Evaluation of Portsmouth Naval Shipyard Cytogenetics and Spermatogenesis Protocol (1982)

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Naval Shipyard Cytogenetics Protocol
Commission on Life Sciences
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NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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COMMITTEE TO REVIEW PORTSMOUTH NAVAL

SHIPYARD CYTOGENETICS PROTOCOL

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EXECUTIVE SUMMARY

At the request of the U.S. Department of Health and Human Services, the Commission on Life Sciences of the National Research Council formed an expert committee to review and evaluate a proposed study entitled, "Evaluation of the Effects of Low-Level Radiation on Peripheral Lymphocyte Chromosomes and on Spermatogenesis in Nuclear Dockyard Workers," which was drafted by the National Institute for Occupational Safety and Health (NIOSH). The committee assessed the scientific justification for such studies in general as well as the specific proposal to study workers exposed to radiation in naval shipyards, namely, those at the Portsmouth Naval Shipyard (PNS). The committee also responded to six questions concerning both scientific and operational aspects of the proposed study.

The committee recommends that neither the proposed cytogenetic study nor the sperm study be undertaken at the Portsmouth Naval Shipyard. The major bases for this recommendation are summarized below. The two types of study are considered separately.

PROPOSED CYTOGENETIC STUDY

- The cytogenetic study under consideration will not contribute significantly to scientific knowledge regarding the effects of radiation on chromosomes in humans. Previous studies in humans and animals have provided ample consistent information on the cytogenetic effects of ionizing radiation at comparable doses and dose rates. The proposed study could add little to this information.

- The evaluation of cytogenetic aberrations is useful as a biological dosimeter, but for routine monitoring of whole populations it is more expensive and complex than the physical dosimeters by which the doses of the PNS workers have been recorded.

- The enumeration of cytogenetic aberrations at the level of resolution proposed in this study has no known unique intrinsic value as a predictor of risk of future ill health beyond that of any other dosimetric system.

PROPOSED SPERM STUDY

- Although more information is needed about sperm changes following chronic exposure to ionizing radiation, the sperm study under consideration will not contribute to scientific knowledge of the effects of ionizing radiation on sperm in humans or on ill health related to infertility or genetic changes. The PNS worker population is too small and the recorded doses (especially recent doses) of ionizing radiation to which the individuals have been exposed are too low for scientific study of the question.

- There is a clinical association between fertility and major abnormalities of sperm count, motility, and morphology, but at the subtle levels of change that might occur in an occupational radiation setting, such as at the PNS, no direct tie between sperm effects and health effects can be predicted.

GENERAL FINDINGS AND RECOMMENDATIONS

Because of its recommendations that neither the cytogenetic nor the sperm studies of PNS workers be carried out, the committee commented only on major questions arising from the NIOSH study protocol. If, for any reason, a study is carried out on any radiation-exposed workers, the protocol should be modified in regard to its objectives, population selection, and experimental design.

In response to some of the other specific questions, the committee concludes that:

- Although the mortality data collected in a previous study of the PNS workers would not expedite a cytogenetic study of the same group, the data comprise an important asset for increasing our knowledge of the health outcomes in humans exposed to low-level chronic radiation.

- The committee believes that the results of a study such as that proposed would not be adequately understood by the workers. A major obstacle to such understanding is that in explaining the results of a study, the absence of a proven causal relationship between observations from cytogenetic and sperm studies and adverse health outcomes would be difficult to communicate.

- The committee recognizes that the performance of such studies should not interfere with work at the shipyard to any significant degree, but speculates that they might have adverse psychological effects on worker performance.

EVALUATION OF PORTSMOUTH NAVAL SHIPYARD CYTOGENETICS AND SPERMATOGENESIS PROTOCOL

INTRODUCTION

At the request of the Secretary, U.S. Department of Health and Human Services (DHHS), the National Research Council (NRC) established an expert committee to review and evaluate a proposed study entitled, "Evaluation of the Effects of Low-level [Ionizing] Radiation on Peripheral Lymphocyte Chromosomes and on Spermatogenesis in Nuclear Dockyard Workers." The proposal was prepared by the National Institute for Occupational Safety and Health (NIOSH) to evaluate possible adverse health effects caused by chronic exposure to low-level radiation among nuclear dockyard workers at the Portsmouth Naval Shipyard. The same population group has recently been studied to determine the impact of its occupational exposure on mortality (Rinsky et al., 1981).

The charge to the NRC committee embraces several separate tasks, each of which is addressed in this report:

- The committee was asked to evaluate all relevant scientific issues raised in the study protocol. The resultant evaluation is presented in the following two sections: Cytogenetic Studies and Sperm Studies. These two issues were treated separately for reasons noted below.

- Derived from the first task is a need for a specific critique of the elements of the proposed protocol, which can be found on pages 39-42.

- The committee was also asked by DHHS to respond to six specific questions:

"Is there a need for studies of chromosomal damage or sperm damage ... resulting from low-dose external radiation?

"Does the Portsmouth Naval Shipyard represent an adequate study population? If not, what would be an appropriate population?

"Will the background data developed for the Portsmouth Naval Shipyard mortality study expedite the cytogenetic studies?

"Is the protocol adequate? Are there any modifications that should be made?

"Can the results be adequately explained to the benefit of the workers?

"Does it appear that the time involved and impact of the results on the workers will interfere unreasonably with work at the shipyard?"

These questions are answered specifically at the end of this report. Of greater importance, many of the questions involved evaluations of the scientific basis of the studies. The answers to these questions are amplified in the main discussions of scientific issues.

In undertaking the requested evaluation, the committee was aware of the attitudes and concerns of the DHHS and the Department of the Navy. In its discussions, it tried to address these concerns as they

pertain to the proposed investigation. The committee also had the opportunity to review correspondence from a number of scientists who had been asked for advice by NIOSH during the development of the proposed study. These documents were useful in determining how the proposal was developed and the terms of reference that were considered in its development.

A concern of the committee throughout its evaluation has been how an evaluation of cytogenetic and sperm abnormalities relates to the prediction of adverse health effects. If any health effects arise in humans directly from radiation-induced cytogenetic abnormalities, they are expected to differ from those that might result from sperm cell changes. Thus, studies of the mechanisms would require very different scientific approaches for the two types of abnormalities. Both would require an understanding of the complex factors involved in interpreting scientific data on cellular and tissue effects and relating them to effects on whole laboratory animals and humans. An important requirement of such studies would be to determine whether the measured cytogenetic and sperm cell abnormalities were direct causal factors of adverse health outcomes or were correlated, but biologically independent, outcomes of the radiation exposures. The studies needed to further understanding of these systems are complex in both theory and practice, and reflect the general problems of reliability inherent in studies of humans to predict the risks of ill health following exposure to environmental agents.

The important epidemiological question is: Given that nuclear shipyard workers (among other occupationally exposed groups) are chronically exposed to measurable levels of low-dose radiation, what, if anything, are these doses doing to their health? In order to respond to such a question, health effects must be identifiable and must be related to the radiation exposure.

In a study of federal radiation research (National Research Council, 1981), another NRC committee noted, "From time to time, there will undoubtedly be populations in which exposures to ionizing radiation have occurred and which for various reasons may seem attractive for intensive study. Seldom, however, will these populations be sufficiently large, nor will their radiation doses be well documented and of adequate size to yield statistically significant data on dose-effects relationships and radiation risk." It is against these criteria that the proposed PNS population study must be measured.

The dose response for cytogenetic abnormalities at low-dose chronic radiation levels is fairly well understood, whereas there are few data on semen quality and sperm cell changes in humans under these conditions. However, attempts to relate such <u>biological</u> changes to <u>health outcomes</u> cannot succeed unless both the biological changes and the health outcomes are quantifiable, and dose-response relationships are defined in the low-dose regions.

An avowed purpose of the two aspects of the proposed study protocol is to seek information on the biological effects of low-level chronic radiation in either somatic (lymphocyte) or germline (sperm)

cells to provide a basis for considering any later adverse effects on health. If the cytogenetic damage occurs in somatic cells, it could lead to, or be associated with, somatic effects such as cancer in the exposed individual. If the damage occurs in germ cells, it could lead to reproductive ill health or to genetic effects in future generations. Although the chromosomes in malignant cells are often abnormal, the relationship between induced aberrations and cancer is still not understood. In fact, although there was a radiation dose-related increase in both cancer and chromosome aberrations in the Japanese population that survived the atomic bombings, it has not been possible to relate the two causally in any given individual since those with chromosome aberrations thus far have been reported to be in good health (Awa, 1975).

There are methods for identifying and quantifying changes in semen quality and sperm morphology in populations exposed to radiation, but their use to study large populations of humans exposed to low-level chronic radiation doses is beset with special problems and uncertainties. For instance, unlike studies of cytogenetic abnormalities in humans, studies on semen quality and sperm morphology are hindered by the lack of comparable data on chronic low-dose exposure of humans. Furthermore, although there are reliable procedures for detecting and characterizing structural abnormalities in sperm, it is not yet certain that they can detect the effects of chronic low-dose exposures with the sensitivity needed to define a relationship for health risk estimation.

Finally, like cytogenetic changes in somatic cells, the significance of sperm cell alterations for human health is poorly understood.

Keeping in mind the general scientific concerns and uncertainties expressed above, the committee has described in the following two sections the scientific basis of, and general considerations about, the proposed cytogenetic and the sperm studies of the Portsmouth Naval Shipyard nuclear workers.

CYTOGENETIC STUDIES

EVALUATION OF THE SCIENTIFIC BASIS

Background

When cells are exposed to ionizing radiation, one readily observed dose-related effect is the induction of chromosome aberrations in the cell nuclei. The great majority of these aberrations are formed by as yet unelucidated mechanisms involving the interaction of radiation-induced lesions in such a manner that the linear continuity of the chromosomes is disrupted and rearranged in new configurations. These effects, which have been known for more than 50 years (see review by Lea, 1946), have been demonstrated in a wide array of organisms and biological systems, including humans.

The types of aberrations formed differ, depending upon the stage of the proliferative cell cycle irradiated. Thus, if cells are exposed while they are in the G_1 phase (i.e., the prereplicative part of the cycle before DNA is duplicated), the chromosomes react to radiation as though they were single-stranded structures, even though they consist of a DNA double helix and its associated protein. When cells with broken and/or rearranged G_1-phase chromosomes pass through the S phase of the cell cycle during which DNA synthesis occurs, the chromosomes replicate so that they now are composed of two chromatids[1] that are similarly

[1]A chromatid is one of the two halves of a postreplication chromosome. At the time of a mitotic cell division, the two chromatids of a chromosome separate from one another and pass into different daughter cells, ensuring that each obtains the same genetic information.

affected. This leads to full chromosome aberrations that may be visualized at metaphase. If, however, the cells are irradiated while they are in the S (DNA synthesis) or G_2 (post synthesis) phases of the cell cycle, then the individual chromatids become the units of aberration formation leading to chromatid rather than full chromosome aberrations.

Our understanding of the ways aberrant chromosomes are produced and the kinetics of their formation has been invaluable in the development of both genetics and radiation biophysics. The contribution to the field of genetics came about because rearranged chromosomes constitute a large portion of the spectrum of mutations found in organisms. Ionizing radiation induces large numbers of rearrangements, the study of which has accelerated our understanding of basic genetic principles. The field of radiation biophysics has been benefited because the chromosomes are very large, visible, radiation-sensitive targets that serve as suitable subjects for studies of the kinetics involved in the radiation damage of targets. The results of these studies eliminated the need to make inferences about this from surviving undamaged cells.

Radiation produces chromosome lesions with a probability that is directly proportional to dose, so that those aberrations resulting from a single lesion increase linearly with the dose. Aberrations that result from the interaction of two closely spaced lesions, however, can increase as the square of dose if the two are produced independently,

as is the case with sparsely ionizing radiation, such as x-rays or gamma rays, or they can increase linearly with dose with densely ionizing radiations, such as neutrons, in which both lesions are produced with the same particle. In fact, even with sparsely ionizing radiations, the LET (Linear Energy Transfer),[2] or energy deposition, at the ends of the ionization tracks approaches that of neutrons so that the induction of complex aberrations such as translocations[3] and dicentrics[4] with x-rays can best be expressed by a formula containing both a one-track (linear) and a two-track (quadratic) term:

$$Y = c + \alpha_1 D + \beta D^2,$$

where Y is the yield of aberrations, α_1 is the probability that both interacting lesions will be produced by the tail of a single track, β is the probability of obtaining the pair of lesions independently, c is the background level of aberrations found in unirradiated cells, and D is the dose in rad (or Grey).

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[2]Linear Energy Transfer (LET) is the average amount of energy lost per unit of particle spur-track length. Low LET radiation is characteristic of electrons, x-rays, and gamma rays. High LET radiation is characteristic of protons and fast neutrons. Average LET is specified to even out the effect of a particle that is slowing down near the end of its path and to allow for the fact that secondary particles from photon or fast-neutron beams are not all of the same energy.

[3]A translocation is a symmetrical chromosome aberration in which distal portions of two separate chromosomes exchange.

[4]A dicentric is an asymmetrical chromosome exchange in which the proximal portions that contain the centromeres of the two chromosomes become attached to form a chromosome with two centromeres.

For two lesions to interact, both must coexist in time and space as they do when the dose is administered under acute conditions. If the dose of radiation is given under chronic conditions, however, the lesions can be repaired (eliminated) before having the opportunity to interact with lesions from subsequent ionizations. Consequently, the more the radiation exposure is protracted, the more the two-track contribution to the total yield decreases, and the closer the βD^2 term approaches zero. However, the one-hit contribution represented by $\alpha_1 D$ is, as expected, unaffected by the dose-rate of the radiation.

These fundamental radiobiological relationships apply to a wide array of living organisms including plants, insects, and mammals. A concern of many scientists was whether or not these facts applied to the exposure of humans to ionizing radiation.

Chromosome Aberrations in Human Peripheral Blood Lymphocytes

Twenty-three years ago researchers learned how to utilize the normally nondividing human peripheral blood lymphocyte for cytogenetic studies (Hungerford et al., 1959). Lymphocytes exposed to phytohemagglutinin (or some other antigenic materials) undergo blast transformation and cell division at which time the chromosomes can be analyzed. The peripheral lymphocyte is eminently suitable for radiation cytogenetic studies because the vast majority ($>99.9\%$) of them are in a resting presynthetic stage of the normal proliferative cycle referred to as G_0. As such, their chromosomes behave functionally as single threads of double-stranded DNA and associated proteins, and only

chromosome-type aberrations are produced when the cells are irradiated. In addition, the requirement of an antigenic stimulus for initiation of cell division provides the investigator with a considerable amount of flexibility in that the actual culturing of the lymphocytes can take place shortly after an exposure to radiation up to several weeks after exposure. These characteristics provided the optimal tool for developing an experimental system of human origin that could be used to evaluate the genetic effects of ionizing radiation in that the investigator was provided with a readily accessible homogeneous population of cells with relatively uniform radiosensitivity. Consequently, the peripheral lymphocyte system was soon utilized to determine the cytogenetic response of the cells after *in vitro* (Bell and Baker, 1960; Bender and Gooch, 1962b) and *in vivo* (Bender and Gooch, 1962a; Tough *et al.*, 1960) radiation exposure (see Bender, 1969, for review).

Subsequent to these reports, a considerable research effort was directed toward elucidating the radiobiological response of the peripheral lymphocyte in hopes of using it as a "biological dosimeter" (see reviews by Bender, 1969; Dolphin *et al.*, 1973; Evans *et al.*, 1967; and Gooch *et al.*, 1964). The rationale behind this endeavor was multifaceted: it was intended that the quantitative measurement of chromosome aberrations would serve as a means of verifying exposure of an individual to ionizing radiation, provide an approximate estimation of the total dose absorbed, and possibly provide a basis for estimating genetic and/or somatic health risks. After more than 20 years of

extensive research in the area, several fundamental facts have been established. Briefly stated these are:

- Ionizing radiation induces chromosome aberrations in human peripheral lymphocytes as it does in other cells in that the yield (Y) of such aberrations induced by low LET radiation can be expressed as a function of dose (D) by the equation:

$$Y = c + \alpha_1 D + \beta D^2,$$

and the yield induced by high LET radiation can be expressed by the equation:

$$Y = c + \alpha_2 D,$$

where c represents the spontaneous frequency, and terms α_1 and α_2 are the coefficients of the one-track events, and β is the coefficient of the two track-events.

- The lesions that give rise to chromosome aberrations are susceptible to repair, and the half-life of these lesions is approximately 90 minutes or more, depending on the dose.

- Due to the combination of repair, the need for the existence of two lesions to form an aberration, and the independent production of a significant proportion of these lesions by low LET radiation, the yield of aberrations as a function of dose of protracted (chronic, long-term) exposure is expressed by the equation:

$$Y = c + \alpha_1 D.$$

This simply means that because the two lesions do not coexist in time, there will be no aberrations resulting from two independent ionizing tracks.

- The α_1 term is not significantly influenced by protracted exposure.

- The radiosensitivity of the lymphocyte is the same whether the exposure occurs *in vivo* (within the body) or *in vitro* (in cell cultures).

- The average half-life of the circulating lymphocyte is approximately 3.5 to 5 years, and some lymphocytes persist and may appear in the peripheral blood for as long as 20 to 30 years. Thus, some chromosome aberrations can be detected up to 30 years after their formation.

- The frequency of chromosome aberrations following an acute (short-term) exposure begins to decline about 30 days after exposure and does so exponentially with a half-life of 3.5 to 5 years because (a) gene imbalance is brought about by division of precursor cells containing the aberrations and mechanical interference with the completion of mitosis, particularly of dicentrics that are used to estimate the absorbed dose, and (b) there is a normal attrition of the aberration-bearing lymphocytes.

- The background (or spontaneous) frequency of dicentrics (the type of chromosome aberration analyzed) is approximately 5×10^{-4} dicentrics per cell.

- Based on a multitude of independent in vitro studies, the best estimate of α_1 is approximately 5×10^{-4} dicentrics/cell/rad (0.01 Gy[5]).

- It is possible to detect the effects of small acute doses, i.e., >5 rad (> 0.05 Gy), in the case of both in vitro and in vivo irradiation.

- The midline absorbed body dose is approximately 50% of the skin dose in a person of average build. Therefore, the dose to a circulating lymphocyte is half of the dose to a film badge.

NEED FOR A CYTOGENETIC STUDY AT THE PORTSMOUTH NAVAL SHIPYARD

Based on the facts listed above, the committee believes there are several issues to be considered with respect to the cytogenetic portion of the NIOSH proposal for studies of the nuclear workers at the Portsmouth Naval Shipyard (PNS).

The first issue is the degree to which determination of chromosome aberrations in peripheral blood lymphocytes of radiation-exposed workers at PNS is likely to add substantially to our knowledge of radiation effects or provide answers to scientific questions. There is already a large body of published information on the induction of chromosome aberrations in peripheral lymphocytes of radiation-exposed persons (Evans and Lloyd, 1978). In addition to studies of the accidentally or therapeutically irradiated, a number of studies have involved persons chronically exposed to doses within the established

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[5] 1 Gy = 100 rad.

occupational limits. Among those demonstrating statistically significant increases in aberration frequencies, two studies were especially relevant: the investigation by Evans et al. (1979), who studied a population of 197 exposed and unexposed workers at a nuclear dockyard in Scotland, and that of Lloyd et al. (1980), who reported aberration frequencies for a population of 146 exposed radiation workers from nuclear facilities in the United Kingdom and 50 control workers from a nuclear facility or an adjacent police station. The results of both studies agree with earlier reports (Buckton et al., 1967; Norman et al., 1964; Visfeldt, 1967) that a significantly elevated frequency of chromosome aberrations is observed when workers with cumulative exposures of approximately 10 rad (0.1 Gy) are compared as a group with suitable unexposed control groups, although individual frequencies are rarely sufficiently elevated to attain statistical significance. An important aspect of these studies is that both groups had been exposed to a wide enough range of doses and contained sufficient numbers of subjects to establish a dose-effect relationship, i.e., a significant regression of mean aberration frequency against mean dose. Thus these and earlier studies confirm that radiation exposures within currently acceptable occupational dose limits (International Commission on Radiological Protection, 1977) do produce detectable elevations in the frequencies of chromosome aberrations in lymphocytes. Furthermore, they fulfill an important biological requirement for establishing cause-effect relationships, i.e., a significant dose-effect regression was found.

The Evans et al. (1979) and the Lloyd et al. (1980) studies also agree quantitatively remarkably well. Although there are slight differences in the statistical analyses of the data, and a more rigorous comparison should be made from the raw data, we are impressed that the slopes (± standard deviations) for the linear regressions of dicentrics on recent cumulative dose are $(2.32 \pm 1.01) \times 10^{-4}$ and $(2.22 \pm 0.94) \times 10^{-4}$ dicentrics per cell per rem (cSv[6]) exposure for the Evans et al. (1979) and the Lloyd et al. (1980) studies, respectively.

The two most fundamental biological questions have already been answered: aberration frequencies in peripheral lymphocytes are elevated to a detectable level in groups occupationally exposed to radiation, and the degree of elevation is a function of radiation dose. The committee does not believe an additional study at PNS is justified simply to confirm the earlier findings. The possibility of hidden bias in the earlier studies seems small in view of their excellent conformity with the large body of cytogenetic data on animals. In addition, the similarity of the PNS study to the study by Evans and colleagues of shipyard workers would make discovery of any new biases unlikely. In considering the question of whether the proposed PNS study might be justified in terms of reducing the standard deviation of the slope of the dose-response of lymphocyte chromosome aberration frequencies, we note that if the proposed study were to

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[6] 1 Sv = 100 rems; cSv = 0.01 Sv = 1 rem.

produce a regression slope similar to that of the earlier studies (as might be expected), then the precision of the current average slope from the two existing studies of $(2.27 \pm 0.69) \times 10^{-4}$ dicentrics per cell per rem (cSv) should be improved to only about $(2.27 \pm 0.56) \times 10^{-4}$ dicentrics per cell per rem (cSv).

We believe that further studies of radiation-exposed humans might elucidate the surprising finding of Evans et al. (1979) that aberration yields seem to be strongly dependent on the age of the subject, although this is not addressed in the NIOSH protocol. Older workers appeared to be 5 times more susceptible to radiation induction of chromosome aberrations than were younger workers. Such an age dependence has not been reported in other studies, including those of the Japanese atomic-bomb survivors (Awa, 1975) and should be looked for in the data of Lloyd et al. (1980). The clarification of this finding might be an appropriate area for scientific investigation. Reasons for such an effect might be age-related differences in lymphocyte turnover and repair, phenomena that are best analyzed by mechanistically oriented studies such as aberration decay rates (for turnover) and split-dose effects (for repair). The proposed PNS study would be an inefficient, inappropriate way to confirm the age dependence reported by Evans et al.

Therefore, this committee concludes that there is no scientific need to carry out the cytogenetic study of the PNS workers.

CYTOGENETIC SURVEILLANCE OF INDUSTRIAL RADIATION WORKERS

The above conclusion should not be construed as indicating that there is no place for cytogenetic analyses in the estimation of the radiation doses workers have absorbed. In fact, the induction of chromosome dicentrics in the lymphocytes of exposed people provides the best "biological dosimeter" we have. The kinetics of induction of the chromosome aberrations have been well characterized, and the lymphocyte is long-lived, which allows it to "integrate" the dose accumulated over time.

The correspondence between the biological dosimetry and physical dosimetry (as represented by film badges, for instance) is good. Therefore, the committee believes the use of expensive and time-consuming cytological techniques is not warranted for routine surveillance of badged radiation workers exposed chronically to low doses. Nevertheless, there are circumstances in which cytogenetic analyses should be performed for individual cases. The committee recommends, for instance, that any worker, badged or not, suspected of having been exposed to high doses of radiation should undergo a cytogenetic examination to assess the degree of biological damage. This is particularly important for cases of partial-body exposure in which the physical dosimeter (film badge or thermoluminescent dosimeter) might not be directly relatable to the equivalent whole-body exposure.

Cytogenetic analyses should also be made to estimate doses that cannot be quantified by any other means. For instance, there are times when the physical dosimeters give high readings that might be spurious or when it is suspected that an unmonitored person might have been exposed to radiation. In situations such as these, cytogenetic analyses can be performed to corroborate whether or not the subject was actually exposed.

CHROMOSOME ABERRATIONS AS PREDICTORS OF ILL HEALTH IN HUMANS

The appearance of chromosome dicentrics in an irradiated population can be interpreted as an indication of exposure and, thus, of increased risk for radiation-induced health effects only in that population. The background level of dicentrics found in human peripheral lymphocytes is approximately 5×10^{-4} per cell, indicating that normal adults already have some 6 million circulating lymphocytes with these aberrations. Thus the appearance of a few extra aberrant cells in a sample from an exposed individual need not imply that that person will suffer any adverse health consequences.

SPERM STUDIES

REVIEW OF SPERM TESTS

Assessment of germinal cell function in a human population exposed to ionizing radiation is relevant for three reasons: as a check on heritable genetic integrity, as a check on fertility, and as a sensitive indicator of general toxicity. In the human, oocytes are essentially nonaccessible, whereas semen can be sampled noninvasively albeit with some social sensitivity. Thus, currently available tests of the quantity and quality of human sperm are of special importance, as is the development of future tests.

The PNS protocol includes three tests on sperm: sperm counts, sperm morphology, and the test for double Y bodies. In the following paragraphs these tests are reviewed, related to sperm studies in other mammals, and evaluated as scientific and practical indicators of radiation effects. A fourth test--sperm motility--is not included in the protocol. Since this test is impracticable in a large occupational study and bears an uncertain relationship to radiation effects, it is not considered further in this report.

This section focuses on radiation effects, but about 90 agents other than radiation can be found in the literature concerning effects on human sperm (Wyrobek *et al.*, in press b). Included are occupational and environmental exposures to chemical agents, exposures to experimental and therapeutic drugs, and use of tobacco, alcohol, and marijuana.

Description of Human Sperm Tests

Sperm count usually refers to the number of sperm per milliliter of ejaculate (or to the total number of sperm in the ejaculate) as determined from fresh or frozen samples. Measurements are made with hemocytometers or automated cell counters. Variable continence time and collection of an incomplete ejaculate affect this measurement as do certain medical, occupational, and personal factors (Schwartz et al., 1979; Wyrobek et al., in press b). Normal ranges have been established for several human populations.

Sperm morphology (or seminal cytology) is the visual assessment of the shapes of ejaculated sperm. Although sperm-head shape is usually emphasized, some assessments also include midpiece and tail abnormalities. In general, there has been little agreement on the definition of normal shapes or in the categories of abnormal shapes. This has resulted in much interlaboratory and interscorer variability; however, results of some studies have shown that quantitative approaches to the visual assessment of morphology can be used with considerable success (David et al., 1975; Eliasson, 1970; MacLeod, 1974).

Sperm can be assigned to shape categories. In evaluating the effects of exposure, sperm of exposed men should be analyzed concurrently in a blind study with that of two controls: (1) sperm from unexposed controls and (2) sperm comprising a set of standard reference slides as a check on the stability of scoring criteria (Wyrobek et al., 1981b). Normal ranges have been established for several unexposed populations (Wyrobek et al., in press b).

The Y-body test scores the frequency of fluorescent bright spots in human sperm stained with quinacrine dye (Pearson et al., 1970). Based on studies in somatic cells, each quinacrine bright spot is thought to represent a Y chromosome in the sperm cell. In sperm, the absence of Y chromosomes or the presence of one Y chromosome should reflect the normal segregation of X and Y chromosomes in meiosis, whereas two (or double) Y chromosomes might represent abnormal disjunction of the Y chromosome. However, there is no direct evidence to prove that the bright spots in sperm are Y chromosomes or that their number represents the number of such chromosomes. The Y-body test is new, and only a few populations of exposed men have been studied (Wyrobek et al., in press b). Unlike the other sperm tests, the fluorescent Y-body test has no direct counterpart in the mouse or other common laboratory mammals because the bright quinacrine fluorescence believed to be the Y chromosome is seen only in man and certain apes (Seuânez, 1980). In the field vole, the unusual pattern of heterochromatin in the sex chromosomes allows the identification and counting of X and Y chromosomes in Giemsa-stained spermatids (Tates, 1979).

Studies in Animals

Acute exposure. The effect of x-ray exposure on spermatogenesis has been documented in several animal species. Studies in mice are the most extensive and illustrate the reproducible nature of dose-effect curves for spermatogonial cell killing and for subsequent reductions in mature sperm counts and changes in sperm morphology. In acutely

irradiated mice, spermatogonial stem cells have an LD_{50} of 200 to 300 rad (2-3 Gy), but the LD_{50} of committed spermatogonia falls dramatically to about 25 rad (0.25 Gy) (Withers et al., 1974).

Further along in the process of spermatogenesis the resistance increases progressively so that the LD_{50} for spermatocytes is around 500 rad (5 Gy); for spermatids, more than 1,500 rad (15 Gy); and for mature sperm, more than 60,000 rad (600 Gy) (Meistrich et al., 1978). After acute radiation exposures of several hundred rad, the testis is repopulated from the relatively resistant spermatogonial stem cells (Oakberg, 1968). In acute exposures, reductions in sperm count generally arise from a depletion of committed spermatogonia. At higher doses, sperm depletion is seen in the epididymis, resulting from depletion of spermatocytes after a transit time of 3 to 5 weeks. After acute exposures up to 300 rad (3 Gy), the sperm count reaches a minimum at approximately 6 weeks and returns to control values within 10 weeks (Bruce et al., 1974; Searle and Beechey, 1974).

The spontaneous rate of abnormally shaped sperm is strain-dependent in mice (Wyrobek, 1979). Acute x-irradiation produces dose-dependent increases in the population of abnormally shaped sperm with maximum sensitivity at approximately 5 weeks after exposure (Bruce et al., 1974). The dose-response curve at 5 weeks follows a $dose^{1.5}$ power function with a doubling dose of about 25 rad (0.25 Gy) by visual criteria and about 10 rad (0.1 Gy) if image-processing techniques are used (Moore et al., in press). After peaking at 5 weeks, the fraction

of abnormally shaped sperm falls by 10 weeks to a plateau level, which remains above background as some yet-to-be-well-studied function of dose, strain, and species.

Studies of acute x-irradiation show that rats and bulls are similar to mice in their dose kinetics and patterns of changes in sperm count and morphology (Gillette et al., 1964; Lock and Soares, 1980).

Little is known of the effects of radiation on sex chromosome nondisjunction in mammalian gametes. In studies of the vole, Microtus oeconomus, x-irradiation at doses of 25 rad (0.25 Gy) or more may increase the rate of meiotic nondisjunction in males, although the importance of time that has elapsed after exposure, dose, and the effects on stem cells remain unclear.

Protracted exposures. In a variety of species, protracted radiation exposure can cause permanent reduction in cell numbers at much lower doses than required for acute exposure (Speiser et al., 1973). Very low doses, even when given repeatedly, need not alter spermatogonial numbers. In an extensive study of beagle dogs, exposure to x-rays at 3.0 rad (0.03 Gy) per week led to complete cessation in sperm production within less than a year, whereas 0.6 rad (0.006 Gy) per week or less had little or no effect on sperm counts even after 13 years of exposure (Casarett, 1964). Similarly, studies of spermatogonial killing in mice showed little or no effect after 15 weeks of daily exposure to 1.8 rad (0.018 Gy) of gamma rays (Fabrikant, 1972 and 1976).

Studies in Humans

Acute exposures. Data on human radiation exposure and spermatogenesis are limited. The most detailed account was provided by Heller and associates (Heller et al., 1965; Rowley et al., 1974), who studied the effects of local testicular x-irradiation on sperm production in 67 men who received acute doses of 8 to 600 rad (0.08 to 6 Gy). In some of the men, transient reductions in sperm count were observed at the lowest dose, whereas 15 rad (0.15 Gy) reduced the sperm count in all men to 20% of control by 2 months--an effect that lasted for 6 to 8 months after exposure. Higher doses reduced sperm counts further, and recovery times increased with increasing dose. Sperm counts in man are more radiosensitive than in animals, and the effects are longer lived (many months to years in man compared to weeks in mice exposed to comparable doses). Furthermore, the kinetics of spermatogonial killing and subsequent recovery in man are considerably different from those in mice (Oakberg, 1968; Oakberg and Heller, 1966). In mice exposed acutely to 100 rad (1 Gy), spermatogonia reach a minimum at 30% of normal within 2 to 3 days, and a 50% recovery occurred by the 20th day. In man, the spermatogonia reach a minimum of 10% of normal levels 200 days after irradiation, and recovery to 50% occurred at 600 days. These observations may account for the marked depression in sperm count and long recovery times after x-irradiation of the human testis. Results of studies of victims of radiation accidents are consistent with these observations (Oakes and Lushbaugh, 1952; MacLeod et al., 1964).

Protracted exposures. Limited studies of men receiving protracted radiation therapy suggest that testicular doses as low as 10 rad (0.1 Gy) can lead to reduced sperm counts (Casarett and Eddy, 1968; Lushbaugh and Casarett, 1976).

In a study of 72 occupationally exposed radiation workers (i.e., radiologists, x-ray technicians, radium dial painters, and industrial technicians), Popescu and Lancranjan (1975) found evidence of reduced sperm counts, reduced sperm motility, and increased abnormal sperm forms among the exposed men, compared to 42 controls. Yearly film badge doses ranged from 0.5 to 3.5 rad (0.005 to 0.035 Gy), and occupational exposure periods ranged from 2 to 22 years; however, data from film badges were available only for 5 to 10 years prior to the sperm study. The results of this study are difficult to interpret because there was no consideration of dose-response and it is not clear how well the control and exposed groups were matched.

Limited data resulting from studies of two other groups of occupationally exposed men suggest that such exposures reduce sperm motility and ejaculate volume (Vascov, 1968) and that men with high cumulative doses have numerous testicular abnormalities (Kirilov-Postnikov et al., 1977).

There are no data on the use of the Y-body test in irradiated men.

The Biological and Clinical Implications of Induced Sperm Changes

Major reductions in sperm count and increases in abnormal forms have been linked to reduced fertility in men as well as in domestic and laboratory animals. We do not yet know the extent (if any) to which

fertility is affected by minor changes, such as might be expected after chronic exposures to low doses of radiation. Very limited data suggest that continued occupational exposures in the range of 0.5 and 3.5 rad (0.005 to 0.035 Gy) a year might be associated with subfertility (Popescu and Lancranjan, 1975).

Studies in mice with chemicals that have been tested for mammalian germ cell mutagenicity show a high correlation between the ability of the chemicals to induce changes in sperm morphology and to induce mutations (Wyrobek et al., in press a). Little is known about this relationship in man. Studies of men whose wives either had had spontaneous abortions (Furuhjelm et al., 1962) or were habitual aborters (Czeizel et al., 1976) suggest that diminished semen quality (increased abnormal sperm forms and decreased sperm numbers) may be linked to abortion. These findings remain uncertain (Homonnai et al., 1980). The assessment of Y bodies may be indicative of chromosomal nondisjunction, but this has not yet been validated. It is not clear what sperm effects, if any, can be expected in men exposed to low levels of radiation and the extent to which such changes might be related to adverse health effects.

Statistical Sensitivity of Sperm Tests

The statistical sensitivities of the tests for sperm counts and morphology, as well as double Y-bodies, were recently estimated from data obtained from a group of control men (Wyrobek et al., 1981a). The mean, standard deviation, 95% confidence limits for single observations, and statistical form for each test were:

- sperm count: 129 x 10^6/ml, 137 x 10^6/ml, 12 x 10^6/ml to 578 x 10^6/ml, log-normal;
- percent abnormal sperm: 41.9, 12.4, 17.6 to 66.2, normal;
- percent double Y bodies: 0.8, 0.7, 0.2 to 1.8, Poisson.

To detect a 25% increase in the mean proportion of abnormally shaped sperm (i.e., from 41.9% to 52.4%) at the 5% level with 90% power would require 26 control and 26 exposed men. Y-body analysis would require 80 men in each group to detect a 25% increase, and sperm count would require 230 men in each group to detect a 25% reduction.

Less variation in sperm count and morphology is observed in repeated sperm samplings of individuals than in samplings from a group of subjects. This is especially true for the overall percent and pattern of sperm morphology, which is highly person-specific. In a longitudinal study using sperm morphology, fewer men would be needed to obtain a statistical power similar to that discussed above.

THE FEASIBILITY OF CARRYING OUT MEANINGFUL SPERM STUDIES AT PNS

The proposed sperm study involves a cross-sectional sampling of PNS employees. A single sperm sample would be collected from each of 266 unexposed controls and 266 exposed workers. Sperm counts and sperm morphology studies would be performed on this sample, and Y-bodies measured and correlated initially with exposure and subsequently with the cytogenetic observations.

Data from studies on acute and chronic radiation exposure of humans suggest that a 15% decline in sperm count is a possible result of

recent exposures to 5 rems (0.05 Sv) or greater (see above). Extension of the calculation of Wyrobek et al. (1981a) to a sample size of 266 exposed and 266 control subjects indicates that the sample size in the NIOSH protocol would be sufficiently large to detect a 15% reduction in sperm count at the 5% level with 90% power. However, the PNS sample does not contain 266 men with "recent" exposures to 5 rems (0.05 Sv) or more. Between 1971 and 1977, the records at PNS indicate that only three PNS employees had been exposed to more than 3 rems (0.03 Sv) in any one year. Although the committee did not precisely define "recent," it expects that, at occupational doses, recovery of sperm count should occur within a few years. At present, information on the full distribution of recent exposures among the PNS work force is not available to the committee, but the limited data that are available indicate that the effective recent exposure records would yield a mean dose in the 266 most exposed men of some small fraction of 5 rems (0.05 Sv). Thus, the committee concludes that there is no reasonable chance of detecting meaningful effects on sperm count with the present study design.

For sperm morphology, a similar argument can be used to estimate the resolution of the NIOSH study design. The data of Wyrobek et al. (1981b) lead to the estimate that the 266 exposed and 266 control subjects could resolve the change from 41.9% to 44.0% at the 5% level with 90% power. However, since human sperm morphology has never been calibrated against radiation dose, we have little idea what magnitude of effect to expect, nor how to extrapolate from animal data to man.

Judging from data on mice (Bruce et al., 1974) and rats (Lock and Soares, 1980), a change to background plus 2.1% abnormal sperm at the peak response requires 30 to 60 rad (0.3 to 0.6 Gy) of acute x-radiation or gamma-radiation. Even if the sensitivity of humans is 10 times greater than that of rodents, the exposed PNS population in the NIOSH study would be inadequate to detect such an effect from cumulative doses of about 5 rems (0.05 Sv).

For the Y-body test, 266 controls and 266 exposed subjects could resolve a 0.07% increase in the mean percent of sperm with double Y bodies (0.80% vs. 0.87%) at 5% level and 90% power. Using the data for X and Y chromosome nondisjunction in irradiated M. oeconomus (Tates et al. 1979), we estimate that a dose of more than 50 rad (0.5 Gy) given acutely to the vole would be required to obtain an increase of this magnitude. These data on the vole, with the inclusion of at least an order of magnitude margin of safety, led the committee to conclude that there is no reasonable chance of detecting any meaningful effects on human sperm Y bodies by studying the PNS workers.

An alternative approach that could be considered is a longitudinal study in which repeated sperm samples are obtained from workers before and after exposure to radiation. Since within-subject variation in sperm count and, especially, sperm morphology appear to be substantially less than between-subject variation, a longitudinal study would require far fewer subjects than a cross-sectional study. The difficulty with a longitudinal study at PNS, however, is that the "exposed" group would have to be composed of workers who will be exposed to radiation during the next year or so. It is highly unlikely that sufficient numbers of PNS workers will receive exposures of more than

1 rem (0.01 Sv) during this period. Thus, although a longitudinal study is statistically more efficient than a cross-sectional study, that type of study is not well-suited for the PNS population. The committee concludes, therefore, that although population studies of the association between low-level radiation and sperm changes are important and should be encouraged, it does not appear that a study limited to PNS would lead to meaningful results.

USE OF SPERM TESTS IN MEN EXPOSED TO IONIZING RADIATION

Although there are extensive data from studies in animals, the paucity of data on radiation effects on human sperm demonstrates the need for obtaining reliable information in selected populations. The sensitivities of the sperm tests for counts and morphology seem to be approaching those required to detect low-level exposures. An effort should be made to design effective studies of sperm in men exposed to radiation with special emphasis on defining the dose-response relationship. Such an understanding of effects at low-dose exposures is essential for future applications of the sperm tests as biological dosimeters of spermatogenic injury. The studies could be either cross-sectional or longitudinal.

Cross-Sectional Studies

In order to understand the dose-response characteristics of the effects of chronic low-level radiation, large numbers of exposed men will have to be studied. The committee suggests that nuclear workers in industry, naval shipyards, and national laboratories be inventoried

in an attempt to assess the size of the exposed populations and their cumulative and yearly doses. If statistically adequate populations and dose ranges are identified, a study incorporating statistical sampling methods and epidemiological considerations might then be designed.

Longitudinal Studies

There are advantages to a longitudinal study since changes within an individual over time can be directly observed and compared to assess dose response and recovery. To study the effects of low-level occupational exposures on sperm counts and morphology, the inventory mentioned above could be used to identify a subpopulation of men with high risk of exposure to >1 rem (> 0.01 Sv) during the succeeding year(s). The men would have to be sampled at the beginning of the study. Then after 1 year, all those with significant (>1 rem) exposures would be resampled and compared to a selected, matched subset of men who experienced no significant exposures. Although the initial sampling group may be large, the group selected for comparison will be composed of those for whom repeated samples were collected. The disadvantage of this approach is the uncertainty of how many men would need to be sampled initially to assure a reasonable study sample within a year or more. The inventory of the exposure characteristics of the workers in the nuclear industry should be helpful to evaluate the feasibility of this approach. The costs of a longitudinal study should be less than those of a cross-sectional study, especially if one considers that initial sperm samples do not need to be analyzed unless indicated by subsequent exposure and samples.

PROTOCOL CRITIQUE

The committee concluded that the proposed cytogenetic and sperm studies of PNS workers should not be carried out. In view of these conclusions, the committee felt no need to comment in great detail on the proposed protocols. However, it did review the protocols carefully and found several noticeable defects in their biological aspects, as well as in the experimental design and proposed methodology for statistical analysis.

The proposed investigation of the PNS workers illustrates some of the difficult issues inherent in all studies of the kind under discussion. For example: Should they be cross-sectional or longitudinal? Should they be cohort or case-control? Should they focus solely on radiation damage or on unbiased estimates of interactions? How should concomitant sources of variation be managed? The proposal demonstrates some of these limitations in study design, particularly those problems of bias introduced by certain population sample selection, inadequacy of population sample size, and methods of statistical analyses.

The following comments apply only to some of the difficulties noted.

- There were several places where the protocol was inadequate for a cytogenetic study. For instance, very little detail was provided about methods of culture, fixation, slide preparation, and scoring. These items are significant since variations in methodology have major

effects on results. Furthermore, the study of sister chromatid exchanges (SCEs) was included even though G_0 lymphocytes are notably resistant to SCE induction by ionizing radiation. Several groups of people seem to have been excluded from the study for reasons that would not affect the cytogenetic results.

- The protocol for the semen studies gives no indication of the techniques for quality control and analysis.

- With the exception of cytogenetic effects, most information on biological and health effects in humans exposed to small doses of radiation rests on extrapolations from data obtained at moderate to high doses or involves data on too few persons to establish with reasonable certainty the risks of damage. Well-designed studies of the effects of exposure to ionizing radiation at low-dose or low-dose rate exposures over an extended period could be informative, but such studies are confronted with a formidable array of difficulties. Clinical, epidemiological, and experimental data indicate that any adverse health effects to be expected may be rare or hard to detect within a reasonable period. Moreover, age, health status, socioeconomic level, and lifestyle factors, as well as nonoccupational exposures to ionizing radiation, are important sources of extraneous variation. These confounding variables as well as dosimetric uncertainties could obscure a real effect or suggest a spurious one. Moreover, the occupational exposures to be anticipated in most well-regulated industries will be small and the sample sizes needed to establish statistical significance correspondingly large. Thus, it is not certain whether any study of an

occupationally exposed population, or combination of populations, will produce unambiguous results.

- The committee recognizes that exposure to ionizing radiation in the workplace has been reasonably well controlled in most instances. In view of the exceptions to this generalization noted by the NRC committee that studied federal radiation research (National Research Council, 1981, Chapter 9 and Appendix C), future studies of occupational groups should be considered only under the following circumstances.

Should occupationally exposed worker populations be identified as receiving high doses of ionizing radiation (i.e., from about 10 to 50 rad), and should there be a range of exposures in the population wide enough to provide statistically significant results, such groups would be deserving of thorough study. Not only would the chromosome and the sperm studies be appropriate for such groups, but careful investigation of potential adverse health outcomes would be essential. The latter should include somatic effects, such as cancer, infertility in males and females, and congenital malformations among the offspring of the worker population.

Although the PNS worker population does not appear to meet the necessary criteria for this study, the committee believes that other occupational groups should be evaluated to determine if they should be examined. The committee has not investigated the radiation dose data in other potentially exposed occupational groups. The appropriate

governmental agency should review the dose commitment of workers in the following fields to see if these groups meet the criteria for a useful and productive epidemiological study: uranium miners, workers at land-fill burial facilities for low-level radioactive wastes, special medical and research groups handling isotopes and other radioactive materials, and personnel in nuclear power generating plants.

If any of these or other groups meet the criteria for a useful dose-response study, a thorough evaluation could provide important information for worker health and protection.

SPECIFIC QUESTIONS

The charge to the committee contained six specific questions, which are answered below:

Question No. 1:

"Is there a need for studies of chromosomal damage or sperm damage ... resulting from low-dose external radiation?"

Response:

The committee believes that there is no need for cytogenetic surveys of populations exposed to low doses of ionizing radiation, although cytogenetic studies could be useful under some circumstances. See extended discussion in report, pp. 18-23. The committee also believes that there is a need to study sperm changes, but that the PNS population is inadequate for a scientific sperm study that would answer the most pressing questions. See extended discussion, pp. 33-37.

Question No. 2:

"Does the Portsmouth Naval Shipyard represent an adequate study population? If not, what would be an appropriate population?"

Response:

The committee believes that the PNS workers do not represent an adequate population for these studies. An appropriate population may be constructed following criteria described on pages 36-37 and 41-42 of the report.

Question No. 3:

"Will the background data developed for the Portsmouth Naval Shipyard mortality study expedite the cytogenetic studies?"

Response:

The committee believes that the data will not expedite the cytogenetic study. However, they do comprise an important data set that will improve our knowledge of health outcomes of low-level chronic exposure, for which cytogenetic analyses offer an expensive and inefficient, but valuable "biological dosimeter."

The existence of a clearly identified population and background epidemiological information would be useful in any subsequent study.

Question No. 4:

"Is the protocol adequate? Are there any modifications that should be made?"

Response:

The committee believes that if for any reason this study is carried out, there should be protocol modifications involving objectives, population selection, and experimental design. Such modifications should include:

- clarification of objectives that are scientifically justifiable and respond to needs for worker surveillance;
- selection of populations that have varied dose and dose rates and are large enough for statistical analysis; and

- specification of techniques of both laboratory analysis and data analysis, so that evaluations will be based on a thorough knowledge of the proposed technologies.

Question No. 5:

"Can the results be adequately explained to the benefit of the workers?"

Response:

Based on experiences in England, Scotland, and the United States, the committee believes that the study results would probably not be adequately understood. The absence of a proven detailed causal relationship between the observations from cytogenetics and sperm studies and adverse health outcomes presents a major obstacle to public (worker) understanding of any study results.

Question No. 6:

"Does it appear that the time involved and impact of the results on the workers will interfere unreasonably with work at the shipyard?

Response:

The committee believes that the performance of the study should not interfere with work at the shipyard to any significant degree, since it involves short-term commitments to collect blood and sperm samples. Such a study might have adverse effects on the worker performance for psychological reasons, but this view is only speculative.

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