Genetic Effects*

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HUMAN populations are being exposed to increased amounts of high-energy radiation. How much will this exposure affect the genetic constitution? This is the question we would like to be able to answer.

Since this symposium deals with low levels of radiation, the next question is: Are the differences between the genetic effects of low levels and of high levels of radiation differences of kind or differences of degree?

A committee on the genetic effects of atomic radiation (National Academy of Sciences, 1956) has recommended that an average total exposure of no more than 10 r of additional radiation from all man-made sources be permitted to the reproductive cells of members of the general population during the first thirty years of life; and that no more than a total of 50 r be permitted for individuals within the population up to age 30 and no more than an additional 50 r up to age 40. These total amounts—10 for the average, 50 for the individual up to age 30—are obviously somewhat arbitrary. Nonetheless, they will serve as an operating definition of the line of demarcation between low and high total doses of radiation.

In the last two decades, the official "permissible" dose for whole body radiation has been successively lowered. The most recent tolerance levels for atomic energy workers accepted by

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the United States Atomic Energy Commission, announced on December 11, 1957, are based upon the most recent recommendations of the National Committee on Radiation Protection announced on February 15, 1957. Under these new levels, an atomic worker beyond 18 years of age may receive 5 rems of radiation a year on the average with a maximum of 15 rems in any one year. At no time should the accumulated dose exceed five times the worker's age above 18 years. In previous definitions of the permissible dose, no limits were imposed on the total accumulated dose.

What I am going to say can be summarized as follows. All levels of man-made radiation, high or low, should be avoided, if possible, in order to minimize or avoid the genetic hazards of radiation. On the other hand, we must continually try to weigh the costs of using high-energy radiation against the costs of not using it. This is not an easy task because we do not know enough about the quantitative effects of radiation, particularly at low levels, to estimate the genetic cost with precision. It may be much more important, in our present status, to avoid the two extremes of reaction.

We should avoid the extreme of saying that, since radiation should be avoided wherever possible, we should at once abandon the use of high-energy radiation everywhere. This course is unrealistic because it would deny us the opportunity of exploiting vast stores of energy for the use of mankind. It would also require setting back the clock, since we have already started the exploitation. We should also avoid the other extreme of saying that, since we do not know precisely what is the genetic risk of additional low levels of radiation and are not likely to have provable cases of damage to the human genetic makeup by radiation, we should exert no restraint whatever on the use of high-energy radiation.

Instead, we should continue the policy of "use with caution." The policy should apply to low levels as well as to high levels of radiation. The value of this policy may be judged by considering

some of the results of experiments on the genetic effects of radiation.

It must be clear that most of the assertions about the genetic effects of radiation are based upon experiments with animals. There are two reasons for this: (1) genetic experiments on man are impossible in our culture, and (2) no species is fully capable of studying its own genetics experimentally. Extrapolating from animals to man is at best going to be difficult, at worst impossible. The results of animal experiments will be valuable, both quantitatively and in principle, only if hard-won corroborating evidence can be found in human populations.

Also, we must distinguish between two different effects of radiation: the somatic effects and the genetic effects. When we refer to the effects of radiation on the exposed individuals themselves —whether on their blood, their eyes, their skin or their life span— we are speaking of somatic effects. Or to put it in another way, we are speaking about the effects of radiation on the physiology of *this generation*. On the other hand, when we refer to the effects of radiation on the descendants of exposed individuals—offspring conceived after the exposure—we are speaking about genetic effects. Or we are speaking about the physiology of the *next and later generations*.

WHAT WE NEED TO KNOW

In order to define what we need to know, we must first agree on what we know now. There should be relatively little quarrel with the assertions that:

1. All biological structures and functions are consequences of the interplay of heredity and environment.
2. The principles of mendelian genetics and of statistical genetics apply to man as well as to other organisms.
3. The hereditary material appears to be organized as many pairs of discrete particles, called genes.
4. The gametes which unite in fertilization are the sole transmitters of genes from one generation to the next.

5. Gametes are produced by pre-existing gonial cells in both males and females, and contain one-half of the hereditary material of gonial cells.
6. Genes normally reproduce exact copies of themselves generation after generation.
7. Once in a while, a change in a gene or an error in reproduction occurs; the new gene is called a mutant.
8. High-energy radiation is one of several agents which induce mutations of genes.
9. To be of importance in the reproduction of offspring, the radiation must strike the gonial cells, or any of the cells produced by gonial cells on the way to producing gametes.
10. New identifiable gene mutations, whether spontaneous or induced, are almost always detrimental to the offspring which receive them in reproduction. In the case of recessive mutations, the effect may not be observed until the daughter mutant gene is received in a double dose, that is, from both parents in a later generation.

Taking these ten statements as a starting point, we may raise a series of questions about the genetic effects of radiation. These are questions which can be answered only by means of experimental or observational data.

1. What is the relationship between the dose of radiation and the gene mutation rate? Or what is the probability for a gene to mutate under a given radiation dose?

2. Is there a threshold? That is, is there such a radiation dose that the probability of mutation is zero for lower doses? Is there a safe dose?

3. Is there any genetic difference in applying a given dose all at once or in applying it continuously over a period of time or in applying the same total dose in several smaller doses at intervals of time? Is there a safe rate for accumulating doses?

4. Do mutated genes revert to their original state? If so, with what frequency and after what length of time?

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5. What kinds of effects do the mutant genes have upon the individuals who receive them in later generations?

6. What are the relative effects of the mutant genes in single and double dose, that is, in heterozygotes versus homozygotes?

7. What kinds of effects of mutant genes may be regarded as socially less important and what kinds as socially more important?

8. In the case of quantitative and continuous traits, what will be the magnitude of change in such traits and in what direction —up or down, advantageous or disadvantageous—as a consequence of radiation exposures?

9. Will the radiation-induced mutant genes, if detrimental in effect, tend to persist in a population and, if so, for how long?

10. Since the human population is an outbreeding population with social taboos against marriages of close relatives, what will be the effects of this breeding structure of the population on the manifestation and persistence of mutant genes?

11. Many, if not all, species of animals, including man, living in nature are genetically heterogeneous. Nature appears to favor, in some cases, a persisting polymorphism. What will be the effect of adding new mutant genes to the gene pool of the population? Will they come to expression in unfavorable or deleterious ways? Or will they be sorted over, so to speak, and in combination with genes already existing in the gene pool give rise to new adaptive combinations?

12. How much radiation is necessary to lead to the extinction of a population in a genetic sense; that is, how much radiation is needed to impair the existing genes and the reproductive mechanism so that, several generations after the removal of radiation, the species becomes incapable of reproducing itself?

To discuss all these questions is impossible. Instead I have picked out five of them as most pertinent to the question of the genetic effects of low levels of irradiation. These are the questions about (1) a threshold, (2) cumulative effects, (3) homozygotes and heterozygotes, (4) breeding structure, and (5) quantitative traits.

THRESHOLD

The question of the existence of a threshold may be the most important question in dealing with the genetic effects of low levels of irradiation: Is there a "safe" dose of radiation? Is there such a dose that it and lower doses do not produce gene mutations?

The evidence on this question is far from conclusive. It is necessary to argue from a combination of limited evidence and of some theory about the genetic effects of ionizing radiation.

Spencer and Stern (1948) found that the mutation rate in *Drosophila* spermatozoa was linear with dose, and increased fairly steadily over a range from 25 r to 4000 r. These results are compatible with the theory that mutations are single-hit events (Boag, 1951). Evidence from single doses lower than 25 r is not available now, but may be in the future. Experiments at doses even as low as 25 r are exceedingly difficult because of the extremely large numbers of offspring which must be produced in order to observe the rare mutants.

If the single-hit theory holds at low doses—and there is no reason today to believe otherwise—we can estimate the mutation rate at 1 r or 10 r by using rates computed from results of experiments with larger doses. Extrapolation beyond the range of direct experience is always hazardous, but it is the best that can be done now. By means of such an extrapolation, Russell (1951) found that the radiation-induced mutation rate of mouse genes is about 25×10^{-8} per roentgen per locus in spermatogonia. Put otherwise, this is the same as saying that 1 r of radiation increases the probability that an average gene will mutate by 250 chances in a billion over its spontaneous rate. Ten roentgens would be expected to increase the probability to 2500 chances in a billion. Translating mutation rates into numbers and kinds of abnormal human offspring requires so many assumptions as to make the translation almost worthless. It can be predicted, however, that each increment in the mutation rate will produce a corresponding increment in the proportion of defective births. At

present about 2% of human births are defective for genetic reasons. A small increase in the radiation level, say by 10 r up to age 30, may be expected to add a fraction of 1% to the proportion of defective births in later generations.

It appears, therefore, that there is no totally safe dose of radiation. Lower doses merely produce fewer mutations than higher doses. The decision to accept a given dose of radiation as tolerable from a genetic point of view must, therefore, be reached by weighing the probable genetic effects against the advantages to be gained by tolerating such a dose. It cannot be based upon the view that there is a nonmutagenic dose.

CUMULATIVE EFFECTS

Instead of delivering radiation all at once in a short interval of time, the dose may be given in small amounts over a longer period of time. This poses the question of whether radiation has or does not have cumulative genetic effects, or whether reproductive tissue, like the germinal layer of skin, undergoes healing and repair.

Doses as low as 2.5 r of gamma radiation of *Drosophila* sperm, when repeated over 21 days to give a total of 52.5 r, were found to induce about the same frequency of sex-linked lethal mutations as 50 r given at once (Caspari and Stern, 1948; Uphoff and Stern, 1949). In this sense, fractional doses of radiation are genetically equal to total doses.

In addition to inducing mutations in gametes, radiation may induce mutations in gonial cells—those cells which produce gametes, as well as reproducing themselves. These mutations in gonial cells may be expected to be propagated into one-half of the gametes produced by the irradiated and changed gonial cell. The evidence for the assertion that mutations occur in gonial cells is that, following acute radiation, the rate of retrieval of mutant-bearing offspring does not decline with advancing age (Russell, 1951). This being the case, it is to be expected that the total mutational load will be proportional to the total radiation dose received up to the time of reproduction.

From a genetic point of view, therefore, it is the total accumulated dose prior to reproduction which is important, assuming that the dose is small enough not to impair the physiology of the exposed individual. It appears to make no difference whether the dose is received all at once or whether it is spread out in small amounts over a longer period of time. The mutated genes in the gonial cells do not heal or repair themselves, nor revert to their previous nonmutant state. There is a persisting effect of radiating the gonial cells. Any offspring produced by the exposed individual at any time later in his life may receive a mutation which actually occurred many months earlier.*

HOMOZYGOTES AND HETEROZYGOTES

The taxonomy of genes with respect to location and effect presents a bewildering array of alternatives. For example, with respect to chromosomal location, genes may be autosomal or sex-linked. With respect to other genes, a gene may be allelic or nonallelic, and if nonallelic, it may be linked or not linked with the nonallele. With respect to relationships between allelic genes, there may be dominance, no dominance, or overdominance. Under certain circumstances, there may be reduced penetrance and variable expressivity. Or the resulting phenotype may be lethal, semilethal, viable, supervital, or subvital. Or the degree of effect may be major or minor, or a given gene may affect a discrete character (in which case it is an oligogene) or a continuous character (in which case it is a polygene). Some genes act early in life, others become manifest only very late in life. Some genes will express themselves under one combination of environmental conditions, but not under another.

It is quite impossible to weigh the social importance of induced

* Some data recently reported (Russell, Russell, and Kelly, 1958) indicate that in the mouse, radiation at a low dose rate is much less effective in producing mutations than it is at a high dose rate. This may be attributed to the complexities of spermatogenesis in the higher animals, and it need not be taken as a refutation of the single-hit or no-threshold hypothesis.

gene mutations without considering these elements of gene taxonomy. A sex-linked recessive lethal mutation that kills an embryo during the first third of pregnancy and that has no discernible effects in heterozygotes would have no social consequences other than changing the probability of a male child from one-half to one-third in specific families in which the mother is a heterozygous carrier of the gene. On the other hand, an autosomal gene may be regarded as socially undesirable if, when homozygous, it acts after the reproductive period by causing physical or mental impairment, but not death, and in heterozygotes has none of these effects, but instead endows the heterozygous carriers with a selective advantage which enables them to produce relatively more offspring.

I shall not attempt to discuss this aspect of the problem with complete generality. Rather, I will mention two studies which show the nature of the complications.

Stern and others (1952) compared the viabilities of females of *Drosophila melanogaster* carrying sex-linked lethals with the viabilities of females not carrying lethals. They did this for 75 different lethal genes. They found that, on the average, lethal genes of this type reduce the viability of the heterozygous carriers by about 4%. This study implies that lethal genes in single doses in heterozygotes may have a small but detectable dysgenic effect.

In a study of a human population in East Africa, Allison (1954) found that the heterozygous carriers of a gene which causes sickle-cell anemia in homozygotes are apparently more resistant to malaria than noncarriers. While there is no reasonable doubt about the fact of this association between malaria resistance and heterozygosity for the sickle-cell gene, it is too soon to say exactly what causes the association.

Add to these the cases of maternal-fetal incompatibility, due to the Rh genes, the association of blood group O with duodenal ulcers, and others from the growing literature on the diverse effects of genes in single and double dose. By doing so we may

appreciate the difficulty of predicting what effects new mutant genes may have upon the biological properties of the human population.

BREEDING STRUCTURE

The detection of new mutations is easy or difficult, depending upon whether the mutant gene affects a readily observed trait or not, and upon whether the effect is dominant (that is, detectable in a single dose of the gene) or recessive (that is, detectable only in a double dose of the gene). In laboratory experiments, schemes have been devised which, with varying degrees of efficiency, may be used to detect recessive mutations. All the schemes require matings of specific kinds, and these may require the mating of close relatives.

In natural populations, including the human population, matings of close relatives are the exception rather than the rule. In outbreeding populations, the positive discovery and identification of recessive mutations can, at best, occur only long after the actual event of mutation. The consequence of outbreeding is to permit recessive mutations to be disseminated in the population through heterozygous carriers, irrespective of the selective advantage or disadvantage of the gene when homozygous. Chance alone may govern whether the mutant gene increases or decreases in frequency.

If the new mutant has a discernible effect only when homozygous, that fact will not be discovered until two daughter genes happen to come together in a remote descendant. The probability for two genes that are alike by descent to unite in fertilization increases with an increase in the degree of relationship between the mates. The probability is higher for first cousins and lower for more remote relatives. It is not surprising, therefore, to find in the literature of human genetics that the familial incidence of numerous rare hereditary diseases is higher when the parents are blood relatives and lower when they are not. Indeed this associa-

tion of incidence with degree of relationship is often taken as the basis for asserting that a given disease is caused by heredity.

The meaning of these statements for the problem of predicting the genetic effects of radiation at low or high levels is this: Until we know much more about the breeding structure of the human population, it will be exceedingly difficult to translate the results of laboratory experiments into human terms. There are, however, several recent studies which will assist immeasurably in assessing the genetic future of man (Cavelli-Sforza, 1956; Freire-Maia, 1957; Morton, Crow, and Muller, 1956; Muller, 1956).

QUANTITATIVE TRAITS

So far, we have been speaking about genes with relatively discrete effects and, in general, about traits which are either present or absent in a given individual. Turning our attention to quantitative or continuous traits requires us to consider a still greater array of complications. Not only is the genetic basis of quantitative characters less easy to define but also the interplay of selective advantages and disadvantages of combinations of quantitative traits is less easy to analyze. Further, vicissitudes of the environment appear to have relatively greater effects upon quantitative traits than upon discrete traits. Nonetheless, in the eventual assessment of the genetic consequences of irradiation at low levels, we shall be very interested in what may be expected to happen to the physiological fitness, reproductive capacity, disease resistance, longevity, work capacity, intelligence, and incidence of constitutional disorders in the descendants of an irradiated population.

We have seen a few of the reasons which have impeded the transfer of results of laboratory experiments on discrete characters to human populations. There is almost no chance of being able to predict the genetic effects of radiation on quantitative characters from laboratory experiments with discrete characters. The most obvious difficulty is that the mutation rates of named

genes, highly variable as they are, may not apply to more than a small segment of the vast array of unnamed genes with small quantitative effects.

There are two ways of approaching this problem. Each has merits, but each is also unsatisfactory in some respects. One way is to develop means of detecting genetic trends in human populations, with adequate attention to indexes of health and fitness, in order to discover effects of changes in levels of radiation. This will require more detailed recording of vital, ancestry, marriage, and reproduction statistics than is now common. Answers to important questions will not be found quickly. But this may be the only approach that in the end will satisfy our needs. A plan for doing this is now under trial in Canada (Newcombe, 1957). Similar plans might be developed and put into operation in other countries, including the United States, if we think that changes in the genetic constitution of the populations, reflected in measures of health and fitness, are worth detecting before they may have become irreversible.

The other way of approaching the problem is the empirical approach of trying to determine the effects of radiation on selected quantitative measures of fitness in laboratory populations of experimental animals. Such populations should be selected, if possible, for their similarity to small human populations; that is, the developmental and physiological patterns, the anatomy, the social habits, the breeding structure, the genetic variation, and the selection pressures, should all be as nearly like the human qualities as possible. This is a large order and cannot be achieved with any easy choice.

Several experiments are now in progress in various laboratories which approximate the needs in varying degrees. One of these is at the Jackson Memorial Laboratory. I shall describe its essential features.

We have arranged two groups of populations of mice. The two groups differ in their initial genetic makeup. One group is called "genetically homogeneous" since all mice in it are descended from

a single pair of mice in the 16th generation of inbreeding of the C57BL/10Gn strain. The other is called "genetically heterogeneous" because the mice in it are descended from the generation of double cross hybrids produced by matings of C3H/eBJax, DBA/2Jax, C57BL/6Jax, and BALB/cJax.

In each group, there are twelve populations which are defined by all the possible combinations of four levels of inbreeding with three levels of radiation. The theoretical rates of loss of heterozygosity defined by the four levels of inbreeding range from 8% per generation for double first cousins to less than 0.8% per generation for 16-fold cousins. The levels of radiation are 0, 50, and 100 r applied with body shielding to the gonads of the males in each generation. Each population is defined as consisting of 16 single pair matings in each generation. The offspring of each mating are harvested as they become of age, and the crop is set on shelves for storage until the next round of matings is made up. Meanwhile the males are irradiated and held for 7 weeks before mating. Mates are chosen at random, by using a table of random sampling numbers, but with the restrictions that matings are made within the populations as defined to control the level of inbreeding and that brother-sister matings are avoided so as to simulate the incest taboos of a human population.

The excess animals in any generation may be used for studies on selected traits, changes in which are thought to be of importance to detect if present. These include measures of reproductive performance (size, number, and spacing of litters), measures of life span, measures of activity, and measures of growth rate. These experiments are just now well underway and no data are available at this time.

SUMMARY

A great deal more is to be learned about the genetic effects of radiation. We need to know more about the radiation sensitivity of experimental animals and more about the array of effects produced by mutant genes. We need to know more about the effects

of radiation on model populations of animals, set up to test one or more hypotheses about the manifestation and perpetuation of hereditary variability. Perhaps most importantly, we need to devise adequate but not overwhelmingly expensive methods of assessing the genetic health of the present human populations of the world. Not until we do so can we detect changes in the population and, on the basis of theory and experimentation, predict what course the changes will take.

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