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# Search for mutations affecting protein structure in children of atomic bomb survivors: Preliminary report

**(human radiation genetics/Hiroshima–Nagasaki follow-up studies)**

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**ABSTRACT** **A total of 289,868 locus tests, based on 28 different protein phenotypes and using one-dimensional electrophoresis to detect variant proteins, has yielded one probable mutation in the offspring of "proximally exposed" parents, who received an estimated average gonadal exposure of 31 to 39 rem in the atomic bombings of Hiroshima and Nagasaki. There were no mutations in 208,196 locus tests involving children of "distally exposed" parents, who had essentially no radiation exposure.**

Studies of the genetic effects of atomic bombs have been in progress in Hiroshima and Nagasaki since 1946 (1–5). The first generation of studies was essentially morphological in nature. More recently, profiting from technological developments, studies have been undertaken at the cytogenetic (6, 7) and biochemical (8) levels. We present here a progress report on the results of the biochemical approach at approximately the midpoint of the study. No statistically significant difference between the children of exposed and control parents can be demonstrated at this time (nor was it expected at this juncture in the study). In addition to the timeliness of a progress report, however, the present publication is dictated by three other considerations. (*i*) The current intense interest in the genetic effects of low-level ionizing radiation has prompted a complete re-evaluation of 30 years of genetic studies on the effects of the atomic bombs; the present data can be integrated into that treatment. (*ii*) The control aspects of the data of this study can be combined with similar data from other studies to yield a direct estimate of the rate at which spontaneous mutation results in electrophoretic variants of proteins; this should be useful in planning the feasibility and magnitude of any other genetic studies of this type. (*iii*) A wealth of data on biochemical variants in Japanese has accumulated during the past 7 years; this description should clear the way for the presentation of this information.

## THE PROTOCOL

**The Populations.** The subjects for this investigation initially were drawn from the so-called $F_1$ Mortality Study of the Radiation Effects Research Foundation (RERF), a study of the mortality structure of a cohort of children born alive to "proximally exposed" parents and two suitably matched control cohorts (4, 5). As originally constituted, the first cohort is composed of those children born between 1 May 1946 and 31 December 1958 and for whom one or both parents were within 2000 meters of the hypocenter of the Hiroshima or Nagasaki bombing. One control cohort consists of children born during this same period to "distally exposed" parents—one or both parents were within the city at the time of the bombing but more than 2500 meters from the hypocenter (and therefore received essentially no radiation). The second control cohort consists of children born in one of the two cities during this same period to parents who were not in the city at the time of the bombing. The three cohorts number 18,946, 16,516, and 17,263 children, respectively [departures from the numbers published earlier (4) are due to a later reevaluation of the radiation exposure of some parents]. The first cohort represents all the children fitting this description within that time frame but, because of the physical facts of the atomic bombings, there was an excess of children from whom matching cohorts 2 and 3 could be selected. Only the first two of these three cohorts were drawn upon in the present study.

When this investigation was being planned, it became apparent that, given these numbers and the gonadal exposure to radiation of the proximally exposed parents (see below), it was highly desirable to extend the first cohort to the fullest extent possible, together with a corresponding extension in the second (control) cohort. Accordingly, in April 1976, an extension of the cohorts born to proximally and distally exposed parents was initiated. This is being accomplished by establishing a roster of children born after 1958 to parents included in the Life Span Study (Extended Sample) of RERF, a study embracing all individuals who were within the city limits of Hiroshima or Nagasaki at the time of the bombing and who were alive and still residing there in October 1950. The vital statistics of the members of this study (including births) are being updated for the Life Span Study on a 5-year cycle by reference to the official Japanese family (koseki) records. On the basis of the progress to date, we estimate that 8000 additional children born to proximally exposed parents will be identified; an age-matched cohort of equal size selected from the children born to distally exposed parents is being assembled simultaneously. The total number of children identified as born to proximally exposed parents will thus be approximately 27,000, with a similar number available born to distally exposed parents. The children on whom the present findings are based are drawn from both the original samples and their extensions.

**The Test Systems.** The protocol calls for obtaining a blood sample from as many children in each of these two cohorts as possible and using starch gel electrophoresis to examine a series of proteins of the erythrocytes and plasma with respect to genetic variants, particularly uncommon variants (allele fre-

Abbreviation: RBE, relative biological effectiveness.

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quency <0.01). The well-known genetic polymorphisms encountered for some of these proteins are by definition not the type of variant under consideration. The rationale for concentrating on the uncommon variants is that, relatively speaking, individual uncommon variants are much more apt to have a recent (mutational) origin than are variants whose phenotype occurs in polymorphic proportions. The initial number of proteins surveyed was 22; at present this has increased to 28. The proteins are as follows.

*Plasma proteins.* Albumin (ALB), transferrin (TF), haptoglobin (HP), and ceruloplasmin (CRPL) are in this group. Types were determined by starch gel electrophoresis (9) supplemented by acrylamide gel techniques where necessary.

*Erythrocyte proteins.* The following erythrocyte proteins were examined for variants: hemoglobin $A_1$ (HBA1), hemoglobin $A_2$ (HBA2), acid phosphatase-1 (ACP1), adenosine deaminase (ADA), adenylate kinase-1 (AK1), carbonic anhydrase-1 (CA1), carbonic anhydrase-2 (CA2), esterase A, B, and D (ESA, ESB, ESD), glucose-6-phosphate dehydrogenase (G6PD), isocitrate dehydrogenase (ICD), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), nucleoside phosphorylase (NP), peptidase A and B, (PEPA, PEPB), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase-1 and -2 (PGM1 and PGM2), glucose phosphate isomerase (GPI), triosephosphate isomerase (TPI), glutamic-pyruvic transaminase (GPT), and glutamic-oxaloacetic transaminase (GOT). The basic technique was starch gel electrophoresis. For 18 of the proteins we have supplied references to our techniques elsewhere (10–12). However, data on six of these enzymes (ESA, ESB, ESD, G6PD, GOT, and GPT) are being reported from this laboratory for the first time. These have been studied by the techniques described in ref 13. Although polyacrylamide is rapidly replacing starch as the gel of choice for electrophoresis, it has seemed important to maintain constancy of technique throughout the study, and no change is contemplated. The laboratory technicians were unaware of the radiation history of the parents at the time of the laboratory studies.

**Family Studies.** Because electromorphs encountered in the population in the frequencies of genetic polymorphisms (allele frequency, >0.01) are individually poor candidates for mutations, family studies of these were not routinely undertaken. (We realize that with this convention we are overlooking some rare variants that happen to have the same electrophoretic mobility as a well-known genetic polymorphism.) Whenever a rare electromorph was encountered, the possibility of a mutational origin in the preceding generation was investigated. Its occurrence was verified in a new sample and studies of both parents and available siblings were undertaken. In the event that neither parent exhibited the variant, the appropriate serological studies were undertaken with respect to possible discrepancies between stated and biological parentage. The systems available for this purpose are: ABO, MN, Rh (C, D, E, c, e), Kell, and Duffy. In addition, the following polymorphisms encountered in the 28 test systems are useful for this purpose: HP types 1 and 2, $ACP_1$ types A and B, PGD types A and C, PGM types 1, 2, and 7, ADA types 1 and 2, ESD types 1 and 2, and GPT types 1 and 2.

**Magnitude of Study.** From a study on the cytogenetic effects of the atomic bombs (6), it was estimated in 1972 that attrition in the two cohorts under study—from death, permanent or temporary absence from the two cities, or refusal to participate in the study—would amount to 40%. It was further estimated that, for an additional 20% of the children, one or both parents would be deceased or absent from the city, thus preventing the study of both parents necessary to a rigorous treatment of mutation rates. Finally, it could be anticipated that in a relatively few cases one or both potentially available parents of a child found to have a variant would elect not to participate in the study, thus also usually vitiating the usefulness of the variant as a test of mutation. Total attrition was accordingly estimated at approximately 60%. In the final report on this study we will present a detailed analysis of the attrition, with particular reference to possible sources of bias. We note at this time only that attrition will be at least as high as anticipated. Thus, if the study is continued until all the children born to proximally exposed parents and the matched group born to distally exposed parents have been examined and if an average 25 proteins are examined per participant in the study, on completion of the study each of the two panels of children should yield approximately 500,000 locus tests (each of the proteins studied reflects the composition of either two or four cistrons, except for sex-linked traits).

**Dosimetry.** Based on meticulous histories of survivors' locations at the time of the bombings and the physical facts concerning the atomic bombs, an effort has been made to assign a body surface dose to each survivor (14, 15). The error attached to these individual estimates is considerable because of the understandable uncertainty of many subjects as to exact position and posture at the time of the bombings. In both cities the radiation received consisted of a mixture of neutrons and gamma rays, but the neutron component was relatively much greater in Hiroshima. Because only one parent needed to be proximally exposed for the child to be included in the cohort born to proximally exposed parents, among the parents contributing to this cohort there are some with no radiation exposure. There are also a few parents within 2000 meters at the time of the bombings for whom for technical reasons a dose cannot be calculated; they have been assigned the average dose ascribed to those persons within 2000 meters for whom doses can be calculated. The average body surface doses in rads (gamma and neutron) to all the parents whose children are in the cohort born to proximally exposed parents and who have been studied thus far are: Hiroshima fathers, 31.0 for gamma and 10.6 for neutrons; Hiroshima mothers, 40.2 and 13.3; Nagasaki fathers, 46.5 and 0.8; Nagasaki mothers, 60.0 and 0.8. The distance–dose curves for the atomic bombs indicate a surface dose of <1 rad of $\gamma$ radiation for distally exposed parents.

From the genetic standpoint, our concern is with the effective gonad dose. Two questions arise in estimating the latter: (*i*) what relative biological effectiveness (RBE) should be assigned to the neutron component? and (*ii*) what was the average attenuation of the neutron and gamma surface exposure en route to the gonad? With respect to the first question, on the basis of the genetic effects in a "specific locus" test system of acute neutron doses of the order of 50–100 rads on mouse spermatogonia and mature oocytes (reviewed in ref 16), an RBE of 5 for the neutron component of the atomic bombs was adopted (5). There is now increasing evidence that, at low acute neutron exposures, the RBE for genetic end points is substantially greater than 5. Although there are no data at very low neutron doses in the mouse specific locus test system, other genetic end points in the mouse suggest that at acute neutron doses below 5 rads the RBE is as high as 20 (17). Under certain assumptions, similar or even higher RBE values at low neutron dosages can be invoked from the Japanese experience for several somatic cell end points that may be viewed as manifestations of somatic cell mutations, such as acute leukemia (18) and chromosome aberrations in leukocytes (19). In Hiroshima and Nagasaki, the distribution with reference to neutron dose of the proximally exposed parents whose children have been included in this study (thus far) is: 1–4 rads, 12,474 persons; 5–9 rads, 4163 persons; 10–19 rads, 2216 persons; 20–49 rads, 1822 persons; 50–99 rads, 767 persons;

≥100 rads, 595 persons. With this preponderance of low neutron doses, we now deem it appropriate to analyze the data on the assumption of an RBE of 5 and also of 10.

With respect to the question of attenuation, the dose of radiation received by the gonads is significantly less than the surface dose because of absorption in the intervening tissues. This average attenuation, as determined from studies on models of the human body, is different for gamma and neutron radiation and for females and males. On the basis of attenuation tables prepared by Kerr (20), we have estimated mean gonadal doses as follows: Hiroshima fathers, 20.7 for gamma and 4.2 for neutron; Hiroshima mothers, 17.1 and 1.6; Nagasaki fathers, 30.3 and 0.3; Nagasaki mothers, 24.1 and 0.1. If we apply an RBE of 5 to the neutron component and weight the average according to the relative contributions of Hiroshima and Nagasaki to the total sample, the per locus dose represented in the children of these parents (average of the parents) is 30.8 rem. At an RBE of 10, the dose is 38.6 rem. Kerr's tables apply to adults; because many parents of the children in this study were themselves children at the time of the bombings, attenuation is undoubtedly overestimated and these are conservative dose estimates.

This mean gonadal exposure is, by a significant margin, the largest average dose received by any contemporary group of this size in the world. Furthermore, because the exposure was instantaneous (rather than chronic), it was the type of greatest genetic significance. On the other hand, by the standards of experimental radiation genetics, this is a rather modest exposure. For instance, in the extensive work on mice, the most frequently used surface dose has been 300 rem of gamma radiation, virtually all of which presumably reached the gonads. By contrast, 49% of the parents in the proximally exposed group received amounts of body surface radiation estimated at less than 10 rem—i.e., in the range commonly referred to as low level. At this juncture it should be emphasized that the order of magnitude of this dose and, by extrapolation from experimental mammalian radiation genetics (21), the low probability that this study would yield statistically significant findings, was recognized from the outset. Given the urgent need for human data, however, there was never any real doubt of the wisdom of pursuing this ambitious project, especially since these data can be combined with the findings of other studies on this population to develop a "best estimate" of the genetic significance of this experience.

**Chronology.** This study was first proposed to the various committees responsible for the follow-up studies in Hiroshima and Nagasaki in 1970, at a time when the organization was known as the Atomic Bomb Casualty Commission. In 1972 authorization was given for a pilot study, to be limited to blood samples already reaching the Commission's laboratories through other approved protocols. For the most part these were samples from exposed survivors (and so would not bear on the question of the genetic effects of the atomic bombs), but there were some samples from children of proximally and distally exposed parents being examined in a search for chromosomal abnormalities. Those children were being drawn from the roster of the $F_1$ Mortality Study, which formed the original basis for the present study. This pilot study extended from 1972 to 1975. The findings in the exposed survivors have been presented (10–13). During this period, 1818 samples from children were processed. Beginning in 1975, following the reorganization of the Atomic Bomb Casualty Commission to the Radiation Effects Research Foundation (22), the biochemical study was gradually expanded, to the point where currently each year 3200 samples drawn from members of the two cohorts of children are being processed (plus the necessary repeat samples and samples for the family studies). This report encompasses all the electrophoretic data collected from the beginning of the study in 1972 to September 1979. It should be emphasized that the investigation from start to finish is a *prospective* study in that cohorts were established from birth records (as was true of the earlier genetic studies); ultimately we will account for each child in the cohort.

## THE DATA

In Table 1, the number of different electromorphs of each protein that have been encountered in the study and the total number of all such variants are given. The findings with respect to the well-known genetic polymorphisms of HP, ACP, PGD, PGM1, ADA, GOT, ESD, and GPT will not be presented in this paper. A variant with the mobility of TF $D_{Chi}$ occurs in this population as a "rare variant" ($P = 0.0059$). Because TF $D_{Chi}$ is a widespread polymorphism in Mongoloid peoples, the variant was not considered an appropriate candidate for studies of mutation and is not included in Table 1. A previously unrecognized variant of CRPL, termed CRPL $C_{NGS1}$, found to occur in polymorphic proportions ($p = 0.0128$), is also not included in the variant count. In Table 1, "Total determinations" corresponds to number of locus tests.

Only when both parents of a child with a variant were examined do we have data suitable for a rigorous treatment of mutation rates. Table 1 lists the frequency of such family studies, by system. For each system, an estimate of the number of proteins that have been effectively screened for mutation is obtained by multiplying the fraction of variants for which family studies are complete by the total number of determinations. By this convention, all the determinations of a protein for which no variants have been encountered are credited as contributing to locus tests. Because several of the proteins tested consist of two genetically independent polypeptides and one exhibits sex-linked inheritance, we may consider that thus far there have been 289,868 locus tests for mutation in the children of proximally exposed parents and 208,196 in the children of distally exposed parents. This is a conservative estimate because we have treated the poorly understood esterase A bands as reflections of a single polypeptide.

We apply the term "exceptional child" (Table 1) to a child who has a variant not observed in either parent. In this study we are concerned with the acquisition of a new attribute; apparent losses of attributes, such as could be attributed to parental heterozygosity for a null variant, are not accepted as defining an exceptional child. Once clerical or laboratory error is excluded (by analysis of repeat sample), there are two possible explanations of such children—namely, either mutation or a discrepancy between parentage reported to the study and the true biological parentage. There are two exceptional children among the sample born to proximally exposed parents and one among the sample born to distally exposed parents. However, by virtue of the genetic typings, parental statements, or recourse to the official family records, either unrevealed adoption or other discrepancies between legal and biological parentage could be established for two of these exceptional children. We were thus left with one apparent mutation. This involves a slowly migrating variant of GPT in a child of an exposed mother and an unexposed father. There was no paternity exclusion by history or from typings for the 12 polymorphic genetic systems mentioned above. On the basis of the number of locus tests calculated for this study, these findings correspond to mutation rates of $0.34 \times 10^{-5}$ per locus per generation in the proximally exposed parents and zero in the distally exposed parents.

Table 1. Summary of data on 30 polypeptides in the children of proximally and distally exposed parents.

| System | Symbols | E.C. No. | Cohort born to proximally exposed parents: Total determinations | Variants: No. of types | Variants: Total | Variants both $P_1$ studied | Equivalent locus tests | Exceptional children | Cohort born to distally exposed parents: Total determinations | Variants: No. of types | Variants: Total | Variants both $P_1$ studied | Equivalent locus tests | Exceptional children |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haptoglobin | HP | | 12830 | 3 | 5 | 3 | 7698 | 0 | 9530 | 1 | 1 | 1 | 9530 | 0 |
| Transferrin | TF | | 13034 | 11 | 57 | 39 | 8918 | 0 | 9686 | 10 | 49 | 28 | 5535 | 0 |
| Ceruloplasmin | CRPL | | 13008 | 3 | 9 | 9 | 13008 | 0 | 9672 | 1 | 4 | 2 | 4836 | 0 |
| Albumin | ALB | | 13040 | 3 | 21 | 15 | 9314 | 0 | 9688 | 3 | 14 | 11 | 7612 | 1 |
| Hemoglobin A1* | HBA1 | | 26076 | 2 | 4 | 4 | 26076 | 0 | 19388 | 1 | 1 | 0 | 9694 | 0 |
| Hemoglobin A2 | HBA2 | | 13036 | 0 | 0 | 0 | 13036 | 0 | 9682 | 0 | 0 | 0 | 9682 | 0 |
| Adenosine deminase | ADA | 3.5.4.4. | 13040 | 1 | 1 | 0 | 0 | 0 | 9682 | 0 | 0 | 0 | 9682 | 0 |
| 6-phosphogluconate dehydrogenase | 6PGD | 1.1.1.44 | 13004 | 2 | 6 | 5 | 10837 | 0 | 9682 | 2 | 6 | 2 | 3227 | 0 |
| Adenylate kinase | AK | 2.7.4.3. | 12100 | 0 | 0 | 0 | 12100 | 0 | 9534 | 0 | 0 | 0 | 9534 | 0 |
| Phosphoglucomutase-1 | PGM1 | 2.7.5.1. | 12004 | 4 | 37 | 28 | 9084 | 0 | 9628 | 6 | 25 | 17 | 6547 | 0 |
| Phosphoglucomutase-2 | PGM2 | 2.7.5.1. | 13036 | 1 | 1 | 1 | 13036 | 0 | 9688 | 2 | 5 | 3 | 5813 | 0 |
| Acid phosphatase | ACP | 3.1.3.2. | 12040 | 1 | 1 | 1 | 12040 | 0 | 8862 | 0 | 0 | 0 | 8862 | 0 |
| Triose phosphate isomerase | TPI | 5.3.1.1. | 11366 | 2 | 2 | 2 | 11366 | 0 | 9124 | 2 | 2 | 2 | 9124 | 0 |
| Nucleoside phosphorylase | NP | 2.4.2.1. | 11362 | 2 | 10 | 6 | 6817 | 0 | 9120 | 1 | 5 | 4 | 7296 | 0 |
| Esterase B | ESB | 3.1.1.1. | 10522 | 0 | 0 | 0 | 10522 | 0 | 8288 | 0 | 0 | 0 | 8288 | 0 |
| Esterase D | ESD | 3.1.1.1. | 11094 | 0 | 0 | 0 | 11094 | 0 | 8830 | 0 | 0 | 0 | 8830 | 0 |
| Esterase A | ESA | 3.1.1.1. | 10768 | 1 | 3 | 3 | 10768 | 0 | 8550 | 2 | 3 | 3 | 8550 | 0 |
| Peptidase A | PEPA | 3.4.11.- | 13032 | 1 | 6 | 3 | 6516 | 0 | 9678 | 1 | 5 | 3 | 5807 | 0 |
| Peptidase B | PEPB | 3.4.11.- | 13042 | 2 | 10 | 9 | 11738 | 0 | 9694 | 1 | 6 | 3 | 4847 | 0 |
| Glucose phosphate isomerase | GPI | 5.3.1.9. | 13038 | 6 | 53 | 41 | 10086 | 1 | 9694 | 5 | 37 | 30 | 7860 | 0 |
| Isocitrate dehydrogenase | ICD | 1.1.1.42 | 13038 | 2 | 18 | 15 | 10865 | 0 | 9690 | 2 | 4 | 3 | 7268 | 0 |
| Lactate dehydrogenase | LDH | 1.1.1.42 | 26028 | 3 | 3 | 2 | 17352 | 0 | 19340 | 0 | 0 | 0 | 19340 | 0 |
| Malate dehydrogenase | MDH | 1.1.1.37 | 13038 | 2 | 3 | 3 | 13038 | 0 | 9690 | 0 | 0 | 0 | 9690 | 0 |
| Carbonic anhydrase 1 | CA1 | 4.2.1.1. | 13008 | 3 | 10 | 9 | 11707 | 0 | 9646 | 1 | 1 | 1 | 9646 | 0 |
| Carbonic anhydrase 2 | CA2 | 4.2.1.1. | 13018 | 0 | 0 | 0 | 13018 | 0 | 9656 | 1 | 2 | 0 | 0 | 0 |
| Glucose-6-phosphate dehydrogenase | G6PD | 1.1.49 | 6853 | 2 | 11 | 3 | 1869 | 0 | 5438 | 2 | 10 | 8 | 4350 | 0 |
| Glutamic oxalacetic transaminase | GOT | 2.6.1.1. | 4536 | 1 | 10 | 9 | 4082 | 0 | 4588 | 1 | 14 | 11 | 3605 | 0 |
| Glutamic pyruvate transaminase | GPT | 2.6.1.2. | 4530 | 3 | 14 | 12 | 3883 | 1 | 4568 | 3 | 16 | 11 | 3141 | 0 |
| Total | | | 354,521 | | | | 289,868 | | 270,277 | | | | 208,196 | |

* Hemoglobin $A_1$ and $A_2$ are tetramers of two polypeptides, one of which is common to both proteins. Accordingly, for locus tests we have counted the $\alpha$-polypeptide only in conjunction with hemoglobin $A_1$. The fact that the single variant of $A_1$ in a child of a distally exposed parent is not seen in $A_2$ localizes this variant to the $\beta$-polypeptide and thus permits us to include the $\alpha$-chain determinations for this segment of the data in our total.

## DISCUSSION

This study is directed at the detection of "point" as opposed to "chromosomal" mutation. The result of spontaneous point mutation may range from an amino acid substitution in a protein (with retention of function at a normal or reduced level) to apparent complete absence of the protein (termed a "null mutation"). The basis for the latter can in turn range from amino acid substitution incompatible with enzyme activity to actual physical loss from the genome of the locus encoding for the protein (i.e., a small deletion) or, theoretically, to mutation of a control element. Of the amino acid substitutions, roughly two-thirds will not alter the electrophoretic behavior of the protein, at least under the screening conditions used in this study. The only extensive data currently available on the relative frequency with which spontaneous mutation in germinal tissue results in electrophoretic variants as opposed to nulls for enzymes is based on studies on *Drosophila melanogaster* (23) in which a total of 1,658,308 locus tests equally distributed over five enzymes revealed 3 electromorphic and 17 null mutations. Thus, neglecting mutation resulting in synonymous changes in the code, and assuming in man the same relative frequency of null mutations as in *Drosophila*, for every electrophoretic variant identified as arising from spontaneous mutation, there may be seven or eight other undetected mutations involving the same protein. With respect to radiation-induced mutations, the detailed studies on mutations at the *d* and *s* loci in the mouse suggest that the contribution of nulls, especially those due to small deletions, to the total spectrum of mutation may be even higher than the 5:1 ratio cited above (24–26). The present approach might therefore be expected to detect only 10–15% of point mutations.

Studies on experimental organisms have revealed significant differences in the frequency with which mutation is recovered in relation to such factors as stage of germ cell at time of irradiation and interval between exposure and reproduction. Accordingly, it seems necessary to acknowledge in specific fashion how heterogeneous these data are in the context of experimental radiation genetics. However, such heterogeneity will characterize exposed human populations so that, with the probable

exception of the preponderance of women among the exposed, the heterogeneities in the Japanese situation will be duplicated in most human population exposures.

At this stage the data scarcely lend themselves to statistical manipulation. On the other hand, given the current interest in the topic, some "face value" implications are inevitable. Three other efforts (27, 28) have failed to identify a single instance of spontaneous mutation resulting in an electromorph, the total number of locus tests in the four available series, including the controls of this study, totaling 522,119. On the other hand, five estimates of such mutation frequencies in tribal populations living under primitive conditions, based on an indirect approach, have ranged from $0.1 \times 10^{-6}$ to $32 \times 10^{-6}$ per locus per generation (refs. in ref. 29). The lower estimate is clearly an outlier, based on a small sample from a large population which may be presumed to have expanded markedly in recent years. If that estimate is eliminated, the average of the remaining four estimates is $1.8 \times 10^{-5}$.

A discrepancy between the results of the direct and indirect approaches to estimating rates of mutation that results in electrophoretic variants is emerging. Because the indirect approach has been based largely on tropical tribal populations and the direct on civilized populations in temperate zones, in addition to the possibility that incorrect parameters have been used in the indirect estimates, we must consider the possibility of real differences between the two types of populations. Clearly, however, the true rate of such mutation in civilized populations cannot be the zero now being found from the direct approach. The facts for now—giving greater weight to the results of the direct approach—are consistent with a spontaneous rate for electromorphs of $0.3–0.5 \times 10^{-5}$ per locus per generation. Thus, the single probable mutation observed among the children born to the proximally exposed parents has a high probability of being unrelated to the radiation experience. Stated in another way, there is no suggestion that the locus-rem exposure of between 8,956,921 (neutron RBE of 5) and 11,275,865 (neutron RBE of 10) estimated to obtain for the "parental exposure" subset of the sample thus far examined has increased the mutation rate in these survivors, but *the data do not exclude a doubling or even tripling of the expected rate.* In this context we emphasize that we do not consider this study (or any of the other genetic studies on the children born to survivors of the atomic bombings) as a test of the hypothesis that mutations were produced but rather as an effort to provide a responsible estimate of the magnitude of the genetic effects that must be assumed to have occurred.

The projected extensions of the study will considerably refine the inferences that can be drawn, but the very low control rates being encountered underline our initial concern that this study by itself would not yield significant differences between the two groups. On the other hand, even now the present data can be integrated into a cumulative treatment of all the genetic studies in Hiroshima and Nagaski and also indicate the magnitude of the effort required to evaluate the genetic impact of lesser exposures to radiation and the various chemical mutagens to which the human species is subjected.


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