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The Children of Parents Exposed to Atomic Bombs: Estimates of the Genetic Doubling Dose of Radiation for Humans

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Summary

The data collected in Hiroshima and Nagasaki during the past 40 years on the children of survivors of the atomic bombings and on the children of a suitable control population are analyzed on the basis of the newly revised estimates of radiation doses. No statistically significant effects emerge with respect to eight different indicators. Since, however, it may confidently be assumed some mutations were induced, we have taken the data at face value and calculated the minimal gametic doubling doses of acute radiation for the individual indicators at various probability levels. An effort has also been made to calculate the most probable doubling dose for the indicators combined. The latter value is between 1.7 and 2.2 Sv. It is suggested the appropriate figure for chronic radiation would be between 3.4 and 4.5 Sv. These estimates suggest humans are less sensitive to the genetic effects of radiation than has been assumed on the basis of past extrapolations from experiments with mice.

Introduction

Recently our colleagues and ourselves have reported in detail on the current status of the various studies aimed at understanding the genetic effects of the atomic bombs detonated over Hiroshima and Nagasaki (Awa et al. 1987; Neel et al. 1988; Yoshimoto et al. 1990, and submitted; Otake et al., in press). The purpose of the present communication is to summarize briefly all these studies, in a fashion that will provide a perspective on the genetic effects of ionizing radiation for humans, by utilizing the latest developments in atomic bomb dosimetry. Then, in the closing sections of the present paper, we will examine the magnitude of the genetic effects which, by these studies, can be excluded at specified probability levels, and we will also attempt to generate an estimate of the amount of acute radiation which will have a mutational impact equivalent to the role of spontaneous mutation each generation. This is the convenient but somewhat elusive measure commonly referred to as the genetic "doubling dose."

As will become apparent, for none of the indicators of a genetic effect of radiation is there a statistically significant difference between the children of the parents receiving increased amounts of radiation at the time of the bombings (ATB) and the children of parents who are not thought to have received significantly increased radiation exposures. Since these studies without doubt constitute the single most expensive and extensive genetic undertaking on record, it is important to recognize that the possibility of such an "inconclusive" outcome was clearly foreseen from the outset (Genetics Conference 1947). However, the occasion is so singular in human history that the various advisory groups considering such a study felt there was no alternative but to proceed. What could not be anticipated when the study was initiated in 1947 was the advent of cytological and biochemical technologies that would greatly increase the rigor of the undertaking and, together with new insights into the morphological data collected during the early years of the investigation, would permit a more quantitative assessment of the outcome of the study than originally seemed possible.

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Background

The details of the Genetics Program which evolved in Japan have been presented on several occasions (Neel and Schull 1956; Kato et al. 1966; Schull et al. 1981; Awa et al. 1987; Neel et al. 1988). In the immediate postwar period in Japan, the economic stringencies were such that a rationing program which had been initiated during the war years to benefit pregnant women was continued. This involved registration of the fact of pregnancy at the completion of the fifth lunar month of gestation. By incorporating the registrants into a genetic study, it was possible to mount a *prospective* investigation of pregnancy outcome in Hiroshima and Nagasaki that included reference to sex ratio, congenital defect, viability at birth, birth weight, and survival of child during the neonatal period. After approximately a year of preparation, this study was initiated in February 1948. The questionnaires and examination procedures have been presented in detail elsewhere (Neel and Schull 1956). This more clinical program was terminated in February of 1954, but the collection of data on the sex ratio and survival of live-born infants continued, with ascertainment of additional births now done through city records. In addition, from the birth registrations in these two cities, the sample for the consideration of these latter two indicators was extended backward in time, to include births between May 1946 and January 1948. During the clinical program, in Hiroshima, some 62% of all infants who were stillborn or died in the first 6 d of life were subjected to autopsy. A subset of some 30% of the total 76,617 infants born during this period were reexamined at approximately 9 mo of age, the examination including measurements of growth and development.

Relatively few survivors beyond 2,000 m from the hypocenter were exposed to radiation from the bombs; the mortality within the 2,000-m zone was high. Accordingly, in the original sample of 76,617 children plus subsequent additions, many more had been born to parents who had been >2,000 m from the hypocenter ATB—i.e., parents who seldom received increased radiation from the explosions—than had been born to parents who had been within the 2,000-m radius—i.e., parents whose gonadal doses were relatively large. In 1959, to increase the efficiency of the study of survival, three cohorts were defined from the children born in the two cities since the bombings. One cohort was comprised of all children born between May 1, 1946, and December 31, 1958, to parents one or both of whom had been <2,000 m from the hypocenter ATB (i.e., who were proximally exposed). The second cohort was composed of age-, sex-, and city-matched births to parents one or both of whom had been distally exposed to the bombs (>2,500 m from the hypocenter), and the third cohort was composed of age-, sex-, and city-matched children born to parents then residing in Hiroshima and Nagasaki but neither of whom had been in the cities ATB. Such parents are referred to as NIC (not in city). The latter two cohorts are thus subsets of a considerably larger pool. Children born to parents who had been within the 2,000–2,500-m radius of distance from the hypocenter ATB are omitted from consideration, both because of the exceptional difficulties in estimating the (low) exposures at this distance and because of the work involved in following children who would contribute relatively little information regarding radiation effects. Setting the earliest date for admission into the cohort at May 1, 1946, effectively eliminates from the sample all children in utero ATB.

These cohorts have been periodically expanded to include matched numbers of recent births, the limiting factor in the expansion being the number of births to the proximally exposed parents. In 1984, some 39 years after the birth of the first children conceived following the bombings, there were only three births to proximally exposed parents, and it was decided to close the cohorts, effective January 1985. At this writing the cohort of children ever to be born in Hiroshima and Nagasaki to proximally exposed survivors of the bombings is essentially complete. The original projection was for equality of numbers in all the cohorts, but various developments detailed by Yoshimoto et al. (1990) have altered these proportions. The cohort of children born through 1982 (the cutoff date for analyses) to parents receiving >0.01 Gy of radiation now equals 31,150; there are 41,066 "control" children, whose parents either are not known to have received any radiation ATB or received <0.01 Gy. In addition to the study of the survival of these children, in the 1960s and 1970s special studies were mounted with respect to the physical development of a subsample of these children during their school years. In 1968 a search for cytogenetic abnormalities in those children in the cohorts >12 years of age was initiated, and in 1975 a search in these same cohorts for mutation altering the electrophoretic mobility and/or function of a select battery of proteins was undertaken. A study of cancer incidence prior to age 20 years was initiated in 1985. For all these studies, the necessary controls were drawn from the children of parents not receiving increased radiation ATB.

An unusual feature of the Genetics Program is that

all of its various components have been based on a well-defined population subsampled in various (overlapping) ways. The result is a multifaceted appraisal of the bombs' genetic impact on what is essentially a single cohort. We note that, although these studies lack the elegance that can be achieved in an experimental setting, they have the virtue of being based on a single complex population exhibiting the full range of human heterogeneity rather than on laboratory animals (mostly mice) that are the product of previous genetic selection intended to reduce the genetic variance associated with traits of interest. Furthermore, the subjects of the study have been born over a sufficiently extended time interval that we begin to approximate the effects on an entire generation rather than on a "window" of access to a very limited period in the reproductive histories of the parents.

The Estimation of the Amount of Radiation Reaching the Gonads

Even after more than 40 years of intermittent evaluation, the issue of the type and amount of radiation received by survivors remains subject to considerable debate. This results primarily from continuing discussion of the yield and radiation spectrum of the Hiroshima bomb, the (in)ability of some survivors to reconstruct precisely their shielding from the effects of the bombs, difficulties in evaluating radiation attenuation by complex shielding, the possible existence of inherent and ineradicable biases in the dosage estimation procedures, and continuing uncertainty over the LD_{50} of whole-body radiation for humans. A recent thorough reconsideration of the "dose problem" (Roesch 1987) has resulted in a system for calculating organ doses which has been termed "Dosimetry System 1986" (DS86) which, while state of the art in its treatment of physical dosimetry, cannot overcome the uncertainties inherent in a complex human disaster such as this. This replaces a system known as "Tentative 1965 Estimate, Dose Revised" (T65DR). In expressing DS86 dose estimates, we shall follow the current practice of employing grays (1 Gy = 100 rad) and sieverts (1 Sv = 100 rem).

The DS86 dose estimate is based on (1) the spectrum and amount of radiation released by the bombs, (2) its attenuation with distance and physical shielding, (3) the free-in-air kerma reaching the person, (4) body position ATB, and, given position, (5) the further attenuation, by the intervening body tissues, of the radiation reaching the gonads. About 88%–96% of the parents entering into the various studies place themselves in ATB situations for which the calculation of the DS86 dose has been relatively straightforward, the exact percentage depending on the study in question. The remaining 4%–12% either (*a*) report being in ATB situations for which the calculation of a gonadal dose with the parameters of the DS86 system is not yet possible or (*b*) provide exposure data not sufficiently detailed for the calculation of a DS86 dose. This latter is especially true for some of the data collected in the early years of the study, before the currently employed exposure history was instituted. Fortunately, almost all of these individuals have in the past been assigned the (less demanding) T65DR doses. Inasmuch as gonadal doses must be available for both parents in a genetic study, and since the "DS86 dose unassigned group" tends to involve predominantly the proximally (more heavily) exposed parents, failure to assign a revised dose to this group would result in a sacrifice of, for example, 23% of the children in the first study we will summarize, i.e., the study on untoward pregnancy outcomes. We have accordingly developed an ad hoc procedure for assigning doses to these parents, a procedure which, with other details regarding dosimetry, is fully described by Otake et al. (1990). In brief, an empirical conversion factor has been developed which permits assigning a DS86-like gonadal exposure on the basis of a previously assigned T65DR gonadal exposure. In the two groups the pattern of occurrence of the critical symptoms of substantial radiation exposure (epilation, subcutaneous bleeding, and oropharyngeal lesions) has been used to provide an appropriate verification of the accuracy of the procedure. In the analyses to be presented we will restrict ourselves to those based on DS86 doses only, except where the sacrifice of data seems too great; the nature of the data will be indicated for each analysis. The term "conjoint" as applied to parental exposure indicates the sum of the parental gonad doses.

The radiation released by the two atomic bombs, although very predominantly gamma, included a small neutron component. The integration of these two types of radiation into a single figure requires that dose be expressed in Sv. For the generally low neutron doses now thought to characterize these atomic bomb exposures, we will, in making the transition from Gy to Sv, employ a value of 20 as the Relative Biological Effectiveness (RBE) for transmitted genetic effects (see Grahn et al. 1983, 1984; International Commission on Radiation Units and Measurement 1986). In our previous publications we have presented average estimated

gamma and neutron absorbed doses, to permit those who are so inclined to apply other RBE.

The Data

The data which have been accumulated over the years will be briefly presented under the following eight headings:

1. *Untoward Pregnancy Outcomes*

We define an untoward pregnancy outcome (UPO) as an infant stillborn and/or exhibiting major congenital malformation and/or dying within the first 2 wk of life expectancy. Between 1948 and 1954, data were collected on these outcomes for a total of 76,617 newborn infants in Hiroshima and Nagasaki, on 69,706 of whom the data were sufficiently complete in all respects to permit an analysis with the biomedical and dosage constraints of 1989. Of these, 12,409 were born to proximally exposed parents whose conjoint gonadal exposure, on the basis of use of both DS86 and our ad hoc gonad dose estimates, was 0.36 Sv. Shortly after birth all infants were examined, with reference to congenital defect, by staff physicians; an enumeration of the types of defect scored as "major" and of their frequencies will be found in the work of Neel and Schull (1956) and Neel (1958). (In view of the current interest in "sentinel phenotypes" in monitoring for mutation, it should be noted that, to the extent these were identified, they are included among the UPOs, but subsequent experience [Mulvihill and Czeizel 1983; Czeizel and Kis-Varga 1987] confirms the decision reached at the onset of the study—i.e., that cohort size would not be large enough to restrict this aspect of the study to those phenotypes only.)

Over the years these data have been analyzed in various ways, with no major differences in the conclusions reached. For this presentation, we will for this and subsequent indicators present only the results derived from fitting a linear dose-response model to the occurrence of the various indicators of radiation-related damage, these indicators being treated as binary-response variable (i.e., 1 if the event occurred in a given individual and 0 if it did not). In addition to conjoint parental dose, data were available on as many as six background or concomitant variables which could be assumed to influence the occurrence of the event of interest—namely, city (Hiroshima and Nagasaki; Hiroshima was assigned the value 1, and Nagasaki was assigned the value 0), sex (male and female; males were assigned the value 1, and females were assigned the value 0), maternal age (years), parental age (years), year of birth, and birth rank. Specifically, we have fitted a model of the following form:

$$P_i = \text{constant} + \sum_{j=1}^{6} b_j x_{ij}\,(\text{background}) + b_D\,\text{dose}_i,$$

where P_i is the expected frequency of the event of interest in the *ith* individual ($i = 1,2, \ldots, n$; i.e., the total number of subjects) having background characteristics x_{ij} ($j = 1,2, \ldots, 6$ and dose i). The constant, the b_j, and the b_D are, of course, the parameters to be estimated.

The findings regarding UPOs, based on the extended cohort (DS86 plus ad hoc doses) are shown in table 1 (also see Otake et al. 1990). There were 3,498 UPOs; their frequency in the children of unexposed parents was 5.02%. The general thesis undergirding the search for an increase in UPOs as an indicator of the genetic effects of the atomic bombs is that some portion of this outcome is due to newly arisen, dominantly expressed mutation in the parental generation. We will discuss the probable magnitude of this fraction later. Meantime we note that the regression is small, far from the significance level, but positive, i.e., in the direction anticipated from an increase in dominantly expressed mutations resulting in such an endpoint. To conserve space, the analyses of the roles of concomitant variables—which introduce a certain perspective into the findings—are not presented but can be found in the basic publication; this is also true for the following two indicators:

2. *Deaths among Live-born Infants (exclusive of those resulting from a malignant tumor)*

As noted earlier, the mortality study embraces three

Table 1

Regression of Frequency of Indicator Trait on Conjoint Parental Gonadal Radiation Exposure (Sv)

Trait	Regression
Untoward pregnancy outcome	.00264 ± .00277
Mortality exclusive of cancer	.00076 ± .00154
Incidence of cancer:	
All cases	−.00008 ± .00028
Most genetic subset[a]	−.00005 ± .00013

NOTE.—Calculation of Sv is based on an assumed neutron RBE of 20. The papers from which these regressions were extracted contain additional regressions which pertain to the effect of concomitant variables and which are useful with respect to prespective.

[a] See text.

cohorts of infants live born in the two cities of interest between May 1946 and December 1985. The mortality data are updated on a 4-year schedule, the last update having been completed in 1985. Cumulative mortality in the live-born children of unexposed parents was 4.58%. At that time the average time elapsed since the birth of a "child" in the study was 26.2 years. The study thus provides coverage of a very high percentage of prereproductive mortality. To avoid overlap with the preceding indicator, the mortality during the first 2 wk of life of those children in the study of UPOs is not included in this mortality analysis. Deaths from childhood cancer were analyzed separately. Detailed descriptions of the organization of the study will be found in the work of Kato et al. (1966); Neel et al. (1974); and Yoshimoto et al. (submitted).

Thus far there have been, after the exclusions just mentioned, 2,584 deaths among the 67,202 members of the three cohorts for whom all the requisite information and DS86 doses are available. Of course, death during childhood has many determinants, only one of which would in this situation be radiation-induced, dominantly expressed mutations in the parental generation. (We include in this term the heterozygous effects of nominal recessives.) In a later section we will deal with what fraction of this indicator is to be assigned to mutation in the preceding generation. All the accumulated data have recently been analyzed in multiple ways (Yoshimoto et al., submitted). Only the results of the analysis based on the multiple linear-regression model applied to the DS86 dose cohort are presented in table 1. In this particular study, for the 26,963 children one or both of whose parents received $\geq$0.01 Sv of radiation ATB, the average conjoint parental gonadal exposure was 0.40 Sv.

3. *Malignancies in the F_1*

The rationale for regarding malignancies in the F_1 as a suitable and separate indicator of the genetic effects of the bombs is developed in detail in an accompanying paper in this issue of the *Journal*. In brief, with respect to the children described in the preceding section, studies were undertaken of the mortality and morbidity from malignancies with onset before the age of 20 years, in relation to parental radiation exposure (Yoshimoto et al. 1990). These studies were greatly facilitated by the existence of tumor registries in both Hiroshima and Nagasaki. At the close-out date for the study, 79.7% of the sample, if alive, would have completed 19 years. Thus far there have been 43 cancers in the 31,150 children born to exposed parents, versus 49 in the 41,066 children of unexposed parents. The incidence of cancer in the children of unexposed parents was 1.2/1,000 persons. The results of an analysis employing a linear-regression model and restricted to the DS86 dose cohort are shown in table 1. The regression of all malignancies (fatal and nonfatal) on parental exposure is a slightly negative term, which in fact is virtually zero. The regression was almost the same when the analysis was confined to those tumors for which the evidence that a parental germ-line mutation is a predisposing factor is strongest (i.e., Wilms tumor, the retinoblastoma-sarcoma complex, neuroblastoma, and embryonal carcinoma of the testes). The average conjoint parental gonadal dose for the parents receiving $\geq$0.01 Sv is, of course, the same as for the preceding data set.

4. *Frequency of Balanced Structural Rearrangements of Chromosomes*

In the late 1960s, as techniques for the accurate evaluation of the human karyotype became available, a program designed to detect an increase in chromosome abnormalities in the children of proximally exposed survivors was initiated, directed at children already enrolled in the F_1 Mortality Study (see Awa et al. 1968, 1973; Awa 1975). Two age- and sex-matched samples have been established, one drawn from the children of the proximally exposed and the other drawn from the children of the distally exposed. For practical reasons, no effort was made to obtain the necessary venous blood samples prior to the children's thirteenth birthdays. The sample will accordingly only provide reliable data on the two types of cytogenetic abnormalities which confer relatively little threat to survival among live-born infants—namely, sex-chromosome aneuploids (to be discussed in the next section) and balanced structural rearrangements of chromosomes, such rearrangements being defined as reciprocal translocations, pericentric inversions, and Robertsonian translocations.

The most recent summary of the findings by Awa et al. (1987) with respect to balanced structural rearrangements in the two samples is shown in table 2. These types of abnormalities may either have been inherited from a parent or have arisen de novo in the germ line. Thus far it has been possible to conduct for somewhat more than half of the children so diagnosed the family studies necessary to distinguish between these two possibilities. In each cohort a single mutant child has been identified. Awa et al. (1987), using an adjustment for the fact that family studies are not complete for all propositi, estimate the mutation rate as 1.0×10^{-4}/gamete/generation among both the exposed and the

Table 2

Frequency and Parent of Origin of Balanced Structural Rearrangements in the Cytogenetic Study of Awa et al. (1987)

	Hiroshima		Nagasaki		Total	
	Exposed	Control	Exposed	Control	Exposed	Control
Studied for parent of origin:						
De novo	1	1	0	0	1	1
Inherited:						
Father	2	4	2	4	4	8
Mother	0	1	0	1	0	2
Undetermined	6	3	0	2	6	5
Subtotal	9	9	2	7	11	16
Not studied for parent of origin	5	7	2	2	7	9
Grand total	14	16	4	9	18	25
No. of children studied	4,716	5,112	3,606	2,864	8,322	7,976

Note.—Rearrangements include reciprocal and Robertsonian translocations plus pericentric inversions.

controls—with, for both estimates, the large 95% confidence interval to be expected under these circumstances, namely, between 0.3×10^{-5} and 5.5×10^{-4}. At the time of the publication of their results, DS86 doses were not available. We have now calculated that, on the basis of an RBE of 20 for neutrons, the mean conjoint parental gonad dose for the parents receiving $\geq$0.01 Sv is 0.60. Unlike the results to be presented for the other studies, we have not calculated the regression of indicator on parental exposure because of the paucity of proven mutations.

5. *Frequency of Sex-Chromosome Aneuploids*

The types and frequencies of sex-chromosome aneuploidy in the individuals comprised by these same two samples have been presented by Awa et al. (1987). On the same population base as given in table 2, there were in the sample a total of 43 individuals with sex-chromosome aneuploidy—19 were children of proximally exposed parents and 24 were children of distally exposed parents. Individuals with some of these abnormalities are sterile (XXY), and with others may exhibit decreased fertility (XXX); furthermore, XYY and XXX individuals only rarely have similarly affected children (Evans 1977). Thus a very high proportion of these persons may be presumed to result from primary nondisjunction (i.e., mutation) in a parent, but specific family studies on these individuals were not undertaken. Sex-chromosome aneuploidy is increased in the offspring of older parents (Court Brown et al. 1969). Accordingly, an increase in such findings in the children of proximally exposed survivors could result from either a direct or an indirect effect of radiation, the latter being mediated by an acceleration of aspects of the aging process.

For the estimates of doubling dose that follow, it is necessary to express the findings as a linear regression on dose. We have accordingly extended the analysis of Awa et al. (1987), assigning parental gonad doses on the basis of the DS86 schedule. Table 3 presents the data as tabulated for the derivation of a linear regression, fitted by the method of maximum likelihood. The regression of indicator on dose (Sv) is .00044 $\pm$ .00069, with an intercept of .00252 $\pm$ .00044. To the extent that this abnormality may have been inherited in some cases, the mutation rate for the trait would be overestimated, but there should be no bias with respect to a radiation effect. The estimate of average conjoint parental gonad dose is, of course, that given in the preceding section.

The finding during the clinical phase of the Genetics Program that diagnoses of Down syndrome (trisomy 21) were no more common among the offspring of parents exposed to the bombs than they were among the offspring of unexposed parents (Schull and Neel 1962) is further evidence that the ATB exposure did not produce an increase in aneuploidy in this population.

6. *Frequency of Mutation Altering Protein Charge or Function*

During the past 40 years there have been frequent conjectures concerning hidden (i.e., recessive) genetic effects of the bombs, which effects would only become evident some generations after the bombings. The ad-

Table 3

Data for a Least-Squares-Fit Linear Regression of Sex-Chromosome Aneuploidy on Conjoint Parental Gonadal Exposure, Based on DS86 Doses

Conjoint Parental Gonad Exposure (Sv)	No. of Children	Mean Dose (Sv)	No. of Aneuploids
.0	8,225	0	24
.001–.050	1,346	.024	0
.051–.100	951	.073	2
.101–.500	2,693	.263	9
.501–1.000	1,531	.719	3
1.001–1.500	686	1.227	2
1.501–2.000	295	1.716	1
2.001–2.500	157	2.228	0
⩾2.501	331	3.674	2
Unknown	83		0
	16,298		43

NOTE.—The data have been distributed so as to optimize the distribution for regression analysis.

vent of convenient techniques for the identification of variant proteins in the 1950s and 1960s provided an approach to this question. In 1972 a pilot study of the feasibility of electrophoretic examinations of what ultimately became a battery of 30 serum and erythrocyte proteins was initiated, followed in 1975 by a large-scale investigation which was only recently terminated. In 1979 a smaller-scale search for mutations that are characterized by loss of activity of a series of 11 carefully selected enzymes was initiated (Satoh et al. 1983); this program has also now terminated. The data from both programs have recently been presented in the *Journal* (Neel et al. 1988). The mutations detected by both these programs would be encountered in the heterozygous state and would have no discernible effect on the gross phenotype; that is, they conform to the classic definition of a recessive.

The program centered on the detection of rare (i.e., nonpolymorphic) protein variants, which were then subjected to family studies, to determine whether they had been inherited or were the result of mutation in the preceding generation. Only when both parents of a child with a variant have been studied is it possible to be certain concerning the occurrence of a mutation. Because both parents of a child who is found to possess a rare variant were not always available for study, an adjustment in the data, to "equivalent locus tests" (described in detail elsewhere [Neel et al. 1980]), is necessary. A child exhibiting a variant not present in either parent was termed "exceptional." Extensive tests were then performed to detect discrepancies between legal and biological parentage, resulting in some children being eliminated from further consideration. We have calculated that the a priori probability that a finding accepted as being due to mutation is in fact due to an undetected paternity discrepancy is approximately 4×10^{-8}. There were a total of seven apparent mutations in 1,256,555 locus tests.

The estimated mutation rate for loss-of-activity and electrophoretic variants was 0.6×10^{-5}/locus/generation, in both the proximally and the distally exposed parents, with the 95% confidence interval being 0.2–1.5 $\times 10^{-5}$ for the former and 0.1–1.9 $\times 10^{-5}$ for the latter. (Because disparate numbers of the two types of observations are involved, this is not an estimate of the total locus rate.) The estimated gonad doses in our recent description of this study were recognized as interim estimates, made while the DS86 dose system was still being phased in. We have now reestimated, with the standard procedure, these conjoint gonad doses for parents whose dose was ⩾0.01 Sv, by using the total sample and value of 20 as the RBE for neutrons. The estimated conjoint dose for the proximally exposed 13,544 sets of parents is 0.41 Sv. Although the numbers are minimal for regression-type analysis, we have, in order to utilize these data in the calculations to come, proceeded with such an analysis, organizing the data as shown in table 4. Because the preceding tables are all on the basis of zygotes, we have in table 4 converted the locus determinations presented by Neel et al. (1988) to their zygotic equivalent by dividing the numbers of determinations in each exposure category by 2. The maximum-likelihood estimate of the slope of the regression is $-.00001 \pm .00001$ with intercept of $.00001 \pm$

Table 4

Data for a Least-Squares Fit, Linear Regression of Mutations Altering Protein Structure and/or Function on Conjoint Parental Gonadal Exposure, Based on DS86 Doses

Conjoint Parental Gonad Exposure (Sv)	No. of Tests[a]	Mean Dose	No. of Mutations
.0	264,310	0	4
.001–.050	109,776	.017	2
.051–.500	165,108	.201	0
.501–1.000	48,264	.708	1
⩾1.001	40,820	1.988	0

NOTE.—The data have been distributed so as to optimize the distribution for regression analysis.
[a] The number of locus products examined for mutation will be twice the number of system tests.

.00001. As for the cytogenetic studies, since there were so few positive outcomes no effort was made to evaluate the effect of concomitant variables.

7. *Sex Ratio among Children of Exposed Mothers*

It was on the basis of a sex-ratio decrease among the offspring of irradiated female *Drosophila* that Muller (1927) first demonstrated the genetic effects of radiation, and in the early years of this Genetics Program the sex ratio—i.e., the ratio of male births to female births among the offspring of proximally and distally exposed survivors—received considerable attention (Neel and Schull 1956; Schull et al. 1966). This was based on the hypothesis that, because of the nature of sex-linked inheritance, any deleterious sex-linked mutations induced in irradiated mothers should reduce the sex ratio whereas similar mutations in irradiated fathers should increase the sex ratio. With the recognition that one X chromosome is inactive in the somatic cells of women (the Lyonization phenomenon), it became clear that sex-linked mutations induced in males were unlikely to have a dominant lethal effect in females. Furthermore, the discovery of the occurrence and frequency of sex-chromosome aneuploids in the late 1950s introduced a new variable into the study of the sex ratio, a variable the direction of whose impact on the sex ratio could not be precisely specified at that time.

The cytogenetic findings now reveal that in this sample there is no evidence that radiation significantly alters the frequency of sex-chromosome aneuploids. This potential complication in the use of the sex ratio as an indicator of the production of sex-linked lethal mutations by the atomic bombs is thus disposed of. Whether and to what extent, with Lyonization, there are sex-linked dominant "point" mutations in women remains a somewhat moot question. We suggest that the least equivocal aspect of the sex-ratio data involves the effects of maternal irradiation. In this event, male conceptuses of exposed mothers should be susceptible to the effects of both sex-linked dominant and recessive induced lethal mutations, whereas female conceptuses should not be expected to exhibit reduced survival from any induced "recessives"; and, in addition, Lyonization would temper to some unknown extent the manifestation of sex-linked "dominant" mutations, as is clear from the review of Wettke-Schafer and Kantner (1983). Thus maternal exposure should, in principle, reduce the sex ratio.

The last presentation of the sex-ratio data (i.e., that of Schull et al. 1966), which included some 70% of births ever to occur to proximally exposed parents, revealed that for children born to proximally exposed mothers (fathers unexposed), the regression of offspring maleness on mother's radiation dose was .0027 ± .0040/Sv. The observation is thus insignificantly counterhypothesis. Reanalysis might alter the magnitude but would not change the sign of the regression, but since we see no way to incorporate such an observation into a truly quantitative estimate of either the minimal or probable doubling dose (see below), we have not reanalyzed these data on the basis of the DS86 dose estimates.

8. *Growth and Development of the F_1*

The variables considered in the preceding seven sections are discrete or qualitative attributes, in whose etiology the role of single genetic events such as mutations can be estimated and the analysis of which is relatively straightforward. A body of data concerning the effect of parental exposure on certain quantitative traits is also available. For these traits, the production

of deleterious mutations in the parents in consequence of exposure to the bombs might be expected to depress the mean and to increase the variance—but in no simple manner amenable to a doubling-dose calculation (see below), unless rather restrictive assumptions are made.

During the period when the study population was under surveillance for untoward pregnancy outcomes (see section item 1 above), birthweights were obtained on all 76,617 infants who entered the study. An analysis restricted to the birthweights of live-born infants failed to reveal any suggestive effect of parental exposure (Neel and Schull 1956). A reexamination—with respect to body length, head and chest circumference, and body weight—of 18,498 of these infants between ages 8 and 10 mo also failed to reveal any between-infant differences related to parental exposure (Neel and Schull 1956). Furusho and Otake (1978*a*, 1978*b*, 1979, 1980, 1985) have extended this analysis by relating the annual measurements (stature, weight, and chest circumference) of Hiroshima school children to the irradiation history of their parents. Parental exposure had no certain or even suggestive effect on the means or variances of these measurements. These analyses employed T65DR doses; it has not seemed relevant to reanalyze the material by DS86 dose assignments. Since these data do not lend themselves to the types of statistical analysis that follow, we will not refer to them again.

Implications of the Data

We propose to develop a perspective on the present findings by three approaches. In all three of these approaches, we take it as a given that the exposure resulted in mutations in some survivors of the atomic bombings, inasmuch as, without exception, under controlled laboratory conditions ionizing radiation has produced mutations in every properly studied plant and animal species.

Approach 1

The simplest approach is to content ourselves with the statement that the children of what is almost certainly the most highly irradiated population in the world's history provide, with the present indicators, no statistically significant evidence that mutations were produced in their parents. The indicators employed in this study run the gamut of genetic change, from the effects of nucleotide substitution to the effects of gain or loss of an entire chromosome. The absence of statistically significant findings does not deny the possibility that the exposed survivors sustained an increased germinal mutation rate, but it characterizes this rate as being too small to be detected unambiguously in this sample by the approaches thus far employed. On a more positive note, these studies have produced an extensive body of data against which to evaluate empirically both past and future surmises concerning the genetic consequences of exposure to ionizing radiation. In particular, the studies should prove reassuring to that considerable group of exposed Japanese and their children, without whose magnificent cooperation these studies would have been impossible and who have over the years been subjected to a barrage of exaggerations concerning the genetic risks involved. To stop at this point in considering the implications of these findings would in our opinion be an abrogation of genetic responsibility.

Approach 2

The next level of sophistication in treating the data attempts to determine what the immediate mutational contribution to each indicator is and then, given this contribution, to determine what genetic "doubling dose" can be excluded for each indicator at various probability levels. Where endpoints such as UPOs and early death are employed, the estimates are to some extent time-place specific, depending on the severity of selection against deleterious genetic traits. For this and the next approach we must estimate the contribution of spontaneous mutation in the parents to each of the endpoints of this study. These critical estimates are developed in the Appendix.

In a linear model, the *excess* relative risk (which can by definition be negative), say RR, is (β/α), where α and β are the background frequencies of events of interest, i.e., the constant in the model and the slope of the regression of the frequency of events on dose, respectively. The variance (large sample) of this ratio is

$$V\left(\frac{\beta}{\alpha}\right) = \left(\frac{\beta}{\alpha}\right)^2 \left[\frac{V(\alpha)}{\alpha^2} + \frac{V(\beta)}{\beta^2} - 2\frac{\mathrm{Cov}(\alpha,\beta)}{\alpha\beta}\right].$$

In any specific situation, α and β are, of course, replaced by their sample estimates. Now, by definition, the "doubling dose," D_d, is $\beta D_d = \alpha$ or $D_d = (\alpha/\beta)$, where it will be noted (α/β) is merely the reciprocal of the excess risk per unit dose. Thus, an estimate of the "doubling dose" is $D_d = (1/\mathrm{RR})$. If, now, the background rate, α, is assumed to consist of both a genetic component, C_G, which is augmented by parental exposure to ionizing radiation, and a nongenetic which does not respond to radiation, then the doubling-dose estimate

given above must be multiplied by the proportion of the background events presumed to be genetic.

In our situation, we reject the possibility of a negative risk implied by the negative regressions on parental exposure given earlier for malignancies and protein mutations, regarding these as random departures from some "true," small positive term. We can, however, even with a negative regression, use the variance term given above to calculate what lower value to the D_d can be excluded at stated probability levels for each of these indicators. Thus,

$$D_{d(\min)} = \frac{1}{[(\beta/\alpha)+Z_\alpha\sqrt{V(\beta/\alpha)}]},$$

where Z_α is the normal deviate at the desired probability level.

Table 5 presents the results of the appropriate calculations. As noted, the data on balanced reciprocal translocations, sex ratio, and physical development were not felt to be adequate for this type of calculation. The first three columns in table 5 develop the proportion of the indicator deemed due to mutation in the parental generation, from the Appendix. There follow the values of β and α as derived from the appropriate regression, as presented in earlier sections and in previously published papers. Finally, the lower values of the doubling dose at three probability levels are presented. The minimal doubling doses for the various endpoints at the 95% probability level range from 0.05 to 2.27 Sv.

In previous calculations of the doubling dose, having reached this point in the calculation, we have been persuaded to divide the result by 2, to convert from a zygotic doubling dose to the gametic doubling dose in which radiation effects are more usually presented (see Schull et al. 1981; more recently Neel et al. 1989*a*, 1989*b*). We now believe this correction was incorrect. Consider the UPO data set. The estimated contribution of spontaneous parental mutation to the indicator is the contribution of both sexes. Assume the contributions are equal, so that $\alpha_{GF} = \alpha_{GM}$ and the total contribution is 2α. The regression of the indicator on dose (Sv) will be $\beta_F + \beta_M$, and that on the assumption of equal effects in the sexes will be 2β. Consider a hypothetical situation where $\alpha_{GF} = \alpha_{GM} = .001$ and where $\beta_F = \beta_M = .001$/Sv, i.e., where 1.0 Sv is the doubling dose. As we now approach this problem, $\Sigma\alpha/\Sigma\beta = .002/.002 = 1.0$, and we have the correct estimate of the D_d without reduction by a factor of 2. Since we cannot separate male and female effects, this estimate is the average of the sexes and in the strict sense is valid only when, as for the Hiroshima and Nagasaki situations, approximately equal numbers of the two sexes were exposed. It cannot be applied to either sex alone. Departures from the assumptions of the equality of spontaneous and induced mutation rates in the two sexes will not alter the principle. For instance, were the female to exhibit both spontaneous and induced rates of zero, the ratio of $\Sigma\alpha/\Sigma\beta$ would still be the *average* gametic doubling dose. To restate the now obvious, while the estimate of the impact that spontaneous mutation in the parental generation has on the zygote

Table 5

Estimate of Gametic Doubling Doses That Can Be Excluded at Specified Confidence Levels by These Data

Trait	Observed Total Background (a)	Mutational Contribution Background (b)[a]	Mutational Component (%[b/a])	Regression		Sv at Lower Confidence Limit of		
				β_{Sv}	α	99%	95%	90%
UPO	.0502	.0017–.0027	3.4–5.4	.00264 ± .00277	.03856 ± .00582	.14–.23	.18–.29	.21–.33
F_1 mortality	.0458	.0016–.0026	3.5–5.7	.00076 ± .00154	.06346 ± .00181	.51–.83	.68–1.10	.81–1.32
F_1 cancer	.0012	.00002–.00005	2.0–4.0	−.00008 ± .00028	.00104 ± .00033	.04–.07	.05–.11	.07–.15
Sex-chromosome aneuploids	.0030[b]	.0030	100	.00044 ± .00069	.00252 ± .00043	1.23	1.60	1.91
Loci encoding proteins	.000013[b]	.000013	100	−.00001 ± .00001	.00001 ± .00001	.99	2.27	7.41

NOTE.—See text for further explanation.

[a] Per diploid locus.

[b] Observed zygotic mutation rates.

reflects the contribution of two gametes, the regression term also reflects the contribution of two haploid sets of chromosomes, and the factors of 2 cancel out.

Estimates such as these can only be combined to yield an average estimate on the assumption there is no heterogeneity among the individual estimates, i.e., on the assumption they have been drawn from a common pool. An extensive literature suggests that the true doubling dose for radiation-induced sex-linked chromosomal aneuploids and protein variants (resulting from nondisjunction and nucleotide substitutions, respectively) is probably higher than the doubling dose for the other three indicators (resulting predominantly from insertion/deletion/rearrangement events and unrepaired chromosomal breaks). We have therefore obtained separate estimates for these two types of indicators, using the formula

$$D_d \text{ pooled} = \frac{1}{[(\beta/\alpha)+Z_\alpha\sqrt{V(\beta/\alpha)}]},$$

where Z_α is again the desired probability level and where (β/α) is now the average of the sum of information-weighted (reciprocal-of-variance) individual estimates and where $V(\beta/\alpha)$ is the variance of this average. The minimal doubling dose at the 95% probability level which results from pooling the estimates for UPOs, F_1 cancer, and F_1 mortality is 0.63–1.04 Sv. The similar estimate for the sex-chromosome aneuploids and protein mutations is 2.71 Sv. These estimates must not be misinterpreted. They are the minimal (95%-probability-level) doubling doses derived from each of these five indicators or some combination thereof but are *not* a lower bound to the estimate to be derived in the next section.

Approach 3

Finally, we come to the third and ultimate level in treating these data, an effort to tease out of these findings a reasonable and preliminary estimate of the most likely gametic doubling dose of radiation for these indicators combined. *In the societal context, the doubling-dose estimate most needed is of the amount of parental radiation that in the first generation will result in the same impact of genetic mortality and morbidity as would result from spontaneous mutation in these same parents.* Ideally, the genetic doubling dose is calculated as that amount of radiation which increases the *total* impact of spontaneous mutation by 100%. It covers the spectrum from the impact of nucleotide substitutions to gains or losses of entire chromosomes. Since the components of this spectrum may present differing frequencies, the doubling dose should be an integrated treatment of both the *frequency* and the *radiosensitivity* of each component.

Because the lack of statistically significant findings raises the possibility that we are simply manipulating the "noise" in the study, some would advocate that the analysis be suspended at this point. Again we reiterate the point that unless humans differ from every other properly studied animal, as well as from plants, mutations must have been produced by this exposure; we wish cautiously to explore what the further genetic message in these data might be. At the mutagenic exposures which any substantial proportion of a human population will survive, there may never be a statistically significant demonstration of the genetic effects of the radiation of humans—and certainly there will be none within the next decade, pending the development of molecular strategies. However, the methodological problems presented by the attempt to generate a doubling dose from these data are unique. We present here one treatment, which in time may be superseded by others.

Each of the eight estimators of a genetic effect of the bombs which we have been able to generate is associated with a relatively large error term, and individually these estimators are fragile reeds on which to lean in building a case. Collectively, however, they amount to a major corpus of data. The estimate to be developed, like all such estimates, is of course *specific for these indicators,* which, however, represent our best effort, under the circumstances, to cover the morbidity-mortality resulting from the total spectrum of mutation. It is also, we repeat, time specific and place specific; were infant and childhood mortality greatly reduced (and selection against genetic imperfections presumably less stringent), the doubling-dose estimate might be different.

In an epidemiological study such as the present one there is a problem which the laboratory-based investigator is spared. Whereas in the laboratory one can by suitable doses and sample sizes usually contrive to demonstrate a significant positive response of the genetic endpoint to radiation, epidemiological studies involve a nonmanipulative situation. In the present study, we are confronted with several small negative regressions, with no current prospect of extending the data. Since we accept the proposition that exposure to the bombs produced genetic damage, these findings must be viewed as random fluctuations around some positive value. Inasmuch as these spuriously negative effects are presumably counterbalanced by some spuriously high positive effects, the former cannot be disregarded and must be

folded in to the estimate. There is no precedent for attempting to develop a doubling-dose estimate under these circumstances.

Calculation of the Doubling Dose

We suggest that the population samples for the various studies are sufficiently overlapping representatives of the same cohort that the various regressions can be additively combined to yield a composite picture. The ability to pursue this approach is a unique feature of this study not previously feasible for other studies. In particular, we contrast this approach to that of the murine studies, where estimates derived from different inbred strains have been combined in various fashions to obtain an average doubling dose (see Lüning and Searle 1971).

To implement this approach we need to estimate the total impact on the morbidity-mortality indicators of this study of spontaneous mutation in the parental generation. We will adopt the position that both the impact of mutation resulting in unbalanced chromosomal exchanges (which we presume to be similar numerically to the impact of the balanced chromosomal exchanges we have measured) and the impact of the heterozygous effects of recessive mutations as studied through protein variants are subsumed within the UPOs and prereproductive mortality in the live born (exclusive of cancer). As table 6 shows, a summation of the appropriate estimators of the impact of spontaneous parental mutation given in table 5 yields a figure of between 0.632% and 0.835%. (The total is, of course, not accurate to the third decimal place but results from simply summing the various indicators.) To obtain the most reasonable estimate of the genetic impact of radiation on the totality of the endpoints, we propose simply to combine the regressions of table 5 additively. We justify such cumulation on the grounds that each regression involves the relationship to radiation exposure, in the same cohort, of specific, independently determined genetic events. As table 6 shows, the value is +.00375. Note, however, that this involves the manipulation of negative (counterhypothesis) terms.

The lower estimate of the doubling dose which results from this procedure is .00632/.00375, or 1.69 Sv; the upper estimate is .00835/.00375, or 2.23 Sv. Note that these limits reflect biological uncertainties about the parameters but do not take into consideration the additional error inherent in the estimation procedure, an error which must be relatively large. Recall (1) that, because of the extreme paucity of numbers, we elected *not* to try to incorporate into this regression approach the data on mutation resulting in reciprocal translocations but (2) that the rates were the same in the two populations. Recall also our inability, because of technical reasons, to incorporate into the calculations the sex-ratio results (which are counterhypothesis) and the data on growth and development (which reveal no hint of a radiation effect). Any use of these data in these calculations could only drive the estimate of the doubling dose upward.

We find it impossible at this time to place true confidence bounds on this estimate. In addition to the errors in estimating both the true value of the parameters and their regression on dose, there is the additional factor that our estimates are geared to specific indicators and to a specific time and place. We believe, however, that throughout we have been conservative in our assumptions. Furthermore, we have reported that the parents who were unexposed to the effects of the atomic bombings (who came to Hiroshima and Nagasaki after the bombings, as released servicemen, repatriates,

Table 6

Summary of Regression of Various Indicators on Parental Radiation Exposure and of Impact of Spontaneous Mutation on Indicator

Trait	Regression/Combined Parental Sv	Contribution of Spontaneous Mutation
UPO	+.00264	.0033–.0053
F_1 mortality	+.00076	
Protein mutations	−.00001	
Sex-chromosome aneuploids	+.00044	.0030
F_1 cancer	−.00008	.00002–.00005
	.00375	.00632–.00835

spouses, or immigrants) were slightly younger and had a little more education and somewhat higher occupational ratings than did the exposed (Neel and Schull 1956; Kato et al. 1966; Neel et al. 1974). In addition, the analysis has assumed complete recovery of the mother from her radiation exposure, i.e., has assumed no maternal effect in this regard. To the extent socioeconomic and maternal effects exist, they can only inflate the apparent effects of the bombs. The true value for the genetic doubling dose of radiation under these circumstances is thus apt to be higher rather than lower than our estimate.

Discussion

We have summarized an effort extending over >40 years to quantify the genetic effects of the atomic bombs detonated over Hiroshima and Nagasaki. No statistically significant findings have emerged. Since, however, we can scarcely doubt that some genetic damage occurred in consequence of that exposure, we have taken the observations at face value and have explored the inferences which can be drawn from them. On the one hand, we have generated estimates of the minimal doubling dose, at various probability levels, consistent with each of the five indicators which lend themselves to this calculation. On the assumption that three of the individual estimates of minimal doubling doses (UPO, F_1 mortality, and F_1 cancer) were all fluctuations about some common mean, we have derived an estimate of the average 95% exclusionary power of these indicators, which is a gametic doubling dose of 0.63–1.04 Sv. For protein mutations and sex-chromosome aneuploids, which from the genetic literature may be assumed to exhibit higher doubling doses, the average is 2.71 Sv. Even the lower of these two figures is larger than the value commonly cited as the doubling dose on the basis of the mouse data (see below). On the other hand, by a different approach, we have calculated a most probable gametic doubling-dose estimate, which is 1.69–2.23 Sv. This estimate is somewhat higher than that given in several recent, preliminary reports (Neel et al. 1989*a*, 1989*b*), primarily because of the introduction of the factor of 2 mentioned earlier. In 1981, on the basis of a smaller body of data, and employing somewhat different analytic methods and the now superceded T65DR dose schedule, we suggested that the most likely estimate of the gametic doubling dose of acute radiation was 1.56 Sv (Schull et al. 1981). This estimate was in error by the factor of 2; the value should have been 3.12 Sv. Given (1) that the gonad radiation exposures (Sv) estimated with the DS86 dose schedule are on average about 70% as large as those estimated with the T65DR schedule, (2) the additional data which have become available, and (3) the somewhat more conservative nature of the present analysis, the agreement between the two estimates is satisfactory.

We emphasize once again that these estimates are specific to the mix of gonadal exposures sustained by approximately equal numbers of mothers and fathers, the socioeconomic conditions in Japan during this period, and the collection of endpoints under scrutiny—but that we believe that, with respect to the latter, they are both representative and societally important. We also recognize the obvious—that genetic effects resulting in fetal loss prior to the fifth lunar month of pregnancy would not be detected by this study but that early fetal losses would have both a relatively small emotional impact on the parents and a relatively small cost to society.

The Extrapolation to the Effects of Chronic Radiation

Short of a nuclear disaster of some type, most human radiation exposures will be a mixture of chronic but very-low-level ionizing radiation punctuated by small, low-level bursts of occupational or medical exposure. In the specific locus test system, the fractionated or chronic irradiation of mice yielded, for a given total (high) dose, about one-third to one-quarter as many point mutations in the spermatogonia of males as did acute, relatively high-dose X-ray irradiation (Russell et al. 1958). For point mutations in female mice, and for such other indicators as chromosomal damage, the dose-rate effect is even greater (see review in Searle 1989). The radiation doses received from the atomic bombings were very high energy and very brief—the estimated individual gonadal doses among those receiving increased radiation were 0.01–3.0 Sv, the average being about 0.25 Sv.

The complex issues involved in extrapolating from such a situation to the effect of the low-level, chronic or intermittent exposures which are the usual lot of human populations are well discussed in report 64 of the National Council on Radiation Protection (1980). There are, unfortunately, no experimental data on the spectrum of radiation exposures observed in Hiroshima and Nagasaki. We must therefore employ an indirect approach. We will assume a linear-quadratic relationship for acute radiation dose and genetic effects. The precise relationship varies for the endpoint under study; we will, to be conservative, use the relationship derived for "point" mutations in the mouse, which has a smaller

quadratic component than do other endpoints, such as chromosomal damage. Employing both the gonadal dose distribution observed in the parents entering into this study and the parameters (developed by Abrahamson and Wolff [1976]) for the linear and quadratic components of the radiation effect for specific locus mutations in the mouse, we suggest the dose-rate factor to be applied to this spectrum of radiation and endpoints is about 2. (Technically, the neutron component does not need adjustment for a dose-rate factor, but the neutron component in the dose is so small it can be included in the adjustment with small error.) A major consideration in this suggestion—which is really quite critical to the argument—is the fact that about 40% of the total gonadal dose in the population of parents under study results from estimated individual exposures of $\geq$0.50 Sv, at which point the quadratic component begins to become significant. In our view, given the higher dose-rate factors for many of the other endpoints included in this study, adjustment by a factor of 2 is quite conservative. This leads to a gametic doubling-dose estimate of 3.38–4.46 Sv for chronic or low-level intermittent low LET radiation.

The data have been presented in such a fashion that those who wish either to (*a*) make other assumptions about the contribution that spontaneous mutation in the parental generation makes to the indicator, (*b*) weight the various indicators in some other fashion, (*c*) apply a different RBE to the neutron values, or (*d*) develop alternative statistical approaches are in a position to do so. A question of this magnitude deserves multiple approaches. Two key aspects of this treatment are (1) the assumptions concerning the contribution that mutation in the parental generation makes to the endpoints and (2) the dose-rate factor appropriate to data of this type. The future will undoubtedly bring refinements to these assumptions.

The Discrepancy between This Estimate of a Genetic Doubling Dose and That Projected from Studies on Mice

Thus far the principal human surrogate in efforts to set genetically permissible limits to radiation exposures has been the domesticated house mouse. On the basis of extrapolation from studies on mice (primarily male), various national and international committees have set the doubling dose of acute gonial radiation for humans at 0.3–0.4 Sv, with limits of 0.1 and 1.0 Sv (reviewed in Committee on the Biological Effects of Ionizing Radiations, National Research Council 1980; United Nations Scientific Committee on the Effects of Atomic Radiation 1986). Because of the relatively very large gonadal doses employed in the mouse experiments, doses unrealistically large from the human perspective, the estimate of the effects of the *acute* exposures employed in mice cannot be compared directly with the present estimate of the doubling dose for the atomic bomb survivors, but the extrapolations to chronic or low-level pulse exposures can be compared. For this type of exposure, the United Nations Scientific Committee on the Effects of Atomic Radiation has for some years projected a doubling-dose value of 1.0 Gy (or Sv) (United Nations Scientific Committee on the Effects of Atomic Radiation 1986), whereas the Committee on the Biological Effects of Ionizing Radiations in 1980 suggested a range of 0.5–2.5 Sv (Committee on the Biological Effects of Ionizing Radiations, National Research Council 1980). Our estimate thus points to higher values than do past modal extrapolations from the mouse. The uncertainties in both the mouse extrapolation and human estimate are such that we do not see our way to applying statistical tests to the significance of this apparent difference, but it seems unlikely that at the 5% probability level there is overlap between the upper range of the mouse projection and the lower range of the present estimate.

There are many differences between the derivation of the present estimate and the past extrapolations from the murine data to humans. Elsewhere we are undertaking an extensive review and reanalysis of the murine data, to determine to what extent the difference results from the methodologies employed and to what extent it results from true species differences (Neel and Lewis accepted). Here we note only four salient points: (1) The sex-chromosome aneuploids, whose frequency of occurrence is relatively uninfluenced by radiation, play a much larger role in the present data than they have played in the extrapolations from mice. We argue that these are a very prominent component of the mutational burden in humans and that they must be treated accordingly. Even when these data are excluded, however, the human doubling-dose estimate for acute radiation would be some three times higher than the estimate based on the mouse data. (2) We suggest, from both direct and circumstantial evidence, that the Russell (1951) seven-locus test system, which has quite properly served as the cornerstone in most extrapolations, may have inadvertently utilized an unusually mutable set of loci (see Favor 1989; Neel et al. 1989*b*). (3) The extended period over which these observations have been assembled allows for any attenuation of genetic effects

associated with cell proliferation phenomena. (4) Data on exposed females contribute to the present findings to about the same extent as do data from exposed males; were the human female to exhibit the same paucity of induced mutation in offspring conceived many years after the bombings as is observed in the late litters of irradiated female mice (Russell 1965), this phenomenon would be incorporated into the estimate.

The Future of These Studies

The implications of these findings with respect to the vulnerability of the human species to extraneous genetic insult are such that every effort should be made to confirm or modify the conclusions of this study. Although different mutagens undoubtedly have different modes of action, and although one should not generalize from these studies to the consequences of other mutagenic exposures, the situation in Hiroshima and Nagasaki will provide, for the foreseeable future, humankind's best opportunity to understand the homeostatic properties of the human genome. The Genetics Program at RERF is currently establishing constellations of lymphocytoid cell lines, each constellation derived from a father, a mother, and one or more children. It is planned to develop $\geqslant$500 such constellations from proximally exposed survivors and their offspring and to develop another 500 such constellations from parents receiving no increased radiation and from their children. It is contemplated that, as the necessary techniques achieve the requisite level of efficiency, these lines will be examined with reference to mutations involving selected proteins visualized on two-dimensional polyacrylamide gels (see Neel et al. 1984, 1989*b*) and also at the DNA level with reference to selected DNA probes (see Delehanty et al. 1986; U.S. Congress, Office of Technology Assessment 1986). These studies should substantially extend our understanding of what are usually termed point mutations. In these studies, we strongly advocate the use of a battery of *representative* probes derived from genes of known function, since the relevance to human affairs of mutation in DNA of unknown function, as studied with so-called anonymous probes, is dubious; in view of the evidence for locus differences in mutability in humans (Chakraborty and Neel 1989), these probes must be selected with extreme care. Even with some of the technical improvements in the offing, these studies will be almost as laborious and time-consuming as those that have preceded them. There are no quick and easy answers in this field of investigation.

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Appendix

The Mutational Component in the Indicators

The indicators to be used in the calculation of a minimal and most probable doubling dose are of two types. With respect to protein variants, sex-chromosome aneuploids, and balanced structural rearrangements of chromosomes, the individuals resulting from mutation in the parental generation can be directly identified by the appropriate studies on the parents, and we have already indicated in the text the base-line mutation rates observed in the offspring of the distally exposed. For three indicators—untoward pregnancy outcomes, mortality among live-born infants, and F_1 cancer—we must proceed more circumspectly. We can only rarely among the children exhibiting untoward pregnancy outcomes, early death, or childhood cancer clearly specify which children exhibit this endpoint because of parental mutation; they are usually anonymous in a much larger pool of similar outcomes because of a variety of other causes, some known and some unknown. For these endpoints, a different approach must be pursued. We must attempt to estimate the fraction of these children that results from spontaneous mutation in their parents. The genetic events contributing to these endpoints in the case of untoward pregnancy outcomes and early death can be subsumed under three headings:

1. *Autosomal aneuploidy.* The most important example of this is trisomy 21 (Down syndrome), concerning whose responsiveness to maternal irradiation there is a considerable and controversial literature (reviewed in Cohen et al. 1977; Uchida 1977). The studies on untoward pregnancy outcomes were conducted prior to the advent of modern cytogenetics, but on clinical grounds we have estimated the frequency of Down syndrome to be 0.13% in a series of 9,440 8–10-month-old infants born to unexposed Japanese mothers and fathers (Schull and Neel 1962), a figure in satisfactory agreement with the results of later cytogenetic studies on newborn Japanese infants (0.08%; Maeda 1979;

Kuroki 1983). An effort on our part to locate, some 20 years later, the children found to exhibit Down syndrome in the course of the earlier study revealed a high mortality rate. The other autosomal trisomies which would be encountered in a study such as this (i.e., trisomy 13 and trisomy 18) are much less common (together summing to about 0.04% in Caucasian [de Grouchy and Turleau 1983; Goldstein and Nielsen 1988] and Japanese [Matsunobu et al. 1989] newborn infants), and the affected children, if liveborn, die within the first week. We will take the position that during the period of this study 0.10% of the children born to unexposed parents died in childhood because of autosomal trisomy, almost always because of mutation in a parental gamete. This high death rate reflects the relatively harsh postwar conditions during which the bulk of our cohorts were assembled, and it would not prevail in Japan today.

2. *Unbalanced structural rearrangements of chromosomes.* Children with major deletions or duplications of the autosomes usually exhibit gross abnormality and, if not stillborn, seldom survive for long. Cytogenetic techniques were not available during the years the clinical data were being collected. Presumably, the frequency in this series would be very similar to that observed in the surveys of Maeda (1979) and Kuroki (1983) on Japanese newborn infants—namely, 0.10%. The appropriate studies on Caucasian infants reveal that, among live-born infants, the rate with which live-born infants exhibit such chromosomal abnormalities because of mutation in the preceding generation is 1.6×10^{-4} (Jacobs 1981). The frequency with which abortuses exhibit such findings is 50-fold greater (Machin 1974; Alberman and Creasy 1977; Evans 1977). Accordingly, we surmise that in our series of live- and stillborn pregnancy terminations after 20 wk of gestation, the observed mutation rate would be at least twice that in live-born term infants—namely, 3.2×10^{-4} (0.032%). This is a minimal figure because it is based on "unbanded" chromosomes (see Hook et al. 1989), but a precise correction for nondetection is not possible at this time.

Children with balanced chromosomal rearrangements occurred in our series with a frequency of 0.22%, in good agreement with the findings of Maeda (1979), Kuroki (1983), and the data on Caucasians (Jacobs 1981). Our family studies on the children of unexposed parents revealed a spontaneous mutation rate for this endpoint (based on a single event) of 1.0×10^{-4}/gamete/generation. This estimate is consistent with the results of studies on live-born Caucasian infants (1.9×10^{-4}/gamete/generation; Jacobs 1981). Although this rate should be factored into our thinking, since it sets the stage for children who will in later generations die of the consequences of unbalanced chromosomal abnormality, in many respects this type of mutation presents the same problem in "forward accounting" as do the "recessive" mutations, and it will be further discussed when they are considered. Neglecting this cause of abnormality, we will assume from the data of this and the preceding paragraph that some 0.03% of all newborns will exhibit gross defect and/or die early because of mutation resulting in a chromosomally unbalanced gamete.

3. *Point mutations.* We come now to the very difficult task of estimating the contribution that spontaneous, so-called point mutation in parents makes to death and congenital defect in their children of the immediately following generation. Under the term "point" we include small chromosomal deletions and duplications. It is customary to distinguish between so-called dominant and recessive mutation—with the dominants visualized as exerting immediate impact and with the effect of the recessives for the most part becoming apparent only generations later—but in fact there is no clear dividing line. Dominant deleterious traits arising through mutation may persist for some generations before ultimate elimination by negative selection, but, conversely, "recessive" mutations may have an immediate impact through a deleterious effect on survival in heterozygotes, estimated in *Drosophila* to amount to 2%–4% in the case of newly arisen spontaneous and radiation-induced mutations (Stern et al. 1952; Hiraizumi and Crow 1960; Mukai et al. 1972). The various attempts to identify an average heterozygous effect of induced recessive lethals in mice have yielded conflicting results; Grahn et al. (1972) concluded that "heterozygote disadvantage is probably no more than 5% for mammalian genetic systems." More recently, Russell and Rinchik (1987) have presented additional evidence that some specific deletion-type "point" mutations as heterozygotes have deleterious effects on survival and weaning weight. An estimate of 2% disadvantage would seem to be conservative. It is arbitrary whether those heterozygous effects of "recessives" should be included in the impact of the dominant or recessive component of mutation; we include them with the latter because, in principle, their impact is in general spread over so many more generations than is the impact of the dominants.

In this situation, there are two "benchmarks" with respect to humans: (1) the numerous studies on the rate

with which spontaneous mutation results in a variety of dominantly inherited phenotypes and (2) the more recent data on the rate with which spontaneous mutation results in "recessively" inherited protein abnormalities. An absolutely lower bound to the impact of "point" mutation is provided by studies of the cumulative rate with which mutation results in the genetic syndromes exhibiting dominant or sex-linked inheritance. Thus, considering the results of a variety of specific studies as well as population surveys, the successive Committees on the Biological Effects of Ionizing Radiation of the U.S. National Academy of Sciences (see Committee on the Biological Effects of Ionizing Radiations, National Research Council 1980) and the various United Nations Scientific Committees on the Effects of Atomic Radiation (see the 1986 Report) have in their various publications tended to converge on the estimate that, in each generation, about 0.2% of all newborns will to some extent be handicapped (and sometimes will die prematurely) from spontaneous dominant or sex-linked mutation in their parents that results in identifiable diseases/syndromes.

This figure does not appear to include some of the growing list of syndromes due to chromosomal microdeletions (reviewed in Dallapiccola and Forabosco 1987), syndromes for which early death or sterility prevent a demonstration of genetic transmission. This figure also does not appear to include either any fraction of the cytogenetically normal children with major congenital defect not corresponding to a known genetic entity or the dysplastic children with impaired survival and mental retardation (with whom all pediatricians are familiar and who are so often encountered in institutions), some proportion of which must be due to dominant mutation, chromosomal or "point." The figure also does not include children with "failure to thrive," a category comparable to that of mouse "runts," whose frequency is increased by radiation (Searle and Beechey 1986).

With respect to autosomal recessively inherited disease, we note our own recent estimates, arrived at by electrophoretic studies (see Neel et al. 1986), that spontaneous mutations characterized by amino acid substitutions in the proteins under study occur with a frequency of about 1×10^{-5}/gene/generation, or 1×10^{-8}/nucleotide/generation. If this rate can be extrapolated to the entire genome, then with 3×10^{9} nucleotides/gamete, or perhaps 50,000 functional genes, the implication is that each zygote might receive on average one mutation of this type in the introns of a functional gene, or some 60 nucleotide substitution–type mutations scattered throughout the genome. The ultimate potential for phenotypic ill effects of some of these mutations is best documented by the extensive studies of the hemoglobin loci, with the demonstration that nucleotide substitutions may result in abnormal hemoglobins (e.g., S or C) or in thalassemia.

In addition, enzyme variants characterized by total loss of activity (the basis ranging, at the DNA level, from key nucleotide substitutions or abnormally situated stop codons to insertion/deletion/inversion events) have about the same frequency in populations as do the rare protein variants presumably maintained by the pressure of mutation resulting in nucleotide substitutions (see Satoh et al. 1983). This similarity suggests that these may arise through the mutation of functional genes with at least the same rate (1×10^{-5}/locus/generation) as nucleotide substitutions. The ill effects of these null variants is documented by the many recessively inherited inborn errors of metabolism. If, again, there were 50,000 genes in the haploid genome, the average zygote might carry one newly arisen mutation resulting in either loss of the activity or nonsynthesis of a gene product. We suggest that the high level of child care exercised by humans reduces dramatically the heterozygote effects in our species prior to age 20 years—to perhaps 10%–20% of those observed in experimental animals. It follows that the heterozygous effects of spontaneous point mutation might result in the prereproductive death of approximately 0.2%–0.4% of all children.

In the light of all these considerations concerning point mutations, we will suggest that in this series *at least* 0.20% of the infants reaching the twentieth week of gestation who do not exhibit gross chromosomal abnormality will be characterized by an untoward pregnancy outcome/prereproductive death because of so-called point mutation of all types in the parents—and the total may well be 0.40%. In view of the total frequency of congenital defect/genetic disease documented for Japanese and other populations (Schull and Neel 1965; Baird et al. 1988; Czeizel et al. 1988), and in view of the data on heterozygote disadvantage, this seems a *conservative figure—conservative in the sense that, the lower this estimate, the lower the estimates of the minimal and probable doubling doses.*

When the results of the considerations of these three sections are totaled, we arrive at the following estimate of the impact of spontaneous mutation on untoward pregnancy outcomes/early deaths: approximately 0.10% + 0.03% + (0.20%–0.40%), or 0.33%–0.53%. We assign half of this to UPOs and half to F_1 mortality exclusive of cancer (see table 5). *Inasmuch as UPOs*

among the children of the unexposed had a 5.02% frequency, and inasmuch as mortality among live borns was 4.58%, this estimate attributes 3.44%–5.52% of these events to mutation in the parental generation. (We emphasize this point because of Sankaranarayanan's [1988] recent criticism of our previous doubling-dose calculation [Schull et al. 1981], a criticism based on his failure to understand that only the estimated mutational fraction entered into the calculation [see Neel 1988].) Again we draw attention to the fact that these estimates are for a period of Japanese history (1946–85) during the first third of which approximately 10% of children either were stillborn or died prior to the age of reproduction—i.e., in a period of Japanese history when selection against deleterious genetic traits may be assumed to have been higher than at present.

It is important to recognize that the estimate of 0.33%–0.53% is very specific—it is for the impact, in the *first* postbomb generation, of spontaneous mutation on the endpoints we have measured. Given the frequency we have attributed to "recessive" mutation, the cumulative impact of the recessive component of these newly arisen mutations, in both the heterozygous and homozygous state, will be greater than that in the first generation. How much greater the perceived impact is depends on how selection operates on these recessives. Since, unlike many of the committee reports referenced above, we are not estimating the total impact of an increased mutation rate but a doubling dose for specified endpoints, we do not have to deal with this very difficult issue of the ultimate impact of recessive effects.

With respect to childhood cancer, we observed 49 cases among 41,066 children of parents not exposed to the effects of the bombs, a normative frequency of 0.12%. However, our detailed analysis of these data and the literature on the genetics of childhood cancer suggested that only 3.0%–5.0% of these malignancies (i.e., a frequency of 0.002%–0.005% of all children) had the type of genetic basis (a germ-line mutation) such that their frequency might be increased by parental irradiation (Yoshimoto et al. 1990); this is the frequency to be used for the doubling-dose estimate.

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