In a study of births to related parents who had been exposed to radiation in the bombings of Hiroshima and Nagasaki no consistent effect on the frequency of malformed infants or perinatal deaths was demonstrable. However, this "negative" study helps to make more precise the area where the search for genetic differences must be carried on.

ATOMIC BOMB EXPOSURE AND THE PREGNANCIES OF BIOLOGICALLY RELATED PARENTS

A PROSPECTIVE STUDY OF THE GENETIC EFFECTS OF IONIZING RADIATION IN MAN

William J. Schull, Ph.D., and James V. Neel, M.D., Ph.D.

THE CONCEPTS and particularly the technics of modern epidemiology are surprisingly similar to those which the geneticist associates with what he terms population genetics. It is one of the purposes of this presentation to indicate this parallelism in approaches through a consideration of a study of the genetic effects of ionizing radiation in man. While the data to be reported have not previously appeared in the literature, we shall emphasize methodology here rather than data.

The prospects for genetic damage following exposure to ionizing radiations have been admirably summarized in the reports of the U. S. National Academy of Sciences-National Research Council (1956), the British Medical Research Council (1956), the World Health Organization (1957), and more recently, the United Nations Scientific Committee on the Effects of Radiation (1958). We shall assume that the substance of these reports is now a matter of common knowledge and, we shall, therefore, not review the experimental observations from which one argues that a genetic risk will accrue from the exposure of human beings to ionizing radiations. Assessment of this risk in man has been hampered by a lack of basic information regarding such variables as spontaneous rates of mutation, selection pressure, average overdominance of nominally recessive mutant genes, and others. Furthermore, and fortunately so, the number of human populations exposed to high or moderately high amounts of ionizing radiations are few. One such population, however, comprises the survivors of the atomic bombings of Hiroshima and Nagasaki. The bulk of the results of a comprehensive study of the effect of exposure to the atomic bombs on this population has been reported elsewhere.[1,2] In the major report, attention was confined to observations on pregnancies occurring to unrelated parents. During the same interval of time, namely, 1948-1953, observations were also obtained on some 5,163 registered pregnancies occurring

in Hiroshima and Nagasaki to parents who were related as first cousins, first cousins once removed, or second cousins. There are a number of reasons why the geneticist is inclined to view the latter pregnancies of related parents as potentially a more sensitive index of radiation-induced genetic damage. In its simplest terms, the main line of the argument is that the addition of radiation-induced mutants to the more homozygous, and presumably less elastic, genetic background of inbred children may produce a relatively greater effect than would be apparent if the same mutants were superimposed on the more heterozygous genetic background of noninbred children, that is, children born to unrelated parents. It seemed unwise, therefore, in the analysis of the data from Hiroshima and Nagasaki, to simply pool the present observations with those on noninbred children, particularly since the inbred children were not uniformly distributed over the various exposure classes to be described shortly. The present report serves then to complete the picture of the effects of parental exposure to the atomic bombing of these two cities.

Brief Description of the Program—In the years immediately following World War II, a ration system existed in Japan which permitted pregnant women to acquire certain rationed items by registering the fact of their pregnancy some time after the fifth month of gestation. The economic milieu of Japan in this period was such that registration was virtually complete. There existed, then, a set of circumstances which afforded an opportunity to launch a comprehensive prospectively oriented study of pregnancy terminations following parental irradiation. With the cooperation of the municipal authorities in Hiroshima and Nagasaki, a system was instituted in 1948 whereby at the time of her registration for rations, each pregnant woman or her representative also registered with the Atomic Bomb Casualty Commission (ABCC), an agency of the National Academy of Sciences-National Research Council working in cooperation with the National Institute of Health of Japan. At the time of registration, the first two-thirds of a questionnaire was completed which included such items as identifying information, a brief radiation history of the husband and wife, a short summary of the past reproductive performances, and pertinent details concerning the present pregnancy. Upon the termination of the pregnancy, the attending midwife or physician notified the ABCC, and completed the previously mentioned questionnaire. Irrespective of the type of termination, a Japanese physician in the employ of the ABCC or the Japanese National Institute of Health called to examine the child as promptly after birth as possible. The completeness of this system of reporting and follow-up was checked periodically; these checks revealed that approximately 93 per cent of the births occurring in Hiroshima, and a somewhat higher figure in Nagasaki, were known to the commission. A large proportion of the 7 per cent not ascertained through this scheme subsequently came to our attention through other channels. The latter, unregistered births, while not included in the results to be reported, permit an appraisal of the representativeness of the registered births.

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 the examining physician. This questionnaire was designed to obtain more detailed information on the gynecologic history, maternal illness during pregnancy, past reproductive performance, and economic status. In addition to this questionnaire, blood was drawn from the mother for a serological test for syphilis (on the average, some 5 per cent of these tests were positive). The same supple-

mentary questionnaire was routinely completed on every registration where the terminal digit in the registration number was zero.

A more complete description of the program will be found in Neel and Schull.[1] Information bearing on the reliability of the consanguinity data, and the importance of certain socioeconomic concomitants on the data to be presented has been given elsewhere.[3]

Parental Radiation Exposure—Clearly to extract the maximum information from the situation we have described, an experimental design more sophisticated than the simple dichotomy, exposed-not-exposed, was needed. Available to estimate the exposure of a given individual was information on (1) his or her distance from ground zero at the time of the bombing, (2) the occurrence of epilation, gingivitis and petechiae, symptoms highly correlated with radiation sickness, and (3) the shielding he or she may have experienced. Available also was some knowledge regarding the neutron and gamma dose, in air, at specified distances from ground zero. The latter information was considerably less precise than could be desired, or than is frequently imagined. The distance-dosage relationships have been repeatedly revised in the intervening 13 years, and will most probably undergo further revision. This, then, was the background on which one had to decide how best to recognize varying degrees of radiation exposure.

In general, two solutions to the problem of estimating degree of exposure were available. On the one hand, one could attempt to assign a score to each exposed individual, this score being a function of distance, shielding, and symptomatology. If applicable, this approach would permit the use of somewhat more elegant procedures for estimating the dose-genetic effect relationships than could be used with the second alternative. It has the disadvantage, in practice, of requiring a value judgment in the assignment of almost every score, and as a consequence, the apparent precision in estimating the genetic effect may well be spurious. On the other hand, one could adopt a classificatory scheme. The principal advantage of the latter, perhaps, is that it is the more conservative of the two procedures, and less subject to dating by revisions in the basic distance-dosage relationships. The classificatory scheme evolved to take into account distance, shielding, and symptomatology was as follows:

1. Not present in Hiroshima or Nagasaki at the time of the bombings.

2. Present in one or the other of the two cities, but asymptomatic, and at a distance from ground zero (a) greater than 3,000 meters, or (b) 0-3,000 meters and heavily shielded, or (c) 1,500-3,000 meters and moderately shielded, or (d) 2,000-3,000 meters and lightly shielded.

3. Present but asymptomatic, and at a distance of (a) 2,000-3,000 meters and unshielded, or (b) 1,000-2,000 meters and lightly shielded, or (c) 0-1,000 meters and moderately shielded.

4. Present but asymptomatic, and at a distance of (a) less than 2,000 meters and unshielded, or (b) less than 1,000 meters and lightly shielded.

5. Present but less than 3,000 meters from ground zero, and reporting epilation a/o gingivitis, a/o petechiae.

The structures which were defined as affording heavy, moderate, or light shielding are given in Neel and Schull.[1, p. 44] From a variety of sources of evidence, it has been estimated that these five categories of exposure correspond to doses of approximately 0, 5-10, 40-80, 100-150, and 200-300 roentgens equivalent physical respectively.

The Data—Of the 5,163 registered pregnancies of related parents which were observed, 382 were rejected prior to analysis for a variety of reasons, among these reasons being (1) incomplete information on birth weight, birth rank, maternal age, or parental exposure, (2) relationship of uncertain degree, more remote than second cousins, or closer than first cous-

Table 1—The Distribution of Births to Parents Related as First Cousins, First Cousins Once Removed, or Second Cousins by City and Parental Exposure

a. Hiroshima

Father's Exposure	Mother's Exposure 1	2	3	4–5	Total
1	1,141	293	89	71	1,594
2	91	108	22	9	230
3	40	23	16	6	85
4–5	22	16	3	5	46
Total	1,294	440	130	91	1,955

b. Nagasaki

Father's Exposure	Mother's Exposure 1	2	3	4–5	Total
1	1,481	670	65	33	2,249
2	204	286	13	10	513
3	13	22	7	–	42
4–5	8	8	4	2	22
Total	1,706	986	89	45	2,826

ins, (3) induced pregnancy terminations, and (4) multiple births. Table 1 gives the distribution of the remaining 4,781 births to related parents by parental exposure and city. Because of the paucity of individuals falling into exposure categories 4 and 5, these individuals are combined with group 3 in all subsequent tables (the estimated mean exposure for the combined groups (3-5) is about 100 reps).

Ideally in the analysis of these data, the precise relationship of the parents would be taken into account by a procedure, say, such as regressing the variable in question on the coefficient of inbreeding within each of the various exposure cells, and then testing the homogeneity of the intercepts and regression coefficients so obtained. Unfortunately, the data are insufficient, particularly at the higher combined parental exposures, to make this approach feasible. A less sensitive procedure is to pool the various types of parental relationship within each of the exposure cells, and then contrast the pooled observations. The validity of this latter approach rests on several assumptions, the most important of which is that the average coefficient of inbreeding, as well as the variances of these coefficients, is the same in each of the exposure cells. Table 2 presents the weighted average coefficient of inbreeding for the nine exposure cells. It will be noted that these values range from 0.0391 to 0.0501, but in general, cluster fairly tightly around the mean over-all exposure cells, namely, 0.0459. The within exposure cell variances exhibit a comparable range of variation to that observed among the means. Thus, it would seem that the related marriages encountered in the exposure cells are sufficiently similar in type and frequency that to pool the observations on the various degrees of relationship within exposure cells would not lead to significant confounding of inbreeding effect with the effects due to parental exposure.

Information is available on the following variables presumably indicative of

Table 2—Mean Coefficient of Inbreeding Among the Offspring of Related Parents by Parental Exposure. The Numbers of Observations on Which These Means Rest Are Given in Parentheses

Father's Exposure	Mother's Exposure 1	2	3–5	Total
1	0.0457 (2,622)	0.0466 (963)	0.0451 (258)	0.0459 (3,843)
2	0.0453 (295)	0.0468 (394)	0.0391 (54)	0.0457 (743)
3–5	0.0444 (83)	0.0501 (69)	0.0421 (43)	0.0459 (195)
Total	0.0457 (3,000)	0.0468 (1,426)	0.0438 (355)	0.0459 (4,781)

genetic radiation damage, the sex ratio, the frequency of major congenital malformations, the frequency of stillbirths, the frequency of death in the first week of life among liveborn children, birth weight, and certain anthropometric data obtained approximately nine months post partum on a sample of those surviving to this age. The observations on the sex ratio have been presented elsewhere[2] as has the information on birth weight and the anthropometric measurements.[4] No significant differences among exposure cells in the birth weights (means or variances) or among the anthropometric measurements were demonstrable. The sex ratio data on the offspring of the related parents alone did not reveal a significant exposure effect. However, when taken in conjunction with the data presented on the unrelated parents, there emerged small, but consistent differences in the sex ratio compatible with the effects to be expected if sex-linked lethal genes were induced by the radiation. We present here the observations on the frequencies of major congenital anomalies and perinatal mortality (stillbirths plus deaths in the first week of life).

The specific malformations defined as major are tabulated in Neel and Schull[1]; in substance, any malformation which was incompatible with life, or, if compatible, seriously limited the function of the individual, was defined as a major malformation. The distribution by parental exposure and city of birth of children with major congenital malformations born to related parents is given in Table 3. In the analysis of these data, observations from Hiroshima and Nagasaki have been pooled to increase the numbers of entries in the exposure cells. This assumes that there is no heterogeneity between cities, an assumption which may not be strictly true. However, in an analysis of the effects of inbreeding on pregnancy termination in Japan based upon children born to parents who were either unexposed or lightly exposed (category 2). Schull[3] found no evidence of heterogeneity between Hiroshima and Nagasaki in the regression coefficients when the frequency of major congenital malformations was regressed on the coefficient of inbreeding F (including $F = 0$, that is, the offspring of unrelated parents). There was, however, evidence of heterogeneity in the regression of "early deaths" on the coefficient of inbreeding. The term "early death" does not cover a period of time equal to that encompassed by "perinatal death." The former includes death from the seventh day of life to the end of the first month. Since most deaths in the first month occur, in fact, in the first week, it is reasonable to assume that heterogeneity would also exist between cities with regard to perinatal mortality, and this can be shown to be true.

Analysis of the observations on major congenital anomalies by either the method of Roy and Kastenbaum[5] or regression technics failed to reveal a significant effect of maternal or paternal exposure on the frequency of major congenital malformations. The regression model used was of the following general form:

$$E(p_{ij}) = \bar{p} + b_1 (F_i - \bar{F}) + b_2 (M_j - \bar{M})$$

where $E(p_{ij})$ is the expected proportion of malformed infants in the ij^{th} exposure cell, $\bar{p}$ is the average proportion of malformed infants, b_1 and b_2 are regression coefficients, F_i and $\bar{F}$ (M_j and $\bar{M}$) are respectively the average exposure of fathers (mothers) in the i^{th}(j^{th}) exposure category and the mean paternal (maternal) exposure. In actual computation, a weighted regression was fitted using the arc sin transformation rather than the observed proportions. It must, of course, be borne in mind that the application of regression methods to these data involve an element of approximation. As might be surmised from inspection of Table 3, the regression co-

Table 3—The Distribution by City of Birth and Parental Exposure of Infants with Major Congenital Anomalies Born to Parents Who Were Biologically Related

a. Hiroshima

Father's Exposure			Mother's Exposure 1	2	3–5	Total
	1	N	1,141	293	160	1,594
		m	18	4	1	23
		p	0.0158	0.0137	0.0063	0.0144
	2	N	91	108	31	230
		m	0	1	0	1
		p	–	0.0093	–	0.0043
	3–5	N	62	39	30	131
		m	1	0	0	1
		p	0.0161	–	–	0.0076
Total		N	1,294	440	221	1,955
		m	19	5	1	25
		p	0.0147	0.0014	0.0045	0.0128

b. Nagasaki

Father's Exposure			Mother's Exposure 1	2	3–5	Total
	1	N	1,481	670	98	2,249
		m	25	7	1	33
		p	0.0169	0.0104	0.0102	0.0147
	2	N	204	286	23	513
		m	3	4	0	7
		p	0.0147	0.0140	–	0.0136
	3–5	N	21	30	13	64
		m	0	0	1	1
		p	–	–	0.0769	0.0156
Total		N	1,706	986	134	2,826
		m	28	11	2	41
		p	0.0164	0.0112	0.0149	0.0145

efficients are both negative in sign although neither is significantly different from zero. The surprise with which one greets this failure to demonstrate significant differences among the exposure groups will be in part a function of whether one views natural selection as primarily favoring homozygotes or heterozygotes. At the present time such is the paucity of data on man that one can defend either of these alternatives with equal vigor. In this connection,

Neel[6] has recently presented evidence which can be interpreted as suggesting that some congenital malformations in man may represent the phenodeviants from balanced homeostatic systems. There may, then, be no contradiction between the apparent effect of parental exposure on the sex ratio, and the failure to observe an effect of parental exposure on the frequency of congenital defects.

Table 4 presents the distribution, by city of birth and parental exposure, of

Table 4—The Distribution by City of Birth and Parental Exposure of Infants Dying in the Perinatal Period Born to Parents Who Were Biologically Related

a. Hiroshima

			Mother's Exposure 1	2	3–5	Total
Father's Exposure	1	N	1,123	289	159	1,571
		d	48	15	4	67
		p	0.0427	0.0519	0.0252	0.0426
	2	N	91	107	31	229
		d	4	3	4	11
		p	0.0440	0.0280	0.1290	0.0480
	3–5	N	61	39	30	130
		d	2	8	2	12
		p	0.0328	0.2051	0.0667	0.0923
Total		N	1,275	435	220	1,930
		d	54	26	10	90
		p	0.0424	0.0598	0.0455	0.0466

b. Nagasaki

			Mother's Exposure 1	2	3–5	Total
Father's Exposure	1	N	1,456	663	97	2,216
		d	41	22	1	64
		p	0.0282	0.0332	0.0103	0.0289
	2	N	201	282	23	506
		d	8	3	0	11
		p	0.0398	0.0106	–	0.0217
	3–5	N	21	30	12	63
		d	0	1	0	1
		p	–	0.0333	–	0.0159
Total		N	1,678	975	132	2,785
		d	49	26	1	76
		p	0.0292	0.0267	0.0076	0.0273

infants dying in the perinatal period. It is worth emphasizing that perinatal deaths include those pregnancies terminating after 21 weeks of gestation in a stillborn infant or a child failing to survive the first week of life, and where the child in question did not have a visible, gross abnormality. The elimination of stillborn children or those dying in the first seven days of life with known abnormality precludes the possibility of measuring any genetic damage twice, a situation which might otherwise prevail in view of the high probability that a grossly deformed child will be either stillborn or succumb in the first week of life.

On inspection, the most striking aspect of Table 4 is the apparent heterogeneity between cities. One not only observes a fairly marked difference in perinatal death rates in the two cities, but the increase in mortality with parental exposure which seems to occur in Hiroshima is countered by a decreasing mortality with increasing exposure in Nagasaki. Analysis of these data readily confirms that the heterogeneity is significant. When these data are explored further, one finds that the differences between the nine exposure groups in Nagasaki are not significant whereas in Hiroshima the groups are significantly different. However, when the model previously described is fitted to the Hiroshima data, one finds that though the regression removes a significant amount of variation the variation not removed by the regression is also significantly large. In short, the linear model would appear inadequate to account for more than a small fraction of the observed variation. Interesting too, is the finding that on the linear model the regression coefficients associated with mother's exposure and father's exposure differ in sign. In most circles, the latter would not be considered consistent with our present knowledge of genetic damage following parental radiation. It must be borne in mind that these seemingly anomalous findings may be due to confounded concomitant variation. Unfortunately, such is the paucity of data that it seems unlikely that uncontrolled concomitant variation can be satisfactorily removed. To briefly summarize the findings, there is no demonstrable, consistent effect of parental exposure on the frequency of malformed infants or perinatal deaths among the children born to related parents.

Before we examine the conclusions which seem justified from these data, permit us to restate, for comparative purposes, the findings with reference to the frequency of congenital malformations and of perinatal mortality obtained from the study of offspring of unrelated parents. These were as follows[1]: "Analysis of the frequency of malformed infants by city and parental exposure reveals no significant, consistent effect of parental exposure." "Analysis of the stillbirth data fails to reveal significant differences between cities or consistent significant effects of parental exposure." Finally, "No consistent, significant effect of parental exposure on neonatal mortality emerges from the data obtained in Hiroshima and Nagasaki on deaths occurring in the first six days post partum." The present findings are not, then, at variance with those previously reported for pregnancy terminations to unrelated parents.

What now are the conclusions which we can draw from these data? Can we assert that no genetic damage has accrued from exposure of the populations of Hiroshima and Nagasaki to ionizing radiations? The answer to this question is "no" since it can easily be shown that, with the present numbers, the differences which would have to obtain among exposure groups for significance to result are considerably larger than those expected on a genetic basis. This body of data does not, then, afford an adequate test of the genetic hypothesis. In much the same fashion, one is led to the con-

clusion that these data do not afford a very sharp test of the basic premise that inbred children because of their increased homozygosity are a more sensitive indicator of radiation induced genetic damage than noninbred children since a several-fold difference in response between these groups could go undetected. Must we conclude. then. that these data contribute little. if anything to our understanding of the genetic hazards of ionizing radiation in man? Fortunately no; in point of fact. each "negative" study serves to further isolate the "critical range," that is. that area wherein we must search for genetic differences. A corollary of this would be that each "negative" study further refines the experimental design from which we may ultimately answer our basic problem. Advances in this area of human biology, important and as necessary as they are, will not come easily nor inexpensively, but they will come. Unhappily, however. we are called upon even now to make decisions regarding "permissible doses." These decisions can affect not only the practice of medicine in this country but our national safety as well. Our thinking on these matters is not aided either by those who with head in sand deny the existence of a danger nor those who see in each new increment of fallout x new monsters. Wisdom would certainly dictate that in so far as is consistent with national defense and the sagacious practice of medicine we should keep at a minimum the exposures to which we are subject.

While it may seem that in these closing comments we have strayed somewhat afield from our objective of indicating the similarity of approaches in epidemiology and in population genetics, this is really not so. In both areas, we are frequently confronted with problems of such moment that objectivity is difficult to attain. Yet only in an atmosphere of impartiality can we hope to sort fact from fancy, be this with reference to smoking and lung cancer, or ionizing radiation and our "load of mutations."

REFERENCES

1. Neel, James V., and Schull, William J. The Effect of Exposure to the Atomic Bombs on Pregnancy Termination in Hiroshima and Nagasaki. National Academy of Sciences-National Research Council Publ. 461, 1956.
2. Schull, William J., and Neel, James V. Radiation and the Sex Ratio in Man. Science 128:343 (Aug.), 1958.
3. Schull, William J. Empirical Risks in Consanguineous Marriages: Sex Ratio, Malformation, and Viability. Am. J. Human Genet. 10:294 (Sept.), 1958.
4. Morton, Newton E. Empirical Risks in Consanguineous Marriages: Birth Weight, Gestation Time, and Measurements of Infants. Ibid. 10:344 (Sept.), 1958.
5. Roy, S. N., and Kastenbaum, Marvin A. A Generalization of Analysis of Variance and Multivariate Analysis to Data Based on Frequencies in Qualitative Categories or Clan Intervals. Ann. Math. Statist. 27:749 (Sept.), 1956.
6. Neel, James V. A Study of Major Congenital Defects in Japanese Infants. Am. J. Human Genet. 10:398 (Dec.), 1958.

The authors are associated with the Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Mich.

This paper was presented before a Joint Session of the Epidemiology and Maternal and Child Health Sections of the American Public Health Association at the Eighty-Sixth Annual Meeting in St. Louis, Mo., October 29, 1958.

This work was sponsored by the Atomic Bomb Casualty Commission, Field Agency of the National Academy of Sciences-National Research Council of the United States and the National Institute of Health of Japan. Analysis of these data was sponsored by the Atomic Energy Commission under a grant to the University of Michigan (Contract AT (11-1) -405).