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To the Graduate Council:

I am submitting herewith a dissertation written by John B. Mailhes, entitled "Chromosome Aberration Persistence and In Vivo - In Vitro Dose Responses in Pig Leukocytes Following Exposure to Fission Neutrons." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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CHROMOSOME ABERRATION PERSISTENCE AND IN VIVO - IN VITRO DOSE RESPONSES IN PIG LEUKOCYTES FOLLOWING EXPOSURE TO FISSION NEUTRONS

A Dissertation

Presented to

the Graduate Council of

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In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

John B. Mailhes, Jr.

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ABSTRACT

The rate of induction of chromosome aberrations in pig lymphocytes following 100, 200, 300 and 400 rads of fission neutrons administered in vivo and in vitro was determined. The data from each exposure method were tested for goodness of fit of linear and quadratic models. Numbers of acentric fragments, numbers of rings plus numbers of dicentrics, and the sum of the frequencies of aberration types were well explained by a linear model of the form $Y = a + bD + e$, where D = dose, a = Y-intercept, b = slope and e = random deviation from the least-squares fitted regression.

A statistical comparison between exposure methods, using doses measured in air, indicated a significantly ($P < .01$) higher aberration index was found following an in vitro exposure of the animal's blood than was produced by exposing the entire animal. Equations were developed which permitted the prediction of in vivo damage from the in vitro aberration coefficient.

Dose attenuation and differences in the relative contribution of the neutron and gamma components reaching the target cells were recognized as factors influencing the biologically effective dose received from the two exposure methods. The average body dose was calculated from measurements made across the width of a tissue-equivalent phantom. From these calculations, it was determined that approximately 56% of the dose measured in air at the animal's midline was actually received by the blood cells in vivo. When the in vitro aberration data were corrected to the average body dose, aberrations were still present in greater numbers following in vitro than in vivo irradiation. The possibility that in vivo

mechanisms exist for the preferential removal of damaged lymphocytes was proposed as a plausible explanation for this observation.

When the neutron-to-gamma ratios for the two exposure conditions and the relative biological effectiveness of the neutron component were considered, the level of acentric fragments was comparable between exposure methods. Rings plus dicentrics were, however, still more abundant following in vitro exposures, suggesting that an inherent difference exists between in vivo and in vitro irradiation in rate of induction and repair or that heavily damaged cells are more rapidly removed from the peripheral system. These data cast some doubt also on the validity of assuming that comparable chromosomal damage will be found following in vivo and in vitro irradiation.

By sampling peripheral blood at various postexposure intervals, the response of the aberration level as a function of time was studied. A rapid decrease in the proportion of cells containing aberrations was noted as early as 24 hours postexposure. This initial loss coincided with a decrease in lymphocyte number and continued until the recovery of lymphocyte numbers began at 2 days following irradiation. Subsequent losses were less dramatic, and numbers of acentric fragments reached preirradiation levels in the lower-dose groups by 56 days postexposure; numbers of rings plus dicentrics persisted throughout the duration of the study. Statistical analyses of the data demonstrated the variability of the aberration coefficient with time postirradiation. Mathematical models are presented which describe the predicted yield of numbers of aberrations as a function of time after irradiation.

The hematological data are examined for their possible relation to postirradiation aberration levels. The fate of aberrant lymphocytes following irradiation is discussed in relation to the kinetics of the reticuloendothelial system. Selective removal of damaged cells from the peripheral system is suggested.

It is concluded that different aberration coefficients will be found in blood sampled from pigs at different postexposure intervals. The kinetics of aberration persistence should therefore be considered when lymphocyte aberrations are employed as biological dosimeters.

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CHAPTER I

INTRODUCTION

The characteristic morphology of normal and aberrant chromosomes is amenable to qualitative and quantitative study by means of light microscopy. This affords the cytogeneticist an avenue for evaluating the effects of many physical, chemical and biological agents on the genetic complement. Frequently these agents induce chromosome aberrations and aneuploidy at levels far exceeding those occurring spontaneously.

The fact that irradiation induces chromosome rearrangements, as well as the feasibility of obtaining peripheral blood, has led to many investigations concerning the radiosensitivity of leukocytes. Much of this work has been directed toward obtaining the dose response kinetics of human leukocyte chromosomes irradiated in vitro. These data have been extrapolated to in vivo situations on the assumption that differences in radiosensitivity, i.e., in aberration coefficients were negligible. Since in vivo exposures of humans for the purposes of testing such assumptions are unrealistic, it is desirable to utilize other mammals which are physically and physiologically comparable to man. The present investigation was designed to determine the relationship between in vivo and in vitro exposures in pigs by means of cytogenetic analyses.

The use of chromosome-aberration data for the estimation of dose received during an accidental human exposure has been advocated. In most instances, it has been assumed that aberration coefficients will not vary among samples taken within the first week postirradiation. Although the kinetics of aberration persistence as a function of time

postexposure is not easily amenable to study in humans, such data are readily obtained from other species. It is obvious that if significant variation is found to exist between aberration coefficients obtained from blood sampled at different intervals following irradiation, this will affect estimates of radiation dose received in human accidental exposure.

Lymphocyte chromosomes of pigs exposed to fission neutrons were utilized to study the relationship between in vivo and in vitro exposures as well as to determine the kinetics of aberration persistence postirradiation. Fission neutrons were utilized in order to simulate nuclear explosions.

CHAPTER II

REVIEW OF LITERATURE

A. Persistence of Chromosome Aberrations

Types of aberrations. It is generally recognized that aberrations fall into two main categories; aberrations of chromosomes and aberrations of chromatids. Chromosome-type aberrations are induced prior to DNA synthesis, while chromatid-type aberrations occur during the S and G_2 phases of the cell cycle (7, 10, 43). A third type (subchromatid) may be produced while cells are in early prophase of mitosis or meiosis (43). Within each of these categories, aberrations may be classified also into symmetrical, which involves the interaction of centromeric chromosome portions with acentric fragments, and asymmetrical, which result from centric to centric or acentric to acentric interactions.

The particular type of aberration occurring will directly influence its persistence or lifespan since the probability of elimination at cell division depends upon the aberration type (34, 37, 74, 83). Asymmetrical chromosome aberrations (acentric fragments, rings, and dicentrics) are more readily lost than symmetrical aberrations and are the ones usually evaluated in cytogenetic analyses (62, 69, 76, 77, 82). Of these, acentric fragments or deletions disappear most rapidly as a result of mitosis because they lack a spindle fiber attachment and will usually fail to be included in either of the daughter cells. Thus, after a cell has undergone one or more intervening mitoses, the proportion of cells containing acentric fragments would be decreased.

Acentric fragments were thought to be eliminated at the first cell division following therapeutic irradiation of humans (74). Karyotype analyses performed on lymphocytes obtained from men involved in an irradiation accident were in accord with the premise that the deletion frequency decreases with time as more and more of the cells in the original population are replaced by cell division (14). This replacement of cells containing fragments by karyotypically normal cells may connote stem-cell proliferation. The aberrations may be induced in the stem cells present at the time of exposure and the eventual distribution of aberrant cells in the peripheral blood will depend largely upon the probability of loss during subsequent cell division. This probability is approximately 0.7 for acentric fragments in human leukocytes during in vitro culture (83).

The ultimate result of loss of chromosome fragments is a deficiency of genetic material in one or both daughter cells, depending upon whether a chromatid or chromosome deletion occurred in the progenitor cell (7). This loss of chromatin will be cell lethal if the genetic material is essential for cellular integrity. The eventual biological effect of such an event may be influenced by the developmental stage of the organism. Loss of genetic material in a zygote may result in lethality, whereas in a more differentiated system the deletion may have less dramatic effects.

Asymmetrical-exchange aberrations include rings and dicentrics but not acentric fragments. The acentric fragment that is produced as a result of the formation of a ring or dicentric is not designated as a separate entity but as part of the asymmetrical-exchange aberration. Rings and dicentrics may also be removed from the cell population

during mitosis, but the relative rate of disappearance is expected to be less than that for acentric fragments since the latter lack a spindle-fiber attachment (23, 74). The probability that a given asymmetrical-exchange aberration would survive either the first or second in vitro division has been estimated for human peripheral blood cells (83). The number of dicentrics per cell decreased from 1.2 at the first metaphase to 0.6 at the second and 0.3 at the third. Clearly, the probability is close to 0.5 that a dicentric will survive either the first or second in vitro division, and about 0.25 of surviving both divisions.

Since asymmetrical-exchanges possess centromeres, movement to the spindle poles is possible. Such aberrations, however, have a certain probability of bridge formation at anaphase which will cause restricted movement to the poles and may result in an abnormal complement of chromatin in daughter cells, polyploidy, or cell death (7, 41, 58). Since all of the asymmetrical-exchange aberrations do not result in bridges or interlocking rings, the chromatids may assort normally to the respective poles (29). Factors such as intercentromeric distance and chromatid exchange influence the probability of bridge formation (7).

Although not routinely evaluated in somatic chromosomes analyses, symmetrical exchanges (translocations and inversions) rearrange the normal position of genetic material. This alteration may lead to abnormal cell physiology or even cell death. The relative stability of these aberrations has been supported by the observation that the frequency of lymphocytes containing symmetrical-exchange aberrations in patients receiving therapeutic irradiation does not change significantly for many years following treatment (34). Peripheral blood samples obtained from men immediately following X-ray therapy for ankylosing spondylitis contained approximately

10% symmetrical exchanges, and this level was maintained during the 10 years over which the men were studied. Asymmetrical exchanges, on the other hand, decreased considerably during the first 3 years and continued to disappear after this time but at a slower rate. They did not, however, reach control levels even after 10 years following irradiation (34). Other data from human peripheral blood exposed in vitro indicate that symmetrical exchanges have about a 0.9 probability of surviving either of the first two cell divisions (83).

Extensive reports have indicated that the type of aberration persisting in the peripheral blood may be related to the age of the subject at the time of exposure. Individuals under 30 years of age at the time of the atomic bombings in Hiroshima and Nagasaki contained fewer lymphocytes with translocations and inversions than did comparable persons over 30 years of age. Apart from the possible age effect, it was proposed that the predominance of symmetrical-exchange aberrations (61% of the exposed individuals) in older subjects indicated that most persistent radiation-induced aberrations resulted in no significant loss of genetic material (16, 17).

Although it appears that a large proportion of aberrations are eliminated during mitosis, other phenomena such as interphase cell death and phagocytosis of aberrant (damaged) cells also may contribute to the disappearance rate of aberration-containing cells. A severe decrease in both absolute lymphocyte counts and the percentage of aberrant lymphocytes occurred in monkeys and pigs exposed to X-irradiation. This reduction aroused the suggestion that radiation-induced death or damage of a high proportion of circulating lymphocytes had occurred, which resulted in their subsequent removal from the peripheral blood by the reticulo-endothelial system (95). These authors

proposed also that an absence of long-lived, non-dividing lymphocytes in some species might explain the rapid loss of aberrant cells after irradiation.

The fact that aberration type plays a role in aberration persistence due to differences in the probability of loss at cell division has been demonstrated. Also, cell death and preferential removal of cells containing aberrations may account for the decrease in lymphocyte number following irradiation. Such mechanisms have been supported but the postirradiation persistence level of induced aberrations will, of necessity, depend upon the particular cell system under investigation.

Types of tissue. The differences in aberration persistence actually observed among different cell types tend to be influenced by variations in the mitotic rate of the cells samples (21). In mammals, peripheral blood lymphocytes and marrow cells have been investigated most extensively. Cells from the liver, spleen, kidney, thymus, and lymph nodes, as well as cell strains originating from diverse sources also have been studied. From a genetic viewpoint, however, the most widely utilized cell has been the spermatocyte which is derived from spermatogonia.

The initial aberration-induction rate *per se* among cells in various tissues may not be as different as coefficients calculated from a single cell type after various intervals of time postirradiation. The possibility that inherent differences in radiosensitivity exist among cell types also should be recognized. This latter consideration may be especially important regarding chemical mutagens which may require certain cellular functions for an effect to be manifested (57).

1. Somatic tissues. The majority of the reported works pertaining to aberration-induction rates and to the persistence of aberrations have utilized somatic cells. Classical studies on plants first demonstrated that acentric fragments, dicentrics, and rings disappear after relatively few cell divisions (58, 62). It appears that asymmetrical aberrations also are readily lost from rapidly dividing mammalian systems. Cultured mammalian cells contained stem lines with symmetrical-exchange abberations, but asymmetrical-exchange aberrations disappeared rapidly following irradiation (69, 82). Similarly, in irradiated mice, asymmetrical aberrations are eliminated, whereas symmetrical exchanges persist in rapidly dividing marrow cells (76, 77).

In cells which do not undergo division, aberrations would be expected to persist for the lifespan of the cell. This expectation is supported by the finding of high frequencies of anaphase bridges and asymmetrical exchanges in mouse liver cells induced to divide by carbon tetrachloride months after X-irradiation (1, 30, 78). In phytohemagglutinin (PHA)-stimulated lymphocytes from the spleen and lymph nodes of irradiated monkeys, between 2 and 7% of the dividing cells contained asymmetrical aberrations 223 days after radiation exposure (95).

2. Germinal tissues. The persistence of aberrations in somatic cells of adults may be of relatively minor practical importance, except insofar as they might be correlated with damage to germinal tissue or clinical syndromes. While a relatively large body of information exists comparing somatic chromosome aberrations with time postirradiation, similar information on germinal cells is rather scarce. Germinal cell studies largely have involved aberration induction in spermatogonia as demonstrated by translocations in spermatocytes of Meiosis I.

Cytological examination of spermatogonia in mice up to 600 days following a 600-Roentgens (R) exposure to X rays revealed that the yield of translocations increased from 8.4% when first examined at 60 days to 12.6% at 100 days postexposure. Although a decrease in translocation configurations was found at 250 days post treatment, the persistence of approximately 7% aberrant cells after 600 days suggested that cells containing translocations were not selectively eliminated (60). These values are in accord with another study (59) which indicated that an average of 10% translocations persisted in mouse spermatocytes sampled 10 weeks after exposure to 600 R of X rays. Other observations (2, 88) on the persistence of translocations in mouse spermatogonia also confirm that these abnormalities may be evident for considerable periods of time following exposure.

Although chromosome aberrations have been induced in cells obtained from different tissues and organs, the persistence of aberrations appears to depend upon the relative mitotic rate plus the specific aberrations formed in the cells sampled. It becomes evident, therefore, that both somatic and germinal tissues can harbor aberrant cells for quite long periods of time following induction.

Cloning of aberrations. Clones of aberrant cells especially would be expected with symmetrical-exchange aberrations because their loss due to cell division is rather uncommon (69, 77, 82, 83). Observations on many cell types from various species coincide quite well with this expectation (6, 7, 43, 58, 75). Cells containing chromosome markers represented approximately 15% of all dividing cells in the marrow of irradiated mice. Similar cloning of aberrant cells was observed also in the spleen, lymph node, and thymus of these same mice following X-irradiation (48).

Several studies have demonstrated the presence of what appears to be the same aberration in a higher than expected proportion of the peripheral blood lymphocytes from irradiated humans. Cloning of symmetrical-exchange aberrations was suggested also from karyotype analysis of leukocytes from men involved in a radiation accident (14). Survivors of the atomic bomb blasts in Nagasaki and Hiroshima were evaluated in several studies for leukocyte chromosome aberrations. Leukocytes from heavily irradiated survivors were thought to represent clones of cells containing translocations (15, 16, 17). Groups of three or more cells in each of these four individuals carried what appeared to be the same translocated chromatin material. However, these cultures were incubated for 3 days, and it has been shown by others (27, 83) that leukocytes are capable of undergoing up to three in vitro cell divisions during this culture period. Consequently, leukocytes with symmetrical-exchange aberrations may have several identical copies present in a three-day culture. It is also possible to find identical copies of asymmetrical aberrations but the probability of occurrence is less than that for symmetrical exchanges.

Other investigators studied the frequency of cloning in patients exposed to either acute X rays or chronic radiation from retained thorium. Peripheral blood leukocytes were harvested after 40 to 50 hours in culture when all dividing cells are considered to be in their first in vitro division. Two or more apparently identical cells with symmetrical-exchange aberrations were found in 16 of the 133 patients studied. Such cells have been confirmed in subsequent samples from four of these individuals (33). Aberration clones require cellular divisions which in turn influence the types of aberrations encountered in such clones.

Lymphocyte lifespan. The fact that human peripheral leukocytes contain asymmetrical aberrations years after exposure has been taken as evidence that these cells have not undergone cell division in vivo (34). This persistence of cells with asymmetrical aberrations has been utilized in determining average lymphocyte lifespan, although others (83) have shown that these aberrations may survive cellular division. A study utilizing peripheral blood lymphocytes from women receiving radiation therapy for cervical cancer indicated that the decrease with time in the frequency of cells containing acentric fragments or one dicentric could be represented by an exponential function. The calculated average lymphocyte lifespan was 530 ± 64 days for cells with acentric fragments and 788 ± 98 days for cells containing a single dicentric. Regardless of the function used, it is evident that aberrations decreased with time and that the rate of decrease depended upon whether the aberration contained a centromere (74). These authors concluded that the variation in lifespan of the various aberrations could be attributed to their differential elimination at cell division.

In similar studies of men receiving X-ray treatment for ankylosing spondylitis, the mean lymphocyte lifespan was calculated on only those observations made after recovery of the lymphocyte count (1,400 days posttreatment). It was assumed that this recovery implied that the lymphopoietic system had returned to equilibrium. This method of determining average lifespan may have resulted in an overestimate because populations of short-lived lymphocytes were neglected. When the percentage of first-division cells containing asymmetrical-exchange aberrations was plotted logarithmically as a function of time posttreatment, an exponential decline was found. This implies that a constant proportion of

the remaining aberrant cells were being lost per unit of time. An estimated mean lifespan of 1,574 days with 95% confidence limits of 891 and 6,743 days was obtained. The authors reported that this figure corresponded with a half-life of approximately 3 years, and that, of the lymphocytes present in vivo at any time, about 1% will have existed for 20 years (23). The preceding discrepancy among values for average lymphocyte lifespan (23, 74) may be partially explained by differences in culture time, areas exposed to radiation, sampling times, and the fact that a certain proportion of asymmetrical aberrations may not be lost during mitosis.

Evidence has been presented that some small lymphocytes are long-lived cells with an immunological role (46, 47). It has been established also that these cells respond to PHA by proliferation and that they can remain in a nondividing state for several years until stimulated to divide (28, 31, 61). The fact that asymmetrical aberrations (accompanied by an acentric fragment) have been found in peripheral blood leukocytes indicated that most of these cells were in their first division after exposure to radiation. Rat thoracic duct lymphocytes change rapidly into dividing cells when involved in an immune response (52, 53), and in vitro studies have demonstrated that small lymphocytes respond by proliferation when exposed to antigens to which the donor was previously sensitized (76, 79).

It has been postulated that lymphocytes recirculate between the peripheral blood and lymphopoietic centers in a nonproliferating state if not exposed to the antigen to which they are committed (46, 47). Upon exposure to such an antigen, these small lymphocytes rapidly transform into large proliferating cells which are capable of antibody formation.

The fact that asymmetrical-exchange aberrations and fragments are found in cells years after exposure to a mutagen implies that the immunologically commited small lymphocyte may persist in vivo until such time as it encounters an antigen capable of inducing its proliferation and subsequent division. The lifespan of immunologically committed small lymphocytes will therefore depend upon the frequency with which they encounter the antigen to which they are committed. Consequently, any estimate of the mean lifespan of small lymphocytes may have limited value since such estimates are derived from a heterogeneous population of cells with different lifespans.

Other studies have been conducted in an attempt to test the theory that small lymphocytes proliferate when reexposed to specific antigens (75, 76). The persistence of dicentrics, rings, and acentric fragments was determined in peripheral blood lymphocytes of patients undergoing partial-body therapeutic irradiation. Acentric fragments and asymmetrical interchanges were found at all postirradiation sampling times in PHA-stimulated cultures, whereas tuberculin-stimulated cultures exhibited a sharp decrease at six and one-half months after exposure. Although such studies imply different subpopulations of lymphocytes, the use of different patients with varying symptoms, different doses, different sites of irradiation, and varying sampling times may have influenced the observed differences. Also, abnormal blood cells may have been sampled since half of these patients had been diagnosed as having lymphosarcoma or chronic lymphocytic leukemia.

A more recent report (76) also indicates subpopulations of lymphocytes based upon antigenic response. Blood samples were obtained at one month and 36 months postexposure from patients receiving therapeutic

irradiation. In each of the two patients the percentage of acentric fragments, rings, and dicentrics was lower in cultures stimulated with tuberculin or smallpox antigens than in similar cultures employing PHA. It was estimated that all cultures were sampled in their first in vitro division, although PHA cultures were harvested several days earlier than antigen-stimulated cultures. Additional data (72) were accumulated from patients undergoing radiation therapy for cervical cancer and lung cancer. In first division PHA or pokeweed mitogen (PWM)-stimulated blood cultures, the frequency of cells with asymmetrical exchanges and acentric fragments was 15 to 20% throughout the 3-month period of study. In tuberculin-stimulated cultures, however, cells with similar aberrations fell from 15% to less than 5% within 3 weeks after irradiation. These results do not definitely test the idea of specific subpopulations of immunologically committed lymphocytes, but they do suggest marked differences between antigen-stimulated and PHA-stimulated cultures from irradiated humans. Such differences might be explained if those cells responding to antigens in vitro had been undergoing division in the body with resultant loss of aberrations. The authors suggested also that both committed and uncommited lymphocytes may respond to PHA and that perhaps uncommitted cells are the long-lived, nondividing lymphocytes in the body (76). It would therefore appear that PHA is capable of stimulating a large battery of small lymphocytes committed to different antigens. That is, PHA is a nonspecific, wide-range factor capable of stimulating lymphocyte proliferation in cells committed to different antigens or perhaps even in uncommitted cells.

In calculating values of average lymphocyte lifetime from the persistence of "marker cells", it was assumed that these cells have not undergone mitosis during the period under consideration. The variation among the reported values (23, 74) reflects differences in cell populations sampled as well as the types of aberrations employed in making such calculations.

Species variation. The disappearance rate of aberrations may differ among species due to inherent variations in their ability to repair lesions as well as to remove damaged cells. Variables such as total dose, dose rate, fractionation of dose, sampling times, cell cycle times, and methods of exposure should be considered within and among species in experiments designed to differentiate among species.

1. Aberration persistence in man. Various reports have confirmed that cells containing aberrant chromosomes may persist for years in individuals previously exposed to radiation (15, 16, 17, 23, 34, 49, 74). Accidental whole-body exposures to mixed gamma ray and fission neutron radiation resulted in the presence of asymmetrical exchanges and minutes (interstitial deletions) in the blood at 29 and 42 months post-exposure. Differences in aberration percentages at the two sampling times indicated that aberrations were being eliminated, and that pre-irradiation aberration levels might eventually be reached in the peripheral leukocytes. Karyotype analysis of symmetrical exchanges indicated that these aberrations may be retained indefinitely (11, 13).

Other results from peripheral leukocytes of men involved in a criticality accident, demonstrated a rapid loss of aberrations beginning at 6 weeks following irradiation. The data for the first several

postirradiation periods are compatible with the idea that the "half-life" for replacement by cell division of the leukocyte population studied (those which divide in culture) is of the order of many weeks (14). Recent data from an accidental whole-body exposure to cobalt-60 gamma rays indicate that the yield of centric rings plus dicentrics does not vary significantly over the first 32 days following exposure. A consistent decrease in the number of rings plus dicentrics was noted after about 39 days, which closely paralleled the onset of total leukocyte recovery (20). Another study also has shown that the aberration yield measured in the peripheral blood of patients given whole-body X-radiation does not change appreciably during the first 24 hours after exposure (25).

Other data, however, demonstrate that lymphocyte aberrations begin to decrease shortly after exposure. An exponential decline of asymmetrical exchanges and deletions in lymphocytes began within one to three weeks following therapeutic irradiation for ankylosing spondylitis. Thereafter, approximately 3.5% of aberrant cells disappeared each month until a rather steady level was attained after 5 years (24). Other studies from similar patients indicate that the function relating the number of asymmetrical exchanges and fragments to time after exposure contains a rapid and a slow aberration-loss component. These components are thought to reflect two populations of lymphocytes differing in their relative lifespan or, alternatively, a single population with a more rapid initial loss due to dilution as the lymphocyte population recovered following irradiation (23, 34).

In lymphocytes from tumor patients receiving radium therapy, an initial value of 34.9% of all analyzed cells had structural chromosome aberrations. Within 3.5 years the number of aberrant cells decreased to

8.7%, and during the following 2 years further significant decreases could not be observed (84). Information obtained from humans reveals differences in aberration persistence between marrow cells and peripheral leukocytes. Dicentric and ring chromosomes were found in leukocytes but not in direct marrow cultures 7 years following accidental exposure to mixed fast neutrons and gamma rays. Chromosomal fragments, however, were found in both types of cultures (49). Various aberration types have persisted for at least 20 years in the leukocytes of humans exposed to the Hiroshima and Nagasaki bombings (16, 17).

2. Aberration persistence in other mammals. In a study of aberration persistence among various animal species, it was found that the highest incidence (26%) of asymmetrical aberrations in leukocytes from monkeys occurred in the initial (30 min.) blood samples following partial-body X-irradiation. Samples obtained 18 hours later contained significantly fewer (9.5%) aberrations. When the study was terminated at 258 days postexposure asymmetrical aberrations persisted in the peripheral leukocytes of the two animals at 0.5 and 8% (95). In this same study, a more dramatic decline in the proportion of pig leukocytes containing asymmetrical aberrations was reported. The highest incidence of aberrant cells was observed when cultures were first established 30 minutes following partial-body irradiation. Within 24 hours after exposure, however, cells containing aberrations had essentially disappeared from the blood of all animals and did not reappear in significant numbers during the 50-day period of study. This rapid decrease in number of asymmetrical aberrations in both monkeys and pigs suggests the occurence of radiation-induced death or damage in a high proportion of circulating

lymphocytes and their removal by the reticuloendothelial system. Practically no aberrations were found in peripheral blood cultures obtained from irradiated guinea pigs, rats, and rabbits. This response in the latter species was attributed to technical difficulties as well as to the possibility of the absence of long-lived lymphocytes (95).

When primary cultures were prepared from rat embryos irradiated in utero with varying acute doses of X rays, the number of cells with chromosome aberrations declined to 20% of the initial values within the first 12 hours following exposure. As time progressed, the number of aberrations approached control values (40). This observation agrees with an earlier study (92) in which chromosome aberrations in rat embryos declined rapidly within the first 24 hours following exposure to 400 R of X rays and returned to nearly normal values within several days. The death of damaged cells and chromosome repair were advanced as possible causes of the disappearance of chromosomal aberrations. It appears that chromosome repair, as reported (92), would include the disappearance of aberrations via cell division.

It has been demonstrated also that marrow cells from Chinese hamsters irradiated in vivo with 100 R of X rays contained a maximum number (50.0%) of chromatid breaks 2 hours after exposure, and that these aberrations dropped rapidly to 4.1% within 24 hours (10). This rapid decrease of number of aberrations in dividing cells would be expected and has been verified by more recent data which showed that chromatid aberrations in both marrow and testicular cells of the Chinese hamster were drastically decreased by 12 hours following 100 R of gamma radiation (21). The possibility of a dose-rate effect upon aberration persistence in mice marrow cells has been reported. X rays given at 30 R per minute resulted

in increased aberration persistence when compared with gamma radiation given at a dose rate of 1.45 R per minute (77). Unfortunately, sampling time was inconsistent among the animal groups.

Since peripheral lymphocytes rarely undergo mitosis in vivo, higher aberration persistence levels would be expected than in rapidly dividing cells. Mechanisms such as the selective elimination of damaged cells or interphase death and the possibility of an absence of a long-lived population of lymphocytes will increase the rate of aberration loss. Identical aberration yields were found in blood cells sampled immediately and 24 hours after whole-body irradiation of marmosets (Saguinus fuscicollis) with gamma rays at 3.7 R per minute (18). Results presented in this report are inconsistent with those of a recent study utilizing pigs (66). Lymphocytes were sampled within 20 minutes following whole-body, bilateral exposures to ^{60}Co gamma rays at 50 R per minute. Subsequent samples were obtained periodically up to 20 weeks postirradiation. Within 24 hours after exposure approximately 39% of the cells containing acentric fragments and 22% of those harboring rings or dicentrics had disappeared from the peripheral blood. It was found that the number of aberrations per cell as well as lymphocyte number fell very sharply during the first 48 hours postexposure. This suggested a preferential removal of aberrant cells from the peripheral system. Variation in dose rate also may contribute, in part, to the observed differences, in that many aberrations may have been lost by selective elimination during prolonged exposures. It appears, however, that whole-body irradiation of primates (18) and of humans (14, 20, 25) results in similar aberration persistence kinetics when blood is sampled shortly after exposure.

From the reported data, it appears that the rapid loss of aberrant cells following irradiation is manifested more pronouncedly in non-human mammals than in humans. Determination as to whether real differences actually exist must await experiments designed to adequately test the aberration loss rate among different species. The early, rapid loss of aberrant cells in some species indicates that postirradiation sampling time and the type of cells sampled have important implications concerning the utilization of chromosome aberrations as a biological dosimeter.

Data on lymphocyte kinetics following extracorporeal irradiation of blood (ECIB) in humans revealed that peripheral blood lymphocytes, sampled from the exit side of the coil immediately after the start of irradiation, contained 0.87 dicentrics per cell after an estimated dose of 320 rads. Subsequent samples were obtained from the entrance side of the coil at various intervals during an 8-hour-per day, 8-day treatment regime. The number of aberrations decreased to a low of 0.057 dicentrics per cell after 1.5 hours of continuous treatment and increased to 0.30 dicentrics per cell after 64 hours of intermittent treatment (90, 91). Variability in the aberration level during treatment was attributed to dose accumulation as well as lymphocyte exchange between the extracorporeal shunt, the peripheral system, and the readily-exchangeable extravascular pools.

More recent data on lymphocyte kinetics in nonleukemic patients receiving ECIB for immunosuppressive purposes support the hypothesis of selective removal of aberrant cells (45). During ECIB there was a progressive accumulation of cells containing aberrations which rose to a value of 4.3% after 8 hours. Immediately upon cessation of treatment there was a rapid decline to a lower level, which was maintained for 2-1/2 treatment-free days at a mean value of 1.1% for five consecutive samples.

These data indicate that damaged cells (as determined by dicentric induction) are removed from the peripheral blood by the reticuloendothelial system both during ECIB and after treatment. These damaged cells were replaced by cells from the extravascular pools.

The population of circulating blood lymphocytes in cattle dropped to a constant low level of between 700 and 2,000 cells per mm^3 after 12 hours of ECIB. This level was maintained during the 48 hours of treatment. The lymphoreticular tissues, on the other hand, continued to lose lymphocytes between the twelfth and forty-eighth hours of ECIB. These workers suggested that the apparent steady-state lymphocyte level in the peripheral blood is maintained by a nonsteady-state process in the lymphoreticular organs wherein the new production of lymphocytes is insufficient to replace the cells leaving these tissues. It was noted also that the cellular debris caused by ECIB was harbored within the spleen, lung, liver, and lymph nodes in this order of relative concentration (32).

A correlation may exist between the decrease in number of circulating lymphocytes and the accumulation of cellular debris. This cellular debris may have implications concerning the fate of damaged (aberration containing) lymphocytes. Other workers reported a maximal lymphocytopenia in calves during 12 hours of ECIB. The blood lymphocyte count was reduced to 17% of the level at the beginning of irradiation. These data indicated that the duration of the postirradiation lymphocytopenia in calves was correlated with the total number of lymphocytes killed, and, in some instances, it was many weeks before numbers of peripheral blood lymphocytes returned to preirradiation levels (35, 36).

The notable decrease in aberration rates following periods of ECIB treatment may be explained in part by the loss of aberrant cells as

demonstrated by the accumulation of cellular debris in lymphoreticular organs and by cell mixing of lymphocytes in lymphopoietic centers with those in the peripheral blood. The dose rate and degree of dose fractionation also would be expected to influence the rate of dicentric induction *per se* (7, 43, 58).

Plasma factors. It has been demonstrated that when normal human peripheral blood lymphocytes are cultured in media containing plasma obtained from irradiated individuals, significantly more chromosomal aberrations are found than when lymphocytes are grown in nonirradiated plasma (50, 54). Such results have led to the suggestion that after in vivo irradiation a fraction of the aberrations in small lymphocytes are produced indirectly by some factor in the plasma of the irradiated individual and not directly by the radiation *per se* (49, 50). This "plasma factor" has been reported to be active for as long as 7 years after whole-body irradiation of humans (49). The identity of the factor, however, has not been verified and the possibility that it is a radiation-activated latent virus in the body has been suggested (50, 54). More recent information has shown that a lymphocyte-chromosome-breaking factor can be induced in the plasma of blood irradiated in vitro as well as in vivo. These aberrations were interpreted as being induced at the chromatid level (85).

The existence of plasma factors capable of inducing certain kinds of chromosomal aberrations appears well established and may well influence the persistence of specific aberrations following irradiation. Attempts to isolate and identify such factors are relevant, especially since these agents may predispose individuals to aberration-correlated syndromes.

Lymphocyte kinetics. The postirradiation fate of peripheral blood lymphocytes and their precursors will influence aberration indexes calculated from them. An extensive study involving men undergoing therapeutic X-irradiation for ankylosing spondylitis demonstrated that the mean lymphocyte count was 2,282 cells per mm^3 prior to radiation and fell rapidly to its lowest value of 539 cells per mm^3 within a few days after exposure. Lymphocyte recovery was incomplete until nearly 4 years after treatment (23). Other investigators have stated that peripheral lymphocyte counts made at intervals up to 24 hours and again at 64 hours after ECIB treatment of humans varied little from initial values (90, 91). These data were obtained from two patients with reticulum-cell sarcoma and Hodgkin's disease, respectively, and may well have involved abnormal cell populations. This may explain the apparent discrepancy with other data (32) which indicated a decrease in peripheral blood lymphocytes following ECIB in normal calves. It should be emphasized also that inherent species variation may exist.

Data obtained from monkeys receiving partial-body X-irradiation showed a rapid decline in the absolute lymphocyte count during the first 18 hours following exposure. These values gradually increased and returned to normal levels at about 223 days postirradiation (95). In this same study, hematological data from pigs showed the expected decrease in absolute lymphocyte counts following partial-body X-irradiation. In all irradiated pigs the lowest level was attained within 24 hours, and then the counts quickly returned to normal levels. From these data on monkeys and pigs, it was noted that the severe decline in absolute lymphocyte counts coincided with a rapid decrease in number of asymmetrical aberrations in the lymphocyte population. Comparable information concerning

symmetrical aberrations was not reported. More recent data demonstrate that pig peripheral blood lymphocyte counts also dropped drastically following a bilateral exposure to fission neutrons (average energy = 1.2 MeV). Approximately a ten-fold decrease in lymphocyte counts occurred at 2 and 4 days following exposure to 850 and to 550 rads, respectively (22). It has been reported also that peripheral blood lymphocytes from pigs are rapidly eliminated during the 1.0-to-1.5 hour interval between the beginning of exposure to 14 MeV neutrons and the obtaining of the postirradiation blood samples. Following varying in vivo doses (187 to 587 rads), reductions in total leukocyte counts ranged from essentially none at the lower exposures to an average of 33% at the higher doses. This same study revealed that the proportion of lymphocytes was reduced by nearly 50% at all dose levels when blood was sampled between 1.0 and 1.5 hours after the beginning of irradiation (64).

The observation that absolute lymphocyte counts decrease following in vivo irradiation appears to be consistent for most species, but the relative loss and recovery rates of the lymphocyte population may vary among species. Further evidence concerning the fate of these cells may shed light on the rapid decrease in number of aberrant cells in some species following irradiation.

B. Neutron-induced Aberrations in Mammals

Several workers have investigated chromosome aberration induction in peripheral blood leukocytes following exposure to fast neutrons of various energies. Relative biological effectiveness (RBE) values for acentric fragments in human leukocytes were reported to be 2.6 for 14.1 MeV neutrons and about 4.5 for 2.5 MeV neutrons when compared with X rays,

all irradiations being given in vitro (8, 51). Such comparisons are justified since acentric fragments display essentially linear dose kinetics for both low and high linear energy transfer (LET) radiation (41, 58). An effect of neutron energy upon the kinetics of ring and dicentric induction was suggested (8, 51). The expected linear dose effect was found for 2.5 MeV neutrons, but rings and dicentrics showed equally good fit to linear and dose-square models for 14.1 MeV neutrons. Other data from a criticality accident involving fission-spectrum neutrons in human leukocytes yielded an RBE of approximately 4.5 for chromosome deletions or acentric fragments. The effectiveness of fission neutrons is displayed by the fact that the ring and dicentric data fit a linear model with a slope greater than that for their induction by 2.5 MeV neutrons (14). Similar estimates of fission neutron RBE for peripheral leukocytes irradiated in vivo ranged from 2.5 to 10.2 for three subjects accidentally exposed. It was concluded that the true RBE for in vivo irradiation is probably near 5 or 6 for deletions (51).

Human peripheral leukocytes were exposed in vitro to fission neutrons at 3.41 or 6.75 rads per hour (86, 87). No dose-rate effect was found, and an RBE value of approximately 4.5 was calculated for rings plus dicentrics. From previous studies (42, 86), these workers had reported essentially linear dose-effect kinetics for these aberrations with 250 kVp X rays, thus allowing straight-forward calculation of RBE values.

Uncertainty exists regarding the kinetics of ring and dicentric induction during irradiation with neutrons of various energies, and recent data (64) have not clarified the situation. It was found that the ring and dicentric data from in vivo exposures of pigs to 14 MeV

neutrons were best fitted by a curvilinear dose response function. It was suggested that the in vitro results also would have tended toward the curvilinear had higher dose levels been utilized.

The induction of translocations has been studied in mice by the cytological examination of spermatocytes following either chronic or acute exposure to fission neutrons (89). The yield of translocations increased linearly up to about 100 rads of acute exposure but decreased with higher doses due to cell killing. On the other hand, 12-week chronic exposures gave no indication of decreased yields at higher doses. RBE values of about 3.7 were obtained for acute exposures below 100 rads, whereas values between 20 and 25 were reported for chronic exposures at similar doses.

Anaphase chromosome aberrations have been evaluated in the regenerating livers of mice previously irradiated with fission neutrons (30, 38, 39). It was reported that neutrons were more efficient than X rays in producing these abnormalities and that their frequency was relatively independent of the time intervening between radiation and chromosome examination. Aberration yields induced by X rays, however, decreased with increasing time following exposure. Unlike the case for X-radiation, chronic or fractionated neutron exposures were just as effective as acute ones. Another study (78), however, has indicated an apparent dose-rate effect for neutron-induced chromosomal aberrations in regenerating mouse liver, lower dose rates resulting in lower aberration frequencies. Such apparent dose-rate effects may actually have resulted from the different average energies of the neutrons used for the acute and the chronic exposures (30).

C. In Vivo-In Vitro Radiation Response

Due to the feasibility of utilizing chromosome aberration data for estimates of biological dose following accidental exposure, many workers have studied the response of human lymphocyte chromosomes to in vitro irradiation (5, 7, 9, 12, 18, 19, 42, 43, 44, 71, 72). The results of such studies have not always agreed, and this may be a reflection of technical variations rather than inherent cellular differences. An assumption made in such investigations is that lymphocytes exposed while in the peripheral blood will display a similar radiation response when irradiated in vitro.

Comparative data on rings and dicentrics following exposure to 2 MeV X rays indicate that at low doses (under 50 R) peripheral blood from an individual gives essentially the same results after in vivo or in vitro radiation (26). Extrapolation of the in vivo data to doses higher than 50 R on the basis of the available in vitro data appear uncertain. Agreement between in vivo and in vitro aberration data in humans is enhanced by the finding that an estimated ECIB dose of 320 rads induced 0.87 dicentrics per cell, whereas 0.83 dicentrics per cell resulted from 300 rads in vitro (90, 91).

Recent data (64) involving in vivo exposure of pigs to 14 MeV neutrons and of their blood in vitro demonstrate higher aberration rates in vitro. The possibility of a protective mechanism capable of removing damaged cells in vivo following irradiation was suggested. These authors studied also the effects of gamma irradiation in this same system (66). Acentric fragments were produced in vivo at 78% of the in vitro rate while the value for rings plus dicentrics was 66% following gamma irradiation. It was concluded that the amount of dose-attenuation

afforded the circulating blood by the body is of primary importance in the final evaluation of observed differences between in vivo and in vitro aberration rates. That is, the damage sustained by leukocytes irradiated in vitro is not different from that induced by an equivalent dose delivered to cells in vivo. Other data from marmosets (Saguinus fusicollis) exposed to gamma rays at a dose rate of 3.7 R per minute and whole blood irradiated in the same manner indicated identical aberration yields (18).

Factors such as radiation quality, sampling time, and dose attenuation appear to influence in vivo-in vitro comparisons. The possibility of actual species differences also should be considered. Until aberration-induction kinetics are established for in vivo and in vitro irradiation for various species, extrapolation between the irradiation modes seems unjustified. Inherent in any practical utilization of chromosome-aberration data is their kinetics over time postexposure.

The practical importance of utilizing chromosome-aberration coefficients obtained from human lymphocytes as an indicator of the radiation dose actually received following accidental exposure to fission neutrons has been amply documented. Although it is possible to determine the aberration coefficient in human lymphocytes exposed in vitro, it is not permissible to acquire in vivo data. It is essential, therefore, to establish the relationship between the level of aberrations induced in vitro with those produced after in vivo exposures. This relationship can readily be established in the domestic pig which is physically and physiologically comparable to man. Should significant differences be found between the aberration levels in pig lymphocytes irradiated in vivo or in vitro, the factors responsible for the variability can be evaluated in terms of accidental human exposures. The resulting data will also afford an

insight into explaining the apparent differences between the results obtained from other studies involving in vivo and in vitro aberration data.

Another possible source of variation in the calculated aberration coefficient is that due to the time interval between irradiation and obtaining the postexposure blood sample. Samples of peripheral blood can be easily drawn from pigs at successive intervals after irradiation. This will permit an evaluation of the change in the aberration index as a function of time postexposure. The postirradiation response of the number of peripheral blood cells can be correlated also with changes in the level of aberrations.

CHAPTER III

EXPERIMENTAL PROCEDURES

A. Irradiation Facility

The Health Physics Research Reactor (HPRR) of the Oak Ridge National Laboratory was employed for irradiation of all animals. The HPRR is a Godiva-type reactor designed for use as a neutron radiation facility for research in health physics and related fields. This reactor is an unmoderated assembly which utilizes highly enriched uranium alloyed with 10%, by weight, of molybdenum as the fuel material. It can be operated either in the steady-state mode or with pulses up to 10^{17} fissions per pulse. The neutron:gamma ratio is approximately 6:1, and the neutron spectrum under these conditions is slightly softer than a ^{235}U fission spectrum with average energy of about 1.2 MeV. The peak of the fluence-versus-energy curve is at 0.6 MeV while the gamma ray spectrum from the HPRR has an average energy of about 2 MeV (22). Other reports (3, 68) have dealt with the various facets of the reactor and its associated equipment.

B. Dosimetry

Dosimetry was based upon well established techniques of measuring dose from coexistent neutron and gamma radiation fields. Four separate detection systems, two for neutrons and two for gamma radiation, were utilized. For each radiation (neutron or gamma) both a passive and an active system were employed. The active systems were the Hurst fast neutron counter for measurement of fast neutron dose and the "phil" dosimeter for measurement of gamma dose. The Hurst system employs a

polyethylene-walled, cyclopropane-filled proportional counter and associated equipment for the measurement of fast neutron dose from neutrons with energies above approximately 160 keV (55, 93). The passive systems employed were the Hurst threshold detector system for the measurement of fast neutron dose and a radiophotoluminescence technique for measurement of gamma dose (81).

For the present study, the reactor was operated in a steady state at a power level of 2 kW. Exposure duration varied from 7 minutes (100 rads) to 28 minutes (400 rads) and was given at a constant dose rate of 14.3 rads per minute.

Exposures were expressed as the dose measured in air at the animal's midline. Since the neutron:gamma ratio was about 6:1, a total midline dose (in air) of 100 rads consisted of 100 rads of fast neutrons plus about 17 rads of gamma rays.

The problem of calculating an average tissue dose is complicated by the facts that the peripheral blood cells are constantly in motion during the course of irradiation and that certain body regions contain considerably more blood volume than others. From a biological viewpoint, calculation of an integrated dose across the width of an animal would not consider the transitory nature of the cells evaluated. Also, a more exact calculation of tissue dose becomes especially complicated when the rotation to which the animals were subjected is considered. Consequently, the midline neutron dose (measured in air) was used as the index of exposure. Admittedly, this is not ideal, but sources of error from its use were less than for other expressions. In order to obtain estimates of the radiation dose actually received by the blood cells in vivo, further calculations

were performed (80). Factors such as dose attenuation, radiation back-scatter, and the distance from the side of the animal to the reactor were considered.

Two methods of calculating the dose received by the blood cells were employed and their agreement was approximately 92%. The first method was based upon unilateral dose distribution measurements at various depths in tissue-equivalent phantoms exposed to fission neutrons at the HPRR. From these data, average whole-body dose estimates were computed for bilateral exposures (method 1). A second method considered the partitioning of a 34 cm diameter cylinder (average body width of the animals used) into concentric circles. The neutron and gamma doses were weighted according to the volume in each of the cylindrical rings. These values were summed in order to obtain a volume average whole-body dose (method 2).

C. In Vivo and In Vitro Irradiation

Eight purebred Duroc sows of comparable age and weight were utilized (Table 1). Two animals were exposed at each of the following doses: 100, 200, 300, and 400 rads. Approximately 30 minutes prior to irradiation, heparinized blood samples were obtained from each pig; half of each sample was irradiated in vitro while the other half furnished blood for control cultures.

Aluminum cages, containing the experimental animals were positioned in a semi-circle so that the midline (saggital plane) of the animal was 3 meters from the reactor. The height of the reactor was 1.7 meters above the floor level which provided a horizontal plane with the midpoint of the animal's body. Halfway through the exposure, electric turntables rotated the cages 180 degrees without interruption of reactor operation.

TABLE 1. Body measurements of pigs at time of irradiation

Animal number	Body weight (kg)	Body depth[1] (cm)	Body width[2] (cm)
60-3	166.5	47.5	32.4
54-9	175.5	51.0	31.8
61-3	176.5	48.5	30.0
62-4	201.6	51.5	34.1
54-1	171.0	51.0	33.8
57-10	182.3	53.5	36.5
54-4	205.1	51.3	38.7
70-4	218.0	51.5	34.8
Average	187.0	50.7	34.0

[1]Measured at midpoint between fore- and hind quarters.

[2]Average of measurements taken across the neck, shoulder, behind last rib, loin, and ham.

This facilitated bilateral exposure of the entire animal and required approximately 30 seconds to complete.

In vitro exposures of samples of whole blood from each animal were performed simultaneously with in vivo irradiation. Approximately 7 ml of heparinized whole blood was placed in plastic tubes and positioned 3 meters from the reactor and at a height equivalent to the vertical midpoint of the animal. All in vitro blood samples therefore received the same dose as that measured in air at the animal's midline.

D. Blood Sampling

Peripheral blood samples were obtained from the jugular vein. Prior to venipuncture, the local skin area was swabbed with 70% alcohol. The inside of a 20 ml syringe was coated with heparin[1] prior to obtaining approximately 14 ml of blood. Before the blood was expelled into a sterile, plastic tube, the needle was removed in order to prevent cellular damage in the blood sample. All blood samples were inoculated into culture media within 2 hours from the time blood was drawn from the animal. Samples of peripheral blood were secured from each animal 30 minutes prior to exposure and at the following postexposure intervals: 30 minutes, 24 hours, 48 hours, 1 week, 2 weeks, 4 weeks, 8 weeks, and 12 weeks.

E. Blood Culture and Cell Harvesting

Routine differential leukocyte determinations were performed on all blood samples, and total leukocyte counts were determined by automated instrumentation. One ml of whole blood and 10 ml of complete

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[1]Heparin Sodium - 1,000 U.S.P. units per ml. Fellows Testagar, Detroit, Michigan.

culture medium[2] were incubated in a 3-ounce prescription bottle at 38.5° C. This incubation temperature corresponds with the normal pig's body temperature. Colchicine (final concentration, 10^{-6}M) was added after 46 hours of incubation, and harvesting of cultures began 4 hours later.

The harvesting procedure was as follows:

1. Cultures were mixed gently until the cells detached from the culture bottles and were in suspension.
2. The entire cellular suspension was poured into a graduated 15 ml centrifuge tube and centrifuged at 700 g for 10 minutes.
3. The supernatant was carefully removed down to the 1 ml mark and the remaining cell suspension was mixed gently but thoroughly.
4. The entire contents were drawn into a Pasteur pipette and 3 ml of warm (37° C) distilled water was added to the tube. The cellular suspension was then gently mixed with the water.
5. After 10 minutes in a 37° C water bath the tubes were centrifuged at 375 g for 5 minutes.
6. The supernatant was gently agitated while caution was exercised not to disturb the cellular pellet.
7. Six ml of fixative (3 parts methanol:1 part glacial acetic acid) was added, and cells were suspended by constant agitation with a Pasteur pipette until all cell clumps were eliminated.
8. Tubes were centrifuged at 375 g for 5 minutes, and the fixative was poured off.

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[2]Complete Culture Medium

	Quantity (ml)
TC Medium 199*	7.00
Fetal Calf Serum* (inactivated, 56° C for 30 min)	2.50
Phytohemagglutinin**	0.50
Penicillin (200 U/ml)-Streptomycin (200 mcg/ml)	0.20
$NaHCO_3$ (4%)	0.13

*Grand Island Biological Company, Grand Island, New York

**Laboratory prepared.

9. Steps 7 and 8 were repeated twice, using 4 ml of fixative each time.

10. Four ml of fixative (1 part methanol:1 part glacial acetic acid) was then added, and the cells were suspended gently with a Pasteur pipette. The pipette and centrifuge tube walls were rinsed with fixative to remove adherent cells.

11. Tubes were centrifuged at 375 g for 3 minutes, and the fixative was decanted.

12. After a repetition of steps 10 and 11, the cells were resuspended in a small amount of 1:1 fixative.

13. With a Pasteur pipette, a drop of cellular suspension was dropped onto clean slides which had been dipped in distilled water.

14. Slides were air dried before staining in Orcein[3] for approximately 1 hour. They were then rinsed with tertiary butyl alcohol and mounted with Diaphane.[4]

It has been found that the above technique provides repeatable results and well-spread chromosomes suitable for analysis (4, 63).

F. Chromosome Analysis

Slides were evaluated by systematically scanning coded slides at 200 X magnification until metaphase figures suitable for scoring were encountered. Upon the location of such metaphases, the centromeric number was determined at 1250 X magnification. Only those metaphases with the modal centromere number (2n = 38) and those with 2n - 1 were selected for critical evaluation. All visible anomalies were recorded, but only chromosome-type acentric fragments, minute deletions, centric and acentric rings, and dicentrics were included in the reported data. Acentric rings were differentiated from minute deletions on the basis of

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[3]Gurrs orcein, synthetic (Lapine Scientific, Chicago, Ill.) 1% solution in solvent consisting of: 42.5 ml glacial acetic acid
42.5 ml lactic acid
25.0 ml distilled water

[4]Will Scientific, Inc., New York, N. Y.

relative size. When the diameter of the acentric aberration was greater than that of a chromatid in the same metaphase, an acentric ring was recorded. Acentric fragments were determined by excluding those normally expected to accompany a ring or dicentric in the same metaphase.

In order to study aberration persistence as a function of time post-irradiation, we attempted to evaluate 100 metaphase cells from each animal at each of the nine sampling periods, representing a potential of 7,200 cells. However, due to technical difficulties, somewhat fewer than 100 scorable cells were analyzed from some of the cultures. This was especially evident in the samples obtained 2 days postirradiation due to unfavorable cellular growth in vitro. In order to reduce random error, the aberration data from the 2-day samples were not included in the statistical analysis.

G. Statistical Procedures

Data analyses were facilitated through the use of the Statistical Analysis System (SAS) developed at North Carolina State University.

The mathematical model used in the analysis of variance for aberration data was:

$$Y_{ijkl} = \mu + D_i + A_{j/i} + T_k + (DT)_{ik} + (AT)_{j/ik} + E_{ijkl}$$

where, Y_{ijkl} = the lth aberration, ith dose, jth animal within the ith dose, kth postirradiation time.

μ = an effect common to all aberrations (mean).

D_i = effect due to the ith dose.

$A_{j/i}$ = effect due to the jth animal in the ith dose.

T_k = effect due to the kth postirradiation time.

$(DT)_{ik}$ = effect due to the interaction between the ith dose and the kth postirradiation time .

$(AT)_j/ik$ = effect due to the interaction between the jth animal within the ith dose and the kth postirradiation time .

E_{ijkl} = random error .

This model was deemed appropriate for aberration data obtained prior to lymphocyte recovery (30 minutes and 24 hours postexposure) as well as those data collected during the period of lymphocyte recovery (1 through 12 weeks postirradiation).

The appropriate error term for testing the effect due to the interaction between the jth animal within the ith dose and the kth postirradiation time was obtained by utilizing the mean square (random error) due to replicate observations within each animal at each dose and postirradiation time. All other F ratios, except that for the ith dose, were obtained by dividing the respective mean squares by the variation due to the interaction between the jth animal within the ith dose and the kth postirradiation time. The F value for the ith dose was calculated by dividing its mean square by the variation due to the jth animal in the ith dose.

A cubic polynomial was fitted to each class of aberrations (acentric fragments, rings, dicentrics, rings plus dicentrics, acentric fragments plus rings plus dicentrics within a dose from 1-to-12 weeks postirradiation. The sum of squares due to linear regression, quadratic after linear, as well as cubic after linear and quadratic effects were calculated and evaluated for their influence on number of aberrations per cell. If either of the terms did not contribute significantly to the explanation of observed variation, they were deleted from the model and the data were

reanalyzed according to the most descriptive expression. Prediction equations containing the remaining components were utilized to calculate the expected aberration yield at a given postirradiation time.

The dose-response relationship of each aberration class for in vivo and in vitro exposures was determined by regressing aberrations per cell on a quadratic polynomial. Sum of squares due to linear regression and to quadratic after linear effects were determined and studied for their significance with respect to variation in number of aberrations per cell. The data were reanalyzed according to the model best describing the dose response. Utilizing the resulting components, equations were constructed that allowed the prediction of aberration yield as a function of dose as measured in air at the animal's midline. Such dosimetry enabled the least-squares regression line to be based upon actual dose measurements and not estimated values. This also facilitated the prediction of aberration yield from one exposure method to another.

In order to determine whether statistically significant differences existed between number of aberrations induced in vivo and in vitro, the respective regression coefficients were compared by an appropriate test.[1]

It is especially desirable to predict the in vivo aberration level from comparable in vitro data. A high correlation between the exposure methods enhances the accuracy of such predictions. Consequently, the regression of the number of aberrations per cell from in vivo exposures on in vitro values and the reciprocal regressions were considered.

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[1]"Statistical Analysis", August 1968, page 62, Olivetti Underwood, Programma 101.

Hematological data were evaluated by analysis of variance. The following mathematical model was deemed appropriate:

$$Y_{ijkl} = \mu + D_i + A_{j/i} + Tk + (DT)_{ik} + E_{ijkl}$$

where, Y_{ijkl} = the lth hematological observation, ith dose, jth animal within the ith dose, kth postirradiation time

μ = an effect common to all observations (mean)

D_i = effect due to the ith dose

$A_{j/i}$ = effect due to the jth animal in the ith dose

T_k = effect due to the kth postirradiation time

$(DT)_{ik}$ = effect due to the interaction between the ith dose and the kth postirradiation time

E_{ijkl} = random error

All F values, except that for the ith dose, were calculated by dividing the respective mean squares by the variation due to the interaction between the jth animal within the ith dose and the kth postirradiation time. The F value for the ith dose was obtained by dividing its mean square by the mean square associated with the jth animal in the ith dose.

CHAPTER IV

RESULTS

I. HEMATOLOGICAL CHANGES FOLLOWING IRRADIATION

A. Total Leukocytes

The results of the total leukocyte counts at each postirradiation sampling time are presented in Table 2. These data are also graphically depicted in Figure 1 as the average percent change in total leukocytes from the preexposure values. It is evident that total leukocytes decreased rapidly between 30 minutes and 24 hours postexposure at all dose levels. Subsequent losses of leukocytes occurred until 7 days following irradiation in all dose levels except 100 rads. The leukocyte counts in animals receiving 100 rads began to recover at 2 days postexposure, whereas higher doses effectively postponed total leukocyte recovery until after 14 days postirradiation.

Dose effects were more pronounced from exposures above 100 rads in that greater losses of leukocytes occurred with the higher doses. By 84 days, total leukocytes had recovered to preirradiation values following 100 or 200 rads, whereas recovery was incomplete in the higher-dose groups.

The analysis of variance of total leukocyte counts revealed highly significant ($P < .01$) effects of animals and sampling time, as well as an interaction between dose and sampling time. This interaction is illustrated by the inconsistency of dose effects over time postirradiation.

TABLE 2. Total leukocytes per mm^3 and differential cell determinations on peripheral blood samples obtained from pigs exposed to fission neutrons

Dose (rads)	Animal number		Preirradiation	Sampling time postirradiation							
				30 min	24 hr	48 hr	7d	14d	28d	56d	84d
100	60-3	TL[1]	10,600	12,300	7,765	9,530	9,020	9,950	10,025	13,800	11,800
		%N	47	74	53	60	42	53	49	44	56
		%L	50	25	45	36	54	44	47	50	42
		%E	3	1	2	4	4	3	4	6	2
100	54-9	TL	13,900	11,200	10,025	8,265	9,650	10,880	15,565	15,565	15,185
		%N	47	63	57	60	42	43	61	57	54
		%L	53	36	37	39	55	56	34	40	38
		%E	0	1	6	1	3	1	5	3	8
200	61-3	TL	12,290	12,790	9,145	6,900	6,700	6,600	12,915	12,040	11,400
		%N	48	66	52	57	41	54	58	54	56
		%L	48	34	45	40	59	46	39	44	42
		%E	4	0	3	3	0	0	3	2	2
200	62-4	TL	12,165	12,290	6,760	6,600	6,515	6,410	9,900	12,200	16,825
		%N	47	64	47	45	34	51	49	55	59
		%L	50	33	45	50	63	45	46	37	31
		%E	3	3	8	5	3	4	5	8	10
300	54-1	TL	12,915	14,175	10,150	7,500	7,800	9,525	9,775	9,525	11,200
		%N	43	65	72	58	55	59	64	47	52
		%L	54	34	25	35	44	40	35	47	43
		%E	3	1	3	7	1	1	1	6	5

TABLE 2 (continued)

Dose (rads)	Animal number		Preirradiation	Sampling time postirradiation							
				30 min	24 hr	48 hr	7d	14d	28d	56d	84d
300	57-10	TL	12,790	11,535	9,020	7,100	5,900	7,390	9,525	12,290	11,100
		%N	34	51	73	59	50	41	53	53	44
		%L	64	49	25	38	48	59	44	43	51
		%E	2	0	2	3	2	0	3	4	5
400	54-4	TL	9,200	13,600	8,390	6,385	5,300	9,270	10,405	7,390	9,050
		%N	39	73	70	76	55	44	61	37	24
		%L	61	27	28	20	45	56	35	58	60
		%E	0	0	2	4	0	0	4	5	16
400	70-4	TL	14,600	13,200	9,145	7,100	6,385	7,500	9,445	7,765	9,775
		%N	49	64	74	76	52	54	31	38	46
		%L	49	36	18	19	48	46	69	48	45
		%E	2	0	8	5	0	0	0	14	9

[1]TL = total leukocytes; N = neutrophils; L = lymphocytes; E = eosinophils.

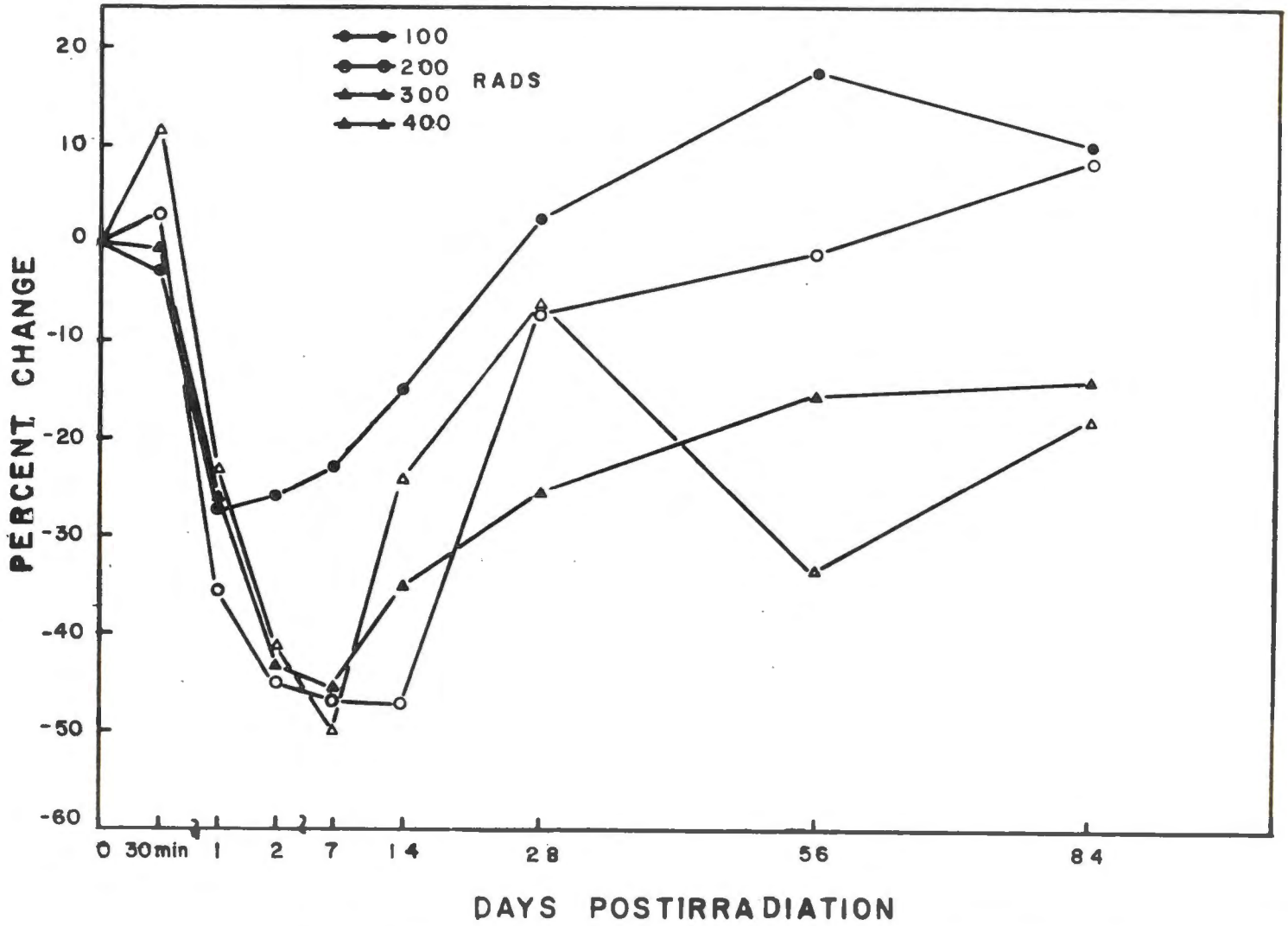

Figure 1. Percent change from preirradiation values in total leukocytes of pigs exposed to fission neutrons.

B. Lymphocyte Number

The values for percent lymphocytes as determined by differential cell analyses are given in Table 2.

The percent decrease, from preirradiation values, in lymphocyte number was plotted as a function of time postirradiation (Figure 2). It is evident that lymphocytes were rapidly disappearing from the peripheral system within 30 minutes following exposure. Subsequent sampling demonstrated that lymphocytes were still being removed through the second day and that the percent decrease was associated with dose when measured at 2 days postexposure. At all dose levels the nadir for lymphocyte number occurred at 2 days following irradiation. Recovery in the number of lymphocytes was evident beyond this time but was still incomplete at 84 days postirradiation.

Statistical analysis revealed that lymphocyte number was significantly influenced ($P < .01$) by variation due to animals, sampling time, and the interaction between dose and sampling time. Dose effects were nonsignificant. The degree of relationship between lymphocyte number and the independent variables was 0.939. This indicates that the components of the mathematical model accounted for a large proportion of the variation in lymphocyte number.

C. Neutrophil Number

The data for percent neutrophils observed at the various sampling times are presented in Table 2 and are plotted in Figure 3 as the percent change from preexposure values. At 30 minutes postirradiation an increase in neutrophil number had occurred which appeared to be influenced by dose. This increase probably accounted for the elevated total

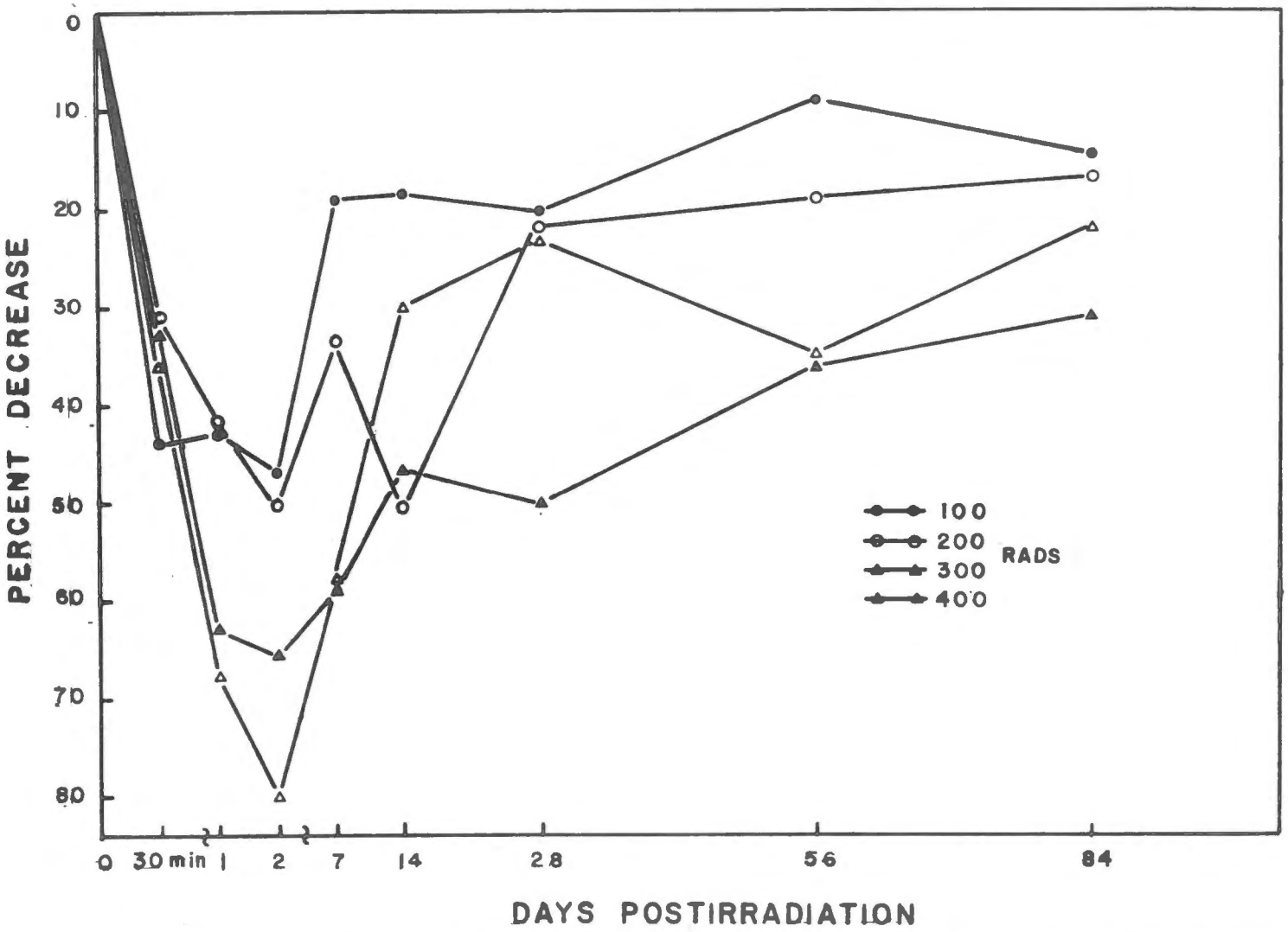

Figure 2. Percent decrease from preirradiation values in lymphocyte number of pigs exposed to fission neutrons.

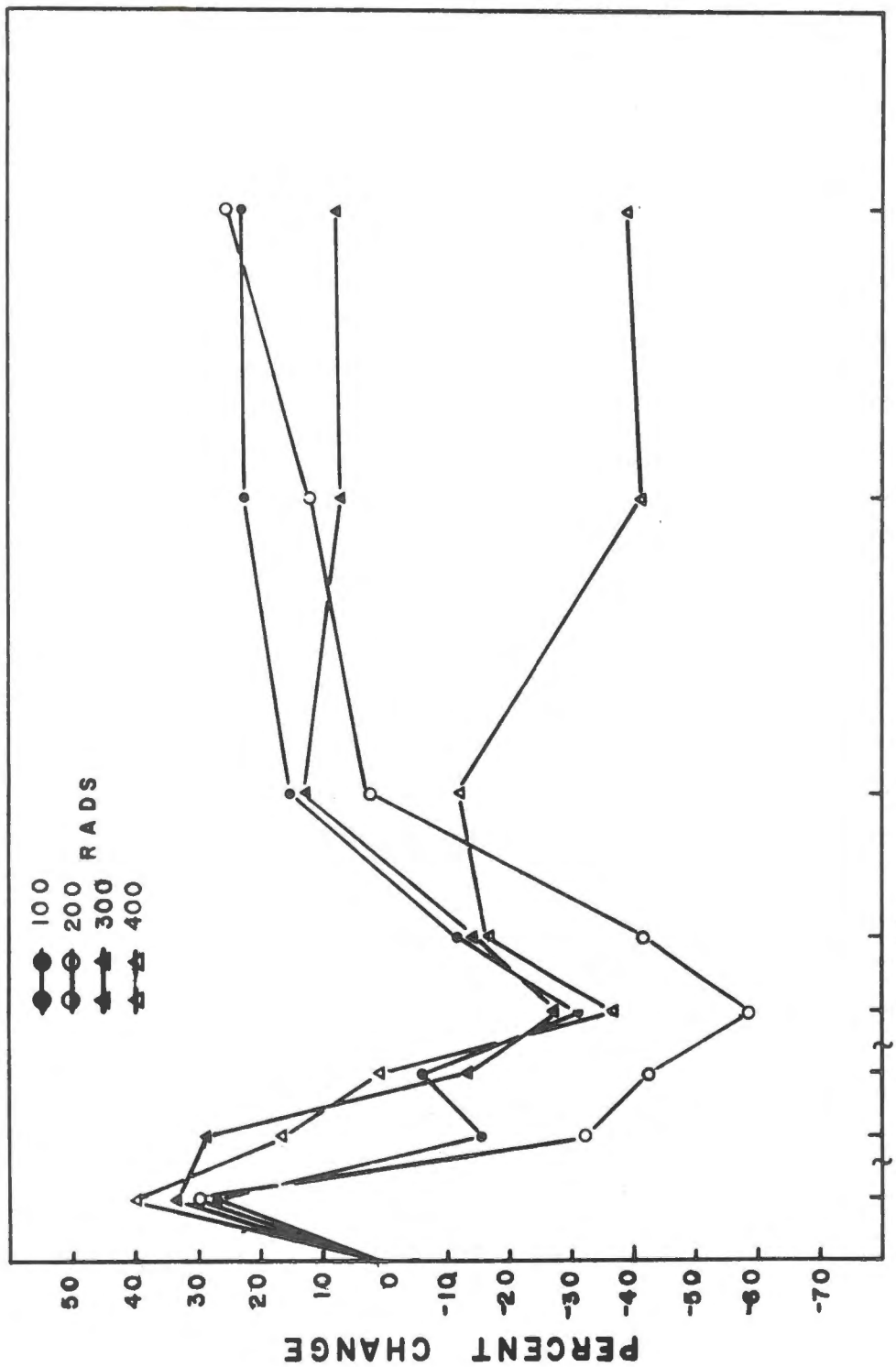

Figure 3. Percent change from preirradiation values in neutrophil number of pigs exposed to fission neutrons.

leukocyte counts in the 30-minute samples following 200 and 400 rads. Subsequently, the neutrophil number decreased to a nadir at 7 days for all dose levels. They began to increase thereafter and exceeded pre-irradiation levels by 28 days postexposure except in animals receiving 400 rads. At 84 days the latter group remained approximately 39% below their preirradiation values.

Variation in neutrophil number was significantly influenced ($P < .01$) by animals, sampling time and an interaction between dose and sampling time. These results are similar to those obtained for total leukocytes and lymphocyte number as was the finding of a nonsignificant dose effect. Also, a high degree (0.895) of association was noted between the independent variables and neutrophil number.

Since the effects of dose were statistically nonsignificant in all cases for the various hematological classes, the data from all doses were averaged in order to describe the relative change in peripheral blood cells as a function of time postirradiation. It is evident in Figure 4 that irradiation caused decreases in the numbers in various classes of leukocytes which later recovered at different rates. Irradiation caused a marked reduction in lymphocyte numbers by 30 minutes postexposure, whereas the number of neutrophils responded with a sharp increase during this same period. The net result of these changes was a slight elevation in the total number of leukocytes during this time period. The nadirs for both total leukocytes and neutrophil number occurred at 7 days postexposure, whereas lymphocytes reached their lowest value at 2 days following irradiation. Complete recovery of neutrophil numbers to the preirradiation value took place between 1 and 4 weeks postexposure in all dose levels except 400 rads. Although recovery of lymphocyte

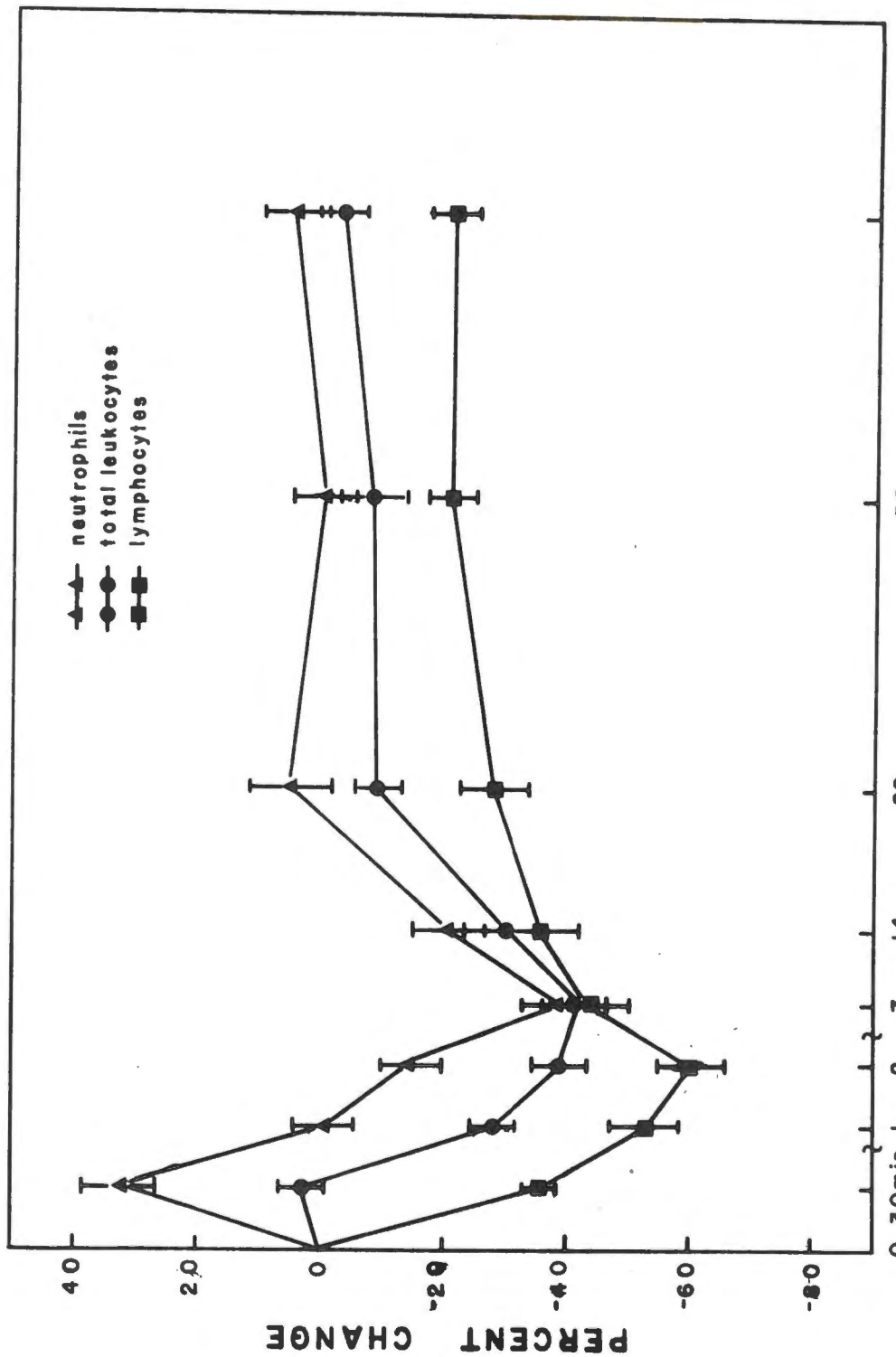

Figure 4. Relative change in total number of peripheral blood cells of pigs exposed to fission neutrons (mean ± S.E.).

numbers began somewhat earlier, the numbers had not returned to normal levels by 84 days. It is obvious from Figure 4 that irradiation had a long-term suppressive effect on the number of lymphocytes but not on the number of neutrophils.

II. IN VIVO - IN VITRO ABERRATION RATES

A. Comparison for Equal Air Doses

In vivo dose response data were obtained from blood samples drawn approximately 30 minutes postexposure, whereas the in vitro data were secured from preirradiation blood samples which had been exposed to comparable doses as measured in air at the animal's midline. In order to determine the dose response kinetics for aberrations induced by each exposure method, the data were tested for goodness of fit of both linear and quadratic models.

The aberration coefficients from the two animals at each dose were pooled when calculating the least-squares regression lines. This was justified by the fact that the variation in aberrations per cell among the animals at each dose was not significantly ($P > .05$) different from the variation within replicate samples from the same animal.

Since the preirradiation or spontaneous aberration coefficients were not included in the regression analyses, no inference is made regarding the prediction of aberration yields for doses lower than 100 or higher than 400 rads (air-dose). The points at which the regression lines transected the ordinates in the prediction equations is merely an extrapolation of the least-squares regression lines between 100 and 400 rads based upon a linear response. The analyses revealed, however, that the calculated Y-intercepts did not differ statistically ($P > .05$) from zero.

This implies that a significant difference could not be detected between the calculated Y-intercepts and the aberration levels found in the preirradiation samples.

The statistical analyses and the comparisons between exposure methods were based upon dose measurements made in air above the animal's midline. This allowed the calculations to be derived from actual dose measurements which can be easily reproduced. Estimates of the average body dose are discussed later and would allow a more accurate determination of the dose actually received by the blood cells. The use of the average body dose estimates in order to calculate expressions for aberration dose response would require, however, that the statistical analyses be based upon estimated values rather than actual dose measurements. Consequently, it is believed that the comparisons between exposure methods are more meaningful and more readily extrapolated to other data when derived from exposures measured in air.

Acentric Fragments

A linear response was found for both types of exposures when acentric fragments per cell were plotted over varying dose levels (Figure 5). The equations which best describe this response are $\hat{Y} = 17.5 \times 10^{-3} + 1.50 \times 10^{-3}$ (D) for in vivo and, $\hat{Y} = -60.0 \times 10^{-3} + 5.72 \times 10^{-3}$ (D) for in vitro exposures. Table 3 contains the aberration levels actually observed under both conditions of irradiation and the predicted estimates obtained by incorporating the administered dose levels into the above formulas. The preirradiation level of acentric fragments ranged from 2×10^{-2} to 4×10^{-2} per cell and was not significantly different ($P > .05$) from the predicted values at zero dose for both types of exposure.

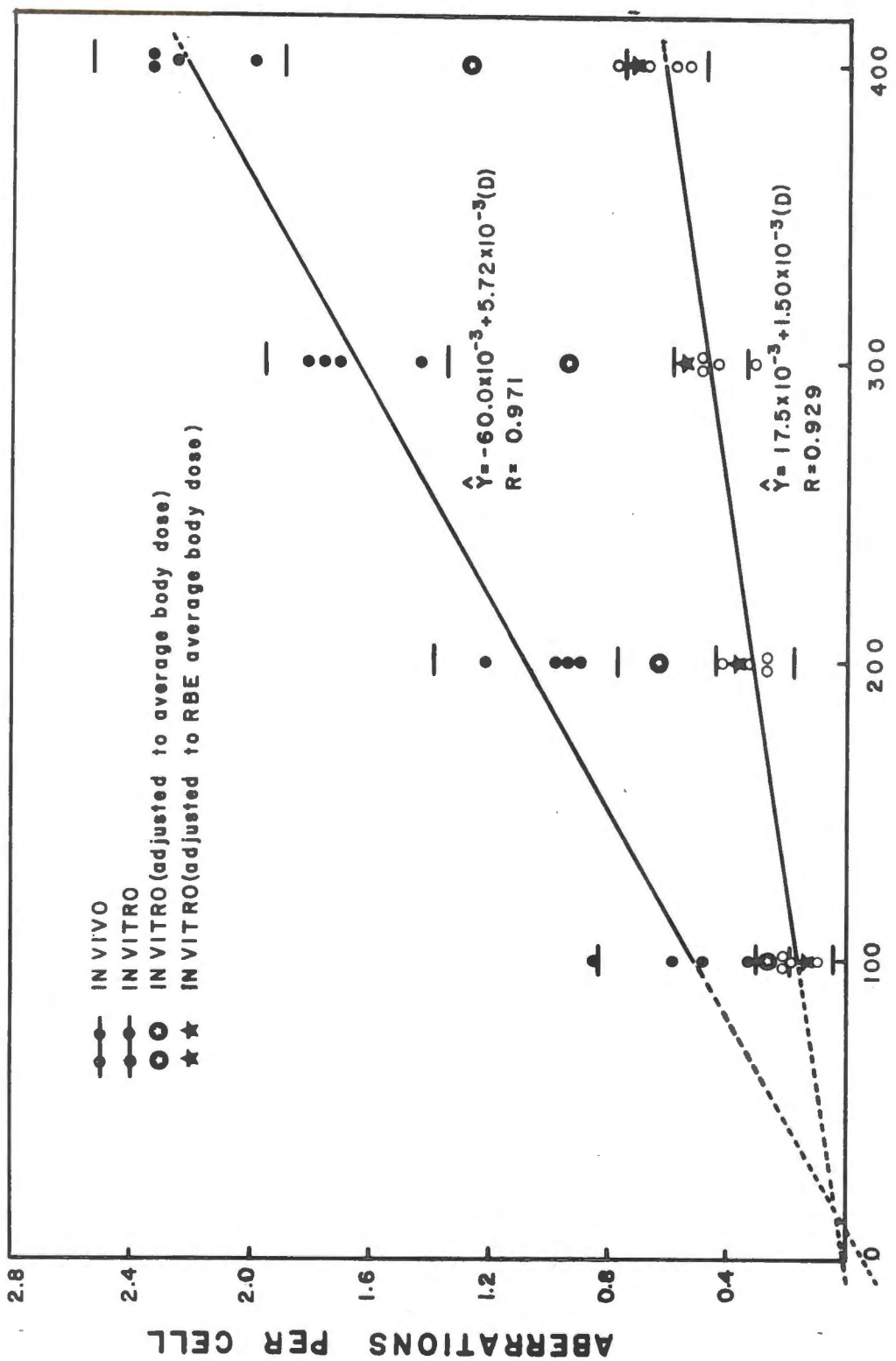

Figure 5. Dose-response curves for acentric fragments among pig leukocytes after in vivo or in vitro exposure to fission neutrons (90% C.L.).

TABLE 3. Observed and predicted ($\hat{Y}$) aberrations in pig leukocytes sampled 30 min after exposure to fission neutrons[1]

Animal number	Preirradiation[2]	Dose (rads)	Acentric fragments		Rings		Dicentrics		Rings + dicentrics		Acentric fragments + rings + dicentrics	
			Vivo	Vitro	Vivo	Vitro	Vivo	Vitro	Vivo	Vitro	Vivo	Vitro
60-3	0.01	100	0.16	0.84	0.06	0.22	0.02	0.72	0.08	0.94	0.24	1.78
	0.00		0.20	0.48	0.04	0.30	0.08	0.68	0.12	0.98	0.32	1.46
54-9	0.01		0.18	0.32	0.02	0.40	0.08	0.58	0.10	0.98	0.28	1.30
	0.02		0.20	0.58	0.06	0.38	0.10	0.72	0.16	1.10	0.36	1.68
		$\hat{Y}$ =	0.168	0.512	0.034	0.354	0.076	0.660	0.109	1.014	0.277	1.525
61-3	0.02	200	0.34	0.94	0.02	0.64	0.18	1.32	0.20	1.96	0.54	2.90
	0.00		0.36	1.22	0.10	0.88	0.08	1.26	0.18	2.14	0.54	3.36
62-4	0.01		0.28	0.90	0.04	0.86	0.16	1.02	0.20	1.88	0.48	2.78
	0.03		0.28	0.96	0.04	0.56	0.22	1.20	0.26	1.76	0.54	2.72
		$\hat{Y}$ =	0.318	1.083	0.065	0.716	0.151	1.227	0.216	1.942	0.533	3.025
54-1	0.04	300	0.44	1.82	0.08	1.26	0.16	1.70	0.24	2.96	0.68	4.78
	0.00		0.46	1.72	0.06	1.10	0.32	1.88	0.38	2.98	0.84	4.70
57-0	0.02		0.46	1.44	0.08	1.04	0.18	1.92	0.26	2.96	0.72	4.40
	0.02		0.32	1.74	0.14	1.10	0.24	1.70	0.38	2.80	0.70	4.54
		$\hat{Y}$ =	0.468	1.655	0.096	1.077	0.227	1.794	0.322	2.871	0.790	4.525

TABLE 3 (continued)

Animal number	Preirradiation	Dose (rads)	Acentric fragments		Rings		Dicentrics		Rings + dicentrics		Acentric fragments + rings + dicentrics	
			Vivo	Vitro	Vivo	Vitro	Vivo	Vitro	Vivo	Vitro	Vivo	Vitro
54-4	0.01	400	0.54	2.26	0.08	1.46	0.30	2.24	0.38	3.70	0.92	5.96
	0.01		0.70	2.00	0.14	1.54	0.40	2.20	0.54	3.74	1.24	5.74
70-4	0.03		0.78	2.34	0.14	1.30	0.22	2.42	0.36	3.72	1.14	6.06
	0.00		0.58	2.34	0.18	1.30	0.28	2.60	0.46	3.90	1.04	6.24
		$\hat{Y}$ =	0.618	2.226	0.127	1.439	0.302	2.361	0.429	3.80	1.046	6.025

[1]Each entry represents the aberrations per cell in 50 metaphases.

[2]Acentric fragments per cell in 100 metaphases (the level of rings and dicentrics was zero in all animals).

Relatively high correlation coefficients of 0.929 and 0.971 were obtained for in vivo and in vitro exposures with dose, respectively. Comparisons between the different modes of irradiation were therefore justified. This was accomplished by statistically evaluating whether differences existed between the regression coefficients. Analysis revealed that a statistically significant ($P < .01$) difference existed between in vivo and in vitro exposures. Such differences were probably influenced by dose attenuation and the relative biological effectiveness of the neutron component of fission neutrons between exposure methods.

It was highly desirable to be able to obtain estimates of the in vivo response based upon a knowledge of the in vitro aberration coefficients. The correlation coefficient of 0.89 between in vivo and in vitro exposures for acentric fragments indicated a high degree of reliability in the prediction of the response to either type of irradiation when the response to the other was known (Table 4). The expression for predicting the in vivo aberration yield ($\hat{Y}$) based upon a known in vitro response (X) is $\hat{Y} = 5.65 \times 10^{-2} + 24.55 \times 10^{-2}$ (X). On the other hand, an estimate of the in vitro aberration coefficient can be obtained from in vivo data by substituting the in vivo response for (X) in $\hat{Y} = 8.59 \times 10^{-2} + 326.85 \times 10^{-2}$ (X). It was obvious that the regression coefficient for the in vitro exposures was greater than that for in vivo irradiation. This difference was statistically significant ($P < .01$). Reliability in the prediction of these values is most effective when both types of exposures are performed on each animal in a randomly selected group.

TABLE 4. Prediction equations for aberrations per cell in pig leukocytes exposed to fission neutrons in vivo and in vitro

	Correlation coefficient	t^2	Degrees of freedom	P
Acentric fragments				
$\hat{Y}$ (in vivo) = $5.65 \times 10^{-2} + 24.55 \times 10^{-2}$ (X)[1]				
	0.89	9.17	28	< .01
$\hat{Y}$ (in vitro) = $8.59 \times 10^{-2} + 326.85 \times 10^{-2}$ (X)[3]				
Rings + dicentrics				
$\hat{Y}$ (in vivo) = $-0.32 \times 10^{-2} + 11.30 \times 10^{-2}$(X)				
	0.90	10.83	28	< .01
$\hat{Y}$ (in vitro) = $46.35 \times 10^{-2} + 722.88 \times 10^{-2}$ (X)				
Acentric fragments + rings + dicentrics				
$\hat{Y}$ (in vivo) = $2.88 \times 10^{-2} + 16.75 \times 10^{-2}$ (X)				
	0.95	15.20	28	< .01
$\hat{Y}$ (in vitro) + $20.93 \times 10^{-2} + 539.24 \times 10^{-2}$ (X)				

[1](X) is aberrations per cell in vitro.

[2]t test for differences between regression coefficients.

[3](X) is aberrations per cell in vivo.

Rings Plus Dicentrics

The response of ring plus dicentric induction over dose levels was linear for both in vivo and in vitro irradiation (Figure 6). A nine-fold difference was evident between exposure regimes with higher aberration levels being found following in vitro exposures. None of this class of aberrations were observed in the preirradiation blood samples.

The calculated "best fit" expression for rings and dicentrics induced in vivo was $\hat{Y} = 2.50 \times 10^{-3} + 1.06 \times 10^{-3}$ (D), whereas that for the in vitro response was $\hat{Y} = 85.0 \times 10^{-3} + 9.29 \times 10^{-3}$ (D) (Figure 6). The point at which the least-squares regression line transected the ordinate was not significantly different ($P > .05$) from zero for both methods of exposure. The slopes of these regression lines were statistically different ($P < .01$). Table 3 contains the observed and predicted ($\hat{Y}$) aberration coefficients for each type of exposure at all dose levels as calculated from the above equations.

A correlation coefficient of 0.90 was obtained between rings and dicentrics induced in vivo and in vitro (Table 4). Coefficients of this magnitude enhance the validity of prediction equations between the different types of exposure. The predicted response of rings and dicentrics in vivo derived from a knowledge of the in vitro response is $\hat{Y} = -0.32 \times 10^{-2} + 11.30 \times 10^{-2}$ (X), where X is the in vitro aberration coefficient. Estimates of the in vitro aberration level based upon in vivo data were calculated by substituting the in vivo results into $\hat{Y} = 46.35 \times 10^{-2} + 722.88 \times 10^{-2}$ (X) and solving for $\hat{Y}$, (Table 4). The two regression coefficients differed statistically from each other ($P < .01$).

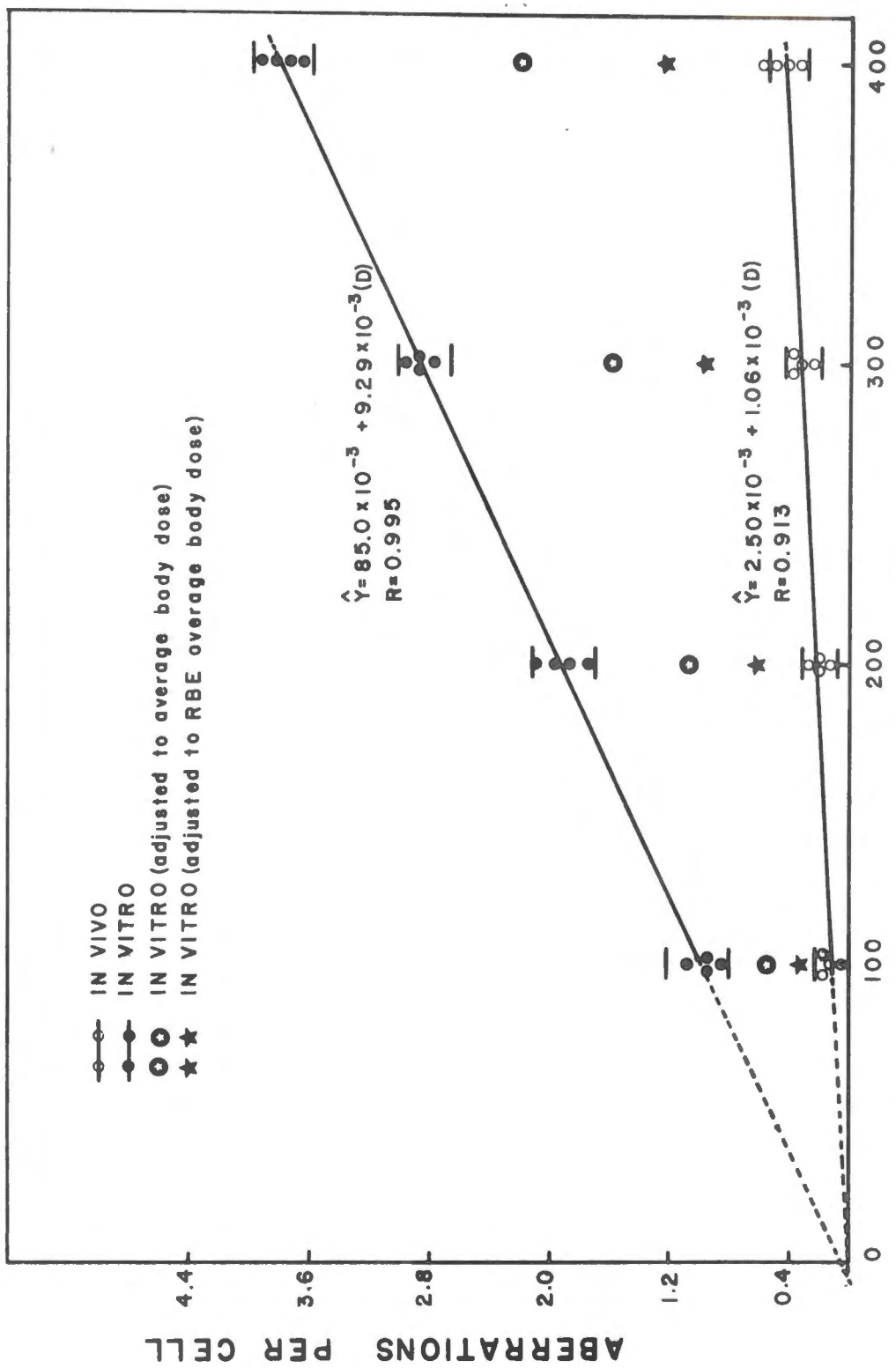

Figure 6. Dose-response curves for rings plus dicentrics among pig leukocytes after in vivo or in vitro exposure to fission neutrons (90% C.L.).

Total Aberrations

This category consisted of the additive effects of acentric fragments, rings and dicentrics. Since each of the aberration types included in the group displayed a linear dose response, their values were pooled in order to obtain equations which describe the change in the number of total-scorable asymmetrical aberrations as a function of dose. Figure 7 displays the kinetics of total aberrations as a function of neutron dose. A linear response for both in vivo and in vitro data was obtained from the regression analyses. Following in vivo exposures, total aberrations increased as $\hat{Y} = 20.0 \times 10^{-3} + 2.56 \times 10^{-3}$ (D) where D represents the dose measured in air at the animal's midline. With in vitro irradiation they increased as $\hat{Y} = 25.0 \times 10^{-3} + 15.0 \times 10^{-3}$ (D). The rate of aberration change was statistically different ($P < .01$) between the different methods of exposure. Table 3, page 53, contains the observed and predicted ($\hat{Y}$) total aberrations per cell for the various doses.

Calculation of the in vivo aberration level from in vitro data followed the form of $\hat{Y} = 2.88 \times 10^{-2} + 16.75 \times 10^{-2}$ (X) where X is the in vitro aberration coefficient (Table 4). Similarly, the predicted in vitro response was found by $\hat{Y} = 20.93 \times 10^{-2} + 539.24 \times 10^{-2}$ (X) if the in vivo data were substituted for (X). The degree of relationship between the in vivo and in vitro exposures was 0.95, and the regression coefficients differed significantly ($P < .01$).

It was evident that the in vitro exposures resulted in a higher rate of aberration induction when compared with similar doses of in vivo irradiation. Since the dose was expressed as energy deposited in air at the animal's midline, dose attenuation and tissue interaction of neutrons would have accounted for a large proportion of the differences observed.

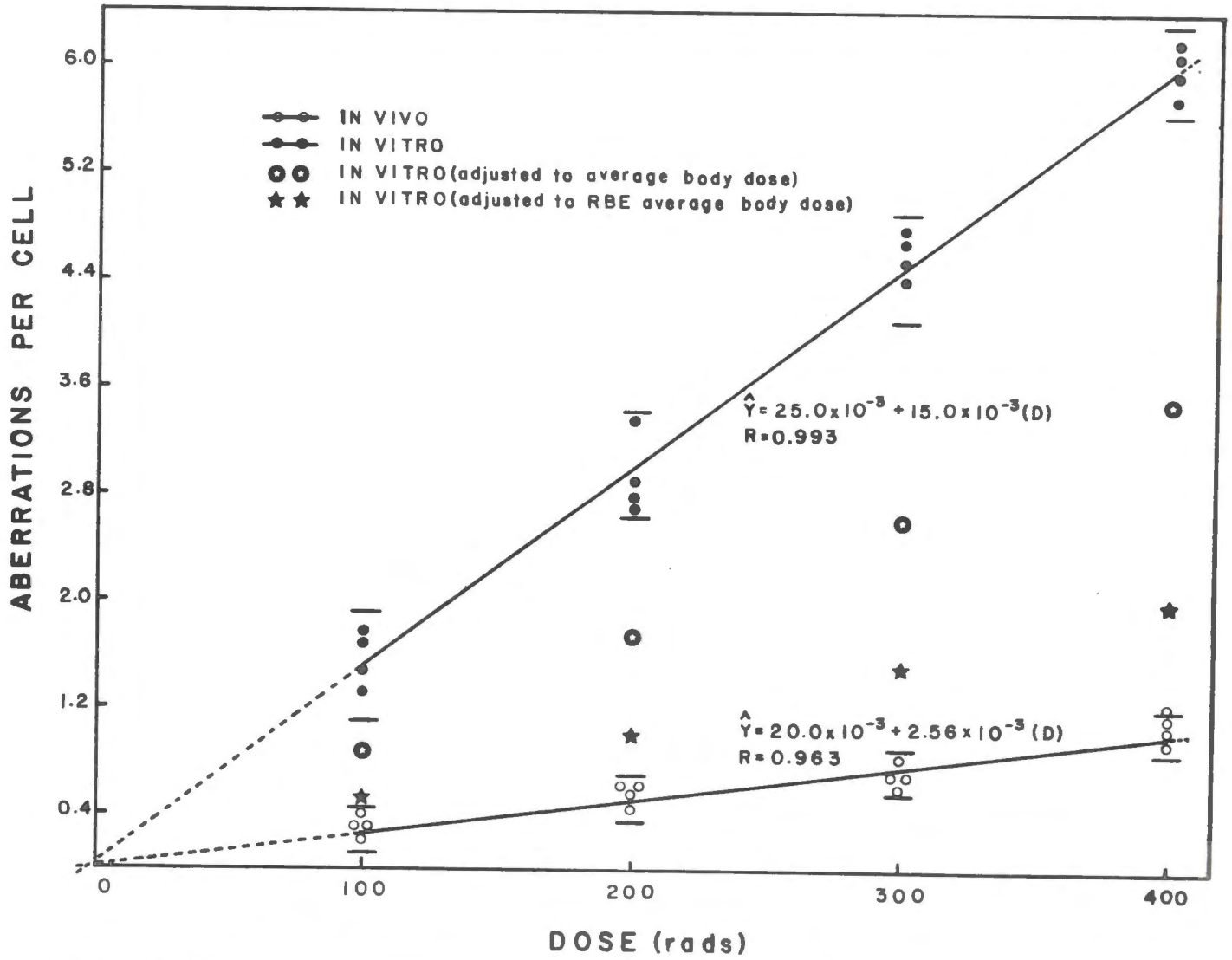

Figure 7. Dose-response curves for total aberrations among pig leukocytes after in vivo or in vitro exposure to fission neutrons (90% C.L.).

B. Estimation of Actual Tissue Dose and Subsequent In Vivo - In Vitro Comparison

Information on the average body dose following in vivo exposures would allow more precise estimates of the dose actually received by the leukocytes. In an attempt to correct for this tissue-interaction effect, estimates of the mean dose actually received by the leukocytes under in vivo conditions were made. The mean tissue dose calculated for a bilateral exposure to fission neutrons was based upon unilateral dose distributions measured at 2-cm depth intervals within a tissue-equivalent phantom. The dose determinations at various tissue depths for the neutron and gamma energy components of the fission-spectrum reactor are presented in Table 5. Since the doses had been measured at the animal's midline, it was necessary to calculate, by inverse square, the dose at the body surface so that tissue depth-dose calculations could be made. For a 100-rad midline fission neutron exposure, these surface doses were 112 rads of neutrons and 19 rads of gamma radiation.

Since there was a relatively large contribution due to gamma rays in the average whole-body dose (Table 5), it would be expected that the in vivo dose-response kinetics for rings plus dicentrics would have a curvilinear component (7, 43). Although a quadratic component was found in the present data, it was statistically nonsignificant ($P > .05$). The second method for estimating whole-body dose was expressed as the volume average dose for a 100-rad fission neutron exposure measured in air at the animal's midline (Table 6). The volume average whole-body dose of 60.89 rads was obtained by summing the volume average dose in each of the seventeen concentric circles within a 34-cm diameter cylinder.

TABLE 5. Estimates of whole-body dose (method I)[1]

Distance inside animal (cm)	Unilateral[2] neutron dose (%)		Bilateral neutron dose (%)	Neutron dose[3] for 100 rad midline exposure	Unilateral[2] gamma dose (%)		Bilateral gamma dose (%)	Gamma dose[4] for 100 rad midline exposure
0	100.0	9.4	54.7	61.3	200.0	54.0	127	24
2	83.0	6.8	44.9	50.2	240.0	60.8	150	29
4	59.0	5.3	32.1	36.0	270.0	68.7	169	32
6	41.5	4.4	23.0	25.8	270.0	77.8	174	33
8	29.8	4.1	16.9	18.9	250.0	87.5	169	32
10	21.5	4.1	12.8	14.3	222.0	97.0	160	30
12	15.7	4.6	10.2	11.4	198.0	111.0	155	29
14	11.7	5.5	8.6	9.6	176.0	125.0	151	29
16	8.9	6.8	7.9	8.8	158.0	140.0	149	28
18	6.8	8.9	7.9	8.8	140.0	158.0	149	28
20	5.5	11.7	8.6	9.6	125.0	176.0	151	29
22	4.6	15.7	10.2	11.4	111.0	198.0	155	29
24	4.1	21.5	12.8	14.3	97.0	222.0	160	30
26	4.1	29.8	16.9	18.9	87.5	250.0	169	32
28	4.4	41.5	23.0	25.8	77.8	270.0	174	33
30	5.3	59.0	32.1	36.0	68.7	270.0	169	32
32	6.8	83.0	44.9	50.2	60.8	240.0	150	29
34	9.4	100.0	54.7	61.3	54.0	200.0	127	24
Average				26.3				29.6

[1]Data from J. W. Poston.

[2]Dose calculated from actual measurements in each half of a tissue-equivalent phantom proximal and distal to the reactor respectively.

[3]Neutron dose to side of animal ≃ 112 rads.

[4]Gamma dose to side of animal ≃ 19 rads.

TABLE 6. Estimates of whole-body dose (method II)[1]

Radius 1	Radius 2	Area (cm^2)	Fraction of total area (cm^2) [2]	Neutron dose for 100 rad exposure	Gamma dose for 100 rad exposure	Total dose (rads)	Volume average dose (rads)[3]
17	16	103.7	.114	55.8	26.5	82.3	9.38
16	15	97.4	.107	50.2	29.0	79.2	8.47
15	14	91.1	.100	43.2	30.5	73.7	7.37
14	13	84.8	.093	36.0	32.0	68.0	6.32
13	12	78.5	.086	30.9	32.5	63.4	5.45
12	11	72.3	.080	25.8	33.0	58.8	4.70
11	10	66.0	.073	22.4	32.5	54.9	4.01
10	9	59.7	.066	18.9	32.0	50.9	3.36
9	8	53.4	.059	16.6	31.0	47.6	2.81
8	7	47.1	.052	14.3	30.0	44.3	2.30
7	6	40.8	.045	12.9	29.5	42.4	1.91
6	5	34.6	.038	11.4	29.0	40.4	1.54
5	4	28.3	.031	10.5	29.0	39.5	1.22
4	3	22.0	.024	9.6	29.0	38.6	0.93
3	2	15.7	.017	9.2	28.5	37.7	0.64
2	1	9.4	.010	8.8	28.0	36.8	0.37
1	0	3.1	.003	8.8	28.0	36.8	0.11
Sum							60.89

[1]Data from J. W. Poston

[2]Total area of a 34 cm diameter cylinder = 907.96 cm^2.

[3]100 rad exposure measured in air at the animal's midline.

The estimates of whole-body dose obtained by considering the dose measured at various intervals within a phantom (method 1) and that determined by partitioning a 34-cm diameter cylinder into concentric cylinders and weighting the dose by the volume of the cylindrical rings (method 2) agreed well (Table 7). The average of both methods, indicated that approximately 58% of that dose measured in air at the midline of the animal was actually received by the average blood cell in the animal (Table 7).

Since the in vitro blood samples were positioned so that they would receive a dose equivalent to that measured in air at the animal's midline, it would be expected from method 1 that the average dose received by the leukocytes in vivo was approximately 56% of that received in vitro. Should the disparity between the aberration coefficients obtained from the different methods of exposure be due entirely to variation in energy deposited throughout the body, correction for these considerations should produce similar biological responses. This, however, was not the situation with respect to lymphocyte chromosome aberrations (Figures 5, page 52; 6, page 58; 7, page 60). The expected number of in vitro aberrations was obtained by reducing the predicted in vitro value by 56%. The in vitro aberration data was adjusted rather than the in vivo data because several assumptions would be required had the in vivo aberration levels been expressed as a function of the average whole-body dose. The first assumption being that the aberration levels of each of the exposure methods could be adequately compared at different doses and secondly, that the dose response below 100 rads is linear. When these expected in vitro values were compared with the in vivo levels, fewer aberrations were still found with in vivo exposures

TABLE 7. Estimates of whole-body dose at various exposures[1]

Fission neutron dose (rad)[2]	Method I			Method II
	Average whole-body neutron dose (rad)	Average whole-body gamma dose (rad)	Total average whole-body dose (rad)	Volume average whole-body dose (rad)
100	26	30	56	61
200	53	59	112	122
300	79	89	168	183
400	105	118	223	244

[1]Data from J. W. Poston.

[2]Midline exposure measured in air.

(Figures 5, 6, 7). This indicated that variation in dose was not the only factor influencing these data. Other mechanisms must be sought to account for such differences. The dose considerations discussed so far fail to take into account the relative biological effectiveness (RBE) of the neutron and gamma ray components of the fission-neutron spectrum. Based upon other data (50, 85, 86), an RBE of four for chromosome aberrations was used to adjust the neutron component. Should the actual RBE be as high as 20, the ratio of in vivo to in vitro dose would be 0.28. This demonstrates that the use of different RBE values would not materially affect the estimated average body doses. This was accomplished by multiplying the average whole-body neutron dose (method 1, Table 7) by four and then adding the average whole-body gamma dose. When this was done for both in vivo and in vitro exposures, approximately 32% of the in vitro dose was estimated to be the amount actually received by the animal's blood cells. The predicted in vitro aberration dose response data was therefore reduced by 32% and plotted in Figures 5, 6, 7.

It was found that the acentric-fragment data agreed quite well between exposure methods when the RBE of the neutron component of fission neutrons was considered (Figure 5). The proportion of rings plus dicentrics per cell, however, was still higher following in vitro irradiation (Figure 6). The fact that a nine-fold difference existed between exposure methods for rings plus dicentrics and about a three and one-half-fold difference for acentric fragments is a result which explains the dissimilarity between irradiation methods with respect to total aberrations (Figure 7).

III. CHROMOSOME ABERRATION PERSISTENCE AS A FUNCTION OF TIME POSTIRRADIATION

Since aberrations were being studied in peripheral blood lymphocytes, it is necessary to consider whether the origin of the cells scored might have an influence on the observed aberration levels. Different lymphocyte populations coexist in the peripheral system following irradiation. The rapid elimination of lymphocytes presumably represents cells which were damaged by irradiation in the peripheral system, whereas the recovery of lymphocyte numbers must be due to cells which were in lymphopoietic centers at the time of exposure. The transition between the decrease and subsequent increase in lymphocyte number occurred between 2 and 7 days postirradiation, and it was believed that this marked the time at which the second population began to exert an influence on the aberration levels obtained. Consequently, the aberration data were analyzed separately according to whether the samples were taken prior to or during lymphocyte recovery.

Although the 2-day samples were of particular interest, they could not be included in the final analysis due to poor culture growth and low lymphocyte numbers (Figure 2, page 46). This resulted in insufficient numbers of metaphases being available for analysis and inclusion of the available data would have resulted in biased estimates of statistical parameters.

A. Aberration Losses Prior to Recovery of Lymphocyte Number

Blood samples obtained 30 minutes postexposure contained a significantly ($P < .01$) higher proportion of cells with acentric fragments

than did samples secured 24 hours following irradiation (Table 8). The analysis of variance revealed significant ($P < .01$) dose effects as well as significant ($P < .05$) variation among animals, while interaction effects were nonsignificant. A multiple correlation coefficient of 0.966 is an indication of the adequacy of the mathematical model in describing the variation in acentric fragments.

Although more acentric fragments were induced by the higher dose levels, their rate of disappearance from the peripheral blood was lower than that noted for the 100-rad exposure. This may be explained, in part, by the more rapid rate of loss in lymphocyte number at 100 rads (Figure 2, page 46).

The level of rings plus dicentrics scored in the 30-minute samples ranged from 0.115 per cell at 100 rads to 0.435 per cell at 400 rads. These aberrations also decreased more rapidly in the 100 rad samples than at higher doses (Table 8).

An analysis of variance indicated that rings and dicentrics were significantly ($P < .01$) influenced by both dose and sampling time. The observed variation was not significantly influenced by animal or interaction effects. A high degree of association ($R = 0.935$) existed between rings plus dicentrics and the independent variables.

If the aberration data were plotted as a function of dose, different kinetics would be observed, depending upon whether the 30-minute or the 24-hour sampling times were utilized. The data from the earlier samples demonstrated linear kinetics, whereas a non-linear dose response was noted from samples obtained 24 hours postexposure. Such variation indicated that aberration coefficients and dose response kinetics were influenced by sampling time postirradiation.

TABLE 8. Aberration yields in pig leukocytes prior to recovery of lymphocyte number

Time post-irradiation	Acentric fragments per 100 metaphases — Dose (rads)			
	100	200	300	400
30 min	18.5[1]	31.5	42.0	65.0
24 hr	6.0	16.0	19.0	52.0
% decrease	68.0	49.0	55.0	20.0
Time post-irradiation	**Rings plus dicentrics per 100 metaphases — Dose (rads)**			
	100	200	300	400
30 min	11.5	21.0	31.5	43.5
24 hr	2.0	14.0	18.0	21.5
% decrease	83.0	33.0	43.0	50.0

[1]Each entry is an average of the values from two animals and represents 200 metaphases.

B. Aberration Losses During Recovery of Lymphocyte Number

In order to obtain a reliable picture of aberration persistence kinetics from 1-to-12 weeks postirradiation, the data from each dose were subjected to regression analyses. The response of the aberration level as a function of time postexposure was tested for "goodness of fit" of the following models:

$$Y = a + bX + e$$

$$Y = a + bX + cX^2 + e$$

$$Y = a + bX + cX^2 + dX^3 + e$$

where Y = the predicted aberration coefficient.

a = the aberration coefficient at 1 week postirradiation.

b, c, d = the calculated aberration coefficients for linear, quadratic, and cubic components, respectively.

X = time postirradiation.

e = random error.

After the expression which best described the response was obtained, the data were reanalyzed according to this model and the resulting coefficients were utilized in the form of regression equations. This allowed the development of formulas for the prediction of aberration yield ($\hat{Y}$) for a given postirradiation time (X).

Acentric Fragments

The least-squares equations which best described the response of acentric fragments from 1-to-12 weeks postexposure were:

100 rads: $\hat{Y} = 68.21 \times 10^{-3} - 4.38 \times 10^{-3} (X)$.

200 rads: $\hat{Y} = 119.61 \times 10^{-3} - 8.08 \times 10^{-3} (X)$.

300 rads: $\hat{Y} = 249.40 \times 10^{-3} - 47.56 \times 10^{-3} (X) + 2.89 \times 10^{-3} (X)^2$.

400 rads: $\hat{Y} = 327.37 \times 10^{-3} - 55.02 \times 10^{-3} (X) + 3.20 \times 10^{-3} (X)^2$ where X is weeks postirradiation.

Tables 9-12 contain the components of the various models tested as well as the results of significance tests for each dose level. Predicted aberration yields ($\hat{Y}$) are compared to those actually observed for the various doses in Tables 13-16.

When the number of acentric fragments per cell was subjected to an analysis of variance, it was found that both dose and sampling time had a significant ($P < .01$) effect upon these aberrations as did the interaction between dose and time ($P < .05$). Other variables were statistically non-significant, and the degree of relationship between acentric fragments and the independent components of the model was 0.915.

It was noted that as dose increased, a more prominent quadratic component appeared in the descriptive equations. This resulted from an increasing number of lymphocytes containing acentric fragments appearing in the peripheral blood between 56 and 84 days postexposure. Such lymphocytes were probably derived from mature cells which were in lymphoreticular organs and were just then being released into the peripheral system. Figure 2, page 46, shows that lymphocyte numbers were generally higher in the last two sampling periods as compared with earlier postirradiation intervals.

Rings Plus Dicentrics

The ring-plus-dicentric data from 100 and 200 rads failed to fit either of the three models tested as determined by an F test (Tables 9 and 10). This failure could have been due to a greater sampling error resulting from the relatively low numbers of these aberrations at these

TABLE 9. Regression analyses of chromosome aberrations in peripheral blood of pigs from 1-12 weeks after 100 rads of fission neutrons

Source	F	P of > F	R	a ($\times 10^{-3}$)	b ($\times 10^{-3}$)	t for testing Ho : b = o	P of >|t|
	Acentric fragments						
Linear	9.54	0.0070	0.612	68.21	- 4.38	- 3.09	0.0070
Linear	9.03	0.0087	0.616	72.10	- 6.55	- 1.11	0.2857
Quadratic	0.14	0.7111			0.18	0.38	0.7111
Linear	8.45	0.0112			- 3.84	- 0.22	0.8228
Quadratic	0.13	0.7199	0.616	69.06	- 0.35	- 0.11	0.9119
Cubic	0.03	0.8651			0.03	0.16	0.8651
	Rings + dicentrics						
Linear	0.06	0.8026	0.061	18.81	0.25	0.25	0.8026
Linear	0.06	0.8036	0.239	25.50	- 3.48	- 0.83	0.5758
Quadratic	0.85	0.6246			0.31	0.92	0.6246
Linear	0.06	0.8057			5.89	0.49	0.6373
Quadratic	0.83	0.6184	0.318	14.97	- 1.51	- 0.68	0.5129
Cubic	0.69	0.5743			0.09	0.83	0.5743

TABLE 9 (continued)

Source	F	P of > F	R	a (x 10^{-3})	b (x 10^{-3})	t for testing Ho : b = o	P of > |t|
	Acentric fragments + rings + dicentrics						
Linear	4.43	0.0491	0.466	87.02	- 4.12	- 2.10	0.0491
Linear	4.31	0.0530	0.496	97.60	-10.02	- 1.24	0.2311
Quadratic	0.57	0.5323			0.48	0.75	0.5323
Linear	4.11	0.0595			2.05	0.09	0.9294
Quadratic	0.54	0.5209	0.511	84.02	- 1.86	- 0.43	0.6768
Cubic	0.30	0.5980			0.12	0.55	0.5980

TABLE 10. Regression analyses of chromosome aberrations in peripheral blood of pigs from 1-12 weeks after 200 rads of fission neutrons

Source	F	P of > F	R	a ($\times 10^{-3}$)	b ($\times 10^{-3}$)	t for testing Ho : b = o	P of > |t|
				Acentric fragments			
Linear	14.60	0.0015	0.669	119.61	- 8.08	- 3.82	0.0015
Linear	16.07	0.0012	0.725	147.82	- 23.25	- 2.51	0.0214
Quadratic	2.81	0.1087			1.17	1.68	0.1087
Linear	15.50	0.0015			- 6.45	- 0.23	0.8158
Quadratic	2.71	0.1159	0.734	128.85	- 2.06	- 0.40	0.6954
Cubic	0.40	0.5409			0.16	0.63	0.5409
				Rings + dicentrics			
Linear	0.60	0.5455	0.180	28.35	1.05	0.77	0.5455
Linear	0.58	0.5357	0.218	34.39	- 2.20	- 0.35	0.7318
Quadratic	0.28	0.6119			0.25	0.53	0.6119
Linear	0.66	0.5669			- 33.36	- 1.89	0.0734
Quadratic	0.32	0.5874	0.469	69.59	6.26	1.93	0.0676
Cubic	3.53	0.0756			- 0.31	- 1.88	0.0756

TABLE 10 (continued)

Source	F	P of > F	R	a (x 10^{-3})	b (x 10^{-3})	t for testing Ho : b = o	P of > |t|
	Acentric fragments + rings + dicentrics						
Linear	9.51	0.0064	0.588	147.97	- 7.03	- 3.08	0.0064
Linear	10.95	0.0043	0.680	182.22	- 25.45	- 2.60	0.0177
Quadratic	3.73	0.0676			1.42	1.93	0.0676
Linear	10.47	0.0053			- 39.81	- 1.34	0.1973
Quadratic	3.56	0.0744	0.687	198.45	4.19	0.77	0.5408
Cubic	0.26	0.6204			- 0.14	- 0.51	0.6204

TABLE 11. Regression analyses of chromosome aberrations in peripheral blood of pigs from 1-12 weeks after 300 rads of fission neutrons

Source	F	P of > F	R	a ($\times 10^{-3}$)	b ($\times 10^{-3}$)	t for testing Ho : b = o	P of > |t|
				Acentric fragments			
Linear	9.78	0.0059	0.593	179.84	- 10.16	- 3.13	0.0059
Linear	14.56	0.0017	0.768	249.40	- 47.56	- 3.88	0.0015
Quadratic	9.80	0.0061			2.89	3.13	0.0061
Linear	14.53	0.0018			- 81.31	- 2.23	0.0386
Quadratic	9.78	0.0065	0.782	287.52	9.40	1.40	0.1766
Cubic	0.96	0.6576			- 0.33	- 0.98	0.6576
				Rings + dicentrics			
Linear	10.12	0.0053	0.599	136.21	- 12.63	- 3.18	0.0053
Linear	14.40	0.0017	0.758	217.72	- 56.46	- 3.69	0.0021
Quadratic	8.60	0.0091			3.39	2.93	0.0091
Linear	20.48	0.0006			- 159.45	- 4.17	0.0001
Quadratic	12.23	0.0032	0.848	334.05	23.24	3.32	0.0045
Cubic	8.18	0.0110			- 1.01	- 2.86	0.0110

TABLE 11 (continued)

Source	F	P of > F	R	a (x 10^{-3})	b (x 10^{-3})	t for testing Ho : b = o	P of > |t|
	Acentric fragments + rings + dicentrics						
Linear	13.93	0.0018	0.660	316.06	- 22.79	- 3.73	0.0018
Linear	25.69	0.0002	0.843	467.13	- 104.01	- 5.03	0.0002
Quadratic	16.18	0.0012			6.28	4.02	0.0012
Linear	35.88	0.0001			- 240.76	- 4.62	0.0005
Quadratic	22.61	0.0004	0.897	621.57	32.63	3.42	0.0037
Cubic	7.76	0.0127			- 1.35	- 2.79	0.0127

TABLE 12. Regression analyses of chromosome aberrations in peripheral blood of pigs from 1-12 weeks after 400 rads of fission neutrons

Source	F	P of > F	R	a ($\times 10^{-3}$)	b ($\times 10^{-3}$)	t for testing Ho : b = o	P of > |t|
				Acentric fragments			
Linear	13.56	0.0020	0.655	250.28	- 13.57	- 3.68	0.0020
Linear	19.67	0.0006	0.793	327.37	- 55.02	- 3.91	0.0014
Quadratic	9.10	0.0077			3.20	3.02	0.0077
Linear	20.93	0.0005			- 110.39	- 2.72	0.0146
Quadratic	9.69	0.0067	0.819	389.90	13.88	1.86	0.0779
Cubic	2.09	0.1644			- 0.54	- 1.45	0.1644
				Rings + dicentrics			
Linear	7.37	0.0136	0.539	130.24	- 7.45	- 2.72	0.0136
Linear	8.71	0.0087	0.658	173.82	- 30.88	- 2.66	0.0158
Quadratic	4.27	0.0518			1.81	2.07	0.0518
Linear	19.93	0.0006			- 133.89	- 5.86	0.0001
Quadratic	9.77	0.0065	0.875	290.16	21.66	5.17	0.0002
Cubic	22.88	0.0004			- 1.01	- 4.78	0.0004

TABLE 12 (continued)

Source	F	P of > F	R	a (x 10^{-3})	b (x 10^{-3})	t for testing Ho : b = o	P of > |t|
	Acentric fragments + rings + dicentrics						
Linear	14.74	0.0015	0.671	380.52	- 21.02	- 3.84	0.0015
Linear	22.72	0.0003	0.814	501.19	- 85.90	- 4.23	0.0008
Quadratic	10.74	0.0046			5.01	3.28	0.0046
Linear	39.29	0.0001			- 244.28	- 5.32	0.0002
Quadratic	18.57	0.0008	0.903	680.07	35.54	4.22	0.0009
Cubic	13.40	0.0024			- 1.56	- 3.66	0.0024

TABLE 13. Observed and predicted ($\hat{Y}$) chromosome aberrations in leukocytes of pigs exposed to 100 rads of fission neutrons[1]

Time postirradiation (weeks)		Acentric fragments		Rings + dicentrics		Acentric fragments + rings + dicentrics	
		Animal number					
		60-3	54-9	60-3	54-9	60-3	54-9
1		0.10	0.08	0.04	0.00	0.14	0.08
		0.04	0.04	0.00	0.02	0.04	0.06
	$\bar{X}$ =		0.065		0.015		0.080
	$\hat{Y}$ =		0.064		0.019		0.083
2		0.08	0.04	0.02	0.04	0.10	0.08
		0.08	0.04	0.02	0.04	0.10	0.08
	$\bar{X}$ =		0.060		0.030		0.080
	$\hat{Y}$ =		0.059		0.021		0.079
4		0.08	0.04	0.02	0.00	0.10	0.04
		0.04	0.04	0.02	0.02	0.06	0.06
	$\bar{X}$ =		0.045		0.015		0.065
	$\hat{Y}$ =		0.051		0.020		0.071
8		0.06	0.00	0.02	0.00	0.08	0.00
		0.02	0.04	0.00	0.04	0.02	0.08
	$\bar{X}$ =		0.030		0.015		0.045
	$\hat{Y}$ =		0.033		0.012		0.054
12		--[2]	0.02	--	0.02	--	0.04
		--	0.02	--	0.04	--	0.06
	$\bar{X}$ =		0.020		0.030		0.050
	$\hat{Y}$ =		0.015		0.024		0.038

[1]Each entry represents the aberrations per cell in 50 metaphases.

[2]Sample lost in preparation.

TABLE 14. Observed and predicted ($\hat{Y}$) chromosome aberrations in leukocytes of pigs exposed to 200 rads of fission neutrons[1]

Time postirradiation (weeks)		Acentric fragments		Rings + dicentrics		Acentric fragments + rings + dicentrics	
		Animal number					
		61-3	62-4	61-3	62-4	61-3	62-4
1		0.08	0.12	0.04	0.04	0.12	0.16
		0.16	0.12	0.04	0.06	0.20	0.18
	$\bar{X}$ =		0.120		0.045		0.165
	$\hat{Y}$ =		0.112		0.042		0.158
2		0.16	0.04	0.00	0.04	0.16	0.08
		0.12	0.12	0.04	0.00	0.16	0.12
	$\bar{X}$ =		0.110		0.020		0.130
	$\hat{Y}$ =		0.103		0.025		0.137
4		0.12	0.02	0.00	0.00	0.12	0.02
		0.12	0.06	0.04	0.04	0.16	0.10
	$\bar{X}$ =		0.080		0.020		0.100
	$\hat{Y}$ =		0.087		0.017		0.103
8		0.00	0.04	0.08	0.00	0.08	0.04
		0.04	0.04	0.06	0.04	0.10	0.08
	$\bar{X}$ =		0.030		0.045		0.075
	$\hat{Y}$ =		0.055		0.045		0.070
12		0.00	0.08	0.02	0.02	0.02	0.10
		0.04	0.04	0.06	0.06	0.10	0.10
	$\bar{X}$ =		0.040		0.040		0.080
	$\hat{Y}$ =		0.022		0.035		0.081

[1]Each entry represents the aberrations per cell in 50 metaphases.

TABLE 15. Observed and predicted ($\hat{Y}$) chromosome aberrations in leukocytes of pigs exposed to 300 rads of fission neutrons[1]

Time postirradiation (weeks)		Acentric fragments		Rings + dicentrics		Acentric fragments + rings + dicentrics	
		Animal number					
		54-1	57-0	54-1	57-0	54-1	57-0
1		0.16	0.26	0.20	0.10	0.36	0.36
		0.28	0.14	0.34	0.22	0.62	0.36
	$\bar{X}$ =		0.210		0.215		0.425
	$\hat{Y}$ =		0.205		0.197		0.412
2		0.24	0.20	0.08	0.04	0.32	0.24
		0.12	0.12	0.08	0.06	0.20	0.18
	$\bar{X}$ =		0.170		0.065		0.235
	$\hat{Y}$ =		0.166		0.100		0.260
4		0.04	0.12	0.02	0.00	0.06	0.12
		0.12	0.06	0.04	0.04	0.16	0.10
	$\bar{X}$ =		0.085		0.025		0.110
	$\hat{Y}$ =		0.105		0.003		0.094
8		0.04	0.12	0.00	0.00	0.04	0.12
		0.04	0.08	0.04	0.02	0.08	0.10
	$\bar{X}$ =		0.070		0.015		0.085
	$\hat{Y}$ =		0.054		0.028		0.093
12		0.12	0.08	0.00	0.02	0.12	0.10
		0.06	0.10	0.02	0.02	0.08	0.12
	$\bar{X}$ =		0.090		0.015		0.105
	$\hat{Y}$ =		0.095		0.021		0.098

[1]Each entry represents the aberrations per cell in 50 metaphases.

TABLE 16. Observed and predicted ($\hat{Y}$) chromosome aberrations in leukocytes of pigs exposed to 400 rads of fission neutrons[1]

Time postirradiation (weeks)		Acentric fragments		Rings + dicentrics		Acentric fragments + rings + dicentrics	
		Animal number					
		54-4	70-4	54-4	70-4	54-4	70-4
1		0.22	0.32	0.24	0.16	0.46	0.48
		0.30	0.28	0.16	0.16	0.46	0.44
	$\bar{X}$ =		0.280		0.180		0.460
	$\hat{Y}$ =		0.276		0.177		0.470
2		0.32	0.28	0.10	0.08	0.42	0.36
		0.20	0.18	0.06	0.14	0.26	0.32
	$\bar{X}$ =		0.245		0.095		0.340
	$\hat{Y}$ =		0.230		0.101		0.321
4		0.08	0.16	0.02	0.04	0.10	0.20
		0.12	0.12	0.06	0.04	0.18	0.16
	$\bar{X}$ =		0.120		0.040		0.160
	$\hat{Y}$ =		0.159		0.037		0.172
8		0.04	0.20	0.04	0.12	0.08	0.32
		0.12	0.12	0.10	0.08	0.22	0.20
	$\bar{X}$ =		0.120		0.085		0.205
	$\hat{Y}$ =		0.092		0.088		0.202
12		0.14	0.10	0.08	0.06	0.22	0.16
		0.06	0.18	0.04	0.02	0.10	0.20
	$\bar{X}$ =		0.120		0.050		0.170
	$\hat{Y}$ =		0.128		0.057		0.171

[1]Each entry represents the aberrations per cell in 50 metaphases.

dose levels. Consequently, the response of rings plus dicentrics per cell at either 100 or 200 rads could not be adequately described by the equations utilized. It appeared, however, that the response at these doses best fit a cubic polynomial as evaluated by the F test and the larger coefficient of multiple correlation (R) (Tables 9 and 10). The predicted values ($\hat{Y}$) found in Tables 13 and 14 have therefore been calculated on the basis of a cubic expression, given linear and quadratic effects, but no inference is made regarding their validity.

The failure of these aberrations to be described by the equations used was certainly influenced by the fact that the majority of rings and dicentrics disappeared within the first week postexposure and sampling error distorted the biological picture with respect to those remaining. The increase in these aberrations noted at 12 weeks after 100 rads and the rise at 8 and 12 weeks following 200 rads may be the result of aberrant cells originating from lymphopoietic stem-cells appearing in the peripheral blood.

Regression analyses on data from the 300-and 400-rad exposures definitely fit the cubic polynomial (Table 11, page 76, and Table 12, page 78). These data could have been described also by the linear or quadratic expressions, but the closest relationship, as indicated by the coefficient of multiple correlation (R), was attributed to the cubic polynomial given linear and quadratic components. Consequently, the calculation of the predicted values for rings plus dicentrics per cell (Table 15 and 16) was based upon the following expressions:

$$300 \text{ rads: } \hat{Y} = 334.05 \times 10^{-3} - 159.45 \times 10^{-3} (X) + 23.24 \times 10^{-3} (X)^2 - 1.01 \times 10^{-3} (X)^3.$$

400 rads: $\hat{Y} = 290.16 \times 10^{-3} - 133.89 \times 10^{-3} (X)$

$+ 21.66 \times 10^{-3} (X)^2 - 1.01 \times 10^{-3} (X)^3$.

The analysis of variance for rings plus dicentrics per cell indicated that dose, sampling time, and the interaction between dose and sampling time were all statistically significant ($P < .01$). Effects attributable to animals and the interaction between animal and sampling time were nonsignificant at the 5% level of probability of chance occurrence. A multiple correlation coefficient of 0.930 was obtained.

Total Aberrations

Total aberrations were defined as the sum of the acentric fragments, rings, and dicentrics. A high degree of association ($R = 0.950$) was found among total aberrations and the independent components incorporated into the model subjected to an analysis of variance. Significant ($P < .01$) effects upon total aberrations could be attributed to dose, sampling time, and interaction between dose and sampling time.

At the lower doses (100 and 200 rads) total aberrations over time postexposure were best described as a linear function. Although the correlation coefficient was slightly larger for the higher order expressions, the F tests exceeded ($P > .05$) for the quadratic and cubic components (Table 9, page 72 and Table 10, page 74). Consequently, the following equations were believed to best describe the kinetics of total aberrations from 1-to-12 weeks postexposure for the two lower doses:

100 rads: $\hat{Y} = 87.02 \times 10^{-3} - 4.12 \times 10^{-3} (X)$.

200 rads: $\hat{Y} = 147.97 \times 10^{-3} - 7.03 \times 10^{-3} (X)$.

Use was made of these linear models to predict the total aberrations to be expected at various postirradiation sampling times (Tables 13 and 14, pages 80 and 81).

Higher order equations most adequately described total aberration persistence at both the 300-and 400-rad exposure levels (Tables 11 and 12, pages 76 and 78). The following expressions were obtained based upon F tests and the comparatively high multiple correlation coefficients:

300 rads: $\hat{Y} = 621.57 \times 10^{-3} - 240.76 \times 10^{-3} (X) + 32.63 \times 10^{-3} (X)^2 - 1.35 \times 10^{-3} (X)^3$.

400 rads: $\hat{Y} = 680.07 \times 10^{-3} - 244.28 \times 10^{-3} (X) + 35.54 \times 10^{-3} (X)^2 - 1.56 \times 10^{-3} (X)^3$.

Tables 15 and 16 contain the predicted $(\hat{Y})$ and observed values for total aberrations at various postirradiation sampling times for 300 and 400 rads, respectively.

These data indicated that the kinetics of acentric fragment loss from 1-to-12 weeks postirradiation was linear at 100 and 200 rads but followed a quadratic response at 300 and 400 rads. This difference in kinetics may be attributed partially to an increased number of lymphocytes appearing in the peripheral blood at 12 weeks postexposure in the 300- and 400-rad samples (Figure 2, page 46). If these lymphocytes were present at irradiation and contained acentric fragments, their later release into the peripheral blood would elevate the proportion of observed aberrations. Tables 15 and 16 show that an increased proportion of acentric fragments was observed when blood was sampled 12 weeks postexposure.

The inclusion of rings plus dicentrics into an analysis of aberration persistence necessitated higher order (cubic) equations in order to explain their response postexposure. Such expressions probably consider the interplay of lymphopoietic centers as demonstrated by their ability to release aberrant cells into the peripheral blood following irradiation. These cells, if they have undergone mitosis, may contain rings or dicentrics without fragments.

The variation in dicentrics without fragments as a function of post-irradiation time is found in Figure 8. An abrupt rise in the number of cells containing dicentrics without fragments occurred at 28 days post-exposure. This coincided with the increased number of lymphocytes found at this same time and suggested that these cells were the daughters resulting from mitoses in lymphopoietic centers. The decrease found at 56 and 84 days postirradiation was attributable to sampling error.

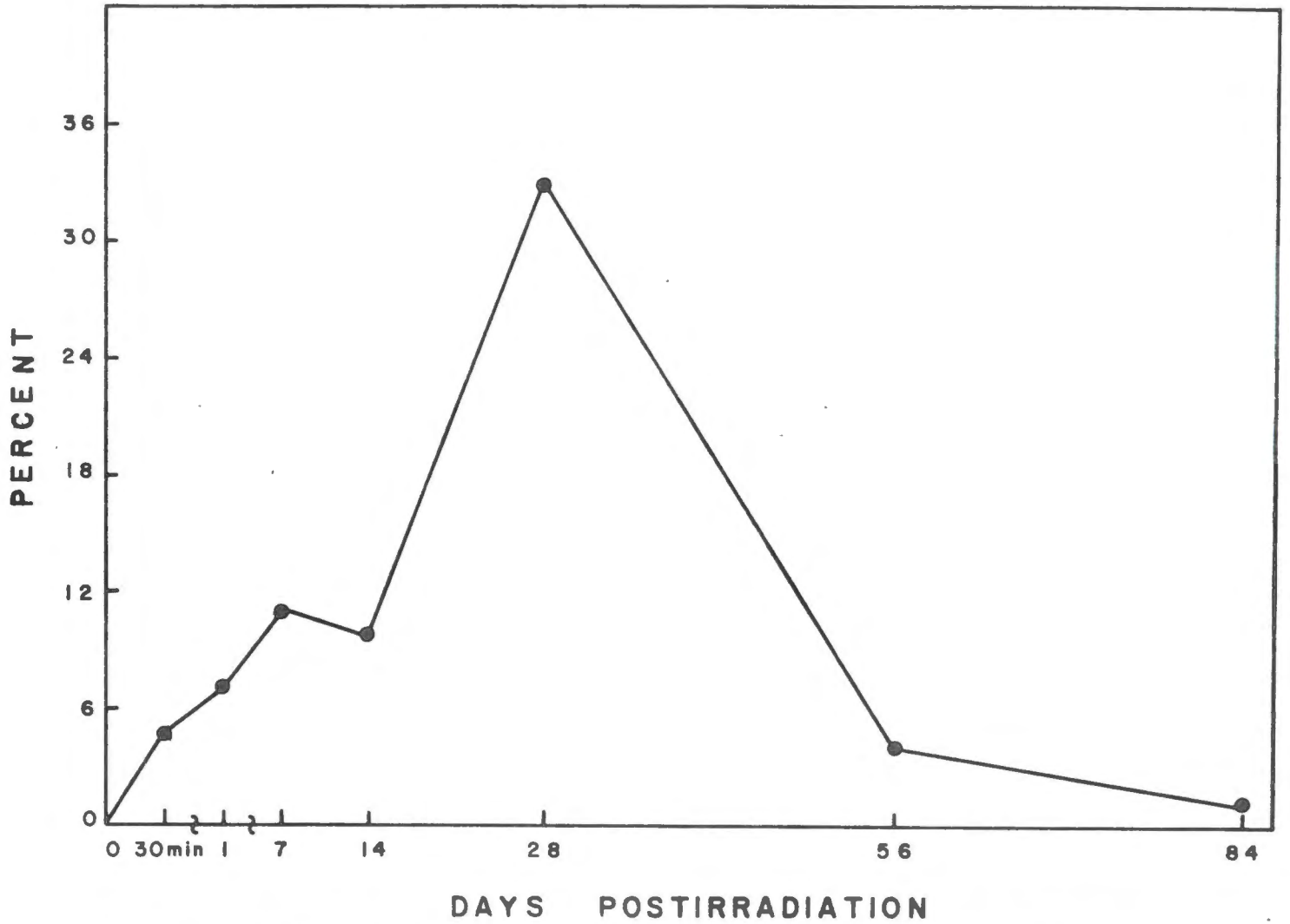

Figure 8. Percent of dicentrics not accompanied by acentric fragments in peripheral leukocytes of pigs exposed to fission neutrons. (average of all 4 doses).

CHAPTER V

DISCUSSION

It has been amply documented that small peripheral lymphocytes respond to phytohemagglutinin (PHA) by proliferation and subsequent division during in vitro culture (28, 31, 52, 53, 61, 70, 76). Consequently, it is these cells that are evaluated for chromosome abnormalities when peripheral blood is cultivated. Since small peripheral lymphocytes rarely undergo mitoses in vivo (28, 31, 61) unless antigenically stimulated (46, 47), chromosome aberrations would be expected to persist for the lifespan of such cells. Upon this premise, estimates of lymphocyte lifespan have been calculated for humans (23, 74). Although chromosome aberrations have been utilized as indicators of average lymphocyte lifespan, they have been extensively investigated also as a basis for a biologic radiation dosimetry system. Several reviews on the radiosensitivity of lymphocyte chromosomes and their use as biological dosimeters have been published (7, 41, 43).

Following accidental radiation exposure of humans, it is highly desirable to obtain adequate estimates of the biological injury received. In order to do this, a system is needed that will be representative of the total body and one which is readily accessible. Chromosome analyses of peripheral lymphocytes have been used for this purpose with reliable results (5, 13, 14, 20, 25, 72). In many of these and similar studies, it has been assumed that the aberration coefficients do not vary appreciably within the first week or so following exposure. Also, in following the practice of irradiating human blood in vitro in order to

extrapolate to the in vivo condition, researchers have often neglected to consider the basic mechanisms that may affect the response of cells under these two conditions and that these may influence the observed aberration levels. A knowledge of the response of lymphocyte aberrations as a function of time postirradiation is required in order to derive accurate dose estimates from aberration coefficients. Also, in order to predict accurately the in vivo radiation damage from in vitro data, the biological relationship of these two types of effects must be established. This relationship is most readily established by simultaneous exposures of animals along with a sample of their blood. The accumulation of such data is not feasible in humans except in cases of therapeutic whole-body exposures. Consequently, we must rely heavily on data obtained from animals which are physically and physiologically comparable to man.

The fact that a linear model best described all types of aberrations studied as a function of dose was in keeping with the high probability of inducing more than one lesion by a single high-energy particle. The finding of statistically nonsignificant quadratic components in the dose-response models is probably a reflection of the larger biological effectiveness of the neutron component as compared to the contribution from gamma rays. Earlier studies also have demonstrated linear dose-response kinetics for human leukocytes exposed to fission neutrons (14, 86, 87). This type of function indicates that dose rate will have little, if any, influence on the aberration coefficients and the dose-response kinetics obtained with such relatively high-linear energy transfer (LET) irradiation.

Direct comparisons between the aberration coefficients obtained from samples irradiated in vivo and those obtained from samples irradiated in

vitro indicated significantly more aberrations in the in vitro samples when the dose was measured in air at the animal's midline. Such results agree with other data from pigs exposed to 14 MeV neutrons (64) or ^{60}Co gamma rays (65), but differ from the identical aberration yields from in vivo and in vitro irradiation reported for marmosets exposed to gamma rays (18). These differences may be resolved by the fact that dose attenuation is a major factor in large animals but of relatively minor significance in smaller animals such as the marmoset. Also, the existence of inherent differences in radiosensitivity and aberration persistence must not be overlooked. Since it is obvious that the radiation dose to the blood cells in the pig will differ from that received in vitro due to the effects of tissue interaction and inverse-square dose relations, comparisons between exposure methods become more meaningful when these factors are considered.

In a large animal such as the pig, dose attenuation within the body is obviously a primary factor (22), and this necessitated the calculation of an average body dose. However, even when average body dose was considered each of the aberration classes was still smaller in the in vivo samples than in the in vitro samples. Assuming the method of expressing the average in vivo dose to be adequate, one is left with the conclusion that aberrations were removed from the peripheral system rather rapidly before the first postexposure sampling, or, alternatively, that chromosome repair mechanisms had become more efficient in vivo. Other workers have proposed that aberrant lymphocytes are selectively eliminated from the peripheral blood following exposure (45, 64, 66, 95). Another factor that must be considered in assessing the results is the larger neutron-to-gamma ratio that existed for the in vitro exposures. Since

the relative biological effectiveness (RBE) for fission neutrons is approximately four (14, 51, 86, 87), it would be expected that more aberrations would be induced following in vitro exposures because the neutron component was rapidly attenuated in vivo. That both of these explanations may be valid was borne out by the observation that the level of acentric fragments but not that of rings and dicentrics was quite similar between exposure methods if the RBE of the neutron component was considered. Others have shown the importance of RBE considerations with respect to chromosome aberrations (14, 51, 86, 87). The fact that rings plus dicentrics were still present in greater numbers following in vitro irradiation suggested that these aberrations were more rapidly eliminated than were fragments prior to obtaining the first postexposure blood sample. This observation, however, is more readily explained by the fact that the adjustment for in vivo dose did not take into account the nine-fold ratio existing between exposure methods for rings plus dicentrics as compared with the three-fold ratio for fragments.

The aberration levels expected after correcting the average body dose for the RBE of the neutron component were similar to those obtained from the estimated mid-line body dose (Figures 5-7, pages 52, 58, 60). This becomes of interest since the mid-line tissue dose is readily calculated and may well represent the average dose to the blood cells.

These data point out that the different aberration coefficients obtained for in vivo and in vitro irradiation are dependent upon the method of expressing radiation dose. It is evident also that the aberration type influences the conclusions to be derived when comparing methods of exposure in pigs. The finding that a rapid decline in number of rings plus dicentrics occurred immediately postexposure parallels the

observation of a rapid elimination of these aberrations following irradiation in humans (45), pigs (66, 95) and monkeys (95).

The significant decrease in number of chromosome aberrations between 30 minutes and 24 hours postirradiation deserves special attention. It is believed that a selective elimination of aberrant cells occurred since the relative percent decrease in number of chromosome aberrations was greater than that for the decrease in lymphocyte number. Others have indicated that aberrant cells are rapidly lost from the peripheral blood following irradiation (45, 64, 66, 95). It has been shown also that cellular debris accumulates in lymphoreticular organs following an exposure (32, 35, 36, 45). This debris may well result from an increased removal of damaged cells.

Earlier studies (95) on the disappearance rate of aberrations in pig lymphocytes following partial-body irradiation demonstrated that cells containing aberrations had virtually disappeared within 24 hours after exposure. The present data show that the rate of elimination is neither as rapid nor as nearly complete as that reported for partial-body irradiation. It is to be expected, however, that the concentration of aberrant cells in the peripheral blood will be rapidly diluted following partial-body exposure due to mixing with cells from nonexposed regions. Such a dilution effect would not be envisioned following total-body irradiation, and other mechanisms such as a selective removal of damaged cells must be proposed.

The rapid removal of aberrant cells from the peripheral system following exposure of pigs to total-body irradiation (66) and after extracorporeal irradiation of humans has been documented (45). Contrary to this is the finding of identical aberration yields in samples taken

immediately and 24 hours after whole-body irradiation of marmosets by gamma rays (18). No change in aberration yields have been obtained also during the first 24 hours following whole-body irradiation of humans (25). Other data from accidental whole-body exposures of humans revealed that aberration levels tend to remain stable during the first 5-to-6 weeks postirradiation (14, 20). When compared with the reported information (11, 13, 14, 18, 24, 66, 84, 95), the present data support the hypothesis that the kinetics of aberration loss following whole-body exposures differ between man and the pig. The basic reasons for this apparent difference remain to be elucidated. It is obvious, however, that the rapid elimination of aberrant lymphocytes postirradiation must be considered in certain species, especially when one compares the radiosensitivity among species. Recent information from humans undergoing extracorporeal irradiation of blood (ECIB) has demonstrated that the loss rate of aberrant cells was rapid and that damaged lymphocytes were removed by the reticuloendothelial system following irradiation (45). Although this study may not be entirely comparable with aberration persistence data on man following total-body irradiation (14, 20, 25), it does appear that the phenomenon of an early disappearance of aberrant lymphocytes may be common to man and pigs. If this conjecture if further substantiated from experiments especially designed to study differences in aberration persistence among species, reliable estimates of aberration coefficients will need to be based upon data in which the kinetics of aberration loss are considered. In studies reporting no change in aberration levels within the first 24 hours (18, 25), the results may be explained by a rapid exchange between peripheral cells and those in tissue pools as noted by others (32, 35, 45) since the minimal exposure

times were approximately 30 minutes. The finding that the aberration yield per cell was the same at the beginning and at the end of a course of ECIB (45) supports the above suggestion.

The early, rapid loss of aberrant cells in some species indicates that sampling time postirradiation must be considered when one utilizes chromosome aberrations as a biological dosimeter. In the present experiment, different dose-response kinetics were obtained depending on whether the 30-minute or 24-hour aberration data were used. This again demonstrates the importance of sampling time and may explain some of the variability among the reported aberration coefficients following in vivo exposures (7, 41, 43).

The rapid decrease in number of aberrant cells within the first 24 hours was followed by a more gradual decline at subsequent time periods. Although the level of induction of fragments was higher, they generally disappeared more rapidly than did rings plus dicentrics. This may be explained by the appearance in the peripheral blood of rings and dicentrics from lymphopoietic centers. It has been adequately verified that a higher proportion of these aberrations survive mitosis as compared with fragments, since the latter lack centromeres (34, 37, 74, 83). The increase in the proportion of dicentrics without accompanying fragments at 28 days and the fact that lymphocyte numbers were rapidly recovering during this time interval support the contention that a higher percentage of the daughters from lymphopoietic stem cells contain rings and dicentrics than contain acentric fragments. This would then explain the more rapid decrease in fragments during the period of lymphocyte number recovery.

The early loss of aberrations was accompanied by a decrease in number of peripheral lymphocytes during the first 2 days following irradiation. Repopulation of lymphocyte numbers occurred thereafter but failed to reach preexposure levels during the course of study. Rapid loss of peripheral lymphocytes and a gradual return toward normal values appears to be a common sequence of events following irradiation (22, 23, 32, 45, 64, 95).

Of foremost importance, in regard to the aberration coefficient, was the dramatic loss of lymphocytes during irradiation and the first 30 minutes postexposure. These cells obviously respond rapidly to trauma, and this indicates that the peak of aberration induction may have occurred even earlier than our first postirradiation sampling time. That is, selective removal of damaged cells influenced the aberration index which was in turn dependent upon the rate of lymphocyte loss.

As lymphocyte numbers return toward the preexposure values, lymphopoietic centers may produce both aberrant and nonaberrant cells. The effect of the latter would be to decrease the proportion of aberrant cells in the peripheral system, whereas aberrant cells (largely without fragments) would tend to maintain a stable level of aberrations. Other mechanisms involving plasma factors also have been proposed for explaining the maintenance of number of chromosomal aberrations following irradiation (49, 50, 54). The present results indicate that there are two stages of lymphocyte populations involved in the kinetics of aberration persistence in swine. Other workers also have implied the existence of two lymphocyte populations in humans following irradiation (23, 34). The initial stage involves the rapid removal of damaged (aberration-containing) cells; whereas the lymphocyte-recovery stage is gradual and

lymphopoietic centers are an important factor. The present data are in agreement with the idea of a short-lived population of lymphocytes and a long-lived population which is dependent upon lymphopoietic centers.

The fact that the number of aberrations decreases rapidly postexposure in some species and does not rebound to "initial" induction levels is evidence against the idea that aberrant cells are reversibly removed from the blood. It seems more likely that a large proportion of damaged cells are phagocytized and eliminated from the peripheral population. The proportion not removed may survive for the normal lymphocyte lifespan (23, 74) and account for the established fact that lymphocyte aberrations persist for many years following their induction (15, 16, 17, 23, 34, 49, 74).

The significant variation found among animals in several instances in the present experiment indicates the necessity of randomly selecting samples large enough to allow inductive reasoning about the population under consideration. The significant interaction between dose and sampling time demonstrates that the initial effect of irradiation was overshadowed by physiological events occurring postexposure. This interaction makes it likely that the practice of sampling blood following considerable intervals of time after exposure will lead to erroneous estimates of biological dose computed from chromosome aberration data.

CHAPTER VI

SUMMARY

The rate of induction of chromosome aberrations in pig lymphocytes following 100, 200, 300 and 400 rads of fission neutrons administered in vivo and in vitro was determined. The data from each exposure method were tested for goodness of fit of linear and quadratic models. Numbers of acentric fragments, numbers of rings plus numbers of dicentrics, and the sum of the frequencies of aberration types were well explained by a linear model of the form $Y = a + bD + e$, a = Y-intercept, b = slope, where D = dose and e = random deviation from the least-squares fitted regression.

A significantly ($P < .01$) higher aberration index was found following an in vitro exposure of the animal's blood than was produced by exposing the entire animal. Equations were developed which permitted the prediction of in vivo damage from the in vitro aberration coefficient.

Dose attenuation and differences in the relative contribution of the neutron and gamma components reaching the target cells were recognized as factors influencing the biologically effective dose received from the two exposure methods. The average body dose was calculated from measurements made across the width of a tissue-equivalent phantom. From these calculations, it was determined that approximately 56% of the dose measured in air at the animal's midline was actually received by the blood cells in vivo.

When the neutron-to-gamma ratios for the two exposure conditions and the relative biological effectiveness of the neutron component were

considered, the level of acentric fragments was comparable between exposure methods. Rings plus dicentrics were, however, still more abundant following in vitro exposures, suggesting that an inherent difference exists between in vivo and in vitro irradiation in rate of induction and repair or that heavily damaged cells are more rapidly removed from the peripheral system. The possibility that in vivo mechanisms exist for the preferential removal of damaged lymphocytes was proposed as a plausible explanation for this observation. These data cast some doubt also on the validity of assuming that comparable chromosomal damage will be found following in vivo and in vitro irradiation.

By sampling peripheral blood at various postexposure intervals, the response of the aberration level as a function of time was studied. A rapid decrease in the proportion of cells containing aberrations was noted as early as 24 hours postexposure. This initial loss coincided with a decrease in lymphocyte number and continued until the recovery of lymphocyte numbers began at 2 days following irradiation. Subsequent losses were less dramatic, and numbers of acentric fragments reached preirradiation levels in the lower-dose groups by 56 days postexposure; numbers of rings plus dicentrics persisted throughout the study. Statistical analyses demonstrated the variability of the aberration coefficient with time postirradiation. Mathematical models are presented which describe the predicted yield of numbers of aberrations as a function of time after irradiation.

The hematological data are examined for their possible relation to postirradiation aberration levels. The fate of aberrant lymphocytes following irradiation is discussed in relation to the kinetics of the

reticuloendothelial system. Selective removal of damaged cells from the peripheral system is suggested.

It is concluded that different aberration coefficients will be found in blood sampled from pigs at different postexposure intervals. The kinetics of aberration persistence should therefore be considered when lymphocyte aberrations are employed as biological dosimeters.

REFERENCES

REFERENCES

(1) Albert, M. D. 1958. X-irradiation-induced mitotic abnormalities in mouse liver regenerating after carbon tetrachloride injury. I. Total-body irradiation. J. Nat. Cancer Inst., 20: 277.

(2) Ashwood-Smith, M. J., E. P. Evans, and A. G. Searle. 1965. The effect of hypothermia on the induction of chromosomal mutations by acute X-irradiation in mice. Mutat. Res., 2: 544.

(3) Auxier, J. A. 1965. The health physics research reactor. Health Physics, 11: 89.

(4) Banner, M. W. 1969. Personal communication.

(5) Bender, M. A. 1968. Somatic chromosomal aberrations: Use in the evaluation of human radiation exposures. Arch. Environ. Health, 16: 556.

(6) Bender, M. A. 1969. Cytogenetic changes after radiation exposure. p. 139-154. In R. J. M. Fry, D. Grahn, M. L. Green, and J. H. Rust (ed.), Proc. of a colloquium, Univ. Chicago. Taylor and Frances Ltd., London.

(7) Bender, M. A. 1969. Human radiation cytogenetics. p. 215-275. In L. G. Augenstein, R. Mason, and M. Zelle (ed.), Advances in Radiation Biology. Academic Press, New York.

(8) Bender, M. A. 1970. Neutron-induced genetic effects: A review. Radiat. Bot., 10: 225.

(9) Bender, M. A., and M. A. Barcinski. 1969. Kinetics of two-break aberration production by X-rays in human leukocytes. Cytogenetics, 8: 241.

(10) Bender, M. A., and P. C. Gooch. 1961. Spontaneous and X-ray induced somatic chromosome aberrations in the Chinese hamster. Int. J. Radiat. Biol., 4: 175.

(11) Bender, M. A., and P. C. Gooch. 1962. Persistent chromosome aberrations in irradiated human subjects. Radiat. Res., 16: 44.

(12) Bender, M. A., and P. C. Gooch. 1962. Types and rates of X-ray induced chromosome aberrations in human blood irradiated in vitro, Proc. Nat. Acad. Sci. U. S., 48: 522.

(13) Bender, M. A., and P. C. Gooch. 1963. Persistent chromosome aberrations in irradiated human subjects. II. Three and one-half year investigation. Radiat. Res., 18: 389.

(14) Bender, M. A., and P. C. Gooch. 1966. Somatic chromosome aberrations induced by human whole-body irradiation: The "Recuplex" criticality accident. Radiat. Res., 29: 568.

(15) Bloom, A. D., Y. Nakagome, A. A. Awa, and S. Neriishi. 1970. Chromosome aberrations and malignant disease among atomic bomb survivors, Hiroshima-Nagasaki. Atomic Bomb Casualty Commission. Technical Report 2-70.

(16) Bloom, A. D., S. Neriishi, A. A. Awa, T. Honda, and P. G. Archer. 1967. Chromosome aberrations in leukocytes of older survivors of the atomic bombings of Hiroshima and Nagasaki. Lancet, 2: 802.

(17) Bloom, A. D., S. Neriishi, N. Kamada, T. Iseki, and R. J. Keehn. 1966. Cytogenetic investigation of survivors of the atomic bombings of Hiroshima and Nagasaki. Lancet, 2: 672.

(18) Brewen, J. G., and N. Gengozian. 1971. Radiation-induced human chromosome aberrations. II. Human "in vitro" irradiation compared to "in vitro" and "in vivo" irradiation of marmoset leukocytes. Mutat. Res., 13: 383.

(19) Brewen, J. G., and H. E. Luippold. 1971. Radiation-induced human chromosome aberrations: "In vitro" dose rate studies. Mutat. Res., 12: 305.

(20) Brewen, J. G., R. J. Preston, and L. G. Littlefield. 1971. Radiation-induced human chromosome aberrations. III. Aberration yields following an accidental whole-body exposure to ^{60}Co γ-rays. Radiat. Res., In press.

(21) Brooks, A. L., and F. W. Lengemann. 1967. Comparison of radiation-induced chromatid aberrations in the testes and bone marrow of the Chinese hamster. Radiat. Res., 32: 587.

(22) Brown, D. G., D. F. Johnson, and J. A. Auxier. 1971. Unilateral and bilateral exposure of swine to fission neutrons. Health Physics, 21: 537.

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

(24) Buckton, K. E., P. Jacobs, W. M. Court-Brown, and R. Doll. 1962. A study of the chromosome damage persisting after X-ray therapy for ankylosing spondylitis. Lancet, 2: 676.

(25) Buckton, K. E., A. O. Langlands, P. G. Smith, and J. McLelland. 1967. Chromosome aberrations following partial- and whole-body X-irradiations in man. Dose response relationships. p. 122-135. In H. J. Evans, W. M. Court-Brown, and A. S. McLean (ed.), Human Radiation Cytogenetics. North Holland, Amsterdam.

(26) Buckton, K. E., A. O. Langlands, P. G. Smith, G. E. Woodcock, and P. C. Looby. 1971. Further studies on chromosome aberration production after whole-body irradiation in man. Intern. J. Radiat. Biol., 19: 369.

(27) Buckton, K. E., and M. C. Pike. 1964. Time in culture. An important variable in studying "in vivo" radiation-induced chromosome damage in man. Intern. J. Radiat. Biol. 8: 439.

(28) Carstairs, K. 1962. The human small lymphocyte: Its possible pluripotential quality. Lancet, 1: 829.

(29) Conger, A. D. 1965. The fate of metaphase aberrations. Radiat. Bot., 5: 81.

(30) Conger, A. D., and H. J. Curtis. 1968. Abnormal anaphases in regenerating mouse livers. Radiat. Res., 33: 150.

(31) Cooper, E. H., P. Barkhan, and A. J. Hale. 1963. Observations on the proliferation of human leucocytes cultures with phytohemagglutinin. Brit. J. Haematol., 9: 101.

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

(33) Court-Brown, W. M., K. E. Buckton, and A. O. Langlands. 1967. The identification of lymphocyte clones with chromosome structural aberrations, in irradiated men and women. Intern. J. Radiat. Biol., 13: 155.

(34) Court-Brown, W. M., K. E. Buckton, and A. S. McLean. 1965. Quantitative studies of chromosome aberrations in man following acute and chronic exposure to X-rays and gamma rays. Lancet, 1: 1239.

(35) Cronkite, E. P., C. R. Jansen, H. Kanti, R. Rai, H. Cottier, and T. M. Fliedner. 1963. The combined application of lymph duct drainage in extracorporeal irradiation of the blood in the study of lymphopoiesis. p. 126-156. Proc. of Guinness Symposium on Cell Proliferation. Blackwell Scientific Publications, Oxford, England.

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

(37) Curtis, H. J. 1963. Biological mechanisms underlying the aging process. Science, 141: 686.

(38) Curtis, H. J., and C. Crowley. 1963. Chromosome aberrations in liver cells in relation to the somatic mutation theory of aging. Radiat. Res., 19: 337.

(39) Curtis, H. J., J. Tilley, and C. Crowley. 1964. The cellular differences between acute and chronic neutron and gamma ray irradiation in mice. p. 143-155. In Biological Effects of Neutron and Proton Irradiations. Vol. I. IAEA, Vienna.

(40) Eicke, J., and O. Hug. 1970. Dose and time dependence of radiation-induced chromosome aberrations in rat embryos. Studia Biophysica, 2: 73.

(41) Evans, H. J. 1962. Chromosome aberrations induced by ionizing radiations. p. 221-321. In G. H. Bourne and J. F. Danielli (ed.). International Review of Cytology. Vol. 13. Academic Press, New York.

(42) Evans, H. J. 1967. Dose-response relations from "in vitro" studies. p. 20-26. In H. J. Evans, W. M. Court-Brown, and A. S. McLean (ed.). Human Radiation Cytogenetics. North-Holland, Amsterdam.

(43) Evans, H. J. 1967. Radiation-induced chromosome aberrations in human cells. Annex C, Report of the United Nations Scientific Committee on the Effects of Atomic Radiation, General Assembly, 24th Session. p. 98-155. United Nations, New York.

(44) Evans, H. J. 1968. The response of human chromosomes to X-irradiation: "in vitro" studies. Proc. Roy. Soc. (Edinburgh), B. II: 132.

(45) Field, E. O., H. B. A. Sharpe, K. B. Dawson, V. Anderson, S. A. Killman, and E. Weeke. 1972. Turnover rate of normal blood lymphocytes and exchangeable pool size in man, calculated from analysis of chromosomal aberrations sustained during extracorporeal irradiation of the blood. Blood, 39: 39.

(46) Fitzgerald, P. H. 1964. The immunological role and long life-span of small lymphocytes. J. Theoret. Biol., 6: 12.

(47) Fitzgerald, P. H. 1967. The life-span and role of the small lymphocyte. p. 94-98. In H. J. Evans, W. M. Court-Brown, and A. S. McLean (ed.). Human Radiation Cytogenetics. North Holland, Amsterdam.

(48) Ford, C. E., P. L. T. Ilbery, and J. F. Loutit. 1957. Further cytological observations on radiation chimeras. J. Cellular Comp. Physiol., 50 (Suppl. 1): 109.

(49) Goh, K. O. 1968. Total-body irradiation in human chromosomes. Cytogenetic studies of the peripheral blood and bone marrow leukocytes seven years after total-body irradiation. Radiat. Res., 35: 155.

(50) Goh, K. O., and H. Sumner. 1968. Breaks in normal human chromosomes: Are they induced by a transferable substance in the plasma of persons exposed to total-body irradiation? Radiat. Res., 35: 171.

(51) Gooch, P. C., M. A. Bender, and M. L. Randolph. 1964. Chromosome aberrations induced in human somatic cells by neutrons. p. 325-342. In Biological Effects of Neutron and Proton Irradiations. Vol. I. IAEA, Vienna.

(52) Gowans, J. L., D. D. McGregor, D. M. Cowen, and C. E. Ford. 1962. Initiation of immune responses by small lymphocytes. Nature, 196: 651.

(53) Gowans, J. L., and J. W. Uhr. 1966. The carriage of immunological memory by small lymphocytes in the rat. J. Exp. Med., 124: 1017.

(54) Hollowell, J. G., and L. G. Littlefield. 1968. Chromosome damage induced by plasma of X-rayed patients: An indirect effect of X-ray. Proc. Soc. Exp. Biol. (N.Y.), 129: 240.

(55) Hurst, G. S., W. A. Mills, F. P. Conte, and A. C. Upton. 1956. Principles and techniques of mixed radiation dosimetry. Radiat. Res., 4: 49.

(56) Johnson, D. R., and J. W. Poston. 1967. Radiation dosimetry studies at the Health Physics Research Reactor. ORNL. Bull. 4113.

(57) Kihlman, B. A. 1966. Actions of Chemicals on Dividing Cells. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

(58) Lea, D. E. 1955. Actions of Radiation on Living Cells, 2nd ed. Cambridge University Press, Cambridge.

(59) Léonard, A., and Gh. Deknudt. 1967. Relation between the X-ray dose and the rate of chromosome rearrangements in spermatogonia of mice. Radiat. Res., 32: 35.

(60) Léonard, A., and Gh. Deknudt. 1970. Persistence of chromosome rearrangements induced in male mice by X-irradiation of premeiotic germ cells. Mutat. Res., 9: 127.

(61) MacKinney, A. A., F. Stohlman, and G. Brecher. 1962. The kinetics of cell proliferation in cultures of human peripheral blood. Blood, 19: 349.

(62) Mather, K., and L. H. A. Stone. 1933. The effect of X-irradiation upon somatic chromosomes. J. Genet., 28: 1.

(63) McFee, A. F., and M. W. Banner. 1969. Personal communication.

(64) McFee, A. F., M. W. Banner, and M. N. Sherrill. 1970. Chromosome aberrations in swine leukocytes after "in vivo" or "in vitro" exposure to 14 MeV neutrons. Radiat. Res., 44: 512.

(65) McFee, A. F., M. W. Banner, and M. N. Sherrill. 1971. Induction of chromosome aberrations by "in vivo" and "in vitro" gamma irradiation of swine leukocytes. Radiat. Res., In Press.

(66) McFee, A. F., M. W. Banner, M. N. Sherrill, and J. B. Mailhes. 1971. Disappearance rates of radiation-induced chromosome aberrations from swine leukocytes. Radiat. Res., In press.

(67) Millard, R. E. 1965. Abnormalities of human chromosomes following therapeutic irradiation. Cytogenetics, 4: 277.

(68) Mihalozo, J. T. 1963. Superprompt-critical behavior of an unmoderated, unreflected uranium-molybdenum alloy reactor. Nucl. Sci. and Eng., 16: 3.

(69) Mizutani, M., Y. Ohnuki, Y. H. Nakanishi, and C. M. Pomerat. 1961. Proc. Conference on Research on the Radiotherapy of Cancer. Madison, Wis., American Cancer Society. p. 51. New York.

(70) Moorehead, P. S., P. C. Nowell, W. J. Mellman, D. M. Battips, and D. A. Hungerford. 1960. Chromosome preparations of leukocytes from human peripheral blood. Exp. Cell Res., 20: 613.

(71) Norman, A. 1967. Multi-hit aberrations. p. 53-57. In H. J. Evans, W. M. Court-Brown, and A. S. McLean (ed.). Human Radiation Cytogenetics. North-Holland, Amsterdam.

(72) Norman, A., R. E. Ottoman, M. Sasaki, and R. C. Veomett. 1964. The frequency of dicentrics in human leukocytes irradiated "in vivo" and "in vitro". Radiology, 83: 108.

(73) Norman, A., M. S. Sasaki, R. E. Ottoman, and A. G. Fingerhut. 1965. Lymphocyte lifetime in women. Science, 147: 745.

(74) Norman, A., M. S. Sasaki, R. E. Ottoman, and A. G. Fingerhut. 1966. Elimination of chromosome aberrations from human lymphocytes. Blood, 27: 706.

(75) Nowell, P. C. 1965. Unstable chromosome changes in tuberculin-stimulated leukocyte cultures from irradiated patients. Blood, 26: 798.

(76) Nowell, P. C. 1967. Chromosome aberrations and immunological memory. p. 99-105. In H. J. Evans, W. M. Court-Brown, and A. S. McLean (ed.). Human Radiation Cytogenetics. North-Holland, Amsterdam.

(77) Nowell, P. C., and L. C. Cole. 1963. Reduced incidence of persistent chromosome aberrations in mice irradiated at low dose rates. Science, 141: 524.

(78) Nowell, P. C., D. E. Craig, F. A. Matthews, and L. C. Cole. 1965. Chromosome aberrations in liver and marrow of mice irradiated with fast neutrons, gamma rays, and X-rays: Effect of dose rate. Radiat. Res., 24: 108.

(79) Pearmain, G., R. R. Lycette, and P. H. Fitzgerald. 1963. Tuberculin-induced mitosis in peripheral blood leucocytes. Lancet, 1: 637.

(80) Poston, J. W. 1971. Personal communication.

(81) Reinhardt, P. W., and F. J. Davis. 1958. Improvements in the threshold detector method of fast neutron dosimetry. Health Phys., 1: 169.

(82) Ruddle, F. H. 1961. Chromosome variation in cell populations derived from pig kidney. Cancer Res., 21: 885.

(83) Sasaki, M. S., and A. Norman. 1967. Selection against chromosome aberrations in human lymphocytes. Nature, 214: 502.

(84) Schmid, E., and M. Bauchinger. 1969. Über das zeitliche verhalten strahleniduzierter chromosomenaberrationen beim menschen. Mutat. Res., 8: 599. (English abstract).

(85) Scott, D. 1969. The effect of irradiated plasma on normal human chromosomes and its relevance to the long-lived lymphocyte hypothesis. Cell Tissue Kinet., 2: 295.

(86) Scott, C., A. L. Batchelor, H. Sharpe, and H. J. Evans. 1967. RBE for fast neutrons and dose-rate studies using fast neutron irradiation. p. 37-52. In H. J. Evans, W. M. Court-Brown, and A. S. McLean (ed.). Human Radiation Cytogenetics. North-Holland, Amsterdam.

(87) Scott, D., H. Sharpe, A. L. Batchelor, H. J. Evans, and D. G. Papworth. 1969. Radiation-induced chromosome damage in human peripheral blood lymphocytes "in vitro". I. RBE and dose-rate studies with fast neutrons. Mutat. Res., 8: 367.

(88) Searle, A. G., E. P. Evans, C. E. Ford, B. J. West, and D. G. Papworth. 1968. Studies on the induction of translocations in mouse spermatogonia. I. The effect of dose rate. Mutat. Res., 6: 427.

(89) Searle, A. G., E. P. Evans, and B. J. West. 1969. Studies on the induction of translocations in mouse spermatogonia. II. Effects of fast neutron irradiation. Mutat. Res., 7: 235.

(90) Sharpe, H., G. W. Dolphin, K. B. Dawson, and E. O. Field. 1967. Chromosomal aberrations in lymphocytes from an extracorporeally irradiated patient. Lancet, 2: 1338.

(91) Sharpe, H. B. A., G. W. Dolphin, K. B. Dawson, and E. O. Field. 1968. Methods for computing lymphocyte kinetics in man by analysis of chromosomal aberrations sustained during extracorporeal irradiation of the blood. Cell Tissue Kinet., 1: 263.

(92) Soukup, S. W., E. Takacs, and J. Warkany. 1965. Chromosome changes in rat embryos following X-irradiation. Cytogenetics, 4: 130.

(93) Wagner, E. B., and G. S. Hurst. 1959. Gamma response and energy losses in the absolute fast neutron dosimeter. Health Phys., 2: 57.

(94) Wagner, E. B., and G. S. Hurst. 1961. A Geiger-Mieller γ-ray dosimeter with low neutron sensitivity. Health Phys., 5: 20.

(95) Weber, W. T., and P. C. Nowell. 1965. Studies on long-lived small lymphocytes in the Rhesus monkey and some other mammals. J. Reticuloendothelial Soc., 2: 326.

APPENDIX

APPENDIX

Since the preirradiation or control aberration levels were not included in the dose response analyses, it was decided to reanalyze the aberration data with the inclusion of the preirradiation aberration levels. The acentric fragment data and the ring plus dicentric data, for both methods of exposure, were subjected to least-squares regression analyses using Poisson variances and weights. The response of the number of aberrations as a function of dose was tested for "goodness of fit" of the following models:

$$Y = a + bD + e$$

$$Y = a + cD^2 + e$$

$$Y = a + bD + cD^2 + e$$

where, Y = the predicted aberration coefficient.

a = the preirradiation aberration coefficient.

b = the calculated aberration coefficient for the linear component.

c = the calculated aberration coefficient for the quadratic component.

D = irradiation dose (measured in air).

e = random error.

The equations which best described the response of acentric fragments were:

$$\text{In Vivo}: \hat{Y} = 12.9 \pm 2.7 \times 10^{-3} + 1.47 \pm 0.06 \times 10^{-3} \ (D).$$

$$\text{In Vitro}: \hat{Y} = 12.3 \pm 4.0 \times 10^{-3} + 5.27 \pm 0.2 \times 10^{-3} \ (D).$$

The ring plus dicentric data gave the best fit to the following equations with a = o (the regression was constrained to pass through the origin) :

In Vivo : $\hat{Y} = 1.04 \pm 0.05 \times 10^{-3}$ (D).

In Vitro : $\hat{Y} = 9.6 \pm 0.1 \times 10^{-3}$ (D).

These results indicate that when the preirradiation level of aberrations were included in the regression analyses, very minor changes resulted in the linear regression coefficients of the models describing the change in acentric fragments and rings plus dicentrics for both exposure methods as a function of dose. It is believed that inclusion of the preirradiation aberration levels into the analyses gives a more reliable prediction of the Y-intercepts.

The finding of similar linear regression coefficients between the two methods of data analyses demonstrate the adequacy of these coefficients in describing the response of aberrations as a function of those.

VITA

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