EXPERIENTIA

Vol. XIII - Fasc. 6 Pag. 217–260 15. VI. 1957

Genetical Effects of Radiation and Chemicals

By CHARLOTTE AUERBACH, Edinburgh*

Editor's Note. The progress of nuclear physics and nuclear chemistry has led up to the present atomic age. Now the science of genetics has shown that the ionising rays which are set free in atom disintegration processes, are capable of changing the genes and thus leading to irreversible damage to the hereditary material. The two following contributions by well-known experts of radiation genetics and chemico-genetics stress implicitly the responsibilities which arise from the inseparable connection between the effects of atomic energy and genetics, as also between chemicals and genetics.

During the last few years civilized man has become radiation conscious. Most people realise that exposure to ionizing radiation—be it from atom-bomb fall out, from radioactive waste-products, from X-rays or any other source—is dangerous to the individual and his progeny; but about the nature and degree of this danger only few are in a position to form an opinion. It must be admitted that at present even the expert can do no more than determine the limits of probability within which various harmful effects of radiation may take place, but this does nevertheless eliminate the two extreme attitudes: exaggerated anxiety, usually based on misunderstood press articles or lectures, and facile optimism, caused by ignorance of the biological facts and principles involved. It is the aim of the present article to acquaint the reader with this biological background insofar as it relates to genetical damage, i.e. damage incurred by the gametes of the exposed individual and manifesting itself in the progeny. For this purpose, it will first be necessary to describe briefly the genetic material and its functioning.

The genetic material.—Ionizing radiations affect chemical substances, in particular organic molecules. If the effects are on the molecules of the genetic material, inherited changes are produced. In all higher organisms the genetic material is organised into microscopically visible structures, called chromosomes. These are present in the nucleus of every cell and become visible when the cell divides. Numbers and shapes of chromosomes are a fixed property of a given species; thus maize cells contain 20 chromosomes, mouse cells 40, etc. In a sexually produced organism the chromosomes consist of two sets, one of which is derived from the father, the other from the mother. Every chromosome in the paternal set is matched by a similar chromosome in the maternal set. The only exceptions are the sex-chromosomes which determine the sex of the individual. In many species these chromosomes form an unequal pair in one sex. In mammals, the female has an accurately matched pair of chromosomes; they are called X-chromosome. The male has only one X- chromosome. Its partner is a so-called Y-chromosome, which differs from the X in size and shape and carries very few genes.

The most remarkable property of the chromosomes is their capacity for replication. In between any two cell divisions, an exact replica of each chromosome is laid down close to the original; when division takes place, original and copy separate and go into different cells. As a result, all cells which arise by division from the same ancestral cell contain the same genetic material. There is one important exception to this rule: in the divisions which precede the formation of gametes, partner chromosomes pair and the two members of a pair go into different cells, so that the gametes contain only one set of chromosomes.

Breeding experiments, corroborated by cytological findings, have firmly established that each chromosome is differentiated lengthwise into many minute segments—called genes—which are characterized by their various effects on development. Genes, interacting with environment, determine the multitude of traits which go into the making of an individual: its outward appearance, its physiological functions, its disease resistance, its ability to perform biosyntheses, its behaviour and mental endowment.

Allelomorphs.—Many or perhaps all genes within a species exist in a variety of forms, called allelomorphs. Allelomorphs occupy corresponding loci on corresponding chromosomes. They also influence the same developmental process, but they do so in ways that may differ qualitatively or quantitatively. A series of allelomorphic genes underlies the differentiation of mankind into the four bloodgroups of the ABO system; black and red cats differ in the possession of one or the other allelomorph of a gene concerned with the formation of coat colour; small and large strains within a species carry different allelomorphs, often of a number of genes determining growth rates. When a gene produ-

* Institute of Animal Genetics, Edinburgh.

ces an abnormality of, say, the skeleton, we infer the existence of a normal allelomorph which is concerned with the normal development of, in this instance, the skeleton.

As the chromosomes are present in pairs, so are the genes which they carry. Every sexually produced individual has a duplicate outfit of genes, one set of paternal, the other of maternal origin. An exception is formed by those genes in the X-chromosome for which the Y carries no allelomorphs. These genes are called sex-linked, and the XY sex has only one set of them. In the domestic cat, the allelomorphic genes for black or red coat colour are sex-linked. For a tomcat there are only two possibilities in regard to these genes: he may have a 'red' gene and a red coat, or a 'black' gene and a black coat. A female cat has three possibilities: she may have two 'black' genes and be a black cat; she may have two 'red' genes and be a red cat; she may also have one 'red' and one 'black' gene, in which case she is a tortoiseshell. Black and red females are said to be *homozygous* for the 'black' or 'red' allelomorphs respectively; a tortoiseshell female is said to be *heterozygous* for the 'black' and 'red' allelomorphs. It will be seen that a mammalian male cannot be heterozygous for a sex-linked gene, although—just like a female—he may be homozygous or heterozygous for any other gene.

Heterozygosity does not usually result in individuals which, like the tortoiseshell cat, display a mosaic of the two contrasting gene effects. In fact, this is a rare situation. Often the heterozygote is intermediate in appearance between the homozygotes: a mulatto is heterozygous for two or more pairs of allelomorphs which in his parents determine black and white skin colour respectively. There are also many cases in which only one allelomorph produces a noticeable effect in the heterozygote. The gene for albinism interferes with the formation of pigment in skin, hair and iris; its normal allelomorph controls a biochemical process which is essential for pigment formation. Mice or rabbits which are homozygous for the albino gene have white fur and red eyes; heterozygotes are indistinguishable from coloured homozygotes for the normal allelomorph. In such cases, we call the allelomorph which is effective in the heterozygote *dominant* over the other, *recessive* allelomorph. Many harmful recessives may be carried in heterozygous condition by perfectly health individuals. Congenital deafmutism is often due to a recessive gene. In families in which such a gene occurs some individuals are deaf because they are homozygous for it; many more are outwardly normal heterozygotes carrying the gene for deafness together with its normal allelomorph. When these persons produce gametes, the two partner genes separate and half the gametes contain the gene for deafness. When two heterozygotes marry, some of the children receive the harmful recessive from both parents and are deaf. The chance that marriage partners may be heterozygous for the same kind of harmful gene is higher for relatives than for unrelated persons; for relatives may have received this gene from a common ancestor. This explains why cousin marriages result in defective children more frequently than do marriages between people not so closely related.

All possible gradations are found between genes which are completely dominant over their allelomorphic partners and others which produce intermediate effects in the heterozygote. Refined methods of investigation often allow the detection of a seemingly recessive gene in the heterozygote. In the fly *Drosophila*, as in man, the male has the genetical constitution XY and carries only one set of sex-linked genes, while the female is XX and carries genes in duplicate. Many sex-linked genes are known in *Drosophila* which harm or kill the male (so-called sex-linked deleterious or lethal genes), but can be carried in heterozygous condition by normal healthy females. In two independent investigations the reproductive ability of females which carried a sex-linked lethal in heterozygous condition was compared with that of normal homozygous sisters; in both cases, the average performance of the heterozygotes was slightly, but significantly, inferior to that of the normal homozygotes. The only reason for doing these tests on *sex-linked* lethals was one of convenience; it is highly probable that the result would have been similar if, instead, heterozygotes for lethals on another chromosome would have been compared with normal homozygotes. The possibility that in man also, genes which are drastically harmful to the homozygote—or to a man carrying them on his single X-chromosome—may be slightly disabling in heterozygotes is an important point to consider in the assessment of genetical radiation damage.

The action of radiation on the genetic material.—Radiation produces two types of change in the genetic material, both of which occur also spontaneously in nature, but at very much lower frequencies: chromosome breaks and mutations.

CHROMOSOME BREAKAGE.—In suitable material, e.g. plant cells with large chromosomes, chromosome breakage and the secondary processes resulting from it can be microscopically demonstrated. Once a chromosome break has been produced, it may have one of three possible fates:–(1) It may remain open, and the cell now contains a fragmented chromosome. Such a cell survives as long as it does not divide, but for reasons which are not yet fully understood it usually dies in the attempt at division. Even if the cell manages to go through one or a few divisions, the chromosome fragments are apt to be lost on the way, so that cells are produced which no longer possess all the genes necessary for survival. Thus, by various means, fragmented chromosomes kill dividing cells. (2) The broken pieces may heal together in the old way. This usually

leaves the cell unharmed. (3) The broken pieces may join in a novel way, resulting in a 'chromosome rearrangement'. Two important types of rearrangement are 'deficiencies' and 'translocations'. A deficiency arises when a chromosome has been broken in two spots and the two terminal pieces fuse, leaving the middle piece to be lost at one of the next divisions. A small deficiency may not be lethal to the cell because the function of the missing genes may be adequately performed by their allelomorphs on the partner chromosome. The larger the deficiency, the larger the chance that among the lost genes there are one or more which have to be present in duplicate for survival; large deficiencies are therefore usually lethal. A translocation is an exchange of pieces between two broken chromosomes. This may or may not result in death of the cell, depending on the way in which the broken pieces have joined. However, even translocations which are compatible with life interfere with the formation of normal gametes; for when the chromosomes pair in preparation for gamete formation the translocated pieces have difficulties in finding partners, and gametes are produced which no longer possess one and only one chromosome and gene of each kind. Individuals which carry a translocation in heterozygous conditions have therefore greatly reduced fertility.

MUTATIONS.—Mutations are changes which arise suddenly and produce variation which is inherited according to MENDEL's fundamental laws of heredity. It is true that changes which fulfil these requirements may also be brought about by chromosome rearrangements. Thus translocations produce inherited semisterility, deficiencies may produce all kinds of inherited abnormalities, and the mere fact that a chromosome rearrangement creates new neighbourhood relationships between genes may affect the functioning of the genes by what is called 'position effect'. There are geneticists who attribute all hereditary changes to chromosome rearrangements. In cases where microscopical and genetical techniques fail to show any chromosomal abnormality, it is postulated that the rearrangement is too small for detection. This theory is difficult to disprove, but the majority of geneticists believe that in addition to chromosome rearrangements there exists a class of hereditary changes which is due to chemical changes in individual genes. Theoretically, the question whether such true gene mutations exist is of great interest; for practical purposes it is convenient and sufficient to distinguish between large, easily detectable chromosome rearrangements and changes affecting only one or a few neighbouring genes, and reserve the term 'mutation' for this latter class.

Mutations may affect every aspect of the living individual: its structure, its physiology, its cellular biochemistry, its mental faculties and behaviour. They may be lethal like deficiencies; they may cause infertility like translocations. The vast majority of mutations are harmful—very few indeed are beneficial. This may seem a contradiction if one considers that without mutation there could have been no evolution. The contradiction resolves itself when one realises that a living species in its present habitat is a product of evolution, and, as such, is finely adapted to its environment and mode of life. Mutations are random events which are likely to disturb the balance between the organism and its environment, and between the multitude of subtly interwoven life processes within the organism.

The spread of mutations through a population.—The speed with which a newly arisen mutation becomes manifest and the manner in which mutated individuals are distributed over the succeeding generations depends on whether the mutated gene is dominant or recessive, sex-linked or unrelated to sex. A dominant mutation becomes manifest in the first individual carrying the mutated gene. A recessive mutation may be carried latent for many generations until mutated individuals appear among the offspring of two heterozygotes. A recessive sex-linked mutation has a better chance of manifesting itself; although, in a species like man or *Drosophila*, it may remain hidden for several generations in heterozygous females, it shows up in the first male which carries it on its single X-chromosome.

Mutation is a process from which there is no recovery. The replicas of a mutated gene or a rearranged chromosome are a similarly mutated gene or a similarly rearranged chromosome. Although the individual cannot rid itself of its harmful mutated genes and chromosome rearrangements, the population as a whole can do so and must do so if it is not to die out as a result of the harmful mutations which very slowly, but inexorably, accumulate in every living species. Every time a genetically afflicted individual dies childless before the end of its reproductive life, the genes which caused this untimely death are removed from circulation among the next generation. The same happens when a gene, by curtailing fertility, reduces the number of offspring to which it might have been transmitted. Eventually, a balance is established between the rate at which harmful genes arise *de novo* through mutation and the rate at which they are lost through early death or infertility. In a population which has reached this equilibrium, every kind of mutated gene is present in a frequency which is determined by the rate at which it arises and the rate at which it is discarded. It has been estimated that in the present human population, every individual carries at least 8 harmful genes.

Quantitative radiation genetics.—It is not sufficient to know what kind of genetical changes are to be expected from exposure of the germ cells to radiation. One also wants to know the absolute and relative frequencies with which the various types of effect are produced by a given dose and kind of radiation. For some

genetically well-analyzed species, in particular *Drosophila* and maize, such predictions can be made with reasonable certainty; applied to our own species, most of them have a very large margin of uncertainty. There are, however, certain general quantitative rules which hold good in all investigated species and which almost certainly are applicable also to man. These are rules relating the overall frequencies of certain broad classes of genetical change to dose and type of radiation.

I. *Dose-effect relations*

Mutations.—(1) The frequency of gene mutations, including that of very small rearrangements, is directly proportional to dose as measured in roentgen(r) units. This rule has been found to hold down to the lowest dose tested (25r), and there is no reason to assume that it will not apply to even lower doses.

(2) The frequency of mutations is determined by the total amount of r units received by the gonads and is independent, within wide limits, of wavelength and intensity of delivery, i.e. the reciprocal of the time over which a given dose is spread. It also remains unchanged when a given dose is administered in several fractions, separated by periods of rest, instead of in one continuous exposure.

From these observations the conclusion has been drawn that a gene mutation is the result of a single ionization—or of a small cluster of ionizations—which takes place inside a gene or very close to it. This interpretation has remained essentially correct, although we have come to realise that very often short-chain chemical processes are intercalated between ionization and mutation. It leads to conclusions which, although they are implicit already in the statements (1) and (2), are so important that they will be re-stated explicitly as—

(3) There is no lower threshold to the production of mutations by ionizing radiation. Every dose produces mutations in proportion to its magnitude.

(4) The number of mutations which a given dose produces in the germ cells of an individual is independent of how the exposure is spread in time. The same number of mutations is produced in the germ cells of a woman who, at the age of 24, receives one exposure of 250 r to her gonads as in those of a woman who, from the moment of conception to the age of 24, was exposed to chronic irradiation amounting to a yearly gonad dose of 10 r.

(5) The number of mutations which a given dose produces in the germ cells of a population is independent of how the exposures are distributed over individuals. 250 r received by the gonads of one individual produce the same number of mutations as 0·1 r received by the gonads of 2,500 individuals.

To be strictly accurate, (4) and (5) would need to be corrected for differences in sensitivity between various types and stages of germ cell, but the correction factors are probably never large and cannot obscure the essential features of these important conclusions.

Chromosome breaks and rearrangements.—(6) The frequency at which chromosome breaks originate increases linearly with dose and follows the rules which have been listed for mutations.

(7) Large chromosome rearrangements, such as translocations or large deficiencies, behave differently. A chromosome rearrangement is a secondary effect of chromosome breakage; it requires the presence in the same cell of two chromosome breaks. Neutrons may produce the two required breaks along the same track of protons; neutron induced rearrangements are, therefore, directly proportional to dose. With less densely ionizing radiation, in particular X-rays and gamma-rays, the two breaks have to be produced independently, and the frequency of rearrangements increases approximately as the square of the dose; approximately and not accurately, because of complications on the biological level, such as inviability of cells in which many breaks have occurred. Because of this dose-effect relationship, rearrangements become negligibly rare at low doses.

(8) The frequency of rearrangements is not, like that of mutations, independent of the intensity at which a given dose is administered or of the choice between continuous or fractionated exposure. At low intensities and after fractionation, rearrangements are less frequent than would be expected from the overall dose. This is so because chromosome breaks remain open and rejoinable for a limited time only. When the intensity of radiation is low or when the dose is fractionated, the second break will often be produced when the first is no longer available for the formation of a rearrangement. In certain types of cell, notably in mature spermatozoa, chromosome breaks can be stored indefinitely without losing their rejoining ability; the frequency of X-ray induced rearrangements in spermatozoa is, therefore, independent of radiation intensity and degree of fractionation of dose.

II. *The relative frequencies of different types of genetical effect*

Physical as well as biological factors influence the proportions with which different types of genetical effect appear after irradiation. Thus, at high doses, rearrangements are relatively more frequent than at low ones because the dose-effect curve is steeper for rearrangements than for mutations. All types of rearrangement are very rare after irradiation of early germ cells, spermatogonia or oogonia. In the mouse, with 20 chromosome pairs, translocations form a larger proportion of all rearrangements than in *Drosophila* with 4 chromosome pairs. In *Drosophila*, X-rays produce changes which by all known criteria are true gene mutations; in maize, X-rays seem to act only destructively on the chromosomes, and, on closer analysis, all

apparent gene mutations were found to be small deficiencies.

Yet, there is one relation which we may expect to hold for all organisms, including man. This is the vast preponderance of harmful and lethal mutations. The theoretical reasons for expecting just such a preponderance have been briefly mentioned before. In practice, it has been found in every tested species. In the best analysed species, *Drosophila*, by far the largest class of mutations consists of harmful genes which, without producing any visible abnormality, lower viability and reduce fertility. The next largest class is recessive lethal mutations, i.e. mutations which kill homozygotes (or males carrying such a mutated gene on their single X-chromosome), while heterozygotes are normal or only slightly inferior to normals. Visible mutations, with only slight or no harmful effect, are much rarer, and the majority of them are recessive. The relative scarcity of dominant as compared with recessive visibles is a general observation, made also for chemically produced and naturally occurring mutations. This is not the place to discuss the various hypotheses which have been put forward in explanation.

III. *Sensitivity differences between germ cells*

It has already been mentioned that not all types of germ cells are equally sensitive to the mutagenic action of irradiation. In the mouse as well as in *Drosophila*, a given X-ray dose produces more mutations in spermatids than in any other stage of male spermatogenesis; in both species, mature spermatozoa are somewhat less sensitive than spermatids, and spermatogonia are the least sensitive stage. This parallelism between two widely divergent species suggests a fundamental sensitivity pattern of the animal testis. It seems reasonable to infer the existence of a similar pattern in the human testis. Information on the production of mutations in female germ cells is still too incomplete to warrant generalisations.

More important than differences in sensitivity between germ cells of the same species is the possibility that the frequencies of mutations which a given radiation dose produces in a given type of germ cell may differ between species; for at present our only means of arriving at some quantitative estimate of radiation damage to the human species consists in extrapolation from lower forms of life, especially *Drosophila*. It is, therefore, of the utmost importance that mouse germ cells have been found to be considerably more sensitive to the genetical effects of X-rays than *Drosophila* germ cells. It is certain that a given X-ray dose produces many more chromosome breaks and translocations in the mouse than in *Drosophila*, but this might be simply a consequence of there being more chromosome material to be broken. In addition, however, there is evidence that, gene for gene, a dose of X-rays produces more mutations in the mouse than in *Drosophila*. The magnitude of this difference is still being examined; its existence can hardly be doubted. It seems probable that in this, as in all other respect, man is closer to the mouse than to *Drosophila*.

Genetical effects of radiation on human populations. Let us first consider persons who have received a fairly high dose of radiation to their gonads, such as the atom bomb survivors in Hiroshima and Nagasaki, or women whose ovaries have been exposed to a high X-ray dose. There is a popular idea that all kinds of monstrosities may turn up among the children of such persons. This is entirely wrong, for dominant mutations which might produce such abnormalities after irradiation of one of the parents are the rarest type of mutation. Recessive mutations with visible effect on the homozygote are much more frequent, but for these to become manifest in the children of irradiated parents, the same mutation would have to be produced in both spermatozoon and ovum, and the chance for this to happen is infinitesimally small. It is, therefore, not surprising that there was no significant increase in the incidence of freaks or monstrosities among children of persons who survived the Japanese atom bomb explosion, and that persons whose gonads have been X-rayed may have healthy and normal children. These findings cannot be taken as evidence that radiation has not caused genetical damage of a less immediately detectable nature.

Among women who were pregnant at the time of the atom bomb explosion, a number gave birth to physically or mentally defective children. This, however, was not a genetical effect of the radiation, but a direct effect on the developing embryo, comparable to an X-ray burn which develops after exposure of the skin. Should the affected children grow up to reproduce they will not transmit the damage which they themselves suffered. It is true that they may transmit genuine genetical changes—mutations or chromosome rearrangements—which were induced in their foetal gonads; but, if so, there will be no resemblance—except perhaps a fortuitous one—between these genetically caused abnormalities and the original radiation damage. Thus, irradiation of pregnant mouse females often results in young with abnormally formed tails. Among the progeny of these abnormal mice, X-ray induced mutations may appear, but these are in no way more likely to affect the tail than any other part of the body.

Not only the frequency of abnormal children, but also that of miscarriages was increased among Japanese women who had been pregnant during exposure to the atom bomb: again, this was due to direct action of the radiation on the developing embryos, and frequency of abortion was normal among the rest of the surviving population. At first sight this is surprising to the geneticist. Embryonic death through dominant lethality is the most easily observed geneti-

cal effect after irradiation of male or female mice. It is highly probable that the atom bomb produced many dominant lethals in the human gametes which were exposed to it. Presumably, these lethals escaped detection because, like dominant lethals in the mouse, they killed the embryos at very early stages. Thus dominant lethality, although almost certainly a fairly frequent result of heavy irradiation, is not likely to cause human suffering or unhappiness. The same is true for translocations which, again by analogy with the mouse, are expected to be frequent among the children of heavily irradiated persons. The affected children will have drastically reduced fertility, and this condition will be transmitted as a dominant genetical change. In a species which habitually practises birth control, this is not likely to cause concern.

The kind of radiation damage wich need cause concern is the production of harmful or lethal recessives which may be carried through a great many generations before they claim their victims. A recessive lethal will kill the first individual which received the mutated gene from both parents, or the first male which received it on his X-chromosome. A recessive harmful gene only increases the probability that those who carry it in homozygous condition will die early; eventually, however, it too will result in what has been called 'genetic death', and on the way to this end it will have produced a track of individuals who in some way, physically or mentally, are slightly inferior to what they would have been without this gene. In addition, it is likely that in man, as in *Drosophila*, many genes which are lethal or drastically harmful to the homozygote are slightly harmful to the heterozygote. Since many more persons inherit a harmful gene from one parent than from both, the slight effects on the heterozygotes will in their totality outweigh the much more drastic, but much rarer, effects on the homozygotes. Moreover, this slight deterioration will take effect even in the immediate progeny of the irradiated individuals.

Eventually, and at the cost of human frustration and suffering, the genetical effects of a single heavy dose of radiation will become eliminated from the population. This is not so for a population which is exposed to chronic low doses of irradiation. Because of the linear proportionality between radiation dose and mutation frequency, chronic irradiation at an approximately constant dose rate will, in effect, result in a mutation frequency which is permanently increased above its previous value. Under these conditions, harmful mutation will go on accumulating in the population until, after a great many generations, a new equilibrium is reached at which the rate of elimination of harmful genes through genetic death again equals the origin of these genes through mutation, and at which all harmful genes are present in higher frequencies than before. This applies to drastic hereditary defects such as certain types of blindness or deafness or mental disease. It applies equally to the more intangible effects of genes which cause very slight disabilities of body or mind. Human variation is so great that it will hardly be possible to pinpoint those persons whose mental or physical abilities are slightly impaired by such genes; but over the population as a whole the deterioration will eventually become noticeable. Medical progress may to some extent mask it, but it cannot prevent it. Moreover, the picture of a human society in which most people are kept alive through constant medical supervision and treatment is not an attractive one.

The question is sometimes asked whether radiation can produce hereditary changes which have not occurred before and which may either cause new kinds of disease or may, through their very novelty, be a stimulus to further human evolution. The answer to this question is: 'No'. Ionizing radiation from natural sources has been producing mutations ever since the origin of life on earth, and all types of mutation which can be produced by its agency must already have occured repeatedly.

Another question which is often raised is whether the benefit from useful mutations may not outweigh the damage done by the harmful ones. The answer is again: 'No'. In all investigated species harmful mutations are so overwhelmingly in excess of beneficial ones that planned genetical improvement through induced mutations is practicable only in species like grasses or bacteria in which one can afford to discard thousands of inferior progeny for the sake of a few superior ones. There is no reason to believe that the ratio of beneficial to harmful mutations will be different in the human species.

The control of genetical radiation damage.—The increasing use of ionizing radiation in medicine and industry makes it imperative to decide on permissible doses to which the individual and the population may be exposed without the risk that the harmful genetical effects may offset the benefit to be derived from X-rays, isotope therapy, nuclear power and other uses of radiation. Only a compromise solution is possible, for there is no lower threshold to the mutagenic action of radiation, and even if we were to abandon completely the use of ionizing radiation, cosmic radiation and naturally occurring radioactive substances would continue to produce genetical changes. We still lack data on which we could base an even moderately accurate estimate of the genetical effects of a given dose of radiation on human genes. Calculations use extrapolations from experiments on lower animals and include premises on which geneticists differ among themselves.

It was, therefore, surprising and encouraging that two independently prepared reports—one issued by the British Medical Research Council, the other by the

U.S.A. National Academy of Sciences—arrived at very similar limits for the estimated doubling dose, i.e. the dose which would double the present mutation rate. Both reports put the most probable limits at between 30 and 80 r received by the gonads from conception to the age of 30. In the final result, such a dose, if given generation after generation, would double the incidence of genetically caused defects, although for recessives this may take a very great number of generations. It has been calculated that in Great Britain there would be 200 more cases each of maniac depressive insanity and schizophrenia, and 1,500 more of severe mental deficiency among the first generation of 20 million births, and that similar increases would take place in each generation until the limiting value of twice the initial number was reached. For hereditary traits which show continuous variation about the normal, an increased mutation rate results in increased proportions of the extreme variants on either side of the mean; but if the trait has already been subjected to much selection towards one extreme, as is likely for human intelligence, it is mainly the minus variants which will be increased in number.

The maximum dose which the British report considers tolerable from a genetical point of view is 50 r to the gonads in excess of the natural radiation up to the age of thirty, and this should be received by not more than 1/50 of the total population. The American report recommends that records of the accumulated life-time exposure to radiation should be kept for every individual, that the average exposure of the population's reproductive cells to radiation above the natural background should be limited to 10 r from conception to age 30, and that individual persons should not receive a total accumulated gonad dose of more than 50 r up to age 30, and not more than 50 r additional up to age 40.

At present, by far the largest source of ionizing radiation impinging on human individuals is X-radiation used in medicine, especially in diagnosis. In Britain, X-ray diagnosis, mainly of hip, lumbar spine, lower abdomen and pelvis, adds at least 22% and possibly much more, to the average amount of natural radiation (3 r) which the gonads receive during the first 30 years of life. In the United States, this figure is considerably higher. H. J. MULLER, the discoverer of the mutagenic effects of X-rays, has been urging for many years that medical X-ray doses should not be higher than absolutely necessary, that wherever possible alternative methods of diagnosis and treatment should be used, and that in both patient and radiologist the reproductive organs should be shielded. The genetical dangers from the use of nuclear fission are at present negligibly small. It has been estimated that if nuclear weapon testing should continue at the present rate, this would raise the normal background radiation by only about 1%. Yet, since even the slightest increase in the amount of background radiation will result in a proportionately increased incidence of harmful mutations, every precaution should be taken to keep all extra radiation from atomic energy processes as low as possible.

Mutagenic chemicals.—The search for mutagenic chemicals is almost as old as the modern science of genetics; but up to 1940 no clear proof for the production of genetical changes by any chemical had been obtained. During the last war this impasse was broken by the independent discovery of two mutagenic substances: mustard gas and urethane. These initial findings were soon followed by others, and at present the list of mutagenic substances is much too long for enumeration in a short article. It contains many chemicals which are related to mustard gas, and many carcinogens and anti-carcinogens. Of particular interest in the present context are three types of compound: (1) simple compounds like manganese chloride, hydrogen peroxide and formaldehyde, (2) alkaloids like morphine and atropine, and (3) purines like adenine, caffeine, theophylline. Some of these substances occur naturally in metabolism or are taken up by man in stimulants or medicines. It is too early to say whether, and in what concentrations or overall amounts, they can affect the chromosomes in human gametes. Most of them have so far proved mutagenic only in bacteria and flowering plants. Some have been found effective also in *Drosophila*. None have been tested on animals other than *Drosophila*. Yet in view of the rapid increase in the variety and amounts of chemical substances to which civilized man is exposed it is well to consider not only the harm which some of them may cause to individuals, but also the possibility that some of them may harm future generations by effects on human germ cells. Unfortunately, there is no cheap and easy way of testing for this possibility, for there is no reason to assume that chemicals, like X-rays, reach and attack the chromosomes in the germ cells of all organisms equally well. At the moment, small rodents are the only mammals which can be used in mutation work. Even this requires incomparably more money, space, time and effort than work on *Drosophila;* but the issue is important enough to justify this expenditure in money and scientific manpower.

Zusammenfassung

Die populären Auffassungen der durch Bestrahlung erzeugten genetischen Schädigungen irren gewöhnlich weit vom Ziele, und zwar in entgegengesetzten Richtungen. Oft hört man die Behauptung, dass bestrahlte Eltern die Gefahr laufen, Missgeburten oder sonstwie anormale Nachkommen zu haben. Noch öfter werden die genetischen Gefahren ionisierender Strahlen als unbeträchtlich und nicht beachtenswert hingestellt. In Wirklichkeit sind diese Gefahren sehr ernst zu nehmen. Sie betreffen aber nicht Individuen, sondern die menschliche Rasse als Ganzes. Über ihre Natur sind wir gut unterrichtet, über ihr Ausmass können wir uns

gegenwärtig nur sehr ungenaue Vorstellungen machen. Genetisch unterschwellige Strahlungsdosen gibt es nicht; die gesetzlich festgesetzte Toleranzdosis muss daher einen Kompromiss darstellen zwischen gegenwärtigem Nutzen und zukünftigen Gefahren.

[1] *The Hazards to Man of Nuclear and Applied Radiations.* Medical Research Council (London, H. M. Stationery Office, 1956).

[2] *The Biological Effects of Atomic Radiation:* a Report to the Public from a Study by the National Academy of Sciences. (National Academy of Sciences-National Research Council, Washington, D.C. 1956).

[3] C. AUERBACH and A. ROBERTSON, New Biology *20*, 30 (1956).

[4] H. J. MULLER, Bull. Atomic Scientists *11*, 329 (1955). (With many references to original papers.)

[5] M. WESTERGAARD, *Impact of Science on Society 6*, 63 (1955).

Chemical Mutagenesis in Relation to the Concept of the Gene

By M. WESTERGAARD, Copenhagen*

Attempts to enhance the mutation rates in plants and animals through chemical treatment go back to the pioneer work of MCDOUGAL (1911) in plants and to MORGAN's experiments with *Drosophila* in 1910. Although some earlier experiments gave suggestive results (especially the work of the Soviet scientists SACHAROV 1931–1936, LOBASOV 1934, and others[1]), the real break-through came at the end of the Second World War in three different laboratories. In the Soviet Union, RAPOPORT (1946) demonstrated the mutagenic effect of formaldehyde on *Drosophila* and, in the years 1946–1948, this brilliant geneticist tested more than 20 chemicals for mutagenic effect on *Drosophila*, among others epoxides, dimethyl- and diethylsulphate, diazomethane, ethyleneimine, acrolein, and other unsaturated aldehydes, etc.[2]. In Germany, OEHLKERS injected ethylurethane and potassium chloride into the flower buds of the plant *Oenothera*, whereby various types of chromosome mutations were induced[3]. These observations were extended to *Drosophila* by VOGT in Germany and by RAPOPORT. Simultaneously, in Scotland, AUERBACH, working in collaboration with the pharmacologist ROBSON, showed that a war gas, 'mustard gas', greatly enhances the mutation rate in *Drosophila*[4].

Without exaggerating it may be said that these discoveries started an avalanche. To-day the list of mutagenic chemicals runs into many hundreds, ranging from inorganic salts to the most complex organic molecules[5].

From the beginning, chemical mutagenesis was undoubtedly stimulated by its contact with cancer chemotherapy. Very soon 'mustard gas', and especially 'nitrogen mustard', was used in therapy against certain types of leukemia. In the last 10 years, many hundred chemical compounds have been tested as tumour inhibitors, and many of these new compounds were made available to geneticists to be tested for mutagenic activity[6].

The fact that many mutagenic chemicals are completely unrelated with regard to chemical structure, physico-chemical and pharmacological properties, may give the impression that the whole field is in a rather chaotic state and that the study of chemical mutagenesis has been, to some extent, a disappointment with respect to the amount of fundamental biological and genetical information which has been obtained so far. The present author does not share this pessimism. Firstly, it should be remembered that this new branch of genetics is hardly more than 10 years old. Secondly, it seems possible even now to arrange the available data in such a way that they do not only stimulate further research but also provide new and important information about both fundamental and applied aspects of genetics. In order to draw such a pattern it is necessary, however, to keep two things in mind: (1) That the genetical mutation concept is very heterogenous, ranging from point-mutations to polyploidy, and (2) that mutagens can act either directly on the gene, or indirectly by inhibiting antimutagens in the cells and thus interfering with the mutagen-antimutagen balance. The hydrogen peroxide-catalase-potassium cyanide system may be cited as an example of this type of interaction: H_2O_2 is mutagenic. Catalase is an antimutagen which destroys the mutagenic effect

* Institute of Genetics, University of Copenhagen, Denmark.

[1] H. STUBBE, Handb. Vererb. Wiss. II F (1938). – N. W. TIMOFÉEFF-RESSOVSKY, Biol. Rev. Cambridge *9*, 411 (1934).

[2] For references to RAPOPORT's publications (which are all in Russian) cf. I. H. HERSKOWITZ, Amer. Nat. *75*, 181 (1951).

[3] F. OEHLKERS, Z. Ind. Abst.-Vererb.-Lehre *81*, 313 (1943).

[4] CH. AUERBACH, Nature *157*, 302 (1946).

[5] Cf. CH. AUERBACH, Biol. Rev. Cambridge *24*, 355 (1949). – E. BOYLAND, Pharmac. Rev. *6*, 345 (1954).

[6] O. G. FAHMY and MYRTLE FAHMY, Proc. 5. Internat. Congr. Radiobiol., Stockholm 1956 (in press).