IONIZING RADIATION: LEVELS AND EFFECTS

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Annex F

EFFECTS OF RADIATION ON THE IMMUNE RESPONSE

CONTENTS

Introduction

1. For many years it has been realized that whole-body irradiation has profound effects on the immune response of experimental animals, and, more recently, this has also been demonstrated in man. Since many types of radiation are now being frequently used in clinical treatment of patients and in experimental research, it is essential that more detailed information on the effect of irradiation on the different phases of immune responses be obtained. This particularly applies to the effects of single or multiple low doses of radiation, as it is becoming increasingly clear that the complex process of immunity is composed of several distinctly separate events, some of which involve very radio-sensitive cells.

2. The essential aim of a review of this type is to provide a means of estimating the risks to man from radiation-induced lesions in the immune system. At

the present time numerical risk estimates cannot be made in relation to the immune system. This annex will therefore merely attempt to evaluate the order of magnitude of the immune system's radio-sensitivity, on the basis of experimental and clinical observations involving mostly high radiation doses. As much of the experimental work is drawn from animal species other than man, some attention will be paid to species variation in order to evaluate the significance of extrapolation to man.

3. It is essential to realize that an analysis of radiosensitivity of the immune system is not simply a study of the radio-sensitivity of one cell type. The immune response as a whole comprises several distinctly different types of response with different cell types and modes of expression. These will therefore be examined separately and, in the course of this analysis, reference will be made to the possible medical uses of suppressing immunity by radiation to assist organ transplantation, and to the use of immunological methods in tumour therapy. This annex will cover each of the major types of the immune response which are detailed in the following paragraphs.

4. Detailed information on the events leading up to the release of circulating antibody has been obtained in the past few years. In many systems an antigen-processing step is obligatory before the antibody-forming machinery can be brought into action. Furthermore in many antibody responses the early events subsequent to antigen processing also may involve a collaboration between two ontogenically distinct haemopoietic cell lines. Thus at least three different cell types can be involved prior to the development of the actual antibody-forming cell. Since all three types are frequently obligatory for certain antibody responses, suppression of any one by irradiation will profoundly affect the over-all antibody response. As these three components may involve cells of different differentiation stages, it is possible that they may show differential radio-sensitivities. This review will accordingly attempt to analyse the radio-sensitivity of the humoral antibody response in terms of the sensitivities of the different components comprising the response.

5. The time interval between antigen administration and irradiation greatly affects the subsequent changes induced by radiation. Whereas it is more commonly found that radiation suppresses immunity, under some circumstances enhancement of certain aspects can be generated. Stimulation may be related to certain over-corrections in the controlling mechanisms following irradiation. A specific analysis of this point will be made, as it is relevant for consideration of radiation therapy in man.

6. Radiation induction of some animal tumours is thought to be mediated through an activation of latent viruses. As it has been clearly demonstrated that many of these tumours carry strong tumour-specific transplantation antigens, it is possible that a factor in the induction of tumours by radiation is the associated immune depression, which in turn permits a normally suppressable potential malignancy to become expressed. Since these experiments usually involve fractionated doses of radiation of the order of 100-200 rads, it is important to consider this phenomenon in terms of possible relevance to human neoplasia.

7. The effect of radiation on the state of immunological tolerance may also be in either direction, towards breaking the tolerant state or helping in the induction of tolerance. In recent years a new concept of two zones of antigen dosage for the induction of tolerance has emerged. Some studies suggest that low-zone tolerance may be involved in normal immunological homeostasis and that breaks in this mechanism may lead to auto-immune disease. It is therefore relevant for human studies to consider the effects of radiation on the state of tolerance, as radiation-induced alterations in this state may lead to auto-immune phenomena.

8. This annex will attempt to consider the effects of radiation in three main areas: (*a*) the normal immune response, specifically examining the various components of resistance to infection, the antibody-forming mechanism, transplantation immunity and delayed hypersensitivity; (*b*) effects of radiation on experimental tumour induction associated with effects on the immune state; and (*c*) the two zones of immunological tolerance, with specific reference to possible auto-immune consequences after alteration of the normal homeostatic condition. For definitions of immunological terms, the reader is referred to a glossary of immunology (231).

I. The general components of the immune response

A. Resistance to infection

9. Immunity has been associated with resistance to infection. In this context we are considering the ability of the body as a whole to check the large number of infectious agents and parasites that perpetually threaten life and health. The term infection is used here to describe the situation in which an organism enters into a relationship with the host such that the host's cells or tissues are frequently damaged. Resistance describes the relative ability of the animal to counteract the infection and not to succumb to the invading organisms.

10. Resistance has been frequently divided into natural and acquired resistance. Natural resistance generally refers to the resistance of animals not specifically immunized, or exposed, to the infection, whereas acquired resistance refers to the state of resistance which develops in animals following active or passive immunization or following exposure to the infection at a sub-clinical level. In general, acquired resistance is specific for a particular organism while natural resistance may be relatively non-specific. This implies that acquired resistance is therefore mediated by a specific immune response either cellular or humoral in nature. It is at this point that considerable confusion arises within the immunological literature. To students of infectious disease, cellular immunity refers to the form of acquired anti-microbial resistance in which the host's mononuclear phagocytes show increased destructive capacity for ingested organisms. This form of cellular immunity can be transferred with cells but not with serum (15). Although it is evoked by way of a specific immunological reaction, it is frequently non-specific in its anti-microbial effects for the period of a few weeks following antigenic challenge but, once established, it will be specific for the original immunogen (160, 258, 318).

11. Another use of the term cellular immunity refers to those immunological reactions that are mediated directly by lymphocytes and are not dependent on

secreted antibody. This includes most forms of transplantation immunity and delayed hypersensitivity and will be discussed in section I B. Recent studies have tended to bring these two alternative views of cellular immunity closer together, as lymphocytes as well as macrophages have now been shown (319) to have an important role in at least some types of resistance to infection, in that lymphoid cells play an inductive role in the immune response which is then primarily effected by the macrophages. In transplantation immunity, however, both lymphocytes and macrophages can be involved in the actual effector stage of killing target cells.

12. For the purpose of this report, this section and section II will deal with resistance to infection in which the animal as a whole is studied, or in which other processes apart from specific antibody formation or lymphocyte-mediated immunity are involved.

13. Resistance to infection is a broad field and has been the subject of several excellent books and reviews (67, 321, 402, 441, 626). It includes defined cell responses in which the macrophage is the essential cell type, antibody formation, possible role of eosinophils, and various non-specific phenomena. Specific antibodies can neutralize toxins, neutralize viruses, or prevent their entry into susceptible cells. With complement, and possibly lysozyme, lysis of bacteria can occur. Antibodies can promote phagocytosis of microorganisms by polymorphs, as can natural antibodies in natural resistance. A large role in natural resistance may be played by such non-immunological factors as unbroken cutaneous or mucous surfaces: free fatty acids with antibacterial properties on the skin; the sweeping action of cilia in the bronchial tree and by lysozyme and other humoral factors (204, 267, 513).

14. From the time of Metchnikoff (360), it was strongly felt that acquired resistance to infection resulted from "the perfecting of the phagocytic and digestive powers of the leucocytes". The important role of the macrophage has indeed been well documented (402), and little more need be said in this introductory section about the importance of this cell type, other than to stress one point concerning its heightened activity in acquired resistance. Although the formation of specific antibody can be an important factor in acquired resistance, it is also clear that macrophages from infected animals can show an intrinsic elevated functional activity, although non-specific methods of stimulating increased lysozymal activity of macrophages will not lead to increased functional activity against specific organisms. This is indicated by the fact that cells from infected mice will completely inactivate *Salmonella typhimurium* organisms within 15 minutes, whereas normal cells only partially inactivate, and do so in a much slower time (51). Furthermore, cells from animals infected with *Listeria monocytogenes* or *Salmonella typhimurium* are equally microbicidal for *Salmonella typhimurium*, despite the absence of demonstrable anti-*Salmonella* antibody in the serum or absorbed on cells of the *Listeria*-infected mice (51). It has also been reported that in the infection of mice with *Salmonella enteritidis*, immunization with a live vaccine (294, 386, 487) or convalescent immunity (431) achieves high resistance against further infection with a virulent strain of the same bacteria. It was noted in this immunity that cultured macrophages derived from either the peritoneal cavity, the subcutaneous tissue or the liver of immunized mice, resisted the cell degeneration caused by *in vitro* infection with virulent bacteria regardless of the presence of antiserum in the culture medium. Furthermore, the serum obtained from immunized mice did not show any passive immunization against fatal infection and had no inhibitory effect on the intracellular growth of virulent bacteria in macrophages cultured *in vitro* (385, 387, 487, 492). Such resistance was referred to as cellular immunity and was also described in some other infections with cytophilic bacteria such as tuberculosis, brucellosis and listerosis (188, 322). In more recent studies (320), it has been shown that acquired resistance may depend upon the activation of host macrophages through a product resulting from the specific interaction between sensitized lymphoid cells and the pathogen or its antigenic products. This finding may help bring together the two interpretations of the term cellular immunity, in suggesting that the enhanced macrophage response is dependent upon the cellular lymphocytic response.

B. Cellular and humoral immune responses

15. Before embarking on a detailed analysis of the effects of radiation on the immune response, it is essential to stress that "the immune response" is a rather general term embracing several different types of immune reactions observed in animals and man. Any consideration of the effects of radiation must, therefore, be made separately for each type of response and in some cases for the separate components of a given type of immune response. This does not imply that the different clinical forms of immunity, hypersensitivity and allergy are all mediated by different mechanisms, but rather that there are a few basically distinct mechanisms of immunity within which there may be many slight variations expressed in different species or under different conditions.

16. The two basic types of immune responses are: (*a*) humoral-antibody formation, which involves the production of circulating antibody molecules found either in serum or in other body fluids; and (*b*) lymphocyte-induced cellular immunity, in which the actual site of the immune reaction contains both lymphoid cells and macrophages.

17. Although there are many results and experiments which support this basic dichotomy, the most striking demonstration that these are two distinctly separate forms of immunity comes from studies in experimental chickens (106, 603) in which the differentiation of the immunoglobulin-synthesizing plasma-cell system is under separate ontogenic control (the bursa of Fabricius) (206, 392) from that of the lymphocyte-mediated cellular immunity (26, 271, 611). Thus, by embryonic bursectomy, animals can be obtained which are totally agammaglobulinæmic and cannot form any antibody (612) but which have normal delayed hypersensitivity (609) and transplantation immunity. This experimental demonstration of two separate types of immune response is also clearly evident in several human clinical syndromes, in which either antibody formation or cellular immunity is selectively depressed (105, 141, 407, 450, 474, 496).

18. A schematic outline of the immune response is given in table 1. Three of the major distinguishing features of the dichotomy of immunity are listed. Antibodies found in serum and other body fluids are primarily synthesized and secreted by cells of the plasmacytic series (57, 133, 304) and also by lym-

phocytic cells (*B* lymphocytes) (119, 220), which ultrastructurally, have the endoplasmic reticulum characteristic of an active protein-secreting cell.

19. The differentiation of the plasmacytic cell line is controlled by the bursa of Fabricius in chickens (105, 603). Several candidates for a bursal equivalent in mammals have been proposed, including Peyer's patches (104), appendix (23), tonsil (451), diffuse intestinal epithelium (179), and even skin (180). There is, however, no universal acceptance of any of these as bursal equivalent sites. For example, the immunological role of the appendix of rabbits seems to be directed only towards the differentiation of IgM-synthesizing cells and not towards the differentiation of cells synthesizing other classes of immunoglobulins (104, 233, 298). In contrast, the bursa of Fabricius has an important role in the differentiation of cell systems involved in the synthesis of all classes of immunoglobulins. It has also been reported that Peyer's patches in the rat (101) and rabbit (249) are directly involved in the synthesis of IgM antibody when antigen is directly injected into the patch or when Peyer's-patch cells are treated with antigen *in vitro*.

20. In the earlier work by Cooper *et al.* (104), it was observed that combined removal of the appendix, the *sacculus rotundus* and all the Peyer's patches in rabbits followed one month later by whole-body exposure to 650 rads resulted in the partial, but not complete, depression of antibody-forming capacity when challenged 21 days after irradiation. Thus, in at least some animals, restoration of the antibody-forming capacity following near-lethal whole-body irradiation did occur in the absence of the postulated bursal equivalent. In a comprehensive examination of germinal centres in the rabbit appendix, it was concluded (409) that it is essentially the germinal-centre compartment which is responsible for the delivery of antibody-forming-cell precursors and that, contrary to the view of Good *et al.* (214), the germinal centres of the gut-associated lymphoid tissue represent plain germinal centres like those in the spleen and lymph nodes.

21. By contrast, cellular immunity is induced by lymphocytic cells which are also found in the immediate vicinity of the active immune lesion, together with macrophages, as for example, in the infiltrate underlying a rejecting skin homograft (49, 497) or in various organs in auto-immune diseases (323). The actual mechanisms of lymphocyte-mediated pathological changes will be considered in a later section in relation to radio-sensitivity. The differentiation of the lymphocyte-dependent line in cell-mediated immunity is thymus-dependent in most animal species studied, including man (213, 215, 373), although in sheep this could not be demonstrated.

22. Humoral immunity is manifested in a variety of different clinical and experimental forms which can broadly be considered as either the production of antibody, resulting in high serum titres of antibody and a state of elevated resistance to certain infections, or as the production of certain molecular classes of antibody which are capable of initiating immediate hypersensitivity reactions and some auto-immune disorders. Antibody molecules can be subdivided into different immunoglobulin classes (90, 171, 194, 306), for example, in man, IgM, IgA, IgG, IgD and IgE which have in common the basic molecular form of two light (*L*) and two heavy (*H*) polypeptide chains (158, 461) but which differ in that different structural genes code for the constant regions of the *H* chains of the various classes (195). Certain biological properties of antibody molecules are mediated through sites on the C terminal half of the heavy chain (433), and since the classes differ in their heavy chains, a given biological effect is usually mediated by only one or a limited number of immunoglobulin classes. These properties include the fixation of antibody molecules to mast cells, which is at the basis of the anaphylactic and reaginic hypersensitivities (433) and is associated with certain specific immunoglobulin classes (IgE-mediated reaginic hypersensitivity in man (268, 274), gamma-G1-mediated anaphylaxis in mice (34, 428, 435) and guinea-pigs (434), and another separate unidentified antibody-mediated reaginic hypersensitivity in mice (595)). Another form of hypersensitivity leading to tissue damage is the Arthus reaction (involved in serum sickness and some glomerulonephritis) mediated by those classes of immunoglobulins that are capable of forming a precipitating complex with antigen in tissue sites (for example blood-vessel walls), which then fix complement components (88).

23. The role of antibody in the rejection of antigenic tumours has not yet been fully elucidated. Cytotoxic antibodies are those antibodies which fix complement and cause lysis of tumour cells. These have been suspected to be active against dispersed leukæmic cell suspensions *in vivo* (16). On the other hand, it has also been shown by Hellstrom and Hellstrom (247) that some serum factors can protect tumours *in vitro* from lymphocyte-mediated tumour destruction. The nature of these serum factors and their role *in vivo* remains to be elucidated. It is by no means clear whether these blocking serum factors are the same as enhancing antibodies, which have been conventionally demonstrated by their ability to enhance tumour growth after prior injection into recipients which are then challenged with tumour cells (275). Although one study (598) suggested that enhancing antibodies were electrophoretically fast migrating (and possibly IgG1), two other studies (266, 543) implicated IgG2 molecules, which are also capable of fixing complement.

24. Cellular immunity is broadly recognized in two basic forms: (*a*) rejection of tissue allografts such as skin or kidney, or (*b*) delayed hypersensitivity reactions, best typified by the Mantoux reaction to old tuberculin or PPD in individuals sensitized to tubercle bacilli. As mentioned in the previous paragraph, there are reports suggesting that not all forms of transplantation immunity are mediated directly by lymphoid cells. Tumour allografts presented in the form of single-cell suspensions can be rejected by circulating cytotoxic antibody (16), and immunological damage to some organ transplants such as kidney has also been claimed to be antibody-mediated (299).

25. The morphological and hæmatological representation of this distinction of immunity into cellular and humoral is diagrammatically represented in figure I in which it is indicated that a multipotent hæmatopoietic stem cell has the potentiality to differentiate into any hæmatopoietic cell system. The true stem cell may possibly differentiate initially into two types of stem cell—a lymphoid stem cell (190) and a second type with potential to form other blood elements. On the other hand, Nowell *et al.* (427) reported evidence indicating the existence of multipotential lympho-hæmatopoietic stem cells in the adult rat. In this study, rats were given near-lethal x-ray doses to produce

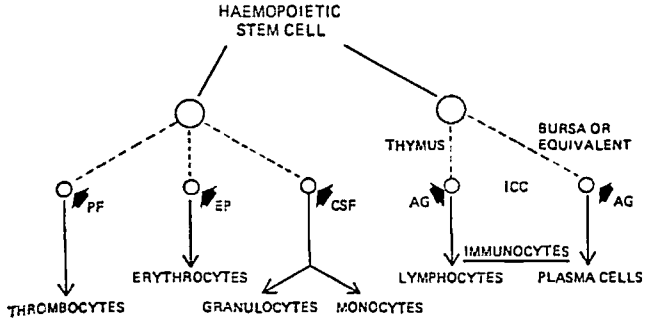

Figure I. Development of the hæmopoietic system from a common stem cell. The first division indicates a possible dichotomy involving a lymphoid stem cell. One type can then differentiate into thrombocytes, erythrocytes, or granulocytes and monocytes, under the induction of platelet factors (PF), erythropoietin (EP) or colony-stimulating factors (CSF), respectively. Immunocyte differentiation is from antigen (AG)-induced stimulation of immunocompetent precursor cells (ICC) which have differentiated under the control of thymus or bursa equivalent (bursa in birds only)

clones of hæmatopoietic cells marked by radiation-induced chromosome abnormalities. Subsequently, bone marrow from these rats was injected into lethally-irradiated mice to form erythropoietic spleen colonies, and peripheral blood lymphocytes from the same rats were stimulated to proliferate in a mixed lymphocyte interaction (MLI), an immunological response to histocompatibility isoantigens. Chromosome markers indicated that in several instances the cells of an erythroid spleen colony and a proportion of the lymphocytes reacting in the MLI were progeny of the same stem cell in the donor rat. In addition, lymphocytes of the same radiation-marked clone were shown to proliferate in response to several different histocompatibility isoantigens, suggesting that immunological specificity is determined during lymphoid differentiation, subsequent to the stem-cell stage.

26. Differentiation of the stem cell into lymphocytic elements is then directed by thymic induction, and differentiation into plasma cells by the bursa of Fabricius or its equivalent, although, as mentioned before, certain cells that are morphologically lymphocytes are also concerned with humoral immunity (*B* lymphocytes). Differentiation of stem cells into the erythroid series involves erythropoietin (182), whereas differentiation into granulocytes and monocytes involves a colony-stimulating-factor effect on a precursor cell (359, 524) and platelet factors are required for thrombocyte differentiation (442). The complete maturation into active immunocytes of lymphoid and plasmacytic immunocompetent cells then involves antigenic stimulation.

C. Stages within antibody formation

27. The injection of an antigen or vaccine into an animal is usually followed by a delay of a few days before detectable circulating antibody appears in the serum. During this period, several discrete steps leading to the production of antibody may be discerned. These can broadly be considered in three parts: (*a*) appropriate processing or handling of the injected antigen so that it effectively reaches the appropriate immunocompetent cell (the afferent limb); (*b*) the proliferation of certain immunocompetent cells and their interaction which, although involving specific antibody-like receptor sites on the surface of these cells, does not involve active antibody secretion (the inductive phase); and (*c*) the final process of differentiation of the plasma-cell line which progressively leads to a cell whose major function is the active synthesis and secretion of specific antibody (productive phase). These stages are schematically depicted in figure II.

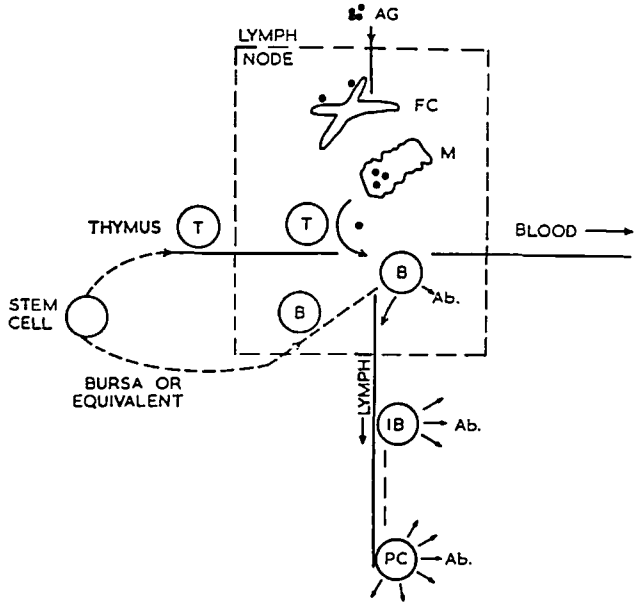

Figure II. Stages of antibody formation within a lymph node. The antigen (AG) often first requires "processing" by macrophages (M) or follicle cells (FC) (afferent stage) and then is transferred in a suitable form to the thymus-derived (T) and/or bursa (or bursal equivalent)-derived (B) lymphocytes for initiation of the immune response (induction). This stage may often require collaboration between the T and B cell types. The B lymphocyte then becomes the progenitor of the antibody (Ab) producing cell clone (efferent stage), first giving rise to immunoblasts (IB) or immature plasma cells, and thence to mature plasma cells (PC)

28. The relevance of this preliminary division of the immune response into separate stages to radiation susceptibility of immunity is that different cell types are involved in these steps and that these may show either over-all differences in sensitivity, or differences at critical stages of their function. The afferent limb involves granulocytic and macrophage cells which directly interact with antigens, and process or simply hold the antigen in a suitable manner for presentation to immunocompetent cells. The inductive phase then involves the presentation of this antigen or of some cellular product specific for the antigen to lymphocytic cells of thymic origin which then proliferate and may interact with another cell type (bursal-derived) which differentiates into the antibody-secreting cell line.

II. Effects of radiation on susceptibility to infections

29. Over the past eight years a vast body of literature has been assembled which repeatedly demonstrates one basic observation. Namely that, if an animal is given a moderate to high dose of radiation and is then challenged with an infectious agent, it will show increased sensitivity to the infectious agent. This observation has been made with virtually all experimental animals (and man), with most known infectious agents, including bacteria, viruses, protozoa, rickettsia and fungi, and with various sources of radiation and doses. Well over 1,000 such independent observations

have been reported and, as it would be extremely repetitious, these will not all be cited in this present document.

30. Of considerable relevance to this review is that increased susceptibility to infection is primarily caused by the decrease in immune responsiveness of the host. As several factors can influence the degree of increased susceptibility, this section will primarily concentrate on examining these variables with examples drawn from the abundant literature in this field. Probably one of the main things to stress is that exactly the same principles apply to radiation-induced immune depression, whether assessed by actual measurements of the immune response, or more indirectly by the death of the animal resulting from increased pathogen growth. In many instances, this latter estimation may be complicated by other factors and accordingly a direct relationship between the radiation parameter and true susceptibility is not observed.

31. Many reviews on the susceptibility of irradiated animals to infections are available (40, 55, 146, 150, 255, 365, 452, 453, 515, 534, 538, 551, 572, 648, 652, 659, 662, 678, 680). Approaches to this problem include assessment of the course of infection after irradiation following challenge with either (*a*) known pathogenic agents; (*b*) conditionally pathogenic agents (normal flora); and (*c*) no challenge but determination of the infection that spontaneously results. In considering the relevance of many of these data to man, it appears that the same principles found in animals also apply in man. For example, in one study (680) it was concluded from an examination of many species that radiation sickness in man closely resembled that observed in monkeys. In a study of patients with late-stage malignancy given whole-body irradiation, it was found (21) that the major cause of early death was infection, principally of gram-negative or fungal origin. Although there are a few other reports describing radiation infection in man, understanding of the basic principles have come from studies in mice, rats, rabbits, guinea-pigs, monkeys or dogs.

32. Antimicrobial immunity against infections in radiation sickness is so markedly impaired that susceptibility is increased not only towards pathogenic agents but also to bacteria which are part of the normal flora. These two aspects will now be considered, followed by examination of several variables such as timing of infectious challenge and radiation, and radiation parameters.

33. For more than 50 years (109) of experimentation in the field of immunology of infections associated with radiation sickness, investigators have determined the sensitivities of irradiated animals to various pathogens. For example, in a study by Yakovleva *et al.* (699) of 14 monkeys given approximately 4 10^{10} paratyphoid *B* organisms orally, only one died of paratyphoid. However, in monkeys also given 300 roentgens (in itself non-lethal in monkeys) four fifths died with paratyphoid five days later. In other studies of this type susceptibility to hæmolytic streptococci increased five times (689) and susceptibility to *S. enteritidis* increased hundreds of times (493) in mice exposed to 350 roentgens. A sharp drop in resistance to influenza virus has been demonstrated in experiments with irradiated mice and rats (682). Similar increases in sensitivity to gas gangrene organisms, to tetanus (673), to icterohæmorrhagic leptospirosis (673) and to tularemia (694) were observed in sublethally-irradiated mice. It is essential to note that any measure of increased sensitivity to an infection following irradiation will be accurate only for the given host, pathogen, and irradiation conditions. The over-all rule, however, is quite clear. The sensitivity of animals to microbes is markedly increased in radiation sickness.

34. In several studies with continuous exposure to low-dose-rate gamma radiation, increased susceptibility to chronic infections has also been observed. In studies in mice with *Listeria monocytogenes*, and using ^{60}Co gamma radiation at a dose rate of 1.0 to 1.5 rads per hour, it was found that the greater the total dose of radiation administered, the greater became the susceptibility (509). Mice receiving 500 rads were three times as susceptible as non-irradiated mice, while those exposed to 2,500 rads were approximately 30 times as susceptible. In an even more prolonged type of study (651), various animals were given continuous ^{60}Co gamma radiation at 1.2-4.3 roentgens per day, for 1.5-2 years. The cause of death of the irradiated animals was totally attributable to auto-infection with the development of septicæmia. Autopsy of these animals did not show the characteristic pattern of acute radiation sickness. The strongest disturbance of natural immunity occurred in young animals and particularly with radiation delivered during intra-uterine development.

35. In irradiated animals, the pathogenicity of conditionally pathogenic micro-organisms is often observed. For example, intravenous injection of doses of *B. proteus*, which are non-lethal in unirradiated mice, led to an increase in number of bacteria in the blood and to eventual death in mice given 400 roentgens three days previously (240). This phenomenon has also been demonstrated with colon and paracolon bacilli, *Pseudomonas aeruginosa*, type III pneumococci and many other bacteria which are non-pathogenic for normal animals.

36. In view of this striking increase in susceptibility of irradiated animals to both pathogenic and conditionally pathogenic organisms, it is reasonable to question whether irradiated animals might also become infected with an agent which characteristically does not infect normal animals of that species. In the main, the answer to this question is no. Species resistance to uncharacteristic infectious agents appears to persist (innate resistance). Thus Kolmer *et al.* (296) were unable to overcome the innate resistance of rabbits, guinea-pigs, rats and ferrets to poliomyelitis virus, despite the fact that the animals were twice irradiated. Many other examples of this type are documented in the review of Petrov (673), and include the agents for anthrax, tularemia, diphtheria, typhus, dysentery, typhoid and leptospirosis. The only exception that might be noted is that sensitivity to non-specific intoxication is increased after the injection of large quantities of microbial mass. It therefore appears that there is a high degree of stability of the animals' innate resistance to the effect of ionizing radiation in terms of certain infectious agents. In all probability, disease not characteristic of a given species does not occur even after irradiation. Irradiation is therefore incapable of abrogating the interrelationships which have been built up during the course of evolution between species of animals on the one hand and of micro-organisms on the other.

37. Although irradiated animals are severely compromised in their ability to undergo active immunization against bacteria and bacterial toxins, they can be satisfactorily protected by the use of passive immunization with antisera. This has been shown with diphtheria (659, 688), tetanus, and gas gangrene (653, 672). Although it has been claimed that irradiation does not change the rate of clearance of passively-transferred antibodies in syngeneic combinations, this has not been specifically evaluated with purified IgM and IgG antibody. In view of other observations on the loss of IgG and IgA through the irradiated gut wall (see paragraph 65), it might be expected that some loss of passive antibody would occur. Indeed, it has been shown that to obtain equal antitoxic effects in normal and irradiated recipients given passive antisera, three to five times more serum must be given to the irradiated recipients (659, 672). As was shown by Kaulen, an increased sensitivity to the complexes of toxin and antitoxin has also been observed in irradiated animals (689).

38. Increased susceptibility to virus infections following irradiation has also been observed frequently with many types of viruses including influenza, smallpox, ornithosis, mouse encephalomyelitis and mouse hepatitis. This is often seen as a shorter incubation period, more virus proliferation or more virus-induced pathogenic lesions, and is observed with sub-lethal doses of 200-500 rads. In several cases, however, the opposite result has been found, namely, a reduction in severity of the disease. On general grounds this might be expected on the premise that cell metabolism is markedly disturbed after irradiation and intracellular virus proliferation may be inhibited. Several examples from the earlier literature (211, 462) concern encephalitis in man, and show that alleviation of symptoms often resulted after radiation, possibly as a result of lymphocyte destruction. Similar results were also observed in studies of lymphocytic choriomeningitis in mice, a type of virus-induced auto-immune disease in mice whose pathogenetic basis is the induction of cell-mediated immunity. Mice exposed to 500 roentgens 24 hours prior to virus inoculation were protected for 48 days (256, 257), the depression of disease presumably being caused by inhibition of proliferation of the pathogenic lymphocytes.

39. Experiments for determining the time of increase in sensitivity to infection after irradiation can be divided into two groups: those showing an immediate increase in sensitivity, and those showing an increased sensitivity only after several days—usually about three days. In the first group, increased sensitivity to infection when given simultaneously with radiation has been shown for trypanosomes, plasmodia, influenza, yellow fever and tuberculosis (673). On the other hand, in a number of cases in which increased sensitivity to infection could be clearly demonstrated if the infectious challenge was given several days after radiation, no increased susceptibility occurred with simultaneous challenge. This includes studies with hæmolytic streptococci, pneumococci, staphylococci and colon bacilli. Irradiation after the infectious challenge leads to results similar to those in the first group (simultaneous administration). Thus, irradiation of mice three days after an inhalation of whooping cough bacilli led to a more serious infection than in control mice (684).

40. What is the reason for the existence of these two distinct timing relationships? The unifying concept is that these different results are related to the duration of the infectious process. Thus, those instances in which simultaneous challenge leads to increased sensitivity all involve chronic infections, whereas acute infections fall into the second group. In confirmation of this interpretation, it has also been found that irradiation after infection will aggravate a chronic infection, and that the difference in the two groups can be brought about with the same pathogen, if it is administered in ways which lead to either an acute or a lingering process.

41. In conclusion of this section, several points might be stressed which are derived from large numbers of individual reports: (*a*) radiation leads to increased susceptibility not only to pathogenic organisms (bacteria, rickettsia, parasites), but also to conditionally pathogenic ones (bacteria); (*b*) species resistance to infections that are not characteristic of that species is usually maintained in irradiated animals; (*c*) increased susceptibility to virus infections also results from radiation exposure, except in those cases where the cellular immune process is actually a part of the pathogenesis; (*d*) increased sensitivity to acute infections is only manifest if challenge is made at least several days after radiation, whereas simultaneous irradiation or irradiation after challenge is also effective with chronic infections; and (*e*) the majority of these consequences are mediated through the effect of radiation on the immune response. Accordingly, the duration of the period of reduced resistance to pathogens follows the period of immune depression and, as discussed in more detail in relation to the immune response itself, depends on many factors, such as the dose of radiation, the dose rate, and the animal species and its individual sensitivity to the particular infection.

42. The delayed consequences of radiation in man with respect to infection are not clearly defined at present. Considerable effort in this regard has been expended at ABCC and to date, with one exception, no relationship between a variety of infectious diseases and radiation has been documented. An analysis of mortality data among members of the Life Span Study Sample in both cities during the period 1950-1960 showed elevated ratios for all causes of death, all natural causes, leukæmia and other malignant neoplasms for persons located 0-1,399 metres from the hypocentre (269). Hiroshima males so located demonstrated a significant excess of deaths due to tuberculosis while Hiroshima females showed an increased frequency of deaths attributable to infectious or parasitic disease other than tuberculosis. These discrepancies were particularly marked during 1951-1952 and seemed to disappear thereafter. Periodic evaluations of the ABCC-JNIH Adult Health Study Sample have shown no clinical, radiographic or laboratory evidence of radiation-related infectious disease. Komatsu *et al.* (297) found no relation between absence from work and exposure dose in a group of male shipyard workers. A review of the ABCC autopsy experience also failed to document a consistent relationship between exposure status and inflammatory processes or infectious disease (22).

43. Finally it must also be stressed that immune depression is not the sole mediator of radiation-induced increased susceptibility to infection. It is almost certainly the major factor, but other components also play a role. Increased permeability of biological barriers has been demonstrated for the skin, the intestines and the blood-tissue barrier. Shortly after irradiation, even before

the development of an acute radiation syndrome, there is a depression of the bactericidal properties of the skin with respect to intestinal bacilli and other microbes applied to it (659). There is a decrease in the complement (655) and properdin levels (679) of the blood. These non-specific aspects have been discussed more fully elsewhere (673).

III. Effects of radiation on antibody formation

A. The afferent limb of antibody formation

44. The afferent limb of the immune response involves the handling of injected antigen in an appropriate fashion to ensure that some of it effectively contacts the immunocompetent cells. It is clear that the first cells to capture antigen are not the ones that synthesize antibody, although some of these cells—particularly monocytes and macrophages—do carry surface immunoglobulins adsorbed cytophilically from the serum (43, 59, 259). The amount of injected antigen is usually many orders of magnitude greater than the amount which ultimately reaches the appropriate lymphoid organ (416), and which then survives the initial degradation within macrophages (4).

45. The initial phase after antigen injection involves a diffuse distribution throughout the tissues without any special associations with the reticulo-endothelial system (4). The duration of this phase depends on the nature of the antigen, as some relatively poor immunogenic materials such as heterologous serum proteins may remain in a diffuse form for days, whereas bacterial products are usually rapidly cleared from the circulation. With particulate material, clearance is extremely rapid. Following its diffuse spread, the antigen is taken up by phagocytic cells, of which there are three main types: polymorphonuclear leucocytes, macrophages, and follicular reticular cells. As these three cells belong to slightly different, though interrelated, cell lines, we will consider their radiation sensitivity separately.

1. *Polymorphonuclear leucocytes*

46. Direct irradiation of polymorphs *in vitro* (498) or irradiation of whole animals appears to have no effect on the ability of polymorphs to phagocytose bacteria (517). However, if phagocytosis is permitted to occur and simultaneously the system is irradiated, increased bactericidal activity of the cell is observed (393). This enhanced killing has been shown to be due to an intracellular effect of irradiation, as irradiation of the cells after phagocytosis of the bacteria is also accompanied by an increased bactericidal activity (395). Furthermore, when active bactericidal fractions of polymorph homogenates are concurrently irradiated, the bactericidal activity is again increased (394).

47. Although the phagocytic capacity of polymorphs from *in vivo* irradiated animals is unaltered, they are not as efficient in killing ingested bacteria as are control leucocytes (647). The total H_2O_2 levels of polymorphs from these irradiated animals are higher, however, than those from normal guinea-pigs and, from the interpretation given above, they might be expected to be more bactericidal, not less. On a more detailed eramination (440), it was found that polymorphs isolated from guinea-pigs three to five days after whole-body irradiation (100 R) showed decreased bactericidal activity, and that addition of foreign particles did not increase H_2O_2 production over resting cells as it did with non-irradiated cells. Although the total H_2O_2 content is elevated, the particle-associated (? lysosome) metabolic H_2O_2 is specifically decreased, possibly as a result of radiation-induced depression in production of H_2O_2 through the hexosemonophosphate shunt. Metabolic H_2O_2 thus seems to be more specifically related to bactericidal activity.

48. These results suggest that direct intracellular effects of radiation on the bactericidal properties of polymorphs can occur, being either suppressive or enhancing, depending on whether phagocytosis takes place at the time of, or later than, irradiation. This may therefore be one of the factors leading to increased susceptibility to infection after irradiation, even at exposures of the order of 100 roentgens. However, there is little evidence to suggest that polymorphs play any decisive role in the induction of antibody formation, although some claims have been made in this regard (521).

49. Irradiation also causes a profound depression of the production of polymorphs in the bone marrow by virtue of the destruction of the hæmatopoietic stem cells which are extremely radio-sensitive. This is clearly seen in an analysis of the *in vitro* colony-forming cells which are the precursors of macrophage and granulocytic progeny and which show a D_{37} survival dose of approximately 85 rads (79, 473). Within 6-8 hours after irradiation, a temporary rise in blood polymorph levels was observed, the mechanism involved being unknown (229). Regeneration of normal levels of *in vitro* colony-forming cells in the bone marrow takes about 16 days after 250 rads (229).

50. Studies in experimental animals have demonstrated that the hæmatopoietic stem cell is the essential precursor cell of the entire hæmatopoietic system, and if all cells of this type were completely inactivated by irradiation, then all activities of the immune system which are dependent on a continual input of differentiating stem cells would eventually fail. However, the reserve of stem cells in the body appears to be such as to outweigh any possibility of its complete eradication with moderate doses of irradiation. Following a dose of 150 rads all parameters of hæmatopoiesis had recovered to at least normal values by 7-8 days (150). On a daily schedule of 50 rads following an initial 150 rads, it required at least a further 250 rads to reduce stem-cell repopulating activity to 5 per cent of control values, which still represents a massive reserve of potential hæmatopoiesis.

2. *Follicular localization of antigen*

51. Primary lymphoid follicles in both spleen and lymph nodes represent rounded densely-packed collections of small lymphocytes in close relationship to a "web" of cytoplasm derived from specialized dendritic reticular cells. The web contains fine cytoplasmic strands with small spaces between them and no definite association with reticulin fibers (377). These cytoplasmic processes set up a very complicated three-dimensional network in the interstices of which many blast lymphocytes are found. The dendritic cells have few free ribosomes and an almost complete lack of lysosomes and of phagocytic inclusions (364) and the very thin cytoplasmic processes can be seen to form closely connected interdigitations with thin processes from primitive lymphocytes. After deposition of antigen in this webbed distribution, a germinal centre may form in the follicle with the original rounded web

being compressed into a crescent cap as the rapidly-dividing lymphoid cells proliferate.

52. These follicular antigen-capturing cells differ markedly from macrophages in their handling of injected antigen. With ^{125}I-labelled flagellar antigens and using electron microscopic autoradiography, it was shown (380) that a substantial proportion of the antigen localized in lymphoid follicles is not actually phagocytosed. These reticular cells retain antigen on the surface of their long dendritic processes where intimate contact is made with lymphoid cells. A similar finding has also been reported for germinal centres in lymph nodes of guinea-pigs injected with ferritin (340).

53. As will be discussed later, medullary macrophage phagocytosis of antigen is virtually unaffected by radiation. However, the retention of antigen in follicles can be profoundly affected by sublethal whole-body x-irradiation. The cytoplasmic fibril web is itself extremely radio-resistant since little direct damage could be observed with doses less than 1,250 rads, and it took 8,000 rads to destroy the structure completely. However, the process of follicular localization and retention of antigen was affected with exposures of 450 roentgens (272). Spleen autoradiographs and whole-organ counts showed that the follicle web was abnormally small, perhaps as a result of its collapse with the radiation-destruction of the lymphoid cells, and that a continuous cortical rim of antigen persisted in the lymph node, possibly indicating a radio-sensitive active process which is normally involved in the movement of antigen from the subsinus region into the follicle. Total retention of antigen in lymph nodes was not reduced, but was severely impaired in the spleen. This latter observation is perhaps more relevant, as initially all splenic antigen localization is in the follicles (420), whereas medullary macrophages are also very prominent in lymph-node antigen localization.

54. Localization of antigen in lymph-node follicles was further studied in rats exposed to whole-body x-irradiation (800 R) (624). This exposure markedly reduced the ability of the lymphoid follicles to retain antigen but did not affect the antigen uptake by the whole lymph node or the uptake by phagocytic cells of the medullary sinuses. It was then found that administration of specific antiserum to the antigen used, or even of larger doses of normal isologous serum, would result in significantly-improved follicular-antigen uptake when assayed 10 days after irradiation. Shielding of the popliteal nodes at the time of irradiation also improved follicular antigen uptake. It was suggested that the follicular antigen-trapping mechanism is extremely sensitive to changes in the level of serum opsonins and that substances present in normal serum act as follicular opsonins. Accordingly, the decreased follicular localization of antigen after radiation may be due to a decline in these opsonic materials, which must therefore be secreted by radio-sensitive cells (? lymphoid cells) in the lymph nodes. This point will be considered in more detail in a following section.

55. What role this impaired antigen trapping plays in the primary immune deficiency of irradiated animals is not clear, particularly as at least some antibody responses can be initiated in the total absence of follicular antigen localization (301). However, follicular trapping may be of considerable importance in the development of immunological memory (564) or continuance of the immune response, and thus its decline could play a major role in antibody depression. A specific study of this possibility has been made (403) in mice subjected to a whole-body exposure of 600 roentgens. The antigen-capture and retention capacity of lymphoid tissue, in particular of the germinal centre stroma, was found to be radio-sensitive, with maximum damage being evident about two weeks after 600 roentgens. Recovery was slow, taking several weeks to be complete. Preliminary electron-miscoscope evidence seems to indicate that the defect in antigen trapping may be attributed to direct damage of the antigen-capturing reticular cells, whereas a role of opsonic factors was not suggested in this study.

3. *Macrophages and the reticulo-endothelial system*

56. Mononuclear cells, of which the macrophage is the common free form and the Kupffer cell is typical of the fixed form, constitute the third and, in terms of antibody formation, the most important group of phagocytic cells. Macrophages take up particulate and soluble antigens within minutes of injection (71). As shown by electron-microscope studies (619), this involves the phagocytic and pinocytic vacuoles becoming surrounded by Golgi vesicles and lysosomes, with fusion to form a complex phagolysosome. Progressive digestion occurs in these vacuoles, but remnants of antigen persist for months. In quantitative studies (585) it has been shown that, although at least 90 per cent of the antigen is actually lost from the macrophage, it still retains its normal immunogenicity.

57. In general, x-irradiation in the LD_{50} range has not been found to affect phagocytosis or antigen degradation of a variety of substances in several species examined (33, 186, 196, 402, 628). Furthermore, macrophages in lymphoid tissue have been noted to be very active in phagocytosing the debris of cells damaged by x-irradiation (62, 514). In one study (445), x-ray exposures of up to 50,000 roentgens caused only a 15 per cent reduction of the engulfing capacity of isolated peritoneal macrophages. The migratory activity of macrophages is also quite radio-resistant (397). The capacity of phagocytes to replicate is, however, as radio-sensitive as that of any other cell population, and although only 1-5 per cent of a phagocyte population appears to be undergoing cell division, this could lead to a decrease in phagocytosis as a function of time following high doses of x rays.

58. In contrast, several reports have indicated that the phagocytic activity of animals can be reduced by whole-body irradiation. Several of these reports show impaired intravascular clearance of bacteria (82) or colloidal material (553) after whole-body irradiation, an impairment that can be considerably reduced by hepatic and splenic shielding during irradiation. Radiation-induced depression of phagocytic activity has also been demonstrated for macrophages from lung (646), intestinal wall (Fridenstein quoted in reference 689) and *in vitro* culture (689). Several reports (142, 196) indicate that a different tissue distribution of injected material may occur following radiation, without affecting the over-all phagocytic removal or rate of clearance. In a recent detailed study (482) the phagocytic activity of rats was significantly impaired after whole-body x-irradiation (800 R). The degree of depression was related to the post-irradiation time interval and was associated with a highly significant decrease in hepatic and splenic phagocytosis. In contrast, the lungs of the irradiated rats showed a significantly greater accumulation of the injected colloid.

59. Several early reports suggested that although no effects on actual phagocytosis or uptake of antigen by cells were induced by radiation, other subtle changes in the irradiated macrophages might occur. Donaldson *et al.* (147) and Kakurin (647) found that macrophages from irradiated animals had a depressed ability to digest intracellular material, and Gordon *et al.* (216) observed that reappearance of live organisms in the blood of irradiated rabbits occurred after a period of normal clearance, although Benacerraf *et al.* (41) found a normal breakdown of a denatured protein in the Kupffer cells of irradiated mice.

60. These results variously suggest that actual phagocytosis may in some instances be affected by irradiation (possibly mainly in liver and spleen) whereas in other cases, although the engulfment of material is normal after irradiation, changes in the normal intracellular digestion of the ingested material may occur as a result of radiation-induced enzymatic changes to the cell. These two stages will now be considered separately, in terms of the radio-sensitivity of phagocytosis as associated with opsonin changes, whereas changes in the actual fate of the ingested antigen will be considered in the light of the subsequent ability of macrophage-processed antigen to trigger the antibody response.

4. *Opsonins and immunoglobulins*

61. It is well established that serum or plasma factors, called opsonins, can augment the phagocytosis of both soluble and particulate material (479, 481). Accordingly, it is possible that depression of phagocytosis by radiation could be mediated through depression of opsonic activity or concentration. In some reports, sublethal irradiation has been shown to depress natural antibody formation with a rapid rate of decline of serum levels (550). This short half-life suggests that the globulins may have been IgM macroglobulins, which in some cases have been formally shown to be responsible for opsonic activity (469). Furthermore, there is mounting evidence that lymphocytic cells may synthesize small amounts of IgM molecules (56, 590, 604, 606, 607) which may be responsible for some or all of the serum opsonin. As these cells are relatively radio-sensitive, opsonic concentration in serum might thus be expected to decrease following radiation. The capacity of spleen cells for the total synthesis of IgG and IgM immunoglobulin was studied quantitatively with cells cultured *in vitro* (668). Mice received a dose of 500 rads and their spleens were extracted for culturing 1 to 12 days later. The rate of immunoglobulin synthesis was reduced by 80 per cent for a period of one to six days, but by the ninth day had over-compensated to a value of 70 per cent in excess of the control.

62. Decreased opsonin activity was proposed to be the most plausible mechanism for radiation-induced changes in follicular antigen uptake (624). Normal serum was shown significantly to improve follicular antigen uptake in irradiated animals as was specific antibody to the antigen. Shielding of the lymph node could lead to protection by either preventing direct damage of the cells, or by preserving some lymphoid cells which could continue to release opsonin. Furthermore, follicular localization of antigens is greatly accelerated by the passive transfer of specific antibody (417). The finding (250) that autologous immunoglobulins themselves tend to localize in follicles suggested that the opsonin coating might be a critical factor for follicular trapping. These results indicate that a serum factor, presumably specific antibody, whether "natural" or immune, is very important for follicular antigen localization.

63. In certain cases serum obtained from irradiated animals was slightly more effective in augmenting phagocytosis than normal serum (483, 490), and in another study radiation of young chickens failed to inhibit the production of natural opsonins (519). These apparent contradictions with other reports might indicate that generalizations are not valid in this context, and it might be proposed that, for some antigens and some species, the opsonic factor is produced by a long-lived, more radio-resistant cell, perhaps a mature non-dividing plasma cell.

64. In a detailed study (482) of rats in which the over-all clearance of a gelatinized ^{131}I-labelled triolein was shown to be reduced after irradiation and to be specifically associated with decreased splenic and hepatic phagocytosis, analysis of serum opsonins has been performed. Opsonization of the test material prior to its injection into irradiated rats significantly enhanced its clearance and therefore appeared to suggest a recovery of phagocytic activity. However, the enhanced clearance was found to be due to greater localization of the colloid in the bone marrow and no changes were found in liver or spleen. This emphasizes that even in the presence of elevated opsonic serum activity, x-irradiated animals can still manifest reticulo-endothelial depression in liver and spleen. This suggests that radiation-induced depression of phagocytosis in liver and spleen may be due to a direct effect on the macrophages in these sites. It also implies that different phagocytic cells may manifest variable radiosensitivities and therefore that their tissue distribution is required to analyse a given situation.

65. If all opsonin activity was associated with IgM macroglobulin, it might be expected in at least some cases to find lower IgM levels in irradiated animals. However, whereas IgG and IgA are depressed in mice exposed to 700 roentgens, IgM levels are virtually unaltered (38). Similar effects have been found when only the gut of mice was irradiated, whereas whole-body irradiation with the gut shielded caused only a slight change in serum immunoglobulins (37). Hence the characteristic early responses of the total serum-immunoglobulin levels to irradiation appear to result from x-ray damage to the intestinal epithelium and the consequent increased immunoglobulin secretion into the gut.

5. *Macrophage-antigen transfer studies*

66. The various reports described above have dealt primarily with specific studies of irradiated macrophages and their handling of antigens. In this section we will consider studies in which the process has been extended further in relation to immunity, and ask: in systems where macrophages are important for the elicitation of antibody formation, will irradiation of the macrophages interfere with the inductive phase of the immune response? This question can best be approached through the use of cell-transfer studies. Using radiation doses which will significantly suppress antibody formation in the whole animal, several groups have investigated whether normal macrophages will restore antibody-forming capacity to these animals.

Since cell suspensions are rarely completely homogeneous for a given cell type, it is important to control for possible contamination of macrophage preparations by immunocompetent lymphocytes.

67. Studies by Gallily and Feldmann (177) have indicated that the essential function of the macrophages in the induction of humoral-antibody formation can be destroyed with a sublethal exposure of 550 roentgens. Normal C57BL mice were given whole-body irradiation (550 R) and were found to be virtually incapable of making antibody to *Shigella* antigen. However, when macrophage preparations which had been pre-incubated *in vitro* with *Shigella* were given to irradiated mice, considerable antibody production then occurred. This activity was not due to contaminating lymphocytes since transfer of pure macrophage preparations also gave similar results and, if mice exposed to 900 roentgens were used as recipients, no restoration took place. In this latter case, if lymph-node cells were combined with the *Shigella*-treated macrophages and transferred to the irradiated recipient, antibody production could then occur.

68. A critical experiment was then performed with donor macrophages which were themselves derived from irradiated mice (table 2) (177). These were incubated *in vitro* with *Shigella* and transferred to recipients that had been exposed to 550 roentgens. Almost complete depression of the ability to transfer a capacity for antibody production occurred with irradiated (450 R) donors, and a significant depression occurred even with donors exposed to 150 roentgens. Macrophages irradiated *in vitro* and then incubated with *Shigella* also had lost the ability to aid in the induction of antibody formation. These results strongly indicate that with *Shigella* antigen, sublethal doses of irradiation will markedly interfere with induction of humoral-antibody formation as a result of a direct effect on an intracellular process (rather than inhibition of phagocytosis) of the macrophages.

69. A similar conclusion was reached by Pribnow and Silverman (465) who showed that both BCG-sensitized macrophages and normal lymph-node cells were required to restore antibody-forming capacity to rabbits exposed to 450 roentgens, whereas neither cell population alone would do so. It appears that in these rabbits 450 roentgens affected the lymphoid cells as well as the macrophages, whereas in Gallily and Feldmann's experiments with mice 550 roentgens did not sufficiently deplete the lymphoid component (compartment) but markedly affected the macrophages.

70. In some other studies, no radiation damage could be shown to the macrophages required for the induction of an antibody response. The critical difference may be solely in that a different antigen has been used. Ellis *et al.* (163) investigated the restoration of antibody response to sheep red blood cells in rats given different doses of x rays. Their results showed that even with lethal doses of radiation (1,000 rads), syngeneic lymphocytes were able to restore an impressive hæmolysin response in the irradiated animals, thus indicating radio-resistance of the host macrophage, as other studies have clearly indicated that macrophage processing or treatment of antigen is essential for the antibody response to sheep red cells (507). Gershon and Feldmann (201) investigated the response to sheep red cells in mice and could find no reconstitution of sublethally-irradiated mice with macrophage-ingested sheep red cells, again suggesting that another non-macrophage cell type had been acutely depressed by irradiation even with sublethal doses.

71. Mitchison (382, 383) has shown that a suspension of bovine serum albumin (BSA) containing macrophages is extremely efficient in priming mice for an antibody response to BSA, much more so than the free BSA. It was reported that the ability of this macrophage-bound BSA to prime mice was relatively radio-resistant. Spitznagel and Allison (523) also showed that macrophage-phagocytosed BSA (MBSA) is far more immunogenic for mice than comparable doses of free BSA. When the MBSA was given to mice exposed to 600 roentgens 24 hours previously, no antibody response occurred. If the recipients were also given 20 million normal lymph-node cells, good anti-BSA responses developed, suggesting that either MBSA can substitute for macrophage-processed antigen, or that only lymphoid depletion had occurred in the irradiated recipients and that macrophage activity is radio-resistant.

72. Although it is now quite clear that macrophages do play an important role in the induction of immune responses to many antigens, particularly in those cases involving large particulate antigens, the mechanism whereby they act is by no means elucidated. In view of the controversy in the literature on their radio-sensitivity, which in essence seems to say that for some antigens macrophages are very radio-sensitive and for others are very resistant, it is difficult to pinpoint a specific radio-sensitive stage in macrophage-antigen handling in general.

73. Various studies have recently indicated that RNA fractions from macrophages that have ingested antigens will transfer the ability to make antibody to normal lymphoid cells (183, 184, 185). In many cases this may be due to the presence of an antigen-RNA complex (25) containing minute amounts of antigen, which alone would not be immunogenic. The alternative possibility is that a true messenger RNA fraction coding for the specific antibody can be obtained from the macrophage preparation and transferred to normal lymphoid cells. Similar results have been obtained with mRNA fractions derived from macrophage-free lymphocyte preparations (12) and this raises the possibility that the mRNA fractions obtained from macrophages may in fact have been derived from a small contaminating population of lymphocytes. Such a possibility has been borne out in at least two reports, both involving allotype markers as evidence of transfer donor immunoglobulin to messenger (7, 39). Recently, Yamaguchi *et al.* (635) reported that a minimum dose of immunogenic RNA, which was derived from spleens of mice immunized with *Salmonella* flagellar antigens and was capable of transferring the immunity against the test antigen to normal mice, did not reveal an evidence of antigen contamination. In this study, it was shown that this immunogenic RNA fraction failed to initiate a secondary response to the test antigen when injected into animals that had been primed with immunogenic RNA or *Salmonella* flagella, while normal mice treated with immunogenic RNA were able to initiate a secondary response upon challenging injection of the test antigen. Regardless of the nature of the material presented by the macrophage to the lymphoid cell (free antigen, an antigenic fragment, an RNA-antigen complex or antigen-free RNA), there still remains the problem of how the material reaches the reactive lymphoid cell. In one study with hæmocyanin

(585) in which the material bound to macrophages was extremely immunogenic, it was proposed that the superior activity of the macrophage-bound antigen might be associated with a membrane-bound fraction which would have a far greater probability of contact with lymphoid cells, in much the same way as dendritic follicular cells are thought to interact with lymphoid cells. However, in experiments with larger hæmocyanin molecules, macrophage-associated antigen was less immunogenic than free antigen (443).

74. At the present time, the possible role of macrophage depression in reduction of the inductive phase of the immune response with relatively low doses of radiation appears to be uncertain. Since its importance has been strikingly demonstrated in at least one system (177), which is perhaps the most closely related to human resistance to infection of all the experimental systems studied, further studies with many different antigens, particularly bacterial antigens or organisms, rather than "laboratory antigens" such as heterologous serum proteins and erythrocytes, should be made in order to determine whether antigen processing by macrophages is a radio-sensitive phase which might account for radiation-induced depression of the immune response to many antigens.

B. The inductive phase of the antibody response

75. The antibody-forming plasma cell is a highly differentiated cell with the major function of secreting antibody and having virtually no prospect for further division. As will be discussed later, this cell is relatively radio-resistant. However, it is clear from a large body of data on the suppressive effect of radiation on antibody formation that there are earlier stages before the formation of the actual antibody-forming cell which are acutely radio-sensitive. In this section we will consider the origin of the antibody-forming cells and the radio-sensitivity of these precursor cells at stages before and after antigen-induced differentiation. Recent evidence of a collaboration between two cell types in the induction of many humoral-antibody responses has been obtained (84, 85, 122, 371, 384), and it is therefore most important to consider separately the radio-sensitivity of these two components. However, as most of the literature on radiation sensitivity of primary antibody formation was produced prior to the formulation of this recent collaboration concept, only a general consideration of this separation will be possible. Before discussing the actual radio-sensitivity of the early antibody response, it is relevant to consider briefly the origin of the immunocompetent cells.

1. *Radiation and the genesis of the immunocompetent cells*

76. In following the complete lineage of the antibody-forming cell, there is a striking demarcation into two stages. These are illustrated in figure II and are functionally distinguished as pre- and post-antigenic stimulation. In this section we are concerned with the radio-sensitivity of the precursor cells which have not yet been confronted with antigen. The true self-perpetuating cells, the hæmatopoietic stem cells, reside principally in the fœtal liver and in the bone marrow in adult animals and to a lesser extent in the spleen. Cells then travel via the circulation and may enter the thymus (390). In a manner which is as yet not entirely elucidated, these stem cells are induced to differentiate along the lymphoidal line and are thus rendered immunocompetent. A similar process (103, 389, 604) occurs with stem cells which enter the bursa of Fabricius in chickens, and the as yet unidentified bursal equivalent in man and other mammals. However, in this latter instance, differentiation into an immunocompetent cell involves the synthesis and expression of IgM molecules on the cell membrane (103, 566, 606). This IgM molecule may act as the recognition unit for antigen (45, 100, 468, 535, 539, 606). Particularly during early life (414), but also to a lesser extent throughout later life (311, 618), the potentially-immunocompetent cells then leave the thymus or bursa (or its equivalent) and form the recirculating pool of lymphoid cells (219) that move from peripheral lymphatic tissue via the lymph into the circulation and back into the lymphoid tissue. It is at this latter level that antigenic stimulation occurs and induces the formation of the true immunocytes, the effector lymphocyte of cellular immunity, and the antibody-producing cell. Since proliferating cells are the most susceptible to radiation destruction, three levels of acute radiation-sensitivity are suggested in this differentiation scheme.

77. The first level is represented by the hæmatopoietic stem cell which is capable of repopulating the bone marrow, the thymus and ultimately the peripheral lymphoid tissue of irradiated animals (363). This cell type is self-perpetuating, as has been shown by the *in vivo* colony-forming assay of Till and McCulloch (569), and is extremely radio-sensitive, with a D_{37} of around 95 rads (351, 511). Lethally-irradiated mice can be restored by injections of hæmatopoietic cells derived from *in vivo* hæmatopoietic colonies (571) and these recipient animals will eventually regain the capacity for humoral-antibody formation. However, as implied in figure III, recovery of immunocompe-

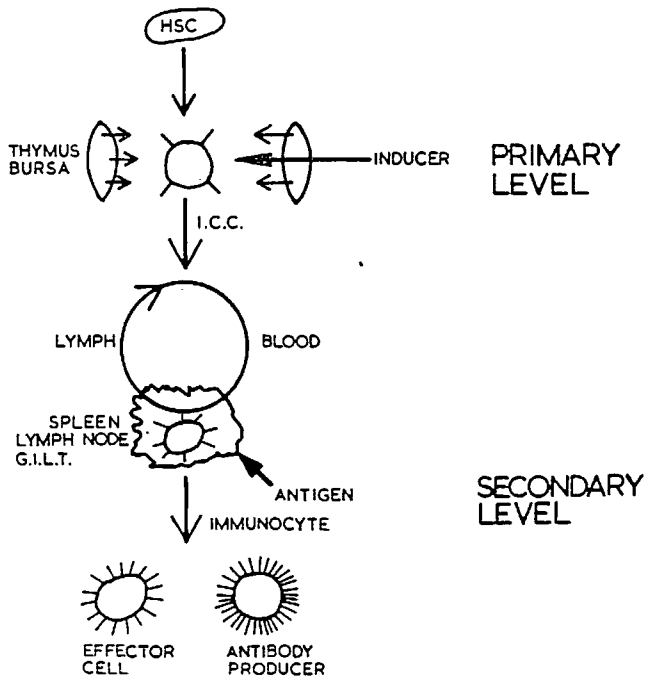

Figure III. Genesis of immunocytes. Hæmopoietic stem cells (HSC) are first directed toward immune pathways by inducing agents in primary lymphoid organs. They then emerge as immunocompetent cells (ICC) which enter the lymphocyte recirculation route passing through the lymphoid organs (including gastro-intestinal lymphoid tissue G.I.L.T.) from blood to lymph and back. It is in these peripheral organs that the second antigen-directed level of differentiation takes place, leading to the development of the antibody-producer or the effector lymphocyte of cellular immunity

tence after irradiation and injection of hæmatopoietic stem cells will only occur if the host animal has an intact thymus gland or a source of thymic inducer (114, 207).

78. The second stage of proliferation involves the lymphoid cells within the thymus (358) and the bursa of Fabricius which have been derived from the proliferating stem cell. X-irradiation causes a rapid involution of the thymus, and was in fact once used as a treatment for the spurious "*status thymolymphaticus*", a condition observed when a large thymus shadow was seen in the chest x-ray of a child (58). This procedure is not only of no benefit, since it is now well realized that the thymus is normally at its largest size in early life (358), but is actually dangerous as some irradiation of the adjacent tissue may lead to development of malignancy (see annex H). X-irradiation of the human thymus causes a rapid thymic involution and shrinkage, with regrowth occurring within a week (70). This effect not only involves a depression of the relatively-high proportion of dividing cells in the thymus, but also a direct lymphocytolysis of thymic lymphocytes (148). The stress of x-irradiation, resulting in an increased cortisol secretion (439), which is known to rapidly lead to thymic-lymphocyte destruction, makes a small contribution to this process.

79. This indirect action of x-irradiation on the adrenal gland leading to some steroid release might possibly affect the immune response more directly (apart from thymic cell destruction). Several studies (36) have demonstrated the sensitivity of antibody production to corticosteroids, and this has also been demonstrated recently *in vitro* (238), with the thymus-derived lymphocytes possibly representing the target for this effect (see also paragraph 298).

80. The third stage of active cell proliferation comes after presentation of the antigen to the immunocompetent cell. This rapidly leads to cell proliferation and accordingly to radio-sensitivity of this phase. This aspect will be considered in two sections: (*a*) the radiation sensitivity data, in which the immune response as a whole is discussed, and (*b*) the limited data available on the recently-demarcated two-component cell collaboration in antibody formation.

81. Radiation can therefore affect the differentiation sequence at three main points of cell proliferation: the hæmatopoietic stem cells; the early-differentiated cells in the thymus; and the antigen-stimulated immunocompetent cells. As all of these cell types may look morphologically like small lymphocytes, examination of the radio-sensitivity of lymphocytes as a distinct morphologically-defined population does not permit a clear demarcation of possible differential radio-sensitivities in these three compartments.

2. *Radio-sensitivity of the early primary immune response*

82. One of the most radio-sensitive phases of the immune response appears to be associated with the process of early induction (415, 546). Many authors (75, 145, 332, 333, 516, 532, 544, 673, 688) have reported on measurements of the radiation sensitivity of the antibody response, and, although somewhat different systems were studied in each case, their radiation sensitivities were similar and clearly indicated that cell proliferation must be an essential feature of the early immune response (330, 333). As it would be redundant to consider all the reports on radio-sensitivity of the early antibody formation (reviews in references 332, 531, 534, 540, 550, 551, 673, 688), we will consider in some detail only a few cases which clearly demonstrate the magnitude of the radio-sensitivity of the early phase.

83. The existence of an early radio-sensitive phase which rapidly moves into a radio-resistant phase was clearly shown by Dixon *et al.* (145) who irradiated rabbits two days prior to the injection of ^{131}I-BGG. A slight inhibition of the antibody response was observed with exposures of 75 roentgens or less, whereas 125 roentgens resulted in a considerable depression and 200-300 roentgens prevented the formation of all but traces of detectable antibody.

84. Makinodan *et al.* (333) tested the ability of spleen cells transplanted into lethally-irradiated mice (800-900 R) to produce hæmagglutinin against sheep erythrocytes when the donor spleen cells were derived from mice which themselves had been subjected to varying doses of radiation three hours before preparing the cell suspensions. The results showed that 37 per cent of the original antibody-forming activity remained after 130 roentgens. Based on the straight-line portion of the inactivation curve, the D_0 value was calculated to be 70 rads. Using a somewhat similar system, Celada and Carter (75) obtained a value of approximately 47-57 rads for this parameter. The immunization of mice with sheep erythrocytes one day after irradiation with an $LD_{50/30}$ reduces to 1 per cent of normal the number of antibody-producing cells accumulated in the spleen (662a, 676). Determination of the dose-effect relationship for spleen cells irradiated *in vitro* and subsequently placed *in vivo* together with sheep erythrocytes yielded a value of D_0=125 rads for the case n=1 (692). The radiation inactivation of immunity as shown by Makinodan *et al.* (333) and Simic *et al.* (510) is graphically represented in figure IV. As

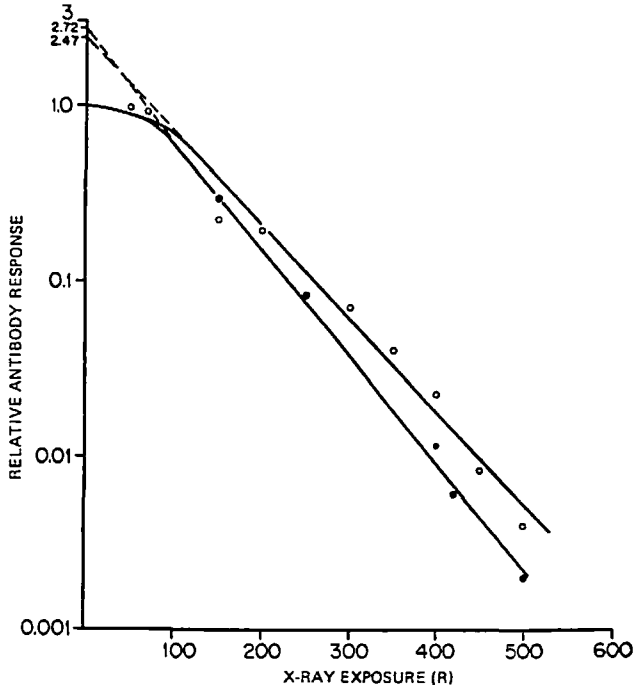

Figure IV. Inactivation of the immune response in rats (510) (open circles) and mice (333) (solid circles). Spleen-cell suspensions from donor mice given the indicated x-ray exposures were transferred to lethally-irradiated syngeneic recipients together with antigen. The resulting immune response is plotted relative to that given by control unirradiated donors

emphasized above, these data strongly indicate that the most radio-sensitive cellular event in the initiation of an antibody response is cell proliferation.

85. In several other biological systems (28, 46, 112, 459, 525, 652), it has been claimed that resistance to radiation is increased in animals repeatedly exposed to ionizing radiation. The population of immunocompetent cells in the body is one that is in a state of flux, showing a continual exponential increase until young adulthood, followed by a decrease with advancing age (335). If the concept of increased radio-resistance after pre-irradiation were to apply to this cell population, it would imply either that cells in a state of flux are more radio-resistant or that their capacity to repair radiation-induced damage is more efficient.

86. In an analysis of this possibility, Petrov and Cheredeev (454) studied the radio-sensitivity of splenic lymphoid cells derived either from normal spleens or from mice which had been given a whole-body dose of 500 rads 14 days previously. In each case, samples of the lymphoid cells were irradiated *in vitro* and transferred to irradiated recipients together with antigen. The immunocompetence of the population was then assessed by the resulting numbers of plaque-forming cells six days later. The dose-effect curve for the population of spleen cells taken from the pre-irradiated mice is characterized by D_0=220 rad (D_{37}=325 rad) and n=10.2. For the normal spleen cells, D_0=188.3 rad (D_{37}=125 rad) and n= 0.8. This study therefore appears to indicate the induction of radio-resistance in lymphoid cells by pre-irradiation. A subsequent study (466), however, did not confirm this observation, although in this study whole-body irradiation of only 250 rads was used. Price and Makinodan (466) suggested that the results obtained in the former study might be related to other observations (662a) which show that the recovery of a normal splenic lymphoid population is a slow process, only partly completed in 30 days.

87. In more recent studies (455), the basic observation of Petrov and Cheredeev (454) has been confirmed, but it has also been found that it can be abolished by the prior addition of normal lymph-node cells to the pre-irradiated spleen cells. This suggests that the pre-irradiated spleen contains limiting numbers of radio-resistant lymphocytes which must then collaborate with the actual antibody-forming-cell precursors or with the progeny of hæmatopoietic stem cells. As the latter are in great excess in the spleen 14 days after receiving 500 rads (but not nearly as much in spleens after 250 rads), considerable reduction in their number by the second radiation treatment can occur without reducing the actual level of immunocompetence, which is dictated by the limiting number of lymphoid cells, possibly of the thymic-derived type. The important conclusion is that these experiments still do not prove that pre-irradiation induces radio-resistance in the cell lineage of the antibody precursor.

88. In most of the studies on radio-sensitivity of the humoral-antibody response, the methods used involved estimation of the amount of specific antibody present in the serum of animals following exposure to known doses of radiation. However, it is by no means certain that an assay for a serum antibody will give a value that is directly proportional to the number of surviving cells producing this antibody, unless it is first demonstrated that the doses of radiation employed have an all-or-none effect on the rate of production of antibody by individual cells, and that irradiation affects neither the rate of removal of antibody from the circulation nor the concentration of various serum factors which might alter the sensitivity of the assay. These objections apply mainly to whole-body irradiation studies rather than to the cell-transfer model of Makinodan. Recently, several techniques (120, 265, 273) have been developed which circumvent these problems by readily permitting the enumeration of antibody-releasing cells in a cell suspension.

89. This approach was used by Kennedy *et al.* (286) in a study of the radiation sensitivity of the ability of normal mice to respond to sheep erythrocytes. A typical result is shown in figure V, in which

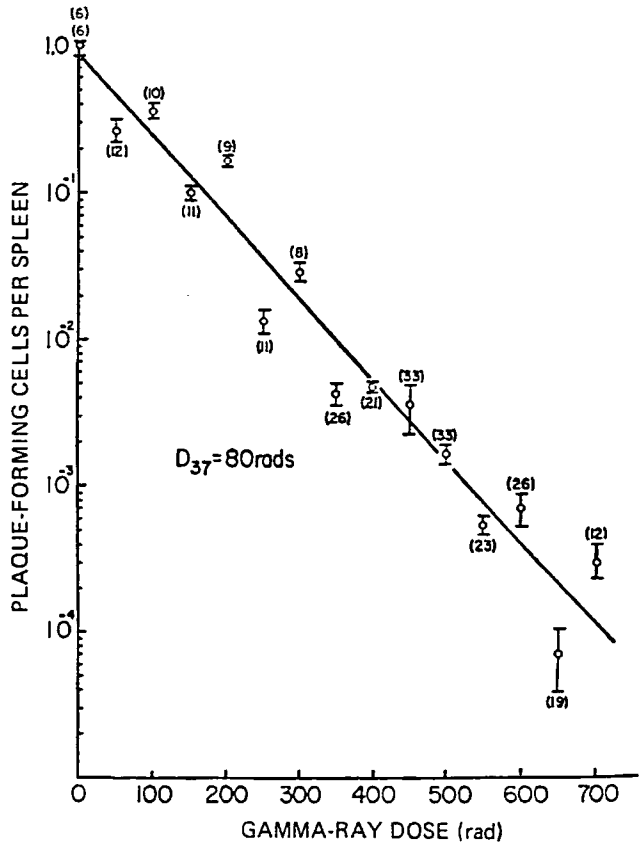

Figure V. Plaque-forming ability of the mouse as a function of radiation dose (286) when antigen (4 10^8 sheep erythrocytes) was given 10 days after irradiation and assays for plaque-forming spleen cells were made 4 days later. The results are plotted relative to the plaque-forming response of control unirradiated mice (control value 2.4 10^4 plaque-forming cells per spleen)

the plaque-forming ability of the mouse is shown as a function of the radiation dose given two hours before the injection of antigen. A D_{37} value of approximately 80 rads has been found in this experiment. The parameters of this curve were found to be unchanged for two different doses of antigen and for two different time intervals between antigen injection and assay for plaque-forming cells. These results therefore indicated that no significant repair of radiation damage to the plaque-forming cell system had occurred during the 2-hour to 10-day interval following irradiation. In a few experiments in which antigen was given 17 or 24 days after irradiation, some recovery of immune capacity was observed.

90. The D_{37} values reported by these various studies are all in the vicinity of 50-100 rads. This degree of radio-sensitivity suggests that it depends on continued cell proliferation because no cellular process other than proliferation is known which shows a radio-sensitivity of this order although interphase death of lymphocytes may also be of some importance (see paragraph 152). This interpretation is based on studies of the type reported by Puck and Marcus (467) and by others (251, 568). The effects of x-irradiation were quantitatively studied by Puck and Marcus with single cells of a human cervical carcinoma (*HeLa*) grown under conditions in which 100 per cent of the unirradiated cells reproduced in isolation to form macroscopic colonies. Survival of single cells (defined by the ability to form a macroscopic colony within 15 days) yielded a typical two-hit curve when plotted against x-ray dose. The exposure needed to reduce survivors to 37 per cent was 96 roentgens. This radiation sensitivity is tens to hundreds of times greater than that of any micro-organism studied in similar manner.

91. In considering the high radio-sensitivity of the early response, Kennedy *et al.* (286) suggested that a relatively small number of cells normally present in the mouse give rise by proliferation and differentiation to the large number of plaque-forming cells at the height of the immune response. The survival curves indicated that for values of fractional survival of 0.001 or more there was still enough residual immune capability for the system to react to an injection of antigen with the formation of antibody. In other words, the immune system could suffer at least a thousand-fold depletion of the proliferative capacity of its cells without completely losing its capacity to respond to antigen by the production of plaque-forming cells. This may not be true for all antigens, as it will depend on the number of precursor cells for the appropriate antigen.

92. Various investigations have compared the radio-sensitivity of the IgM *versus* the IgG humoral response. In general terms, as the IgG response usually appears later in time than the IgM, it might be expected to be more radio-resistant (as radio-resistance of the immune response as a whole appears to increase with time). Alternatively though, if the earlier IgM response were essential for the development of IgG response, the IgG response might indirectly appear more radio-sensitive than the IgM. In an examination of this problem, all combinations have in fact been found experimentally and will be briefly considered.

93. Several groups (406, 472, 541) have reported that the IgM response is more radio-resistant than the IgG response. Whole-body x-irradiation was administered to rabbits 20 hours prior to antigenic stimulation with polio virus. Antibody formation in rabbits exposed to 600-650 roentgens showed (541) a delay in IgM-antibody formation with a lower but more persistent titre than controls, and virtually-complete inhibition of IgG-antibody synthesis over a period of 2½ months after antigen.

94. The effect of x-irradiation on the sequential formation of immune globulins was studied (472) using flagellar antigens in rabbits given increasing whole-body doses. A delay in the appearance of IgG antibody was observed, whereas only a slight diminution in the timing or amount of IgM antibody was noted. In another study (406) on the regenerative potential of the immune response after irradiation, a preferential suppression of the IgG antibody was observed in mice exposed to 200, 400 or 600 roentgens. That is, the capacity to form 7S antibody was more heavily suppressed than the capacity to form 19S antibody, and this preferential suppression persisted throughout the recovery phase after irradiation. This was even more striking in thymectomized mice given 850 roentgens and isologous bone marrow, in which recovery of the 7S-antibody response was virtually abolished whereas the 19S response recovered substantially. X-irradiation may impair conditions necessary for the differentiation of 7S-producing cells, possibly by damaging the mechanism of antigen retention in lymphatic-tissue germinal centres, as discussed previously. These centres do in fact seem to be more closely related to the production of 7S antibody in the primary response (234). Alternatively, the IgM and IgG response may involve totally separate progenitor cells, and the IgM progenitors might either be more numerous or more radio-resistant than those of the IgG progenitors. Indeed, evidence consistent with this interpretation has been reported by Shearer *et al.* (501). These investigators considered that precursors of IgM and IgG plaque-forming cells are distinct populations and that the frequency of the former population in the normal spleen of a mouse is seven times higher than that of the latter. However, several studies (308, 424) have suggested that the IgM-IgG line is a single differentiating line of cells.

95. In a study with *Salmonella* antigen in rats (272), both the IgM and the IgG phases of the primary response were markedly inhibited by 450 roentgens given a day before antigen, and both phases could be restored by an injection of 200 microgrammes of Colchicine given at the same time as the antigen. The final possible combination of radio-sensitivity was reported by Berlin (44) who irradiated mice one to four days before immunization with influenza vaccine and observed markedly low IgM-antibody titres and a high degree of sensitivity of IgM antibody, as indicated by a D_{37} of 74 rads.

96. This latter value indicates that the radio-sensitivity of IgM cells is of the same order as that of the over-all immune response previously mentioned. Since all the data mentioned on greater sensitivity of the IgG response have been concerned with measuring IgG titres in sera obtained from irradiated animals, it should be noted from earlier discussion that, following irradiation, the IgG globulin is more selectively lost from serum than the IgM antibody. Accordingly, a greater radio-sensitivity of the IgG response might be falsely deduced from measurements of serum titres. A complete solution of this problem will again require the use of a direct cell-plaque estimation technique which is capable of determining both IgM and IgG plaque-forming cells following varying doses of radiation.

97. The effect of internal irradiation delivered from intravenously administered radio-active (^{32}P) colloidal chromic phosphate on the primary immune response of rabbits to sheep erythrocytes and typhoid antigen has been studied (636). When 14 days of internal irradiation from 520, 624 and 780 microcuries preceded a single immunizing injection, antibody responses to both antigens were significantly depressed, as shown by delayed appearance of antibody, decreased antibody synthesis rates, and lowered maximum antibody titres.

Splenic participation in the immune response of rabbits given 488 microcuries or more was judged non-operative. The spleens of these rabbits were estimated to have absorbed 7,000 to 14,000 rads during the 14 days preceding immunization. This result therefore parallels the marked suppression of primary antibody response with a single antigen injection after splenectomy in rabbits (545). In both cases, the impairment of antibody production could be corrected by the use of multiple antigen injections, which would induce the participation of non-splenic sites in antibody production. This might well indicate that intravenously administered radio-active colloid primarily affects the lymphoid tissue of the spleen, and has less effect on the circulating lymphocyte pool.

3. *Cell collaboration in the humoral immune response*

98. The irradiation studies described above indicate that cell proliferation is an early event following antigenic stimulation. The simplest view would be that the immunocompetent cell is stimulated by antigen (perhaps *via* macrophage) and directly proliferates and differentiates to become the antibody-forming cell (328, 423). Clonal expansion has been directly demonstrated in studies by Playfair *et al.* (458) and Kennedy *et al.* (287). These workers devised a method for the enumeration and characterization of cells which, on appropriate antigen stimulation, could produce a clone of antibody-forming cells. These they termed the "antigen-sensitive cells". They are detected by the injection of normal lymphoid cells into a lethally-irradiated animal. A proportion of the injected cells reach the spleen and settle there. A stimulus of antigen (sheep erythrocytes) then triggers a certain specific proportion of the injected cells to proliferate and differentiate into antibody-forming cells. Provided a small enough inoculum is given, these form discrete areas in the recipient spleen, which can be detected by laying thin slices of the spleen on agar containing the antigenic red cells. After allowing for diffusion of antibody and attachment to the red cells, hæmolysis is induced by the addition of complement. This system therefore appears to show a direct proliferation of cells following antigenic stimulation, because most studies are compatible with the concept that a single cell is the progenitor of the clone and ultimately produces many antibody-forming cells. It does not, however, establish that the cells that initially react with antigen are the ones that give rise to the clones observed. In fact, the "simplest" view described above has been controverted by later work demonstrating cell interaction in immune responses, interactions that produce specific second-order effects on the cells whose progeny eventually produce the antibody.

99. Collaboration between thymus or thymus-derived lymphocytes present in thoracic-duct lymph, and non-thymus-derived precursors of antibody-forming cells has been implicated in the immune response of mice to sheep erythrocytes (84, 85, 122, 371, 372). Neonatal thymectomy impairs the response of mice to sheep erythrocytes. This can be reversed by inoculating thymus or thoracic-duct lymphocytes simultaneously with the red cells (370). In this system, the identity of the antibody-forming cells was determined by using anti-H-2 sera in allogeneically-reconstituted hosts and chromosome-marker analysis in a syngeneic system (421). These techniques demonstrated that the antibody-forming cells were in general derived *not* from the inoculated lymphocytes, but from cells already present in the thymectomized hosts. In an attempt to identify the origin of the true precursor of the antibody-forming cells, a synergistic effect between thymus and bone-marrow cells on transfer into lethally-irradiated mice was demonstrated (85, 378). By means of a chromosome marker it was again shown (421) that all the antibody-forming cells produced were derived from the bone marrow. These series of experiments therefore indicated that, at least with some antigens, collaboration of thymus-derived cells with bone-marrow-derived cells is required to initiate the antibody response.

100. Although more recent studies (366) have further extended the list of antigens for which cell collaboration seems to be essential for antibody production, it is doubtful that this is an obligatory phenomenon for the initiation of all antibody responses. For example, current data would perhaps suggest that, although cell collaboration is important for heterologous antigens such as gamma globulin, albumins, erythrocytes and some haptens, it may not be involved in responses to many bacterial antigens. Recent studies with a congenitally-athymic strain of mouse have clearly shown that whereas this strain is quite incapable of making antibodies to heterologous erythrocytes, normal IgM-antibody production to several bacterial antigens occurs, although IgG-antibody responses are considerably depressed (110).

101. Various studies have demonstrated that although the thymus-derived cells do not become the actual antibody-producing cells, they do directly proliferate in response to antigenic stimulation. By the use of chromosomally-marked cells, Davies *et al.* (123) have demonstrated that thymus-derived cells will directly proliferate in response to either sheep red cells or an allogeneic skin graft, and will not become the antibody-forming cells (124). This has been extended in another study (500) showing by means of a limiting dilution assay that proliferation of the thymus-derived cell produces more cells which can collaborate with bone-marrow-derived cells and induce the latter into antibody formation. Similarly, using tritiated-uridine or thymidine markers, a proportion of injected thymus cells was observed (371) to transform directly under antigenic stimulation into blast-like pyronophilic cells which then divided into smaller lymphocyte-like cells. To confirm this interpretation, Koller *et al.* (295) assessed whether any significant frequency of mitosis would follow the antigenic stimulation of immunologically-incompetent (thymectomized) mice. Although the results are rather sparse, they do indicate that nearly all the mitoses seen in lymphoid sites after antigenic stimulation of thymus-grafted mice were indeed dependent upon the immunocompetence of the injected animal. This might be interpreted as indicating that the bone-marrow-derived cell does not proliferate unless it is somehow "stimulated" by the antigenically-stimulated thymus-derived cell. This interpretation would imply that a thymus-derived cell is the only cell type directly stimulated to proliferate by antigenic challenge. If so, this cell would represent a major radio-sensitive cell type involved in the radiation suppression of the inductive phase of the antibody response.

102. Studies on cell-to-cell interaction in the initiation of humoral-antibody responses in rabbits have given somewhat different results. In this species, antibody response to sheep erythrocytes of animals treated

with 800 rads can be restored by injection of allogeneic bone marrow from normal rabbits (2). Furthermore, with the use of allotypic markers of immunoglobulins, it was shown that the antibody-forming cells are derived from the irradiated host and not from the donor marrow (470). These results suggested that antibody-forming precursor cells are relatively radioresistant, while antigen-reactive cells which, in the rabbit, are found in bone marrow (1) are more radiosensitive. Several other alternative explanations might be given, however. As allogeneic marrow was used, it is possible that augmentation might result from a graft-*versus*-host reaction as has been proposed recently (283). It is also possible that the peripheral tissues of rabbits contain different proportions of thymic-derived lymphocytes than mice, and therefore that restoration is made by *T* lymphocytes in the bone marrow.

103. Several experiments have indicated that the lymphoid cells that reside in the thymus are very radiosensitive whereas the supporting thymic epithelial cells are not. The recovery of thymic epithelium after irradiation is the major thymic factor in effecting the full recovery of immunocompetence in the animal. As mentioned previously, virtually all thymocytes are destroyed by an x-ray exposure of 500 roentgens (573), leaving a residual stroma of reticular epithelial cells. Morphological observations indicate that these latter cells are resistant to exposures as high as 5,000 roentgens. Although several studies show that the irradiated thymus is capable of some lymphoid regeneration (within 3-4 days after 400 roentgens (166), within 1-2 weeks after 500 roentgens (367) and within 2 weeks after 850 roentgens (114)), normal regeneration is grossly impaired after exposures of 2,000 roentgens. Although lymphoid regeneration in thymus grafts exposed to 2,000 roentgens *in vitro* was observed after 11 days (154), two other studies (50, 125) showed that, despite almost normal lymphoid repopulation, the functional activity inducing immunological competence had not returned after three weeks.

104. A more direct evaluation of the radio-sensitivity of thymus-derived and non-thymus-derived cells would be to irradiate *in vitro* either suspension separately and then to attempt cell-collaboration experiments with the other cell type being unirradiated. Several recent reports on this type of experiment appear to give conflicting results. Claman and Chaperon (84) found that in thymus-marrow synergism in mice, both cell populations are sensitive to irradiation. Miller and Mitchell (370) also showed suppression of thymus-cell induction of antibody formation in bone-marrow-derived cells when the thymus cells were exposed *in vitro* to 1,000 roentgens. In contrast to this report, Goldie and Osoba (212) have reported synergism between heavily-irradiated (up to 2,500 rads) and non-irradiated normal spleen or lymph-node cells of the mouse in the development of plaque-forming cells to sheep erythrocytes *in vitro*.

105. It has been amply demonstrated that in the adoptive secondary response to hapten-protein conjugates in mice, co-operative interactions are mediated by hapten-specific and carrier-specific lymphoid cells (384). More recent observations (366) have confirmed that these correspond to bone-marrow and thymic-derived cells, respectively. Using this hapten-specific and carrier-specific cell-interaction system, Katz *et al.* (282) have studied the radio-sensitivity of the carrier-primed cells in guinea-pigs. The transfer of lymphoid cells, from strain-2 guinea-pigs immunized to bovine gamma globulin (carrier cells) into syngeneic recipients immunized with dinitrophenyl ovalbumin, was found to enhance markedly the recipient's secondary anti-dinitrophenyl response to challenge with dinitrophenyl bovine gamma globulin. This function of the carrier bovine gamma-globulin-specific cells was found to be resistant to 5,000 rads. However, the capacity to transfer immunological memory to bovine gamma globulin or to be stimulated by antigen to synthesize DNA *in vitro* was abolished by as little as 500 rads.

106. Similar results have also been obtained with an *in vitro* system (288). A primary immune response of normal spleen cells to trinitrophenylated sheep erythrocytes (TnpRBC) was studied *in vitro* and the number of anti-Tnp plaque-forming cells was determined. The number observed could be greatly enhanced by prior inmunization of the donor spleen *in vivo* with the carrier erythrocytes, or by using normal unprimed spleen cells in combination with spleen cells from mice that were immunized to the carrier erythrocytes. If these latter added carrier-primed cells were first treated *in vitro* with 1,000 or 4,000 rads before their addition to the normal spleen cells, they were still capable of enhancing the anti-Tnp response of the normal spleen cells. The immune response of the carrier cells themselves to erythrocytes was totally abolished by the irradiation. This observation also therefore demonstrates radio-resistance of thymus-derived helper cells.

107. These studies clearly indicate that in the transfer of immunological memory, where cell division is required, irradiation will abolish this function. However, in a primed system, reactive carrier cells are clearly able to co-operate with hapten-specific cells, without the need for division of the reactive carrier cells. This may therefore entail a presentation of the antigen by the carrier-primed cell to the hapten-specific cell, a task which can satisfactorily be performed by a lethally-irradiated cell. It is also possible that the reactive carrier cells may normally continue to divide, but that helper activity is needed only briefly at the initiation of the response. The experiments in which thymic cell function was destroyed by irradiation all involve primary immune responses. In this situation the virgin thymic cell on confrontation with antigen must proliferate in order to collaborate, and this is therefore a radio-sensitive step.

108. Although no direct data on radio-sensitivity in terms of collaboration potential have been obtained for the bone-marrow compartment, some data may be cited from avian studies. This is based on the view that the mammalian bone-marrow-derived cell is in effect "bursa-differentiated". The bursa is the primordial site of origin for cells that synthesize immunoglobulins in birds and the immunoglobulin specificity in the antibody-forming cell of mammals is of bone-marrow-type origin (270). Although embryonic bursectomy by hormones or surgery will totally prevent all potential antibody and immunoglobulin synthesis (102, 612), surgical bursectomy at hatch is not as effective in this respect. This is presumed to be due to the movement of bursal cells into peripheral tissues prior to hatching (103). If sublethal whole-body irradiation is given to newly hatched bursectomized chickens, much greater immunodepression is observed, even with doses of 250 rads (106). This suggests considerable radio-sensitivity in this cell line, although

the number of cells available in the periphery may only be very small at this stage, even in the normal animal. If the bursa of Fabricius is exposed *in vivo* to 1,000 roentgens at one and seven days of life (613) massive destruction of the bursal lymphoid follicles occurs without eventual normal regeneration. A diminished antibody response then results in most birds. Further studies are clearly needed to define the radiosensitivity of the bone-marrow component, and in chickens to confirm whether bursal cells play this role.

4. *Timing of irradiation and antigenic challenge*

109. The effect of irradiation on the immune response can be studied when the antigen is given before, at the time of, or after irradiation. In the latter case, where antigen follows irradiation, immunodepression is usually observed. The studies on radio-sensitivity of the inductive phase described above all deal with antigen given within a fairly short time after irradiation, namely, up to a few days later. When antigen is given many days or weeks after irradiation, this in essence is a study of the regeneration of the immunocompetent population and depends on many factors including stem-cell differentiation.

110. As discussed above, regeneration of the immune response appears to be thymus dependent, at least for certain antigens. Thymectomized, lethally-irradiated, bone-marrow-protected mice will respond only poorly to many antigens given even months after the irradiation (368, 406). The primary antibody-forming potential recovers very slowly from irradiation and this process does not require the presence of antigen, although impairment of the antigen-retention mechanism may be a factor in the delay of recovery of expression of immunity (403). In cell-transfer studies, the size of the immunocompetent pool was shown to be reduced for three to five months after sublethal x-ray exposure. It is not completely clear whether the renewal of the immunocompetent cell pool is partly due to a self-renewal system normally maintained in a steady state which slowly recovers after irradiation, or whether new competent cells are formed by differentiation from true progenitor cells. Some evidence (542) suggests that the resting antigen-reactive cell is not a rapidly dividing cell, as massive doses of the mitotic poison vinblastine yield only a slight reduction in the numbers of antigen-reactive cells. Thus most of these cells are in the G_0 state and are rapidly induced to divide by antigen. Although the relatively large amount of data on the importance of the thymus for the regeneration of immunocompetence tends to suggest a major role of differentiation of stem cells to immunocompetent precursors, the removal of the thymus does not totally deprive the animal of its capacity to regain immunocompetence even after severe suppression. Thus the recovery in thymectomized mice or rats given 400-600 roentgens was only moderately retarded in comparison with non-thymectomized irradiated animals (9, 153, 406). After higher exposures (850 or 500 + 500 R) the effect of thymectomy is much more marked, but nevertheless still not absolute, as particularly 19S responses still eventually recover (406). It therefore appears possible that in the absence of the thymus some regeneration of the immunocompetent cells (*x* cells or *PCl* cells) (444, 499) might occur from other surviving *x* cells, or from a more primitive precursor pool.

111. The classical studies of Taliaferro *et al.* (550) have clearly revealed that the timing of irradiation relative to the injection of antigen is of crucial importance in determining the amount of antibody eventually produced by an animal. When antigen was given prior to x-irradiation, an actual increase in the titre of antibody produced by the animal was noted. With an x-ray exposure of 500 roentgens given two days to two hours after the antigen, enhanced peak titres were the rule, though the latent period was lengthened. If, on the other hand, antigen was given one hour after irradiation, there was a slight inhibition, whereas the response to antigen was drastically inhibited if the injection took place 24 hours after irradiation. On the basis of these and other studies, Taliaferro proposed two types of radiation-induced enhancement. In the first type, seen with x-ray exposures between 25 and 300 roentgens given two days to two hours after injection, there is a heightened peak titre accompanied by a shortened latent period, and an abnormally high rate of antibody synthesis. In the second type, observed with exposures from 500 to 700 roentgens also after injection, there is an increased peak titre, but a lengthened latent period and a slower rise to peak titre.

112. Since the original observations of this phenomenon (338, 547, 549), many other workers have amply confirmed radiation-induced enhancement of antibody formation. Perhaps one of the most important of these studies was a detailed analysis by Dixon and McConahey (144). Before considering this in depth, mention of a few other confirmatory reports will be made.

113. A series of rabbits were given diphtheria toxoid, followed by x-irradiation (850 R) one, two or four days after injection (664). The synthesis of antibody took place in a large number of cells that were present for significantly longer periods of time than in unirradiated, immunized rabbits. In the early periods after irradiation, there was a tendency towards a reduction in the proportion of young forms of cells containing antibody. From the eighth to the fourteenth day after immunization, irradiated animals showed considerably greater numbers of young forms of antibody-containing cells than unirradiated animals in the same periods after immunization. After irradiation, the synthesis of antibody took place in the same types of cells as in unirradiated animals, although degenerative changes in the nucleus and protoplasm were seen in a large percentage of cells and the amount of antibody in the cell was altered. The percentage of lymphocyte-like cells in the antibody-forming population was considerably increased. These results are in contrast to a similar morphological study (665) made when antigen was given *after* irradiation, and somewhat opposite cellular changes were observed.

114. Antibody formation was analysed by the plaque method in mice treated with a dose of 660 rads one to two days after immunization with sheep erythrocytes. It was shown that exposure to radiation does not halt the increase in number of plaque-forming cells (677). The number of such cells accumulated in the spleen was only half that of controls. After irradiation, the number of antibody-producing cells continued to increase, becoming at least tenfold greater. This would suggest that after several mitotic cycles (before irradiation was given) the antigen-stimulated immunocompetent cells become capable of maturing (differentiation?) to antibody-producing cells without any, or only a limited number of, cell divisions. The general laws

governing suppression or stimulation of the immune response as a function of the relative timing of irradiation and immunization have also been discussed in detail by several authors (659, 675, 690).

115. In another study with rabbits exposed to 500 roentgens at various times before or after antigen, it was found (289) that essentially unimpaired responses occurred in animals irradiated immediately before, immediately after, or 12 hours after antigen. Marked depression was observed with irradiation given 12 or 24 hours prior to antigen. With irradiation 12 hours after antigen, there was usually a slight initial depression up to the sixth day after antigen, but the eventual peak titres rose to levels above those in the non-irradiated control. (This exactly agrees with the Taliaferros' observations on their second type of enhancement.) Radiation damage of the spleen was characterized by the complete degeneration of the lymphoid follicles, with survival of much of the peri-arteriolar lymphocyte sheaths. In the irradiated animals in which antibody responses were unimpaired (radiation after antigen), normal plasma-cell reactions localized in the surviving peri-arteriolar lymphocyte sheaths were observed two to three days after stimulation.

116. Irradiation after antigen is not always associated with antibody titres higher than in controls. In some instances (187), it is rather that the degree of immunodepression observed is not as great as when irradiation precedes antigen. Rats exposed to 500 roentgens at various times after antigen all showed some immunodepression which was greater when irradiation was given a few hours after antigen rather than four days after. Mice given a whole-body x-ray exposure of 710 roentgens immediately before or after antigen were severely immunodepressed (200), whereas radiation given five or more days after the antigen had only a slight enhancing effect on antibody formation.

117. Further studies (548) on the radiation enhancement of antibody formation with exposures from 25 to 100 roentgens have confirmed the original report that injection of antigen four hours to a week, or even one month, after irradiation in this dose range will lead to a prolonged production of hæmolysin and to transient high peak titres. It was suggested that after injury, the cells show an over-compensatory activity of a duration proportional to the x-ray dose. This stimulatory effect of small doses did not seem to be directed against the developmental and proliferative activities of immunocompetent cells or of memory cells, as in neither case was an effect observed during the latent period preceding antibody detection in serum.

118. These various reports taken together seem to indicate that radiation-induced enhancement of antibody formation is more marked in certain cases than in others. A detailed analysis of this problem by Dixon and McConahey (144) has revealed that many variables can indeed affect the degree of enhancement induced by radiation. They observed that (*a*) the degree of optimum stimulation varied from one antigen to another; (*b*) the time interval between antigen and irradiation differed in terms of optimum stimulation for different antigens; and (*c*) the optimal radiation dose also differed for the various antigens. In general, it appeared that the more rapid the antibody response the earlier x-irradiation may be given to enhance the response. For example, with soluble bovine gamma globulin (BGG) the interval between antigen stimulus and peak antibody response was from 10 to 11 days, and x-irradiation gave maximum enhancement when administered 2½ days after antigen. With heat-aggregated BGG as antigen, the interval between stimulus and maximum antibody was only seven to eight days and x-irradiation gave maximum enhancement when administered as early as two hours after antigen, although comparable enhancement could be elicited with irradiation one and two days after antigen injection.

119. It was proposed on the basis of these results that x-irradiation given early in the immune response destroys the majority of lymphoid cells, leaving behind depleted lymphoid tissues. Of the remaining lymphoid cells, many of which are primitive or immature, some are presumably responding to antigen and some are not. Those responding to antigen would be expected to proliferate more rapidly than those unaffected by the antigen. The cellular proliferation after antigenic stimulation and irradiation may then be more rapid and extensive than after either alone. During this exaggerated proliferation, the rapidly dividing antigen-stimulated cells can far outstrip their non-stimulated counterparts, resulting in the observed increased antibody formation and in large numbers of antibody-containing cells.

120. A second view has been proposed by Makinodan and Price (336) and is based on the concept of feed-back control mechanisms, that is, that the maximum immune expression of an individual, as measured by peak serum-antibody titre or number of antibody-synthesizing cells, need not necessarily reflect his full immunological potential. Several experiments clearly indicate that the immune system is capable of enhanced responses that are much greater than are achieved in conventional immunization schemes. Animals given 10,000 roentgens to exteriorized spleens with the rest of the animal shielded, will make a markedly-augmented antibody response to intravenously-injected particulate antigens (510). Cell-impermeable diffusion chambers containing antigen and spleen cells from pre-immunized mice, when implanted in irradiated recipients, can generate 10 times more antibody-producing cells per unit number of spleen cells than *in situ* immunization (331). In recipients made immunologically inert by the use of drugs, transfused histoincompatible spleen cells will generate more antibody than compatible spleen cells (489).

121. These and other studies therefore imply that the level of antibody response found in a conventionally-immunized animal reflects activation of only a fraction of its full immunological potential. Makinodan and Price (336) suggested that an immune response can be augmented most readily by radiation if it can cause a sufficient amount of cell destruction and thereby create a *milieu* for proliferation and differentiation of more immunocompetent cells than normally would participate in a response. However, it is essential that the dose of radiation be low enough so that the percentage of immunocompetent cells destroyed be less than the percentage normally expressed in the response.

122. Berenbaum (42) has determined the number of antibody-forming cells at various stages of the immune response when 450 roentgens were given between 1 day before and 20 days after antigen. At all times a rapid fall in the number of antibody-forming cells occurred, which was followed by a rise towards the levels found in unirradiated controls. Further stimulation of newly emerging antigen-reactive cells may have occurred from antigen lodged in depot sites in the

spleen. There was no evidence that radio-resistance rises after the antigen is given. As one of the first demonstrable effects of antigen injection is an increase in the number of antigen-reactive cells, an increased pool of antigen-reactive cells is exposed when irradiation follows antigen. Even if this post-antigen pool is reduced by radiation to the same extent as a pool that has not been exposed to antigen, the absolute number of surviving antigen-reactive cells may be considerably greater.

123. A third explanation for this apparent change in radio-sensitivity of the antigen-stimulated *versus* unstimulated immune response is to consider that there is a difference in the radio-sensitivity of the cells at these two stages, or that a better repair mechanism exists in the immunocompetent cell than in other proliferating cells. As previously discussed, there is little support for this view, although one recent report (638) has shown a basic change in the radiation-dose-response relationship when the immune system is irradiated before or after antigen injection.

124. Using a modification of the plaque technique (638a), Zaalberg and Van der Meul (638) found a significant difference in the effect of irradiation on plaque-forming capacity to sheep erythrocytes depending on whether the antigen was given 24 hours after or 1 hour before, or after, irradiation. The results given in table 3 show that when antigen is given after irradiation, a dose-dependent depression in immune response occurs. Shown graphically in figure VI, this

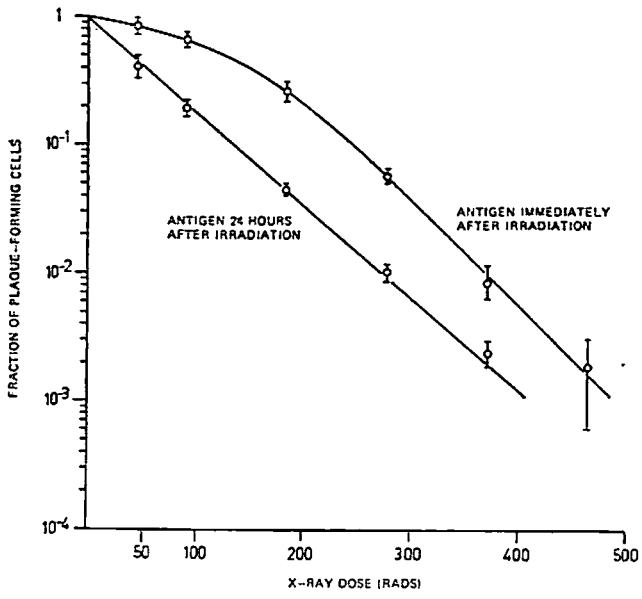

Figure VI. Radiation dose-response relation of IgM plaque-forming cells in mouse spleen (638). The points represent the number of plaque-forming cells determined four days after immunization, expressed relative to unirradiated mice. The bars represent 95 per cent confidence limits. The upper curve was obtained with mice injected with antigen within one hour after irradiation, and the lower curve with mice injected 24 hours after irradiation

is a linear depression. However, when antigen is given one hour before *or* after irradiation, an enhanced response is seen with 50 rads, and a totally-different dose-dependence is found (figure VI). As the radiation damage had already occurred in the animal given antigen one hour after irradiation, a repair mechanism might be postulated. It was suggested that the high level of radio-sensitivity of the non-stimulated small lymphocytes is connected with its relatively-inactive metabolic state. It is therefore not capable of repairing the radiation damage leading to interphase death. However, provided the antigen encounters the cell very soon after irradiation, it may stimulate the cell to repair the radiation damage, possibly by causing changes similar to those previously shown (622) to be capable of preventing interphase death.

125. From a practical point of view, the enhancement of antibody formation by x-irradiation has several interesting facets. It is a convenient laboratory tool for manipulating the immune response, in that antisera of very great potency can be obtained far more rapidly than in normal prolonged hyper-immunization schemes. For clinical medicine, these results serve as a caution against any attempts to inhibit the immune response of patients with x-irradiation if they have recently received the antigen. This may be of particular relevance to homograft situations such as kidney graft, where this type of model might imply that enhanced rejection rather than depression could result, if the antibody response is indeed participating in the graft destruction. Alternatively, if antibody production were acting to enhance graft survival (in the "blocking" sense referred to in paragraph 23), increased antibody production would not be disadvantageous. Also, in situations such as the response to a pathogen or a tumour antigen, an exposure to x rays after the administration of the antigen might greatly facilitate the immune process. As emphasized by Dixon and McConahey (144), the timing relationship of irradiation and antigen injection for either suppression or enhancement of the immune response depends on several factors, including the actual antigen used. Accordingly, this approach to achieve more efficient antibody responses to pathogens or tumours would be most dangerous to apply to man at the present time.

126. Although it might be expected that immunocompetence would be fully restored within months after irradiation, studies on antibody formation in survivors of atomic-bomb-irradiation have occasionally indicated persisting immunological changes. In several studies on blood-group antibody (254), bactericidal activity (253), or serum agglutinin to TAB vaccine (506), no appreciable difference in serum antibody titres of the survivors and controls was found. However, as these studies were performed at least 10 years after the atomic bombing, a more sensitive retrospective indicator for effects of irradiation on antibody formation was sought. Studies of Davenport *et al.* (121) have suggested that serum-antibody levels which appear in response to influenza-virus infection in a specific age group are highest against the strain of virus of the initial infection. Thus, following inoculation with influenza-virus vaccine antigenically related to the virus of primary infection, lower levels of serum antibody against the primary virus should develop in the heavily exposed subjects in comparison to the non-exposed controls.

127. The effect of atomic-bomb radiation on antibody production was studied (276) among persons living in 1961 who were exposed while *in utero* to the atomic bomb in either Hiroshima or Nagasaki. Patterns of the antibody levels in the group beyond three kilometres from the hypocentres suggested that the primary infection in these individuals was from a virus of type A1. Significantly reduced A1-type serum-antibody

levels were noted in pre- and post-vaccination sera of subjects within two kilometres from the hypocentre in Nagasaki. Depression in the pre-immunization sera was not, however, observed in the Hiroshima subjects. In both series, the heterotypic antibody response to Asian-influenza vaccination among those within two kilometres clearly demonstrated a considerably-poorer response to FM1. In subjects within 1.6 kilometres, antibody responses to type-A1 viruses were almost completely suppressed. In Hiroshima, subjects within two kilometres also failed to increase their serum-antibody response to Gotoh virus which is a variant strain of type-A1 virus. In the case of serum response, as tested with other type-A viruses, the results were somewhat more varied. All subjects showed a strong response to PR8 virus, although the response to the Weiss type was poor. The over-all development of serum antibody to the Asian virus following vaccination showed the opposite result, a somewhat better response being observed in subjects within two kilometres than in those beyond three kilometres. While this result might indicate involvement of the overcompensation mechanism discussed above, this is unlikely to have persisted for such a long period of time.

128. A further factor affecting the radiation-induced depression of the primary immune response is the exposure rate. A study was undertaken (74) to determine whether an exposure-rate-dependent suppression existed for antibody synthesis, and to establish the range of exposure and exposure rate over which an effect could be seen. Adult mice were exposed to ^{60}Co gamma radiation in doses of from 200 rads to 1,100 rads at exposure rates ranging from 4 R min^{-1} to 100 R min^{-1}. Irradiated mice were injected with rat- and sheep-erythrocyte antigen at various times before or after irradiation, and the titre of circulating antibody was determined. Greater suppression of antibody production occurred at the higher exposures rates, particularly when the total dose was in the sublethal range, 600 and 700 rads. Rate-dependent suppression of antibody production was dependent upon the type and dose of antigen, the route of antigen administration, and the time interval between antigen administration and radiation exposure. When antigen preceded irradiation by 12 hours and the dose was 700 rads, the suppression at 72 R min^{-1} was 64 times that at 8 R min^{-1}. The exposure-rate effect was demonstrated at the cellular level by culturing irradiated spleen cells in irradiated (950 rads) syngeneic recipients. In this experimental system both primary and secondary antibody formation were differentially sensitive to exposure rate. At the level of maximum exposure-rate sensitivity for formation of antibody against sheep erythrocytes (700 rads), responses were depressed with increasing exposure rates up to 40 R min^{-1}, whereas insignificant additional depression occurred after exposure to higher rates, up to 100 R min^{-1}. The exposure-rate dependence of radiation mortality was determined, and the responses of mortality and immune suppression were compared. No correlation was observed.

C. The productive phase of antibody formation

129. A great deal of data obtained through histological correlative studies (170), studies on antibody content of cells (305), and observations of direct *in vitro* antibody formation by single plasma cells in microdrops (413) has shown that plasma cells are important antibody producers. However, it has also been recognized that other cell types of different morphology can secrete antibody. Many of these include DNA-synthesizing cells (305, 413) and other smaller lymphocyte-like cells were also found to be active. The majority of these differ from the main bulk of small lymphocytes in having a distinct rim of cytoplasm rich in RNA and are now known to be non-thymic-derived *B* lymphocytes. More detailed electron-microscopic studies of antibody plaque-forming cells have appeared more recently (57, 119).

130. In studies combining detection of antibody formation at the cell level with ability to synthesize DNA (328, 423, 446), the conclusion was reached that every antibody-forming cell which arose during the primary or secondary response was the result of a recent mitotic division. It is quite clear that antigen-reactive cells usually enter a proliferative phase and divide, probably several times, to produce mature antibody-forming progeny. Multiplication and the expression of specialization (differentiation) occur over the same interval. Thus, some actual antibody-secreting cells (plasmablasts and immature plasma cells) still retain the capacity for division. However, after a sequence of about six to eight mitoses, division stops and fully specialized non-dividing end-cells dominate the scene.

131. In a detailed analysis (446) of the rate of cellular proliferation and recruitment in the spleens of mice undergoing a primary immune response, it was concluded that, although cellular proliferation during the lag phase is the dominant event, many recruitment events also occur with an exponential increase. It was found that (*a*) antigen-induced cellular proliferation begins about 12 hours after antigen injection: (*b*) plaque-forming cells begin to significantly appear after a lag of about 24 hours; (*c*) most, if not all, of the precursors of the plaque-forming cells during the lag phase are proliferating; (*d*) the number of these cells increases in a staircase fashion suggesting a considerable degree of synchronous growth; (*e*) a series of recruitment events occur in phase with division of plaque-forming cells (this possibly involving the cell-collaboration phenomenon); and (*f*) cells responsible for these recruitments are themselves proliferating before they transform into plaque-forming cells. Similar findings have been reported for both 19S and 7S plaque-forming cells from spleen-cell cultures undergoing secondary anti-sheep RBC response in millipore diffusion chambers (486).

132. In this section of the productive phase we are therefore concerned with cells which are actually synthesizing and releasing the antibody molecules. In terms of cell-collaboration concepts, this refers to the bone-marrow-derived (bursa-induced?) compartment in which the proliferative events referred to above may be induced either simultaneously with or, more probably, only after antigen-induced thymus-cell proliferation. The productive phase therefore is heterogeneous, in that it involves some immature blast cells, some of which are capable of several further division cycles and would be relatively radio-sensitive, fully differentiated plasma cells, the "background" antibody-forming cells present in animals not deliberately immunized, and finally, although not strictly an active secreting cell, the memory cells involved in the elicitation of the secondary response. Virtually no direct data are available on the immature plasma cell. Its contribution to the total serum antibody would be rather small

and direct single-cell experimentation would be required to assess its radio-sensitivity. We shall therefore concentrate on the major antibody-producing cells and the secondary response.

1. *Plasma cells and the active immune response*

133. Various early studies on whole-body x-irradiation of animals after antigen injection clearly indicate that a depression of antibody formation does not result (as discussed under enhancement). Thus, Dixon *et al.* (145) showed that 800 roentgens given three days after antigen had no suppressing effect on the antibody response. Mice given about 650 or 775 rads from a ^{60}Co source at the time of peak serum-antibody formation to tetanus toxoid showed (228) only slight depression or no change in their antibody level 5 to 10 days later. Rabbits immunized with bovine serum albumin were given 450 to 550 roentgens during the steady-state phase of antibody production after either primary or secondary antigen challenge (83). Irradiation during the primary-response steady state produced a continuous fall in antibody levels, but was without effect when given during the declining phase of the secondary response. This would indicate that, at least in some cases, the steady state of persisting serum antibody, particularly after a primary response, is maintained by a balance between proliferation of differentiating cells (probably involving many immature plasma cells) and the half-life of the antibody molecules. In another study, rats received bacterial antigens and gave a prolonged and sustained antibody response (302). Whole-body irradiation during this steady-state phase did not affect the antibody titre for at least a period of several weeks after irradiation. This, in comparison to the report previously mentioned, probably indicates that in other systems, that is, with different antigens, the steady state of persisting antibody production may involve only the mature non-dividing element, and does not require the continuing recruitment of other immunocompetent cells.

134. From these and many other similar studies it its clear that, as the phase of detectable serum antibody develops, the over-all immune response appears to become much more radio-resistant. However, this type of study could involve many factors. Particularly with the use of different antigens, there may well be marked differences in the proportion of mature non-dividing cells and newly-recruited dividing antibody-producing blast cells in the steady-state level. Radio-sensitivity of the antibody molecules, their loss through the gut, the actual radio-sensitivity of the antibody-forming cell or of the antigen depots, are all further factors affecting serum titres in an animal given whole-body x-irradiation.

135. Before considering the effect of irradiation on antibody-producing plasma cells, it is relevant to determine whether irradiation can directly affect the product of the plasma cell, namely, the antibody molecule. The effect of ionizing radiation on the hæmolytic activity of rabbit IgG and IgM hæmolytic antibodies was studied (477). Protein fractions of rabbit serum were irradiated in a beam of 2-MeV electrons generated in a Van de Graaff electrostatic accelerator or by a beam of 5-MeV protons generated in a linear accelerator. The antigen-binding capacity of hæmolysin, unirradiated and irradiated, was measured by determining the number of sheep erythrocytes required to neutralize (absorb) hæmolytic activity. Other investigators who have studied the inactivating effect of ionizing radiation on biologically active proteins of known molecular weight, have found a good correlation between the apparent target size of the active molecule and the size of the whole molecule. Inactivation curves with these other systems were linear. However, with the IgM system, non-linear radiation inactivation curves were obtained. For IgM, 10 per cent of hæmolytic activity was retained with a dose of 14 10^6 rads and for IgG 30 per cent remained after 18 10^6 rads (2-MeV electrons). Some structural characteristics of IgG and IgM hæmolytic antibodies were then deduced by target theory analysis of the relation between the dose of radiation and inactivation of the molecule. Destruction of a single target with a molecular weight of 52,000 in the IgG molecule was sufficient to destroy hæmolytic activity. These data are consistent with a model of the IgM molecule containing more than three sub-units, each of molecular weight from 1.6 to 1.8 10^5. In this model, each sub-unit was capable of combining with antigen, and two adjacent sub-units were required for the fixation of complement (C′) and for hæmolytic activity. In the context of the present discussion these results clearly indicate that no inhibitory effect on antibody molecules themselves is detected within the dose range used in experiments with antibody-forming cells.

136. In most cases mature plasma cells are relatively short-lived, surviving for two to four days only. However, a small but important minority, perhaps one in a thousand of all antibody-forming cells created during the proliferative phase, live for many months and maintain continued antibody production (375). These long-lived cells will therefore become of increasing importance in the maintenance of serum-antibody levels once the productive phase is reached. In assessing their possible radio-sensitivity, Miller and Cole (376) gave rats and mice a secondary stimulus of TAB vaccine, followed by injections of tritiated thymidine twice a day for four days. Thirty days later two groups of mice were given 850 rads and 500 rads, respectively, and one was left unirradiated; the rats received 850 rads, controls being unirradiated. Large numbers of persisting labelled plasma cells were found in lymph nodes after irradiation. No difference could be found in the numbers or distribution of labelled plasma cells in lymph nodes from irradiated animals compared to lymph nodes from those non-irradiated. This ability of plasma cells to survive irradiation may partly explain the radioresistance of established antibody production.

137. It has been shown (334) that exposure of spleen cells in millipore diffusion chambers to 10,000 roentgens during the plateau phase of secondary antibody formation results in a decrease in the total number of cells and in a relative increase in the proportion of plasma cells. Based on the incorporation of amino-acids into specific antibody, Vann (591) showed that spleen cultures exposed to 10,000 roentgens continue to synthesize antibodies at a normal rate. This indicates that the antibody-synthesizing polyribosomal units, which contain the messenger RNA for specific antibody-peptide synthesis, as well as enzymes required for protein synthesis, are not only stable but remarkably radioresistant. In a further analysis of the ultrastructural changes in irradiated antibody-forming cells exposed in chambers to 10,000 roentgens, Sado (484) showed by plaque assay that 3 out of 10 nucleated cells were specific-antibody formers four days after the irradia-

tion (10 per cent in the unirradiated control). The half-life of irradiated antibody-producing cells was not different from that of unirradiated cells. Electron-microscope studies showed pronounced nuclear damage, but fully developed endoplasmic reticulum rich in ribosomes. A low but significant number of blast and mature plasma cells were still capable of incorporating tritiated thymidine several days after exposure to 10,000 roentgens. Based on several studies, it was suggested that these cells represent those which were in the *S* phase at the time of irradiation and were incapable of generating further progeny. This study indicates an extremely high radio-resistance of plasma cells according to all parameters.

138. The direct effect of x-irradiation on antibody-plaque-forming cells *in vitro* has been studied by Kennedy *et al.* (286). Four days after the injection of antigen (sheep red blood cells), spleen cells from mice were taken and irradiated *in vitro*. They were then plated for content of antibody-forming cells as assayed by the Jerne plaque technique. As shown in figures VII-IX, doses of less than 2,000 rads had no effect

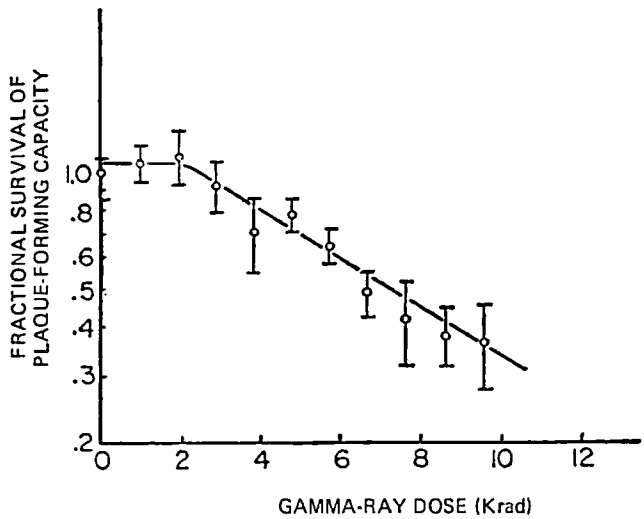

Figure VII. Plaque-forming ability of spleen cells from immunized mice as a function of radiation dose *in vitro*. Four days after the injection of antigen, the spleen cells from 12 mice were pooled, irradiated and assayed, 10 aliquots being used for each point. The 95 per cent confidence limits shown were calculated from variation in the number of plaques per plate (286)

on the capacity of plaque-forming cells to form plaques and doses in excess of 2,000 rads, up to 10,000 rads, had only a moderate effect. Although an accurate D_{37} could not be obtained, approximately 9,000 rads were required to reduce plaque-forming capacity to 37 per cent of its initial value.

139. In a more recent study, Sado *et al.* (485) determined the characteristics of the survival curves of 19S- and 7S-antibody-producing cells irradiated *in vivo*. In this study, the antibody-producing cells were derived from spleen-cell cultures undergoing secondary anti-sheep erythrocyte responses in cell-impermeable diffusion chambers and their numbers were assessed three days after irradiation by a modification of Jerne's hæmolytic plaque procedure. The results indicated that the number of 19S-antibody-producing cells decreased exponentially with increasing doses, giving a survival curve with a D_0 of 6,200 rads and an n_0 of 1.0. On the other hand, the survival curve for 7S-antibody-producing cells gave a shoulder portion at exposures below 15,000 roentgens which was followed by an exponential decrease with increasing doses, indicating that there exists a threshold for inactivation of this type of antibody-producing cell. This survival curve gave a D_q dose of 8,000 rads, a D_0 of 4,250 rads, and an n_0 of 1.62.

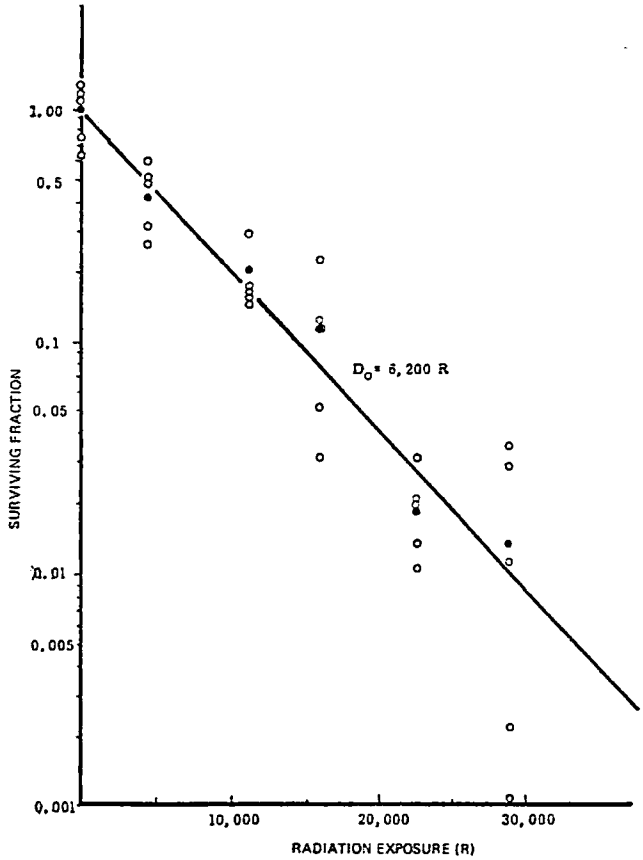

Figure VIII. Survival curves for direct plaque-forming cells. Each circle represents a value obtained from individual chambers and the solid circles represent the mean. Mice bearing diffusion chambers containing immunized spleen cells were irradiated with the indicated doses and the chambers then transferred to new hosts. Plaque-forming cells in the chambers were then assayed three days later (485)

140. In contrast to these situations involving direct irradiation of plasma-cell populations, some results with cell-transfer situations have indicated a depression following radiation. For a quantitative estimate of the radio-resistance of the productive phase of the immune response, mice of the C57BL strain were immunized with non-pathogenic leptospiræ (667). After 14 days of antibody production the spleens were removed, and a cell suspension was prepared and then irradiated *in vitro* with doses ranging from 100 to 20,000 rads. After this the cells were placed in culture *in vivo* (i.e. injected into irradiated recipients), and after six days the antibody titres in the blood of the syngeneic recipients were measured. In this case the dose-effect curve consists of two parts. The first part—in the 100-800-rad dose range—has characteristic values of $D_0 = 260$ rads and $n_0 = 1.3$. The second part of the curve is less steep: an increase in the dose from 1,000 to 20,000 rads produces no substantial additional depression of antibody formation in the cell suspension. Irradiation with a dose of 800 rads depressed the antibody formation by 81 per cent, and 20,000 rads by 93 per cent. The conclusion drawn from this was that in the pro-

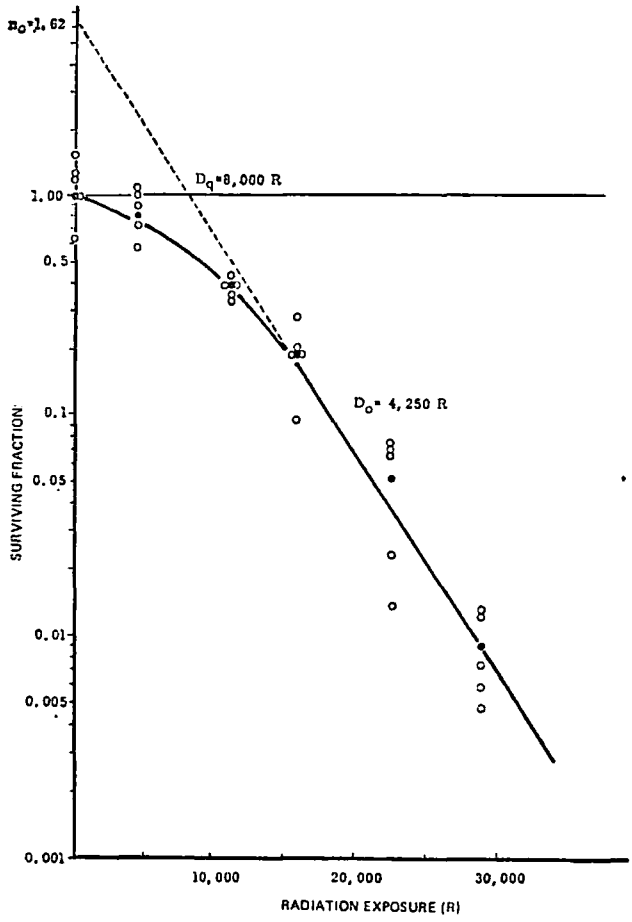

Figure IX. Survival curves for indirect plaque-forming cells. Other details as for Figure VIII

ductive phase the population of producing cells is heterogeneous: some are in the blast stage and require cell division in order to develop, while others are mature non-multiplying plasma-cell elements. The results were analogous when the synthesis of antibodies in an *in vitro* culture after the inclusion of tagged amino-acids was considered (668).

141. In normal spleens of unimmunized animals, there are varying numbers of cells which will form antibody plaques against several red-cell antigens. These are referred to as background plaques and may represent persisting plasma cells from previous immunizations (spontaneous or induced) to cross-reacting antigens. The numbers of these background plaque-forming cells are unaltered (241) when measured two and seven days after x-ray doses of up to 200 rads. Doses of 500 rads and 900 rads caused some slight decrease in background plaques (approximately 20 per cent at two days and 30 per cent at seven days after 900 rads). The lack of sensitivity to radiation at whole-body doses of 50 to 100 rads indicates that maintenance of normal levels of background plaque-forming cells is not dependent on rapid proliferation, and that the average lifetime of these cells is greater than seven days. This result is also consistent with the relative radio-resistance of the mature plasma cell.

2. *The secondary antibody response*

142. The secondary antibody response is elicited in an animal after the second injection of antigen. This may be given at a time well after the first injection when the primary response has completely disappeared, or earlier, when persisting antibody is still present. The three main hallmarks of a secondary, memory or anamnestic response, are a shortened latent period (time between antigen injection and appearance of serum antibody), a higher peak titre, and a greater and earlier contribution of IgG rather than IgM to the antibody population. All three of these criteria are not always manifest in a secondary response, and generalizations are not very relevant in this regard as differences occur with different species, antigens, doses, timing, etc.

143. In general, when the secondary response is considered in its entirety, without attempting to separate the true secondary from a decaying primary, or without giving attention to the quality (avidity) of antibody, the secondary response is quantitatively more radio-resistant (figure X). Thus Dixon *et al.* (145) found

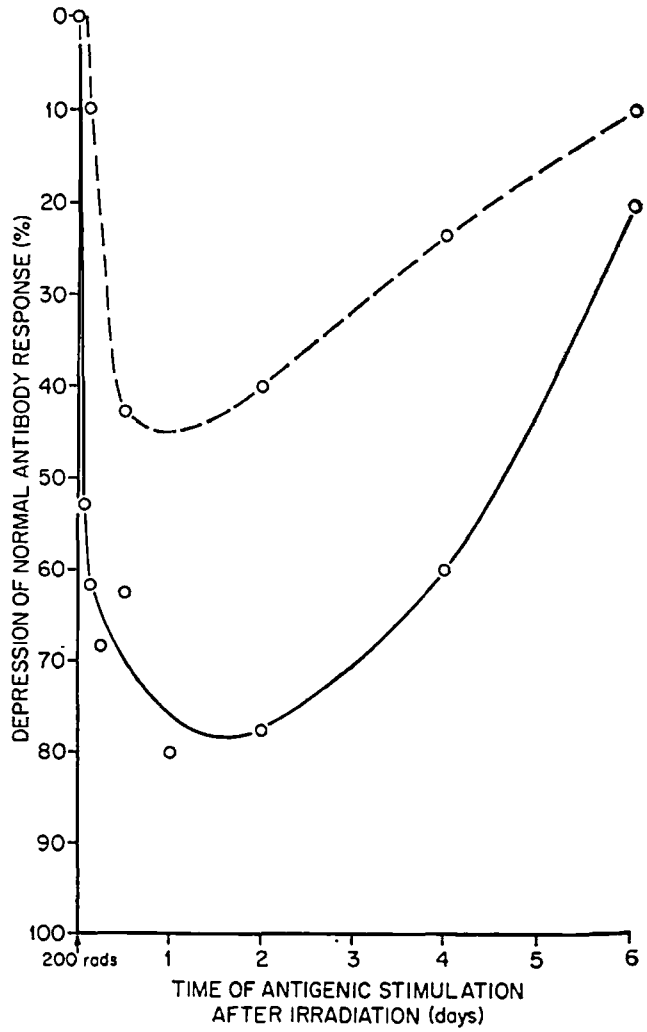

Figure X. Relative radio-sensitivity of the primary (solid line) *versus* secondary (broken line) response (532). In these studies the effect is estimated in the whole animal by measuring over-all antibody response (for both primary and secondary, the results are plotted relative to control unirradiated animals given primary or secondary immunization, respectively)

little effect of 400 roentgens two days before the secondary antigen injection; but some delay occurred with 800 roentgens (400 R was fully effective in the primary). Similarly, Silverman and Chin (508) found

no effect on the anamnestic response in rabbits given 400 roentgens 24 hours before second injections of egg albumin. However, in the earlier studies of Taliaferro *et al.* (549), it was reported that the specific anamnestic response to Forssman antigens was as susceptible to x-ray damage as the initial response. Crosland-Taylor (113) also found that the secondary response of rabbits to tetanus toxoid was radio-sensitive but differed from the primary response in that an exposure of 400 roentgens had to be given two or more days before the antigen to reduce the peak titre. Porter (460) found that 550 roentgens given to rabbits during the latent period between the first and the second antigen injection destroyed or markedly inhibited secondary response. Following this further, Thorbecke *et al.* (565) showed that whole-body irradiation (450-500 R) given to rabbits 21 days after a primary injection produced a permanent partial inhibition of the booster response, whereas irradiation eight days after the primary injection resulted in some inhibition followed by a rapid recovery. This recovery appeared to be correlated with the destruction and reappearance of secondary nodules in the white pulp of the spleen.

144. These earlier studies therefore seemed to indicate that the secondary response could be inhibited by pre-irradiation, but perhaps not to the same degree of sensitivity as the primary response. Detailed measurements of radio-sensitivity using the cell-transfer system were then made by Makinodan *et al.* (333). When the antibody-forming activity of spleen cells is assayed on a given day after an antigenic stimulus, the logarithmic relation between antibody titre and viable-cell number is linear up to a certain cell dose, and the slopes of these regression lines are not significantly different regardless of whether the response is primary or secondary. The slopes of these regression lines remained unaltered even after sublethal x-irradiation, and the magnitude of the decrease in the primary and secondary antibody-forming activities of spleen cells after a given dose of x rays was approximately the same. These findings therefore suggest that the apparent difference in radio-sensitivity between primary and secondary antibody responses among intact animals exposed to sublethal whole-body doses of radiation is mainly due to the difference in absolute number of competent cells surviving after radiation treatment. It follows then that radio-sensitivity of secondary antibody-forming capacity of intact animals can be best shown with those given a minimum pre-immunization treatment.

145. In further extending their original observations made in 1952, Taliaferro and Taliaferro (548) have shown that, in rabbits immunized with sheep erythrocytes, the anamnestic response to Forssman antigen was still depressed when sheep erythrocytes were injected two to six months after exposure to 500-700 roentgens. The results demonstrate that the IgM response is of equal radio-sensitivity in the primary and secondary response when maximally depressed by 500-700 roentgens, but that the anamnestic response is more radio-sensitive than the primary response during the phase of recovery from these high doses. These authors accordingly suggested that the memory cells themselves might be more sensitive to radiation than the initial immunocompetent cells.

146. It has been suggested (499) that the cell pool responding in the secondary response is a specific differentiation product of the memory-cell pool induced by antigenic stimulation. As the antigenic stimulation subsides, its expansion ceases and it does not regenerate after injury if the stimulus is lacking. Since its self-generating capacity may be limited, it can be permanently reduced in size or even abolished by irradiation. This general conclusion (406) is based on earlier studies of the regenerative potential of antibody formation after irradiation. It was shown (404) that the minimum antigen dose necessary to initiate a near-maximum antibody response is about 10^5 times greater for irradiated than for unirradiated spleen cells. Accordingly, various factors affect post-irradiation recovery of the ability to give a secondary response. These include the type and amount of priming antigen, the primary x-irradiation interval, and the x-ray dose (405). Recovery of the memory-cell pool after irradiation does occur provided the antigen persists until uncommitted progenitor cells again become available and are stimulated to form memory cells.

147. A recent report (552) has described the radio-sensitivity of the *in vitro*-induced primary and secondary antibody responses to a bacteriophage antigen. In this culture system both types of responses could be compared in an identical environment. Radiation-induced depression of the secondary response initiated *in vitro* with lymph-node cultures from immunized rabbits was clearly demonstrated with 500 rads given 3 hours before or 24 hours after antigen. Peak antibody production was both delayed and reduced. The radio-sensitivity of the secondary response was as great as, if not greater than, that of the primary response. This type of direct study, taken with the recent reports described above, clearly demonstrates the equal radiation sensitivity of the actual antigen-induced cells whether they be of virgin or memory type. The actual expression of the radio-sensitivity of the primary or secondary response, as measured by serum titres in the whole animal, involves other factors, which in turn are mainly related to the number of virgin or memory cells that are respectively irradiated.

148. In a previous discussion of the enhancing effect of radiation on antibody formation, Makinodan and Price (336) considered the phenomenon in terms of the actual immunological expression in relation to the full potential response that would be possible. They also discussed the apparent paradox of radio-resistance of the secondary response in these terms. Previous studies had clearly shown that although the difference in the magnitude of response between individuals undergoing primary and secondary responses might be only twofold, the secondarily-stimulated animal actually possesses up to 100 times more potentially responsive immunocompetent units (331). In other words, the ratio of immunological expression to potential is much larger in a primary than in a secondary response. This in turn implies that, for a given dose of radiation, even though both primary and secondary cells have equal radio-sensitivities, a much greater number of unused potentially reactive cells remain in the secondarily-challenged animal. A sample calculation of this nature has been made by Makinodan and Price (336) and is shown in table 4. In this example it is seen that although 300 roentgens reduced a primary response to 5 per cent of control, no effect was observed on the secondary response.

IV. Effects of radiation on cellular immune reactions

A. Cellular components involved in cellular immunity

149. As originally outlined in this review, immune responses are broadly divisible into those involving humoral antibody mediated by plasmacytic and some lymphocyte-like cells, and cellular immunity mediated by lymphoid cells. The small thymic-derived lymphocyte (*T* cell) is the cell involved in immune reactions such as delayed hypersensitivity and graft rejection. This cell may undergo various changes and appear as a pyrinophilic blast cell which then may give rise again to lymphocyte-like progeny (523a). Although this cell is known not to secrete appreciable amounts of immunoglobulin molecules, it is likely that the recognition unit on the cell surface, which is responsible for specific reaction to antigen, is an immunoglobulin molecule (30, 35, 225, 343, 425), or possibly only a free light chain or light-chain component. Many studies of this problem are currently in progress, and at least agree that the density of immunoglobulin molecules on the surface of the *T* cell, if present at all, is only of the order of 1 per cent of that on non-thymic-derived lymphocytes. The *T* lymphocyte is part of the recirculating pool and is markedly depleted by thymectomy, particularly neonatal thymectomy (374, 379). Those lymphocytes which are involved in cellular immunity are directly derived from the thymus and carry surface marker antigens such as theta, which distinguishes them from the non-thymic lymphocytes that are precursors of antibody-forming cells. The phenomenon of cell collaboration has been repeatedly stressed in discussions on antibody formation. Although cell collaboration may be equally relevant for cellular immunity, there is at present only slight direct evidence of such interactions (72), involving two thymic-derived cells. There is no evidence for collaboration between thymic-derived cells and bone-marrow (bursa equivalent?) derived cells in cell-mediated immunity (91, 536, 577). This section will first examine morphologically-defined lymphocytes, as a heterogeneous population, and then discuss in functional terms specific cellular immune responses.

B. Lymphocytes, lymphoid tissue and radiation

150. Organized lymphatic tissue and individual lymphocytes are extremely radio-sensitive. This fact was recognized within a few years of the discovery of x rays, and has been the subject of numerous detailed reviews over many years (54, 156, 165, 408, 495, 663, 670, 675). The striking effect of a single lethal whole-body dose of x rays on the mouse lymph node is indicated in figure XI. The effect of a large acute exposure to ionizing radiation is to destroy the cortical masses of tissue lymphocytes and the dividing cells in the germinal centres of the lymph node, leaving intact the stroma, blood vessels, mature plasma cells and reticulo-endothelial cells (95). A few lymphocytes usually remain and these will be considered later. This pattern is typical of all organized lymphatic tissue.

151. Regeneration of lymphatic tissue usually involves reappearance of parenchymal elements in the same order as in the original ontogenic development, that is, collections of cortical lymphocytes appear first, and are followed by germinal centre formation. As previously discussed in relation to immunological re-

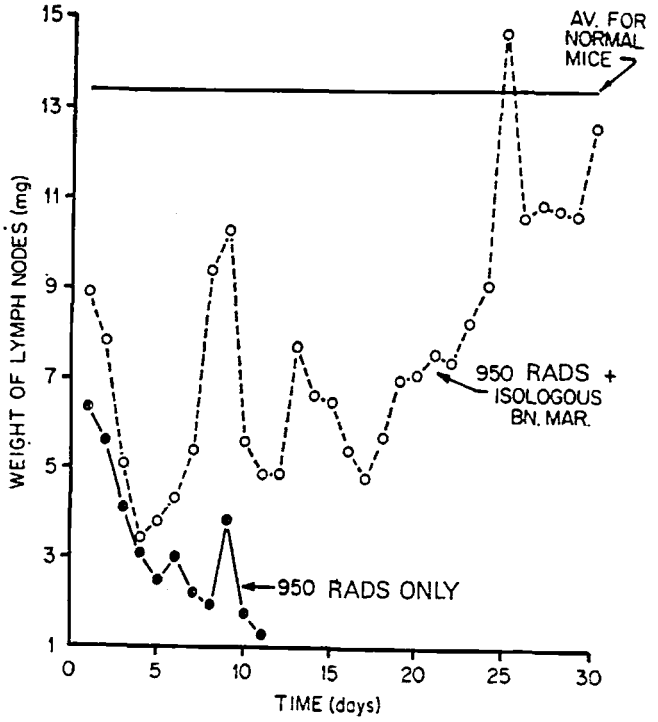

Figure XI. Weight changes in four peripheral lymph nodes in mice exposed to supralethal whole-body doses of x rays. Also shown are the weight changes in lymph nodes in mice similarly irradiated but given isologous bone marrow afterwards. The temporary weight increase between 5 and 10 days after the treatment was caused by extramedullary granulopoiesis. Each point represents five mice (95)

generation, the regeneration of lymphoid tissue and of functional activity depends on the presence of an intact thymus. Extramedullary myelopoiesis in lymphatic tissues preceding lymphoid regeneration has been observed (54) but is of unknown significance. Regeneration of lymph nodes after local, rather than whole-body, irradiation is extremely rapid, presumably because of the influx of normal cells from the unirradiated areas (98). If a high local dose (e.g. 3,000 rads) is given, an extreme secondary atrophy develops in subsequent weeks, apparently following vascular damage and destruction of the original stroma (165).

152. In considering the effect of radiation on lymphocytes, it is important to distinguish between two general mechanisms. Firstly, lymphocytes are virtually the only cell population of the body to show interphase death from radiation, and this may play an important role in radiation-induced depression of immunity. On the other hand, as has been previously discussed (paragraph 90), the doses of radiation that affect the primary antibody response suggest that a main effect of radiation is on cell division.

153. The biochemistry of necrosis has not been studied to the same extent as the morphology of necrosis and there is no firmly established biochemistry of radiation necrosis in lymphatic tissue. Bacq and Alexander (27) consider interruption of energy supply and enzyme release as two main theories on the nature of the early biochemical lesion in cells exposed to ionizing radiation. Major consequences of radiation exposure are the interference with the biosynthesis of nucleic acids and chromosome breakage. It is generally considered that the inhibition or delay in DNA synthesis is the single most important biochemical change

in lymphatic tissues caused by radiation and it may be presumed that ionizations are in some way affecting those portions of the cell genome that control DNA synthesis itself.

154. Normal human blood lymphocytes have been found (527) to show extreme sensitivity to x-irradiation *in vitro*. A statistically significant sensitivity to x-irradiation was shown with two and five roentgens, producing, respectively, 13 to 21 per cent and 35 per cent effect (scored by morphological and motility changes). In other studies *in vivo*, as little as four hours after receiving a radiation exposure of 100 roentgens, the peripheral lymphocyte count is 25 per cent of normal in four- to seven-month-old rats (495). In addition to a reduction in count, the lymphocyte shows direct changes with pyknotic nuclei beginning to appear in four to six hours in lymphocytes exposed *in vitro* to 100-400 roentgens. It should be noted that the extreme sensitivity of lymphocytes to two and five roentgens was only observed with cells irradiated *in vitro*. It is possible that this represents an artificial condition in so far as the cells are not in their normal environment, and that these results might therefore not be relevant to *in vivo* irradiation, whereas *in vivo* results may also depend upon abscopal effects (e.g. as discussed in paragraph 298).

155. Lymphocytes within the gut epithelium in mammals have been termed theliolymphocytes, and it was proposed that they constitute a specialized type in that the gut epithelium may function as the first-level lymphoid organ. It has been shown (178) that on the whole they are as radio-sensitive as blood lymphocytes, although the number of theliolymphocytes is restored to normal values much earlier than blood-lymphocyte levels after irradiation. This may indicate a selective radio-resistance of the unknown source of the theliolymphocytes, or a preferential localization of the regenerating precursor cells.

156. Some persisting lymphocytes are still seen in lymph nodes of animals given whole-body irradiation in the lethal dose range. These cells may represent either a random fraction surviving the particular dose of radiation, or a specific population of more radio-resistant lymphocytes. Some *in-vitro*-culture studies with phytohæmagglutinin-stimulated lymphocytes have pointed to the existence of a separate resistant population (93). It has been shown that some small lymphocytes can persist for at least a year (375) and some of these may be responsible for immunological memory (222, 426). Several studies have accordingly been carried out to determine whether there is a difference in the radio-resistance of the long-lived and short-lived lymphocyte. In two reports (169, 621) no change in the proportion of long-lived to short-lived lymphocytes was found in blood lymphocytes or thoracic-duct lymphocytes after 215 or 300 rads. In another study (376) where doses of 500-850 rads were used, lymphocytes were examined in the local lymph nodes draining an antigen-injection site. Despite a marked generalized destruction of lymphocytes, the nodes examined contained significantly higher proportions of the long-lived lymphocytes (identified by tritiated thymidine introduced at time of antigen stimulation one month prior to irradiation). It was felt that these cells were probably not part of the circulating pool of small lymphocytes, and the results therefore do not necessarily contradict the other two reports which are concerned with the recirculating pool. It was therefore proposed that at least some types of long-lived lymphocytes are relatively resistant to quite high doses of x rays.

157. There is a clear-cut dose-response relation for lymphatic-tissue damage and repair when the dose is delivered over a short interval. However, the dose rate as well as the total dosage is important. In two studies (108, 198) on transplantation of foreign bone marrow, dose rates of 1-4 rads per minute were much less effective in immune depression than dose rates of 29-54 rads per minute, although the same cumulative dose was given. Dose rates in the range of 1.1 to 1.8 rads for eight hours per day had only a moderate effect on morphological changes in lymphatic tissue, as it often took several months to produce discernible changes.

158. A paradoxical finding on radiation exposure and thymic destruction has been reported by several authors (574, 575, 597). Whereas increasing exposure usually leads to enhanced lymphoid destruction, when it reaches the kiloroentgen range an opposite effect is observed. Thus rat thymus given between 10 and 30 kiloroentgens *in vivo* showed less damage (by morphology and weight) than in animals exposed below 10 kiloroentgens. With 30 kiloroentgens, virtually no thymus weight reduction was observed, whereas maximum depression in thymus weight occurred with approximately one kiloroentgen. A similar phenomenon has also been observed with thymus irradiation *in vitro* (574). Within the lymphatic-tissue system all sites appear to be equally radio-sensitive. The thymus, however, regenerates faster than other lymphatic tissues, presumably because it is the site of differentiation of new lymphocytes from immigrant stem cells.

159. X-ray exposures in the 10-200-roentgen range produce stimulation of adrenocortical secretion as judged by depletion of either adrenocortical sudanophilic material or total adrenocortical cholesterol (148). Accordingly, it is possible that x-irradiation may damage the lymphocyte through an indirect corticosteroid-mediated effect. In a study on atrophy of lymphoid organs in unoperated and adrenalectomized mice given different doses of radiation, it was found (149) that acute involution of lymphatic tissue (that is, steroid-independent lymphocyte destruction) occurred in both groups of animals with x-ray exposures from 25 to 200 roentgens, but that with 10 roentgens destruction of lymphoid tissue was more pronounced in intact mice than in adrenalectomized animals.

160. Thoracic-duct lymphocytes enter the splenic white pulp *via* the blood and, after traversing a pathway within the splenic pulp, subsequently re-enter blood (192). This suggests that local continuous irradiation of the spleen would lead to a marked fall in the recirculating lymphocyte pool and therefore of the primary immune status of the animal (221). This has been studied (191) by attaching a ^{32}P-impregnated polythene strip to the antihilar surface of the rat spleen. This resulted in a profound drop (to 15 per cent in four days) in the output of small lymphocytes from a thoracic-duct fistula. No other type of blood cell was affected. It appears that the lymphopenia was brought about by radiation death of small lymphocytes (possibly mainly interphase death) passing through the spleen from the blood. Other studies (230) on isolated lymph nodes had previously shown that large acute doses of radiation do not impair the organ structures essential for the recirculation of lymphocytes at least in the

immediate period, although later effects have been noted (see paragraph 289).

161. Lymphopenia has also been produced by chronic extracorporeal irradiation of the blood (111), by intra-atrial implantation of a beta-emitting source (31) and by intralymphatic infusions of radio-isotope-labelled agents (159, 567, 620). In this latter instance studies in man with intra-lymphatic infusion of ^{131}I-lipidiol have shown that even with a unilateral lower-limb infusion an appreciable volume of lymphoid tissue is irradiated, and histological examination of lymph nodes revealed widespread destruction. Many workers have proposed that depression of lymphopoiesis accounts for the lympho-cytopenic state. However, in the experiments with the ^{32}P-soaked strip (191), the lymphopenia occurred far too rapidly (50 per cent fall in one day) to be accounted for by depressed lymphopoiesis. A direct radiation death of the recirculating small lymphocytes seems far more likely. Leukæmic lymphocytes also appear to be markedly radio-sensitive and accordingly chronic extracorporeal blood irradiation may be of potential value in removing leukæmic cells. Several of the relevant findings from a recent international symposium on chronic extracorporeal blood irradiation are summarized below.

162. Reports at the experimental level clearly indicate the efficacy of chronic extracorporeal blood irradiation in producing lymphopenia. This may either be due to radiation destruction of the lymphocytes or to their inability to recirculate after irradiation. It was felt that there was still a bewildering amount of variability in technique for a relatively small amount of clinical information. In general, the experience with different clinical situations after chronic extracorporeal blood irradiation could be summarized as follows:

Acute myelocytic leukæmia: rare hæmatological remissions. Survival does not appear to be greatly changed;

Chronic myelocytic leukæmia: relatively few cases reported. No remission reported. White-cell count rises again rather rapidly;

Acute lymphocytic leukæmia: again relatively few cases reported and generally poor results;

Chronic lymphocytic leukæmia: best results with chronic extra-corporeal blood irradiation are in this disease. There have been clinical but no hæmatological remissions. Some decrease in spleen and lymph-node size has occurred.

C. Delayed hypersensitivity

163. Delayed hypersensitivity reactions can be readily induced in man and various laboratory animals. The guinea-pig is the classic species favoured for studies of this type of immune reaction. Studies on delayed hypersensitivity *in vivo* suffer from the disadvantage that the reaction can only be assessed semi-quantitatively at best, and that little information is available on the relation between the sensitivity of development of the skin lesion and the number of sensitized lymphocytes. Accordingly, the possibility of detecting accurately small radiation-induced changes is more limited than for antibody production, particularly when in the latter case actual numbers of antibody-forming cells are measured. At present, there is no universally accepted technique for enumerating sensitized cells involved in delayed hypersensitivity reactions comparable to the plaque type of assay. (Although one recent plaque type of assay has been reported (53) it is rather complex, and has yet to be fully confirmed.) This absence of a satisfactory plaque assay implies that a rather substantial reduction in the immune reaction probably has to be induced before it becomes observable by current methods such as measuring indurated skin lesions.

164. In several early studies, the induction of delayed hypersensitivity was not markedly inhibited by irradiation in doses which suppressed antibody formation. Thus 300 rads given 18 hours before sensitization with diphtheria toxoid resulted in a period of pure delayed hypersensitivity up to the twenty-first day post-sensitization without any antibody being detectable. When 300 rads were given 18 hours after sensitization, delayed hypersensitivity lasted for the usual period (488). This was confirmed (584) with another antigen, ovalbumin, which again showed retention of delayed hypersensitivity in the absence of antibody formation. However, when high radiation exposures (800 R) were given to rabbits before sensitization, complete suppression of delayed hypersensitivity was observed. A single exposure of 200-250 roentgens to guinea-pigs failed (494) to suppress the acquisition of allergic contact dermatitis to dinitrochlorobenzene, which is a manifestation of delayed hypersensitivity. Radiation will also depress the development of hypersensitivity to tularin and brucellin (661).

165. A febrile reaction is often associated with the state of delayed hypersensitivity. In guinea-pigs given 200-300 roentgens before sensitization, a febrile response occurred on systemic challenge with antigen in both irradiated and control groups (584). This reaction occurred in animals showing suppression of antibody response but not of delayed hypersensitivity.

166. Most types of experimental allergic autoimmune diseases such as experimental allergic encephalomyelitis appear to involve predominantly a cellular immune response (437). Administration of 150 roentgens 18 hours prior to antigen was reported (181) to result in an increased severity of experimental allergic encephalomyelitis in guinea-pigs, rather than a depression. There was no significant diminution of delayed sensitivity to the original brain material used for inoculation. However, in another study (438) of allergic encephalomyelitis induction, 400 roentgens (whole body) given to rats prior to sensitization with spinal cord and adjuvant suppressed the encephalomyelitis. This suppression was dose-dependent and was observed in two strains of rats sensitized by either of two routes. A reduced production of complement-fixing antibodies occurred, but there was little, if any, suppression of delayed immunologic reactivity as based on tuberculin skin testing. In two other reports, x-irradiation enhanced rather than depressed the development of experimental allergic encephalomyelitis in guinea-pigs (13) but suppressed it in rabbits (94). These apparent contradictions in radiation effect on the induction of experimental allergic encephalomyelitis may be due to differences in species, doses of x rays, etc. The studies of Paterson (437) would seem to indicate that a reduction of cytotoxic-antibody formation might explain the reduced clinical disease. It might be speculated that reduced antibody formation could also lead to the enhanced severity observed in guinea-pigs. Since certain types of antibodies (enhancing antibodies) may protect animals from the disease (437)

it is possible that these, rather than a cytotoxic antibody, are normally produced in the guinea-pig with the immunization scheme used. Accordingly, radiation-induced depression of this type of antibody formation would lead to an apparently more aggressive immune cellular response that would further the disease process.

167. Some contradictions also exist in the literature regarding the question of radiation sensitivity of the transfer of delayed hypersensitivity. Experiments with donor cells irradiated *in vivo* or *in vitro* and with normal recipient animals have been described. As regards irradiation of the donor *in vivo*, it was shown (118) that a whole-body x-ray exposure of 150 roentgens diminished the tuberculin reaction of sensitized donors when irradiation was given prior to antigen. Comparable x-irradiation of recipient animals four days before cell transfer from either irradiated or non-irradiated donors also produces a diminution in the tuberculin reactivity of the recipient animals. Irradiation of sensitized cells *in vitro* prior to transfer has also been reported (24) to reduce the resulting reaction, provided the exposure is above 1,500 roentgens. Exposure to 1,000 roentgens did not affect transfer. Since the small lymphocyte is very sensitive to radiation, it might be expected that reduction of transfer by irradiated donor cells would occur very readily.

168. Three possible explanations for this apparently high radio-resistance of sensitized cells might be considered: (*a*) that, as discussed by Makinodan *et al.* for antibody production, the immunized cell population is as radio-sensitive as unimmunized cells, but contains so many specifically sensitized cells that high doses of radiation are needed to eradicate enough of the component donor cells; (*b*) that the sensitized memory cell responsible for the transfer of delayed hypersensitivity may belong to a different category of lymphocytes (possibly of the type described by Miller and Cole (376)) and be inherently radio-resistant. This might imply that its radio-resistance has in fact been induced by the antigenic stimulation, as suggested by Stefani (526); and (*c*) by analogy with the experiments of Katz *et al.* (282) on the radio-resistance of carrier-primed cells, it may be that the donor-sensitized cells in transfer of delayed sensitivity also collaborate with host cells, and that this collaboration involves very little, if any, donor-cell proliferation, and is therefore relatively radio-resistant.

169. X-irradiation (550 R) of recipient rats (89) before transfer of sensitized cells totally prevented the expected delayed reaction, provided skin-test challenge was given soon after the cell transfer and irradiation. The passive transfer by sensitized cells of experimental allergic encephalomyelitis into recipients was also inhibited (309) by x-irradiation of the recipients 24 hours before cell transfer. Complete inhibition occurred with 700 or 1,000 roentgens, partial inhibition with 400 roentgens and no inhibition with 100 roentgens. These two experiments strongly suggest that a host component involving cell proliferation is essential for successful passive transfer of delayed sensitivity of experimental allergic encephalomyelitis. This is consistent with various studies (review by Bloom and Chase (52)) which clearly indicate that the majority of infiltrating cells in the delayed-hypersensitivity lesion are host-derived cells.

170. One of the more classical hallmarks of delayed-hypersensitivity reactions is that they can be passively transferred by cells but not by serum (52). A recent report (157), however, has described the passive transfer of delayed hypersensitivity to PPD by plasma from BCG-immunized x-irradiated (800-1,000 R) donors. Neither plasma from non-irradiated BCG-sensitized donors, nor plasma from x-irradiated non-immunized donors, could transfer PPD sensitivity to normal recipients. Passive transfer of PPD sensitivity was also achieved by normal spleen cells which had been incubated *in vitro* with plasma from immune x-irradiated donors. Repeated washing of these cells failed to remove their ability to passively transfer PPD sensitivity. It was suggested that some factor of unknown nature which is normally bound to cells was released into the plasma by irradiation and could then bind to host cells *in vivo* or *in vitro* and "confer reactivity". Such a factor could theoretically be an immunoglobulin-type molecule with appropriate specificity, a nucleic acid coding for a polypeptide with the specificity, a transfer factor of one of the types recently reviewed by Lawrence (303), or a very immunogenic antigen. This is a complex problem as a failure to detect migration-inhibition factors in supernatants of sensitized lymphocytes irradiated *in vitro* was also recently reported (19).

D. Transplantation immunity

1. *Experimental allograft rejection*

171. The feasibility of pretreating prospective recipients with ionizing radiation to promote survival of foreign grafts was clearly demonstrated by Murphy in 1914. This work appears to have been forgotten until the early 1950s when, following on the pioneer studies of Medawar (355) on the immunological basis of transplantation rejection, Dempster *et al.* (131) showed suppression of skin homograft rejection by pretreatment of the recipients with x-irradiation. An exposure of 250 roentgens given to rabbits before the application of skin grafts from another rabbit markedly prolonged the survival of the grafts. The second-set response, however, was unaffected by this dose of radiation.

172. Prolonged survival of skin grafts with only minor genetic differences can be induced by pretreating recipients with ionizing radiation in non-lethal doses. A moderate delay in primary homograft rejection was observed (362) in mice given 400 rads, although the depression of antibody formation was far greater. Prolonged rejection of male-skin grafts on female syngeneic mice has also been induced by exposing recipients to 300 roentgens (285). Exposures of 1,000 roentgens were far more immunosuppressive on both primary and secondary graft rejections (63).

173. The effect on graft survival of extracorporeal gamma irradiation (ECI) of the circulating blood of calves before and after skin homografting has been described in 13 calves (77). In all the ECI-treated calves, the normal acute and violent skin-homograft-rejection process occurring at 9 to 10 days was modified to a slow and mild process with an increase in rejection time by 1 to 11 days. In one calf where thoracic duct lymph was drained for eight days and cell-free lymph was returned to the animal followed by four days of ECI to the lymph, the skin-graft-rejection time was 40 days.

174. These results clearly indicated that the homograft-rejecting capacity could be depressed by prior irradiation, although the relative radio-sensitivity of

primary *versus* secondary graft rejection was not clear. Tyan and Cole analysed this problem in a series of papers in which different variables were considered, such as radiation dose, method of presensitization, comparison of hæmagglutinin production *versus* graft rejection, and comparison of xenogeneic (heterograft) with allogeneic (homograft) grafts. It was found (580) that the second-set response of mice presensitized by means of allogeneic or xenogeneic skin grafts was more resistant to a lethal dose (850 rads) of x rays than the first-set response. This was also shown (578) with mice given sublethal irradiation (670 rads). Differential radio-sensitivity of the xenogeneic and allogeneic reactions was also observed but in opposite directions in primary *versus* second-set rejections (579). The method used for presensitization can also affect the apparent radio-sensitivity of the second-set-rejection mechanism (576). Thus, if spleen cells in Freund's adjuvant are used for presensitization, the resulting homograft response is as radio-sensitive as that produced by application of skin grafts. However, if the spleen cells are anatomically separated from the Freund's adjuvant in the recipient, a more radio-sensitive response develops, this difference possibly being due to a differential proportion of proliferating and mature cells induced by the two régimes. Consideration of hæmagglutinin production and skin-graft rejection by irradiated mice also tends to suggest that these two immune responses are mediated by separate cell lines (581), as has been discussed previously.

175. Accurate measurements of radio-sensitivity of the homograft immune mechanisms are again difficult unless a quantitative cell assay can be used. Two approaches to this problem have been reported. In one case (76), for estimating second-set rejection, recipient mice are primed with donor homologous (transplantation) antigens and then given irradiation and an injection of spleen cells of donor type which have been previously sensitized to sheep red cells. The ability of the transferred cells to form anti-sheep-red-cell antibody in the recipient is then dependent upon radio-sensitivity of the cellular immune response of the recipient. When recipients were given 500 roentgens, only a few animals responded, indicating an almost complete failure to take on the part of the infused homologous cells, that is, evidence of a still functioning host immune response. With 700-850 roentgens the antibody responses by the donor cells were intermediate and, with 900 roentgens, titres comparable to isologous controls were observed (complete homograft suppression).

176. Further quantitative evidence of the radio-sensitivity of homograft immunity came from a second assay method (75) in which the killing effect of parental (P1 or P2) cells was studied in irradiated, immunologically inert (P1 × P2) F1 recipient mice, by determining the decrease of anti-rat agglutinins synthesized by P2 cells. The data showed that the homograft-rejecting capacity is more radio-resistant than the agglutinin-forming capacity. Slight strain differences were also observed. The LD_{37} values for agglutinin formation by C3H and C57 cells were 58 and 47 rads, respectively. The corresponding value for homograft-rejecting capacity (C3H cells) was calculated to be 78 rads, which is in the range of radio-sensitivity calculated for cells in the inductive phase of the humoral antibody response. This suggests that cell proliferation is also the major radio-sensitive step in the development of a homograft response.

177. One problem with this interpretation is that the particular assay system used has not been proved to represent graft rejection by a direct *T* lymphocyte cellular process, and that cytotoxic or protective antibody formation might also be involved. In fact, in a further extension of this assay method (73), evidence was presented that the reaction could proceed through a porous membrane. Critical studies of the radio-sensitivity of the actual effector cells that mediate cellular immunity are still needed, and several suitable methods for this have recently become available. These involve *in vitro* assays directly measuring cytotoxic effects of sensitized lymphocytes on target cells (64, 65, 205, 447). Recent data suggest that there are two categories of specific cytotoxic lymphocytes, one of which retains cytotoxicity after doses of 2.000 rads, whereas the other is much more radio-sensitive, being markedly inhibited after doses of around 500 rads. There is also some evidence to suggest that stimulation by antigen renders the cytotoxic lymphocytes more radio-resistant (224).

178. Inactivation of stem cells has also been used as a target assay for homo-transplantation activity of lymphoid cells (681). When both lymphoid and hæmopoietic cells are grafted from CBA and C57Bl mice to lethally-irradiated F1 hybrids, the lymphocytes of CBA genotype inactivate 90-100 per cent of the colony-forming elements of C57Bl type, which is detected by the reduction in spleen colony formation (677). CBA donors were irradiated with $LD_{50/30}$ doses of gamma rays, after which the ability of their spleen cells to inactivate the stem cells of C57Bl mice was investigated. One hour, one day, seven days and fourteen days after irradiation the index of inactivation was 0 to 10 per cent. A partial re-establishment of lymphocyte homograft activity was observed after 30 days. Normal values were not obtained until 60 days after irradiation.

2. *Hæmopoietic grafts*

179. Bone-marrow transplantation to a lethally-irradiated recipient within a syngeneic system will produce complete restoration of the hæmopoietic system and thus, in situations in which the radiation damage causes lethality due to hæmatopoietic damage, the survival of the animal. This effect was well studied in laboratory animals for many years and is known to be due to the repopulation of hæmatopoietic tissues in the depleted host by the injected cells and their descendants (189, 310, 314, 315, 587, 644).

180. When marrow transplantation is performed in allogeneic situations, two problems are encountered. Firstly, if the bone marrow is foreign to the host, then the immune competence of the host must be sufficiently depressed by irradiation or by other means to permit the survival of the injected cells. It was estimated (570) that, when a major histocompatibility difference is involved, the minimum dose of radiation (followed by homologous marrow) necessary to permit survival of the injected cells, and therefore tolerance to the donor, lies between LD_{13} and LD_{90}. With minor histocompatibility differences, lower radiation doses are effective (126). In studies in mice, insufficient radiation leads to marrow-graft rejection and an early (within 5-21 days) mortality (570). This occurs even in the high sublethal range, presumably because the graft-rejection mechanism appears to be more radio-resistant than the animal's own hæmopoietic stem

cells. Thus, although the number of cells that persist is sufficient to reject allografts, the animal's own hæmopoiesis is suppressed below limits required for its survival.

181. The second problem with allogeneic grafts occurs when the host carries a major transplantation antigen not present in the donor's genotype. This results in a late mortality (21-60 days) when bone-marrow cells are injected into allogeneic lethally-irradiated recipients (e.g. parental strain into F1 hybrids). This type of mortality is attributed to an immunological reaction against the foreign host antigens by the homologous lymphoid elements (or their progeny) that have been introduced with the grafted bone marrow (92, 99, 136). The immunological nature of both of these problems is now well documented (162, 512) and will not be extensively reviewed. Instead, a brief consideration of hæmopoietic transplantation in larger animals and man will be undertaken, particularly in terms of the radiation conditions and doses required for adequate immunosuppression of the recipients. Several other factors concerned in this problem, such as dose rate, will also be discussed in relation to the animal experiments.

182. In a study of the survival of irradiated rats injected with allogeneic bone marrow, Courtenay (108) found a relation between survival and the x-ray dose rate. The study suggested that the lower rates of 0.28 and 1-4 R min^{-1} were less effective in depressing the host's immune response than the higher rate of 29 R min^{-1}. This was confirmed by Gengozian (198) who irradiated mice at several different exposure rates so that they received a total of 900 roentgens over the whole body. Within two hours they were injected with rat bone marrow. The higher the exposure rate, the greater was the success of the grafts. The results strikingly indicated that in mice given 900 roentgens at a rate of 3.75 R min^{-1}, virtually no take of donor cells occurred. This phenomenon was further studied by Gengozian *et al.* (199) who gave mice lethal whole-body exposures of 900 and 1,200 roentgens at different exposure rates followed by allogeneic or xenogeneic bone marrow transfusion. With both grafts and both total doses, mice exposed at 3.75 or 19.8 R min^{-1} did not show permanent survival. In fact, with 900 roentgens at 3.75 R min^{-1} no increased survival could be shown. Mice given 1,200 roentgens at high rate (39.7 R min^{-1} or 53.4 R min^{-1}) had a permanent take of grafted marrow.

183. It is to be emphasized that in these experiments the difference between dose rates is not large. Previous work (547) has stressed the importance of high dose rates for immunosuppression in comparing chronic (days, weeks) with acute (minutes, hours) irradiation. The distinction drawn in the experiments with bone-marrow transplantation is between a time of delivery of only 22 minutes (1,200 R at 53.4 R min^{-1}) and one of about 5½ hours (1,200 R at 3.75 R min^{-1}). These findings may have great relevance to clinical attempts at allogeneic marrow transplantation after whole-body irradiation, because many of the irradiators used on humans operate at low exposure rates. A survey of various clinical studies (20, 346, 352, 555) reveals that radiation is often delivered at exposure rates ranging from 0.5 to around 5 R min^{-1}, so that even though a total dose of 800 to 1,800 rads may be given, it is delivered at a very low rate. As will be discussed in the following paragraphs, most attempts at allogeneic bone-marrow transplantation in man have been relatively unsuccessful. Similarly, the difficulties in obtaining successful foreign-marrow grafts in large animals are well documented and again may be related to the low dose rates used, usually of the order of 4-20 R min^{-1}.

184. As a result of the geometry of large animals and of man relative to the radiation sources used, the absorbed tissue dose and the tissue-dose rate may be even lower. Exposure rates greater than those found (199) satisfactory for transplantation of bone marrow in the mouse may therefore be necessary for success in large animal studies. These considerations clearly indicate the need for careful evaluation of this basis for transplantation failure, as opposed to the more conventionally accepted graft-*versus*-host reaction.

185. Grafts of bone marrow from donors which differ at major histocompatibility loci can survive for about a month or two if the prospective recipients are pretreated with sub- and mid-lethal doses of radiation (197). Rejection of the foreign graft, which is related to the recovery of the host's immune system, can occur with a severe reaction leading to the death of the recipient. This effect is often referred to as the "mid-lethal killing effect" and is observed in these situations in mice where a mid-lethal dose is used together with strongly-antigenic donor bone marrow (587). The effect may be analogous to the enhancing effect of irradiation on the antibody response, and accordingly may have an important bearing on clinical attempts with allogeneic marrow transplantation after total body irradiation in so far as, with the doses and dose rates of irradiation used, this mid-lethal killing effect may be involved in apparent failure of takes of allogeneic donor marrow. A similar problem may also occur with situations of minimal donor-host genetic differences, since Barnes and Mole (32) showed that the injection of a minimal number of lymph-node cells from H-2 compatible mice into sublethally-irradiated recipients (CBA) caused a significant fraction to die 2-18 months later of a lymphoid deficiency (? graft *versus* host) syndrome.

186. The lethal effects of whole-body irradiation (1,800 R) of dogs can be overcome by administration of the dog's own marrow taken before irradiation (227). However, when allogeneic bone marrow is used, permanent takes are extremely rare. When methotrexate is given early in transplantation, controlled studies (558) show that an increased number of long-term survivors results. Survivors for five months to four years have been observed after whole-body exposures of 1,200-1,800 roentgens and injection of marrow with methotrexate (561). In some animals, mild secondary disease developed and then subsided. In studies with dogs given 1,200 roentgens and cross-circulated (168) with normal dogs of opposite sex or injected with marrow of opposite sex (167) donor-type mature granulocytes were readily evident in the irradiated partner. In this study also, methotrexate was of some value in diminishing the secondary disease (168). In view of the previous discussion of dose rate, it is to be noted that in these studies with dogs dose rates of less than 10 rad min^{-1} were used and the effect of methotrexate may have been to aid the immunodepression of the host. However, the clinical symptoms are claimed (562) to be different in dogs dying of graft rejection than in those dying of secondary disease, and care should be taken to clarify this in all cases.

187. In contrast to much of the experience in dogs and man, bone-marrow takes appear to be relatively successful in primates. In recipients given whole-body doses in the range of 550-930 rads and 4.2 10^8 allogeneic bone-marrow cells, takes have occurred in at least 95 per cent of the cases (116). However, at this stage the major problems with primate-bone-marrow transfusions are encountered. In mice and rats, although takes of bone marrow require suppression by reasonably-high radiation doses, permanent chimeras are then relatively frequently established. In primates, on the other hand, secondary disease is a far more common problem (135). This difference may be partly due to the numbers of cells required to protect the lethally-irradiated recipient. Estimates (588) range in the order of 5 10^6 cells per kilogramme for mice, 40 10^6 cells per kilogramme for monkeys and of the order of 100 10^6 cells per kilogramme for man. If it is assumed that comparable proportions of immunocompetent cells exist in the bone marrow in the different species, and that a similar absolute number of immunocompetent cells will initiate the graft-*versus*-host process, then it is possible that the excessive severity of secondary disease in primates is mainly due to the larger absolute numbers of immunocompetent cells. On the other hand, by varying the number of allogeneic bone-marrow cells used in transfusion, Vos (599) has shown that mouse bone marrow indeed contains less immunologically active cells than monkey bone marrow. Dicke (137) has also shown that mouse bone marrow contains far fewer phytohæmogglutinin-sensitive cells than monkey bone marrow.

188. Attempts at bone-marrow transplantation after whole-body irradiation in *Rhesus* monkeys which have received multiple transfusions only rarely leads to acceptance of the graft, in distinction to the almost invariable takes in non-immunized monkeys (593). This was also demonstrated with prior transfusions of blood from third-party donors. Decreased takes may well be due to the existence of a heightened state of the immune response in the recipients prior to irradiation, which, by increasing the number of immunocompetent cells, would accordingly lead to an increased number of reactive or potentially-reactive immunocompetent cells surviving after irradiation. The time interval between transfusion and irradiation (minimum 30 days) is probably too long to account for the decreased takes being caused by increased levels of antibody resulting from irradiation-induced antibody enhancement. In presensitized recipients, this problem might be overcome by giving the recipients higher doses of radiation with a view to a more complete eradication of the population of immunocompetent cells. However, if some of this population should involve the more long-lived radio-resistant subpopulation, irradiation at a sufficiently high total dose would not be feasible. Before considering the various approaches to amelioration of the secondary disease problem, a brief report on human bone-marrow transplantation is relevant.

189. Bone-marrow grafts were first introduced in man in patients with leukæmia (344) and in the victims of the radiation accident in Vinca, Yugoslavia (348). Although it has been clearly indicated that marrow grafts can take initially, the secondary disease problem in man is very severe, as its onset is generally very early, when the aplasia from total irradiation at the high dose of 800 rads is still uncorrected. Several groups have studied the effect of marrow infusion in patients with leukæmia. Using dose rates of up to 2 rad min^{-1}, total exposures of 1,200 to 2,000 roentgens do not appear to induce early gastro-intestinal complications. However, even in this exposure range it was found (556) that, although initial takes of allogeneic marrow (usually from related donors) occurred, survival of the patient was only of 2-4 weeks duration. Death was either from infection or, occasionally, from recurrent leukæmia. It appears that extremely high doses of radiation would be needed to completely eradicate the leukæmic cells.

190. With the increasing frequency of marrow transplantation in man, the syndrome of graft-*versus*-host disease as seen in man is becoming well defined. In a study by Meuwissen *et al.* (361), the following clinical findings were noted in 7 of 13 patients treated by marrow transplantation. The classical syndrome of fever, rash, liver disease and diarrhea was present in most instances although exceptions were noted. Skin showed destruction of the basal cell layer, a predominantly mononuclear cell infiltration, acanthosis, and dysparahyperkeratosis. A most striking finding was the frequency of localized or generalized ulceration of gastro-intestinal mucosa. Bone-marrow abnormalities occurred less regularly, and included hypoplasia and infiltration with plasma cells and histiocytes. Although elevation of liver enzymes occurred regularly during the graft-*versus*-host reaction, serious liver lesions were rare at *post-mortem*. It is noted that some patients showing these changes included those in whom an HL-A-matched marrow transplant had been given.

191. Although occasional remission of leukæmia has been observed (557, 560) with whole-body irradiation in a sublethal exposure range of 325 or 880 roentgens, most studies have involved higher exposures combined with marrow transfusion. In several studies with identical twins, the leukæmic individual was given 800-1,600 roentgens and marrow from the normal twin. In each instance (557, 559), a remission was achieved but the improvement was followed by a discouraging early return of the leukæmia (554). These studies confirm that the lethal effect of high doses of radiation in man can be counteracted by marrow transfusion, but again indicate that the same doses are not sufficient to eliminate all the leukæmic cells. A series of reports on allogeneic-marrow grafting in irradiated leukæmic patients was reviewed in 1965 (554). It was stressed that large numbers of cells must be given and that addition of immunosuppressive therapy to the radiation treatment is advantageous. Over-all success, however, is rare.

192. The seven years since that review have not seen any major improvement. A summary by Mathé (347) showed that 17 out of 24 marrow grafts had taken in the recipients. However, of the 17, 10 died with acute secondary disease, 3 with a later subacute or chronic secondary disease, and 4 with recurrent leukæmia. Several workers, including Mathé (345), have considered the possibility that presensitization of the recipient by repeated blood transfusions might occur and some evidence for this is suggested. It has been indicated (345) that administration of Imuran during the period of the transfusions reduces or annuls this immunization.

193. To stress the point again, it is the eradication of secondary disease which appears to be the major

problem for human-bone-marrow transplantation. Thus, although low dose rates are usually used, it appears that a sufficient depression of host immunity has been achieved. It is possible, however, that the low dose rate may partially account for the incomplete eradication of the host leukæmic cells, particularly as recurrences occur even after very high total radiation doses. Approaches to prevention of secondary disease consist mainly of (*a*) pre- or post-treatment of the host and (*b*) direct efforts to reduce or remove immunocompetent cells from the donor inoculum.

194. Treatment of recipients includes the addition of other immunosuppressive agents in the few days after marrow grafting, in an attempt to suppress the proliferation of the injected immunocompetent cells. Some success in this regard has been achieved with cyclophosphamide, amethopterin or antilymphocyte serum (396, 589), although it now appears that cyclophosphamide only postpones the onset of secondary disease and does not suppress it sufficiently to allow long-term survival.

195. Suppression of the induction of secondary disease by manipulation of the donor cells can be approached in various ways. The essential aim is to transplant an inoculum which contains sufficient numbers of hæmopoietic stem cells without containing any immunocompetent cells with specificity against the host. The ideal source of cells is a bone-marrow donor of identical histocompatibility type, at least for "major" antigens, and considerable effort is currently being invested for the complete histocompatibility typing of man. However, this can only be a complete solution provided there is ready availability of all types of donor marrows, which involves problems of procurement and storage.

196. The other approaches are all concerned with using allogeneic bone marrow lacking immunocompetent cells. Again, a possibility for the use of an unmanipulated cell suspension exists. In the adult, hæmopoietic stem cells are found predominantly in the bone marrow. However, in fœtal life the liver is the major site of hæmopoiesis and, if taken at an early stage, liver does not contain any immunocompetent cells, as the thymus induction of immune differentiation has only barely commenced. Studies in mice (582, 583, 586) clearly show that fœtal-liver-cell suspensions will not induce secondary disease in primary irradiated hosts. However, they become differentiated to immunocompetent cells provided the host has an intact thymus, and at the same time become tolerant to the host's histocompatibility antigens. The use of fœtal-liver-cell suspensions for the treatment of irradiated *Rhesus* monkeys has been studied by Van Putten *et al.* (594) who found that more than one complete fœtal liver was required per recipient. With the use of pooled fœtal-liver-cell suspensions, 4 10^8 to 11 10^8 cells per kilogramme gave repopulation in one fifth of the monkeys after 800 roentgens and in three fifths after 900 roentgens. It was concluded that fœtal liver is relatively less effective than bone marrow, possibly because the initial immune attack of the bone-marrow competent cells on the recipient aids in depressing the host's immunity. However, studies in mice (388) clearly show that fœtal liver is extremely rich in hæmopoietic stem cells provided it is taken at an appropriate fœtal age, and it is possible that the optimal age of monkey fœtal liver was not used. Perhaps the main drawback of fœtal liver cells as potential donor cells for use in clinical medicine is the problem of availability. If large numbers of cells are needed, this approach could only be realistically achieved through the use of pooled donors with a successful storage method. It was concluded (594) that the pooled frozen liver cells of roughly 50 human fœtuses of 20-26 weeks of age would be required for one adult. As such a large amount of material cannot be transfused safely, the use of fœtal liver cells was thought to be clinically unrealistic. On the other hand, it is possible that a more judicious choice of fœtal age for the liver source, and possibly fractionation of the cell populations giving more effective cells might permit the use of this approach.

197. If allogeneic bone marrow is to be the source of donor cells, the next general approach is either to purify the stem cells, or to selectively kill or remove the immunocompetent cells. Various physical cell-separation methods (for example, gradient centrifugation) with mouse bone marrow have shown (138, 388) that fractions with enriched hæmopoietic stem-cell activity can be obtained, although as yet not in pure form. In one study (139) it was also shown that certain fractions could be obtained which contained up to 50 per cent of the original stem-cell activity, but produced no secondary disease even with large numbers of cells. This approach is therefore promising and should be attempted with primate cells, provided suitable *in vitro* assays for measuring separately the activity of stem cells and immunocompetence can be developed.

198. By the use of the *in vitro*-colony-forming method (61) as a measure of stem-cell activity, recent data with monkey bone marrow strongly suggest that this approach may be very successful (391). Separation of *Rhesus* monkey bone marrow by buoyant density gradient has demonstrated a reproducible and homogeneous light density distribution profile of cells capable of forming hæmopoietic colonies in agar culture. An average hundred-fold enrichment of these cells was obtained, with the most enriched fractions containing the majority of these cells in the original marrow inoculum. Although assays for immunocompetent cells have not been performed on these inocula, it is most probable that the content of these latter cells would be considerably reduced, particularly in those fractions where up to 33 per cent of the cells are *in vitro*-colony-forming cells.

199. Recent studies (343) of graft-*versus*-host reactions in mice have indicated that the receptor site on the mouse immunocompetent cell that is responsible for recognition of the foreign histocompatibility antigens is an immunoglobulin molecule, possibly either a free L chain or a new type of immunoglobulin. Pretreatment of adult mouse spleen cells with rabbit antisera against mouse-immunoglobulin light chains completely prevented the cell suspension from inducing a graft-*versus*-host reaction. Viability of the cell suspension after this treatment was indicated by dye-exclusion tests with trypan blue. However, of even greater relevance was the observation that hæmopoietic stem-cell activity, as measured by the mouse spleen-colony assay, was unaffected by the anti-light chain serum treatment (605). This general approach should be extensively applicable to clinical work if it proves reliable in experimental studies. The treatment involves a short (approximately

1-2 hours) incubation of the cells with an appropriate concentration of the antiserum, followed by cell washing and then transfusion.

200. Specific removal of immunocompetent cells reactive to host-histocompatibility antigens could be achieved by treating them with the specific antigens and in some manner then effecting their removal. This might be approached by determining whether the specific immunocompetent cells formed rosettes or aggregates on mixing with allogeneic cells. If this occurred, the aggregates could be removed by some method involving particle size. Another potential method might be based on the studies of Ada and Byrt (3) which showed that the potential for the formation of specific antibody to a bacterial antigen could be removed by pre-incubating a normal cell suspension with a very heavily ^{125}I-labelled preparation of the antigen, thus inducing radiation killing in those cells which specifically bound the antigen. The potential to produce antibodies to other non-cross-reacting antigens was not destroyed, indicating that the radiation effect was only induced at very close range, presumably through the labelled antigen held on the cell surface (337). For this approach to be applicable to man, it would be necessary to purify specific histocompatibility antigen which can be successfully labelled with ^{125}I, and to apply a concomitant histocompatibility-matching scheme, so that the appropriate cells are removed prior to their transfusion into the recipient. This latter necessity, however, could be avoided, at least theoretically, if a radio-labelled antigen preparation were to be prepared from each recipient and then applied to the donor cells before transfusion. This method offers the potential advantage of involving only a relatively short pretreatment of the donor cells before transfusion.

201. Other measures that have been attempted to eliminate the graft-*versus*-host reaction include (*a*) an *in vitro* exposure of donor cells to antigens of the prospective recipients before use in transfusion (107), (*b*) sublethal irradiation of donors before collection of their bone marrow (117), a procedure which, however, would also considerably reduce the hæmatopoietic stem-cell activity of the marrow, and (*c*) control of the radiation dose rate (199, 562).

202. At the present time, none of the above methods has yet been shown to work completely satisfactorily, which probably indicates that several variables are involved. This is substantiated by the studies of Congdon *et al.* (96, 97) who undertook a comprehensive "4-factorial" study in mice, assessing the effects of variation in the interval between whole-body irradiation and injection of allogeneic bone marrow, the number of bone-marrow cells, the age of bone-marrow donor and sex. On the basis of these experiments the 90-day mortality could be reduced tenfold by controlling these factors. These results indicate that, combined with other approaches mentioned above, complete elimination of the secondary-disease mortality is a very realistic possibility.

203. At present, there is very little information bearing directly on the question of possible cell collaboration in cellular immunity as related to radiation sensitivity. Several aspects of the induction of a graft-*versus*-host reaction by bone-marrow cells are at present confusing. The basic immunological question is whether the bone-marrow population contains immunocompetent *T* lymphocytes which will immediately initiate the graft-*versus*-host reaction on injection into recipients, or whether maturation of the potentially-immunocompetent cells in marrow (stem cells) is required. If the latter is true, or if a cell-collaboration step is involved, the process may take place in the host and the radiation dose rate used in man and other primates may be insufficient to prevent a rapid expression of secondary disease by the donor cells. This possibility is based on the following experiments.

204. Adult thymectomized irradiated mice given syngeneic bone marrow are immunologically unreactive. When an allogeneic thymus graft was also placed in the recipient, the mice recovered their immune capabilities and rejected the thymus graft itself (369). At no time was there any evidence of repopulation of the allogeneic graft. This indicates that the injected syngeneic bone-marrow cells already carried the potential to react against the histocompatibility antigens of the allogeneic graft but first required something from the graft, probably humoral in nature (432), to express this activity. Thymus grafts irradiated *in vitro* (2,000 R) failed to restore neonatally-thymectomized mice to full immunological capacity (367), thus suggesting the existence of a radio-sensitive stage in the synthesis, release or activity of the thymic factor. These experiments therefore suggest that bone marrow contains an immunocompetent cell capable of reacting against histocompatibility antigen, provided a thymic factor is available.

205. If this is also applicable to the injection of bone-marrow cells into allogeneic recipients, it implies that the host animal must provide a thymic factor for the injected cells to be able to induce the graft-*versus*-host reaction. As this effect of the host thymus may be radio-sensitive, it is quite possible that the relatively-late onset of secondary disease in lethally-irradiated mice is due to radiation damage to the host's thymic epithelium, and that this must first recover before the injected cells can attack. As the dose rates used in man and primates are of a low order (<5 R min^{-1}), it is quite possible that this radio-sensitive phase of the thymic effect has not been sufficiently destroyed, thus allowing for immediate maturation of injected stem cells to immunocompetence. This concept would suggest that higher dose rates might also be advantageous in delaying thymic epithelial restoration and therefore development of immune competence. It is possible that local thymic irradiation might even produce a sufficient delay to permit the injected cell population to become tolerant to host antigens.

206. Experimental verification of this concept could come from a direct demonstration that removal of the host thymus prevented the induction of secondary disease in irradiated mice given allogeneic cells. Such an experiment has been reported by three groups, but the interpretation of the results is difficult, because adult thymectomized lethally-irradiated mice given syngeneic bone marrow also develop a wasting disease as a result of lymphoid aplasia. Even if the secondary disease were prevented in thymectomized allogeneic recipients, the mice could still die of wasting through lymphoid aplasia. In one study (592) with heterologous combinations, a marked reduction in the incidence of secondary disease was observed in thymectomized mice, and in two other studies a marginal prolongation of life was observed (209, 537). A critical test of this hypothesis would require the use of germ-free irradiated thymectomized recipients, to avoid wasting disease from lymphoid atrophy.

3. *Organ grafts*

207. Human renal allograft transplantation has become a major accepted form of clinical therapy for certain kidney diseases. Inherent in any successful organ grafting is the prevention of a host immune response from rejecting the graft. This can basically be approached in two ways: (*a*) by avoiding presenting the recipient with an effective foreign antigen, and (*b*) by suppressing the host's immune response. Irradiation has been a valuable tool for immunosuppression over the past 10-15 years, but it is certainly not the ultimate ideal approach. The majority of current approaches to suppression do not involve irradiation, and accordingly the field of organ transplantation is currently of less direct relevance to the topic of radiation and immunity. The main current efforts are aimed at (*a*) developing histocompatibility typing in order to select the most closely matched graft possible and therefore to limit the degree of foreignness in the donor graft, and (*b*) achieving immunosuppression by means of drug therapy, anti-lymphocyte serum, or immunological tolerance. However, as radiation has been used very frequently in the past, certain aspects which involve different uses or types of radiation will be discussed here. In terms of the two approaches to suppression of homograft immunity mentioned above, radiation has been aimed at either (*a*) the graft itself, or (*b*) the immune system of the host. These will be considered separately.

208. An impressive body of data clearly indicates that local irradiation of the kidney soon after transplantation is of definite value in delaying acute rejection (261). An example drawn from the kidney-transplant registry is shown in figure XII. The influence of local

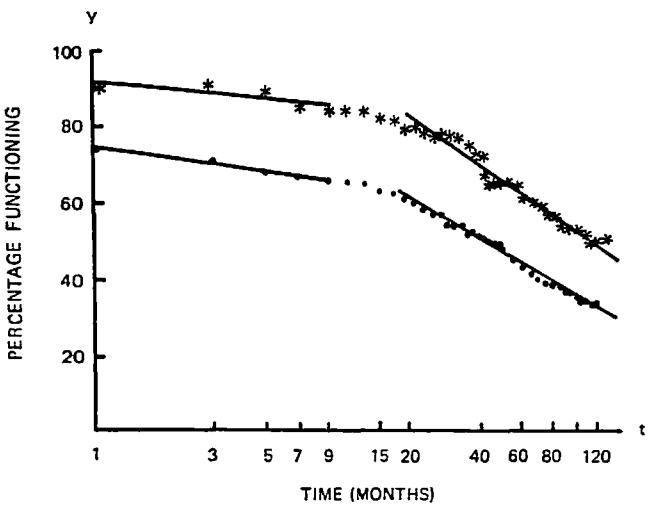

Figure XII. Effect of local kidney-graft irradiation on survival of the graft. The figures are drawn from the kidney-transplant registry (8), and plot the percentage of functioning grafts against time for both irradiated (stars) and non-irradiated (closed circles) grafts

irradiation can be seen as early as one day after grafting. Local graft irradiation is usually performed by fractionation of some substantial dose of radiation, e.g. 1,000 rads, into smaller doses of not less than 150 rads, which are given at some appropriate interval. Before considering the possible mechanism involved, it is of interest to mention a few of the direct experiments on local irradiation. Some complications of local irradiation will be discussed in the section on delayed effects (VII.C).

209. In 1953 Dempster (130) claimed that the pyrinophilic reaction in the transplanted kidney could be reduced by irradiating it prior to transplantation. Approximately 250 rads were given to the kidneys while still in the donor. However, in other early reports or experimental studies, high doses given to the donor did not influence the characteristic reaction (193, 260). In a further study in dogs, local irradiation was given as six fractions of 150 roentgens every two days (631) to the graft *in situ*. The mean kidney survival in the irradiated group was 23.4 days compared to 9.9 days in the controls. This prolongation was confirmed clinically (261) in a review of many patients, and again experimentally in a further trial in dogs (631). With experimental heart transplants in rats a similar schedule of repeated local irradiation (6 × 150 R) of the graft starting immediately after transplantation also produced a slowed interstitial infiltration of the graft by lymphocytes, and its longer survival (430).

210. The critical question in relation to the radiation effect is whether radiation is destroying the actual immunogenicity of the donor graft or is suppressing the early phase of the host response. There is some experimental evidence for both points of view. The former interpretation is based on the notion that the circulating lymphoidal cells of the graft constitute the major immunogenic stimulus. It has been shown that the mixed lymphocyte reaction can be inhibited when one of the component cell populations is exposed to 1,000 roentgens and it was suggested that this acts by destroying the capacity to stimulate the allogeneic lymphocytes (300). However, this was not consistent with another study using 2,500 roentgens in which no loss of activity was observed (326). In transplantations of allogeneic tissues together with adult leucocytes onto the chick chorioallantoic membrane, rejection only occurred if the transplant contained a relatively large component of reticular tissue. Treatments such as gamma-irradiation, which reduce the amount of reticular tissue in the graft, protected it from transplantation damage (300).

211. In another study (161) it was shown that in a direct graft-*versus*-host reaction induced by parental strain cells injected in the kidney of an F1 rat, several factors were operative. Since Gowans has reported (218) that sensitization of lymphocytes is a consequence of their perfusion through an isolated allogeneic kidney, and since rat kidney cells have been used as target tissues for *in vitro* cytotoxic immune reactions (625), it is likely that kidney parenchymal cells may offer an immunogenic stimulus to specific cells. However, by themselves, the donor parental strain cells cannot do much damage to the host kidney or even generate more than a very sparse local infiltrate. It therefore appears that a host component is necessary for the full development of interstitial infiltration and parenchymal destruction. This was deduced from experiments which showed that whole-body irradiation of the hosts 24 hours before the injection of allogeneic parental cells resulted in an inhibition of the subsequent reaction, to a degree commensurate with the radiation damage sustained by the lymphoid system of the host.

212. These results in general suggest that local irradiation of the kidney after transplantation may be beneficial and that several factors are possibly involved,

including both destruction of any donor lymphoid cells which may act as a strong immunogen, and destruction of early-infiltrating host cells, many of which may be acting in a non-specific destructive manner, perhaps in a fashion analogous to the recruitment of normal host lymphoid cells in delayed-hypersensitivity reactions (52).

213. Even apart from other more serious considerations of the disadvantages of using radiation, in immunological terms whole-body irradiation for suppression of organ-graft rejection is by no means an ideal approach. If radiation were to be the sole agent for immunosuppression, the accompanying problems of bone-marrow transplantation would also have to be solved as the dose required to create sufficient immunosuppression would lead to marrow aplasia. Accordingly, only sublethal exposures are practical, which, although aiding in immunodepression, may still be expected to provide significant radiation damage. Before the advent of the more recent methods of immunodepression, whole-body irradiation at doses of the order of 400 rads was used with some possible success (232, 357). Additional localized irradiation of the spleen and the right lower abdomen has also been given to depress immunity and to obliterate the lymphatic field draining the transplant (633).

214. Under certain experimental circumstances, whole-body irradiation has *facilitated* the destruction of renal grafts. Studies in inbred rats (174) with renal grafts placed into immunologically-tolerant hosts afforded a means of examining the rejection process under controlled conditions. Graft rejection could be induced by the injection of large numbers of competent syngeneic lymphoid cells. If whole-body irradiation (550 R) was also given, graft rejection was greatly facilitated, in that fewer injected cells were needed to induce rejection, and graft destruction was hastened. Total-body irradiation *per se* was occasionally followed by the destruction of skin homografts. This effect may have occurred through a variety of mechanisms, such as (*a*) depletion of lymphoid cells in host organs allowing better seeding of the injected cells; (*b*) enhanced cell growth and preferential mitosis in the presence of antigen; (*c*) alterations in the target cells rendering them more susceptible to rejection, or (*d*) reduction or suppression of the state of tolerance in the host. This latter mechanism will be discussed in more detail in a later section.

215. Various other means of achieving radiation-induced immunological depression have been reported (29). Although it is through the discovery of many immunosuppressive drugs that the results of organ transplantation have greatly improved, in certain instances it may not be advisable to use these agents, and resort to radiation-induced depression may still be required. For example, it has been reported that a severe toxic reaction to Imuran and prednisolone may occur in Japanese people, and alternative immunosuppression by intralymphatic administration of radioactive isotopes (^{198}Au) to destroy lymphoid tissues was attempted (505). This method resulted in a reduction of peripheral lymphocytes and a decrease in serum gamma globulin. A useful reduction in dosage of Imuran and prednisolone thus became possible, safeguarding against the development of post-operative complications in these patients. Several other studies with intralymphatic radio-active materials have been reported. Wheeler *et al.* (620) also used intralymphatic colloidal gold (^{198}Au) combined with splenectomy and direct injection of the isotope into the mesenteric lymph node of dogs. A marked selective lymphopenia was observed in the dogs for three to five weeks. The rejection of homologous renal transplants was delayed. Severe lymph-node destruction was produced and it was felt that, combined with Imuran, additive immunosuppression occurred.

216. Intralymphatic injection of ^{131}I-ethiodal into dogs was followed by a marked lymphopenia for four weeks, with progressive reduction in lymph-node size (567). Repeated doses were found to have a cumulative effect. Radio-active chromic phosphate (^{32}P) given by direct intralymphatic injection into dogs has also been used (81) and produced a severe destruction of a majority of lymph nodes with subsequent lymphopenia. However, although antibody production against human serum albumin was significantly inhibited in this series, the reactivity to the allografted heart was not altered. It was noted that intralymphatic injection of radio-active material leads to selective destruction or change only in lymphoid tissues. All other organ systems appeared quite normal following intralymphatic injection, in contrast with intravenous injection of ^{32}P which affects all systems. The intravascular implantation of a high-energy beta-emitting source (^{90}Y) into dogs was also shown (630) to produce a profound lymphocytopenia. Within 12 hours, levels fell to 0 to 10 per cent of pre-implantation values and remained low for three weeks. Ten animals given renal homografts (with the implant as the only source of immunosuppression) showed a mean functional survival of 16.9 days (controls 5.3). Biopsies showed minimal cellular infiltration.

217. These approaches suffer from the disadvantage that it is very difficult to control the radiation dose. Their use is still in the experimental stage and they are not at present recommended for human clinical use.

218. Another approach to the reduction of the circulating immuno-competent lymphocyte pool is through extracorporeal blood irradiation (ECIB). This approach was first developed by Heymans in 1921 (252) and subsequently refined extensively by Cronkite *et al.* (111) who described a method of producing a profound lymphopenia in calves by shunting the blood around a ^{137}Cs or ^{60}Co source. In dogs, a short course of ECIB produces a significant lymphocytopenia, and repeated daily doses give a prolonged lymphocyte suppression (324). Two patients also were so treated and in one case some reversal of the early acute rejection process was reported. However, no effect on the later rejection process was observed. In a study (476) of 11 patients waiting for renal transplantation, to whom approximately 9-37 kilorads were given by ECIB, lymphocytopenia was observed in only three. An impairment of renal-homograft rejection in dogs given continuous ECIB has also been observed by Wolf *et al.* (632). Several other reports (262, 356) have indicated that ECIB has been successfully applied in the treatment of rejection crises. In a recent detailed study (448) of 18 patients on ECIB before renal transplantation, followed by drug-mediated immunosuppression, a significantly smaller number of rejection crises occurred in the irradiated patients as compared to 60 controls given only the same drug treatments, and in general survival rate was higher.

219. Despite the encouraging results of currently-used schedules of ECIB in human renal transplanta-

tion, some recent data suggest that there may be a need to further evaluate different schedules (78). In this study, two alternative schemes of ECIB were used in goats and, following one of these, the mean goat renal allograft survival was doubled as compared to control goats. This success was achieved without the help of immunosuppressive therapy or the benefit of donor-host matching. The preliminary results with two goats indicate that a combination of pre- and post-transplant ECIB might be better than pre-transplant ECIB. This study also showed that, as with other techniques of immunosuppression, the degree of blood lymphocytopenia following ECIB does not correlate with the transplant survival.

220. The general lack of close correlation between lymphopenia and improved allograft survival following ECIB, raises the possibility that more subtle inactivation changes may have occurred in the remaining lymphocytes, or that there may be an alteration in the proportion of *T* and *B* lymphocyte types. This latter possibility was investigated (614) by means of lymphocyte-transformation tests with blood samples taken before and after ECIB. The response to phytohæmagglutinin was unchanged, while the response to purified tuberculin and allogeneic cells was reduced per unit number of lymphocytes after ECIB. These results were interpreted as indicating that the fraction of thymic derived cells left in the peripheral blood after ECIB was unchanged, but that the immunological functions of these cells was impaired. Similar results were observed after irradiation of the blood *in vitro* with single doses of from 100 to 500 rads.

221. In all these reports, it is fairly clear that ECIB, like intralymphatic irradiation, implant irradiation or even local lymphoid-organ irradiation, will reduce the level of the recirculating lymphocyte pool and reduce the incidence and severity of the early rejection crises. As a complete and permanent immunosuppressive régime it is clearly not enough, but may still be useful as an adjunct to immunosuppression by other means, perhaps particularly during rejection crisis. Again it should be stressed, however, that in view of the damaging effects of radiation, a goal of transplantation research should be to avoid and replace its use wherever possible.

V. Radiation and immunological tolerance

A. Two antigen dosage zones for tolerance induction

222. The concept of immunological tolerance as first proposed by Burnet and Fenner (69) was based on the discovery by Owen (436) that erythrocyte mosaicism existed in dizygotic twin cattle and persisted for a long period of time. This mosaicism results from an interchange of primordial hæmopoietic cells through vascular anastomoses between the co-twins. It was the persisting nature of this chimeric state that led Burnet and Fenner to the hypothesis that the immunological system of the organism becomes non-reactive to antigens with which it comes into contact in embryonic life and that the normal function of this mechanism is to ensure the non-antigenicity of self components. Further evidence of the anomalous situation in dizygotic twin cattle was found in the acceptance of skin homografts between the partners (17, 48) and the phenomenon was termed immunological tolerance. Experimental demonstration of the production of tolerance was then made by injection of embryos with homologous cells (47).

223. Since these earliest demonstrations of tolerance, a vast literature on the subject has developed and has been the subject of various reviews (152, 239, 307, 518). As much of this is not relevant to this present topic, we shall be concerned in this section only with the recent development of the concept of two zones of antigen dosage in which tolerance can be induced, as one of these zones may be involved in maintaining the normal homeostatic mechanism and thus preventing anti-self reactivity. Theoretically, a disturbance in this system, such as might be induced by radiation, could lead to breakdown of tolerance and the production of auto-immunity. This will be considered in section V (D).

224. In normal adult mice, the repeated administration of high doses of antigen paralyses the immune system and leads to a progressive decline in reactivity. Lower doses of antigen (for example, 0.1 to 1.0 mg of bovine serum albumin) lead to immunization and to stabilization of the serum antibody at a high level. The studies of Mitchison (381, 382), Dresser (151), Shellam and Nossal (504) and Ada and Parish (5) have now revealed that another antigen-dose zone for the induction of tolerance exists with amounts of antigen below the immunizing dose. The actual dose range involved appears to differ for different antigens used. With bovine serum albumin, repeated doses of 1-10 microgrammes will induce a partial tolerance but, with *Salmonella* flagellin, the picogramme range is more effective.

225. Although the detailed mechanism of tolerance, particularly of low-zone tolerance, is not completely understood, the existence of such a phenomenon may be of some fundamental importance. The results suggest that tolerance might be the most likely result of an interaction between an antigen-sensitive cell and a molecule of antigen, although it must be noted that low-zone tolerance has not been demonstrated to occur after challenge with low doses of living infectious agents. Immunity appears to require the presence of more antigen, either because it has a higher threshold or because it requires antigen-processing by macrophages or localization of antigen on the dendritic processes of reticular cells. Some recent studies (244-247, 456) have suggested that true allogeneic tolerance of *T* lymphoid cells may not exist, and that the phenomenon may be explained by the presence of a blocking factor which prevents the cell-mediated attack by *T* lymphocytes. On the other hand, recent data by Rouse and Warner (478) demonstrates the induction of allogeneic tolerance in agammaglobulinæmic animals, which indicates that the formation of blocking antibodies cannot be the sole explanation for allogeneic tolerance.

B. Induction of tolerance

226. It appears likely that tolerance induction and immunity induction are alternative effects of the antigen on particular lymphoid cells. One injection of antigen may drive some of the cells in one direction and other cells in the opposite direction (381, 419). The great usefulness of x-irradiation in regard to tolerance induction relates to the use of adult animals, when moderate to high immunogenic materials are used. In the absence of x-irradiation, the antibody formation which results from antigen-reactive cells being driven

towards immunity masks or blankets any simultaneous tolerance induction in other individual cells. When sublethal irradiation precedes antigen injection, many of the antigen-reactive cells are killed in proportion to the dose of radiation. Recovery of the immune system then occurs mainly by recruitment of stem cells from the bone marrow which, under thymic influence, are induced to become antigen-reactive cells. This recovery phase in essence resembles the immunological maturation around the time of birth, and many studies have clearly indicated a greater ease of tolerance induction in new-born than in adult animals (152, 518), even with the low-zone tolerance model (6, 418). As the recruitment and differentiation of new antigen-reactive cells after irradiation is a progressive occurrence, paralyzing antigen concentrations must be maintained for some time in these tissues. Several studies have been performed on the detailed kinetics and exact requirements for tolerance induction after x-irradiation and these will be briefly reviewed.

227. In normal adult rabbits, repeated infusions of large amounts of heterologous plasma proteins can induce a state of specific immunological unresponsiveness. This normally lasts for about 3-4 months. However, if the rabbits had been given 400 roentgens two days before the start of the antigen infusions, the tolerant state persisted for at least 10-11 months (143). These studies were then extended by Nachtigal and Feldmann (399) who assessed the influence of two variables on tolerance induction, namely, (*a*) dose and timing of irradiation, and (*b*) dose of antigen. Evidence was presented that the degree of unresponsiveness was a function of the time interval between x-irradiation and the beginning of antigen administration. If antigen was given 24 hours or 16 days after irradiation, complete tolerance was produced, whereas 42 days later administration of the antigen led to only partial tolerance. In this system, doses of antigen that would be immunogenic in normal animals were found to bring about tolerance in the irradiated rabbits. In another study (471) adult rabbits were given either 10 or 100 milligrammes of bovine serum albumin 24 hours after irradiation. Antibody response to the lower dose was suppressed but not that to the higher dose. It was further shown that the 10 milligramme dose had in fact established a state of specific immunological unresponsiveness.

228. Kinetic studies (400) in rabbits given 550 roentgens and human serum albumin revealed that tolerance can be induced with small amounts of antigen which in non-irradiated animals would constitute small immunizing doses. This only occurs when the antigen is injected over a prolonged period. Thus 20 milligrammes given in a single injection applied shortly after x-ray treatment did not induce tolerance. This result is contrary to the overloading concept of tolerance induction, since cellular depletion is most severe immediately after irradiation and the overloading of cells with antigen would be most pronounced at that time. Tolerance was most effectively induced in the x-irradiated rabbits when administered in small doses spread over the post-irradiation period. Tolerance induction could occur even when the antigen treatments were started four weeks after irradiation. Moreover, it appeared that smaller amounts of antigen are required for tolerance induction in this period, which suggests that susceptibility to tolerance does not develop immediately following inactivation of immunocompetence by x rays and that it may perhaps be a transient phenomenon appearing closer to the immune recovery phase. In other words, this would indicate that tolerance induction is acting on a cell at a certain stage of differentiation which is present particularly in new-born animals and during the recovery phase after irradiation.

229. The effects of small amounts of proteins given over the course of ten weeks immediately following whole-body irradiation (600 R) has been examined in mice (383). Four different proteins acted in much the same way, all but one showing a similar threshold dose of antigen for tolerance induction. Doses of antigen given three times a week are more effective in paralyzing than doses more widely spaced or than a single injection. The only exception to this statement is that in rabbits a single injection of bovine serum albumin was shown to paralyze after irradiation (312), but this may be due to the relatively slow elimination of bovine serum albumin from the circulation of the rabbit.

230. The concept of radiation-enhanced immunological tolerance might be applicable to problems of graft rejection. A soluble histocompatibility antigen prepared either directly from the potential kidney donor or from another source of histocompatibility-matched (to the donor) material, might be used to induce tolerance in the recipient, at least for the initial period when graft rejection is most likely to occur. The graft itself might then act as a continuous source of transplantation antigen to permit the maintenance of the tolerant state. The critical problem then is to be assured of inducing tolerance rather than immunity in the recipient. Theoretically there are two approaches. As it is rather unlikely that enough material will be available to induce high-zone tolerance in adult, either low-zone tolerance or radiation-induced tolerance would be required. As the former is rarely an absolute and total tolerance, and to err on the side of too much antigen might easily provoke an immune response, the use of sublethal irradiation of the recipient two to three weeks before the transplant, combined with repeated injections of the soluble antigenic material, would be more likely to result in specific tolerance. Indeed, studies in mice (285) have clearly shown that non-lethal exposures (e.g., 150 R) can be used as an excellent facilitating agent in inducing skin-graft tolerance to weak histocompatibility antigens. Further doses of radiation would not be advisable, in part because radiation-induced breakage of tolerance might then occur (see next section).

C. Breakdown of tolerance by radiation

231. The state of immunological tolerance persists for only a certain finite period unless continuing, albeit low, levels of antigen are maintained. There are several factors involved in the loss of the tolerant state, the two major ones perhaps being the decrease in antigen concentration and the emergence of new immunocompetent cells *via* the differentiation pathway. Regardless of whether or not there is such an entity as a reversibly tolerant cell, new immunocompetent cells are constantly arising throughout life. Thus if tolerance is to be maintained, there must be sufficient antigen still present to make tolerant each new immunocompetent cell as it arises. Thus, measures that reduce the rate of appearance of new competent cells, such as thymectomy, prolong the state of tolerance (86, 87). On the other hand, if antigen were to be more rapidly eliminated

or an excessive production of immunocompetent cells were stimulated, then breakdown of tolerance would occur much faster.

232. If the continued presence of antigen is indeed required for the maintenance of the tolerant state, it was predicted by Denhardt and Owen (132) that x-irradiation of tolerant animals would result in a loss of the tolerant state. This would be expected either from possible radiation destruction of the cells storing antigen, or by the excessive proliferation of stem cells which occurs following irradiation. In the first experimental test of this idea (132) rabbits made tolerant to bovine serum albumin were given 300 roentgens and immunized with bovine serum albumin 16 days later. No evidence of a break in tolerance could be detected. In a similar experiment but using 450 or 1,000 roentgens, Weigle (616) also could not find any break in tolerance. However, this particular tolerance model represents one of the most stable tolerance situations known and may therefore be the most resistant to change.

233. In studies with rats, Nossal and Larkin (422) induced tolerance to mouse red blood cells by starting injections at birth, and then gave lethal irradiation when the animals were adult. The rats were then given bone marrow from a tolerant donor, and on immunization with mouse red cells were shown to be capable of antibody production. This was then extended (327) to a simpler system in which the tolerant rats were given sublethal irradiation. Tolerance breakdown was again observed with the formation of substantial amounts of antibody. Similar data were also obtained by Stone and Owen (530) using rats tolerant to sheep erythrocytes. These results also showed that the loss of tolerance could not be demonstrated unless the antigenic challenge was given at least 6-18 weeks after irradiation. The results of both groups indicate that the cells emerging by proliferation and differentiation after irradiation are less likely to be made tolerant by antigen and perhaps are more prone to stimulation towards antibody formation, thus aiding further in tolerance breakdown by immune elimination of any residual antigen. Breakdown of transplantation tolerance has also been demonstrated; partial tolerance across H-2 barriers was induced in mice at birth, and tolerance was completely abrogated by exposure to 350-450 roentgens (173).

234. Attempts to break tolerance induced with antigen doses in the low-zone range have recently been described (503). Tolerance to flagellin was induced in rats by repeated daily doses of 10 microgrammes for several weeks. These animals were then given normal thoracic-duct lymphocytes with or without added irradiation of the recipients prior to cell injection. Challenge with antigen was also made at the time of thoracic-duct cell injection. Irradiation alone did not produce any loss of tolerance in the three weeks following injection. In view of the preceding reports, this may well have been too short a time to allow for recovery. However, the injection of normal thoracic-duct cells combined with host irradiation led to a breakdown in tolerance, even when only the recipient's spleen was irradiated. In this instance, irradiation may have aided by creating some lymphoid atrophy in the lymphoid organs of the host, thereby permitting a more successful colonization of the injected normal mice by the transfused lymphoid cells. In general, it appears that, regardless of the exact detailed mechanism of tolerance breakdown, the effect is essentially an *acceleration* of the anticipated eventual breakdown. Thus tolerance situations that are inherently more stable and permanent may be relatively more difficult to break by radiation.

D. Implications for auto-immunity

235. It has been frequently pointed out that the immunocompetent cell population is often called on to produce antibodies or cellular immune reactions against materials which are of a nature very similar to that of the tissues of the animal itself. This includes recognition of histocompatibility antigens, allotypic forms of immunoglobulins including those derived by maternal-fœtal transmission (522, 608, 627), various tissue-specific iso-antigens (e.g. those within the thymus, TL, theta, etc.) (60) and various tumour-specific antigens (e.g. Prehn, (463)). The cell population of the body must therefore have some means of distinguishing these from self-antigens or of preventing the continual emergence and activation of potentially autoreactive cells (66). If the maintenance of the normal state of immunological homeostasis (non-reactivity to self) involves a tolerance type of mechanism which eliminates or inhibits anti-self reactions, then agents which break down induced tolerant states might behave similarly with potential anti-self reactions and play a role in the induction of auto-immune diseases. A precedent for this argument comes from studies that show that injection of related antigens can break down a state of immune tolerance. Weigle (615) showed that injection of either human serum albumin or chemically-modified bovine serum albumin into rabbits tolerant to bovine serum albumin will break the state of tolerance to a certain extent. He then (617) extended this observation in showing that auto-immune disease in rabbits could be induced by the injection of a similarly chemically-modified self protein. Hence it is reasonable to consider the possibility that, as radiation can break tolerant states, particularly weak states, it may also be capable of breaking self-tolerance, that is, of inducing an auto-immune disease.

236. Recent studies with an *in vitro* system of mouse spleen cells and a fragment of a bacterial flagellin, have shown that specific tolerance can be induced purely *in vitro* with either a high-zone (140) or a low-zone dose of antigen, provided that an optimal concentration of antibody is present in the latter case (176). It is the critical ratio of antigen to antibody that determines the capacity to induce tolerance in the antigen low-zone dose range. If this mechanism is also applicable *in vivo*, a source for this critical amount of antibody must be envisaged. Such a source could either be found in the so-called natural antibody, or be induced by the initial dose of antigen. Ada *et al.* (6) have in fact reported a concomitant antibody production to occur during induction of low-zone tolerance *in vitro*. It was therefore considered by Feldmann and Diener (176) that such a mechanism of low-zone tolerance may be operative in the maintenance of self-tolerance. Possibly the small amount of antibody synthesized by the antigen-reactive cell, and normally exposed on the cell surface, may serve this purpose. Regardless of the actual source of this antibody, it might be proposed that radiation-induced proliferation of the stem-cell system and differentiation towards potential antibody production might alter the balance between the normal homeostatic levels of self-antigens

and of their respective antibody. Such an event might then swing the system in either direction. Excess antibody would possibly not provoke any break in control of self-reactivity as it would perhaps continue to mediate feed-back inhibition at the central level (175). However, the alternate direction of increased antigen levels, perhaps as a result of radiation-induced release of antigen, might trigger an auto-immune process. Further experimental studies on the relevance of the different current mechanisms of tolerance to the normal homeostatic control are clearly warranted.

237. In the light of these general considerations on radiation and auto-immunity, it is of considerable importance to examine any available human data that may relate to this problem. The most suitable material would derive from an examination of the immunological consequences of exposure to the atomic bombs of Hiroshima and Nagasaki. Interest in this area at the Atomic Bomb Casualty Commission is of relatively recent origin, and much of the attendant data is as yet incomplete. However, various observations have been made and should be considered. Two studies have been carried out in an examination for effects on auto-antibodies. In connexion with a study for the presence of thyroid disease, the Hyland thyroglobulin autoprecipitation test and the Wellcome thyroglobulin hæmagglutination test were applied to approximately 1,100 sera. No relation between agglutination titres and radiation experience was observed. In a study of rheumatoid arthritis, examination of sera by the latex agglutination test for rheumatoid factor was made. Again, no relation between the findings of this test and exposure to radiation was apparent (281). A further index of auto-immunity that has been studied concerns the spleen weight. The ratio by weight of the spleen to the entire organism has been used to document experimentally-induced auto-immune disease, although other causes may lead to the same observation. One study of this parameter, made prior to the availability of the T65D dose estimates, showed no radiation-related effects with respect to spleen index (18).

238. On the whole, the available data on incidence of auto-immune findings in individuals exposed to radiation is sparse, but does not at present indicate any significant connexion. It should be strongly noted, however, that, in animal species, the maximum radio-sensitivity is in the early young adult period, and accordingly the incidence of auto-immune changes among highly-irradiated persons who were exposed at relatively young ages will be of particular interest. The available studies on the effect of spleen shielding (described elsewhere) certainly indicate that the maximum effect may be in persons exposed in the second and third decades of life. Thus, a future relationship with radiation of, for example, spleen index and collagen disease, may well become apparent, but probably only in a select age group. Detailed studies on cellular criteria of auto-immune immunological activity should also be sought for, as these may more directly relate to the actual disease process.

239. In considering the possible relationship between auto-immunity and radiation, it is also relevant to consider this association in terms of the various concepts relating to radiation and ageing. Much of the attention placed on studies of ageing has related to the use of parameters of ageing in non-dividing cell populations and static tissues on the *a priori* assumption that these are most intimately concerned with ageing. On the other hand, it is possible that a more indirect biological principle may be operative, which involves proliferating cells. Such a theory has been expounded by Walford (602) in propounding an immunologic theory of ageing. This theory basically considers that ageing is due to somatic-cell variation, particularly of those factors which determine self-recognition patterns among cells. In higher animals the cells of the reticulo-endothelial system are especially involved. Ageing in these species is brought about by the unleashing of self-destroying processes of the nature of auto-immunity or transplantation disease. The initial cause of the somatic-cell variation, whatever it may be, is extrinsic to this pathogenetic mechanism, although cell variation may be further stimulated by auto-catalytic immune processes. If irradiation increases the rate of somatic-cell variation, and therefore the potential development of an auto-immune state, and if at the same time is immunosuppressive, it will tend to inhibit the auto-immune tendencies of the somatically-variant cells. Thus irradiation may have two opposing effects on the onset of auto-immune disease, one accelerating and one retarding. The actual result might therefore depend on the balance of these two factors and in turn depend upon the type of radiation, total dosage, dose rates, age of animals at time of irradiation, species, nutrition, and many other factors. In particular, if age is a factor, it may well relate to the greater radio-sensitivity of the young animal. If ageing is an auto-immune process, then in adults the process may well be sufficiently underway to be autocatalytic, and irradiation at this time would not lead to any greater observable rate of change. This conclusion (from Walford, (602)) is indeed similar to that reached by Anderson (18) in considering the preliminary data available on the immunological effects of radiation on atomic bomb survivors.

240. Another connexion of auto-immunity with irradiation lies in the possibility that radiation-induced somatic mutations in lymphoid cells might enable these to directly react with self-components (14). Spleens from inbred mice were taken seven days after lethal whole-body irradiation. Cell suspensions were injected intracutaneously into the skin of normal syngeneic mice. A marked reaction was observed which did not occur with either allogeneic or syngeneic cells taken only one day after irradiation. It was speculated that this represented acquisition of self-reactivity induced by the radiation. However, as mouse skin is a rather sensitive site for these types of local reaction mediated by various pharmacological agents, considerably more studies with precise controls are needed for a confirmation of this observation.

241. In addition to these previously mentioned speculative aspects of radiation and auto-immunity, it has also been recognized that radiation-induced tissue damage might lead to the release of normal self antigens, which then induce the formation of auto-antibodies (155, 659). These might then play a role in the general pathology of radiation damage, although this has not been conclusively confirmed.

242. Irradiation has also been shown (639, 645, 649, 654, 673) to produce changes in the antigenic structure of tissues. This is also often followed by the appearance of auto-antibodies (646, 654, 659). An important role has been ascribed to these auto-antibodies in the development of radiation sickness (658). In the opinion of one author (645) the complement-fixing auto-antibodies against denatured protein, formed

under the action of external irradiation, are capable of neutralizing the toxic products of tissue disintegration and are a vital factor in protecting the organism against the effects of irradiation. Similarly, with internal irradiation by daily intake of a mixture of rare-earth and alkaline-earth radio-isotopes, rats were shown (697) to develop auto-antibodies for tissues of the kidney and liver. Disturbance in enzymic function of the liver preceded the detection of auto-antibodies, which in turn preceded the development of morphological changes in liver and kidneys.

243. Two hypotheses have been formulated concerning the role of auto-immune processes in the pathogenesis of acute radiation damage. The first is the auto-allergy hypothesis (657, 661), which assumes that the development of an anti-tissue immunological reaction caused by the action of cell-destruction products on the immunological apparatus leads to the appearance of anti-tissue cytotoxic antibodies and autohæmolysin-forming cells in the blood. This in turn leads to the development of general and local increases in sensitivity to autologous, allogeneic, and xenogeneic tissue products. The second hypothesis is the immunogenetic concept of the consequences of radiation damage (674, 675) which assumes the following sequence of events: mutagenic effect of radiation→relative increase in the anomalous cells which have an immunological competence against normal tissue antigens→accumulation of clones of these "forbidden cells" with the development of tolerance to them→auto-immune aggression of the forbidden clones against the normal tissues as in the graft-*versus*-host reaction.

244. These preceding paragraphs have considered the general question of radiation as it may relate to auto-immunity, and possibly in turn to ageing. In general, there is very little information available either in animal models, or from human studies. As was discussed in relation to the acute radio-sensitivity of the immune response at the young adult period, it may still be some time before the effects on the immune system that might be expected from atomic bomb exposure will become evident, and further studies on these patients are continuing. There are however several results, particularly from animal studies, that are consistent with the present hypothesis, that irradiation may lead to a breakdown in the balance of self-tolerance, which in turn may lead to auto-immune disease.

VI. Immunological aspects of radiation-induced carcinogenesis

245. It is a well-established fact that irradiation can lead to an increased incidence of cancer. A general review of cancer induction in animals is provided in annex G. Radiation neoplasia in man has been known for an even longer period of time and there is a vast literature covering the field. The reader is referred to annex H for a detailed discussion of human data.

A. Immunological surveillance and enhancement

246. In this report on radiation and immunity, the connexion with cancer stems from the interactions of the immune response with malignant cells, and therefore we will be concerned solely with those aspects of cancer and radiation which may involve immunological mechanisms or interactions. This will be confined to a detailed examination of a few of the mouse tumours in which the ætiology of the malignancy may involve immune processes activated or suppressed by radiation.

247. The general concept of *immunological surveillance* is based on the observation that tumours can present to the host a foreign antigen which is capable of stimulating an immune response directed against the tumour. It was first proposed by Thomas (563), and then considerably expanded by Burnet (68), that one of the main functions of the body's cellular-immunity system is in fact to control and eliminate potential malignancies. This thesis is essentially based on the factual observations that some tumours are antigenic. It should be noted, however, that although it is well established that the immune response can affect the growth of an established tumour, there is little direct evidence (except for virally-induced tumours in mice) to indicate that immunosuppression will increase the incidence of primary tumours, despite several recent investigations of this possibility. Furthermore, although an elevated incidence of certain malignancies has been observed in immunosuppressed kidney transplant patients and in immunodeficiency disease patients, this has not been found in a large series of immunosuppressed auto-immune disease patients.

248. Several reviews (246, 290, 429, 464) have dealt in depth with this area and the types of tumour-specific antigens might be summarized as follows:

(a) *Antigens associated with viruses*: these are well described in mice and represent a virus-directed product which is ultimately found either within the cell or on the cell membrane. All tumours induced by a given virus carry the same virus-associated tumour-specific antigen, for example, the G+ antigen of the Gross murine-leukæmia virus (293, 529). In man, the Epstein-Barr viral antigens carried on and in Burkitt-lymphoma cells and in nasopharyngeal carcinoma cells appear to be the most likely parallel known at present to the mouse-leukæmia viruses (134);

(b) *Tumour-specific antigens induced by chemical carcinogenesis*: in this instance, a series of tumours induced by the same chemical carcinogen may all have tumour-specific antigens, but with the exception of occasional cross reactions, these are mostly different antigens from one tumour to another. It should be noted that carcinogen-induced tumours may have virus-associated tumour antigens, which may be the consequence of later super-infection of the tumour by latent leukæmia viruses, although this relationship is still uncertain;

(c) *Embryonic antigens*: these are not strictly speaking tumour-specific antigens, but are antigens normally present only in embryonic life and expressed by the tumour in the adult host. The human-colon embryonic antigen carried by all tumours arising in the gastro-intestinal tract is one of the best known examples of this type (210), although some other recent data cast doubt on the colon specificity of this antigen (313). It is not yet clear whether some of the instances of tumour-specific antigens presently classified in groups a and b may not in fact belong in group c.

249. In many of these cases, it can be directly demonstrated that an immune response develops in the host bearing the tumour (244). Alternatively, immunization of normal animals with various forms of killed or altered tumour cells will provoke a state of immunity such that subsequently-transplanted tumour cells will

be rejected. Many of these studies have been performed with serially-transplanted mouse tumours, principally virus-induced tumours, which are quite strongly antigenic. Other studies have been performed with carcinogen-induced primary tumours or radiation-induced sarcomas and in these cases the antigenic stimulus often appears weaker. On the basis of these studies, it is a reasonable hypothesis that the first malignant cell arising in the primary tumour, carrying the tumour-specific antigen, presents an immunogenic challenge to the host's wandering lymphocyte pool of cells. Whether such stimulation is then mediated directly or via antigen-processing mechanisms is not known. It is envisaged that a continuing eradication of emerging, potentially-neoplastic cells must be occurring in the body, and that if a tumour clone is to develop, it must override or evade this potential antagonism. It is important to stress that this type of study has not been extensively performed with spontaneous primary tumours (although some data are available, Stjernsward and Vanky (528)), and that at present we are extrapolating from the data with virus- and carcinogen-induced tumours in the mouse.

250. The relevance of this discussion of neoplasia and immunity to radiation and immunity is based on the following set of premises: (*a*) many tumours are antigenic and therefore may initiate an immune response in the host against the tumour cells (cellular and/or humoral response); (*b*) many carcinogens, both chemical and viral, can induce an immune depression; (*c*) upon induction of a tumour, an interaction develops in which the growth rate of the tumour is pitted against the developing immune response. Various studies have shown that the immune response can both retard the growth rate of tumours, and be of particular importance in limiting the metastatic spread of malignant tumours. The relationship of the immune response to tumour growth is therefore pictured in an analogous fashion to the balance between immunity and infection, and just as radiation depresses immunity and permits a greater spread of infection, so it is proposed that radiation depression of immunity may permit more rapid tumour growth and spread. The essential question relevant to this report is therefore whether radiation-induced depression of immunity is a key factor in the mechanism of the radiation induction of cancer.

B. Radiation and tumours in mice

1. *Effect of radiation on antigenicity and the immune response*

251. Radiation may act on the immune response to tumours by a possible effect on the antigenicity of the tumours, or by altering the host's immune response itself. In regard to the first point, normal lymphocytes exposed to 1,200 roentgens *in vitro* fully retain their antigenicity, as assessed by their ability to stimulate a mixed lymphocyte reaction (164). Irradiated mouse lymphoma cells were assayed by two different methods for growth activity in syngeneic mice (341, 342). No difference was observed between cells exposed to 100 roentgens and controls, but with 1,000 roentgens increased reaction against the tumour was very evident. It appears that the irradiation may have exposed the antigenic sites of the tumour cells to a greater extent, or alternatively may have selectively enriched the tumour population in antigenic cells by selectively removing the less antigenic ones.

252. There are many observations indicating that mouse tumours are antigenic to their syngeneic strain and that irradiation, like many other forms of immunodepression, will permit a more rapid tumour growth or an earlier induction of tumours (172, 208, 292, 610). For example, in a study (475) on methylcholanthrene-induced sarcomas in mice, whole-body irradiation prior to transplantation resulted in marked increase in tumour growth. A dose of 400 rads give a maximum effect, and enhanced growth rate could be detected in mice in which the tumour was transplanted four months later, although the maximum effect was observed with transplantation 24 hours after irradiation. Unfortunately, there are few studies yet available dealing with primary or spontaneous tumours and radiation-induced immune depression. However, a similar result of an earlier appearance of primary carcinogen-induced tumours has been reported in mice immunodepressed by neonatal thymectomy (223).

253. Osteosarcomas which arose in mice following administration of ^{90}Sr have been shown to carry tumour-specific transplantation antigens, in that immunization of recipients with 15,000 irradiated tumour cells will result in a lower incidence of takes of transplanted tumours providing the recipients are also exposed to 400 roentgens one day before transplantation (410). These experiments confirm a previous suggestion (411) that radiation-induced sarcomas may be antigenic, and that this antigenicity may be a factor in the development of the primary tumour. Infection of ^{90}Sr-treated mice by BCG at a time close to the expected appearance of the first bone tumours resulted in a delay of the development and a significant decrease of the total incidence of such tumours, which may have been due to an increased stimulation of the immune system by the BCG.

2. *Radiation and mouse leukæmias*

254. One of the strongest arguments relating radiation-induced immune depression to tumour induction comes from a study of radiation-induced mouse leukæmias. Before considering this argument in detail, it must, however, be emphasized that the model system used is not ideal, as it involves neoplasia of a component cell type in the immune system. The changes that have been attributed to the host immune system might alternatively be explained by direct interference with the potential neoplastic line of cells. However, in the absence of any more suitable model, but with this reservation, it is relevant to consider this model system (see also annex G).

255. In any attempt to propose a pathogenic mechanism for radiation-induced lymphosarcomas and lymphatic leukæmias in mice, two main experimental observations must be considered (277, 278): (*a*) there is a far greater incidence of tumours when the dose is fractionated with successive increments spaced a few days apart; and (*b*) the entire body must be irradiated since shielding of the spleen or bone marrow, or injection of normal bone marrow after whole-body irradiation, drastically reduce tumour incidence (280, 316). Three separate factors appear to be involved: injury to the normal sites of storage of the latent virus with its concomitant release; injury to the thymus followed by regeneration; and injury to the bone marrow which in turn interferes with the thymic regeneration, thereby producing a maturation arrest in which large numbers of blast cells are exposed to oncogenic virus. Lym-

phoma induction can also be achieved by the direct injection of the leukæmogenic filterable agent from irradiated C57 mice into a thymus graft carried by a thymectomized irradiated host (236). If the host is not irradiated, leukæmia will not result, suggesting that something more is required than the active virus in large numbers and the presence of large and medium thymus lymphocytes. Haran-Ghera (235) and Haran-Ghera and Peled (237) have given evidence to suggest that the other essential factor in leukæmogenesis may be irradiation-induced immunological depression. Tests on the immunological reactivity of irradiated mice were performed by evaluating the production of antibodies to *Shigella* antigen. The four weekly whole-body exposures of 170 roentgens used for leukæmia induction resulted in marked immunological depression, with the minimal antibody production in these mice persisting for about one week following irradiation, and coinciding with the timing of the demonstration of release of filterable agent into bone marrow. Inoculation of normal bone marrow immediately after irradiation was, therefore, suggested to re-equip the immune system, and accordingly reduce tumour incidence. An alternative explanation is that it leads to repopulation of the host thymus, thus interfering with the maturation arrest of thymic cells.

256. It therefore appears that in leukæmia induction a transient radiation-induced depression in host immunity (possibly mainly homograft immunity, Haran-Ghera, (235)) is an important factor, combined with the activation or release of a latent virus, in permitting expression of the neoplastic transformation that occurred in the appropriate thymus cell. A similar phenomenon may well pertain to other tumour-induction systems in that host immune depression may permit the proliferation and expression of other non-radiation-induced neoplastic transformations.

257. In these preceding paragraphs we have been considering radiation-induced tumour induction in a mouse strain that rarely develops lymphoid leukæmia unless it is irradiated. In studies with a high-leukæmia-incidence strain of mice, AKR, a novel immunological approach to the ætiology of the tumour was proposed (601). In this strain, all mice eventually succumb to leukæmic development and it has been shown that the Gross virus probably acts as one of the ætiological agents of the AKR lymphomas (600). In an analysis of the immunological status of the AKR mice, it was proposed (601) that an immune attack, rather than immune depression as we have previously been discussing, may play an ætiological role in AKR-leukæmia development. Using a cytolytic plaque assay with AKR embryonic cells (600) it was shown that both spleen and lymph-node cell suspensions from AKR mice taken in the preleukæmic adult period will exhibit an immune type of cytolytic activity against syngeneic AKR cells. As young AKR mice are tolerant to the G antigen, it was suggested that the development of a partial or complete breakdown of tolerance to the G antigen occurs in the preleukæmic period. Secondary, immunologically-mediated damage of virus-infected G+ thymic lymphoid cells may then be the ultimate process that precipitates leukæmia development in the AKR mice. Recent evidence (637) suggests that a comparable sequence of events may occur in the development of mammary cancer following neonatal infection by the Bittner virus. Thymectomy reduced the incidence of mammary cancer in C3H MTV-positive mice (mammary-tumour-virus positive) and thymus grafts to such mice restored a high mammary-cancer incidence. When adult C3H MTV-negative spleen cells were injected into thymectomized C3H MTV-positive mice, a high incidence of mammary cancer was observed. It seems likely that in this tumour also, the injection of non-tolerant spleen cells precipitated tolerance breakdown, leading in some manner to the development and/or emergence of mammary cancers.

258. These latter two experiments do not involve irradiation. However, their basic premise is that loss of tolerance may lead to the development of an immune response which itself is directly involved in the ætiology of the neoplasia. Since irradiation has been shown to break the tolerant state, particularly in situations where tolerance will eventually be lost in any case, it is not unreasonable to consider that radiation-induction of neoplasia might sometimes involve a break in the state of tolerance against a vertically-transmitted oncogenic agent, which would then swing the balance towards an immune attack against those cells expressing the particular tumour-specific antigen. This in turn may lead in some manner to a proliferation or destruction of the target cells which, perhaps by altering the normal proportions of blast and mature forms, greatly increases the proportion of cells that are acutely sensitive to malignant transformation.

259. Another effect of radiation on leukæmia incidence in mice has recently been reported (317). The same radiation dose which enhances leukæmogenesis in an unirradiated mouse will counteract leukæmia development, if given to a mouse which was previously irradiated but has not yet developed leukæmia. This indicates that the preleukæmic interval between recovery from the first dose of radiation and the development of the tumour includes a vulnerable radio-sensitive stage in the preleukæmic cell line. It was proposed that the target cell for transformation may be acutely radiosensitive in this phase. However, in terms of the immune-attack theory of Wahren and Metcalf, it might be proposed that the first dose of radiation has broken a state of tolerance to a vertically-transmitted leukæmia virus. Following this, a phase of a developing immune response to the viral antigen occurs, which may be essential for neoplastic development. A second dose of radiation in this period would largely suppress this newly-emerging state of immunity against the viral antigen, and therefore suppress tumour development.

260. It must also be considered that radiation may have other effects on viral release akin to lysogeny, such as has been shown for lambda phage in bacteria (see for instance reference 127). If radiation has such an effect on vertically-transmitted oncogenic viruses in animals, it may well be of considerably greater ætiological importance than any of the other more speculative immunological considerations.

C. Radiation and immunotherapy

261. Viable tumours frequently carry exposed tumour-specific antigens on their surface membranes which renders these cells vulnerable to an immune reaction against the tumour antigen. This observation suggests that elimination of the cancer cell by an induced immune reaction might be a feasible means for therapeutic elimination of the cancer. This has now become a most intensively investigated area. Most of the current work is concerned with basic immunological

approaches and does not touch on the field of radiation. Approaches to immunotherapy are reviewed elsewhere (11, 520). Two aspects of this field are, however, relevant to this present report: (*a*) the use of irradiation of donor cells as antigens and the effect of host irradiation on tumour immunity, and (*b*) the use of radio-labelled antitumour antibodies.

262. Growing tumours may lead to the establishment of a type of paralysis to the tumour antigens in the host. If an immune response against the tumour is to be elicited, a more potent immunogenic stimulus must be given to the host. Furthermore, if immunization is to be attempted, it must be in a manner such that the serum blocking factors described by Hellstrom and Hellstrom (242) are not increased but rather that cellular immunity is primarily activated. When tumours carry unique tumour-specific antigens, the autologous tumour may have to be used as the immunizing antigen. Since re-inoculation of the viable autologous tumour may lead to its regrowth, the tumour cells must first be exposed to some treatment that destroys their viability without affecting their immunogenicity. X-irradiation appears to meet these requirements in most cases. Lymphoid cells appear to retain their normal immunogenicity after irradiation (164), although some reduction in activity has been reported (353). While some investigations have not shown any effect of irradiated autologous cells alone in inducing tumour rejection (349), others have observed a significantly increased immunogenicity of irradiated isologous tumour cells (341, 342). In one study (226), a sample of fibrosarcoma was removed from rats and exposed to 10,000 roentgens *in vitro*. The irradiated cells were then given back to the autologous animal and the remaining large mass of the primary tumour was locally exposed (*in vivo*) to 2,000 roentgens. A striking regression in growth of the primary tumour occurred in many cases. Injection of irradiated autografts alone had no effect without local irradiation. It was suggested that with large masses of tumour tissue, local irradiation (even 2,000 R) does not kill all cells. A certain proportion will remain. The growth of the surviving fraction, however, may be considerably inhibited by the immune response initiated by the irradiated autologous graft.

263. Tumour-specific cytotoxic antibodies were also produced in man by the immunization of 13 patients with their own irradiated melanoma cells (263). The longest response lasted 14 days, but again the procedure had no apparent effect on the course of the disease in these patients. In a study with the Morris hepatoma in rats, the immunogenicity of the hepatoma cells was considerably increased when the cells were combined with a pertussis vaccine (629). Irradiation of the hepatoma or liver homogenate did not seem to interfere with the immunizing properties of the tumour.

264. In recent studies, attempts have been made to determine whether rat reticulo-endothelial cells are capable of producing a cellular anti-tumour agent against Yoshida-sarcoma cells in tissue culture (491). In these studies, it was found that an effective antigenic cell component was released into the tissue-culture medium from tumour cells after three days of culture in a diffusion chamber. The same cell components were obtained from cultured medium of tumour cells after x-irradiation. Optimal doses of radiation capable of releasing this agent ranged from 2,000 to 4,000 rads. In other studies (412) this same anti-tumour agent, derived from incubation of macrophages in the supernatant fluid from irradiated tumour cells, could be transferred to lymphoid cells when they were cultured in the same tube. These studies therefore indicate the possibility of producing immunogenically-active tumour-specific antigens in culture by irradiation of cultured cells, and also of activating immunocompetent cells *in vitro* and perhaps of then obtaining *in vivo* destruction of tumour with these cells.

265. Immune sera prepared against tumour-specific antigens can occasionally be shown *in vivo* to reduce the growth rate of tumours (217) and in these cases it is most likely that cytotoxic complement-fixing antibodies are involved. However, in many cases this is not found, and antibody-mediated enhancement of the tumour is more likely. It is clear, from these experiments, that anti-tumour antibodies can localize on the surface of tumours *in vivo*. If the antibody carried with it a high source of radiation, then selective radiation killing of the tumour cells might occur. This finds a good precedent in the work of Ada and Byrt (3) who showed that ^{125}I-labelled antigen bound to the surface of antigen-reactive cells specifically killed those cells without affecting normal cells. In man, cancer-specific antibodies have been produced, but there is little evidence that they have any inhibitory or destructive effects *in vivo* (263, 264). In a report on the production of a specific precipitin to a renal cancer in man, Nairn *et al.* (401) suggested the idea that specific antibody to tumours might localize on the surface of the tumour cells and act as a homing carrier for radiotherapeutic or chemotherapeutic agents. This was then demonstrated in mice by Ghose *et al.* (203) who treated Ehrlich-ascites cells *in vitro* with an ^{131}I-labelled antibody to the tumour. On inoculation of these cells into mice, the tumour did not grow. In another series of investigations (128, 129, 325), it was shown by means of radio-labelled antibodies that antibody molecules to human brain tumours could be localized *in vivo*, and further studies on this approach are in progress. Radio-labelled antifibrin antibodies have been shown (350) to localize preferentially in certain cancer lesions, as the deposition of fibrin often occurs in these areas. This indicates a possible means of delivering local radiation to fast-growing tumours.

266. Another recent approach has been the demonstration (202) that antibody-treated Ehrlich-ascites cells are rendered more radio-sensitive than control tumour cells. This may be an effect mediated through antibody fixation on the membrane and interfering with the cell-membrane permeability, making some of the x-ray effects more damaging (596). Doses of radiation that did not greatly influence the subsequent growth rate of normal rabbit-serum-treated tumour cells severely inhibited the antibody-treated cells. It was suggested that this phenomenon may be related to the correlation of "observed durability of the response to chemotherapy in a Burkitt lymphoma" with the observed frequency of preferential binding of a globulin fraction on the tumour cells surface (291).

VII. Effect of variation of condition of irradiation on immunological responses

267. In the preceding paragraphs, much of the available data on the effects of radiation on the different types of immune responses has been considered primarily from the point of view of the nature of the immune response. In this section emphasis will be

given to the different ways in which radiation may be presented to the individual, and their subsequent effects on the immune response.

268. It must be stressed that in most of the cases where experimental studies have clearly shown effects of radiation on some type or component of the immune response, relatively few studies are available on the effects of changing the conditions of irradiation, and in particular dose, number of exposures, or type of radiation. In most studies, the aim has been primarily related to an immunological problem and the type of data that would be most relevant for estimates of risks from radiation is simply not available.

A. Small doses

269. Studies of radiation inactivation of antibody-forming capacity have usually given D_{37} values of around 60-100 rads. Thus doses of radiation in this range, when given to the whole animal, have usually shown some significant degree of suppression of antibody formation. A 75 per cent reduction in antibody-forming plaques was found (286) in mice given 50 rads 10 days prior to antigen. Dixon *et al.* (145) also found a significant reduction in antibody formation with 75 roentgens, and 125 roentgens gave considerable depression. The results of Makinodan and Price (336) show 65 per cent of a normal primary response following 100 rads. In dose-effect studies, which have usually started at either 50 or 100 rads, increasing exposure to radiation yields proportionately more suppression of antibody formation (e.g., table 4 and figure V). The phenomenon of interphase death of lymphocytes *in vivo* discussed in paragraph 152 is probably not involved with doses of radiation to the whole body below 100 rads, although it must be observed that there are few direct data on the effect of different types of radiation or of dose rate on interphase death of lymphocytes.

270. The effect of radiation exposures in the 100-roentgen range may not be solely on the immunocompetent cell. Decreased bactericidal activity was observed in polymorphs isolated from guinea-pigs 3-5 days after whole-body exposures of 100 roentgens (440). Some depression in antibody formation was also observed (177) when mice exposed to 550 roentgens were given macrophages from donors that had received only 150 roentgens (table 2). These two studies therefore reinforce the point that depression of the immune response as a whole by radiation in the 100-roentgen range is not solely due to interference with the proliferation of immunocompetent cells.

271. Radiation-induced enhancement of the immune response has also been observed with relatively low doses. A heightened peak titre, shorter latent period, and a high rate of antibody synthesis were all observed in rabbits given 25 rads two days to two hours after antigen injection (547). Prolonged production of haemolysins was also observed when rabbits were given 25 rads even one month before injection of antigen (548). In a similar system with mice a dose of 50 rads was also shown to give an enhanced response when given one hour before or after antigen (table 3). These results show that single doses of radiation in the range of 25 to 50 rads may either depress or enhance the antibody response, the direction being determined mainly by the time relation between injection of antigen and exposure to radiation.

272. Data on the effects of single radiation doses below 25 rads are very sparse, and in most instances no significant effects were observed on the immune response as measured in the whole animal. However, a change in the morphology and motility of small lymphocytes following *in vitro* exposures of 2-5 roentgens was reported (527), although the possible *in vivo* significance of an effect at this dose might well be doubted.

273. A key problem in attempting to extrapolate from the effects of high doses of radiation on the immune response to the effects of low doses, is that the immune response as a whole is composed of separate components which differ in their radio-sensitivities. Accordingly, at moderate to high doses, several components may be affected, but at low doses only one may be susceptible. The real question is therefore whether *any* of the essential components are indeed radio-sensitive with exposures below 50 to 100 roentgens. The studies mentioned above dealt primarily with the 25 to 100 roentgen range, and it is indeed apparent that some significant, albeit relatively minor, effects are observed with 25 roentgens. However, as the D_{37} value (calculated from experimental curve) for the actual antibody-forming cell series is around 75 rads, very little significant effect on this component will occur with single exposures below 25 roentgens. As mentioned above, the phagocytic cell series is also affected, but only marginally, by 50 to 100 roentgens, and again no significant effect would be expected with exposures less than 25 roentgens. For antibody production, this only leaves the lymphocyte cell series to be considered and, in view of the extreme radio-sensitivity of this cell series as a whole, it is possible that some depression in the antibody response might occur in situations where the full available complement of thymic-derived "*T*" cells are needed in eliciting a primary response. Clearly, further direct studies are needed to provide information relevant to this question. A more appropriate question, however, concerns the effect of multiple or continuous low doses of irradiation on the immune response and, in particular, whether the immune-response potential will eventually decline or adapt to continuous low-level exposure. This will be considered in the next section.

B. Fractionated and prolonged doses

274. It is perhaps appropriate to first consider the hæmatopoietic stem cells, and to note that if all cells of this type were completely inactivated by irradiation, then the immune system would also eventually fail, at least in the ability to mount primary responses as, in this case, a continual input of differentiating stem cells proceeds throughout life. It is quite clear that this possibility is remote except with relatively high doses. With a daily dose of 50 rads after an initial dose of 150 rads, a further 250 rads was required to reduce the stem-cell repopulating activity to 5 per cent of control values which still, however, represents a large reserve of potential hæmatopoiesis. Furthermore, the observation that adult thymectomy alone only leads to a depression in some immune responses several months later, suggests that a large reserve of differentiated immunocompetent cells already exists in the body.

275. The amount of actual data available on the effects of continuous or repeated exposure at low dose levels is again quite limited, but does at least provide an order of magnitude of exposure for which suppression is found. In rabbits given 4-5 roentgens per

day to a cumulative exposure of 356 to 2,039 roentgens, no functional disturbances of antibody formation in response to three injections of paratyphus vaccine (642) were evident. However, with 21 roentgens per day up to a total of 2,000 roentgens a partial inhibition was observed. Also, in monkeys, a daily exposure of 1.34 roentgens (up to a total of 675 R) did not affect the production of antibody to tetanus toxin (641). In another study on mice, rats, guinea-pigs and rabbits (651) given a daily exposure of 1.2 to 4.3 roentgens for 1½ to 2 years, animals were investigated for bactericidal activity in the blood. The strongest disturbance of natural immunity occurred in young animals and particularly with radiation delivered during intrauterine development. Some depression of immunity occurred with cumulative exposures in the range of 300 to 450 roentgens. In a study (533) on pathogen-free mice exposed to 1-4 roentgens per hour, the ability of irradiated animals to produce antibody to some but not all antigens was inhibited by sublethal doses.

276. In a study (671) on the effect of low doses of radiation given each day for five days, pregnant rats with fœtuses at 16 days of embryonic development were irradiated with 4-65 roentgens per day for five days. There was found to be a resulting inhibition of agglutinin production to typhoid vaccine when the immunization was performed at 2-5 months of age. Some reduction of phagocytic activity of blood leucocytes was also observed.

277. In considering data on the effects of fractionated low doses, it is important to bear in mind the studies previously discussed in paragraphs 128, 157 and 182, that stress that dose rate as well as absolute dose is quite important in determining the degree of radiation-induced inhibition of immunity. As many of these fractionated or continuous radiation studies were performed with low-dose rate irradiation, it is difficult to assess the actual effect of a fractionated dose that would have caused considerable suppression if it had been given in a single dose.

278. One of the main issues that is relevant here, is whether repeated small doses or low-level continuous irradiation give rise to an accumulation of damage, or whether restoration following small doses is complete and thus adaptation to repeated irradiation occurs for the immune system. This problem is at the heart of the matter in attempting to assess risk estimates for man in terms of effects on the immune system. Although there are considerable data on the over-all susceptibility of the immune response to higher doses of radiation, there is very little that is of direct relevance to this central question. Some speculation is therefore justifiable.

279. It is most likely that the immune response as a whole would readily adapt to repeated low doses of irradiation. Studies assessing the over-all potential expression of responding cells by comparison with those that actually respond (336) stress the enormous reserve that is held unexpressed. Thus, if an individual normally expresses only 10 per cent of his actual immunological potential, then this cell population could readily tolerate up to a 90 per cent loss in that system from continual irradiation without any apparent loss of immune responsiveness. In this connexion, it is also relevant to consider the hæmatopoietic stem cell which is continually entering into differentiation throughout life and feeding more potentially-immunocompetent cells into the system. Unlike some other tissues, the immunocompetent population of cells is not produced only once in ontogeny but rather depends heavily on continual replacement. Furthermore, as the hæmatopoietic stem cell itself is in large reserve, considerably high levels of radiation would be required to limit the potential input into the immune system.

280. It is essential, however, to remember that the expression of an immune response is not a single one-hit event dependent on antigen directly stimulating one cell. From studies on the relative radio-resistance of thymic-derived carrier cells essential for collaboration in many secondary responses, it would not be expected that much of an effect would be exerted on this cell type by repeated or continuous irradiation. The cell type which is more likely to control the over-all immune response is the macrophage, as this cell type may not be renewed as frequently as immunocompetent cells, and in many instances active processing by this cell is obligatory if the immune response is to proceed. As several studies mentioned above have suggested that some interference with antigen processing may occur at moderately-low single exposures (100 R), it is possible that cumulative damage to these cells might result from repeated or continuous exposures to low doses. Further studies on this aspect are clearly required, with particular emphasis on a comparison of the effect of continuous or repeated irradiation on immune responses which do or do not require macrophage participation.

281. It is important to distinguish this concept of adaptation to repeated low-level irradiation from the implication of acquired radio-resistance in the antibody cell series. This latter view was first proposed in studies with mice (454) but, as has been discussed previously (paragraphs 85 and 86), can be explained by changes in the proportions of interacting cells. Instead, the concept of adaptation implies that cells of the antibody-forming precursor series are all equally radio-sensitive, and are being continually replaced throughout life, and that at any given time there are many more potentially-immunocompetent cells to a given antigen than are needed to produce the usual level of immune response. In the studies of Kennedy *et al.* (286) it was in fact suggested on the basis of plaque-forming response data, that the immune system could suffer at least a thousand-fold depletion of the proliferative capacity of its cells without completely losing the ability to respond to an antigen by the production of plaque-forming cells. This actual number will vary for different antigens, and further studies of this type would be most relevant to this problem.

282. There is one instance in transplantation immunology where fractionated radiation doses appear to be of some value in depressing the host's potential immune rejection of the graft. Local irradiation of organ grafts (kidney or heart) *in situ* appear to aid in prolonging graft persistence. These studies (see paragraphs 208 to 212) usually involve 150 rads given six times at two daily intervals. The actual mechanism of prolongation is obscure, but is likely to be associated with destruction of invading host cells which are continually infiltrating the graft.

C. Whole-body and local irradiation: delayed effects

283. Most of the studies described in this report have dealt with early effects on the immune system

after whole-body irradiation. In some studies, *in vitro* irradiation of cells has been used, and these are often valuable in determining immediate and acute effects on a given cell type. Local irradiation of an organ *in vivo* can, however, be complicated by other problems. In many instances, local radiation applied to various organs of the immune system, such as lymph nodes, spleen, or even extracorporeal radiation, have been shown to lead to lymphopenia and reduction in immunocompetence by depleting the recirculating lymphocyte pool. However, in cases where solid organs are irradiated, the fixed structural cells of the organ are also irradiated, and it is reasonable to question whether long-term effects may be observed in these cases, even though the lymphocyte content of the organ may be completely restored by entry of new cells.

284. The special case of local irradiation of renal allografts in man, dog and goat was discussed above, and in this instance with the doses used (6×150 R) no deleterious effects appear to have been observed in the parenchymal tissue of the kidney, although in many of these experimental situations, prolonged observations were not made.

285. In many centres there is increasing use of cadaveric kidneys for renal transplantation. As the kidney from the cadaver source frequently shows some degree of acute tubular necrosis (ATN), it is pertinent to consider the effects of radiation on the regenerating tubular epithelium. In a recent study of this problem with kidney grafts in dogs, it was shown (354) that therapeutic doses of local graft radiation (600 rads) given immediately following the onset of acute tubular necrosis significantly delay recovery of renal function from the ischemic insult. These authors therefore caution against indiscriminate use of local kidney radiation without signs of immunologic injury to the kidney which would merit its use.

286. Following whole-body irradiation at a dose of 1,250 rads, little direct damage was observed to the cytoplasmic fibril web of the reticular structure in lymph nodes of rats. When doses of up to 8,000 rads were used, the entire structure showed considerable necrosis and destruction (272). With local irradiation of lymph nodes, regeneration of lymphoid content is extremely rapid, presumably because of entry of immigrant unirradiated lymphoid cells. However, following 3,000 roentgens, an extreme secondary atrophy develops several weeks later, apparently following vascular damage and destruction of the original stroma (165).

287. The effect of radiation on the popliteal lymph node of sheep on its output of lymphocytes has been described (230). Chronic fistulæ were established in the different ducts, and the nodes received x-ray exposures of 800 to 2,000 roentgens. A significant fall in lymphocyte output occurred, but was not accompanied by any gross change in the morphology of the cells. Five preparations were also antigenically stimulated 6 to 140 hours after the nodes had received 2,000 rads. The resulting increases in antibody titre and characteristic cellular changes showed that irradiation had not significantly altered the immunological performance of the nodes. This strongly indicates that the functional capacity of the node is dependent on the entry of recirculating lymphocytes and not on a fixed cellular population. Late effects on the node are discussed in paragraph 289. A similar conclusion was reached from a study of the regeneration of lymph nodes from whole-body irradiated mice (643).

288. In this aspect of delayed changes following irradiation, data from the clinical use of radio-therapy in Hodgkin's disease and malignant lymphomas are most relevant. Radio-therapy offers a significant chance for cure of Hodgkin's disease (449). In retrospective studies of the recurrence rate (279), defined as the probability of reappearance of disease in a radiation-treated field as the first new manifestation of disease, a correlation with the median dose was clearly evident. With a median exposure of 500 roentgens there was a 78 per cent recurrence rate, with a median exposure of 1,000 roentgens the rate fell to 60 per cent, and with 4,000 roentgens in weekly fractions of 1,000 roentgens (using megavoltage energy beams) only 2 per cent recurrence was observed in 300 fields at risk. As these figures are based on single fields, it was stressed that the chance of success is an independent variable for each field, and accordingly the use of higher doses (4,000 rads) becomes of even greater statistical importance. With this type of treatment, it is obvious that considerable care must be taken to shield the lungs and other vital tissues. The judicious use of lead shielding, monitored carefully, has proven that this problem can be successfully avoided. But what of late complications in those areas which are irradiated?

289. Severe leucopenia or thrombocytopenia has rarely been a problem (279), presumably because of adequate shielding of some bone marrow which has sufficient hæmatopoietic stem-cell reserve. In terms of survival rates, the data in table 5 strongly vindicate the use of radical (3,500-4,000 R) radio-therapy. This is particularly evident when it is realized that virtually no cures of Hodgkin's disease in stage III had been reported before this study. Subsequently, some evidence of late necrotic changes may occur in lymph nodes in the treated fields. In one study (634) some calcification was observed in intrathoracic lymph nodes 1-14 years following irradiation of the mediastinum at exposures between 1,000 and 6,000 roentgens. This calcification is probably due to post-irradiation tissue necrosis. However, it must be stressed that this possibility of some minor post-irradiation changes in lymph nodes most certainly does not outweigh the enormous value of carefully-administered radical radio-therapy for this disease. Hall and Morris (230) observed that the irradiated lymph nodes of sheep eventually showed a definite increase in the thickness of the capsule and of the connective tissue trabeculæ. They suggested that the lymph node may eventually lose its capacity to transmit recirculating lymphocytes. In an earlier report (10) with irradiated rabbit popliteal lymph nodes marked fibrosis was seen three weeks following irradiation.

D. Radio-isotopes

290. In various experimental studies radio-isotopes have been used to deliver radiation at localized sites in the lymphatic system. This includes such methods as the application of ^{32}P-impregnated polythene strips to the surface of the spleen (191), intra-atrial implantation of a β-emitting source (31), and intra-lymphatic infusions of radio-isotope-labelled agents (159, 567, 620). Perhaps one of the greatest dangers in applying these types of treatments to man is that it is relatively difficult to calculate effective dosages to organs in the body. In one study (80) with endolymphatic radio-therapy (ERT) for therapy of malignant lymphomas,

some attempts were made at dosimetry. ERT may use either ^{198}Au, ^{90}Y, ^{32}P, but more frequently ^{131}I. With 50 millicuries of ^{131}I injected, it was estimated that a dose of 842 rad mCi^{-1} was given to the lymph node, and of only 10, 2 and 8 rad mCi^{-1} to lungs, liver and spleen, respectively, but of 48 rad mCi^{-1} to the thyroid. Possible complications from ^{131}I accumulation (after excessive doses) in the thyroid can be minimized by administration of Lugol's solution preceding ERT (80).

291. Several experimental studies on the effects of various radio-isotopes on immune responses have been reported. Moderate inhibition of immunity after chronic uptake of small doses of ^{90}Sr was observed even a year later. A single subcutaneous injection of 0.5 mCi kg^{-1} of ^{210}Po, or a single intraperitoneal injection of 0.05 mCi kg^{-1} of ^{90}Sr to guinea-pigs did not affect the primary response, but led to a marginal reduction in secondary immunization (666). Single intraperitoneal injections of tritium oxide in doses of 0.3 Ci kg^{-1} (total dose 400 rad) to dogs (693) led to depression in immunological activity which correlated with the clinical manifestation of radiation disease. Depression of phagocytic activity and agglutinin formation was observed in rabbits given simultaneous subcutaneous injections of ^{60}Co or ^{131}I together with secondary immunization (698b). Intravenously-administered ^{32}P colloidal chromic phosphate in a dose of 780 microcuries to rabbits (636), was calculated to yield 14,000 rads during the 14 days between isotope and antigen injection. This resulted in marked depression of antibody formation. This effect could be counteracted by multiple antigen injections, which might indicate that the major effect of intravenously-injected isotope was on the spleen, and that by multiple injections non-splenic sites then participated in the response. However, in another study (81), the injection of ^{32}P chromic phosphate showed some effect on all organ systems when injected intravenously, whereas a selective destruction or change only in lymphoid tissues occurred following intralymphatic injection.

292. The literature also contains a large amount of data concerning the effects of various incorporated radio-isotopes (^{210}Po, ^{89}Sr, ^{90}Sr, ^{32}P, ^{131}I, ^{198}Au, ^{65}Zn and an unseparated mixture of nuclear-fission products) on immunogenesis when experimental animals are vaccinated with various other bacterial antigens: *Salmonella breslau* (683), *Proteus vulgaris* (669), typhoid-dysentery vaccine (698b) and brucellosis vaccine (666). It has also been shown that antibody formation is decreased when animals damaged by ^{210}Po, ^{131}I, ^{45}Ca or ^{65}Zn are immunized with tetanus and diphtheria anatoxins or gamma globulin (691). In a number of investigations it was found that antibody formation was reduced when animals damaged by ^{32}P, ^{131}I, ^{137}Cs and ^{144}Ce were immunized with rickettsial and viral antigens (smallpox-vaccine virus and influenza virus) (640, 695, 696). Reduced antibody formation in animals damaged by ^{45}Ca, ^{90}Sr and ^{137}Cs was observed when the animals had absorbed total doses of the order of 220-270 rads (698). A depression in the formation of antibacterial and antiviral antibodies in rats damaged by ^{144}Ce was observed when the isotope had been introduced intra-abdominally, even at relatively low total absorbed doses to the critical organs—liver, skeleton and spleen—apparently because of severe damage to the reticulo-endothelial cells (696).

293. According to some authors (698) the changes in immunogenesis which result from incorporated radio-isotopes have several phases: periods of depressed antibody formation alternate with phases of normalization and stimulation. It is also important to point out that internal irradiation is accompanied by a very marked suppression of the secondary immunological response in a number of cases; this suppression is more marked than the depression of the primary immunological reaction (666, 696). It is assumed that when animals are irradiated internally, the long period over which the dose is accumulated slows down the restorative processes. Under these conditions, continued exposure to radiation causes a marked suppression of the immunological response when the animal is revaccinated. Internal irradiation with ^{144}Ce may stimulate the formation of plasma cells (685, 687) and there have even been cases of mitosis among them. The sub-microscopic organization of the plasma cells undergoes only slight changes (some disturbances in the structure of the nucleus and the mitochondria), which confirms the structural hypothesis concerning radio-sensitivity to the effect that resistance to radiation is due to the presence of enough organoids in the cells to keep reparative processes at a high level. The irradiation both accelerates differentiation of the plasma-series cells and stimulates the development of the endoplasmatic reticulum (686, 687). It has been shown by electron-microscopic immunocytochemistry (using peroxidase as an antigen) that, despite the normal ultrastructural organization of the plasma cells, an antigen-antibody reaction no longer develops in ultra-thin sections after irradiation. A generalized discussion of these data is available in a monograph by Klemparskaya *et al.* (660).

294. In various other studies radio-active gold, bismuth, silver, yttrium and iodine have been used, either as the "naked" radio-active material itself, or coupled to a carrier. Probably the main point with which to conclude this section, is to stress again that the major problem with this type of irradiation is the great difficulty in controlling radiation dose, and thus although it may be valuable in experimental research studies, it should be considered hazardous in clinical studies.

E. Indirect effects

295. It is well recognized that lymphocytes, particularly thymic cortical lymphocytes, are very sensitive to lymphocytolysis by x-irradiation, by corticosteroids and some other steroid hormones. This raises the theoretical possibility that destructive effects observed on the immune system through the use of external agents such as radiation, may in fact operate by causing a release of endogenous steroid hormones which in turn actually cause the destructive effect on the lymphocyte. A distinction between the direct action of radiation and steroid-mediated destruction could be made (*a*) by assessing effects of *in vitro* radiation of lymphoid cells, and (*b*) by the use of adrenalectomized animals. Actual data on this subject are again sparse and, where available, have been obtained without consideration of the separate components of the immune response.

296. *In vitro* studies (238) have shown that the antibody-forming response can be inhibited by corticosteroids only very early after antigenic challenge. Resistance to steroid inhibition develops rapidly with time, and as this steroid-resistant phase coincides with the lag phase of cell proliferation, steroid inhibition is clearly active only on non-dividing lymphoid cells, prior to their antigen-induced proliferation. This would in turn imply that doses of radiation which are known

to mediate suppression of the immune response by inhibition of cell division, are clearly not operating through steroid mediation. Indeed, in a study (149) on atrophy of lymphoid organs in unoperated and adrenalectomized mice, no difference in involution was observed with exposures from 25 to 200 roentgens.

297. The possibility of steroid effects mediating lymphocytolysis is more likely with exposures around 10 roentgens. X-ray exposures in this range will produce stimulation of adrenocortical secretion, as judged by depletion of either adrenocortical sudanophilic material or total adrenocortical cholesterol (148), and by increased cortical secretion (439). On the other hand, some *in vitro* effects of radiation on lymphocytes have been observed with two to five roentgens (527). Again it might be concluded that although further studies are necessary, there is little likelihood that steroids play an important role in mediating radiation-induced immune depression.

298. It it also possible that abscopal effects may exert a positive influence on the lymphoid system rather than a negative effect. It has been shown (339) that exposure of the head of rats to 1,000 roentgens will increase the rate of incorporation of thymidine into DNA in the thymus. With 250 roentgens it was found that the effect is detectable within two hours, reaches a maximum at 19 hours, and then disappears after four days. No change in DNA incorporation into spleen was observed in these animals. This observation may well relate to another study which demonstrated that neonatal thymectomy of mice results in early degranulation of acidophilic cells of the anterior pituitary (457) and it was suggested that the thymus is a target gland of the hypophysis. It is therefore possible that thymic cell turnover is directed and controlled by a neuro-endocrine factor probably at the hypothalamic-hypophyseal level and that irradiation of the head affects this system.

F. Comparative studies in animals and man

299. That radiation has profound destructive effects on the immune response of experimental animals is quite clear but, because of the paucity of data in man, it is essential to question whether direct species comparisons are possible in order to extrapolate from the experimental findings to a realistic risk estimate for man. This is of particular importance in evaluating those situations where considerable benefit to the patient from radio-therapy must be compared to the long-term risks.

300. Much of the experimental data on radiation suppression of immunity has been obtained in small laboratory animals, such as mice. We are therefore attempting to extrapolate from an animal with approximately 3 10^8 potential immunocompetent cells to man with approximately 10^{12} cells of this type. This absolute difference is probably one of the main factors which might argue against the feasibility of direct extrapolation, in the sense that, whereas a single exposure of 700 roentgens in the mouse may depress the primary immune response to an antigen to 5 per cent of control, the degree of depression may not be nearly as large in man. Several factors will be important in determining whether or not this is so. For each antigen, the absolute number of initial responding cells, as a proportion of the actual potential number capable of responding, must be related to the degree of eventual response required to deal with the particular type of antigenic challenge. Thus, if 90 per cent of cells are destroyed with a given dose of radiation, an immune response which requires most of the original potential response to be expressed may be approximately as severely compromised in man as in the mouse.

301. This factor will certainly vary from antigen to antigen but it does not even imply that what is true for a particular antigen as studied in mice need also be true for man. As stressed previously, the secondary immune response as a whole appears far more radio-resistant than the primary response, simply because there are more cells available to react with that antigen. This is most relevant for species comparisons, as the natural experience of cross-reacting antigens often determines whether a state of immunity may have been developed to a particular antigen. For example, natural antibodies to various bacteria may be present in man but not in mice, so that first direct challenge in these two species may in fact respectively measure a secondary *versus* a primary response.

302. Where direct measurements of radio-sensitivity of the immune response have been made in different laboratory animals, similar results were almost invariably found. For example figure XIII shows the radio-sensitivity of the immune response as a function of the time relative to antigenic challenge for mouse,

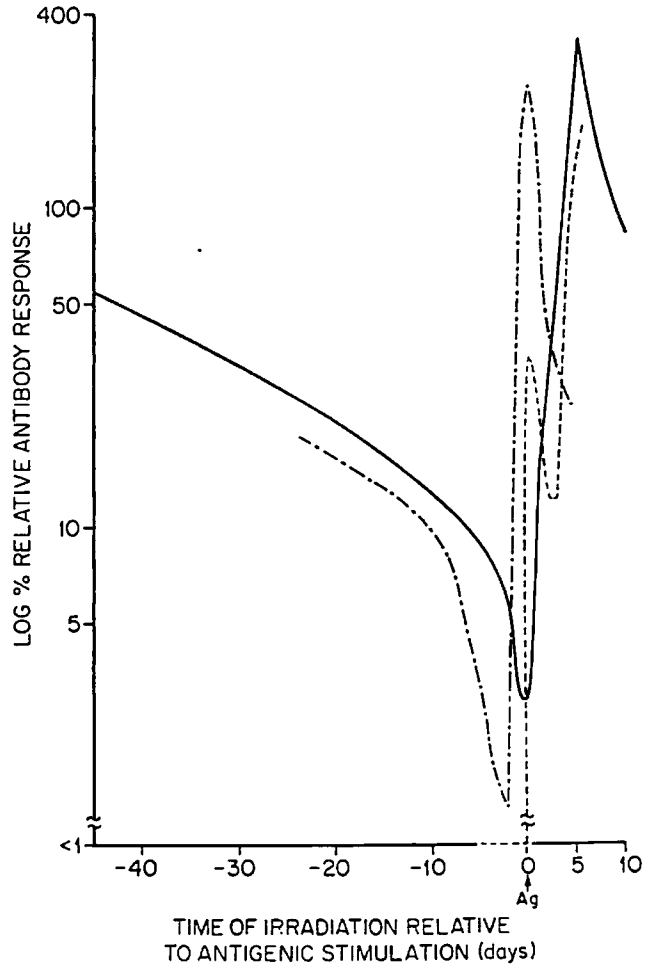

Figure XIII. Radio-sensitivity of the immune response as a function of time of irradiation relative to antigenic stimulation, for three animal species (336, 510, 550): mouse (710 R) (—); rabbit (500 R) (— · —); and rat (500 R) (- - -)

rat and rabbit. Similar results were observed in all cases for both radiation depression and enhancement. In terms of the actual radio-sensitivity of the immunocompetent cells, the data available would strongly predict that no essential difference will be observed among species, including man. Direct assessment of this will be possible with the use of *in vitro* techniques for studying the immune response of human cells.

303. One of the other major problems in comparing radiation studies in different species, particularly the radio-therapeutic studies in man, is the wide range of dose rates and radiation quality used in these studies. A further example of the dramatic effect of dose rate on immune depression is shown in figure XIV, in which

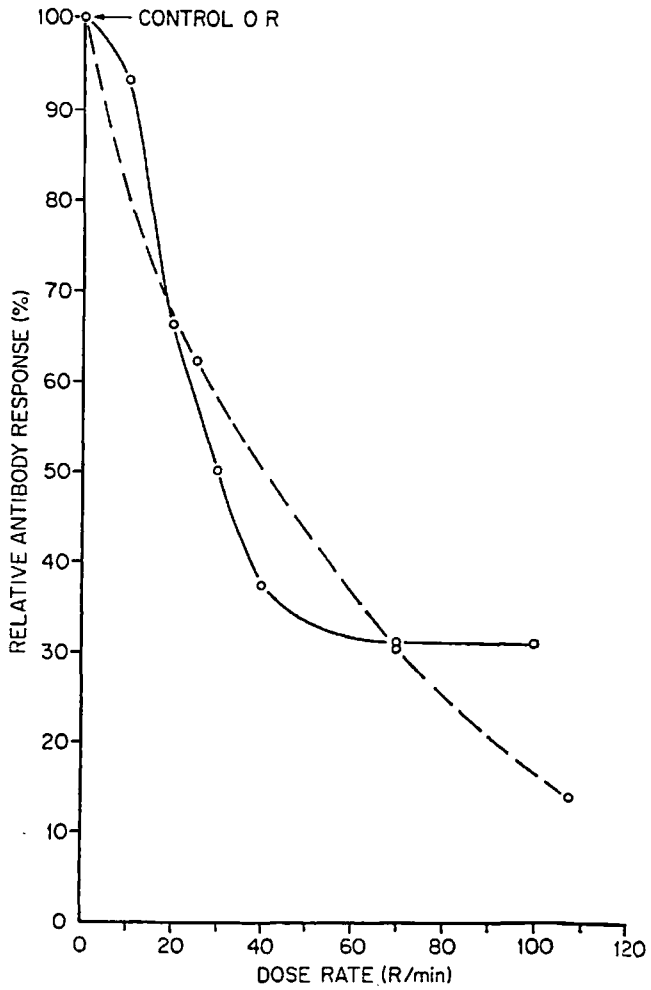

Figure XIV. Effect of exposure rate on the relative antibody response of mice (—) given a total exposure of 700 R, and of rats (- - -) given a total exposure of 500 R (197, 336, 510)

mice were given a cumulative exposure of 700 roentgens, and rats one of 500 roentgens. As a curve of this type is not available for man, and since so many factors enter into the calculation of the actual dose rate received by the relevant tissue, extrapolation of effects from animals to man simply on the basis of cumulative dose is very likely to be inaccurate. Despite this possible problem, however, studies with experimental primates have shown that whole-body irradiation in the range of 550 to 930 roentgens did permit initial successful takes of allogeneic bone marrow in at least 95 per cent of cases (116). This is in the same dose range as for similar experiments in mice, and would therefore seem to indicate that extrapolation from animals to man, at least for this type of immune response, may not be subject to any wide errors.

304. Clearly we need more direct data on the radiosensitivity of human immune responses before it can be concluded that the wealth of experimental animal work can be safely extrapolated for estimates of human risk. Such basic data on the radio-sensitivity of the various components of the immune system could be obtained from *in vitro* studies of antigen processing, of lymphocyte-mediated cellular immunity, and of antibody induction and proliferation. With this as a basis, it might then be more practical to consider other possible factors that might modify direct extrapolation, such as nature of antigen (primary or secondary response depending on previous cross antigenic experience), dose rate, etc.

305. Despite some uncertainty at present on this matter, it should be stressed that in many radio-therapeutic studies such as with Hodgkin's disease, and for eradication of leukæmia followed by marrow transplantation, the benefit to the patient of successful radiotherapy (and marrow transplantation if feasible) may well outweigh the perhaps minor but uncertain risks in regard to long-term effects of radiation on the immune response.

VIII. Summary and conclusions

A. Proposals for further investigation

306. There is virtually no well-controlled careful assessment of the radio-sensitivity of the antibody response in man. As this may be extremely relevant to certain aspects of the problem of radiation and neoplasia, some effort should be made to ascertain radiation sensitivity *in vitro* of the different human lymphoid cells involved in different stages of immune responses, with due consideration to effects of different forms of radiation, dosage, and dose rate.

307. It is clear from the many experimental studies that assessment of the true radio-sensitivity of the cells involved in antibody formation *in vivo* may be complicated by many factors. These include differential sensitivities of the various components, for example, macrophages *B* and *T*, lymphocytes and plasma cells, and differential effects on the catabolism of different antibody proteins. Accurate measurements of the radiosensitivity of the various human lymphoid cells involved in the development of an immune response should be made *in vitro*. With the various cell-separation systems available and the methods at hand for inducing primary-antibody formation *in vitro* and for quantitating numbers of antibody-producing cells, it should be possible to carry out the following determinations: (*a*) *macrophages*: with certain antigens, macrophages are required for the primary induction of antibody formation. Through the use of a macrophage-free test system, assays could be performed on the effect of addition of macrophage preparations which were previously subjected to different radiation exposures; (*b*) *antigen-reactive cells and antibody-forming precursor cells*: in similar fashion, both thymus-derived and bone-marrow (bursa?) derived cells could be separately irradiated at various doses and then recombined with the appropriate unirradiated cell type and studied for ability to collaborate towards antibody production *in vitro*. In man, bone-marrow cells and thymus cells (obtained

from fragments at surgery), could be assayed, together with *T* cells and *B* cells derived from normal human blood and fractionated by some of the immunological techniques now available; and (*c*) *plasma cells*: antibody-plaque-forming cells from any primary induction system with human lymphoid cells could be assayed for radio-sensitivity *in vitro*.

308. In studies with *Shigella* antigen and mice, the radio-sensitivity of the macrophages has been stressed. This type of study in experimental animals should be extended to other antigens, since, if this is a major factor with bacterial antigens, then susceptibility to infection following sublethal irradiation with doses above 100 rads might primarily involve interference with macrophage function. Active immune responses against pathogenic bacteria might then be induced in man by the use of appropriately modified forms of the bacterial antigen (for example, small soluble proteins rather than whole bacteria or flagella) which do not require macrophage processing. This would apply even in situations where irradiation had been previously encountered by the individual.

309. In the field of transplantation there are at least two areas in which radiation can be of considerable value. In organ transplantation, it is clear that immunosuppression from whole-body irradiation with sublethal doses is not feasible. However, both extra-corporeal irradiation of the blood, and local graft irradiation have been shown to be of value, particularly in acute reactions. Further experimental studies with these techniques, preferably in large animals, should be continued, and in particular should evaluate alternative schedules of irradiation. The use of other techniques, such as intralymphatic injection of radio-active colloids in suppression of allografts, also need further evaluation, with much emphasis on a more accurate determination of doses received by various cells and tissues.

310. In marrow transplantation, several promising approaches to the elimination of the secondary disease syndrome must be actively pursued if elimination of leukæmic cells by radiation, followed by marrow transplantation, is to be a practical form of therapy. Such approaches include comprehensive multifactorial studies, fractionation of immunocompetent cells from hæmatopoietic stem cells, and elimination of immunocompetent cells by appropriate anti-serum pretreatment. The possibility also exists that host thymic factors (? epithelial in origin) are involved in inducing differentiation of donor marrow stem cells into immunocompetence. This host component might be vulnerable to radiation suppression and thereby result in a depression of the initiation of secondary disease. Further studies of this phenomenon are needed to determine whether this could lead to a practical approach to the elimination of secondary disease.

311. If the concept of immunological surveillance is applicable to most forms of cancer, it might be expected that irradiated individuals would show an increased susceptibility to all types of cancer, approximately in proportion to their normal incidence although, if serum blocking factors are antibodies, then depression of this factor could result in an effective increased efficiency of cell-mediated tumour regression. These aspects are however quite speculative at present, as the original observation of an increased incidence of reticulo-endothelial and lymphoid tumours that occur in immunosuppressed kidney transplant patients is not observed in immunosuppressed auto-immune patients. However, as there is now available a considerable amount of background knowledge (480) on production of antibodies in man to defined antigens under controlled circumstances, a careful examination of various groups of irradiated subjects for antibody production might therefore be undertaken. This should include patients receiving intralymphatic radio-isotopes, as well as all known survivors previously subjected to whole-body irradiation. The basic question is whether, if immunodepression were demonstrated, this result would help in assessing the probability or risk of subsequent tumour development. Studies on cell-mediated immunocompetence would perhaps be of even greater value.

312. The concept of loss of the tolerant state after irradiation leads naturally to a consideration of possible auto-immune consequences. If the normal absence of anti-self reactivity is due to a continuing equilibrium between a potential aggressor cell and the right balance of self-antigen (perhaps in combination with natural antibody), then an alteration in the equilibrium might lead to antibody production as a result of radiation effects on the population of lymphoid cells. As few relevant data are available, further examinations of the sera of surviving patients who have received whole-body irradiation (from atomic bombings or, for example, from treatment for ankylosing spondylitis) for various auto-antibody levels will be of great interest. Again, there is considerable background information available on the incidence of various auto-antibodies in normal subjects of differing ages (623). It already appears that incidence of auto-antibody formation increases with age, and whether this is indeed a part of the ageing process itself is of great interest. Particularly as radiation is also thought to have some accelerating effects on the ageing process, auto-antibody incidence and titre estimations would be of considerable value. If radiation-induced life-span shortening is associated with, or mediated by, effects on the immune system, then it is likely that increased manifestations of auto-immunity may occur predominantly in the sub-population of those exposed to radiation in young life. Accordingly, more intensive surveys for cellular as well as humoral auto-immune activities would be most warranted in exposed individuals.

313. The potential involvement of radiation in immunotherapy of neoplasia is of great interest. Several relatively new approaches are available, which will require extensive evaluation. If a tumour gives rise to an impairment of the host's immune response to its own tumour antigens, a drastic immunogenic stimulus will be required to overcome this state. This might well be aided by a break of the tolerant state brought about by sublethal irradiation of the recipient, followed by administration of a sample of the autologous tumour pre-irradiated *in vitro* in order to stimulate additional lymphoid elements. This type of approach should be monitored not only by examination of the growth of the primary tumour but also by attempting to directly assess the state of anti-tumour immunity (cellular and humoral) of the host by the various *in vitro* assays which are currently under development in several laboratories.

314. Eradication of tumour cells might also be approached by the use of tumour-specific antibodies which would localize on the tumour-cell surface. If the antibodies were heavily labelled with a radio-

isotope, a lethal radiation dose to the cell might thereby be delivered. However, the major problem may rather be in effectively contacting tumour cells in a solid tumour with the antibodies.

B. Radiation, resistance to infection, and antibody formation

315. One of the best illustrations of the injurious effects of ionizing radiations on immunity is that showing decreased resistance of irradiated animals (usually in the 200-600-R exposure range) to the pathogens of infectious diseases. This has been demonstrated countless numbers of times with many different pathogens of bacterial, viral, rickettsial and fungal types. In general, species resistance is maintained after irradiation, although in some studies there are examples of partial elimination of congenital resistance to certain infectious agents.

316. Decreased resistance to infection varies considerably for different infections, species, types of infections (acute or chronic) and radiation parameters. Part of this variation may depend on the type of assay system, for example it appears that radiation-induced decreased resistance to infection occurs primarily after several days, rather than immediately, and challenge with a very acute infectious agent at the time of radiation will not show any decrease in resistance. Challenge with an agent that induces a more chronic and prolonged infection will be more likely to show decreased resistance.

317. Radiation-induced decreased resistance to infection is primarily mediated by the decrease in the host's specific immune response, although other non-specific factors may also be of importance, particularly macrophage handling of antigen and granulocyte functions. As this review is primarily concerned with the immune response, evaluation of the susceptibility of its components cannot be very readily ascertained by simple whole-animal studies with challenge of infectious agents. Accordingly, a more detailed examination of the separate components of immunity has been made.

318. Phagocytosis of antigens and antigen degradation are relatively radio-resistant with doses of the order of 1,000 rads. Some changes in granulocyte activities have been reported even with relatively low doses (100 rads) but the significance of this for the eventual immune response is probably minor. In several studies, however, although irradiated macrophages successfully phagocytosed antigen, they did not appear to be capable of processing it in a manner which is obligatory for the initiation of the immune response. This effect was observed even with doses of 150 rads. Antigen-handling in lymphoid follicles appears to be particularly important for the development of the secondary response and radiation inhibition of this function may be a factor in antibody depression.

319. Depending upon dose, dose rate and time of irradiation relative to antigen injection, the immune response may show either a shortened lag phase and higher antibody levels (particularly with relatively low doses), or a lengthened lag phase and reduced antibody response. This increase in the antibody response appears whenever the radiation dose is low enough (observed with 25 rads) and antigenic stimulation delayed enough to allow the steady-state population of precursor cells to recover. The radio-sensitivity of the hæmatopoietic stem cells and the precursor immunocompetent cells, as indicated by their D_{37} values, is in the range of 60 to 150 rads. It is during the lag phase of the immune response that cell co-operation appears to occur in some antibody responses, and although the actual precursor cell of the antibody producer is most sensitive, conflicting data on the thymic-derived cell (T) exist. It appears that in primary responses, if the T cell must proliferate to produce sufficient numbers for collaboration, then it will appear radio-sensitive, whereas in its carrier function in secondary responses proliferation may not be as important, and radiation in doses of up to 2,000 rads does not seem to interfere with this function.

320. The logarithmic phase of antibody production is only moderately radio-sensitive, because of the mixture of both proliferating immature plasmablasts and the highly radio-resistant mature non-dividing antibody-synthesizing cells. Finally, no significant depression of antibody secretion is observed in the populations of cells irradiated (with doses up to several thousand rads) in late logarithmic, plateau and decline phases, most of which are mature plasma cells.

321. The secondary antibody response has often been described as radio-resistant in studies which assess the over-all antibody production in whole animals. However, many of the differences between the primary and secondary responses can be accounted for by the numbers of potentially available cells which can be called upon for the particular immune response. Thus a comparable reduction by radiation in the percentage of cells in a primary and secondary response will still leave in absolute numbers many more surviving cells in the secondarily-stimulated animal.

C. Radiation and transplantation

322. Although it is clear that the lymphocyte population which is involved in cellular immunity is *in general* relatively radio-sensitive, there are few direct assessments of the actual radio-sensitivity of all components involved in graft rejection. Furthermore, there are indications that some of the lymphocytes that mediate cytotoxic cellular immune responses are relatively radio-resistant.

323. It is now quite definite that prolongation of foreign organ grafts cannot be obtained solely by whole-body irradiation of the recipient, at least without using lethal doses of radiation, which would then require simultaneous marrow transplantation. Local irradiation of the graft *in situ* (e.g., kidney), usually administered in fractionated doses, has been used as giving some definite advantage for graft survival in the early stages. Indiscriminate use of radiation may, however, be of considerable disadvantage since, if radiation is administered in a form such that radiation-induced augmentation of the immune response occurs, this might lead to accelerated graft rejection, which might also negate any further attempts at transplantation.

324. Various other methods of irradiation have been attempted in order to suppress the recirculating pool of lymphocytes which is of prime importance in graft rejection. These include extracorporeal irradiation of the blood (ECIB), intravascular insertion of radio-active implants, intralymphatic injection of radio-active colloids, and application of a radio-active strip to the

surface of the spleen. As these latter approaches are mainly experimental and suffer from some problems of irradiation of other tissues, and because it is very difficult to calculate doses at various points in the body, further investigations are needed before these techniques can be used extensively in man. ECIB, however, has been found to be of advantage with renal allografts, particularly in acute rejection crises. The combined use of radiation therapy and other immunosuppressive régimes (drugs, anti-lymphocyte serum) often leads to considerably greater immunosuppression and graft prolongation.

325. Lethal doses of whole-body radiation have been attempted only in bone-marrow transplantation. If these patients could indeed be restored by marrow transplants, then a direct application of this technique to leukæmia might be possible. Although it is by no means certain that there is a systemic factor involved in the induction of the malignancy, it must be pointed out that if this did exist, donor-derived marrow cells could eventually become malignant and the major problem of marrow transplantation would indeed not be the immunological problem. However, there is a major immunological obstacle to marrow transplantation in man, namely, the graft-*versus*-host reaction (secondary disease) which is now clinically well documented. Initial takes of marrow appear to be satisfactory, indicating that irradiation has prevented early host rejection. The key immunological problem now is to prevent the subsequent secondary disease complications, and several recent promising reports in this field have been published, although care must be taken in extrapolating from animal graft-*versus*-host reactions, as the content of *T* cells in marrow clearly differs from species to species.

D. Radiation, tolerance and cancer

326. In considering the interaction between a developing primary tumour and the antagonistic host immune response, it is likely that any factors which affect the host immune response may alter the current balance, and tend to favour or inhibit the growth of the tumour, again depending in turn on the relative proportions of cell-mediated immunity, humoral immunity and blocking factors. Experimental studies have demonstrated that immune suppression will lead to an increase in the growth rate of transplanted tumours, and will permit greater and more rapid metastatic spread of tumours. This in turn affects the time of observation of macroscopic tumour development and the survival of the host. Whether immune suppression actually leads to an increase in the number of primary malignant clones is not yet clear. It is on this critical point that the concept of immune surveillance rests, and further work is still needed, particularly with spontaneous primary tumours, rather than the more highly antigenic viral induced tumours. Alternatively, however, if the case for human virus induced tumours becomes fully established, the role of immune depression in the ætiology of tumours must be carefully evaluated.

327. Radiation-induced augmentation of immune tolerance has been demonstrated with various systems. In the field of transplantation, non-lethal doses (300-500 rad) have been used in this manner to facilitate the induction of skin-graft tolerance to weak histocompatibility antigens. On the other hand, a state of persisting immunological tolerance can be broken by radiation and converted into an active immune response. This may well have relevance to certain types of tumour induction.

328. If radiation can break the state of induced immunological tolerance, it may also do so with self-tolerance. This could lead to the development of autoimmune disease. Experimental or clinical information on this aspect is very sparse, and limited studies with atomic-bomb survivors have not at present indicated any increased incidence of auto-antibodies in this population. However, it is also quite probable that such increased incidence may only be observed in that subpopulation of individuals who were exposed at a young age, and further studies on this aspect should continue.

329. In conclusion, it must be emphasized that accurate determinations of the radio-sensitivities of the various cell types involved in immune responses in man are not at present available. It appears reasonable to extrapolate from animal studies in relation to the actual radio-sensitivities of the cell types, although it is important to note that the expression of an immune response frequently involves an interaction of cell types, and that the proportions of these cells in the tissues of man may not be the same as in other species. Even less is known in man about the possible role of radiation effects on immunity in relation to cancer and autoimmune disease.

330. These considerations indicate that accurate risk estimates for man on the effect of radiation on immune responses cannot at present be made. Further studies on the radio-sensitivity of individual cell types and long-term studies on immunological changes in relation to cancer and auto-immunity may eventually lead to the realistic assessment of these risks.

TABLE 1. CLASSIFICATION OF IMMUNE RESPONSES

	Humoral-antibody formation		*Cellular immunity*	
Reactive cells ...	Plasma cells and *B* lymphocytes		*T* lymphocytes	
Ontogenic control of differentiation	Bursa of Fabricius (avian), bursal equivalent (mammal) *and* thymus		Thymus	
Primary mediator of measured immune response .	Antibody-secreted immunoglobulin		Lymphoid cells (possibly through cell-surface-bound immunoglobulin) and macrophages	
Secondary mediators of response	Complement components, histamine, serotonin, SRS		Migration inhibition factor Transfer factors Lymphotoxin, etc.	
Clinical and experimental forms of immunity ...	Serum antibody IgM, IgA. IgG etc.	Immediate hypersensitivity: Anaphylactic reaginic	Transplantation immunity	Delayed hypersensitivity: Arthus type

TABLE 2. ANTIBODY PRODUCTION BY 550 R IRRADIATED MICE FOLLOWING INOCULATION OF MACROPHAGES FROM NORMAL AND IRRADIATED DONORS INCUBATED WITH *Shigella* ANTIGEN (177)

Radiation exposure to macrophage donors[a] *(R)*	*Treatment of recipients*	*Agglutinin titre* 5 days: *Number of animals*	5 days: log_2	8 days: *Number of animals*	8 days: log_2
0	Macrophages[b]	20	2.0	47	5.7
150	Macrophages	15	0.5	34	2.8
300	Macrophages	15	0.4	31	2.2
450	Macrophages			12	1.5
600	Macrophages			12	0.8
750	Macrophages	5	0	10	0.8
	Shigella (0.1 ml of 0.1 per cent suspension)	12	0.5	36	0.8

[a] Animals were exposed to x rays two days after thioglycolate injection. Two days later the peritoneal cells were harvested.

[b] 15 10^6 macrophages incubated *in vitro* with *Shigella* antigen.

TABLE 3. THE EFFECT OF THE TIME OF ANTIGEN INJECTION ON THE IMMUNE RESPONSE TO SHEEP ERYTHROCYTES OF SUBLETHALLY IRRADIATED MICE: PLAQUE FORMATION IN THE SPLEEN AS A FRACTION OF THE CONTROL[a]

(95 per cent confidence limits)

Irradiation dose (rads)	*Antigen 1 hour before irradiation*	*Antigen 1 hour after irradiation*	*Antigen 24 hours after irradiation*
50	1.645 (1.354-1.930)	1.698 (1.390-1.956)	0.774 (0.617-0.916)
100	0.914 (0.751-1.087)	1.086 (0.900-1.282)	0.533 (0.417-0.623)
200	0.600 (0.408-0.729)	0.697 (0.564-0.837)	0.142 (0.102-0.185)
300	0.133 (0.095-0.159)	0.107 (0.079-0.139)	0.032 (0.026-0.038)

[a] Plaque formation determined 3 days after antigen injection.

TABLE 4. EXPECTED RESULTS ON THE EFFECT OF TOTAL-BODY X-IRRADIATION ON PRIMARY (1°) AND SECONDARY (2°) ANTIBODY RESPONSES BASED ON THE POPULATION-DENSITY FEED-BACK-CONTROL THEORY[a] (336)

X-ray exposure (R)	*Relative suppression[b] (survival percentage)*	*Number of surviving immunocompetent units[c]*		*Number of immunocompetent units differentiating into antibody-synthesizing cells*		*Responses (per cent of normal)*	
		1°	*2°*	*1°*	*2°*	*1°*	*2°*
0	0	100	4,000	100	200	100	100
100	65	65	2,600	65	200	65	100
200	18	18	720	18	200	18	100
300	5	5	200	5	200	5	100
400	1.5	1.5	60	1.5	60	1.5	30
500	0.3	<1	12	<1	12	<1	6

[a] It is assumed that the ratio of immunological expression to immunological potential is 1.0 in a primary antibody response and 0.05 in a secondary antibody response. This is based on the following two observations: (*a*) there can be as many as 10 to 100 times more immunocompetent units responsive to an antigen in the spleens of maximally primed mice than in those of nonprimed mice, and (*b*) the difference between primary and secondary antibody responses against foreign red blood cells can be as small as twofold.

[b] Data obtained from Makinodan, Kastenbaum and Peterson (333).

[c] For convenience it is assumed that there are 100 immunocompetent units in nonprimed individuals. If so, there should be 4,000 immunocompetent units in maximally primed individuals.

TABLE 5. RESULTS OF X-RAY THERAPY IN HODGKIN'S DISEASE (279)

X-ray therapy and stage of disease	*Total number of patients*	*Number of deaths*		*Patients continuously free of disease*	
		With disease	*Without disease*	*Number*	*Duration (months)*
IB and IIB					
Limited fields	10	4	0	2	
Extended fields	10	0	1	7	
IIIA					
1,500 R	6	2	0	3	30,39,43
3,500-4,000 R	5	1	0	4	14,24,43,47
IIIB					
1,500 R	9	5	0	0	
3,500-4,000 R	17	7	1	7	15,15,15,22 23,25,51

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