Genetic Danger of Small Radiation Doses for Man and Their Effect on the Heredity of Monkeys and Rodents

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1. Introduction

The problems of radiation genetics of man are exceptionally important. The genetic danger arising from radioactivity even on small dose levels, as shown by recent data,[1-3] appears to be serious if large masses of the earth's population are subjected to it. It is also obvious that, among the factors which increase the artificial radiation, the main attention is directed to experimentation with nuclear weapons, since this leads to a systematic increase in radiation on the earth, thus subjecting all mankind to its influence. The harmful effect of changes of genetic structures in embryonic cells manifests itself in the following generations, beginning with the first one and continuing through to very distant ones. Also, it is necessary to consider the effect of radiation on the genetic structures in somatic cells, whose number is enormous in each individual. In this case, the genetic changes may lead to the emergence of somatic effects in individuals directly subjected to radiation and, sometimes, for a long time after the exposure. Among such genetically specific somatic effects, the many malignant new growths (neoplasms) and, in particular, leukemia are extremely menacing.

The importance and complexity of the problem requires a careful quantitative analysis of the effect of small and other radiation doses on the heredity of man. However, the data obtained directly on man can be only haphazard in character. Therefore, to evaluate the genetic danger of radiation various species of mammals are used for experiment. In the United States of America, at Oak Ridge Atomic Energy Research Center,[4, 5] and in Great Britain, at Harwell center, enormous radiation-genetics research programs are conducted on mice. This research is of great significance, since it is possible to apply to mice the necessary genetic methods for the quantitative and qualitative study of the mutation process in relation to the doses, magnitude, and forms of ionizing radiation. The conclusions derived from this work usually are used for the calculation of the frequency of genetic changes in man arising from the effects of radiation. However, it remains unclear to what extent this sensitivity toward radiation is similar or different among various species of mammals, nor is it possible to judge the genetic danger of radiation in man from the data on mice. In consideration of these doubts, Arsen'eva and Tinyakov[8, 9] in 1956 (at the Radiation Genetics Laboratory, Biophysics Institute, Academy of Sciences USSR) began a study on radiation cytogenetics of the monkey as a form extremely near to man. Although the study did justify itself completely by the disclosure that monkeys are radiogenetically two to three times more sensitive than mice, it was still necessary to obtain direct data on the effects of small or other doses of radiation on the hereditary structure of man. This necessity was pointed out by the studies of Kerkis and others (at the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences USSR) who showed that in radiogenetic sensitivity, not only in closely related species of monkeys but also in individual lines of one and the same species, there can be notable differences.

Direct experiment on the action of radiation on the chromosomes of man was possible due to the success of cytology in mastering study methods of mitoses in cell cultures of human tissue. These studies were done abroad by Bender[10] and Puck.[11] However, quantitative data obtained by these authors were not extensive. The work of Dubinin, Kerkis, and Lebedeva (in press, carried out at the Institute of Cytology and Genetics, Siberian Branch, Academy of Sciences USSR) yielded extensive material on the action of small doses of radiation in the emergence of chromosome rearrangements in cell cultures of human embryonic tissue. More than that, the tissue cultures on application of the single cell-clone method, analogous to the one used in microbiology, proved to be suitable for the analysis of the rate of individual types of mutation. Thus, Lieberman showed by this method that the mutation rate of resistance against the antibiotic puromycin in a cell culture of human fibroblasts is equal to 4.10^{-6} in each cell generation. Even though the study data are as yet only the first steps in this exceptionally interesting and important field, they at once reactivated the problems of analyzing the influence of radiation on the heredity of man in general and the influence of small radiation doses in particular. Thanks to these studies, we can now propose the basic criterion of the quantitative aspect of the genetic danger of radiation in man and of the data necessary for the solution of problems in regard to the character of the influence of small doses, the magnitude of doses that double the rate of the natural mutation process, and also the more fundamental comparison between the radiation action on the heredity of man and that of other mammalian species.

2. Natural Mutation Process in Man

To evaluate the influence of radiation on the heredity of man, it is necessary first of all to arrange the data regarding the natural mutation process. Taking into consideration the relative constancy of the natural mutation rate, it is accepted at the present time to assume as one of the characteristics of radiogenetic sensitivity of organisms a radiation dose bringing about the same extent of hereditary variation as would a natural mutation process. Usually the unit used is the radiation dose which produces mutations at the rate of the natural mutation phenomenon (the "doubling dose"). Factual material and general considerations on the natural mutation process in man can be found in the works of many authors.[13-22] In the majority of cases, the evaluation of the natural mutation rate in man is carried out by studying the rate of emergence of dominant or sex-linked inheritable diseases and anomalies. The evaluation of recessive anomalies is much more difficult to carry out, since these show up only in the homozygous state, and to discover heterozygous carriers of such recessive mutations is difficult and not always possible.

A serious difficulty in the exact measurement of the natural mutation rate of human genes is the circumstance that a disease of the same outward manifestation can have been caused by the action of different, but according to their manifestation, analogous factors. Thus, for example, the existence of two sex-linked types of hemophilia is known,[20] whereby the introduction of human blood serum of one type may correct the defect of hemophilia in an individual having a different type of hemophilia. One of these types of hemo-

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philia is encountered four times more frequently than the other. Only 10–20 years ago, it was never taken into consideration that, as in the given case, upon the appearance of an outwardly similar disease, two different genes had mutated. This example shows that to determine the mutation rate it is necessary to use both clinical and genetic data, the former to differentiate between the various types of disease and the latter to judge on the inheritance of the specific defects. This is true also for such diseases as diabetes insipidus, which is a recessive, sex-linked disease. It has been shown that in a series of cases such anomaly can be cured by the introduction of hypophysis extract (pitressin) with a disease of which (hypophysis) the anomaly is possibly connected. In some affected individuals the introduction of pitressin did not influence their condition, since the primary defect was of a different nature. These data show that the material on the natural mutation rate in man must be treated critically. In particular, errors tending to increase the mutation rate are possible, due to summation of a series of genes that together give rise to the same disease. The rate of emergence of diseases which arise as a result of mutational appearances of dominant autosomal genes can serve as a better criterion for the calculation of the mutation rate. Nachtsheim[23] gathered voluminous material on the mutation rate of Pelger's anomaly, the peculiarity of which is the impediment of granulation of the neutrophilic nuclei. This characteristic is well expressed in heterozygotes. The notion about the mutation rate which leads to the appearance of both dominant and recessive characteristics may be distorted in cases when the heterozygotes have an increased viability, as happens with the heterozygotes in the gene which determines the crescent shape of erythrocytes in sickle-cell anemia. In this case, the heterozygocity increases immunity against malaria, due to which the organism proves to be more viable.

Serious difficulties in determining the true mutation rate in man appear when, outwardly, one and the same disease has in one case a hereditary and in the other case an environmental character. For the characterization of such a change, work on the determination of the mutation rate of the gene determining retinoblastoma is significant. Studies carried out in England and in the USA established that the mutation rate of this gene was 14 to $23 \cdot 10^{-6}$. However, in 1957, Vogel'[20] showed that a great number of cases of retinoblastoma were either phenocopies or were based on the appearance of somatic mutation. According to the data of Vogel', the true mutation rate of this gene in sex cells is equal to 4 to $7 \cdot 10^{-6}$.

The methods of indirect evaluation of the mutation rate of specific genes in man are associated with an analysis of genetic regularities characteristic of a population. On free crossbreeding within a population, a fixed, equilibrium relationship is established between the mutation rate and the negative mutation rate prevailing in that population. The rate of surviving mutation is dependent on the strength of influence of natural selection. Calculation of the relative viability of the mutants, which is determined by their ability to produce descendants, allows calculation of the concentration of a given mutant within a population based on the mutation rate of the given locus.

At the present time, regardless of the extensive literature,[24–32] we cannot establish precisely to any extent the true average natural mutation rate in man for individual genes. According to available data, the rate of dominant mutations is on the average lower than the rate of recessive mutations, and this is true of both sex-linked and autosomal mutations. However, it is obvious that this is not a true difference but connected with the methods of calculation of mutations. If we return to the comparison of the rate of dominant mutations then also their averages have great differences. Moreover, the data on most of the mutating loci—achondroplasia ($45 \cdot 10^{-6}$) and retinoblastoma ($23 \cdot 10^{-6}$) are just as doubtful, since it has been shown that in these cases the mutation of several loci giving outwardly similar diseases has not been taken into account. Probably the most precise are the determinations of the rate of such dominant mutations as tuberous sclerosis ($8 \cdot 10^{-6}$), aniridia ($5 \cdot 10^{-6}$), microphthalmia ($5 \cdot 10^{-5}$), precisely established clinical forms of retinoblastoma ($4 \cdot 10^{-6}$), and partial albinism. From these data it follows that, although the natural mutability in man, on the average, is notably higher than in mice and drosophilae, it is not of such a magnitude that tens and hundreds of mutations could arise from one gene for each million gametes. The true average natural mutation rate in man for one gene, taking into consideration necessary corrections, varies apparently from $1 \cdot 10^{-6}$ to $1 \cdot 10^{-5}$, constituting a value of approximately $5 \cdot 10^{-6}$, i.e., one mutation for 200 thousand gametes. This determination is largely arbitrary, of course, but at the present time it is the most probable, and we shall proceed from it to the further analysis of the problems of radiation genetics in man. It must be noted that, although the establishment of a true average for the mutability in man is extremely important, all data obtained so far, with all the significance they may have, are insufficient. Thus, Fritz-Niggli,[17] in contrast to the average mutation rate for each gene in man as accepted by us, assumes that it is equal to $2 \cdot 10^{-5}$. The solution of this problem comprises a great task in human genetics.

When the quantity $5 \cdot 10^{-5}$ is used as the average mutation rate for each gene, it must be stressed that there are great differences in mutability of the various genes. The category of the frequently mutating or the so-called mutable genes in drosophila, corn, purslane, and other organisms attracted attention for a long time. Moreover, it seemed that the phenomenon of mutability of genes was of purely theoretical interest. However, the recent works on Mediterranean anemia have shown that in man the phenomenon of a high mutability of genes can have a practical significance. The mutation rate of sickle-cell anemia proved to be exceptionally high, equal to $1 \cdot 10^{-2}$. The result of such an enormous mutational stress in the human population in those areas where this anomaly is encountered is that the population is continually being saturated with acutely negative hereditary changes. However, such high mutability so far is known only for the gene of sickle-cell anemia, which in the homozygous state leads to death in early childhood. Knowledge of the overall mutability in man has a great practical significance, since a number of hereditary diseases are the result of the mutation of genes associated with their appearance. According to Kemp's[34] résumé the sharply expressed hereditary diseases in man appear due to mutation of at least 500 different genes. Besides, many lethal and semilethal mutations not having a characteristic disease syndrome are not included in this number. Morton, Crow, and Muller,[35] analyzing the increased hereditarily determined disability and mortality among the descendants of marriages between relatives in various Western countries where the population has a stable character and where marriages between relatives of one or another degree take place frequently, showed that on the average each individual has three to five mutations which, although harmless in heterozygotes, show lethal or semilethal properties in the homozygous state.

The number of "genetic victims," i.e., people lost due to the influence of hereditary factors in the early stages of development or exhibiting various hereditary diseases at one or another age, is determined by a series of complicated processes which are brought about in the human population. The continuous mutation process supplies new mutational changes which, in the result of crossbreeding of individuals, combine and form new features and combinations of characteristics. Mutational changes that determine the characteristics harmful for the individual are discarded from the population, whereby the rate of their elimination depends on the character of their action and manifestation. The

dominant mutations which lead to the destruction of the organism before the onset of sexual maturity or impede the reproduction of the individual having the given genes are discarded first. The genes not sharply influencing propagation and viability or the recessive ones acting only in the homozygous state are eliminated at a considerably lower rate.

The number of known hereditary diseases of man at the present time is very great. Muller[36–38] thinks that any further increase in the rate of appearance of hereditary anomalies already known or the emergence of new ones will constitute a serious danger to the health of humanity. A direct study of the occurrence and frequency of emergence of new hereditary diseases in the human population has shown that the natural mutation process is responsible for the appearance of a great number of genetic victims. Unfortunately the possibilities of medical intervention to reduce the appearance of the majority of the hereditary anomalies are as yet limited. Therefore, humanity is forced to carry the "burden," and each generation has to yield its victims due to the action of causes that cannot be eliminated: the natural radiation background of the earth and the biochemical disturbances of the intracellular metabolism bringing about chemical changes in the chromosomes.

What is the extent of that injury which has been accumulated up to the present in the human race by the appearance of natural mutation? Kemp[34] points out that in Denmark approximately 3% of the population is born with serious inborn hereditary defects. Stevenson[39] indicates this to be 7% for Ireland. According to American data,[20] this figure in the USA is 3–4%. Thus, of the adult population now living in the USA, about 100 million children are born in each generation, of which about 2 million suffer a clearly expressed hereditary disease bringing about mental imperfections, epilepsy, neuro-musculatory defects, blood defects, and other anomalies. Considering the fact that for a series of diseases the determination of the role of hereditary factors is extremely difficult, it can be assumed that, on the average, about 4% of the children are born with acute hereditary defects.

A series of new works which have shown the significance of chromosome rearrangements and disturbances in a number of chromosomes associated with certain diseases in man should be noted. As was shown in 1959 by Lejeune and others and then in 1960 by Ford, Mongoloid idiocy is determined by the presence of an extra small chromosome (22nd) in the karyotype of the diseased individuals. Such diseased individuals proved to be trisomics in regard to this chromosome, and instead of the 46 chromosomes possessed by normal humans they have 47. The syndrome of Kleinfetter's disease appears in persons having an additional sex chromosome (type XXY), which was shown in 1959 by Jacobs and Strong. As was shown in 1959 by Ford and others, Turner's disease appears in persons having lost one sex chromosome (type XO). Marfan's disease, according to data by Puck and others in 1960, is associated with the exchange of the factors in a pair of chromosomes. Finally, Ford and others showed in 1959 that in a series of cases the spontaneous formation of cancerous neoplasms may be associated with a whole complex of chromosome rearrangements.

3. Mutations Brought about by the Action of Ionizing Radiation

All living organisms, including man, are subject to the action of ionizing radiation emitted by sources in the surrounding medium or inside the tissues of the organism in the form of radioactive isotopes. In man a substantial portion of the natural mutation is brought about by the influence of the natural radiation sources, and it is apparent that an increase in the ionizing radiation dose must lead to an increase in the individual mutation rate and possibly also to the appearance of new, previously unobserved mutations.

The study of radiation genetics using drosophila and then other organisms led to the basis of the existing principles on the nature of radiation action on heredity which state that isolated mutations of genes can arise as the result of the appearance of isolated ionizations or of clusters of them in the cell, out of which follows the recognition of the absence of a threshold dose bringing about mutations. Since mutations after their emergence have a stable character and due to autoreproduction of chromosomes are transmitted to all subsequent cell generations or are conserved in temporarily nonmultiplying cells, the result is that chronic irradiation with small doses has a cumulative character.

These principles of radiation genetics are exceptionally important; they show that in the action of radiation on the hereditary structure a genetically ineffective dose does not exist. No matter how small the radiation dose, it causes the appearance of some small amount of ionization in the cell which then leads to the appearance of a certain genetic effect. Relative to man, for whom radiation shows a harmful genetic effect, the result of the principles of the absence of a threshold of mutability and the cumulative consequences of the influence of small doses means that genetically harmless doses cannot exist. Any minimal dose brings about a hereditary effect. The extent of this effect in conformity with the law of proportionality of mutations to the dose will be correspondingly small. However, when not just individual persons or small groups of individuals, but the whole of humanity is subjected to the chronic influence of such radiation doses, the danger rises sharply.

However, it is necessary to point out that recognition of the absence of a threshold dose for the mutability until recently was not based on direct experiments. The conclusion on the absence of a threshold dose was made as an extrapolation of the linear relationship between the mutation rate and dose, whereby this same relationship was obtained by an analysis of the influence of doses from a few hundreds to 5–10 thousand r. Furthermore, in individual experiments with drosophila the influence of 25 to 50 r was studied. However, the influence of the very small dose range from fractions of a roentgen to approximately 20 r remained uninvestigated.

Therefore, new data on the mutagenous effect of doses in the range from 5 to 20 r are of interest.

Glembotskiy, Abeleva, and Lapkin (in press) studied the influence of gamma rays in doses from 5 to 20 r on drosophila melanogaster. Mainly investigated were the point mutations, including gene changes. It was established that gamma-ray irradiation of sperm and spermatids of drosophila with a dose of 5 r brings about the appearance of point mutations. Dividing the irradiation into doses of 5 r, each with $1\frac{1}{2}$-hr intervals between sessions, gave a cumulative effect. The number of mutations proved to be equal to the number of mutations caused by a single irradiation with 20 r.

The analysis of the action of equivalent small doses of fast neutrons showed that their genetic effect, even at a small dose level, is $1\frac{1}{2}$–2 times higher as compared with the action of gamma rays.

Dubinin, Kerkis, and Lebedeva (in press) studied the influence of x rays in doses of 5, 7, 10, 20, and 50 r on the chromosomes in cell cultures of human embryonic tissue. It was established that all doses, among them also 5 and 7 r, bring about the production of a chromosome rearrangement. That the small doses even in this case have a cumulative effect follows from the fact that in all the irradiations from 5 to 50 r the number of chromosome rearrangements for 1 r was one and the same and fluctuated around the value 0.24%.

M. A. Arsen'eva and G. G. Tinyakov (in press) studied the influence of x rays in doses of 10 r on embryonic cells of the monkey Macaca mulatta. A distinct effectiveness of this dose in the bringing about of chromosome rearrangement was established. Kerkis, Ronichevskaya, Rukavishnikov, and

Naumenko (in press) studied the influence of x rays in a dose of 4 and 0.5 r on the gonads and bone marrow of individual types of rodents. In a series of cases it was shown that the dose used brought about a definite amount of chromosome rearrangement in the nuclei of the tissue cells of these animals.

Thus, the analysis of the mutagenic effect of small doses in the range from 4 to 20 r showed conclusively that the conclusion made on the basis of the linear relationship between the mutations and the dose on the absence of a threshold dose for mutability was entirely justified. On the basis of further experiments, it will be possible to clarify the problem of smaller doses and the problems of cumulativeness under chronic irradiation with small doses.

The basic conclusion that, under the influence of radiation, there do not exist small, harmless doses for the heredity of man, and that any minimal dose brings about a corresponding hereditary, radiation-caused effect obtains a new confirmation in the experiments described.

As has already been shown on the basis of the relationship of mutation rate and dose the basic data on the action of radiation on the mutation process are drawn now from the studies carried out on various organisms (bacteria, plants, drosophila, mice). However, the direct data obtained on man are very scant. Extremely interesting facts are described by Turpin, Lejeune, and Rethore,[41] who examined children whose parents had been subjected to radiation therapy without their gonads having been protected. These authors gathered data on the correlation of the sex in children of the same parents before and after the irradiation of the father and the mother. It turned out that, among the children born before the irradiation of the fathers, the fraction of boys was 0.514. Among the descendants of irradiated fathers, this fraction was somewhat higher and equaled 0.555. Such a result corresponds to what had been expected, since the irradiated X-chromosome of the fathers is transmitted only to their daughters; the action of the harmful dominant mutations arising in them must decrease the number of girls and, at the expense of this, relatively increase the number of sons. The opposite result was obtained in descendants of irradiated women, where the fraction of boys among the children born was 0.463, i.e., was decreased in comparison with the control by 0.083. The deficiency of boys is interpreted as the result of the manifestation of lethal, sex-linked mutations, i.e., localized in the X-chromosome.

In the study of the relationship of sexes in the descendants, taking into consideration the time of birth of children after irradiation of the parents, the decrease in the number of girls with the increase in time elapsed after the irradiation is noted. The interest in all of these data consists in their pointing to a method for the direct determination of the genetic action of ionizing radiation on man; their statistical basis, however, is insufficient.

Voluminous, statistically reliable data, as indicated in the foregoing, were revealed in experiments on radiation genetics of mice whose embryonic cells proved to be 20–25 times more sensitive toward the action of ionizing radiation than those of drosophila.[4, 5, 7] Based on these materials and in contrast with the data on the natural mutation rate, the National Research Council on Radiation of the United States of America[40] and the Medical Research Council of Great Britain[42] came to the conclusion that the radiation dose doubling the mutation rate is 50 r. However, it remained unclear to what extent the data on the radiogenetic sensitivity obtained with mice precisely reflect the character of the influence of radiation on the heredity of man, since it has been shown that different forms of mammals can substantially differ in their radiostability.

Kerkis, Renichevskaya, Rukavishnikov, and Naumenko (in press) studied the genetic radiosensitivity of cells of different mammalian forms in regard to the influence of small radiation doses. In unirradiated guinea pigs the rate of naturally arising chromosome rearrangements in spermatogonia was equal to 1.05 ± 0.14%, but 24 hr after irradiation with a dose of 4 r it was 11.4 ± 1.1%. In rats the rearrangement rate of chromosomes in controls was equal to 1.1 ± 0.2%, but after irradiation with a dose of 4 r—3.4 ± 0.5%. In mice the chromosome mutation rate in controls was equal to 0.17 ± 0.06%, but after irradiation with 4 r—0.8 ± 0.3%. In rabbits the spontaneous chromosome rearrangement rate in spermatogonia equaled 0.8 ± 0.2%, but after irradiation with 4r—1.1 ± 0.1%. On conversion of the disease rate of the nuclear apparatus in the four forms studied in regard to the action of radiation doses of 1 r, the values were obtained as shown in Table 1.

Table 1 Number of chromosome rearrangements in nuclei of spermatogonia cells of various forms of rodents on irradiation with a dose of 1 r

Animal	Cells with chromosome rearrangement, %
Guinea pig	2.56
Rat	0.57
Mouse	0.16
Rabbit	0.07

Different forms of mammals thus have substantially differing radiogenetic sensitivity of chromosomes in spermatogonia. The same authors established a similar order in the degree of radiosensitivity in the chromosome rearrangement rate in nuclei of blood-forming cells of the bone marrow. According to data in the literature, the forms investigated are arranged in the same order by the magnitude of the lethal dose which brings about the destruction of the animals by radiation disease upon total irradiation.

Considering the possibility of marked differences in radiogenetic sensitivity of different forms of mammals and the difficulties arising in connection with this in a simple transfer of data obtained in experiments with mice to man, in 1956 scientists at the Laboratory of Radiation Genetics, Institute of Biophysics, Academy of Sciences USSR began a study with monkeys, owing to their nearness to man. Arsen'eva and Tinyakov[8, 9] carried out a comparative study of radiation sensitivity of the monkey Macaca mulatta and mice in the dose range from 10 to 600 r. The study of the rate of chromosome rearrangements and changes of histological character of testes of monkeys and mice in normal animals and after the action of ionizing radiation showed a higher cytogenetical radiosensitivity of the embryonic epithelium of monkeys in comparison with mice. From these data it follows that the radiosensitivity of monkeys exceeds that of mice two to three times. In Table 2 are the results of an analysis of embryonic cells of monkeys and mice carried out on the tenth day after irradiation according to the work of Tinyakov and Arsen'eva,[8, 9] and then 24 hr after irradiation according to the work of Dubinin, Arsen'eva, and Kalyayeva (in press). Taking into consideration the growth time of spermatocytes of the first order, one can assume that in both cases the first mitosis after the radiation action has been analyzed. The data in Table 2 show the similarity of the number of affected cell nuclei for 1 r after 24 hr and on the

Table 2 Number of chromosome rearrangements in the cell nuclei of spermatogonia of type B in the primary spermatocytes of mice and in the embryonic epithelium of monkeys for 1 r

	Cells with chromosome rearrangements, %	
	After 24 hr (dose 200 r)	After 10 days (doses of 10, 50, 100, 200 400, and 600 r)
Mouse	0.076	0.078
Monkey	0.122	0.114

tenth day of analysis. Radiosensitivity of the embryonic epithelium of monkeys is almost twice as high as that of mice.

The great radiosensitivity of the monkey gonads in comparison with mice is indicated by the histological pictures in the irradiated testes. In the testis tissues of monkeys, even after the action of a 10-r dose, one can observe characteristic changes, which is not true of the testis tissues of mice. Prolonged sterility in monkeys sets in after the action of a dose of 100 to 200 r. In mice, sterility begins after a dose of 400 r, and it ends much earlier. The discovery of the fact of the great cytogenetic radiosensitivity of monkeys had a serious significance. It made it possible to approach the evaluation of the influence of small doses on the heredity of man and to give new calculations of the radiation dose doubling the rate of appearance of natural mutations in a new way. Based on the data of radiation cytogenetics of the monkey and critical consideration of the data available in literature on the quantitative characteristics of the natural mutations in man, Dubinin[2] could substantiate that the quantity of energy of ionizing radiation doubling the rate of appearance of natural mutations in man could be as small as 10 r. Such an evaluation of the influence of small radiation doses on the heredity of man showed the serious danger for the heredity of man caused by an overall increase in radiation if this increase were to touch great population masses.

However, despite the importance of the work on the radiation cytogenetics of monkeys and the great significance of the studies on the radiation genetics of other forms of mammals, it was necessary to obtain direct data on the influence of small radiation doses on the hereditary structure of man. The rate of nuclear changes in human cells under the influence of radiation under tissue culture conditions was investigated by Bender[10] and Puck,[11] but the involved methods of calculation of chromosome ruptures in the metaphases made it impossible for these researchers to obtain sufficiently voluminous material in the quantitative sense. Data characterizing the influence of doses of 5, 7, 10, 25, and 50 r on the rearrangement rate of chromosomes in the nuclei of embryonic cells of man (in tissue cultures) were obtained by Dubinin, Kerkis, and Lebedeva (in press) and compiled in Table 3.

To establish the dependency of the chromosome rearrangement rate on the dose, it is necessary to disregard the influence of the natural mutation process. Changes caused, on the one hand, by small doses of ionizing radiation and, on the other hand, by the natural mutation process are independent occurrences, and, since these values are very small, their statistical error is great. This being taken into consideration, the components of the induced mutation (A) are calculated according to the formula ($A = \sqrt{B^2 - C^2}$) where B is the total value of both processes, i.e., the value observed in the experiment ($A + C$), different for different radiation doses, but C is the rate of the natural mutation process observed in the control. The calculation showed that the rate of chromosome rearrangement for 5 r is 0.85%; for 7 r, 1.47; for 10 r, 2.60; for 25 r, 7.50; and for 50 r, 13.00%. Conversion of the data of all the doses studied shows that the number of chromosome rearrangements caused by the action of the energy in 1 r is very close for all doses (Table 4).

In agreement with the forementioned work of Bender and Puck, the rate of chromosome ruptures in a human cell in a tissue culture upon the action of 100 r turned out to be equal to from 0.03 to 2. In our case the calculation was given not according to the number of ruptures, but according to the number of chromosome rearrangements. However, on small dose levels, the calculations of ruptures and rearrangements have basically a single-stroke formation mechanism. The difference lies only in the fact that the changes of the cell independently from the number of aberrations in our experiments served as units of measurement. According to Bender and Puck, for 100 r all 100% or 33% of cells were changed. For 1 r the number of changed cells was 1% and 0.33%, respectively. In our experiments for 1 r, 0.24% of changed cells were obtained. The somewhat greater number of rearrangements observed by Bender and Puck is because they analyzed the rearrangements in the metaphases where that part of the changes which escape observation in the anaphase stage is visible. However, this difference does not influence the calculation of the doubling dose, since in our case the number of radiation rearrangements and the number of natural mutations was equal to their analysis in the anaphase stage under identical conditions.

In the experiments described the structural mutations of chromosomes were studied; it is known, however, that this type of change makes up only a part of all mutation categories arising due to the action of radiation. Besides, mutations arise not only in embryonic but also in somatic cells. The malignant neoplasms and, particularly, leukemia owe their appearance to somatic effects which change the genetic constitution of the cell. Stewart and others[43] have shown that a dose in the order of 1 to 3 r after its action on a 7 to 8 month human fetus doubles the appearance of leukemia after birth. According to Lejeune and Turpin,[44] the action of ionizing radiation on the fetus during the first four months of pregnancy has a still more powerful effect in causing leukemia.

Data obtained in experiments with human tissue cultures have the significance of a principle. Although the precise determination of the doubling dose is the subject of future investigations, it is apparent from the new experimental facts obtained in these studies that the concept introduced by Dubinin,[2] which assumes 10 r as the radiation dose doubling the mutation rate in man, can be considered as basic in direct experiments. Moreover, it is possible that this value is less than 10 r. As shown in the experiments by Dubinin, Kerkis, and Lebedeva with a tissue culture of embryonic cells of man, the rate of the natural appearance of chromosome rearrangements was 1.42 ± 0.17%, but upon the action of doses from 5 to 50 r the rate of chromosome mutations was 0.24% per 1 r. Thus the doubling dose in this case was equal to 6 r.

The magnitude of the doubling dose in the case of the action of fast neutrons which are formed in atomic and hydrogen bomb explosions must be considered specially. At the present time it has been shown experimentally that neutrons have an increased genetic effectiveness in comparison with the action of rarely ionizing gamma and x rays. Generally the genetic effectiveness of fast neutrons is 10 times higher or more. One roentgen of neutrons according to their genetic

Table 3 Frequency of anaphase and telophase with chromosome rearrangements in the irradiation of fibroblasts of human embryo

Dose, r	Control	5	7	10	25	50
Number of normal anaphases and telophases	3526	2098	2838	3236	643	676
Number of anaphases and telophases with chromosome rearrangements	51	32	55	99	50	104
Total number of anaphases and telophases studied	3577	2130	2893	3335	693	780
Percent of anaphases with nuclear changes	1.42± 0.17	1.48± 0.26	1.92± 0.14	2.96± 0.30	7.2± 0.82	13.3± 1.21

effectiveness correspond to 10 r of x rays or gamma rays. It follows, therefore, that if the doubling dose of gamma or x rays for man is 10 r, then this dose of fast neutrons is about 1 r in all. If it is taken into account that various types of mutations (the dominant lethal, the recessive lethal, the translocation, etc.) require different doubling doses in the range from 7 to 80 r, then the dose of fast neutrons in this case will be equal to from 0.7 to 8 r. Thus the neutrons of atomic weapons and atomic energy have a very high genetic effectiveness. This circumstance should attract the most serious attention.

4. Inadmissibility of an Increase in the Radiation Background on the Earth

At the present time, in the medium surrounding man, numerous changes are taking place as the result of enormous infiltration of the most diversified technology into industry and way of life. One of the most important aspects of these changes is the increase in the ionizing radiation level. The experimental detonations of atom and hydrogen bombs have specially great significance in this respect, since the radioactive fallout from them is spread very far, finally settles on the whole earth, and leads to a substantial increase in the radiation level.

A responsible task is ahead for radiation genetics, namely, to give a scientific prognosis of the biological consequences which might be expected from the intensifying increase of the artificial radiation level in the future. Many articles have been devoted to this problem by foreign authors.[45–63, 65, 66] Unfortunately, this prognosis cannot yet be precise, since there are too few experimental data that would allow an objective and precise evaluation of possible damage to the heredity of man. However, we cannot disregard even an approximate evaluation which would allow us to find the right direction and to obtain some quantitative coefficients. The data of such a sample evaluation, considering the grandiose character of the recent changes in atomic energy, will have great significance. Also, the work in this field serves as a basis for more precise and more thorough prognoses in the future.

When evaluating the danger of ionizing radiation on the heredity of man, it is very important to take into consideration that the danger of the action of small doses will become threatening in case the increased background embraces sufficiently large groups of population, for example, the population of whole countries, continents, and the whole world. By a chronic influence of small radiation doses on individual people and on small population groups, the damage to heredity, of course, also takes place, but it cannot acquire any significant influence on the heredity of the generation as a whole due to the low absolute rate of appearance of mutations. Therefore, when evaluating phenomena associated with the use of atomic energy, attention should be directed primarily to the factors leading to changes in radioactivity in the medium enveloping the bulk of humanity. Such a factor at the present time is the testing of atomic and hydrogen weapons.

Repeated attempts to minimize the significance of the increase of the radiation level in the medium inhabited by man in the result of testing of thermonuclear weapons have been taking place abroad during recent years.[67–70] Attempts were made to bypass the problem that even a very small percent of people with hereditary damage in absolute numbers means a great quantity of people with hereditary disease. Recent official sources in the United States of America[40] indicate in their publications that the level of natural radiation in the Northern hemisphere of the earth is equal to 3 r per person; after a 30-year life period this will be increased by 1 r if the nuclear detonations are continued at the 1953–1955 level.

What is the implication of such an increase in the radioactive background if it should continue indefinitely? Under the condition that 10 r double the mutation rate, addition of 1 r will cause hereditary anomalies in 800 thousand people out of 200 million in each succeeding generation. For the total population of the earth, numbering 2.75 billion, the increase in the radioactivity background on the earth by 1 r will mean the appearance of about 10 million people affected by serious hereditary anomalies per generation. Of a serious significance is also the appearance of malignant neoplasms caused by radiation, including leukemia.[71–74] Thus, the increase by only a fraction of a roentgen in the radiation background in the medium enveloping man threatens humanity with the appearance of a great number of mutilated lives in future generations. Continuation of testing of nuclear weapons will lead to an increase in the increased radiation background and will ruin the heredity of a vast number of people.

Table 4 Chromosome rearrangement rate in the human cell nuclei in a tissue culture at different radiation doses converted to 1 r

Dose, r	Changed cells converted to 1 r, %
5	0.17
7	0.21
10	0.26
25	0.28
50	0.26
Average	0.24

All this points to the exceptional importance of the problems whose solutions are dealt by radiation genetics. Along with this, it should be stressed that a scientific solution of problems about the danger of ionizing radiation for the heredity of man is very complex. At the present time only the order of magnitude of this damage has been established. All the quantitative data given have a preliminary and approximating character. They by no means can pretend to play a role of exhaustive quantitative characteristics of the damaging effect of the ionizing radiation on the heredity of man. However, this does not lessen the significance of these data as a basis for a serious alarm about the genetic consequences to be expected in case of uncontrollable increase of radiation in the medium surrounding man.

With all the variations in the quantitative evaluation of the radiation-genetic effect, all of them show that the maximal reduction of radiation in the medium surrounding man is required. These evaluations are built on the assumption of the absence of a threshold dose and the nonexistence of some small, for the heredity of man, harmless radiation dose. However, it is necessary to have precise knowledge about the influence of radiation on the heredity of man without which it is impossible to evaluate reliably and definitely the danger of these changes for the heredity of man. The urgent necessity to conduct wide studies in this direction is quite evident. In this connection, the study of radiation genetics of mammals and particularly of monkeys, as well as the study of cytogenic changes taking place in irradiated human tissue cultures, is very important. To make this work successful, it is necessary to accumulate new facts concerning the nature and regularity of the natural and the induced mutation process in man, the analysis of the genetic structure of the population, and the clarification of the form of transmission from generation to generation of mutations on which the various hereditary diseases and other deviations from the norm in man depend.

However, the data in radiation genetics even today strongly substantiate the inadmissibility of a further continuation of atomic and hydrogen detonations. Experimental facts show that the effect of small radiation doses in the case of their influence on the whole of humanity harbors a threat to future generations and may be the reason for malignant neoplasms

in the generation that has been subjected to radiation. Testing of atomic and hydrogen weapons should be discontinued immediately and forever.

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Editor's Note

This paper was reviewed by Herman M. Slatis, Division of Biological and Medical Research, Argonne National Laboratory, Illinois. Dr. Slatis considers it a well-written summary of current American and Russian thought and research in a complex field. Some of the Russian work is in fields that are not well represented outside of the Soviet Union, and their research on small doses of radiation is of particular importance.

—Igor Jurkevich