RADIATION GENETICS

Neel, J. V. and *W. J. Schull:* Acta genet. *6*, 183–196, 1956

Department of Human Genetics, Medical School,
University of Michigan, Ann Arbor, Michigan, U.S.A.

STUDIES ON THE POTENTIAL GENETIC EFFECTS OF THE ATOMIC BOMBS

By J. V. NEEL and W. J. SCHULL

The study to be described was undertaken as one facet of a comprehensive attempt to obtain information concerning the various possible delayed biological effects of exposure to an atomic bombing. So well known are the genetic effects of irradiation that inevitably one of the foremost questions in the minds of those considering the possible late consequences had to do with the characteristics of the children of exposed parents. We shall report on certain efforts made during the year 1946–1955 to answer the following two questions:

1. Can there be observed, during the first year of life, any differences between the children born to parents, one or both of whom were exposed to the effects of the atomic bombings of Hiroshima and Nagasaki, and the children born to suitable control parents, and
2. If differences do exist, how are these to be interpreted?

Data pertinent to these two questions were collected through the agency of the Atomic Bomb Casualty Commission (ABCC) of the National Academy of Sciences—National Research Council of the United States and with the assistance of the National Institute of Health of Japan. The study has involved the efforts of many, many people, to all of whom grateful acknowledgment is made in the detailed presentation now in press.

The possible observable genetic effects of irradiation upon the first generation born after an atomic bombing are many and varied. These include changes in the sex ratio, the frequency of stillbirths, the frequency of congenital malformation, infant mortality, etc. *Each of these indicators of genetic damage is also influenced by a number of other factors; there are*

no known unique yardsticks of genetic damage. **Under these circumstances, the crux of any program of study was the feasibility of establishing control material which insofar as possible differed from the irradiated only with respect to the radiation factor. The kinds and quantity of data to be collected were shaped by a number of considerations, practical as well as scientific. Notable among these were the expected "smallness" of the radiation effects, and the expected "largeness" of non-radiation sources of variation.**

Brief Description of the Program

Briefly, the plan of attack on the problem was as follows: In the post-war years there existed in Japan a ration system such that pregnant women upon registration of their pregnancy following the completion of the fifth month of gestation could acquire access to certain rationed items. With the cooperation of the city administrators of Hiroshima and Nagasaki, a system was instituted in 1948 whereby at the time of her registration for ration purposes, each pregnant woman or her representative in these two cities also registered with the ABCC and completed the first two-thirds of a questionnaire which included such items as identifying information, a brief radiation history of the husband and wife, a short summary of the past reproductive performance, and pertinent details concerning the present pregnancy. At the time of the termination of the pregnancy, the midwife or physician in attendance notified the Commission and completed the aforementioned questionnaire by answering certain questions pertaining to the characteristics of the child and delivery. More specifically, information was requested on the following possible indicators of a genetic difference between the children of control and irradiated parents: sex, birthweight, stillbirth, and presence of malformation. Fig. 1 is an English translation of the questionnaire.

Fig. 1. **English Translation from the Japanese Investigation Sheet for Births after Atom Bomb. Printing of July, 1949.**

1. Name of city and investigation sheet number □□□□□□
2. Day, month, and year of registration
3. Expected date of birth □

Husband *Wife*

4. Name
   (Maiden name in case of wife)
5. Birth dates of husband and wife
6. Age
   (exact number of years and months) □□ □□

7. Present in Hiroshima or
Nagasaki at time of bombing
8. Location at time of bombing
(street and number) □
9. Distance from hypocenter □□ □□
10. Indoors □
11. Type of building □
12. Did you have or not
have subcutaneous bleeding □
13. Did you have or not
have gingivitis □
14. Did you have or not have
bloody diarrhea □
15. Did you have or not
have epilation □
16. Did you have or not
have fever □
17. Did you have or not
have burns □
18. Did you have or not have
external injuries □
19. Date, month, and year of beginning cohabitation
20. Number of months interruption of cohabitation
21. Total number of months cohabitation □□□
22. Number of months cohabitation before August, 1945 □□□
23. Number of pregnancies before August, 1945 □□
24. Number of spontaneous stillbirths before August, 1945 □
25. Number of therapeutic abortions before August, 1945 □
26. Number of months cohabitation after August, 1945 □□□
27. Number of pregnancies after August, 1945 (including present) □□
28. Number of spontaneous stillbirths after August, 1945 □
29. Number of therapeutic abortions after August, 1945 □
30. Total number of pregnancies □□
31. Total number of spontaneous stillbirths □
32. Total number of therapeutic abortions □
33. Marriage of blood relations (first cousin,
one and one-half cousins, second cousins, etc.) □
34. Present address and occupation of husband
35. Day, month, and year of beginning of last menstrual period of wife
36. Day, month, and year of birth expectation (according to calculation sheet)
37. Present month of pregnancy
38. Day, month, and year of termination of birth □□

39. Number of weeks of pregnancy			□□
40. Course of labor:	Spontaneous Duration	Induced Use of instruments	□□
41. Condition of newborn	Live birth after 38 or more weeks Miscarriage 20 weeks or under Stillbirth 30–38 weeks	Premature birth under and including 38th week Stillbirth 21–29 weeks Stillbirth after 38 weeks	□
42. Multiple birth (2, 3, etc.)		Order of birth	□
43. Sex of newborn		44. Weight (grms)	□ □□□
45. Presence or absence of malformation			□
46. Type of malformation (give details)			□□□□□□
47. Date of death of newborn child			□□
48. Date of termination of any pregnancies after January, 1948			□
49. Remarks			
50. Name and address of attendant at birth			

Regardless of the type of termination, a Japanese physician in the employ of the Commission or the Japanese National Institutes of Health called to examine the child—at once, if there was a report of an abnormal termination or on a somewhat more leisurely schedule if the termination was reported as normal. The completeness of this system of reporting and follow-up was checked periodically by contrasting the number of births reported to the Commission with the number of births reported to municipal authorities. These studies indicated that approximately 93 per cent of births occurring in Hiroshima, and a somewhat higher percentage in Nagasaki, were known to the Commission. A large proportion of the 7 per cent not ascertained through the registration scheme subsequently came to our attention through other channels. These latter births, which we have termed the unregistered series, are not included in the results to be reported. They have been of value, however, in determining the magnitude of any bias introduced by the failure of the registration program to be exhaustive.

In the event that a pregnancy terminated abnormally, as in a stillbirth or a child with a congenital malformation, a supplementary questionnaire was completed in the patient's home by a physician in the employ of the ABCC. This questionnaire covered in some detail gynecologic history, maternal illness during pregnancy, past reproductive performance, and economic status. In addition to this questionnaire, blood was obtained from the mother for a serological test for syphilis. This same supplemental questionnaire was routinely completed on every registration for which the terminal digit in the registration number was zero, that is to say, for every tenth registration.

The possibility had to be recognized that for a variety of reasons some malformations would not be diagnosed at birth. Accordingly, in 1950, a program was inaugurated to bring into the central clinical facility at age nine months as many of the children examined shortly after birth as possible. This afforded an opportunity to check on diagnostic oversights, to make supplementary diagnoses, and to collect more information on infant mortality. In addition, certain anthropometric measurements (height, weight, head and chest circumference) were obtained as an index of general physical development. Clinical facilities did not permit a 9-month follow-up on every registered termination, hence it was necessary to sample the terminations. This was accomplished by the simple expedient of calling in babies for examination according to the terminal registration digit of the pregnancy. Where a child who was included in the sample could not be examined, an attempt was made to establish why, in an effort to detect possible sources of bias. Other evidence pertinent to the question of irradiation effects was obtained from a study of early pregnancy terminations (those pregnancies terminating before the pregnant woman was eligible to register), and from the autopsying of as many as possible of the stillborn infants and those infants dying during the first few days of life.

The Evaluation of Parental Radiation Exposure

To analyze the data it was necessary to classify each pregnancy termination with respect to the exposure of the two parents. Five categories of exposure were recognized for each parent; hence a given pregnancy termination could be scored in one and only one of twenty-five exposure cells, the appropriate cell being determined by the conjoint parental exposure. The five exposure classifications are as follows:

1. Not present in Hiroshima or Nagasaki at the time of the bombing.
2. Present in one or the other of the two cities but at a distance from ground zero (a) greater than 3000 meters, or (b) 0–3000 meters and heavily shielded, or (c) 1500–3000 meters and moderately shielded, or (d) 2000–3000 meters and lightly shielded.

3. Present at a distance of (a) 2000–3000 meters and unshielded, or (b) 1000–2000 meters and lightly shielded, or (c) 0–1000 meters and moderately shielded.
4. Present but at a distance (a) less than 2000 meters and unshielded, or (b) less than 1000 meters and lightly shielded.
5. Present but less than 3000 meters from ground zero and exhibiting one or more of the following three symptoms of radiation sickness: epilation, petechiae, gingivitis.

"Heavy" shielding denotes presence in concrete or brick building or air raid shelter at the time of the bombing. "Moderate" shielding includes being within a street car, train, or car, behind a wall or under the eaves of a house on the side away from the hypocenter. Finally, "light" shielding includes those individuals giving their location as in a Japanese-style building or in a trench or behind a post or tree. From a consideration of what has been published concerning the distance-dosage curves of a "nominal" atomic bomb, the degree of shielding afforded by the structures enumerated above, and the levels of irradiation necessary to induce radiation sickness and/or leucopenia, it is estimated that these five categories of exposure correspond to doses of approximately 0, 5–10, 50–100, 100–150, and 200–300 roentgens equivalent physical respectively. The distribution of registered births by parental exposure is given in Table 1. Because of the relatively few individuals falling in Categories 4 and 5, these categories were combined for purposes of analysis.

Table 1a. Distribution of births by parental exposure (all births)

HIROSHIMA

		Mother's Exposure					
		1	2	3	4	5	Total
Father's Exposure	1	20192	6089	2499	462	855	30097
	2	1726	2145	488	100	142	4601
	3	697	452	594	54	75	1872
	4	155	127	93	34	27	436
	5	290	156	87	21	63	617
Total		23060	8969	3761	671	1162	37623

Table 1b. Distribution of births by parental exposure (all births).

		NAGASAKI					
		Mother's Exposure					
		1	2	3	4	5	Total
Father's Exposure	1	16721	10398	851	121	492	28583
	2	2497	4654	314	39	97	7601
	3	269	309	118	14	22	732
	4	50	56	14	6	1	127
	5	111	138	26	2	22	299
Total		19648	15555	1323	182	634	37342

Extraneous Sources of Variation

As has been stated, all of the possible indicators of genetic damage utilized in this study are influenced by a variety of factors other than exposure. It was necessary, therefore, to undertake a detailed comparison of the parents of the infants comprising the various exposure subclasses with respect to certain possible differences which might influence the outcome of pregnancy. Time does not permit more than the briefest sketch of the result of this comparison. Consanguinity, maternal age, parity, economic status, frequency of positive serological test for syphilis, frequency of induced abortions and dilatation and curettage of the uterus, and the frequency of repeat registrations were among the factors studied. Of these, significant differences could be shown to exist among exposure subclasses with respect to the frequency of consanguineous marriages (the rate tended to decrease with increasing parental exposure), maternal age (the mean age tended to increase with increasing parental exposure as did the variance), and parity (mean and variance increase with increasing exposure). Each of these three factors exerts a rather appreciable effect on pregnancy outcome, with any of them quite probably equaling if not exceeding as a source of variation the expected effect of irradiation. The observed differences in maternal age and parity are such as to lead to higher frequencies of malformation, stillbirths, and neonatal deaths in the cells corresponding to the parents more heavily exposed even in the absence of an effect of exposure. Hence maternal age and parity differences could in these data lead to spurious irradiation effects. On the other hand, the observed differences in the frequency of consanguineous marriages are such as to lead to an increase in indicator values in the cells corresponding to the unexposed or lightly exposed parents and hence to a possible obscuring of irradiation differences. Among the other non-radiation sources of variation only year of birth requires special comment. The bettering of the economic situation in Japan in the post-war years can be shown to be inversely correlated with the frequency of births to heavily exposed parents. To the extent that the bettering of the economic situation would be reflected in improved nutrition, year of birth can be shown to be of importance in determining the effect of parental exposure on birthweight.

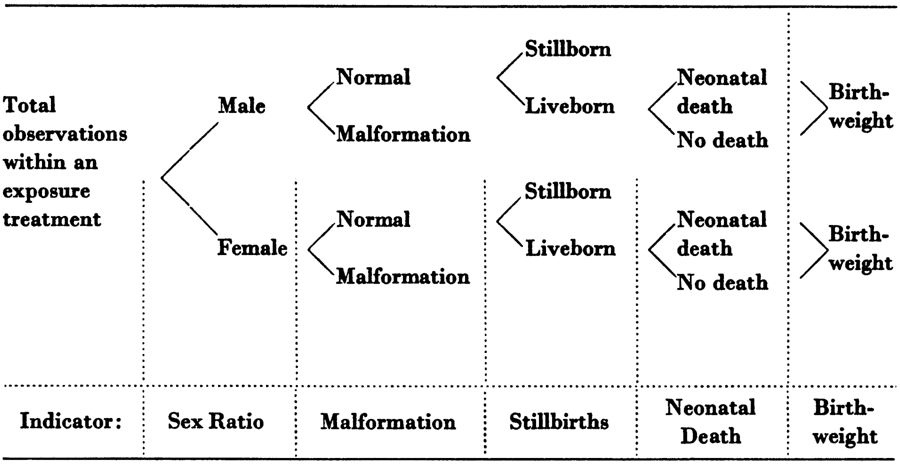
In the main the differences between exposure cells with respect to these non-radiation sources of variation are most pronounced between, on the one hand, the cells in which neither or only one of the parents were exposed and, on the other hand, the cells wherein both parents were exposed. Thus limiting one's attention to those cells where both parents were exposed minimized non-radiation sources of variation, and yet affords a good measure of the effect of irradiation since mean dose will vary from approximately 10 to 400–500 roentgens equivalent physical.

Statistical Methods

In view of the multiple problems which arise when one attempts to employ survey data in an analytical fashion, it is doubtful whether, given a body of survey data, any two competent statisticians would evolve essentially the same approach. While the basic question to be asked of the data is a relatively simple and straightforward one, namely, is there a difference between the outcome of pregnancy in irradiated and non-irradiated parents, the attempts to answer the question are complicated by three factors: (1) the possibly overlapping nature of some of the indicators, (2) extraneous (concomitant) variation, and (3) disproportionate numbers of observations in the various exposure cells. The first of these factors was readily met by a pyramidal handling of the data. Under the scheme employed, the first attribute to be handled was the sex ratio. This was followed by the frequency of malformation. In this and all subsequent partitions, sex was taken into account. All grossly malformed infants were then excluded, and the frequency of stillbirths obtained. The stillborn infants were discarded in turn, and birthweights distributed on the remainder. The order of the testing is indicated in Fig. 2.

But while it was relatively easy to handle the data so as to minimize the problem of overlapping indicators, making allowance for concomitant variation and disproportionate cell numbers was more difficult. Time does not permit even a sketchy presentation of the statistical techniques. Of the various types of concomitant variation enumerated above, three were

Fig. 2



felt to introduce potential biases of such magnitude that some effort at statistical adjustment was necessary. These were consanguinity, maternal age, and parity. Variation as regards the amount of consanguineous marriage among the parents of the children falling into the 25 radiation subgroups was met by the simple expedient of eliminating all children of consanguineous marriages from consideration. Variation in maternal age and parity was handled by a covariance analysis or by increasing the ways of classification, depending upon whether the variable was continously or discontinously distributed. In the latter case, information from the different ways of classification was pooled only after such pooling could be shown to be justified by the absence of significant interaction among the ways of classification. The techniques employed in testing for interactions will be presented in detail in the full publication now in press. The numbers of observations available in testing the various indicators are presented in Table 2, a and b.

With respect to the third of the analytic complications mentioned earlier, namely, disproportionate numbers of observations and the consequent non-orthogonality of the contrasts, in an analysis of variance we have relied primarily on the method of "fitting constants" described by *Wilks* [1938], with, in the case of the anthropometric studies on children aged 9 months, logical extensions of this method appropriate to the multi-variate analysis of dispersion.

This very brief description of the statistical methods, while mathematically entirely inadequate, may have at least served to indicate the main lines along which the analysis proceeded and the extremely labori-

Table 2a. An accounting of the number of observations considered at representative stages in the analysis of the "at birth" data and the number of rejected observations with the cause of rejection.

	Available Observations			Rejected Observations		
	Hiroshima	Nagasaki	Total	Hiroshima	Nagasaki	Total
Total infants seen	38421	38205	76626			
Rejected because the pregnancy was unregistered, parental exposure was unspecifiable, consanguinity or other observations were incomplete . .				3478	1868	5346
Considered for consanguinity .	34943	36337	71280			
Rejected consanguinity . . .				2113	2920	5033
Considered for maternal age .	32830	33417	66247			
Rejected multiple births . .				365	451	816[1]
Considered for sex ratio	32465	32966	65431			
Considered for malformations .	32465	32966	65431			
Rejected malformations . .				313	281	594
Rejected congenital heart disease				44	53	97
Total				357	334	691
Considered for stillbirths . . .	32108	32632	64740			
Rejected stillbirths				472	482	954
Considered for neonatal deaths .	31636	32150	63786			
Rejected neonatal deaths . .				414	480	894
Considered for birthweight . .	31222	31670	62892			

[1] In Hiroshima one set of registered triplets and 181 sets of registered twins occurred; in Nagasaki there was one set of registered triplets and 224 sets of registered twins.

ous computations involved. In closing this section we would like to express our particular appreciation for statistical help from *C. R. Rao* and *H. L. Lucas*.

Results

The results of the study are summarized in Table 3. For most of the indicators, two separate analyses have been presented, one including (the 4×4 case) and one excluding (the 3×3 case) those exposure cells wherein one or both parents were unexposed. The latter was deemed necessary because of the criticisms which can be leveled at use of the unexposed parents as controls. The comparisons given in this table are, in several instances, without correction for age-parity differences between exposure subclasses, a point we will refer to from time to time.

Table 2b. **An accounting of the number of observations considered at representative stages in the analysis of the "9-months" data, and the number of rejected observations with the cause of rejection.**

	Available Observations			Rejected Observations		
	Hiroshima	Nagasaki	Total	Hiroshima	Nagasaki	Total
Total infants on whom there exists some follow-up study .	14768	12324	27092			
Rejected inadequate exposure history, infant not part of 9-months program, etc.. . . .				3422	1882	5304
Total infants considered under the 9-months program . . .	11346	10442	21788			
Rejected consanguinity . . .				694	828	1522
Rejected incomplete measurements				140	308	448
Considered for neonatal death .	10512	9306	19818			
Rejected neonatal deaths . .				484	458	942
Considered for malformation . .	10028	8848	18876			
Rejected malformations . .				183	195	378
Considered for anthropometrics	9845	8653	18498			

Table 3a. **A summarization of the comparisons of the various indicators with parental exposure when (a) all exposure cells are considered (the 4×4 case), and (b) only those cells where both parents were exposed are considered (the 3×3 case).**

Indicator	Parental Exposure: Fathers		Parental Exposure: Mothers	
	4 × 4 case	3 × 3 case	4 × 4 case	3 × 3 case
Sex Ratio	.30–.50 ↑	.90–.95 ↑	.10–.20 ↓	.95–.98 ↓
Malformation: at birth	.50–.70 ↑	.80–.90 ↓	.50–.70 ↑	0.99 ↑
at 9 months .		.30–.50 ↓		.02–.05 ↓
Stillbirth	.20–.30 ↑	.80–.90 ↑	.001–.01 ↑	.30–.50 ↓
"Neonatal" Death	*	.20–.30 ↓	*	.02–.05 ↓
Death in 9 months		.95–.98 ↑		.50–.70 ↓
Birthweight Means:				
males-Hiroshima	.10–.25 ↓		>.25 ↓	
females-Hiroshima	>.25 ↓		>.25 ↓	
males-Nagasaki	>.25 ↑		.10–.25 ↓	
females-Nagasaki	>.25 ↑		.10–.25 ↑	
Anthropometrics:				
generalized means	<.001	.25–.50	.02–.05	.05–.10

* No general test.

Table 3b. **A summarization of the comparisons of the various indicators with parental exposure when (a) all exposure cells are considered (the 4×4 case), and (b) only those cells where both parents were exposed are considered (the 3×3 case).**

Indicator	Combined Parental Exposure	
	4 × 4 case	3 × 3 case
Birthweight Variances:		
males-Hiroshima	.10–.25	
females-Hiroshima	<.001	
males-Nagasaki	.10–.25	
females-Nagasaki	.10–.25	
Anthropometrics generalized variances:		
males-Hiroshima	.10–.25	>.25
females-Hiroshima	.10–.25	>.25
males-Nagasaki	.10–.25	>.25
females-Nagasaki	.05–.10	.05–.10

The figures in the columns refer to probability levels. The arrows indicate the direction of the difference between irradiated and control values. The arrow is directed upwards if there exists a continuing increase in the attribute or measurement under consideration as mean exposure increases, or downwards if the converse obtains. In the event the attribute or measurement bobbles, as it were, with increasing exposure, the direction of the arrow was determined by pooling exposure classes until a decision could be reached. The observations were weighted by the mean exposure of the class from which they were drawn. It will be at once apparent that most of the analyses have failed to reveal apparently significant relationships between indicator and parental radiation history. There are, however, a few specific points that merit discussion. In the order in which they appear in the table, the first finding at the level of significance is with respect to the frequency of malformation at age 9 months in relation to maternal exposure, in the 3×3 case. The downwards-directed arrow indicates a decrease in the frequency of malformation among the children of the more heavily irradiated, a finding which under most hypotheses would not be taken as evidence for increased mutation production. The second significant finding is an apparent increase in stillbirth frequency among the children of the more heavily irradiated mothers for the 4×4 case, but not for the 3×3 case. When, however, age and parity corrections are introduced, the apparent maternal exposure effect disappears for the 4×4 case. The third finding at the level of significance is neonatal death rate in relation to maternal exposure for the 3×3 case. Significance here stems largely from a rather striking depression of the death rate among the infants born to mothers in Exposure Class 3. There is, however, a slight increase in the death rate in Exposure Class 4–5. The fourth and fifth findings concern the generalized means of the anthropometric examinations conducted at age 9 months, in relation to both maternal and paternal exposure for the 4×4 case. The disappearance of these apparent effects for the 3×3 case raises questions concerning their validity. The sixth and final significant findings concerns birthweight variances among female infants born in Nagasaki, with parental exposure considered jointly for the 4×4 case. This effect is not borne out by the three other comparable analyses of birthweight variances.

In summary, then, there emerge from this analysis no really clear indications that the radiation history of the parents has affected the characteristics of their children here under consideration. It should in this connection be pointed out that 5 of the 6 findings which give some indications of significance involve the element of maternal exposure, a fact which in view of the possibility of maternal somatic effects suggest the need for particular caution in reaching conclusions. In order to avoid all possible misunderstandings we hasten to state that under no circumstances can this study be interpreted as indicating that there were no genetic consequences of the atomic bombings. The interpretation is simply that *conclusive* effects could not be demonstrated.

In a preliminary communication concerning this study (*Neel* et al. [1953]), it was reported that there appeared to be a significant relationship between sex and parental exposure history, but no other positive findings. This relationship does not appear in the present analysis, although the direction of deviation is still the same. Among possible reasons for the disparity the following should be considered: (1) the different (improved) classification of parental exposure employed in the present analysis, and (2) the accumulation of additional data.

Thus far in our analysis we have been concerned with attempts to demonstrate a positive effect of exposure to the atomic bombs on the indicators selected for study. There is, however, another aspect of these data. They permit us to place upper limits on the effects which may have been induced but not demonstrated by these studies. In other words, we can place confidence limits on our observations. The approach employed has been to compute the power functions for our several tests, having first taken several steps to simplify the statistical computations. The most important of these steps involves limiting the computation to those exposure cells with father's and mother's class 1 or 2 vs. those cells with father's and mother's class 3, 4, or 5. This step, by ignoring a portion of the data, has the effect of making our approach appear statistically less powerful than it really is. At any rate, on the basis of these computations, it can be stated that our data are adequate to give assurance at the 90 per cent level that we would be able to detect the following:

(i) a decrease in the sex ratio, following maternal exposure, in excess of an absolute change of 1.6 per cent;

(ii) an increase in the sex ratio, following paternal exposure, in excess of an absolute change of 4 per cent;

(iii) an alteration of the malformation rate in excess of two times the control value; and

(iv) an alteration of the stillbirth and neonatal death rates in excess of approximately 1.8 times the control value.

In concluding, we should like to be the first to recognize the unsatisfactory situation in which this study leaves us, with respect to drawing firm conclusions concerning radiation-induced genetic changes in human populations. There seems to be agreement at all levels that with the advent of the atomic age, one of the most pressing questions in the entire field of human biology, including medicine, concerns the genetic problems created by the exposure, for various reasons, of the human race to increasing amounts of high energy irradiation. The complexity of this problem is self-evident. The final evaluation on which a valid course of action can be based will depend on studies of many types, all carefully evaluated and cross-correlated. This study will have achieved its objective if in the ultimate synthesis it supplies one of the sets of observations to be taken into consideration.