals than in the few surviving controls. It has not been possible to find cells of mouse origin in the hematopoietic tissues of the treated rabbits (oral communication). In addition no secondary mortality was observed. In the light of these observations the reviewer is tempted to speculate that a temporary proliferation of mouse cells has occurred, the duration of which having been sufficient to allow for a recovery of the host's hematopoiesis. It may of course be argued that the above results provide some evidence in favor of a humoral factor, but, since cell-free extracts were certainly not involved, this conclusion seems a bit presumptuous. In any case these experiments are in need of a careful re-investigation, the more so because the radiosensitivity of rabbits is subject to considerable variation.

f. Bone Marrow Cell Suspensions. Quantitative investigations on the therapeutic effect of bone marrow suspensions have been carried out with isologous, homologous, and heterologous host-donor combinations. In CF₁ mice optimal results were reported by Jacobson *et al.* (53) with 5 to 10×10^6 isologous nucleated cells. Urso and Congdon (121) obtained 30 to 50%

protection in CF₁ mice using 6×10^5 isologous nucleated cells, whereas van Bekkum and Vos (101) needed only 10^5 isologous viable nucleated cells to obtain 100% protection in irradiated CBA mice. All these data refer to intravenous administration. The lower figure reported by the last-mentioned authors is not significantly biased by their exclusion of dead (eosin-permeable) cells because these never exceeded 15% of the total number of nucleated cells. The rather large number of cells required by Jacobson *et al.* may indicate some inhomogeneity of their CF₁ mice, but this assumption rests on mere speculation.

A minimal degree of protection has been obtained with as few as 50,000 isologous bone marrow cells (101). If one considers that the majority of these are mature differentiated cells, the true number of stem cells whose descendants proliferate to form a complete hematopoietic system must be amazingly small.

Using inbred Wistar rats and a dose of 650 r (LD₉₅), van Bekkum and Vos (101) observed about 20% protection with 5×10^5 isologous bone marrow cells. As many as 10^8 cells were required to give complete protection. In irradiated (700 r = LD₁₀₀) Sprague-Dawley rats therapeutic effects amounting to 70% recovery were reported by Fishler *et al.* (58) with isologous bone marrow cells, the number of which was not estimated. After 800 r isologous bone marrow had no effect on survival (58, 122), which failure may have been due to the preponderance of the intestinal damage in the irradiated rats.

Van Bekkum and Vos (101) have investigated the quantitative aspects of treatment with isologous, homologous, and heterologous bone marrow using CBA, C57BL and (CBA \times C57BL) F₁ mice and inbred WAG rats. Their results have been discussed earlier and are illustrated in Fig. 5.

g. The Irradiation. The majority of successful bone marrow transplantsations have been performed in animals subjected to whole-body irradiation with a dose exceeding the LD₁₀₀. After doses which cause the intestinal syndrome, the administration of bone marrow obviously no longer affords protection.

In the case of the foreign bone marrow, but not with isologous cells, there is a lower limit to the irradiation dose after which transplantation and consequent recovery may be obtained. It has been noted by several investigators (104, 123, 124) that the injection of foreign bone marrow is ineffective and sometimes even harmful after irradiations in the mid-lethal dose region (the so-called MLD effect, see Fig. 7). Congdon *et al.* (125) have shown that under these circumstances the usual intense proliferation of donor cells takes place initially, followed by a sudden destruction of the hematopoietic graft around the seventh day after transplantation and subsequent death of the animals. There can be no doubt that this course of

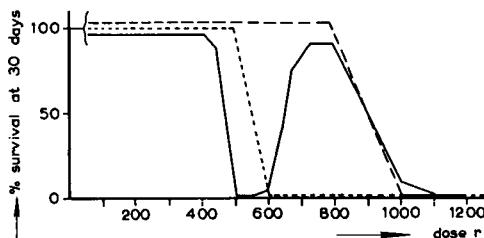


FIG. 7. The MLD effect. The results of bone marrow transplantation after various doses of total-body irradiation in mice (104). - - - no bone marrow. - - isologous bone marrow. — homologous and heterologous bone marrow.

events is the result of an early recovery of the host's immunological defense system, and it may be compared as such to a transplantation reaction. This interpretation is consistent with the observation that the MLD effect is absent in irradiated F₁ hybrids after treatment with parent strain bone marrow, whereas it is apparent, though to a lesser degree, in the reverse combination (126). It is of interest that Cole failed to observe the MLD effect in (L × A) F₁ mice which received Sprague-Dawley rat bone marrow (127). No explanation can be given at present for this discrepancy, but Cole's findings necessitate additional experiments concerning the occurrence of the MLD effect in other mouse strains and especially in F₁ hybrids.

As matters stand at present the lymphoid cells are considered to be responsible for the immunological reactions in general. Since the destruction of the lymphoid tissues is considerable even after a midlethal dose of irradiation, one is forced to presume that a comparatively small number of surviving cells may be able to cause a rejection of the foreign bone marrow graft. This has been borne out by the following experiments (101, 104). Mice were subjected to a lethal dose of whole-body irradiation and injected with a number of heterologous bone marrow cells which was known to afford maximal protection. In addition varying numbers of *isologous* lymph node cells were given intravenously. As few as 4×10^5 lymph node cells were sufficient to abolish the therapeutic effect of the rat bone marrow. A similar effect was obtained with 8×10^5 isologous thymus cells. Although these observations point to an excellent transplantability of lymphoid cells in irradiated recipients, they also stress the capacity of mouse thymus cells to produce an immunological reaction.

Few investigators have attempted bone marrow transplantation after irradiation with other kinds of ionizing radiations besides X-rays. Satisfactory protection was reported with isologous bone marrow after a lethal dose of irradiation in mice (128). In the same paper Lorenz and Congdon described a beneficial effect of bone marrow transplantation in mice which had received a lethal dose of radon. Hematopoietic cells seem to be less

effective in the protection of animals irradiated with fast neutrons (129-131). There is some evidence that the damage to the intestinal mucosa is relatively more severe after irradiation with neutrons, which would explain the failure of bone marrow treatment.

4. EXPERIMENTAL AND CLINICAL APPLICATIONS OF BONE MARROW TRANSPLANTATION

It is scarcely two years since the recognition of the transplantability of hematopoietic tissue in irradiated animals, and already a great number of applications, among them clinical ones, have appeared in the literature. Mention should be made of the work of Hollcroft *et al.* (132), who investigated the possibilities of this procedure as an aid to experimental radiation therapy as early as 1953—that is, several years before the mechanism of the therapeutic effect of bone marrow had been elucidated. They obtained an increased survival time of leukemic guinea pigs by whole-body irradiation with large doses followed by bone marrow injections. At the same time they investigated the effect of heavy irradiation of localized tumors in combination with shielding of a portion of the bone marrow. The results of this treatment were not encouraging.

The ideas advanced by Hollcroft *et al.* have been extended by other workers and may be summarized as follows: In the treatment of malignant diseases it would be possible to administer much larger and even lethal doses of whole-body irradiation or cytotoxic drugs if the ensuing bone marrow aplasia could be alleviated by bone marrow transplantation. In fact lethal effects of Myleran, CB1348 and TEM have been successfully counteracted in mice by the administration of bone marrow (133-135). In the case of malignant reticuloses, e.g., leukemia, a complete destruction of the malignant cells might be attempted, followed by a replacement of the destroyed hematopoietic cells with normal donor cells. It is even conceivable that an actively proliferating hematopoietic graft would contribute to the final elimination of any surviving malignant cells. In addition it has been suggested that the production of antibodies directed against the malignant host cells by the graft might be of value in the cure of the malignant process.

So far most of the work on tumor treatment has yielded results that are rather disappointing. In view of the complex experimental setup and the necessity of using transplantable tumors, which are usually exceedingly resistant to radiation, these failures are to be expected. A list of the results published so far is represented in Table III. The transplantation of homologous bone marrow after irradiation has resulted in the death of the animals from secondary disease. In many of these mice the tumor growth was found to be successfully inhibited. Barnes and Loutit (136) suggested

TABLE III
SUMMARY OF MAIN FINDINGS IN RECENT WORK ON EXPERIMENTAL TREATMENT OF TUMORS BY WHOLE-BODY IRRADIATION AND BONE MARROW TRANSPLANTATION

Authors	Tumor	Interval between tumor inoculation and treatment	Treatment	Bone marrow donor	Results
Barnes <i>et al.</i> , 1956 (99)	Transplantable leukemia in mice	7 days	1500 rads. X-rays in 25 hours (LD_{50}) 950 rads. in 14 minutes (LD_{50}) 950 rads. X-rays (LD_{98})	Isologous Isologous Homologous (also from donors previously immunized against the tumor)	Prolongation of life, in many cases longer than 3 months, 100% death in about 1 month All animals died from secondary disease, some without evidence of tumor Many survivals at 190 days
Barnes and Louitt, 1957 (136)	Transplantable leukemia in mice	7 days			
Trentin, 1957 (137)	Gardner mouse lymphosarcoma	0-8 days	880 r X-rays	Isologous	
Ambrus, 1958 (138)	Spontaneous and transplanted leukemia in mice	—	Lethal dose of X-rays	Isologous	30-day survivals in spontaneous leukemia when irradiated under special conditions
Cole and Ellis, 1958 (139)	Transplantable mouse myeloid leukemia	16 hours	X-rays, 700 r or 870 r	Homologous	All mice died 8-20 days after irradiation; no gross evidence of leukemia
Maddock and Djerassi 1958 (140)	Five different transplantable tumors	24 hours	X-rays 600-800 r	Isologous	Increased survival time due to irradiation; more extensive growth of tumor in bone marrow-protected mice
Schwartz, 1958 (141)	Transplantable mouse lymphoma	—	X-rays, single and fractionated exposures 800 r ($LD_{100} \cdot 700$ r) X-rays	Isologous Isologous lymphoid cells	Prolongation of survival; few long-term remissions
de Vries and Vos, 1958 (142)	Transplantable mouse lymphoma	4-6 days			Significant number of long-term survivals
Mathé and Bernard, 1958 (143)	Spontaneous mouse leukemia	—	600-1100 r	Homologous Heterologous Homologous bone marrow or embryonal liver cells	No long-term survivals Prolongation of survival; some long-term survivals

that this might be due to the general wasting of the animals, but de Vries and Vos (142) produced evidence in favor of a direct effect of the grafts' immunological reaction against the malignant cells. In addition the latter workers obtained a survival exceeding 3 months in a number of animals by injecting large numbers of isologous lymph node cells in combination with isologous bone marrow into irradiated (CBA \times C57BL) F₁ mice carrying a transplantable C57BL lymphosarcoma. It could not yet be established whether this effect is due to a competition between normal and malignant lymphoid cells or to the presence of an antigenic difference between the tumor and the host.

Much additional experimentation is clearly needed before a proper evaluation of the possibilities of bone marrow transplantation in the treatment of malignancies in human subjects can be made. Nevertheless clinical trials are already underway, and a few preliminary reports have appeared (98, 144, 145).

It is perhaps too much to hope that a permanent complete replacement of the hematopoietic tissue by homologous cells will ever be feasible in man because of the dangers involved in heavy whole-body irradiation and the as-yet-unknown effects of immunological reactions. But in many cases even a temporary proliferation of donor cells could conceivably be life-saving. Heller and Yakulis (105) have described the case of a man with Hodgkins disease whose marrow had become aplastic after the administration of Tetramine. The intravenous injection of pooled marrow from four donors was followed by recovery, and large numbers of donor-type erythrocytes could be detected in the patient until 98 days after the transplantation. Thereafter the donor-type cells were gradually displaced by the patient's own erythrocytes. Although this case cannot be completely evaluated because of previous blood transfusions, there is reason to assume that the bone marrow transplantation has prevented the patient's death from pancytopenia.

In mice Russell *et al.* (146) have successfully treated hereditary anemia by whole-body irradiation followed by transplantation of isologous bone marrow from normal mice. The treated mice maintained stable relatively high hematocrit levels for at least 160 days, which strongly suggest a successful transplantation of the normal marrow.

Another application of bone marrow transplantation seems to present itself in cases where the transplantation of a vital organ, e.g., a kidney, is required. This approach is based on the consideration that the transplantation of bone marrow into irradiated animals leads not only to a replacement of the host's hematopoietic system by donor-type cells but to a similar replacement of the host's immunological defense system as well. Strong support for this concept comes from the results of skin transplantsations

in radiation chimeras. Several investigators (79, 147, 148) have shown that permanent growth of bone marrow donor-type skin occurs in homologous radiation chimeras, and Brocades Zaalberg *et al.* (149) have even succeeded in transplanting rat skin into mice which carried functional rat bone marrow. These positive results disprove the postulate which claims a biochemical incompatibility to be partially responsible for the failure of heterologous skin grafts and stresses the importance of immunological factors in tissue transplantation. The question whether the tolerance of radiation chimeras toward bone marrow donor-type skin has to be attributed to a general or specific immunological paralysis or merely to the absence of any host-type immunological response will be dealt with in the next paragraph.

The possibilities suggested by the results of skin grafting are evident. There is every reason to expect that other organs, e.g., kidneys, may be transplanted as successfully as skin. At present, however, no reports of such attempts in animals are available, probably because this type of surgical technique is extremely difficult to perform in the mouse and because the production of radiation chimeras in the larger animal species has not been studied sufficiently. The application of these techniques in the treatment of human patients may hardly seem to be justified because it involves a whole-body irradiation with a lethal dose of X- or γ -rays. Nevertheless this treatment has been attempted in at least two patients who had lost all functional kidney tissue, and, although their lives could unfortunately not be saved, it has been reported that the whole-body irradiation added less to the patient's suffering than had been expected (150). It is the author's opinion that experimental work with primates, which are more suitable for clinical observation than rodents, is urgently needed, because the results will certainly decrease the necessity for clinical experimentation.

5. COMPLICATIONS OF FOREIGN BONE MARROW TRANSPLANTATION; THE SECONDARY DISEASE

The clinical exploration of the various possibilities of bone marrow transplantation is being severely restricted by the fact that homologous donors are virtually the only available source of bone marrow. Autologous bone marrow may be employed in specific cases, e.g., when a substantial portion of the hematopoietic tissue is free of the disease under treatment, and this procedure is being investigated in at least one center. Isologous cells, which would be the material of choice in nearly all cases, are available only in those rare instances where the patient has a healthy identical twin.

On the basis of the mouse data provided by van Bekkum *et al.* (101), and with body weight ratios taken as the extrapolation factors, the number of isologous bone marrow cells required to treat a patient of 60 kg would

be between 3×10^8 and 10^9 . Since an adult's rib may furnish between 1 and 2×10^9 hematopoietic cells, the isologous bone marrow transplantation seems to be entirely feasible.

The great majority of cases, however, will have to resort to homologous bone marrow only. Since experience with homologous bone marrow transplantation is almost entirely restricted to animals, the various complications so far encountered have to be taken into full consideration. Two of these have already been discussed and will be mentioned only briefly here.

The MLD effect which has been observed in mice warrants considerable caution in the administration of homologous bone marrow to sublethally irradiated patients. Since the precise value of the LD₁₀₀ in man is not known, some risk remains even after dosages as high as 600 r. In the treatment of victims of radiation accidents who have received a dose of radiation which is supposed to be lethal, these considerations will scarcely be an absolute contraindication, since no other effective treatment is available to them.

There are some indications which suggest that the MLD effect in mice is less outspoken if the bone marrow has been injected 24 hours after the irradiation. A possible explanation of this phenomenon is provided by earlier observations of Williams *et al.* (95), who found a decreased antibody formation to soluble antigens when the challenge was performed 24 hours after irradiation as compared to immediately afterward.

The second difficulty involved in homologous bone marrow transplantation is the requirement for enormous numbers of hematopoietic cells. Extrapolations from mouse data indicate that about 4×10^{10} cells will be needed for optimal protection of man. The procurement of these numbers from one single donor, though not impossible, imposes serious problems of organization. The reader is referred to published discussions on these subjects (145).

The third and, up to now, by far the most serious complication of foreign bone marrow transplantation is the secondary disease. Its occurrence has been acknowledged by all workers in this field, and the disease has been called foreign bone marrow disease or homologous disease. The present author prefers the term secondary disease for reasons which will become clear in the course of this treatise.

The first symptoms of secondary disease appear after the period of radiation mortality—that is, usually during the fourth or fifth week after foreign bone marrow transplantation. They consist chiefly in emaciation, diarrhea, and skin lesions. The weight loss occurs after a recovery of the post-irradiation decrease of body weight and therefore rightly deserves the designation secondary. The secondary weight loss is accompanied by

the excretion of unusually large amounts of loose wet feces, the color of which is lighter than normal. This symptom is generally called diarrhea. One or two weeks after the start of these symptoms the animals begin to die, showing extreme emaciation. The peak of this secondary mortality lies between the thirtieth and the seventieth days after irradiation. The mortality may amount to 100 %. When it is less, the diarrhea has usually subsided by day 100, and few deaths occur afterward. Another characteristic symptom of the secondary disease is localized in the skin. The irradiation-induced graying of the hair is delayed, and the fur shows a ruffled appearance. A variable number of animals show a localized or generalized loss of hair, and the exposed skin may develop abrasions and ulcers. These skin lesions have been found to persist after day 100.

Some confusion has arisen as to the typical pathological changes associated with the secondary disease. This is due to the variation of both nature and severity of the symptoms between different host-donor combinations. Moreover, during the early part of the period of secondary disease, especially in those experiments in which the irradiation dose was rather low, a number of animals may show a delayed type of bone marrow graft rejection. The latter reaction is most outspoken in the so-called MLD effect, but even after lethal doses of X-rays some heterologous chimeras have been found to die with a total aplasia of the bone marrow which occurs chiefly during the third and fourth weeks postirradiation (151). This delayed bone marrow aplasia should be sharply distinguished, however, from the secondary disease. After higher doses of irradiation the former syndrome is virtually absent, and the incidence of secondary disease is at least as great as after low lethal doses. With this in mind, we find the typical pathological changes of secondary disease to be colitis, emaciation, dermatitis, extensive atrophy of the lymphatic tissues, a high incidence of local inflammatory processes, and late hepatic necrosis.

The pathogenesis of the secondary disease has been subject to a great deal of discussion. Barnes and Loutit (118) were first to point out that the disease occurs only after the transplantation of homologous or heterologous bone marrow, and they suggested an immunological reaction between the host and the graft as the underlying cause (152). This idea has been generally accepted, although serious controversy still exists concerning the question whether a host-versus-graft or a graft-versus-host reaction is the main causative mechanism.

The possibility of the foreign bone marrow graft's being able to react immunologically against the host's antigens was suggested by the following observations: Firstly, evidence has accumulated to show that in radiation chimeras the lymphoid tissues are also repopulated by donor-type cells. Secondly, the results of skin graft experiments have indicated that the immunogenetic nature of the bone marrow donor determines the toler-

ance to foreign skin. Finally, Weyzen and Vos (153) have demonstrated the presence of rat proteins in the γ -globulin fraction of the serum of mice carrying a functional rat bone marrow graft. This finding was confirmed by Grabar *et al.* (154) and is suggestive of an antibody production by the grafted cells.

Several authors have attempted to obtain definite proof of a detrimental graft-versus-host reaction by employing an immunogenetic approach. It should be recalled that Snell demonstrated that the fate of a tissue graft is determined by a number of dominant genes called histocompatibility genes (155). Differences in these histocompatibility genes between donor and recipient may result in a rejection of the graft. The F_1 hybrid possesses the alleles of both the parent strains and will consequently not be able to produce antibodies against tissues of either of the two parent strains. Conversely, the parent strain host will produce antibodies against the F_1 hybrid graft. It is obvious that the use of inbred strains and their F_1 hybrids in various host-donor combinations is theoretically the method of choice to distinguish between a host-versus-graft and a graft-versus-host reaction (Fig. 8).

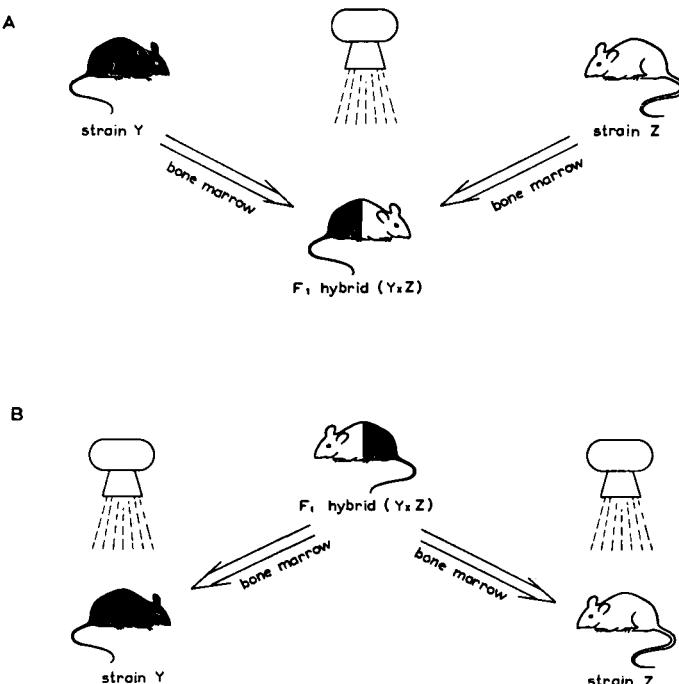


FIG. 8. Schematic representation of bone marrow transplantation experiments with F_1 hybrids and parent strain mice. *A*, F_1 hybrid host-parent strain graft. *B*, parent strain host- F_1 hybrid graft.

Unfortunately, conflicting results were obtained by the three investigators who have employed this procedure. Van Bekkum *et al.* (101) reported the absence of secondary mortality both in CBA and C57BL mice receiving (CBA \times C57BL) F₁ bone marrow as well as in the reverse combinations. Uphoff (156) found no secondary disease in F₁ hybrids after isologous bone marrow transplantation and a high incidence of secondary disease and mortality after the transplantation of parent strain bone marrow. In the one exception where parent strain marrow was equally harmless as isologous marrow, both parent strains had an identical H₂ locus. In a subsequent paper Uphoff and Law (157) showed that differences in a single histocompatibility gene (H₂) between the donor and the recipient provide sufficient basis for a severe form of secondary disease. These results are extremely important in providing evidence in favor of a graft-versus-host reaction as the cause of secondary mortality. The same authors did not, however, present evidence of an equally convincing nature to show that secondary mortality is absent in irradiated parent strain mice which receive F₁ hybrid bone marrow. Their data contain only one group of this particular combination, which showed 24% mortality between the thirtieth and sixtieth postirradiation days compared to 26% mortality during the same period in a group receiving treatment with isologous bone marrow. In any case this mortality was considerably lower than after homologous bone marrow transplantation. Furthermore the authors state specifically that the mice treated with isologous and F₁ hybrid marrow showed no signs of secondary disease.

Trentin (123) reported the occurrence of secondary mortality in F₁ hybrids treated with parent strain bone marrow. His data show a great variation, however, in secondary mortality between comparable experimental groups as well as an incidence of secondary mortality in parent strain mice receiving F₁ hybrid marrow, which is difficult to ignore. Nevertheless, Trentin concluded that the incidence of secondary mortality was higher in F₁ hybrids treated with parent strain marrow than in the reverse combination.

These partially conflicting results have been explained by van Bekkum *et al.* (126, 158), who obtained evidence in favor of the hypothesis that secondary mortality is caused by the combined effects of a graft-versus-host immunological reaction and late radiation damage. The latter factor was suggested by their observation that secondary mortality occurs after isologous bone marrow transplantation when the dose of irradiation is sufficiently increased. It is noteworthy that these animals showed no typical symptoms of secondary disease, except for a slight diarrhea. In CBA mice up to 70% mortality occurred after the thirtieth day following 950 r and treatment with isologous bone marrow, but no secondary mor-

tality was observed in similarly treated CBA mice subjected to 675 r. Both Trentin and Uphoff reported a variable incidence of secondary mortality in isologous bone marrow-treated mice, but they did not discuss this phenomenon to any extent.

By increasing the X-ray dose, extensive diarrhea and some secondary mortality was induced by van Bekkum *et al.* in their F₁ hybrid mice when parent strain marrow was used for treatment, but no detrimental effects were observed after the injection of isologous marrow. It should be recalled that these authors found no secondary disease in the same combination after a lower lethal dose of X-rays (700 r).

Additional data to support the participation of a graft-versus-host reaction came from a number of investigators who showed detrimental effects of homologous spleen and lymph node cells administered to irradiated mice (120, 159, 160). When large numbers of lymphoid cells are injected the mice die within 8 days, but after the injection of smaller numbers death occurs later and some mice develop symptoms resembling secondary disease, with the exception of diarrhea. No such effects could be produced in irradiated parent strain mice by the administration of large numbers of F₁ hybrid lymphoid cells. Similarly, symptoms resembling secondary disease, as well as mortality, were obtained both in sublethally irradiated and in nonirradiated F₁ hybrid mice after the administration of parent strain lymphoid cells (120, 158, 161).

It was also found that the majority of F₁ hybrids died in the course of 4 months after their parabiotic union with a parent strain mouse. The F₁ hybrids showed gradual emaciation, and at autopsy a severe atrophy of the lymphoid tissues was found. Some hybrids developed characteristic skin lesions (120, 158).

The multitude of experimental data which have been discussed in the foregoing pages permit the following tentative conclusion concerning the pathogenesis of secondary disease: The diarrhea is most likely the result of a combination of late irradiation damage to the large intestine and a graft-versus-host reaction. The skin lesions have been observed in every situation where a graft-versus-host immunological reaction has been evoked, and radiation is not requisite for its development. Secondary mortality may be induced by each of the two factors (late radiation damage and the immunological reaction), either acting alone or in combination. It is probable that a decreased immunological reactivity of the chimeras constitutes a third factor which leads to a deterioration of the animal's condition. This would explain the rather high incidence of infections during the period of secondary disease. This assumption rests on the decreased reactivity of radiation chimeras toward skin transplants which are normally incompatible to both the donor and the host (149), on the finding of atrophy

of the lymphoid system (151, 162) and on the observation of beneficial effects of the feeding of antibiotics during the period of secondary disease (163).

It follows from the explanation presented above that the unfavorable long-term effects of homologous bone marrow transplantation *as compared to treatment with isologous bone marrow* are due mainly to an immunological reaction of the graft versus the host. The majority of investigators in this field are in essential agreement on this point. This does not imply, however, that a host-versus-graft reaction is completely absent under these conditions. On the contrary, there are some indications that such a reaction does occur, but it does not in itself contribute significantly to the secondary mortality.

Essentially the opposite point of view is being held by Congdon and Makinodan and their co-workers. Briefly, they consider the secondary disease which follows foreign bone marrow transplantation to be the result of a delayed host-versus-graft reaction. According to Makinodan (164), the delay of the immunological response of the host is caused by the lethal dose of irradiation. This would mean that there is no basic difference between the MLD effect and the secondary mortality. It is difficult to accept this argument, because the condition of the foreign bone marrow is excellent in the majority of the mice dying from secondary disease, contrary to the pathological changes which accompany the MLD type of mortality. In the latter case an acute degeneration of the grafted bone marrow has been consistently found. Among the other arguments which were put forward by Makinodan *et al.* in defense of the host-versus-graft hypothesis are their failure to detect circulating antibodies against host erythrocytes in mice carrying rat bone marrow and their failure to confirm the observations of Weyzen and Vos (153) and Grabar *et al.* (154) concerning the presence of rat proteins in the γ -globulin fraction of the serum of rat-mouse chimeras. The generalized atrophy of the lymphoid organs in mice suffering from secondary disease has been attributed by Congdon and Urso (162) to exhaustion, resulting from an overproduction of antibodies directed against the graft. This interpretation neglects the available evidence showing that these atrophic lymphoid tissues are populated by donor-type cells.

None of the arguments discussed above provides direct evidence for a host-versus-graft mechanism as the primary cause of secondary disease and secondary mortality, and the standpoint of Makinodan, Congdon, and co-workers seems no longer acceptable in the light of the experimental results discussed in the preceding pages.

In considering the hazards of homologous bone marrow transplantation in man, it should be kept in mind that the secondary disease has been

investigated nearly exclusively in irradiated mice. Very little reliable information is as yet available on the long-term complications of foreign bone marrow transplantation in other species. No evidence of secondary disease was observed by Odell *et al.* (165) in their homologous rat chimeras, but it may be confidently assumed that in this case immunogenetic differences between the host and the donor rats were relatively small.

Porter and Murray (166) have reported late deaths in irradiated rabbits which carried homologous bone marrow. The findings of diarrhea and weight loss in the main group of delayed deaths (7 animals which died between the thirtieth and fortieth days postirradiation) agree with the observations made in mice. At autopsy the bone marrow appeared normal or hyperplastic and the lymphoid tissues showed extreme atrophy. The immediate cause of death was reported to be an infection, usually a diffuse bilateral bronchopneumonia. A relatively large proportion of the rabbits remained alive and well with the marrow graft intact for over 32 weeks after the irradiation. It seems, therefore, that secondary disease and subsequent mortality does occur in rabbits, but the incidence is lower than in most homologous mouse experiments. The rabbits were described by Porter as "not inbred in the genetic sense," which might suggest that a certain degree of homogeneity cannot be excluded. At least one case of delayed death was reported in a group of monkeys after irradiation and protection with homologous bone marrow (167). It is not known whether this monkey carried the foreign bone marrow.

Obviously these data do not allow any reliable prediction to be made concerning the incidence of secondary disease and mortality after homologous bone marrow transplantation in man. Under the present circumstances it appears necessary to consider this complication as great a hazard to man as to mice.

Some partially successful attempts have been made to preclude or combat the secondary disease in mice. The use of fetal liver and spleen in the treatment of irradiated mice has been reported to result in significantly less secondary mortality in some homologous host-donor combinations (168-170). These results agree with the idea of the secondary disease being due to an immunological reaction of the graft against the host, because fetal hematopoietic tissue is supposed to be less reactive immunologically than adult tissue. This had led some investigators to the rather premature conclusion that fetal tissue is the material of choice for treating human patients. Since very little is known of the protective potentialities of homologous fetal cells as compared to adult hematopoietic cells, the practical application of this idea may involve serious risks.

The continuous oral administration of aureomycin was found to be

beneficial in reducing the secondary mortality in mice (163). It is to be expected that additional efforts will be directed in the near future to the alleviation of these secondary complications.

C. STIMULATION OF HEMATOPOIETIC REGENERATION

For many years substances which might stimulate hematopoiesis have been of interest in the treatment of agranulocytosis, aplastic anemia, and other conditions associated with bone marrow failure. It is only logical that this group of agents should be tested in the postirradiation bone marrow syndrome. The effects of substances which are supposed to stimulate myelopoiesis will be reviewed first.

In 1952 a lipoprotein nucleic acid complex called "reticulose" was claimed to cause an accelerated recovery of the white cell counts in irradiated rabbits (171), but Smith *et al.* (172) failed to obtain a reduction of radiation lethality in mice and rats by postirradiation treatment with this substance. Other nucleic acid preparations were reported to stimulate myelopoietic recovery in sublethally irradiated rabbits (173) and to counteract leukopenia in irradiated patients (174). So far the mechanism of these effects on myelopoiesis has not been investigated.

The citrovorum factor and the leukocyte promoting factor, both logical therapeutic agents, were ineffective in reducing X-ray mortality in rats and mice, respectively (175, 176). In the experiments with citrovorum factor leukocyte counts were made as well, which did not show any effect of the treatment.

A slight beneficial influence on X-irradiated mice has been reported from treatment with batyl alcohol (*dl*- α -octadecylglycerol ether) by Edlund (177). The compound was injected subcutaneously in arachis oil on alternate days for 2 weeks postirradiation, resulting in a 30% decrease of mortality. Larger dosages of batyl alcohol increased the mortality. In view of the fact that the X-ray dose was only an LD₅₀, this protection is not very impressive. These experiments have been repeated under similar conditions by others (178) who were unable to confirm Edlund's findings. The effect of alkoxyglycerols on X-ray-induced leukopenia in patients was found to be variable (179, 180).

Although the therapeutic results obtained so far with batyl alcohol are rather disappointing, the investigations of Linman *et al.* (181) indicate that a further careful study of the potentialities of batyl alcohol may be worth while. The latter authors found batyl alcohol capable of stimulating erythropoiesis and thrombopoiesis in normal rats. The first-mentioned response seemed to be chiefly an acceleration of erythroblastic cellular divisions without associated hemoglobin synthesis. From similarities in chemical, physical, and physiological characteristics of batyl alcohol and

plasma erythropoietic factor the authors conclude that these two compounds may be closely related. Even if the beneficial effects of this type of compound after irradiation will not be impressive, work along these lines may bring some much needed understanding of the process of hematopoietic regeneration.

Atabrin, which seems to produce leukocytosis when administered in large doses to various animal species (182), has been the subject of a few therapeutic trials. Kelley *et al.* (183) studied the effect of the addition of atabrin to the diet starting in some experiments before and in others after the irradiation. The X-ray mortality in chicks, mice, and rats was not significantly altered, but the decrease of the white cell counts was less in the treated animals. The latter effect was also produced when atabrin administration was started after the irradiation. It is interesting that the X-ray-induced inhibition of feathering in chickens has been overcome by the addition of atabrin to the diet (184).

The effects of pre- and postirradiation injections of bacterial endotoxins have been the subject of a series of investigations by W. W. Smith and co-workers. The authors began to investigate substances which increase the resistance of mice to experimental infections. Since infection is one of the major complications of the bone marrow syndrome, it seemed possible that some protection might be obtained with these compounds. Some increase of survival was indeed observed after treating irradiated mice with inorganic particulate matter (185), a treatment which was known to increase the resistance to infection in normal mice. Following the report of Mefferd *et al.* (186), who claimed a slight beneficial effect of pretreatment with the bacterial pyrogen Piromen on radiation mortality of mice, Smith *et al.* (187) investigated the effects of endotoxins derived from three different gram-negative bacteria in ten strains of mice and in hamsters and rats. The preparations, which contained mainly lipopolysaccharide, were injected intraperitoneally either immediately after or 24 hours before irradiation. In many experiments a considerable decrease of mortality was obtained. In mice the results were better when the endotoxin was injected before the irradiation, but hamsters responded more favorably to treatment after the irradiation. These beneficial effects proved to be reproducible, but it was found that the time of injection is very important, which is clearly illustrated in Fig. 9. The authors also drew attention to considerable differences in the beneficial effects of endotoxin treatment between various animal species.

Initially Smith *et al.* (188) postulated that the effects of endotoxins in irradiated animals were primarily associated with a decreased susceptibility to infection. Subsequent careful studies of hematopoietic recovery in the treated animals has revealed, however, that the induction or stimulation

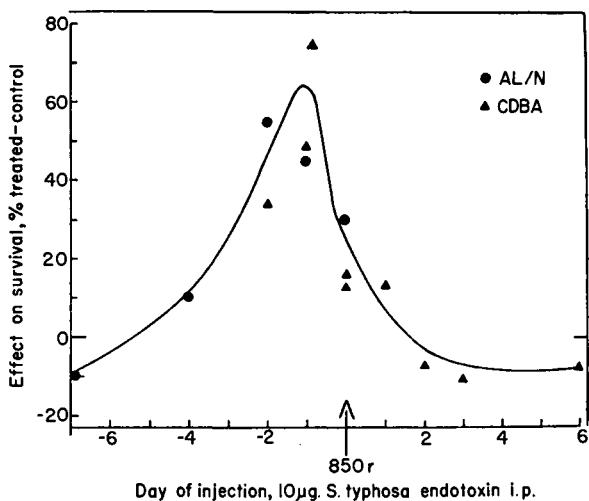


FIG. 9. The influence of time of injection relative to irradiation with 850 r on the beneficial effect of an endotoxin injection. From Smith *et al.* (187).

of hematopoietic regeneration is more likely to be the principal action of the endotoxins (189). In the treated animals the cellularity of the bone marrow was greater and spleen weights recovered earlier than in the nontreated controls. The mechanism by which endotoxins induce hemopoiesis remained obscure. At present the main point of interest appears to be whether endotoxin stimulates precursor cells directly or by way of some other reaction of the organism. It seems possible that the reduction of radiation mortality of rabbits and mice which has been produced by pretreatment with pyrogens and bacterial vaccines (186, 190) bears some relation to the effects of endotoxin. Here again the dosage schedules seem to be of critical importance to the outcome of the experiments. It is noteworthy that bacterial pyrogens have been reported to induce extramedullary hemopoiesis in rabbits (191).

Previously Smith *et al.* claimed that the postirradiation injection of particulate matter, e.g., ground glass and Portland cement, causes a significant increase of survival, but this treatment apparently did not influence marrow cellularity or the leukocyte count.

Another insoluble material which has been investigated with regard to radioprotection is zymosan, a preparation consisting of polysaccharides of the cell walls of yeast. The experiments with zymosan were initiated because of the finding by Pillemer and co-workers that treatment with this substance under certain conditions increased the properdin levels as well as the resistance against experimental infections (192). It was subse-

quently reported that injections of properdin before or after the irradiation increased survival of mice and rats (193). The degree of protection depended on the dose, the route of injection, and the time of administration. The protective action of zymosan could not be confirmed by Haley *et al.* (194), and the properdin investigations have not been published by Pillemer's group in sufficient detail so far. Several authors have reported a decrease of serum properdin levels after whole-body irradiation (193; S. Michaelson, personal communication). The interpretation of this observation remains uncertain because at present the significance of properdin levels with regard to nonspecific resistance against infectious organisms is far from clear.

A stimulation of the erythropoietic activity may influence radiosensitivity as well as recovery after irradiation. When bone marrow hyperplasia was induced by high-altitude exposure, rats were found to be more sensitive to the lethal effect of X-rays although the ensuing anemia was less pronounced (195). On the other hand, long-term acclimatization to high altitudes renders the animals more resistant (196). Another method to stimulate erythropoiesis consists in the administration of small amounts of cobalt salts. Pretreatment with cobalt for 5 and 8 days before the irradiation combined with administration of cobalt for 15 days subsequent to the irradiation increased the tolerance of mice to whole-body irradiation (197). No beneficial effects were obtained either by treatment with cobalt for 36 days before irradiation or for 15 days after irradiation, which indicates again that the dosage schedule is essential for the outcome of the experiments. Long-term cobalt feeding has been reported to increase radiation mortality in rats (198).

Rekers (199) infected rats with *Bartonella muris* and observed an increase of radioresistance at about 10 days after the infection. This effect may be related to the erythrocyte damage produced by the infective agent, which most likely evokes a stimulation of erythropoiesis. In irradiated dogs and rats erythropoiesis could be markedly stimulated by bleeding before or immediately after whole-body irradiation (200). The amount of blood removed by the bleeding amounted to 25% of the total volume. When the bleeding was performed at 24 hours after the irradiation, no effect on erythropoiesis resulted. Since a sublethal irradiation dose was employed, the effect of the bleeding on survival has not been studied.

The administration of folic acid after total-body irradiation did not influence the mortality and the blood picture of swine (201). Similarly Goldfeder *et al.* (202) failed to observe any influence of folic acid treatment on the hematopoietic system of irradiated mice, although a significant prolongation of the survival time was reported in the treated animals. In contrast Mücke *et al.* (203) have described a decrease in leukopenia in

irradiated mice after treatment with folic acid and vitamin B₁₂. It should be kept in mind that beneficial effects of vitamins may be expected in irradiated animals if the diets used during the preirradiation period are partially or wholly deficient in these particular factors.

The data gathered in this section are admittedly scattered, and no valid conclusions can be drawn from many of the reported experiments. They provide sufficient evidence, however, to indicate the possibility of accelerating recovery by stimulation of the blood-forming tissues. A further exploration of these possibilities is definitely needed, but a systematic approach depends on the advance of our knowledge of the factors which regulate the proliferative activity of the hematopoietic tissues.

D. EFFECTS OF CELL-FREE TISSUE EXTRACTS

For several years the therapeutic effect of hematopoietic tissue preparations was thought to be due to a humoral factor. As has been described in a previous section, this hypothesis has been definitely disproved. The various claims that cell-free extracts of hematopoietic tissue afford protection when administered after irradiation have been subjected to careful reinvestigation by several groups of workers (71, 72, 204, 205). The results were equivocal in showing that cell-free preparations were devoid of therapeutic activity and reversely that effective preparations contained large numbers of intact cells. In contrast, Ellinger (206, 207) has reported protection of guinea pigs against radiation death by cell-free mouse spleen extracts. The therapeutic effects in Ellinger's experiments were rather small, and the experimental conditions were such that a nonspecific influence on recovery is by no means excluded. It should be noted that the experimental conditions employed by Ellinger are extremely well standarized, so that even small effects on recovery can be recorded reproducibly. Several years ago the same author obtained increased survival by post-irradiation treatment with physiologic saline (208).

Panjevac *et al.* (209) have claimed an increased survival of irradiated rats as a result of treatment with rat nucleic acid preparations. Both RNA and DNA preparations from rat spleen and liver were found to have a slight therapeutic effect. Rabbit nucleic acids were devoid of activity. The data show that none of the X-ray doses employed was lethal to 100% of the untreated animals. The conditions required to demonstrate this effect seem to be rather critical, as in the case of treatment with bacterial endotoxins and particulate matter. In this connection it is of interest that Panjevac and co-workers used highly polymerized nucleic acids. Goldwasser and White (178) have described a slight therapeutic effect of a hog mucosa extract free of low molecular protein, but so far these results have not been confirmed by others.

In conclusion it may be stated that the various active extracts discussed in this section are weak therapeutics at best. At present the only reasonable explanation for the reported therapeutic effects seems to be that nonspecific effects are involved.

IV. Effects of Parabiosis

In 1951 Brecher and Cronkite (64) succeeded in protecting lethally irradiated rats by postirradiation parabiosis with a nonirradiated partner. They attributed this effect to a therapeutic factor which presumably entered the irradiated animal by way of the vascular junctions between the partners. The advances made in the field of bone marrow transplantation have provided a more plausible explanation, namely, that recovery is due to a seeding of normal hematopoietic cells from the nonirradiated partner. This explanation is in accord with the results of Binhammer *et al.*, who studied the effect of parabiosis at various time intervals before and after the irradiation (210). Optimal results were obtained when the parabiosis was effectuated within a few hours after irradiation and maintained until the tenth postirradiation day. If parabiosis was performed after a long interval, the results were less favorable, although some therapeutic effect remained when the interval was as long as 4 days (Fig. 10). Since the establishment of a vascular anastomosis between the partners takes probably 2 to 3 days, it would seem that the introduction of hematopoietic cells as late as 7 days postirradiation may save some of the irradiated animals. Obviously the parabiotic state is quite different from the condition achieved by bone marrow transplantation in single animals, because the

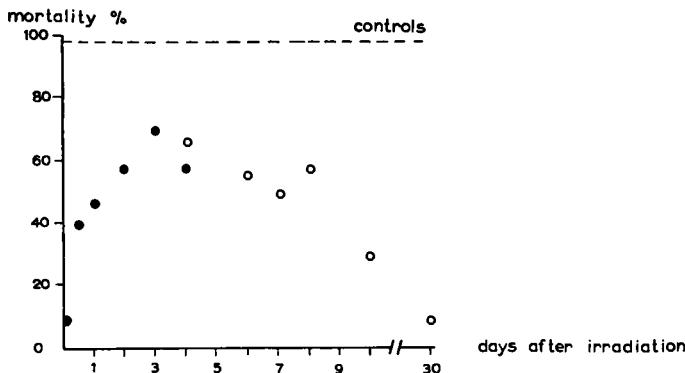


FIG. 10. The effect of parabiosis with a nonirradiated partner after total-body irradiation in rats. X-ray dose: $LD_{95(30)}$. The data are derived from Binhammer *et al.* (210). ● day of operation, parabiosis continued until the thirtieth postirradiation day. ○ end of parabiotic state; the parabiosis was effectuated immediately after irradiation.

irradiated twin partner may receive additional protection from the circulating peripheral blood cells and possibly from other factors derived from the nonirradiated partner.

A significant protection was also found when the parabiotic junction was established immediately after irradiation, after which the twins were separated on the fourth day. If the time required for the establishment of a vascular union is considered, this leaves only 1 or 2 days for the introduction of normal hematopoietic cells into the irradiated partner. This is in full agreement with Jacobson's results in spleen-shielded irradiated mice, in which a significant protection remained when the shielded spleen was removed as early as 5 minutes after the completion of the irradiation (65).

V. Hormones in the Treatment of Irradiated Animals

It is well known that the radiosensitivity of the whole organism as well as that of several tissues may be influenced by endocrine factors. Clinicians have noted that the radiosensitivity of the skin varies with sex and age. In females menstruation and pregnancy have been reported to decrease the tolerance of the skin. Various other endocrine disturbances seem to be associated with alterations in skin radiosensitivity (211).

A considerable number of investigations deals with the effects of the removal of endocrine glands on the survival of irradiated animals. In the case of the adrenals and the pituitary gland, deprival of these organs usually results in increased mortality, whereas substitution with the specific hormones completely or partially restores radioresistance.

This kind of data has added little to our understanding of the recovery process, since the profound metabolic disturbances resulting from the removal of these endocrine glands would be expected to decrease the animal's resistance to any kind of stress. The present discussion will be limited to the relatively few instances of an apparently specific influence of hormones on recovery from radiation damage. It should be kept in mind that many of the reported beneficial effects of hormones were obtained with dosages which greatly exceed those required for the induction of physiological responses. It is therefore conceivable that pharmacological or even toxic effects of the hormones were involved.

In view of the widespread cellular destruction and loss of proteins which have been observed after the irradiation, the trial of somatotrophic hormone (STH) in the treatment of irradiated animals seemed to be a sensible approach. Another justification was provided by the claim that STH stimulates hemopoiesis. Selye *et al.* (212) investigated the effects of two daily injections of 1.5 mg of STH in rats which received two X-ray doses of 670 r each, with an interval of 6 days. Survival was not influenced, but the body weights and the weights of thymus, spleen, liver, and adrenals were significantly greater in the surviving animals of the treated group.

In mice, STH similarly failed to reduce radiation mortality (213), even when the hormone was combined with streptomycin. Spellmann *et al.* (214) found small doses of STH to counteract the detrimental effect of daily injections of saline in irradiated mice, whereas large doses of STH enhanced mortality. Pretreatment for 2 weeks prior to irradiation decreased the mortality significantly. In swine exposed to an LD₈₀ the daily administration of 100 mg of STH increased the mortality to 100% (215). The treated animals died sooner and lost weight more rapidly than the controls. The pathological findings were essentially the same in both groups. Contrary to these negative results, Lacassagne and Tuchmann-Duplessis (216) reported a protective as well as a curative action of STH in irradiated rats. In the guinea pig STH increased radioresistance only when the treatment was given prior to irradiation. The doses of STH used by the French workers were reported to be almost lethal.

Although the range of hormone dosages and schedules which has been investigated seems to be rather incomplete, the results obtained so far with STH do not suggest that this treatment will have much therapeutic value.

Mention should be made of a specific action of gonadotropic hormone after irradiation. Anterior pituitary gonadotropin induces an accelerated recovery of the seminiferous epithelium of irradiated testes if injections are started at or shortly after irradiation and continued for several weeks (217). No restorative effect is shown by testosterone propionate on the X-rayed seminiferous epithelium, but the interstitial cells and the accessory sex organs are stimulated as usual.

Both ACTH and cortisone were found to be without therapeutic value or to increase radiation mortality (218-222). The latter effect may be related to the decreased resistance toward infections which follows treatment of normal animals with these hormones. In contrast, ACTH and cortisone seem to improve the condition of irradiated patients according to some authors (223, 224), which is probably due to the nonspecific action of ACTH to decrease the sense of sickness. After local irradiation cortisone treatment has been reported to reduce fibrosis of the lungs in one case (225) as well as ulceration and erythema of the skin in guinea pigs (226).

Deoxycorticosterone acetate reduces X-ray mortality when administered before irradiation, but a similar effect has been obtained when injections were started after the irradiation (227, 228). This hormone is effective only in the lower X-ray dose range and fails to influence the mortality after an LD₁₀₀.

The influence of thyroid hormones and of suppression of the thyroid gland activity has been discussed earlier in their relation to the metabolic state. No beneficial effects on recovery were encountered.

The results obtained with the sex hormones, in particular with estradiol,

deserve some more attention. As early as 1938, Lawrence and Gardner (229) studied the effect of pretreatment with estrogen for 9 to 11 days on radiation mortality in mice (LD_{85}). An intramuscular injection of 16.6 μ g of estradiol benzoate increased survival to 57 %. Additional results were reported in 1943 by Treadwell *et al.* (230). The beneficial effect of estradiol (0.08 mg intramuscularly) when administered before irradiation could be confirmed. In addition it was found that the injection of estradiol benzoate immediately after the irradiation caused an increase of mortality as well as a shortening of the survival time. These observations were confirmed and extended by Patt *et al.* in 1949 (231). They found that the schedule of administration of estradiol benzoate is critical. With an LD_{72} of X-rays in mice, single intramuscular injections (0.166 mg) given between 5 to 15 days prior to irradiation decreased mortality to about 30 %. No effect on mortality was obtained when the hormone was administered 25 or 35 days prior to irradiation. Postirradiation treatment increased mortality up to 100 %. Pretreatment with estrogen was found to increase adrenal weight by 35 % to 40 % at 4 days after irradiation, which suggested that the protection was mediated by the adrenal glands. This hypothesis was tested in a series of experiments with adrenalectomized mice, but the results did not support this idea. An investigation of the peripheral blood revealed that the dose of estradiol employed causes leukopenia in normal animals, with the maximal depression around 10 to 14 days after injection, which period coincides with the period of decreased radiosensitivity. After irradiation the leukocytes decrease to an equal extent in both estrogen-treated and control mice, but recovery occurs earlier in the former group. Estrogen-treated animals manifest a smaller decrease in erythrocyte counts and regain normal values faster. The effect of estradiol may be rather specific for irradiation because no protection was observed against toxic doses of nitrogen mustard. Pretreatment with progesterone (0.1 to 0.2 mg) or testosterone (0.1 to 1 mg) did not protect irradiated mice. A prophylactic as well as a therapeutic action was described for estradiol benzoate and for diethylstilbestrol in irradiated mice by Mirand *et al.* (232). They injected 0.1 mg daily for 7 days, and it is possible that the large total dose administered may account for the discrepancy with the negative results of postirradiation treatment obtained by others.

It has been known for many years that estrogens influence ossification. In 1934 Kyes and Potter (233) described variations of ossification in the bone marrow of female pigeons. The osseous modifications of the marrow were cyclic and paralleled functional changes of the ovary. The degree of ossification was found to be related to the size of the largest ovarian follicle. These observations were confirmed by Bloom *et al.* (234, 235), who noted in addition that the transitory bone formation takes place in the granulopoietic portion of the marrow, leading to an almost complete disappearance of

myelopoiesis. Erythropoiesis is not greatly affected. Furthermore hypercalcemia and ossification of the marrow could be induced in male pigeons by treatment with estradiol benzoate. In mice, estrogens increase the breaking strength of the long bones by increasing the degree of ossification (236). In young mice daily injections of 250 µg of diethylstilbestrol cause a marked reduction of the number of leukocytes in the peripheral blood (237) and a decrease in the ratio of spleen to body weight. The erythrocyte counts were not affected by the hormone treatment. In addition the estrogen produced a marked retardation in growth, but this does not sufficiently explain the severe leukopenia. Previously other workers have reported anemia in dogs after large doses of estrogens (238, 239).

In this respect it seems of interest that Rugh and Clugston (240) found female mice to be most radioresistant during the 24-hour period of estrus which was postulated to be due to the substantially higher secretion of estrogens during this period.

It can certainly not be said that the mechanism of the protective action of estrogens has been clarified, but there are strong suggestions that the effect is related to the variations of hematopoietic activity which follows the treatment with estrogens. There is no doubt that a significant protection can be obtained by pretreatment with these hormones.

The influence of the male sex hormones on radiation mortality has also been investigated. Negative results of pre- and postirradiation treatment with testosterone propionate were reported (231, 232, 241). In Spellman's experiments, however, testosterone cyclopentylpropionate decreased mortality of irradiated male mice when 0.1 mg was injected every 14 days after the irradiation (242). Still better results were obtained when an additional injection was given 10 to 14 days before the irradiation. It is not known whether these beneficial effects have to be ascribed to the anabolic action of the treatment. No studies are as yet available on the effect of steroid hormones with predominantly protein anabolic properties.

VI. Specific Therapy of the Gastrointestinal Syndrome

Symptoms associated with injury to the gastrointestinal tract may accompany the characteristic bone marrow syndrome of the acute radiation disease. In the case of whole-body irradiation the severity of these symptoms varies with the dose of irradiation and with the species. Supralethal doses are required in most species to produce a characteristic gastrointestinal syndrome. Early vomiting occurs in man, monkeys, and dogs; other species, e.g., rats, show anorexia immediately after the irradiation. This is followed by protracted diarrhea, which leads to dehydration and a disturbance of the electrolyte balance with death in 3 to 5 days. In mice the pattern of the disease is remarkably uniform in the dose range between 1000 and 10,000 r. There is some evidence that the small intestine is the

most sensitive part of the gastrointestinal tract and that fluid and electrolyte losses occur chiefly by way of this part of the gut. Conard (243) provided some indirect evidence suggesting that a relative resistance to acute gastrointestinal death might be related to the presence of a large colon, as, for instance, in guinea pigs and rabbits. Presumably reabsorption of fluids in the colon could counteract the losses which occur through the walls of the small intestine to a certain extent. In accordance with this idea are the observations of Swift and Taketa (244), who found that shielding of a small exteriorized section of the duodenum or ileum significantly decreases radiation mortality after a whole-body dose of 1000 r of X-rays in rats. Osborne (245) reported that removal of the irradiated intestine after local radiation of the abdomen had a beneficial effect.

Although some favorable influence, notably on the severity of the diarrhea, has been found as a result of treatment with antibiotics, the mortality has usually not been altered to a significant extent. It should be remembered that even a successful therapy of the gastrointestinal syndrome would not necessarily save the animal's life, since a few days after the critical period a full-blown bone marrow syndrome is likely to develop.

The results obtained with forced feeding of irradiated animals have been equally disappointing, as was pointed out elsewhere in this chapter.

The only really effective method of combating the acute intestinal syndrome seems to be by correcting the fluid and electrolyte balance. Conard *et al.* (246) have shown conclusively that the parenteral administration of balanced electrolyte solutions causes a dramatic improvement in the condition of dogs which suffered severely from diarrhea and vomiting after irradiation with 1300 to 1800 r. The mean survival time increased from 3.7 days in the controls to 7.2 days in the treated animals; some of the latter lived beyond the seventh day, at which time the diarrhea diminished. In the second week a morphologically complete regeneration of the mucosa of the small intestine was observed, but the functional repair was apparently not complete, since diarrhea and anorexia continued into the second week. The bone marrow of these dogs was extremely aplastic.

It would be of considerable importance if these results could be confirmed with other species. Furthermore, the question needs answering whether the subsequent administration of bone marrow might save the animals that have survived the critical phase of the intestinal syndrome. Unfortunately such a combined treatment is at present exceedingly difficult to perform.

VII. Therapeutic Attempts with Miscellaneous Substances

Of the chemical substances which have been investigated for beneficial effects in irradiated animals, many have been discussed in previous sections

of this chapter. There remains a limited number of components which deserve some attention, either because of their therapeutic properties or because of the interesting ideas which led to a therapeutic trial with these components.

The experiments of Ellinger (208) underline the necessity for appropriate control groups when median lethal radiation doses are employed in the study of therapeutic effects. Ellinger observed a reduction of the mortality of X-irradiated mice (LD_{60}) subsequent to the daily injection of 0.3 ml of physiologic saline for 6 days. The explanation for this therapeutic effect of saline injections is not at all clear. It should be noted that the magnitude of the effect is rather small and that a decrease of mortality occurs under certain specific conditions only. It does not seem likely that the effect is due to a correction of the mineral and fluid balance. In this respect it is of interest that the administration of plasma expanders after whole-body irradiation had little or no influence on recovery in the few cases that have been investigated (247, 248). One plasma expander, polyvinyl pyrrolidone (PVP), has been investigated because of its supposed enhancement of the excretion of toxic products. It was postulated that PVP administration might reduce mortality by the removal of radiation toxins from the body. Burger *et al.* (249) reported a beneficial effect in lethally irradiated rats resulting from treatment with PVP after the irradiation. Unfortunately these results could not be confirmed by a number of investigators (250, 251), and in 1955 Burger (252) reported his failure to reproduce his former results. It has also been reported that *in vivo* dialysis of X-irradiated dogs was ineffective in prolonging life (253). Although it may well be that the above procedures are not effective in removing radiation toxins, it is the author's opinion that the role of these hypothetical radiation toxins in the development of radiation sickness and mortality is at best a doubtful one. If so, any treatment that is aimed at neutralizing these toxins may be expected to yield negative results.

The "flavenoids" (vitamin P) have received some attention in the treatment of irradiated animals because these compounds are supposed to decrease the fragility and permeability of the capillaries. Some successes have been claimed in early experiments with regard to the treatment of the hemorrhagic syndrome and the reduction of radiation mortality in dogs (254, 255) and guinea pigs (256). Other investigators have failed, however, to confirm this in guinea pigs and mice (257-259). A few years later it was claimed that the tolerance to irradiation was increased in patients as a result of the administration of these compounds during the course of radiation therapy (260). The same authors reported that the injection of "flavenoids" (extracted from citrus fruit) prior to and after exposure increased the tolerance of rats to contact radiation (261). Un-

fortunately in the latter papers the nature of the "flavenoids" is insufficiently described and the therapeutic action of vitamin P cannot be fully accepted without additional and more convincing evidence.

The effect of daily injections of vitamin K has been investigated in irradiated guinea pigs (262). In the group that was subjected to an LD₃₉₍₁₅₎ postirradiation administration of vitamin K (4 mg/day) reduced the mortality to 5 %. After an LD₇₃₍₁₅₎ the group treated with vitamin K showed a mortality of only 40 %. No appreciable reduction of mortality was obtained after an LD₉₇. Although the number of animals was limited to 30 in the largest experimental groups, the survival data are supported by the autopsy findings. Vitamin K administration resulted in a definite reduction of the hemorrhagic manifestations in the gastrointestinal tract and the testes at the LD₃₉ level. The radiation-induced changes in the hematopoietic tissues were not discernably modified by the treatment. These observations indicate that vitamin K has a slight but definite beneficial action on the recovery of irradiated guinea pigs. At present it is not possible to explain the mechanism of this effect. Although there seems to be no reason to doubt the adequacy of the diet on which the guinea pigs were kept before and after the irradiation, with respect to the vitamin K level it is necessary to ascertain whether the therapeutic effect occurs in animals which have been fed rations supplemented with vitamin K for some time before the irradiation. In other words, one should like to know whether the findings might reflect an irradiation-conditioned subclinical vitamin K deficiency.

A great number of drugs has been investigated for control of symptoms of radiation sickness in patients undergoing radiotherapy. In evaluating these clinical trials it should be kept in mind that the administration of a placebo is generally reported to give relief of symptoms in a surprisingly high proportion of cases. In Stoll's investigation (263) of the therapeutic effects of chlorpromazine and related compounds, between 53 and 75 % of the cases responded favorably to the administration of inert tablets, as compared to 75 to 89 % of the patients given the most effective drug under study. It is unfortunate that many of the clinical studies do not meet the minimum requirements for a conclusion to be drawn. Anyhow, it is the rather general opinion that chlorpromazine and other similarly acting compounds inhibit postirradiation nausea and vomiting (263-265). This is entirely in accord with the results of a careful analysis of the emetic action of whole-body irradiation by Chinn and Wang in dogs (266) and by Brizzee in monkeys (267). These authors showed that destruction of the emetic chemoreceptor zone, which is situated in the brain stem, completely prevents postirradiation vomiting. Chlorpromazine is supposed to act as an inhibitor on this chemoreceptor zone.

The convulsions which develop after high doses of radiation (6000 r) in guinea pigs have been suppressed by pretreatment of the animals with sodium phenobarbital (268). All signs of hyperexcitability were effectively reduced after 7500 r, and the survival time increased from 17 hours to 89 hours in the treated group. Similarly sodium diphenylhydantoinate controlled the convulsions and increased the median survival time several-fold in mice, when administered prior to irradiation with dosages between 55,000 and 150,000 r (269).

In the author's opinion this prolongation of survival has little to do with recovery. The degree of destruction which results from these formidable doses of radiation seems to exclude this possibility. The recovery of the nervous tissues from radiation damage has been explored only superficially, and no means to promote these processes are known at present. In searching for these factors it will probably be more advantageous to employ much lower doses of radiation or preferably local irradiation of the cerebrum.

VIII. Conclusion

Investigations into the treatment of irradiated animals have been restricted largely to the lower lethal radiation exposures, which result in the development of the bone marrow syndrome in most mammalian species. By far the most outstanding achievement during the past decade in this area is the development of bone marrow transplantation to the stage of clinical experimentation.

So far, attention has been focused mainly on the complete replacement of the recipient's hematopoietic system by donor cells. The resulting condition has many interesting aspects, of which the increased possibilities of homologous tissue transplantation are the most obvious. The clinical application of these findings is severely tempered by the necessity to expose the recipient to a lethal dose of whole body irradiation. Therefore a systematic search for less-drastic means of conditioning the recipients is obviously indicated.

Although the production of permanent radiation chimeras offers many fascinating problems, this cannot in itself be the primary object of bone marrow transplantation in the treatment of lethal radiation disease. The latter requires the survival of the irradiated animal, and with genetically unrelated donors and recipients the continued presence of a functional bone marrow graft has been shown to be undesirable. It would seem more advantageous if the foreign graft would be permitted to survive only for the period required for a spontaneous recovery of the host's hematopoietic tissues. The occurrence of so-called total reversals shows that at least in a proportion of the animals and after the lower lethal radiation doses com-

plete regeneration of the host's hematopoiesis is entirely feasible. It is not inconceivable that after higher doses of radiation—when the frequency of reversion is much lower—regeneration of the host's bone marrow is suppressed by the graft, which might proliferate more activity under these conditions because of a more complete inhibition of the host-versus-graft immunological reactions.

The regenerative capacity of the hematopoietic cells of the host after these higher radiation doses in animals that are kept alive by a foreign bone marrow graft is essentially unknown. In view of the above speculations an investigation of this problem seems worth while.

It is perhaps fortunate that, apart from bone marrow transplantation, rather promising results have been obtained with a number of factors which appear to act by stimulating the regeneration of hematopoiesis. Admittedly, much additional information is still required with regard to the nature of some of these factors as well as of the exact mechanism of their therapeutic action. In the case that a sufficiently active factor would emerge from these studies, however, the clinical application would seem to be less restricted than that of bone marrow transplantation, because the occurrence of severe late complications is not to be expected as a result of the former treatment. There is also a possibility that these two forms of treatment may be combined to act in a complementary fashion according to the ideas outlined above.

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