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LYMPHOCYTE REPOPULATION AND RESTORATION OF CELL MEDIATED IMMUNITY FOLLOWING RADIATION: WHOLE BODY AND LOCALIZED IRRADIATION*

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INTRODUCTION

Shortly after the turn of the century Benjamin and Sluka (1908) discovered that whole body irradiation (WBR) of animals prior to the administration of an antigen would suppress antibody production. Hektoen (1915) confirmed this pioneering observation and correlated the suppression of antibody production with damage to the thymus, lymph nodes and spleen. The first observation on what was later to be called "cell mediated immunity" was made by Murphy and Taylor (1918) who discovered that irradiation of animals prior to the transplantation of tumors allowed their prolonged growth. They correlated the growth of the foreign tissue with the suppression of lymphocyte production. Dempster et al. (1950) discovered that skin allografts survived substantially longer in rabbits that had been given WBR as compared to non-irradiated rabbits. Thus the influence of radiation upon cell mediated immunity was established early in the century.

In this paper there will be a selective review of: (1) effect of radiation on lymphocyte survival in vivo; (2) effect of whole body and local irradiation on lymphocytic populations and their restoration; (3) effect of whole body irradiation on cell mediated immunity; (4) effect of extracorporeal irradiation of the blood and lymph upon lymphocytic populations; (5) effect of local, whole body and extracorporeal irradiation on allograft rejection; (6) influence of whole body and extracorporeal irradiation of the blood on hemopoietic cell proliferation.

In order to better understand the topics to be discussed, an appreciation of the heterogeneity of lymphocytic populations, their

migration pathways and proliferation is required. It is common knowledge that lymphocytes are being produced in the bone marrow, thymus, lymph nodes and spleen. It is generally believed that lymphocytic stem cells arise in the yolk sac and bone marrow during embryonic life and produce progeny which populate the thymus in the embryo. Bone-marrow-derived lymphocytes repopulate the thymus during regeneration from radiation injury and probably provide a continuous steady state source of stem cells for the thymus throughout life. The thymus produces a large number of cells, a substantial fraction of which migrates into the peripheral tissues such as lymph nodes, spleen and the gut associated lymphoid tissues (GALT). There is controversy about the degree of intrathymic death of newly produced cells. Most thymic and a variable proportion of thymus-derived (T-cells) cells are characterized by the presence of membrane associated alloantigens such as the theta.

Bone marrow derived cells that have probably bypassed the thymus during their transit to peripheral lymphoid organs are considered equivalent to the cells of the avian Bursa of Fabricius and are called B-cells. These cells are characterized by their easily detectable surface immunoglobulins. In the peripheral lymphoid tissues, B-cells are intermixed in varying degrees with T-cells.

The paracortical area of lymph nodes has been termed the "thymic dependent area" (Parrott and de Sousa 1971). T-cells are predominant in this area. However, T-cells are also found in the outer cortex and in the medullary cords to a lesser extent. In the spleen, T-cells are

found most commonly in the dense white pulp, but they are also present in the loose white pulp and the red pulp of the spleen (Iorio et al. 1970). The predominant cell in GALT is a T-cell (Joel et al. 1972 and Chanana et al. 1974).

About 5-10% of lymphocytes emerging from lymph nodes are newly produced while 90-95% are lymphocytes either recycling from blood to lymph through the postcapillary venules and the paracortical areas of the lymph node or are on terminal tissue migrations. A comparable recycling of lymphocytes proceeds through the spleen and the GALT (Ford and Simmonds,1972). In addition to functional differences in lymphocytes such as T-cells (cellular immunity) and B-cells (humoral immunity), the heterogeneity of lymphocytes concerns life span of a few hours to several years (Everett et al. 1964; Norman et al. 1965; Buckton et al. 1967; Robinson et al. 1965), the tissue of origin and proliferative and migrational behavior.

The proliferation of lymphocytes per unit time is enormous. Joel et al. (1974) estimate that in rodents about 5×10^4 cells are produced per milligram of thymus per hour. The average volume of small lymphocytes in man is approximately 250 μm^3. Due to normal involutionary changes, lymphocytes will represent only a fraction of thymus weight around and after puberty. Assuming a thymus weight in man of 15 grams (Hammar, 1926), arbitrarily estimating lymphocytes to be 10% of thymic weight and production rate comparable to that of rodents, a standard man of 70 kg would produce 1.8×10^9 or 0.45 gm of thymic cells per day. The production rate in bone marrow and spleen is not well established.

One can estimate a minimum production in lymph nodes since 5-10% of thoracic duct lymphocytes are flash labeled with tritiated thymidine and hence are in the production pathway. The output of thoracic duct lymphocytes in man is 10^7 /kg/hr (Yoffey and Courtice 1956) or 70 x 10^7 /hr in standard man of 70 kg. The daily output therefore is 1.68 x 10^{10} lymphocytes. Based on a thymidine labeling index of 5%, 0.084 x 10^{10} is the daily output of lymphocytes in the production pathway. Each lymphocyte in DNA synthesis produces two cells so the production is 0.168 x 10^{10} new cells or 0.42 g per day in lymphoid tissues drained by the thoracic duct. This minimum value for lymphocyte production and lymph nodes and daily thymic production adds up to approximately 0.9 g of lymphocytes/day. Bone marrow, and splenic production will add to this by an undetermined amount.

Let us assume that there are about 10^4 antigens for which T-cells have a unique genetic recognition capability. There are 1.8 x 10^9 thymocytes being produced/day in standard man and accordingly there are 1.8 x 10^5 cells/day being produced that can recognize a specific antigen. If the mean life span is even 1 day, the total body content in the steady state will be 1.8 x 10^5 cells that can recognize a specific antigen. Accordingly, to eliminate the last T-cell in the mature pool that can recognize a specific antigen requires greater than a 5 log kill. The practical problem with alloantigens is apparently much more extreme. Ford and Atkins (1973) have shown that at least 7% of T-cells in their parent to F_1 hybrid system respond to strong alloantigens and are removed

from thoracic duct recirculation by tissues. This would require greater than a 9 log kill for extermination. The problem is further complicated by regeneration of thymic stem cells. With longer mean life spans, the total number increases proportionately and the eradication process is more impractical.

Anatomic studies indicate that T-cells recirculate rapidly from blood through the paracortical areas of the lymph node. A similar migration takes place through the spleen (Ford and Simmonds 1972). In addition, the presence of lymphocytes in afferent lymph proves that there is also a continuous flow of lymphocytes through tissues in general. Whereas it is not possible to estimate the actual concentration of T-cells per g of tissue, the steady migration from blood to lymph through tissues is unquestioned.

Field et al. (1972) estimate that the total exchangeable pool of lymphocytes is about 30 times that of the blood lymphocytes or 45×10^{10} cells. With an average cell volume of 250 μm^3 this would represent 112.5 g of lymphocytes. From the studies of Buchti et al. (1970) it would appear that not more than 10% to 60% of the lymphocytes are easily mobilizable from the diverse lymphoid tissues. Assuming a mean value of 25%, the total lymphoid mass would then represent about 450 g. The earlier minimum production of 0.9 g of lymphocytes/day leads one to suggest a maximum average turnover time of tissue lymphocyte pool to be 125 days. In marked contrast are the observations and estimates of Norman et al. (1965) and Buckton et al. (1967) who cultured human blood lymphocytes at intervals following termination of radiotherapy and observed

the incidence of lethal acentric chromosomal aberrations when the lymphocytes were forced to divide in vitro. They estimated mean life spans of 530 and 1574 days respectively. These estimates if applied to the daily lymphocyte production, would result in a prohibitively large pool of total body lymphocytes. It is possible that radiation influenced surviving small lymphocytes so that they are not susceptible to in vivo mitotic stimuli and are thus detected for long periods in the blood by in vitro culture methods that force them into a mitosis. Consequently this technique may overestimate mean lymphocytic life span.

Virtually nothing is known about factors that regulate proliferation of lymphocytes in the thymus and bone marrow. However, it is reasonable to believe that at least a fraction of cells proliferating in the lymph nodes are responding to antigenic stimuli. The studies of Janett et al. (1966); Wagner et al. (1967); Safier et al. (1967); Vincent et al. (1969) and Sordat et al. (1972) indicate that the DNA synthesis and the generation time of proliferating bovine thoracic duct lymphocytes is 4-5 hours and 5-6 hours respectively. These estimates of generation time indicate that in a 60-hr period, population of stimulated lymphocytes can expand by a factor of 1,000 from 10 serial mitoses. The periodic antigenic stimulation of proliferation is superimposed on a continual non-antigenic proliferation. A commensurate periodic death of cells is required to keep the fluctuating population in bounds thus a large fraction of progeny from antigenic stimulation must be short-lived.

At this point some further speculation may be useful. In the case of erythropoiesis and granulocytopoiesis there are reasonably well defined

humoral feedback loops that regulate production rate and concentration of cells in the peripheral blood, namely erythropoietin and possibly the colony stimulating factor. In the case of lymphocytopoiesis, humoral feedback loops are not so well established. Thymic lymphocytopoietic factors such as thymosin and lymphopoietin have been proposed as candidates for humoral regulation (Klein et al. 1966; Metcalf 1958 and Timson 1969). Antigens on the other hand can trigger cells into proliferation, (Hall and Morris 1962; Hall 1967; and Pedersen and Morris 1970).

Let us assume that the steady state production of cells by the thymus is autonomous and determined only by the delivery of stem cells to the thymus. Limited self replication of stem cells within the thymus would be followed by amplifying mitotic divisions within the thymic stroma. Osoba (1974) reviews the evidence for a continuous flow of stem cells to the thymus from the bone marrow both for steady state production and for regeneration after thymic injury. The thymus lacks the capacity for compensatory hyperplasia after partial thymectomy (Borum 1969). Furthermore after partial irradiation of the thymus the regeneration of the irradiated portion is not accounted for by migration from the adjacent thymic tissue (Engeset and Schooley, 1968). One can postulate that an influx of stem cells from the blood is responsible for renewed mitotic activity and restoration of the thymus. Metcalf (1963) has reported autonomous growth of multiple thymic grafts. Furthermore, studies of Ford et al. (1966) also support the notion that there is a steady flow of stem cells into the thymus. Humoral stimuli for thymic

proliferation appear unlikely because there is no compensatory hyperplasia after surgical removal and no thymic enlargement after peripheral lymphocyte depletion. Therefore, the following working hypothesis is proposed. The number of T-cells produced is the product of the stem cell influx into the thymus, intra-thymic stem cell proliferation and the intra-thymic amplification factor. This leads to the notion that thymic size is a function of the stem cell influx and the amount of thymic stroma available in which thymocytes can proliferate. The studies of Borum (1969) and Metcalf (1963) also point out that the medulla is required for proliferation in the thymic cortex. This may be related to the fact that the point of entrance of cells into the thymus appears to be the corticomedullary junction. The thymic production rate is estimated at 1.8×10^9 /day in man. Sainte-Marie and Leblond (1965) proposed that there are 8 serial mitoses from the stem cell for an amplification of 256. With a 6 hour generation time this takes 48 hours which is consistent with transit of labeled cells through the thymus (Craddock et al. 1964). If the 256 amplification factor applies to man and all stem cells are immigrants the influx of stem cells is $1.8 \times 10^9 \div 256$ or 7×10^6 /day. If there is some self replication of stem cells in the thymus the influx is less.

In the steady state the following are evident:

Birth rate (K_B) = Death rate (K_D)

$$K_B = \frac{Ns}{t_S} = \frac{N_L}{t_L} = K_D$$

where N_S = number in DNA synthesis

t_S = DNA synthesis time

N_L = number in thymus-derived lymphocyte pool

t_L = average life span in pool

If it is assumed that there is an autonomous fixed production rate of lymphocytes some notion about the recovery patterns after depletion can be obtained. If the mature pool is depleted by a method that does not impair the autonomous production of cells K_D would decrease in proportion to the depletion. K_B will be greater and repletion commences and will continue until K_D again equals K_B. The difference between K_B and K_D decreases with time hence the rate of repletion decreases giving a repletion curve that rises rapidly at first and then more slowly. With no feedback loops sensing depletion and accelerating production there will be no overshoot in repletion.

Studies on the Radiation Sensitivity of Lymphocytes In Vivo

The notion that lymphocytes are highly radiosensitive is due to the rapid appearance of advanced morphological damage in lymphoid tissues following irradiation that produces interphase death of the lymphocytes. This is also expressed in a rapid decrease in the size of lymphocytic organs such as the thymus, lymph nodes and spleen after total body irradiation. A commonly used measure of radiation sensitivity is the incorporation of radioactive precursors into DNA. However, this is not a true measurement of radiation sensitivity of proliferative cells because they may retain the capability to undergo one or more divisions or at least to incorporate

the tagged precursors into DNA after injury by radiation. The best techniques for measuring suppression of cell mediated immunity are those which measure the reproductive integrity of the cells of interest -- the B- and T-cells after irradiation. In considering the dose-effect relationship based for example on thymic or splenic weight, one is concerned with dynamic overlapping processes of necrosis and removal of dead cells and regeneration. Regeneration commences after a variable, dose-dependent time. 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 (RBE) of neutrons compared to x-rays (Carter et al. 1954). The killing effect of WBR on thymic and lymph node cells has been studied by measuring the weight and number of cells in the lymph nodes and thymus 24 hours following graded doses of radiation (Sato and Sakka, 1969). At this time interval regeneration is insignificant and hence the quantitative measurements represent "killing doses" on combined interphase and cycling lymphocytes. 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. A commonly used measurement of cell proliferation is the incorporation of ^{3}H-thymidine *in vivo* and *in vitro*. It was shown by Fliedner (1967) that 800 R of x-rays to human lymphocytes *in vitro* does not prevent their response to PHA transformation and incorporation of ^{3}H-thymidine. However,

the stimulated cells, when attempting mitosis, had observable mitotically connected abnormalities which in all probability would have resulted in the death of the daughter cells. Similar observations have been made *in vivo* showing that incorporation of ^{3}H-thymidine measures abortive regeneration at an early stage after irradiation and later true sustained regeneration.

A study perhaps pertinent to cell mediated immunity is that of Whitelaw (1965) who was concerned with the relative radiosensitivity of old lymphocytes *vs*. newly produced lymphocytes. In his studies he gave repeated injections of ^{3}H-thymidine over a 2-week period, a procedure which resulted in labeling 33% of the circulating small lymphocytes. Then animals were exposed to 208 R of whole body irradiation. The proportionate decrease in the ^{3}H-thymidine labeled newly formed lymphocytes and the unlabeled older lymphocytes was equivalent. These studies suggest that newly formed lymphocytes committed to allograft rejection or some other form of cell mediated immunity would be equally responsive to radiation as lymphocytes in general. Miller and Cole (1967) immunized mice and rats by giving primary and secondary antigenic stimuli in the footpads. Commencing 16-96 hrs after the secondary antigenic injections they intermittently injected ^{3}H-thymidine into the same footpads. Thirty-one days after the last dose of ^{3}H-thymidine, mice were divided into 3 groups and given either 850R or 500R WBR with one group serving as non-irradiated controls. Despite the marked atrophy of the popliteal and aortic lymph nodes, the percentage of labeled small lymphocytes was significantly higher in the irradiated than in the control animals. Labeled plasma cells also persisted. These studies concerned with humoral immunity

show that the progeny of immunologically stimulated and proliferating cells are able in part to survive the stated doses of radiation. Presumably lymphocytes concerned with cell mediated immunity would also survive in part.

The essential role of specifically sensitized cells in the rejection of allografts has been shown by numerous investigators (Billingham et al. 1954 and Strober and Gowans 1965, as examples). In addition an integral part of cell mediated immunity and in particular allograft immunity is the proliferation of cells either in lymph nodes draining the graft site (Hall, 1967) or within a renal allograft itself (Pedersen and Morris, 1970). The radiosensitivity of cells producing a graft *vs*. host reaction has been determined by Vos (1967). The number of lymphocytic cells killing 50% of the recipients by GVH reaction was determined. Since the number required increased with dose of radiation a dose-effect curve could be established. The D_{37} for lymphocytic cells, irrespective of anatomic site, irradiated *in vivo* or *in vitro* was about 85 R. The D_{37} of anoxic cells is substantially greater -- of the order of 230 R with an oxygen enhancement ratio of 2.7. Makinodan et al. (1962) and Kennedy et al. (1966) estimated the D_{37} for antibody producing cells to be 70 R and 80 R, respectively, independent of the time of injection of antigen and measurement of proliferation potential. Katz et al. (1970) and Kettman and Dutton (1971) have produced data suggesting that there is a very marked radioresistance (upto 5000 rads) of the "helper" function of T-cells. The results of these studies suggest that the T-cell helper function is not dependent upon proliferative integrity. Anderson et al.

(1972) also investigated the radiosensitivity of T-cells. These investigators injected irradiated parental thoracic duct lymphocytes into F_1 heavily irradiated mice. Doses of radiation from 0 - 300 R progressively decreased the capability of the transfused cells to incorporate ^{3}H-thymidine and above 300 R there was no significant incorporation. The helper function of carrier primed thoracic duct lymphocytes was abolished by exposure to 1000 R *in vitro*. In these systems presumably the type of helper function that is being measured has varying radiosensitivity.

The Effect of Whole Body and Local Irradiation on Lymphocytic Organs and Their Repletion:

The heterogeneous family of lymphocytes described earlier has a defined sensitivity to radiation. Schrek (1947) and Trowell (1952) have shown that doses as small as 15-20 rads can kill a small fraction of lymphocytes directly as indicated by pyknosis and other cytological changes. The D_0 for all proliferating hemopoietic cells is circa 100 rads. Exposure to doses of radiation that increases the acceptance time of allografts or suppresses other types of cell mediated immunity are substantially larger and result in a marked depletion of all lymphocytic organs. In addition, germinal centers in lymph nodes and spleen are severely depleted. The population of small lymphocytes in the bone marrow (the probable source of stem cells for repletion of lymphocytic organs) diminishes within 4-5 days after doses of the order of 300 rads whole body irradiation. The lymphoreticular tissue

is almost devoid of small lymphocytes, germinal centers and proliferating lymphocytes in all areas of the body. The depletion of the lymphoreticular tissues is roughly dependent upon the dose of radiation as measured by weight of thymus and spleen and histologic appearance. Histologic depletion is almost maximal within the lethal dose range. Sequentially, commencing within one hour or even less after exposure to an LD_{50} dose of x-ray, extensive pyknosis, necrosis, and karyorrhexis of lymphocytes in the spleen, lymph nodes, and the thymus is visible. The clearance of this necrotic debris is rapid. By 24 hours the organs are depopulated proportionate to the dose and only traces of nuclear debris can be seen in the phagocytes. The residual injury of the proliferating cells is expressed in two ways. The more seriously injured cells are able to go through a few mitotic divisions and account for a temporary diffuse regeneration that is manifested by cells with abnormal size, bizarre shape, mitotic abnormalities, chromosomal bridges, and fragmentation similar to the injury to cells in tissue culture that results in micro-colonies that cannot sustain continued growth. Later sustained regeneration arises from cells that have not suffered a fatal injury to their genetic apparatus. The time between irradiation and commencement of sustained regeneration is dose-dependent and may not commence for 20-30 days after very high exposures. The dose-effect curves for cell killing and reproductive integrity generally show an initial shoulder followed by an exponential decline in the fraction of surviving cells over a large dose range. The D_0 for reproductive integrity is in the vicinity

of 100 rads. After doses of the order of 1,000 rads, the probability of survival of the common hemopoietic stem cell is between 10^{-3} and 10^{-4}. With a production rate of 1.8×10^{9} T-cells per day and an assumed mean life span of 100 days the total body T-cells in 70 kg man would be 1.8×10^{11} hence 1000 rads would reduce this population to 10^{7} - 10^{8} cells. Presumably the reproductive capability of survivors will return after a dose-dependent time interval. However, an unknown fraction of these cells, when triggered into mitosis in vitro culture will show lethal chromosomal abnormalities. Vos (1967) investigated the Elkind type recovery by split dose irradiation. There was a small degree of recovery within 2 hours of the conditioning irradiation. At 4 hours a further increase was observed. Vos observed no appreciable differences in radiation sensitivity of lymph node and spleen cells. The lymph node cells of preimmunized mice were only a trifle more resistant to radiation than cells from normal mice.

In reviews and text books there are many statements about the prolonged time required for lymphocyte repopulation in the diverse lymphocytic organs following WBR. There is very little in the literature in the way of quantitative measurements of histologic repopulation. Systematic studies were, however, performed by many investigators during the development of the nuclear bomb. These are reviewed by Bloom (1948). It is stated that after a mid-lethal dose of 800 R in the rabbit reconstitution of the spleen is by active mitotic proliferation of the lymphocytes and takes from 10 days to 4 weeks and that at lower doses the regeneration is more rapid. Below 175 R there is only transient debris and very little

destruction of the structural pattern which returns to normal in a very short time. In the lymph nodes and intestinal lymphatic tissue the great majority of the nodules are destroyed resulting in a "nodule-free" period until about 3 weeks after irradiation when new nodules begin to form. The thymus atrophies rapidly exposing a condensed epithelial stroma in the cortex and leaving a few surviving lymphocytes in the medulla. For two to nine days after irradiation the shrinkage continues and the connective tissue becomes prominent. The phase of regeneration commences 10 days after irradiation and continues to completion by 4 weeks with repopulation of lymphocytes proceeding outward from the medulla. This is an interesting observation, since this is the point at which an occasional labeled lymphocyte returns to the thymus (Cronkite and Chanana 1970).

Vos (1967) using induction of GVH reaction studied the long term repopulation of immunocompetent lymphocytic stem cells (antigen sensitive T-cells) in sublethally and lethally irradiated animals. After irradiation cell numbers decreased for 1-2 weeks. Repopulation was very slow and was incomplete 100 days after irradiation.

Sato and Sakka (1969), in studies that lasted only 12 days showed that the degree of depletion of murine thymocytes was a function of the radiation dose becoming maximal at 700 R. Takada et al. (1969) studied regeneration for a longer period. They demonstrated that 300-400 R of WBR to mice resulted in precipitous fall in thymic weight which was followed by an increase in the mitotic index and an almost complete restoration of

thymic mass at 10-15 days. This abortive restoration was followed by a secondary fall and later sustained recovery to normal values 30 or more days after irradiation. Injection of syngeneic bone marrow cells or leg shielding diminished the degree of the secondary decrease. These studies were interpreted as indicating a need for a continuous migration of cells from the bone marrow to the thymus for the maintenance of its cell population. In another series of experiments Takada et al. (1971) studied marrow, spleen and thymus regeneration and concluded that the spleen requires stem cells from bone marrow for recovery from radiation injury and that the recovery is delayed until the irradiated bone marrow can seed the spleen with a sufficient number of stem cells. Blomgren et al. (1970 and 1971) and Decleve et al. (1972) also observed the biphasic thymic regeneration and demonstrated the alleviation of the secondary degeneration by bone marrow transplants. Blomgren observed that thymus and bone marrow repopulation with lymphocytic cells were parallel and cyclic. If animals were transplanted with non-irradiated bone marrow, the recovery was continuous rather than cyclic. In addition, bone marrow stem cells in mice recovering from x-irradiation were found to have a decreased proliferative activity since they produced significantly smaller spleen colonies in lethally irradiated recipients than marrow cells from unirradiated mice. The author interpreted this as indicating that bone marrow lymphocytic cells act as thymic cell precursors and that thymic lymphopoiesis is dependent on the presence of these cells. He also concluded that the need for granulocytic production produces competition for stem cells which results in a cyclic variation in the

production of bone marrow lymphocytic cells.

Order and Waksman (1969) have studied cellular differentiation in the repopulating thymus following irradiation. Their studies show that bone marrow cells migrate to the thymus by four days after irradiation. Following this the total number of thymic cells begins to increase rapidly. Bone marrow-derived cells also appear in lymph nodes and Peyer's patches at 6 days. Cells removed from the repopulated thymus at 6 days and injected into irradiated recipients homed promptly on the irradiated bone marrow in considerable numbers. Of great interest is the fact that the progeny in the marrow were all lymphocytic and that the short-term residence of the bone marrow-derived cells in the thymus apparently prevents them from being able to differentiate down the erythrocytic, granulocytic and megakaryocytic pathways.

Benninghoff et al. (1971) irradiated rats with 300 R and then measured the size of the mobilizable lymphocyte pool by draining the thoracic duct lymph. They concluded that the long-lived lymphocyte pool was in the process of recovery by one month after WBR and took 2-3 months for complete recovery.

Volkman and Collins (1968) studied the recovery of delayed hypersensitivity in sensitized mice which were irradiated with 400 R WBR. For 8 days after irradiation delayed hypersensitivity reaction was almost totally suppressed. Following this, restoration commenced but was not complete at 10 days after exposure.

Regeneration after Localized Irradiation:

Engeset and Schooley (1968) observed that the partially irradiated

thymus apparently regenerates without migration of cells from the adjacent non-irradiated thymus. This implies, but does not prove, that thymic regeneration is initiated by migration of cells into the thymus. However, in concert with other studies on thymic regeneration after whole body irradiation with and without bone marrow transplantation one reaches the nearly inescapable conclusion that thymic integrity is maintained by a continuous influx of stem cells from the bone marrow.

The question of repopulation of the locally irradiated lymph nodes has been investigated by Hall and Morris (1964) who have shown that local irradiation of the lymph node only temporarily suppresses output of cells in the efferent lymph. Subsequently, Benninghoff et al. (1969) have shown that the locally irradiated lymph node is very rapidly repopulated following 300 rads. There is severe and prolonged depletion of the same lymph nodes after the same dose of WBR, showing that there is a rapid repletion of the lymph node by the population of long-lived recirculating lymphocytes that had not been irradiated.

Application of Whole-Body Irradiation in Preparing Patients for Kidney Transplantation:

Murray et al. (1960) expressed the issue clearly: "The original requirements for adaptation of the experimental design of 'irradiation, marrow, and homograft' to man appeared to be a heavy dose of x-irradiation to the entire host to destroy its immune mechanism, a source of hematopoietic cells capable of self-reproduction and of subsequent graft from

the marrow donor. These three requirements are difficult to meet in man. The dose of x-ray needed to destroy all the host's immune system is not yet determined. Because of limitations on the amount of marrow which can be obtained from a living patient at one time, the same living donor cannot give the marrow and then donate a kidney within the allotted time interval of 72 hours following irradiation." The results with WBR throughout the world were so discouraging that by 1965 almost all clinical investigators ceased the use of the whole-body irradiation to prepare patients for kidney allografts. This result is not surprising. As discussed earlier 1,000 rad WBR will only decrease the proliferative potential of lymphopoietic cells by 10^{-3} to 10^{-4} leaving 10^{7} or more cells of which perhaps 5-10% are able to engage in allograft reactions and proliferation. In addition, the residual surviving pool of lymphocytic cells is slowly repleted with time.

The extension of allograft acceptance following WBR is related to the decreased number of cells available, the delay in being able to resume proliferation and reduced capacity to sustain proliferation. In view of the huge size of the pool of immunocompetent cells, its capability of slow but sustained regeneration and the large fraction of cells capable of detecting and reacting against alloantigens one wonders why WBR has had any measurable effect. One is led to believe that it must be related to the long term effect of radiation on surviving cells which impairs their reproductive efficiency thus reducing the intensity of the allograft reaction.

Local Irradiation of the Graft: The use of this modality has been reviewed by Cronkite and Chanana (1968). Some investigators used irradiation of the graft to change its antigenicity. This was doomed to failure since it was subsequently shown that histocompatibility antigens are resistant to as much as 13,000 R *in vitro*. Local irradiation was also used to suppress the adjacent lymphatic tissue. This also was unsatisfactory since there is rapid repopulation by lymphocytes from elsewhere in the body as described earlier. Local irradiation of the renal homograft could by killing passenger lymphocytes result in some suppression of the afferent arc of the immune response (Kauffman et al. 1966). In view of the observations of Pedersen and Morris (1970) in which it was shown that the entire cellular process of allograft rejection can take place in the renal homograft, one might expect local irradiation of the kidney to be of some value in control of rejection episodes or postponing the time of rejection. However, this would have a limited effectiveness since the kidney will not tolerate more than approximately 3,000 R before developing radiation nephritis. The usefulness of local irradiation of the kidney has apparently been demonstrated in reversal of acute rejection by Fidler et al. (1973) who found that rejection was completely reversed by several external radiation doses of 150 R for a total of 300-900 R.

Influence of Extracorporeal Irradiation of the Blood and Lymph on Lymphocytic Populations and Their Restoration:

The history of extracorporeal irradiation of the blood (ECIB) and extracorporeal irradiation of the lymph (ECIL) was reviewed by Cronkite

et al. (1964) and Cronkite (1968). The induction of a lymphopenia by prolonged ECIB was established by Cronkite et al. (1962). The effectiveness of ECIB on the depletion of the lymphoreticular organs of the calf was reported by Cottier et al (1964) and elaborated upon by Ruchti et al. (1970). The favorable effect of ECIB before and after skin allografts has been reported by Cronkite et al. (1965) and Chanana et al. (1966, 1969a). The influence of ECIL on skin allografts was described by Joel et al. (1967) and Chanana et al (1969b). The influence of ECIB before and after renal allograft has been reported by Chanana et al. (1971b). Calculation of radiation dose was presented by Slatkin et al. (1963).

The underlying principle of ECIB is to utilize the difference in susceptibility to injury by irradiation between the radiosensitive circulating lymphocyte and the radioresistance of the other formed elements in the blood. Thus, radiation kills the radiosensitive cells which are then removed from the circulation by the lymphoreticular system and does little harm to the other cells. ECIB is accomplished by diverting a fraction of the cardiac output through irradiation fields by means of semipermanent artery-to-vein shunts composed of Teflon and Silastic. The amount of radiation received by a cell during one circuit through the radiation source is referred to as a transit dose and can experimentally be varied from a fraction of a rad to several thousands. Cells remaining in the circulating blood may pass through the irradiator one or more times depending upon their probability of remaining in the circulation, the shunt volume, flow rate and blood volume. ECIB given continuously or in short repetitive

sessions (Sipe et al. 1965) has been shown to produce a lymphopenia, the degree of which is a function of the duration of ECIB, the transit dose, the number of blood volumes passed through the irradiator per treatment and the rate of exchange of tissue lymphocytes with the blood lymphocytes. Depending upon the preceding, the recirculating pool of lymphocytes can be drastically depleted as manifested by a low blood lymphocyte count and a markedly decreased output of cells in the thoracic duct. In quantitative histologic studies performed after 3-50 hours of continuous ECIB, it was shown that the degree of depletion with time of lymphoreticular tissues followed an exponential function with two components. The first component corresponded to a relatively rapid fall and the second to a slow reduction in lymphocyte content. The former is related to the elimination of an easily mobilizable pool of lymphocytes while the latter corresponds to a more sessile mass of lymphocytes which exchange with blood lymphocytes very slowly. Effective elimination of the easily mobilizable pool of lymphocytes by ECIB from all tissues studied was observed within 10-15 hours, indicating that the rate of change with blood is similar for this group of cells in various lymphoreticular tissues. The size, however, of the easily mobilizable vs. the more sessile pool of lymphocytes may vary considerably, the best estimate for the former being less than 10% in the lymph node medulla, 18% in the lymph node cortex and paracortical zone, 37% in the red pulp of the spleen, 55% in the densely populated white pulp of the spleen and 60% in the loosely populated

white pulp of the spleen. Prolonged ECIL results in marked lymphocytopenia and a reduction of lymphocytes in tissues which has not been studied quantitatively.

The size distribution of lymphocytes in the thoracic duct has been studied. The small recirculating lymphocyte is depleted to the greatest degree. However, larger cells are also reduced. Continuous ECIL results in a two-component decrease in the output of thoracic duct lymphocytes. The first component has a half time of about 1.2 days, corresponding to the elimination of the easily mobilizable pool of lymphocytes and the second component with a half time of approximately 30 days presumably corresponds to the more sessile mass of lymphocytes described above. Calculations based on these data, indicate the size of the easily mobilizable pool to be approximately 10 times the number of circulating blood lymphocytes, or 3-5 x 10^9 lymphocytes/kg body weight in the calf.

Recovery from lymphocyte depletion induced by ECIB or ECIL is very prolonged indeed. Depending upon the degree of depletion, blood lymphocyte counts may remain below the pre-irradiation levels for 6 months or more. This is mainly due to a lag in recovery of small lymphocytes. Large lymphocyte levels return to normal in approximately 3 weeks.

These studies indicate that ECIB and ECIL are effective in depleting the body of an easily mobilizable pool of lymphocytes (the recirculating pool). This pool is composed principally of

small, non-dividing, long-lived lymphocytes which in part at least, are thymic in origin. In the calf, studies on the emigration of thymic cells with a specific surface antigen or radioactively labeled cells Chanana et al (1971a) indicate that a sufficient number of lymphocytes leave the thymus to replace the easily mobilizable pool in less than 5 days. Accordingly, a large fraction of thymic migrants in calves do not enter the long-lived recirculating pool.

In view of the preceding one would predict that lymphocyte depletion by ECIB and ECIL might suppress immune response and may prolong allograft acceptance. Repetitive ECIB given prior to skin grafting prolonged the graft acceptance time by 2-3 days and changed the normal violent skin allograft rejection to a chronic milder reaction. With repetitive ECIB continued after skin allografting the acceptance time was further prolonged. When ECIB was combined with small doses of Azothioprine, which in itself had no effect on rejection, the acceptance was prolonged even longer. This suggests that ECIB and immunosuppressive therapy may be synergistic. Thymectomy combined with ECIB was no more effective than ECIB alone.

Continuous ECIL resulted in a longer skin allograft acceptance. Of particular interest is the fact that the effect of ECIL was dependent upon the anatomical location of the graft. When the grafts were placed in the drainage bed of the thoracic duct (posterior grafts), the grafts were retained in general for the duration of ECIL, while skin grafts on other parts of the body (anterior grafts) were rejected provided that: (1) lymphatic-venous communications other than the

thoracic duct are absent; and (2) anterior and posterior grafts are from two unrelated donors. These experiments suggest that viable cells emerging from regional lymph nodes are essential for skin allograft rejection and these cells can be killed by irradiation of thoracic duct lymph. In addition, the entry of lymphocytes committed to allograft rejection into the blood appears to be exclusively through the efferent lymphatics. If the cells could enter directly into the blood stream they would escape irradiation and the posterior skin grafts would be rejected at the same time as the anterior grafts.

ECIB has been used to prepare human beings for renal transplants (Weeke 1973 a and b). The survival of renal grafts was prolonged only slightly but the rejection episodes were reduced and hence the quality of life for the patients improved. It is of interest that after ECIB lymphocytes retain unimpaired reactivity to PHA but their response to antigens and allogeneic cells is suppressed for months after ECIB. (Andersen et al. 1970, and Weeke 1972).

Whole body irradiation exposes all body cells and would be expected to kill a larger fraction of the lymphocytic pool of cells than ECIB. Accordingly, one asks the question of why ECIB has any effect on cell mediated immunity. The answer is not at all clear. It is conveivable that a large fraction of the surviving lymphocytic pool of cells have received a non-fatal injury. These T-cell survivors may be able to recognize antigen but cannot indulge in sustained, vigorous proliferation of clones of cells directed against the alloantigens thus reducing the intensity of the allograft rejection.

Effect of Whole-Body Irradiation on Growth of Cells in Diffusion Chambers Implanted in the Peritoneal Cavity:

Another series of studies have shown that bone marrow and peripheral blood cells grow more rapidly in diffusion chambers implanted into the peritoneal cavity of irradiated animals than when implanted into the normal animals (Boyum et al. 1970, Cronkite et al. 1974, Laissue et al. 1974). The stimulation is directed at pluripotent stem cells (CFU_s of mice); granulocytic, erythrocytic and megakaryocytic series; and the lymphocytic and plasmacytic series particularly when the implanted cells are from the peripheral blood. The enhancement of growth in irradiated recipients may have been related to the suppression of host immune responses in isogeneic and xenogeneic culture systems; alternatively, growth enhancement in autologous irradiated recipients is better understood if specific and/or nonspecific humoral factors are assumed to diffuse into the culture chamber containing normal hematopoietic cells. In fact, serum of such animals obtained at a time when growth in the in vivo culture proceeded at a faster rate than in non-irradiated recipients has been shown to stimulate granulocytopoiesis in-vitro (Laissue et al. 1974). One can perhaps explain erythropoietic, granulopoietic and megakaryocytic proliferation on the basis of specific "poietins" for cell lines concerned acting at the committed stem cell. The apparent humoral stimulus of the pluripotent stem cell to replicate in the diffusion chamber is of great basic interest and potential practical application. One can hypothesize that after WBR the surviving stem cells are receiving a strong humoral stimulation to proliferate and

thus replete all hemopoietic pools in a hierarchical progression most compatible with survival of the animal. After ECIB there may not be depletion at the common stem cell level or a common humoral stimulation to the stem cell and consequently little to accelerate repletion. In addition the remaining pool of lymphocytic cells, not subjected to a lethal dose of irradiation, may have an impaired proliferative response to antigenic stimulation and hence is not taken out of circulation as rapidly. This line of reasoning suggests that there is a feedback loop from the peripheral pool to formative organs, but other lines of logic discussed earlier leads to a probable autonomous proliferation at a set rate. The resolution of these questions is mandatory before further progress can be made in attaining a more effective control of suppression or stimulation of immunity with fewer harmful effects.

SUMMARY

The effects of whole-body, local and extracorporeal irradiation on lymphocytic populations, cell-mediated immunity, allograft rejection and hematopoietic cell proliferation are reviewed. Emphasis in this review is placed on (1) the heterogeneity of lymphocytes with respect to origin, function, migrational patterns, anatomic distribution and life span and (2) on the relatively large production rate for thymic and extrathymic lymphocytes. The conclusion is reached that eradication by irradiation of any functional subpopulation of lymphocytes is a practical impossibility. At least a 5 log kill would be necessary to eliminate specific antigen recognition and even a greater log kill

would be required in case of allograft recognition. It is pointed out that when measuring irradiation suppression of a cell population it is vitally important that the methods employed establish the reproductive integrity of the cells of interest. Incorporation of DNA precursors as an indication of survival may be misleading.

Recovery of lymphoid organs following irradiation is time-dose dependent. Of particular importance, however, is the 'degree' of total body exposure. Locally irradiated lymphoid tissues recover rapidly. Bone marrow shielding markedly enhances recovery due apparently to migration into the irradiated tissues of lymphocytic stem cells.

Total body irradiation as a immunosuppressant prior to kidney allografting in man is not practical. Irradiation of kidney allografts may however be helpful in preventing rejection crises since the entire process of detection, proliferation and attack by lymphoid cells can all occur within the graft.

Extracorporeal irradiation of blood (ECIB) and/or thoracic duct lymph (ECIL) is effective in depleting the body of the easily mobilizable pool of lymphocytes. There remains, however, a relatively large pool of sessile lymphocytes which varies in size in different peripheral lymphoid tissues. ECIB has been shown to be moderatively effective in prolonging skin and kidney allografts in animals. ECIL is more effective in prolonging skin allograft rejection particularly if the skin grafts are placed within the drainage bed of the thoracic duct.

REFERENCES

1. Andersen, V., Bjerrum, O., Ranek, L. Studies on lymphocytes during extracorporeal irradiation of the blood in patients with active cirrhosis. Scand. J. Haemat. 7, 471, 1970.
2. Anderson, R. E., Sprent, J. and Miller, J.F.A.P. Cell to cell interaction in the immune response VIII. Radiosensitivity of thymus-derived lymphocytes. J. Exp. Med. 135, 711, 1972.
3. Benjamin, E. and Sluka, E. Antikörperbildung nach experimenteller Schädigung des hämatopoetischen Systems durch Roentgenstrahlen. Wien Klin. Wschr. 21, 311, 1908.
4. Benninghoff, D. L., Tyler, R. W. and Everett, N. B. Repopulation of irradiated lymph nodes by recirculating lymphocytes. Radiation Res. 37, 381, 1969.
5. Benninghoff, D. L., Girardet, R. and Stackhouse, L. Radiation recovery of long-lived lymphocytes in the rat. Radiation Res. 48, 589, 1971.
6. Billingham, R. E., Brent, L. and Medawar, P. B. Quantitative studies on tissue transplantation II. The origin, strength and duration of actively and adaptively acquired immunity. Proc. Royal Soc. Series B, Biological Sciences 143, 58, 1954.
7. Blomgren, H. Changes of the "radioresistant" thymic lymphocyte population following x-irradiation of mice. Exp. Cell Res. 61, 271, 1970.
8. Blomgren, H. The role of bone marrow of x-irradiated mice in thymic recovery. Cell Tissue Kinet. 4, 443, 1971.

9. Bloom, W. Histopathology of Irradiation from External and Internal Sources. New York: McGraw-Hill Book Co., Inc., 1948.

10. Borum, K. Lack of restoration of thymic tissue following partial surgical thymectomy in the mouse. Acta Path. Microbiol. Scand. 76, 515, 1969.

11. Boyum, A. and Borgstrom, R. The concentration of granulocytic stem cells in mouse bone marrow determined with diffusion chamber technique. Scand. J. Haemat. 7: 294, 1970.

12. Buckton, K. E., Court-Brown, W. M. and Smith, P. G. Lymphocyte survival in men treated with x-rays for ankylosing spondylitis. Nature 214, 470, 1967.

13. Carter, R. E., Cronkite, E. P. and Bond, V. P. The effect of neutrons on thymic and circulating lymphocytes in the mouse. The Amer. Naturalist 87, 257, 1954.

14. Chanana, A. D., Brecher, G., Cronkite, E. P., Joel, D. D. and Schnappauf, H. The influence of extracorporeal irradiation of the blood and lymph upon skin homograft rejection. Radiation Res. 27, 330, 1966.

15. Chanana, A. D., Cronkite, E. P., Joel, D. D. and Schiffer, L. M. Skin allograft survival in calves. Influence of thymectomy and extracorporeal irradiation of blood. Arch. Surg. 98, 803, 1969a.

16. Chanana, A. D., Cronkite, E. P., Joel, D. D., Schiffer, L. M. and Schnappauf, H. Studies on lymphocytes XII. The role of immunologically committed lymphocytes in rejecting skin allografts. Transplantation 7, 459, 1969b.

17. Chanana, A. D., Cronkite, E. P., Joel, D. D., William, R. M. and Waksman, B. H. Migration of thymic lymphocytes: Immunofluorescence and ^{3}HTdR labeling studies. In Morphological and Functional Aspects of Immunity (Eds. Lindahl-Kiessling, K., Alm, G. and Hanna, Jr., M. G.) Plenum Press, New York, pp. 113-118, 1971a.

18. Chanana, A. D., Cronkite, E. P., Joel, D. D. and Stevens, J. B. Prolonged renal allograft survival: Extracorporeal irradiation of blood. Transplantation Proc. 3, 838, 1971b.

19. Chanana, A. D., Schaedeli, J., Hess, M. W. and Cottier, H. Variations in the expression of θ C3H alloantigen on thymic and peripheral lymphocytes of newborn and young adult mice. Cell. Immunol. (in press) 1974.

20. Cottier, H., Cronkite, E. P., Jansen, C. R., Rai, K. R. and Singer, S. Studies on lymphocytes III. Effects of extracorporeal irradiation of the circulating blood upon the lymphoreticular organs of the calf. Blood 24, 241, 1964.

21. Craddock, C. G., Nokai, G. S., Fukuta, H., and Vanslager, L. M.: Proliferative activity of lymphatic tissue cells of rats as studied with tritium-labeled thymidine. J. Exp. Med. 120, 389, 1964.

22. Cronkite, E. P., Jansen, C. R., Mather, G. C., Nielsen, N., Usenik, E. A., Adamik, E. R. and Sipe, C. R. Studies on lymphocytes I. Lymphopenia produced by prolonged extracorporeal irradiation of the circulating of the blood. Blood 20, 203, 1962.

23. Cronkite, E. P., Jansen, C. R., Cottier, H., Rai, K. R. and Sipe, C. R. Lymphocyte production measured by extracorporeal irradiation, cannulation and labeling technique. Ann. N.Y. Acad. Sci. 113, 566, 1964.

24. Cronkite, E. P., Chanana, A. D. and Schnappauf, H. Extracorporeal irradiation of blood and lymph in animals: Its effect on homografts and on lymphoma. New Eng. J. Med. 272, 456, 1965.

25. Cronkite, E. P. Déplétion lymphocytaire par irradiation extra-corporelle du sang et de la lymphe. Nouvelle Revue Francaise d' Hématologie, 8, 643, 1968.

26. Cronkite, E. P. and Chanana, A. D. Ionizing radiation. In "Human Transplantation", Felix Rapaport and Jean Dausset (eds.), Grune and Stratton (pub.) pp. 423-39, 1968.

27. Cronkite, E. P. and Chanana, A. D. Lymphocytopoiesis. In "Formation and Destruction of Blood Cells", T. J. Greenwalt and G. A. Jamieson (eds.), J. B. Lippincott Co. (pub.) pp. 284-303, 1970.

28. Cronkite, E. P., Boecker, W., Carsten, A. L., Chikkappa, G., Joel, D., Laissue, J. and Öhl, S. The use of diffusion chamber cultures in the study of normal and leukemic cell proliferation in man. In "Hemopoiesis in Culture", W. A. Robinson (ed.) p. 185-198 DHEW (NIH) 74-205 Government Printing Office, Washington, D.C. 1974.

29. Decleve, A., Gerber, G. V., Leonard, A., Lambiet-Collier, M., Sassen, A. and Maisin, J. R. Regeneration of thymus, spleen and bone marrow in x-irradiated AKR mice. Radiation Res. 51, 318, 1972.

30. Dempster, W. J., Lennox, B. and Boag, J. W. Prolongation of survival of skin homotransplants in the rabbit by irradiation of the host. Brit. J. Exp. Pathol. 31, 670, 1950.

31. Engeset, A. and Schooley, J. C. Morphological changes following irradiation of a segment of the rat thymus. Proc. Soc. Exp. Biol. Med. 128, 26, 1968.

32. Everett, N. B., Caffrey, R. W. and Rieke, W. O. Radioautographic studies of the effect of irradiation on the long-lived lymphocyte of the rat. Radiation Res. 21, 383, 1964.

33. Fidler, J. P., Alexander, J. W., Smith, E. J., Miller, H. C. and Mutiah, V. Radiation reversal of acute rejection in patients with life-threatening infections. Arch. Surg. 107, 256, 1973.

34. Field, E. O., Sharp, H. B. A., Dawson, K. B., Andersen, V., Killmann, S. A., and Weeke, E. Turnover rate of normal blood lymphocytes in exchangeable pool size in man, calculated from analysis of chromosomal aberrations sustained during extracorporeal irradiation of the blood. Blood 39, 39, 1972.

35. Fliedner, T. M. Experimental studies on PHA-stimulated lymphocytes in autotransfusion of tritiated cytidine labeled lymphocytes in chronic leukemia. In "The Lymphocyte in Immunology and Hemopoiesis", J. M. Yoffey (ed.), pp. 198-206, Edward Arnold, Ltd. London, 1967.

36. Ford, C. E., Micklem, H. S., Evans, E. P., Gray, J. G., and Ogden, D. A. The inflow of bone marrow cells to the thymus: Studies with part-body irradiated mice injected with chromosome-marked bone marrow and subjected to antigenic stimulation. Ann. N.Y. Acad. Sci. 129, 283, 1966.

37. Ford, W. L. and Atkins, R. C. The proportion of lymphocytes capable of recognizing strong transplantation antigens in vivo. In "Micro-environmental Aspects of Immunity", Branislav D. Jankovic and Katarina Isakovic (eds.), Plenum Publishing Corp., New York 1973.

38. Ford, W. L. and Simmonds, S. J. The tempo of lymphocyte recirculation from blood to lymph in the rat. Cell Tissue Kinet. 5, 175, 1972.

39. Hall, J. G. and Morris, B. The output of cells in lymph from the popliteal node of sheep. Quart. J. Exp. Physiol. 47, 360-369, 1962.

40. Hall, J. G. and Morris, B. Effect of x-irradiation of the popliteal lymph node on its output of lymphocytes and immunological responsiveness. Lancet, 1, 1077-1080, 1964.

41. Hall, J. G. Studies of the cells of the afferent and efferent lymph nodes draining the site of skin homografts. J. Exp. Med. 125, 737-754, 1967.

42. Hammar, J. A. Der Menschenthymus in Gesundheit und Krankheit. Das normale Organ. Zschr. mikroskop. - anat. Forsch. 6, suppl., 1926.

43. Hektoen, L. The influence of x-rays on the production of antibodies. J. Infect. Dis. 17, 415, 1915.

44. Iorio, R. J., Chanana, A. D., Cronkite, E. P., and Joel, D. D. Studies on lymphocytes XVI: Distribution of bovine thymic lymphocytes in the spleen and lymph nodes. Cell and Tissue Kinet., 3, 161, 1970.

45. Janett, A., Wagner, H. P., Jansen, C. R., Cottier, H. and Cronkite, E. P. Studies on lymphocytopoiesis IV. Comparison of two approaches for the determination of the generation time in thoracic duct cells without detectible cytoplasmic differentiation. Europ. J. Cancer, 2, 231-236, 1966.

46. Joel, D. D., Chanana, A. D., Cronkite, E. P. and Schiffer, L. M. Modification of skin allograft immunity by extracorporeal irradiation of lymph. Transplantation 5, 1192, 1967.

47. Joel, D. D., Hess, M. W. and Cottier, H. Magnitude and pattern of thymic lymphocyte migration in neonatal mice. J. Exp. Med. 135, 907, 1972.

48. Joel, D. D., Chanana, A. D. and Cronkite, E. P. Thymus cell migration. To be published in Series Hematol. 1974.

49. Katz, D. H., Paul, W. E., Goidl, E. A. and Benacerraf, B. Radioresistance of cooperative function of carrier specific lymphocytes in anti-hapten antibody responses. Science 170, 462-464, 1970.

50. Kauffman, Jr., H. M., Cleveland, R. J., Robertshaw, G. E., Graham, W. H. and Hume, D. M. Inhibition of the afferent arc of the immune response to renal homografts by local graft irradiation. Surg. Gynae. and Obstetrics, 123, 1052-1056, 1966.

51. Kennedy, J. C., Siminovitch, L., Till, J. E. and McCulloch, E. A. A transplantation assay for mouse cells responsive to antigenic stimulation by sheep erythrocytes. Proc. Soc. Exp. Biol. Med. 120, 868, 1966.

52. Kettmann, I. and Dutton, I. W. Radioresistance of enhancing effect of cells from carrier-immunized mice in an in vitro primary response. Proc. Acad. Sci. USA, 68, 699, 1971.

53. Klein, J. J., Goldstein, A. L. and White, A. Effects of the thymus lymphocytopoietic factor. Ann. N.Y. Acad. Sci. 135, 485, 1966.

54. Laissue, J., Chanana, A. D., Cronkite, E. P., Joel, D. D., and Pavelec, M. Culture of autologous bone marrow (BM) in diffusion chambers: Effect of host irradiation. Fed. Proc. 33, 598, 1974.

55. Makinodan, T., Kastenbaum, M. A. and Peterson, W. J. Radiosensitivity of spleen cells from normal and preimmunized mice and its significance to intact animals. J. Immunol. 88, 31, 1962.

56. Metcalf, D. The thymic lymphocytosis-stimulating factor. Ann. N.Y. Acad. Sci. 73, 113-119, 1958.

57. Metcalf, D. The autonomous behaviour of normal thymus grafts. Aust. J. Exp. Biol. Med. Sci. 41, 437, 1963.

58. Miller, J. and Cole, L. J. The radiation resistance of long-lived lymphocytesand plasma cells in mouse and rat lymph nodes. J. Immunol. 98, 982-990, 1967.

59. Murphy, J. B. and Taylor, H. D. The lymphocyte in natural and induced resistance to transplants. III. The effect of x-rays on artificially induced immunity. J. Exp. Med. 28, 1, 1918.

60. Murray, J. E., Merril, J. P., Dammin, G. J., Dealy, Jr., J. B., Walter, C. W., Brooke, M. S., and Wilson, R. E. Study on transplantation immunity after total body irradiation: Clinical and experimental investigation. Surgery, 48, 272-284, 1960.

61. Norman, A., Sasaki, M. S., Ottoman, R. E. and Fingerhut, A. G. Lymphocyte life time in women. Science, 147, 745, 1965.

62. Order, S. E. and Waksman, B. H. Cellular differentiation in the thymus. Transplantation 8, No. 6, 783-800, 1969.

63. Osoba, D. Precursors of thymus lymphocytes. To be published in Series Hematol. 1974.

64. Parrott, D. M. V. and de Sousa, M. Thymus-dependent and thymus-independent populations: Origin, migratory patterns and lifespan. Clin. Exp. Immunol. 8, No. 5, 663-684, 1971.

65. Pedersen, N. C. and Morris, B. The role of lymphatic system in the rejection of homografts: The study of lymph from renal transplants. J. Exp. Med. 131, 936, 1970.

66. Robinson, S. H., Brecher, G., Lourie, I. S. and Haley, J. E. Leukocyte labeling in rats during and after continuous infusion of tritiated thymidine: Implications for lymphocyte longevity and DNA reutilization. Blood, 26, No. 3, 281-295, 1965.

67. Ruchti, C., Cottier, H., Cronkite, E. P., Jansen, C. R., and Rai, K. R. Studies on lymphocytes XVII. Differential lymphocyte depletion in lymphoreticular organs of the calf during continuous extracorporeal irradiation of the blood. Cell Tissue Kinet. 3, 301-315, 1970.

68. Safier, S., Cottier, H., Cronkite, E. P., Jansen, C. R., Rai, K. R. and Wagner, H. P. Studies on lymphocytes VI. Evidence showing different generation times for cytologically different cells in the thoracic duct of the calf. Blood 30, 301-310, 1967.

69. Sainte-Marie, G. and Leblond, C. P. Elaboration of a model for the formation of lymphocytes in the thymic cortex of young adult rats. Blood 26, 765-783, 1965.

70. Sato, C. and Sakka, M. Cell population analysis of radiation damage and recovery of thymus and lymph nodes in mice. Radiation Res. 38, 204-213, 1969.

71. Schrek, R. Darkfield observations on lymphocytes exposed to x-rays and other injurious agents. Proc. Soc. Exp. Biol. Med. 64, 381, 1947.

72. Sipe, C. R., Chanana, A. D., Joel, D. D., Cronkite, E. P. and Schnappauf, H. The influence of varying dose and repetitive short sessions of extracorporeal irradition of the blood on the production of lymphopenia. Radiation Res. 25, 684, 1965.

73. Slatkin, D. N., Jansen, C. R., Cronkite, E. P. and Robertson, J. S. Extracorporeal irradiation of blood: Calculations of the radiation dose. Radiation Res. 19, 409, 1963.

74. Sordat, M., Sordat, H., Cottier, H., Hess, M. W., Riedwyl, H., Chanana, A. D., and Cronkite, E. P. Studies in lymphocytes XV. Analysis of the in vivo division cycle of large lymphoid cells in calf thoracic duct using combined microspectrophotometry and autoradiography. Exp. Cell Res. 70, 146-153, 1972.

75. Strober, S. and Gowans, J. L. The role of lymphocytes in the sensitization of rats to renal homografts. J. Exp. Med. 122, 347, 1965.

76. Takada, A., Takada, Y., Huang, C. C. and Ambrus, J. L. Biphasic pattern of thymus regeneration after whole-body irradiation. J. Exp. Med. 129, 445-457, 1969.

77. Takada, A., Takada, Y., Kim, U. and Ambrus, J. Bone marrow, spleen, and thymus regeneration patterns in mice after whole-body irradiation. Radiation Res. 45, 522-535, 1971.

78. Timson, J. Lymphopoietin. Acta haemat. 42, 23, 1969.

79. Trowell, O. A. The sensitivity of lymphocytes to ionizing radiation. J Path. Bact. LVIV, 687-704, 1952.

80. Vincent, P. C., Borner, G., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., Joel, D. D., Schiffer, L. M. and Stryckmans, P. A. Studies on lymphocytes XIV. Measurement of DNA synthesis time in bovine thoracic duct lymphocytes by analysis of labeled mitoses and by double labeling before and after extracorporeal irradiation of the lymph. Cell Tissue Kinet. 2, 235-246, 1969.

81. Volkman, A. and Collins, F. M. Recovery of delayed-type hypersensitivity in mice following suppressive doses of x-radiation. J. Immunol. 101, No. 5, 846-859, 1968.

82. Vos, O. Radiation sensitivity in post-irradiation repair of mouse lymphatic cells *in vivo* and *in vitro*. A study with a parent to F_1 hybrid graft--vs--host reaction. Int. J. Rad. Biol. 13, 317-333, 1967.

83. Wagner, H. P., Cottier, H., Cronkite, E. P., Cunningham, L., Jansen, C. R. and Rai, K. R. Studies on lymphocytes V. Short *in vivo* DNA synthesis and generation time of lymphoid cells in the calf thoracic duct after simulated or effective extracorporeal irradiation of circulating blood. Exp. Cell Res. 46, 441-451, 1967.

84. Weeke, E. Lymphocyte transformation test before, during and after extracorporeal irradiation of the blood. Acta. Med. Scand. 192, 271, 1972.

85. Weeke, E. The effect of extracorporeal irradiation of the blood in necro kidney transplantation. Three years follow-up study. Acta. Med. Scand. 1973a (in press).

86. Weeke, E. Extracorporeal irradiation of the blood. Further investigation on the effect of varying transit dose, blood flow rate and frequency of treatment on the development of lymphopenia in uremic patients. Acta Med. Scand. 1973b (in press).

87. Whitelaw, D. M. The effect of total body irradiation on the labeling of circulating mouse lymphocytes with tritiated thymidine. Blood 25, 749-758, 1965.

88. Yoffey, J. M. and Courtice, C. F. Lymphocytes, Lymph and Lymphoid Tissue, Edward Arnold, London, 1956.