RADIOSENSITIVITY OF LYMPHOCYTES*

Eugene P. Cronkite, M.D.

Chairman, Medical Department

Brookhaven National Laboratory

Upton, L. I., New York

USA

**Conference on Radiosensitivity held in Freiburg, Germany
September 25-30, 1972.**

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

***Research supported by the U. S. Atomic Energy Commission.**

MASTER

HISTORICAL:

The determination of the sensitivity of any living system to a toxic agent implies the capability of quantitatively measuring both an effect and the exposure with satisfactory units. Accordingly, all studies performed prior to quantitative cellular techniques and the methods of measuring radiation remain qualitative. However, the excellent reports of Heineke in 1903-1905 (1,2) remain qualitatively correct in respect to the fact that radiation can produce severe damage to the hematopoietic system in general and lymphopoietic system in particular.

Bergonié and Tribondeau (3) first enunciated the concept that dividing cells in general are very radiosensitive whereas non-dividing cells are quite radioresistant, requiring very high doses of radiation to kill them or to impair biochemical functions such as oxygen-carrying capacity, protein synthesis and enzyme function. Thus, if the cells of an organ do not divide normally in the adult, that organ may be most radioresistant. If an organ is composed in whole or in part of functional cells that are continuously being replaced at a rapid rate from a precursor pool of dividing cells then that organ in general will be radiosensitive because the immature cells of the system are easily damaged or killed by relatively low doses of radiation. There are two apparent exceptions. The small lymphocyte of the peripheral blood, rarely seen in mitosis, is exquisitely sensitive to radiation. Another cell which does not divide and is very sensitive to radiation is the oocyte. The reasons for these two exceptions are not clear.

MASTER

THE LYMPHOCYTIC SYSTEM:

It is beyond the scope of this paper to describe in detail the entire lymphocytic system, the origin of lymphocytes, their functions and migration pathways. Current doctrine states that lymphocytes arising in the bone marrow produce progeny which populate the thymus in embryo and repopulate the thymus during regeneration. The thymus produces T cells, coats them with antigens (thymus specific and thita) and thereafter they migrate to peripheral tissues in huge numbers. Bone marrow (B cells) cells also migrate to peripheral lymphoid tissue and are intermixed to varying degrees with T cells migrating out of the thymus, the proportion varying with anatomical site. The paracortical area of lymph nodes is populated primarily by T cells. B-lymphocytes appear to become antibody synthesizing cells through an interaction with T cells. The latter apparently are sensitive to antigen and endow the B cells with the capability of producing antibodies in lymph nodes, spleen, and gut lymphoid tissue. About 5-10% of lymphocytes emerging from lymph nodes are cells newly produced in the lymph node - 90-95% are lymphocytes recycling from blood to lymph through the postcapillary venules and paracortical area of the lymph node.

Lymphocytes are also divided on basis of life span. Long- and short-lived small and large lymphocytes exist. The long-lived small lymphocytes perform a ceaseless cycling from blood to lymph. Potentially, immunocompetent lymphocytes are instructed, transformed and proliferating in the lymph nodes, spleen and gut lymphoid tissue, produce clones of cells, dedicated to the production of specific antibody.

In this paper, selected quantitative data on the radiosensitivity of T and B cells will be considered along with the radiosensitivity of cells known to be dedicated to the production of a specific antibody. Consideration will be given to interphase death and loss of reproductive capability also. Lastly the effect of radiation upon the synthetic capability of cells actively synthesizing antibodies will be considered.

A wide spectrum of techniques exists for the measurement of radiosensitivity. The relationship between dose of radiation, weight of spleen and/or thymus, and concentration of cells in the peripheral blood has been used empirically. However, these procedures which measure the result of extensive death of lymphoid cells in tissues have led to the notion of an excess radiosensitivity of proliferating lymphoid cells as compared to proliferating cells of other systems. The notion of excessive radiosensitivity is largely due to the rapid appearance of extreme morphological damage in lymphoid tissues following irradiation from interphase death. This is also expressed in the rapid decrease in the size of lymphatic organs such as the thymus, lymph nodes and spleen after irradiation. Erroneous notions also result from the measurement of incorporation of tritiated thymidine or other DNA precursor into DNA of irradiated cells. As noted later, the incorporation of radioactive precursors into DNA is not a reliable estimate of proliferative capacity after injury by radiation. There are a variety of recently developed in vivo and in vitro methods for measuring the immunological activity of lymphocytes that have been applied to the study of radiation sensitivity, for example rosette- and

plaque-forming cell assays and transfer of graft versus host disease (GVH) in animals receiving graded doses of radiation either before or after antigenic stimulation. These methods measure the reproductive capacity of lymphoid cells or clone formation since the radiosensitivity of the lymphoid cells involved in such proliferative activities may be expressed in terms of the familiar D_{37} , extrapolation values, etc. Viewed in these terms, as will be shown, radiosensitivity of the lymphocytic cells appears not to be different from that of other proliferating hematopoietic cells. However, once the antibody producing system is switched on, the protein synthesizing machinery is exceedingly resistant to radiation.

ATROPHY OF LYMPHATIC TISSUE FOLLOWING EXPOSURE TO LOCAL AND WHOLE BODY IRRADIATION:

In considering the dose effect relationship of weight of the thymus or the spleen after irradiation, one is concerned with a dynamic two-phase process. Following irradiation there is necrosis with atrophy of the lymphocytic tissues. After a variable, dose dependent time, regeneration commences in parallel with continuing necrosis. The quantitative relationship of the degree of weight loss in thymus and spleen to dose of radiation has been used effectively to determine the relative biological effectiveness of neutrons compared to x rays (4).

Sato and Sakka (5) have measured the weight of and number of cells in lymph nodes and thymus after graded doses of whole body radiation. Using these end points, two classes of lymphocytes were identified with different radiosensitivity. The D_{37} was 135 r and 425 r for small lymphocytes and 57 r and 520 r for medium and large lymphocytes in the

thymus. The D_{37} values for small and larger lymphocytes in lymph nodes were 213 r and 200 r, respectively. Since these measurements were made 24 hours after irradiation, regeneration was insignificant and these values represent "killing doses" on combined interphase and cycling lymphocytes.

The comparative atrophy of lymphocytic tissues following local irradiation and whole body irradiation has been used as a method of obtaining data on whether there is an abscopal effect. An example of such a study is that of DeBruyn et al. (6). They observed a substantial difference between weight loss of the appendix in the whole body irradiated animal and the animal in which only the appendix of the rabbit was irradiated. Their interpretation may be incorrect since this work and other similar work was performed before the recycling of lymphocytes and their migration from other parts of the body to gut, lymph nodes and spleen was shown by the elegant studies of Gowans and Knight (7). Hall and Morris (8) have shown that local irradiation of the lymph node only temporarily suppresses output of cells in the efferent lymph. Subsequently, Benninghoff et al. (9) have shown that the locally irradiated lymph node is very rapidly repopulated following 300 rads whereas there was severe depletion of the same lymph nodes after the same dose of whole body irradiation, showing that there is a rapid repletion of the lymph node by the population of long lived recirculating lymphocytes. The contribution of migration of blood lymphocytes into lymph node and the recycling of the long lived small lymphocytes from blood to efferent lymph was further emphasized by subsequent studies of Hall and Morris (10) in which tritiated

thymidine was injected directly into the lymph node. Only about 4% of the efferent lymph cells were labeled. Correcting for ratio of DNA synthesis time to lymphocyte generation time, an upper limit on the fraction of cells in efferent lymph that are produced in lymph nodes is 8-10%.

Rixon (11) has studied the relationship of thymocyte size and the in vitro survival following graded doses of radiation. The survival of cells following all doses of radiation decreased as the cell size increased. The exponential dose survival curves for each classification consisted of two components. The percentage of survival decreased with doses up to 300 r but became independent of dose after higher doses. The larger the cell the more sensitive. Their data suggest that once thymocytes are lethally injured by radiation, the rate of degeneration is determined by the rate of metabolic processes in the cell. Since the small cells are in G_0 or long G_1 they can be assumed to be metabolically relatively inactive. Other work has shown that the largest cells in many cell populations are in late DNA synthesis or G_2 and could be assumed to be very radiosensitive. The D_{37} of cells 7 microns or less in diameter is about 175 r whereas the larger cells have a D_{37} of about 50 r.

THE INFLUENCE OF RADIATION UPON THE UPTAKE OF TRITIATED THYMIDINE INTO LYMPHOCYTES:

The incorporation of tritiated thymidine into DNA of cells is proof positive that these cells have not lost the enzymatic capability of replicating DNA. This indicates that the cells are in DNA synthesis; however, it is not proof that these cells have retained reproductive capability and will multiply themselves indefinitely. In fact, Fliedner (12)

has shown that 800 r X rays to human lymphocytes in vitro does not prevent their response to PHA transformation and incorporation of tritiated thymidine. However, these stimulated cells, when attempting mitosis, show all of the signs of mitotically connected abnormalities which in all probability will result in the death of the daughter cells. Manasek et al. (13) studied the effects of X rays on DNA synthesis in suspensions of hamster spleen cells. The dose response curve was dependent on temperature at time of irradiation and temperature of incubation with tritiated thymidine. In these studies, suspensions of the cells were irradiated following which tritiated thymidine was added to the suspensions. Thereafter DNA was extracted and its specific activity measured. When irradiation and incubation took place at 37°C, a single exponential decline was found with D_{37} of 7 kilorads. When irradiation was at 7°C with an incubation temperature of 37°C, there was a logarithmic biphasic response. The steep slope had a D_{37} of 4 kilorads. These studies clearly indicate that irradiation does not kill the cells in DNA synthesis or about to enter DNA synthesis and that the enzymes responsible for the incorporation of tritiated thymidine into DNA are exceedingly resistant to irradiation. However, these studies do not give any great insight into the actual radiosensitivity of the cells so far as reproductive capacity is concerned. Jackson et al. (14) performed similar experiments in which the thymus was removed from animals, cell suspensions made and irradiated at 4°C. Following irradiation, the cells were incubated with adenine-8- ^{14}C . The incorporation into DNA was determined as CPM/micrograms of DNA. The decrease in incorporation of adenine ^{14}C and decrease in the numbers of living

cells paralleled each other hence the decreased uptake represents direct cell killing. This system shows a much greater sensitivity than that of Manasek et al. in spleen cell suspensions. Ilbery et al. (15) have also studied the incorporation of tritiated thymidine into PHA stimulated cultures following varying doses of radiation. The blood was irradiated with graded doses of radiation, placed into culture using standard techniques and 6 hours before harvesting, tritiated thymidine was added to the cultures. The incorporation of tritium into the cells was then determined by scintillation counting and the results expressed as counts per minute per 10^6 small lymphocytes in the initial culture. Suppression of incorporation was observed at 250 r and at 2,000 r, depending upon time in culture, the uptake was decreased 60-80%.

Tritiated thymidine has been used in another manner to get at the relative radiosensitivity of old lymphocytes versus newly produced lymphocytes. Whitelaw (16) gave repeated injections of tritiated thymidine over a 2-week period, a procedure that resulted in labeling 33% of the circulating small lymphocytes. After exposure to 208 r of whole body irradiation, there was a decrease in the total lymphocyte count from 5,000 to 1,000 per mm^3 . The labeled newly formed lymphocytes and the unlabeled older lymphocytes were equally responsive to this dose of radiation.

Tritiated thymidine has been used to ascertain the relative radioreistance of long lived lymphocytes and plasma cells. Miller and Cole (17) studied animals given primary and secondary immunological stimuli. Thymidine was then injected intermittently into the foot pads from 16 through 96 hours after the secondary antigenic injection. Thirty-one days after the last dose of tritiated thymidine, mice were divided into 3 groups

1) receiving 850 r, 2) 500 r and 3) the non-irradiated controls. Although there was a marked atrophy of the popliteal and aortic lymph nodes, the percentage of labeled small lymphocytes was higher in the irradiated than in the control animals. Labeled plasma cells also persisted. Such studies indicate in fact that cells are able to survive the stated doses of radiation but give little information on the actual radiosensitivity since data are not quantitative nor were dose-effect curves performed.

RADIOSENSITIVITY OF THE IMMUNE RESPONSE:

Not long after Heineke (1,2) discovered radiation induced atrophy of lymphocytic tissues, Benjamin and Sluka (18) pioneered in the study of the suppression of antibody formation by radiation. There is a huge literature on the effects of radiation upon the suppression of the immune responses. This literature is primarily concerned with the effect of irradiation prior to or after the injection of antigen upon both primary and secondary serum antibody titers. These studies are comprehensively reviewed by Stoner and Hale (19). When antigen is given prior to irradiation, there is either enhancement or relatively little effect upon antibody titers. However, when antigen is administered after irradiation, the marked suppression is both radiation-dose and time-dependent. In addition, the nature of the antigen influences the response. For example, when insoluble antigens are given, the continued presence of antigen results in stimulation during the recovery after irradiation. When fluid easily degradable antigens are given, a marked suppression is seen in the primary response. All of the early studies on radiosensitivity of antibody responses tacitly assumed that these were mediated through effects on cellular immunity. Definitive studies on

radiosensitivity of the lymphocytic system and its component cells were not performed until the lymphocyte was clearly demonstrated to be the antibody producing cell and techniques developed to study its reproductive capacity.

THE DIRECT MEASUREMENT OF RADIOSENSITIVITY OF LYMPHATIC CELLS:

Makinodan et al. (20) and Kennedy et al. (21) have shown that proliferation of immunocompetent cells is a prerequisite for the development of an immune response. Makinodan et al. (20) estimated the D_{37} to be 70 r with extrapolation number of 2.7. There was no significant difference between primary and secondary responses in their system involving measurement of serum antibody titer, transplant of viable spleen cells and graded doses of X ray. Kennedy et al. (21) measured the radiosensitivity of antigen-sensitive cells in spleen of mouse to proliferate and produce Jerne plaque-forming cells. The D_{37} was shown to be 80 r, independent of time of injection of antigen and measurement of proliferation potential. In contrast the capability of a plaque-forming unit to produce a plaque was resistant to 2 kilorads.

Vos (22) has also directly investigated the impairment of proliferation (reproductive integrity) of lymphocytes capable of producing a GVH reaction by irradiating lymphocytic cells in the intact animal or in vitro and transplanting the parental cells into fatally irradiated F_1 hybrids protected by bone marrow. The number of lymphocytic cells killing 50% of the recipients by a GVH reaction is determined. Since the number required increases with dose of radiation a dose effect curve can be

established. The D_{37} for lymphocytic cells, irrespective of anatomic site, irradiated in vivo or in vitro is circa 85 r. The D_{35} of anoxic cells is about 230 r with oxygen enhancement ratio of 2.7. Accordingly in discussing radiosensitivity of lymphocytes, care must be taken in in vitro and in vivo experiments to assure comparable oxygen tensions. The question of whether certain factors may alter the radiosensitivity of immunologically competent cells is of interest. Cronkite et al. (23) had shown that after prior fractionated exposure there is a significant increase in the resistance of mice to whole body irradiation so far as mortality is concerned. Petrov and Cheredeev (24) performed experiments indicating that after exposure to a sublethal dose of x-irradiation immunocompetent cells of young adults were more radioresistant than they were before irradiation. Price and Makinodan (25) reinvestigated this problem assaying the relative radiosensitivity of immunocompetent cells in the spleen of unirradiated young adults and aged mice and of spleen cells of young adult mice at varying intervals after sublethal total body x-irradiation. In their system, a mouse was given 250 r whole body irradiation. At various intervals thereafter the spleen was removed and suspensions of spleen cells were irradiated with graded doses of X-ray and then injected into a fatally irradiated mouse along with sheep red blood cells and at an appropriate time interval the number of plaque-forming cells in the spleen of the fatally irradiated mouse was determined. The D_{37} values determined 16 and 18 days after exposure to 250 r were not significantly different from the D_{37} of normal animals of 75 r. However, an assay was not made at time interval comparable to that of Cronkite et al.

of 30 days after last exposure to radiation nor when complete recovery had taken place in the immune response at 25 days or when there was an overshoot at 45 days in studies of Price and Makinodan. Berenbaum (26) bearing in mind the apparent differences in radiosensitivity of the immune response when one measures the titer of antibodies produced against specific antigens, performed studies aimed at determining objectively the radiation sensitivity of plaque-forming cells using the Jerne technique. In these studies it made no difference whether irradiation was given before or after the administration of antigen since in all combinations and observing production of 7S and 19S plaque-forming units there was always a substantial decrease in the number of antibody producing cells following irradiation. However, in these studies, D_{37} values were not determined. Katz et al. (27) and Kettman and Dutton (28) have produced data suggesting that there is a marked radioresistance of the helper function of T-cells, whereas 5,000 r abolished the capability of transferring immunological memory and the capacity to be stimulated by antigen to synthesize DNA. In these studies, huge numbers of cells were transferred. Anderson et al. (29) have further investigated the radiosensitivity of T-cells. In their studies parental thoracic duct lymphocytes were injected into F_1 heavily irradiated mice and the capability of the transferred cells to incorporate tritiated thymidine measured. Doses of radiation from 0-300 r progressively decreased the capability of incorporating tritiated thymidine and above 300 r there was no significant incorporation. Additional studies using a 7S plaque-forming cell analysis in the spleen showed that the helper function of carrier primed thoracic duct lymphocytes was abolished by exposure to 1,000 r in vitro and also

inhibited antibody forming capacity of the primed thoracic duct lymphocyte population. The discrepancy between the studies of Anderson et al. and those of Katz et al. is not clear.

THE RADIORESISTANCE OF ANTIBODY PRODUCTION BY STIMULATED LYMPHOCYTIC CELLS:

Makinodan et al. (30) have demonstrated that the capacity of immunocompetent cells to proliferate and differentiate into antibody synthesizing cells is extremely sensitive to x-irradiation. However, the fully differentiated progenies, consisting mainly of pure plasma cells will continue to synthesize antibody effectively for several days after exposure to radiation doses as high as 10,000 r. Vann and Makinodan (31) have pursued the study of antibody synthesis by heavily irradiated cells. They have shown that after irradiation with up to 10,000 r, the surviving immunocompetent cells are as efficient as unirradiated cells in synthesizing new antibodies. Sado (32) has extended the studies of antibody producing cells grown in millipore diffusion chambers after exposure to 10,000 r. Three of ten nucleated cells were specific antibody producers four days after exposure to X ray, whereas only one of ten in the unirradiated controls were antibody producers showing that cells not committed to antibodies were to a great extent killed. The half-life of irradiated antibody producing cells was not significantly different from that of unirradiated cells. Although immature plasma cells incorporated tritiated thymidine as late as 15 days after irradiation, they did not divide. Electron microscopic studies showed that there is marked nuclear damage but apparently intact endoplasmic reticulum.

SUMMARY AND CONCLUSIONS:

1. Radiosensitivity of cells cannot be estimated well by the measurement of radioresistant phenomena such as DNA and enzyme synthesis, enzyme function and antibody synthesis.
2. Useful quantitative data can be obtained by measurement of prompt lymphocyte killing and suppression of cell proliferation involved in 1st and 2nd immune responses using Jerne plaque and transfer of GVH reaction by cells.
3. The in vivo D_{37} for killing mitotic and interphase lymphocytes during 1st 24 hours after X ray is variable and 2 component
 - a. D_{37} 135 and 425 rads for small thymi
 - b. D_{37} 57 and 520 rads for medium and small thymi
 - c. D_{37} 213 and 200 rads for small 1. node cells.
4. The in vitro D_{37} for killing of thymic cells are
 - 175 r for small thymic cells
 - 50 r for large thymic cells
5. The D_{37} for suppression of incorporation of DNA precursors into DNA is of order of several kilorads.
6. Although dose effect curves have not been performed, there seems to be no difference in response of newly-formed labeled lymphocytes and older lymphocytes to a single dose of X ray.
7. The D_{37} of proliferating immunologically committed cells measured by transfer of GVH reaction and ability of Jerne plaque forming cells to proliferate is of order of 70-80 r when oxygenated. D_{37} of anoxic

cells is 230 r (OER 2.7).

8. The radioresistance of the 2nd response, measured by serum antibody titres, is greater than the radioresistance of this primary response. However, D_{37} of lymphocytes to proliferate in 1st or 2nd response is the same -- about 80 r. The discrepancy is probably explained on the basis of the radioresistance of the antibody synthesizing cells already proliferated by the time of irradiation.

9. Proliferating lymphocytes have same radiosensitivity as other cell renewal systems.

10. Interphase lymphocytes are much more sensitive to killing than other interphase cells except oocytes.

REFERENCES

1. Heineke, H., "Über die Einwirkung der Roentgenstrahlen auf innere Organe. München med. Wchnschr. 51, 785, 1904.
2. Heineke, H., "Über die Einwirkung der Roentgenstrahlen auf Tiere. München med. Wchnschr. 1, 2090-2092, 1903.
3. Bergonie, J. and Tribondeau, L., An interpretation de quelques resultats de la radiotherapy et essai de fixation d'une techniques rationnelle. Compt. Rend. Acad. Sci. 143, 983-985, 1906.
4. Carter, R.E., E.P. Cronkite and V.P. Bond, The effect of neutrons on thymic and circulating lymphocytes in the mouse. The American Naturalist, 87, 257-267, 1954.
5. Sato, C. and M. Sakka, Cell population analysis of radiation damage and recovery of thymus and lymph nodes in mice. Rad. Res. 38, 204-213, 1969.
6. De Bruyn, P.P.H., M.M. Tornova-Svehlik, and J.H. Venter, The radio-sensitivity of the lymphocytes of the rabbit appendix. Rad. Res. 24, 15-31, 1965.
7. Gowans, J.L. and E.J. Knight, The route of recirculation of lymphocytes in the rat. Proc. Roy. Soc. B. 159, 257-282, 1964.
8. Hall, J.G. and B. Morris, Effect of x-irradiation of the popliteal lymph node on its output of lymphocytes and immunological responsiveness. Lancet 1, 1077, 1964.
9. Benninghoff, D.L., R.W. Tyler and N.B. Everett, Repopulation of irradiated lymph nodes by recirculating lymphocytes. Rad. Res. 37, 381-400, 1969.
10. Hall, J.G. and B. Morris. The origin of the cells in the efferent lymph from a single lymph node. J. Exp. Med. 121, 901, 1965.

11. Rixon, R.H., The effect of radiation on the survival in vitro of rat thymocytes of different size. *Rad. Res.* 32, 42-53, 1967.
12. Fliedner, T.M., Experimental studies on PHA-stimulated lymphocytes and autotransfusion of tritiated cytidine labeled lymphocytes in chronic lymphocytic leukemia. In: The Lymphocyte in Immunology and Hemopoiesis, edited by J.M. Yoffey, Edward Arnold, L.T.D. London, 1967, pp. 198-206.
13. Manasek, F.J., S.J. Adelstein and C.T. Lyman, DNA-synthesis in irradiated lymphoid cell: Effects of temperature on the dose-response curve. *Int. J. Radiation Biol.* 10, 189-197, 1966.
14. Jackson, K.L., G.M. Christianson and R.W. Bistline, Irreparable injury produced by x-irradiation of rat thymocytes in vitro. *Rad. Res.* 38, 560-568, 1969.
15. Ilbery, P.L.T., A.B. Rickinson, and C.E. Thrum, Blood lymphocyte replicating ability as a measurement of radiation dosage. *Brit. J. Radiol.* 44, 834-840, 1971.
16. Whitelaw, D.M., The effect of total body irradiation on the labeling of circulating mouse lymphocytes with tritiated thymidine. *Blood* 25, 749-758, 1965.
17. Miller, J. and L. Cole, The radiation resistance of long-lived lymphocytes in plasma cells in mouse and rat lymph nodes. *J. Immunology*, 98, 982-990, 1967.
18. Benjamin, E. and E. Sluka, Antikörperbildung nach experimenteller Schädigung des hämatopoetischen Systems durch Röntgenstrahlen. *Wien klein. Wochschr.* 21, 311-313, 1908.

19. Stoner, R.D. and W.M. Hale, Radiation effects on primary and secondary antibody responses. In: The Effects of Ionizing Radiation on Immune Processes, edited by Charles A. Leone, pp. 183-219, Gordon and Breach Science Publishers, New York 1962.
20. Makinodan, T., M.A. Kastenbaum and W.J. Peterson, Radiosensitivity of spleen cells from normal and preimmunized mice and its significance to intact animals. J. Immunology, 88, 31, 1962.
21. Kennedy, J.C., L. Siminovitch, J.E. Till and E.A. McCulloch, A transplantation assay for mouse cells responsive to antigenic stimulation by sheep erythrocytes. Proc. Soc. Exptl. Biol. Med. 120, 868, 1966.
22. Vos, O., Radiation sensitivity in post-irradiation repair of mouse lymphatic cells in vivo and in vitro. A study with the parent to F₁ hybrid graft-versus-host reaction. Intl. J. Rad. Biol. 13, 317-333, 1967.
23. Cronkite, E.P., C.R. Sipe, D.C. Eltzholtz, W.H. Chapman, and F.W. Chambers, Jr., Increased tolerance of mice to lethal x-radiation as a result of previous sublethal exposures. Proc. Soc. Exptl. Biol. Med. 73, 184-186, 1950.
24. Petrov, R.V. and A.N. Cheredeev, Effect of preliminary irradiation on mice on radioresistance of immunologically competent spleen cells, Nature 220, 1349, 1968.
25. Price, G.B. and T. Makinodan, Radiosensitivity of immunologically competent cells. N.Y. Acad. Sci., 32, 453-457, 1970.
26. Berenbaum, M.C., Radiosensitivity of immunologically activated cells. Nature 209, 1313-1315, 1966.

27. Katz, D. H., W. E. Paul, E. A. Goidl and B. Benacerraf, Radioresistance of cooperative function of carrier-specific lymphocytes in antihapten antibody responses, *Science* 170, 462-464, 1970.
28. Kettmann, I. and R. W. Dutton, Radioresistance of the enhancing effect of cells from carrier-immunized mice in an in vitro primary response. *Proc. Nat. Acad. Sci, USA*, 68, 699, 1971.
29. Anderson, R. E., J. Sprent and J.F.A.P. Miller. Cell-to-cell interaction in the immune response. VIII. Radiosensitivity of thymus-derived lymphocytes. *J. Exptl. Med.* 135, 711, 1972.
30. Makinodan, T., P. Nettesheim, T. Morita and C. J. Chadwick, Synthesis of antibody by spleen cells after exposure to kiloroentgen doses of ionizing radiation. *J. Cell. Physiol.* 69, 355-366, 1967.
31. Vann, D. C. and T. Makinodan, In vitro antibody synthesis by diffusion chamber cultures of spleen cells. I. Methods and effect of 10,000 r on antibody synthesis, *J. Immunol.* 102, 442-450, 1969.
32. Sado, T., Functional and ultrastructural studies of antibody-producing cells exposed to 10,000 r in millipore diffusion chambers. *Int. J. Radiat. Biol.* 15, 1-22, 1968.