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

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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

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[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).

of H_2O_2. If catalase is inhibited by KCN, the mutation rate is enhanced; not because KCN is mutagenic, but because the H_2O_2 concentration in the cells is increased[7].

In order to prove the first point we must, however, try to integrate the mutation concept into the other basic genetical concepts. To this purpose it may be most convenient to define and describe the scope of genetics in terms of the modern language of cybernetics, information theory[8]. Genetics deals with the mechanism of transmission of genetic information from parent to offspring. The first problem—to what extent and in which way is the information which is expressed in the so-called phenotype of the parents transmitted to their offspring—has largely been solved: The genetical information (or rather information specificity) is transmitted from parent to offspring through self-reproducing particulate units, genes, which are organised in the chromosomes of the cell nuclei. The laws for transmission of information are expressed in the first and second Mendelian law and determined by the behaviour of the chromosomes during meiosis and fertilization.

After the solution of the first problem we are at once confronted with a second and most important one: How is the genetic information carried by the genes of the zygote translated to make its impact upon the offspring's phenotype?

It is well known that this is a very complicated process, since the phenotype is shaped through the interaction of two components, heredity and environment. The genetic information contained in the genes must be considered to be mere potentialities. What is actually decoded, and how it is decoded, depends upon the environment. Some of the more promising attempts at studying this interaction may perhaps be found in the work of BEALE and SONNEBORN on antigen differentiation in *Paramœcium*[9].

It is fully realised that this somewhat unconventional approach to the fundamental genetical concepts may be too rigid and dogmatic. Still, it may be of more than just heuristic value to operate with the following three elements in genetical information theory:

(1) A self-reproducing information system, (2) a translation system which decodes the information and transmits it to (3) a receptor system.

It is likely that, if we accept these three elements, our system may be changed spontaneously or experimentally on three different levels: (1) in the receptor system or (2) in the translation system or (3) in the information system. Until now the staring point in all genetic analysis has been the phenotype, although a direct chemical and even physical approach to the information system is within reach (due to the crystallographic analysis of the DNA molecule which led WATSON and CRICK to postulate a most suggestive double helix structure of DNA)[10]. Leaving this aspect aside for the moment, it may be stated that a given phenotypic variant may have arisen as a consequence of changes in either the receptor system, the translation system, or the information system. Fortunately, in most organisms the adequate tools are present for discriminating between these three events, namely the Mendelian crossing experiment, combined with microscopical, cytological observations.

It is again fully realised that it may be stretching a few points, when proposing to identify phenotypic variants due to changes in the receptor system with the so-called phenocopies. A phenocopy (GOLDSCHMIDT) is defined as an environmentally produced, non-inherited imitation of a known mutation[11]. Such phenocopies can be induced by treating the organism with physical or chemical agents during certain sensitive stages of development.

It is also known that phenocopies can be induced rather specifically. By choosing the right chemicals and by proper timing of the treatment, almost 100% of the treated populations may sometimes show the same phenocopy. Also here RAPOPORT's studies on the induction of chemophaenes are outstanding[12]. This field of research may actually have been somewhat neglected by geneticists, and the possibilities for integrating the results of induction of phenocopies into the results of experimental mutagenesis are far from having been exhausted. It is important to keep in mind that phenocopies, which can be rather specifically induced, are not inherited, or, in other words, there is no feedback mechanism from the receptor system to the information system. However, we shall leave the phenocopies and turn to the next level of our three-phase system: Phenotypic variants due to alterations in the translation system.

In this field our present knowledge is possibly most deficient. However, we tentatively propose to identify such changes with so-called cytoplasmic mutations and use the important work of EPHRUSSI and his colleagues in Paris on the induction of the so-called 'petite' colonies in yeast as a model[13]. EPHRUSSI has shown that there are two kinds of 'petite' colonies in yeast, which both lack certain enzymes in the oxidative system. Some 'petites' arise as a result of nuclear mutations and the 'petite' character is then inherited according to a normal Mendelian scheme. Others which are due to cytoplasmic changes are

[7] K. A. JENSEN, I. KIRK, G. KØLMARK, and M. WESTERGÅRD, Cold Spring Harbor Symp. Quant. Biol. *16*, 245 (1951). –

[8] N. WIENER, *Cybernetics* (New York, Paris 1955). – H. QUASTLER (Ed.). *Essays on the use of information theory in biology* (Urbana 1955).

[9] G. H. BEALE, *The genetics of Paramœcium aurelia* (Cambridge 1954).

[10] J. D. WATSON and F. H. C. CRICK, Cold Spring Harbor Symp. Quant. Biol. *18*, 123 (1953).

[11] R. GOLDSCHMIDT, Z. Ind. Abst.-Vererb.-Lehre *69*, 38 (1935).

[12] J. A. RAPOPORT, Amer. Nat. *81*, 30 (1947).

[13] B. EPHRUSSI, *Nucleo-cytoplasmic relations in micro-organisms* (Oxford 1953).

transmitted through the cytoplasm only. The latter, cytoplasmic 'petites' can be induced at a very high frequency by chemical treatment with acridines like euflavine. There is some evidence to believe that, in the cytoplasmic 'petites', something is wrong with the mitochondria of the yeast cells—and we know that the cytochrome-enzymes are located in the mitochondria. Hence, specific changes observed on the phenotypic level can, in this system, be pinned down with great precision to nuclear or cytoplasmic events, and the latter can be specifically induced by selecting the proper chemicals. Again there seems to be no feed-back from the translation system to the information system. EPHRUSSI's work shows, in a beautiful and clear way, how a normal phenotype is produced only when both the information system (e.g. the proper nuclear genes) and the cytoplasmic component (which we here propose to identify with the translation system, ascribing an important role in the latter to the mitochondria) are functioning normally.

Let us now turn to the third level, where changes may result in a phenotypic variant: the information system, mutations proper. This is undoubtedly the category about which our knowledge is most satisfactory. For one thing, combined cytological and genetical studies have shown that all changes of this kind take place in the chromosomes, either in the individual genes (gene or point-mutations) or by changing the linear order or the number of the genes along the chromosomes (deficiencies, duplications, translocations or inversions). The latter types of mutations require at least one and mostly two breaks in the chromosomes. Finally, changes in the number of single chromosomes (aneuploidy) or chromosome sets (polyploidy) may also be responsible for changes in the information system which we classify as mutations. We shall, however, omit the two latter types from the following discussion.

Various genetical and cytological tests are available for the study of spontaneous and induced mutation rates, but it is important to know exactly which type of mutation is scored in each test.

(1) There are purely cytological tests for studying chromosome breakage. Plants with large and few chromosomes like *Allium, Vicia, Tradescantia, Oenothera* and others are mostly used. In the majority of cases, the genetical consequences of the chromosome breaks are not followed up in these tests.

(2) A method which is in a way complementary to the chromosome breakage test, is the so-called *Neurospora* back-mutation test. There are two main types of nuclear mutations (leaving aneuploidy and polyploidy out of consideration), namely (a) mutations which are due to one or two breaks of the chromosomes, and (b) changes which do not require, and do not seem to be associated with, breakages of the chromosomes: Gene- or point-mutations. It has always been difficult to develop a clear-cut test for this latter important group, because the only way of deciding whether a mutation is due to point-mutation or chromosome breakage is by a process of elimination: If a mutation is *not* associated with visible changes in the chromosomes, if it is inherited in a regular Mendelian way, and if the crossing-over values are not distorted, we think we are dealing with a point-mutation. Actually, the existence of induced, at least X-ray induced, true point-mutations has sometimes been disputed, especially by STADLER[14]. The back-mutation test, where it is studied how a mutated gene reverts, through back-mutation, to the original 'wild type' allele, is probably as good a model for a point-mutation as may be expected to be found. We shall therefore consider the back-mutation test as a 'pure test' for point-mutations, complementary to the cytological tests for chromosome breakage.

(3) The classical methods for studying induced mutations, the *Drosophila ClB* test or its various modifications (the 'Muller-5' test) must be considered mixed tests, where the mutation yield is a mixture of mutations due to chromosome breakage and, possibly, point-mutations. However, it is also possible in *Drosophila*, by combined genetical and cytological methods, to score the rate of dominant lethals, and due to the ingenuity of Dr. MULLER and his students a number of stocks are now available in *Drosophila* which allow the separate study of various types of mutations (e.g. MULLER's 'multi-purpose' stock, and the 'maxy-stock'. In the latter, it is possible to score the mutation rate at 10–15 different, single *loci*). However, many flies are needed and much work has to be devoted to obtain quantitative information from such stocks which are only recently being used in the study of chemical mutagenesis.

(4) Very specific and interesting information has been obtained from the so-called 'prophage-activation test' in *Bacillus megatherium* developed by LWOFF and his colleagues at the Pasteur Institute[15]. Some bacteria are lysogenic. They carry a prophage which, when activated, is transformed into a phage which will lyse the bacteria which carry them. It is possible to increase the number of lysogenic cells in such strains (by activating the prophage) by means of radiation or by chemical treatment. It is very striking that the same chemicals which enhance prophage activation also induce back-mutations in *Neurospora*, whereas those which give negative results in the prophage-test are also inactive in the back-mutation test. However, since the yield of lysogenic particles may be 100% in such experiments, it is doubtful, whether we are here dealing with a true mutational event.

[14] L. J. STADLER, Science *120*, 811 (1954).

[15] F. JACOB and E. WOLLMAN, Cold Spring Harbor Symp. Quant. Biol. *18*, 101 (1953).

(5) In one of the classical objects in botanical genetics, *Antirrhinum*, OEHLKERS has developed a method for studying rates of visible mutations after treatment with chemicals. The mutation rates have been enhanced by compounds like urethane and various alkaloids, for instance morphine hydrochloride[16].

(6) Finally, a great deal of work has been done on induction of mutations in bacteria, especially by using screening tests like resistance to penicillin, streptomycin or other antibiotics[17]. Unfortunately, it is not so easy to break down phenotypical changes in bacteria into the three possible categories, phenocopies, cytoplasmic mutations or nuclear mutations, as it is in higher organisms with a better understood sexual mechanism. We shall, however, omit the work on induction of bacterial mutations from the following discussions and confine ourselves mainly to the two complementary tests (1 and 2) for chromosome breakage and for point-mutations.

First a few words about the chemical induction of chromosome breakage. The pioneer work of OEHLKERS has been extended by many authors. In Sweden, LEVAN, TJIO, KIHLMAN, ÖSTERGREN, and others have shown that the chromosomes of *Allium* and *Vicia* can be broken by treatment with, for instance, phenols, quinones, benzpyrene, purines like 8-oxy-caffeine, by coumarin and others. D'AMATO in Italy has studied the effect of acridines, benzidine, acenaphtene and others. LOVELESS, REVELL, and FORD in England have investigated the effect of numerous alkylating agents, including many 'mustard' analogues, epoxides, ethyleneimine, triazine etc.[18]. The latter compounds have also been studied in *Drosophila*, especially by the FAHMY's at the Chester Beatty Research Institute in London[19].

It is suggestive that chromosome breakage may result from a great variety of treatments. Breaks can be induced with a very high frequency by highly reactive, alkylating agents, but also by treatment with chemically very stable (but mostly very toxic) compounds which possibly act as enzyme poisons or which in some other way interfere with cell and chromosome metabolism. In this respect the induction of chromosome breakage has many characteristics in common with the induction of cancer. Also here one gets the impression that, if a cell is irritated long enough even by a very unspecific treatment, it may be transformed into a malignant cancer cell.

One great difficulty in dealing with the induction of chromosome breakage is the fact that parallel with the process of induction of breaks goes the equally important problem of the future fate of the breaks: They may stay open, or they may reconstitute, *e.g.* the break may 'heal', or two different breaks may recombine in a new way. Probably most treatments which induce breaks also interfere with these processes. Very important recent work on these problems has been performed by SHELDON WOLFF at Oak Ridge[20]. He has shown that there are two types of chromosome breaks, namely some which may stay open for 15 min or more, and which require energy for healing. If the ATP system of the cell is blocked by enzyme poisons like dinitrophenol, such breaks will remain open. The second type of breaks will stay open only 2–3 min, and they seem to reconstitute or recombine 'spontaneously', e.g. the healing process does not require energy. The first type of breaks is attributed to breaks in covalent bonds. The second type seems to be due to breaks in ionic bonds involving calcium or magnesium bonds. The possibility that the chromosome nucleoproteins are held together by ionic forces in calcium bonds was first suggested by MAZIA[21]. MAZIA, STEFFENSON, and SHELDON WOLFF have also shown that chelating agents, e.g. versenes (ethylenediamine tetra acetid acid) which remove calcium from the cells, induce chromosome breakage. The recent observation by TATUM and co-workers, that chelating agents also enhance the crossing-over frequency in the green alga *Chlamydomonas*, may have important consequences for our understanding of crossing-over as related to chromosome breakage[22].

Finally, it should be mentioned that there is some evidence for a certain specificity in the chemical induction of chromosome breakage. Compounds like maleic hydrazide[23] and 8-oxy-caffeine[24] seem to induce breaks in the heterochromatin. However, this does not change the main impression conveyed by the work on chemical induction of chromosome breakage, namely that this is a fairly unspecific event; chromosome breakage can be induced by many different chemicals which have quite different physico-chemical and pharmacological properties, and chromosome breakage can probably be induced in various ways.

The results of the *Neurospora* back-mutation test provide a somewhat different picture. This test was worked out in 1946 by GILES at Yale University and, independently, by the present authors[25,26]. It is well known that it is possible to induce so-called auxotro-

[16] E. OEHLKERS, Heredity *6*, Suppl. 95 (1953).

[17] M. DEMEREC, G. BERTANI, and J. FLINT, Amer. Nat. *85*, 119 (1951).

[18] A. LEVAN, Cold Spring Harbor Symp. Quant. Biol. *16*, 233 (1951).

[19] O. G. FAHMY and MYRTLE J. FAHMY, Heredity *6*, Suppl. 149 (1953).

[20] S. WOLFF and H. E. LUIPPOLD, Proc. Nat. Acad. Sci. Wash. *42*, 510 (1956).

[21] D. MAZIA, Proc. Nat. Acad. Sci. Wash. *40*, 521 (1954).

[22] R. A. EVERSOLE and E. L. TATUM, Proc. Nat. Acad. Sci. Wash. *42*, 68 (1956).

[23] J. MCLEISH, Heredity *6*, Suppl. 107 (1953).

[24] B. KIHLMAN and A. LEVAN, Hereditas *37*, 382 (1951).

[25] N. H. GILES, jr., Cold Spring Harbor Symp. Quant. Biol. *16*, 283 (1951).

[26] K. A. JENSEN, I. KIRK, G. KØLMARK, and M. WESTERGARD, Cold Spring Harbor Symp. Quant. Biol. *16*, 245 (1951).

phic mutations in *Neurospora* and other microorganisms. Such mutations are unable to grow on the synthetic minimal medium which promotes the growth of the 'wild type' strains, because they lack a gene (or a gene has been inactivated) which in one way or another controls the activity of a specific enzyme that is essential for the synthesis of an indispensable growth factor, be it a vitamin, an amino acid, or a nucleic acid constituent. Normal growth of the mutant can be restored by adding the proper growth factor to the substrate. Some of these auxotrophic mutations are not stable, but revert with a low frequency to 'wild type', e.g. they are again able to grow on minimal medium without the additional growth factor. By conventional genetical methods, including analysis of linear tetrads, it is possible in *Neurospora* to identify the cause of a reversion, whether it is due to a non-genetic event (phenocopy), to a chromosome mutation, to a suppressor-mutation in a different *locus*, or to a true back-mutation[26]. The back-mutation test is in principle a screening test where the non-mutated (non-reverted) cells are automatically eliminated on the minimal medium. It is therefore possible to test approximately 10^7 spores in a single experiment, and obtain reproduceable dosis-effect curves for various treatments.

The work from the present author's laboratory has mainly dealt with an adenine-requiring auxotrophic mutant which only reverts by back-mutation, whereas GILES started with a number of inositol-requiring mutants which by conventional genetical criteria would seem to be allelic, but which showed different spontaneous and radiation induced reverse mutation patterns. An important extension of this work is due to Dr. G. KØLMARK from our laboratory, who crossed one of GILES inositol-requiring strains with our adenine strain and isolated the double mutant. By using properly supplemented media, KØLMARK has been able to study the back-mutation pattern of the two mutations adenineless and inositolless on the same genetic and physiological background.

Some of the results of treating the single mutant (adenineless) with various chemical agents are shown in Table I. It will be seen that many chemicals are able to enhance the back-mutation frequency. However, although these chemicals are quite different in chemical structure, they have one trait in common: They are chemically very unstable and highly reactive compounds. Most are strongly alkylating agents, some may form free radicals or very reactive ions. In contrast to these positive results with the reactive compounds are our completely negative results with, e.g. phenols, 8-oxy-caffeine, urethane, maleic hydrazide, and others. All the latter chemicals can induce chromosome breakage, and in some cases also lethals or even visible mutations in *Drosophila* and in plants (*Antirrhinum*). They are chemically very stable and, although most of them are toxic, all have failed to enhance the back-mutation rate in *Neurospora*.

It should be pointed out that the list of mutagenic chemicals given in Table I not only includes compounds which might be called abiological, in the sense that it is very unlikely that cells under natural conditions would be exposed to such chemicals, but also hydrogen peroxide and organic peroxides, which are certainly normal intermediates in cell metabolism. The mutagenic effect of hydrogen peroxide and organic peroxides was first discovered in bacteria by STONE and his collaborators in Texas[27], by DICKEY, CLELAND, and LOTZ[28], and by ourselves on *Neurospora*, and later extented to *Drosophila* (with respect to organic peroxides) by Mrs. ALTENBURG in USA[29] and by SOBELS in Utrecht[30]. Its important perspectives are that these reagents link chemical mutagenesis with the study of X-ray and ultraviolet light induced mutations, since hydrogen peroxide and organic peroxides are certainly formed during the radio-chemical processes resulting from irradiation of cells. They also form a bridge between chemical mutagenesis and the problem of the origin of spontaneous mutations. It is known that only a very small fraction of the mutations in *Drosophila*, and perhaps not more than 10–15% in man, can be caused by 'natural' radiation, the rest must be due to chemical mutagens and probably also to thermal agitation. It is known, furthermore, that not only peroxides are formed during normal cell metabolism, but, as RAPOPORT has pointed out, even a strong mutagen like diethyl sulphate is sometimes likely to be formed during metabolism[31].

The study of the mutagenic effect of peroxides has also been helpful in the understanding of one of the most important concepts in mutagenesis, the mutagen-antimutagen concept. If chemical mutagens play an important role in the origin of spontaneous mutations, there must obviously be a buffer system in the cells which counteracts such mutagens, in order to keep the spontaneous mutation rate as low as it is actually observed. At least one such natural antimutagen, namely catalase, is known. The mutagenic effect of hydrogen peroxide stops instantaneously if catalase is added, and in many organisms it has been impossible to induce mutations by hydrogen peroxide treatment, probably because the catalase content of the cells is too high. If this is true, we should expect enzyme poisons like potassium cyanide, which inactivates catalase, to be mutagenic, and this is also the case, as has been shown both in *Neurospora*[26] and more in-

[27] O. WYSS, J. B. CLARK, F. HAAS, and W. S. STONE, J. Bact. *56*, 51 (1948).

[28] F. H. DICKEY, G. H. CLELAND, and C. LOTZ, Proc. Nat. Acad. Sci. Wash. *35*, 581 (1949).

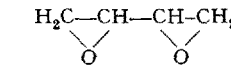
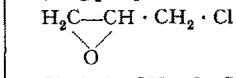
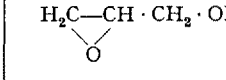
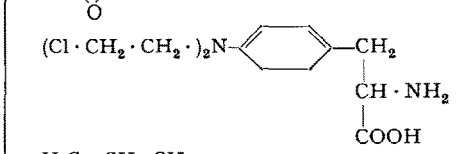
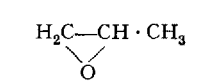
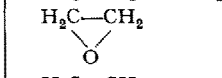
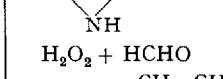
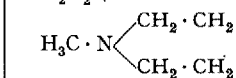
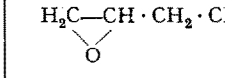
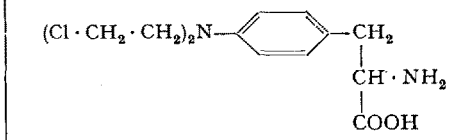
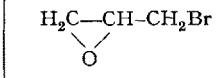
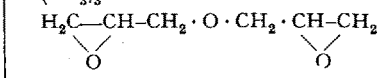
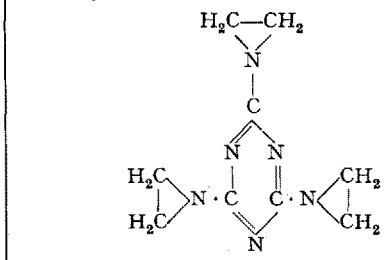
[29] L. S. ALTENBURG, Proc. Nat. Acad. Sci. Wash. *46*, 1037 (1954).

[30] F. H. SOBELS, Nature *177*, 979 (1956).

[31] I. A. RAPOPORT, *Doklady Usesoyuz. Akad. Scl'sko Khoz. Nauk. im I. J. Lenina 12*, 12 (1947).

Table I

The effect of various mutagens on the back-mutation rate of an adenineless strain (# 38701) of *Neurospora crassa*. The figures in the last column give the number of back-mutations per 10^6 treated macroconidia (the average number of nuclei in the macroconidia is 2-3) under optimal conditions. 'Optimal conditions' are defined as the treatment which induces the highest overall number of back-mutations, corresponding to the peak of the curves shown in Figure 1*.

Mutagen	Chemical formula	Mol. conc.	Time min	% survived	Mutations per 10^6 conidia
1. Diepoxybutane		0·2	40	56	*85*
2. Dimethyl sulphate	$(CH_3)_2SO_4$	0·005	30	44	*64*
3. Epichlorohydrin		0·15	45	42	*56*
4. Chloroethyl methane sulphonate (CB 1506)	$Cl \cdot CH_2 \cdot CH_2 \cdot O \cdot SO_2 \cdot CH_3$	0·1	13	58	*51*
5. Glycidol		0·5	60	26	*34*
6. *p*-N-di(β-chloroethyl)phenylalanine CB 3025. *L*-form		0·03	40	100	*22*
7. Propylenoxide		0·5	60	27	*21*
8. Diethyl sulphate	$(C_2H_5)_2SO_4$	0·04	40	68	*18*
9. Ethyl methane sulphonate (CB 1528)	$CH_3 \cdot CH_2 \cdot O \cdot SO_2 \cdot CH_3$	0·1	12·5	14	*17*
10. Ethylenoxide		0·025	15	63	*17*
11. Ethylenimine		0·05	30	75	*16*
12. Hydrogen peroxide + Formaldehyde	$H_2O_2 + HCHO$	0·06 + 0·3	30	20	*4·3*
13. 'Nitrogen Mustard' Di(β-chloroethyl) methylamine		0·0025	25	60	*3·4*
14. 1,2-monoepoxybutane		0·2	40	47	*3·2*
15. *p*-N-di(β-chloroethyl)phenylanine CB 3026. *D*-form		0·03	120	90	*3·1*
16. Epibromohydrine		0·08	45	40	*2·5*
17. Monochloro-'mustard' (β-chloroethyl) dimethylamine	$(CH_3)_2N \cdot CH_2 \cdot CH_2 \cdot Cl, HCl$	0·005	60	80	*1·7*
18. tert. butylhydroperoxide	$(CH_3)_3 \cdot C-O-OH$	0·09	30	50	*1·5*
19. Diepoxypropylether		0·1	20	65	*0·7*
20. Triethylene-melamin (TEM)		0·02	50	55	*0·6*
21. Diazomethane	CH_2N_2	0·03	40	20	*0·6*
22. Trimethylphosphate	$(CH_3)_3PO_4$	0·2	40	96	*0·5*
23. Hydrogen peroxide	H_2O_2	0·2	45	10	*0·4*
24. Formaldehyde	HCHO	0·01	180	80	*0·3*

* Data compiled from K. A. JENSEN, G. KØLMARK, I. KIRK, and M. WESTERGÅRD, Cold Spring Harbor Symp. Quant. Biol. *16*, 245 (1951). – G. KØLMARK and M. WESTERGÅRD, Hereditas *39*, 209 (1953). – G. KØLMARK, Hereditas *39*, 270 (1953). – G. KØLMARK and N. H. GILES, Genetics *40*, 890 (1955). – G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. physiol. *26*, 204 (1956); G. KØLMARK (unpublished).

directly in *Drosophila*, where treating the flies with KCN enhances the mutagenic effect of X-rays[32]. It seems likely that organic peroxidases in the cells have the same function as catalase and act as anti-mutagens. This discovery of the mutagen-antimutagen system has far reaching consequences for the understanding of the origin of spontaneous mutations as well as the action of mutagens. It is seen how chemicals can act not only directly, but also indirectly by destroying or inhibiting natural antimutagens in the cells. Hydrogen peroxide may be called a mutagen 'of the first order' whereas potassium cyanide is a mutagen 'of the second order'.

Some of KØLMARK's results with the double mutant are shown in Figure 1 and in Table II. These results add an interesting new pattern to the picture, namely the relative specificity of mutagens[33]. As was shown in Table I, the adenine-mutant is very sensitive to treatment with, for instance, diepoxybutane. It was a great surprise to find that the inositol-mutant studied was almost resistant to this treatment. This does not mean, however, that the inositol-mutant always has a lower mutation rate. If ultraviolet light is used as a mutagen, the picture is reversed (Fig. 1*a*). However, most chemicals have induced very few mutations in the inositol strain with the remarkable exception of dimethyl sulphate and diethyl sulphate (Fig. 1*b*), the two compounds which were first tested

[32] F. H. SOBELS, Z. Ind. Abst.-Vererb.-Lehre *86*, 399 (1955).

[33] G. KØLMARK, Hereditas *39*, 270 (1955). – G. KØLMARK and N. H. GILES, jr., Genetics *40*, 890 (1953).– G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. Physiol. *26*, 204 (1956).

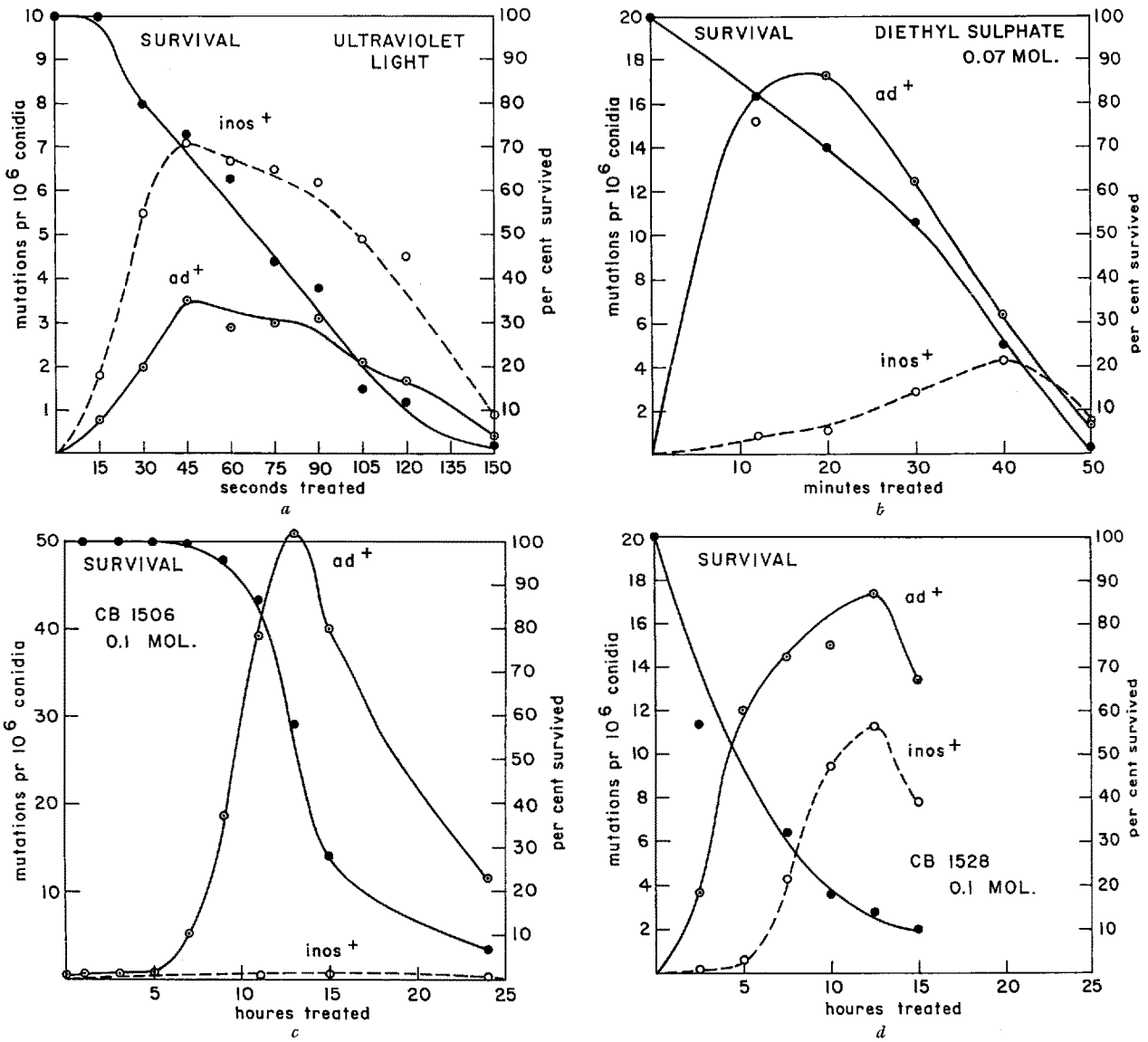


Fig. 1.—The effect of 4 mutagens on the double mutant adenineless (# 38701), inositolless (# 37401). The right ordinate refers to the survival curve, the left ordinate to the number of back-mutations induced per 10^6 treated conidia*. 1*a*: ultraviolet light; 1*b*: diethyl sulphate; 1*c*: chloroethyl methane sulphonate (CB. 1506, cf. table I for chemical formula); 1*d*: ethyl methane sulphonate (CB. 1528, cf. table I). Note that the scale of the left ordinate is not the same in all the graphs.

* Data from G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. Physiol. *26*, 204 (1956), and G. KØLMARK (unpublished).

Table II

The relative specificity of 6 mutagens on the double mutant inositolless (# 27401), adenineless (# 38701). The mutagens are compared under 'optimal conditions'*

	Mutations p. 10^6 conidia		Proportion ad$^+$/inos$^+$
	inos$^+$	ad$^+$	
1. CB 1528**	11·3	17·4	1·5
2. Ultraviolet light . . .	7·1	3·5	0·5
3. Diethylsulphate	4·3	16·8	4
4. Dimethylsulphate . . .	3·4	64·0	19
5. CB 1506**	0·3	51·0	190
6. Diepoxybutane	0·2	89·0	445

* Data from G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. Physiol. *26*, 204 (1956); and G. KØLMARK (unpublished).
** See Table I for chemical structure.

on *Drosophila* by RAPOPORT[31]. More recently, KØLMARK (unpublished) has found an even more striking difference between two methane-sulphonate compounds, the chloroethyl and the ethyl derivates (Fig. 1*c* and *d*). It is very interesting that the only two chemicals which appreciably enhance the reverse-mutation rate of the inositol mutant are both ethylating agents. Especially in the case of diethyl sulphate (Fig. 1*b*), it is obvious that the dosis-effect curves for the response of the two mutants are quite different; the maximum effect appears much later and at a much higher killing rate in the inositol mutant than in the adenine mutant. Reconstruction experiments have shown that selective killing cannot explain these differences[34]. Table II summarizes this pattern of relative specificity of the two mutants after various treatments. It should be stressed, however, that these differences are not to be ascribed to the two *loci* controlling adenine and inositol synthesis, but they are characteristic of the two mutant alleles selected for these investigations, adenine strain # 38701, and inositol strain # 37401. Both GILES and KØLMARK have found other inositol mutants which behave in their reverse-mutation pattern like the adenine strain, and *vice versa*, and it should be kept in mind that many, and probably most, auxotrophic mutants are completely stable, or at least unable to revert through back-mutation. *The back-mutation pattern of a given mutant gene, as defined by its response to various physical and chemical mutagens, depends upon how the gene was originally damaged.*

However, the degree of destruction does not depend primarily upon the mutagen used. Recently, GILES and DE SERRES have shown that even X-ray induced mutations do not represent irreversible losses, because a number of X-ray induced adenine mutants are able to back-mutate, thus refuting the opinion, held by STADLER, that all X-ray induced mutations are loss mutations[35].

Thus, it becomes clear, however, that the classical concept of the gene as a unit of mutation can no longer be maintained. The gene, defined as a functional unit, can be damaged in many different ways, there are many 'sites of damage' within the gene, and each mutation may be characterized by its specific reverse mutation pattern, spontaneous and induced. We shall revert to this most important point later. Two results should still be mentioned in order to complete this account of the back-mutation test.

KØLMARK and GILES[36] and KØLMARK[34] have been able to obtain further information about the mechanism of induction of back-mutations in the adenine *locus*. They have shown that the efficiency of a mutagen on the adenine strain is correlated with its dipole characteristics. By comparing the effect of a number of monofunctional epoxides, they find that the stronger the electro-positive charge of the carbonium ion, the stronger is the mutagenic effect. According to KØLMARK, the same is true for the alkyl esters[34]. This suggests that the mutagen reacts with 'something' which carries an electro-negative charge in (or close to) the adenine gene. Such an ionic attraction may perhaps explain the 'relative specificity' of some chemical mutagens.

Finally, some important recent results obtained in GILES' laboratory should be mentioned. He has developed very precise methods for measuring the degree of phenotypic identity between the back-mutated strains and the 'wild-type' strain from which the mutants originated. The best results come from work on a gene which controls the last step in adenine synthesis, namely the splitting of adenylosuccinic acid into adenylic acid and fumaric acid[37]. The enzyme responsible for this splitting (adenylosuccinase) has been isolated from 'wild-type' strains. However, mutants which are blocked in this terminal step lack this enzyme. In back-mutated, prototrophic strains which originated spontaneously or were induced by mutagenic treatment, the enzyme was again present. Quantitative studies of the enzyme activity showed, however, that the activity in the back-mutated strains was not up to the normal, e.g. 'wild-type' level. Does this mean that the back-mutations are incomplete, and that the original 'wild-type' gene has not been completely restored? This question can probably not be answered, but it might be pointed out that natural selection may come into the picture. This assumption is based upon the following line of reasoning: We start from a 'wild-type' gene which has long been exposed to, and presumably stabilized, through natural selec-

[34] G. KØLMARK, C. r. Trav. Lab. Carlsberg, Sér. Physiol. *26*, 204 (1956).

[35] N. H. GILES, jr., *Brookhaven Symp. in Biol. 8*, 103 (1955).
[36] G. KØLMARK and N. H. GILES, jr., Genetics *40*, 890 (1955).
[37] N. H. GILES, jr., Intern. Genetics Symp. Japan *91*, (1956) and personal communication.

tion. This gene is inactivated and probably partly destroyed by a mutagenic treatment. By further mutagenic treatment, a back-mutation is induced. We may perhaps consider the back-mutated gene as a 'new gene', not yet 'lubricated' and stabilized through natural selection. It will be very interesting to learn whether the enzymatic activity in Dr. GILES' back-mutated strains can be restored to normal level through natural and artificial selection.

A certain optimism with respect to chemical mutagenesis as an important tool for both theoretical and applied genetics was expressed at the beginning of this paper. The reason for this optimism may be summarized in the following paragraphs.

(1) There is no overall theory which can explain the induction of all types of mutations. Each type of mutations (point-mutations, mutations due to chromosome breakage, cytoplasmic mutations, etc.) must be dealt with separately. It is therefore necessary to have specific quantitative methods for studying the different mutational events separately. Such methods are now available.

(2) It has been shown that chromosomes can be broken by many different chemicals which act through various mechanisms (alkylating agents, enzyme poisons, chelating agents, etc.). Point-mutations (using back-mutations as a model), on the other hand, are induced only by a limited group of chemicals which are very unstable and reactive. The release of free energy through the reaction of such compounds with cell constituents may be a decisive factor in the ultimate mutational event. Probably all mutagens which induce point-mutations also break chromosomes, but not all 'chromosome mutagens' are able to induce gene (back) mutations.

(3) A further important step towards the understanding of the action of chemical mutagens comes from the knowledge that mutagens are counterbalanced by antimutagens. The mutation rate may therefore be enhanced by chemicals which act either directly (mutagens of the first order) or indirectly, by destroying or inhibiting natural antimutagens (mutagens of the second order). The hydrogen peroxide-catalase-potassium cyanide system may, as already mentioned, serve as a model for such a mechanism.

(4) A number of chemical mutagens, such as hydrogen peroxide and organic peroxides, and probably others, must be considered 'natural mutagens' and are responsible for at least part of the spontaneous mutations in plants, animals, and man. They therefore deserve the same attention as 'natural radiation' when problems such as the origin of spontaneous mutations are discussed.

(5) We now begin to realize that there is no sharp demarcation line between the physical and chemical approach to the study of mutations; the gap is bridged by the discovery of the mutagenic effect of peroxides and organic peroxides. The recent discovery by SOBELS of the synergistic effect of formaldehyde or potassium cyanide and X-ray treatment on the mutation rate in *Drosophila*, and SHELDON WOLFF's demonstration of a similar effect of versene treatment and X-rays on *Vicia*, should be mentioned in this connection.

(6) We are—and should be—much concerned about genetical radiation hazards, because man-made radiation (from the widespread use of X-rays in medical therapy and diagnosis, and from peaceful and military uses of atomic energy) has increased the natural background radiation by perhaps 25%. However, perhaps not more than 10% of the spontaneous mutations in man are due to natural radiation. It is, in the present author's opinion, quite possible that man's internal as well as external chemical environment has been changed even more drastically by 'man-made chemicals': Internally through the growing use of stimulants, drugs, and antibiotics. Even weed-killers may be concentrated in the human body *via* the vegetables we eat. It is known that most antibiotics and many drugs are mutagenic, because they will break chromosomes in *Vicia*, *Allium*, etc. Maleic hydrazide, which has been widely used in agricultural practice, is also a powerful mutagen. Even more important is perhaps the change in our external chemical environment. Exhaust fumes from automobiles and factories are creating the notorious 'smog' over many big cities, and it is known that organic peroxides are major constituents of this 'smog'—and that peroxides are mutagenic. We may soon have to take as drastic steps to protect our genes from man-made chemical mutagens as we take in trying to protect our descendants from man-made radiation.

However, because of the close (although not yet fully understood) relation between mutagenesis and carcinogenesis, these problems concern our own generation too. Many chemical mutagens are also carcinogenic (although the correlation may not be 100%). Fortunately, there is a more optimistic aspect to the mutation-cancer problem, because some chemical mutagens are also tumour inhibitors and used as such in cancer chemotherapy.

(7) The most important aspect of chemical mutagenesis as a tool in fundamental genetical research is perhaps the new possibility for characterizing individual mutant alleles. The development of adequate screening tests has made it possible to study quantitatively mutation rates, and especially back-mutation rates, of single genes, and the individual mutant allele can now be characterized not only by its spontaneous mutation frequency and by its response to X-rays and ultraviolet light, but also by its response to many chemical mutagens. As we have seen, the consequence of this improvement of our analytical tool has been *that the concept of the gene as a unit of function and of mutation has broken down.*

It should be pointed out that this situation is completely parallel to the one which now exists with respect the reevaluation of our concept of the gene as a unit of recombination. Screening methods (again mainly in microorganisms) have been developed by which it is possible to test for crossing-over events of the order of 10^{-8}. Thereby the phenomenon of intra-allelic crossing-over has been discovered. (In *Aspergillus* by PONTECORVO and his group in Glasgow[38]; in *Neurospora* by GILES[25], MITCHELL[39], and others; in bacteria by DEMEREC[40]; in virus by SEYMOUR BENZER[41].) Recombination within the gene (between what, from a functional point of view, must be considered alleles of the same *locus*) is also a fact in *Drosophila*[42]. In the classical 'white-series' of multiple alleles, 'white' and 'eosin' are now for the first time on the same chromosome. Whether it will be necessary to introduce the new concept of 'Gene Conversion' to explain some phenomena connected with intra-allelic, meiotic interaction (as suggested by LINDEGREEN) remains to be seen (for further discussion see LINDEGREEN[43], MITCHELL[39], EMERSON[44], PRITCHARD[38], SANSOME[45], and WINGE[46]).

In conclusion a few general comments on the impact of these results upon our concept of the gene: Some geneticists think that they have completely invalidated the old concept, but this is not necessarily true. At least to the present author, the situation is rather clear. From the beginning, genetics operated with three gene-concepts: The gene as a unit of function, the gene as a unit of recombination, and the gene as a unit of mutation[47]. Until 10–15 years ago, everybody was satisfied, because the gene as a functional unit was at the same time the recombinational and mutational unit. In recent years, the resolving power of our genetical analytical tools for studying recombination as well as mutation has been increased by a factor of 1000 or more. It is possible to test millions of gametes in much less time than it took before to test hundreds or thousands. This has given much more detailed information about the fine-structure of the gene[48]. However, the tools for studying mutational and recombinational events are now so refined that in using them we interfere more and more with the functional integrity of the gene. As a consequence, the two approaches to the study of the gene, the functional and the mutation-recombination approach, are gradually becoming mutually exclusive. This does not mean, however, that the new data on subgenic mutations and recombinations are inconsistent with the concept of the functional integrity of the gene; but it is not unlikely that we are approaching a situation in biology which has for years been known in physics, namely the principle of complementarity[49]. The results of different approaches to the study of the gene should not be considered mutually exclusive, but complementary.

There is, however, one aspect of present day genetical research which seems somewhat distressing. Classical genetics was a synthesis of results from genetical crossing experiments and visible light microscopy. Today the resolving power of the genetical tools is pushed far beyond that of light microscopy, which is practically useless in the study of phenomena such as intra-allelic crossing-over, point-mutations etc. The electron microscope may have sufficient resolving power, but it has been unable to serve its purpose in genetics because of many technical difficulties with fixation and dehydration. In the meantime, new tools such as cytochemistry, crystallography, and the use of isotopes, provide unforeseen possibilities for studying genetical mechanisms on a molecular level. The WATSON-CRICK model of the DNA-molecule, with its possibilities for explaining DNA replication, has already been mentioned. It is, however, still an open question how far we dare generalise from this model. It is true that the brilliant isotope experiments of HERSHEY and others have shown that, in bacterial viruses, the genetical information must be in the DNA[50]. However, we also know that in plant viruses like TMV (Tobacco Mosaic Virus) the genetical information is probably in the RNA[51]; in higher organisms with organized chromosomes it may well be the nucleo-proteins which are the carriers of genetical information. Despite the many brilliant and stimulating papers on the genetical function and replication of DNA as visualized from the WATSON-CRICK model[52], the next major breakthrough in genetics may come when the cytological approach can again keep pace with genetical analysis; when the electron microscope takes its proper place in genetical analysis, when we can study the submicro-

[38] R. H. PRITCHARD, Heredity *9*, 343 (1955).

[39] MARY B. MITCHELL, C. r. Trav. Lab. Carlsberg, Sér. Physiol. *26*, 285 (1956).

[40] M. DEMEREC *et al.*, Carnegie Publ. *612* 1 (1956).

[41] S. BENZER, Proc. Nat. Acad. Sci. Wash. *41*, 344 (1955).

[42] E. B. LEWIS, Amer. Nat. *89*, 73 (1955). – M. E. MCKENDRICK and C. PONTECORVO, Exper. *8*, 309 (1955). – Cf. also the classical 'lozenge' case in *Drosophila:* M. M. GREEN, Proc. Nat. Acad. Sci. Wash. *35*, 586 (1949).

[43] C. C. LINDEGREN, Science *121*, 605 (1955).

[44] S. EMERSON, C. r. Trav. Lab. Carlsberg, Sér. Physiol. *26*, 71 (1956).

[45] EVA SANSOME, C. r. Trav. Lab. CARLSBERG, Sér. Physiol. *26*, 315 (1956).

[46] Ø. WINGE, Heredity *9*, 373 (1955).

[47] S. BENZER, in '*The Chemical Basis of Heredity*' (Baltimore 1957).

[48] It should be pointed out that already in 1940, MULLER discussed this problem of the divisibility of the genetic material. See H. J. MULLER and D. RAFFEL, Genetics *25*, 541 (1940).

[49] N. BOHR, *The Unity of knowledge* (Doubleday, New York 1955), p. 47. – M. DELBRÜCK, Trans. Connecticut Acad. Arts. Sci. *38*, 173 (1949).

[50] Cf. A. D. HERSHEY and M. CHASE, J. Gen. Physiol. *36*, 39 (1952).

[51] H. FRAENKEL-CONRAT and R. S. WILLIAMS, Proc. Nat. Acad. Sci. Wash. *41*, 690 (1955).

[52] M. DELBRÜCK and G. STENT in '*The Chemical Basis of Heredity*' (Baltimore 1957).

scopic basis for processes like intra-allelic crossing-over, forward- and back-mutations, 'gene conversion' etc. This day may not be far off.

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Zusammenfassung

Die vorliegende Arbeit gibt einen Überblick über neuere Untersuchungen chemischer Mutationsauslösungen mit besonderer Betonung der Induktion von Chromosomenbrüchen in Pflanzen und Rückmutationen bei *Neurospora*. Die Arbeit zeigt, dass jedes Allel ein charakteristisches Mutationsverhalten zeigt, das durch die Reaktion auf physikalische und chemische Mutagene definiert wird. Diese Ergebnisse werden unter dem Aspekt diskutiert, dass ein Gen die Einheit der Funktion, Rekombination und Mutation darstellt.

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

Der Einfluss der «Lorentz-Kontraktion» der Erde auf den Gang der Quarzuhren. II

Aus Vergleichungen von Quarzuhren mit Pendeluhren, die am Observatorium in Neuenburg angestellt wurden, ergab sich, dass die Standunterschiede zwischen den zwei Uhrentypen innerhalb von nur etwa $\pm$ 0,01 s einen linearen Verlauf nehmen, ihre Gangschwankungen also nahe gleich sein müssen. Da es nun möglich ist, für eine Pendeluhr eine theoretische Gangformel aufzustellen, welche die Wirkung der «Lorentz-Kontraktion» auf die Schwingungsdauer des Pendels ausdrückt, so kann also dieselbe Formel auch bei Quarzuhren Anwendung finden.

In meiner Abhandlung «Die beobachtete Gangschwankung der Quarzuhren und die «Lorentz-Kontraktion» der Erde»[1] habe ich die von mir abgeleitete Formel angegeben. Sie enthält in ihrem weit überragenden täglichen bzw. jährlichen Hauptglied den Faktor $\sin 2\varphi$, wo φ die Polhöhe der Beobachtungsstation bezeichnet. Daraus folgt, dass die von der «Lorentz-Kontraktion» der Erde hervorgerufene Gangschwankung in südlichen Breiten das umgekehrte Vorzeichen haben müsste wie in nördlichen, während natürlich eine etwaige Jahresschwankung in der Rotationsdauer der Erde überall gleich herauskommen würde. Die Jahreskurven der relativen Uhrkorrektionen von Quarzuhren an nördlichen und südlichen Stationen, bezogen auf das Mittel der Uhrkorrektionen an allen Stationen, werden sich daher spiegelbildlich verhalten.

Dass dies vollkommen zutrifft, habe ich schon in einer früheren Arbeit mit dem gleichen Titel[2] gezeigt, auf die ich hier bezüglich aller Einzelheiten verweise. Es konnten dort die an den Stationen Greenwich, Potsdam, Buenos Aires und Mount Stromlo erhaltenen Jahreskurven für 1948, 1949, 1950 wiedergegeben werden.

[1] L. COURVOISIER, Astronom. Nachrichten *281*, 259 (1954).
[2] L. COURVOISIER, Exper. *9*, 286 (1953).

Inzwischen liegen nun aber auch die im «Bulletin Horaire du Bureau International de l'Heure» (Paris) verzeichneten relativen Uhrkorrektionen für 1951, 1952, 1953, 1954 vor, und sie sollen hier in entsprechenden graphischen Darstellungen als Fortsetzung der schon publizierten Jahreskurven folgen. Für die nördlichen Kurven wurde das jeweilige Mittel N der in Greenwich und Potsdam beobachteten relativen Uhrkorrektionen benutzt, für die südlichen das Mittel S der Beobachtungen an den zwei Stationen in Buenos Aires (Geodätisches Institut und Naval Observatorium). Die nachstehende Tabelle enthält die Monatsmittel dieser relativen Uhrkorrektionen, die dann in den vier Figuren für jedes Jahr graphisch ausgeglichen sind.

Wie man sieht, kann gar kein Zweifel darüber bestehen, dass auch bei diesen neuen Jahreskurven der relativen Uhrkorrektionen von Quarzuhren die an den südlichen Stationen erhaltenen Kurven, abgesehen von der natur-

Monatsmittel der relativen Uhrkorrektionen (0,001 s)

$$\left(N = \frac{G + P}{2}, \qquad S = \frac{BAg + BAn}{2}\right)$$

Monat (Mitte)	1951		1952		1953		1954	
	N	S	N	S	N	S	N	S
Januar	+ 5	− 14	+ 20	− 10	+ 22	− 28	+ 21	− 22
Februar. . . .	+ 16	− 8	+ 18	− 8	+ 28	− 23	+ 26	− 21
März	0	0	+ 12	+ 3	+ 26	− 28	+ 23	− 20
April	− 2	+ 8	+ 6	+ 6	+ 8	− 22	+ 19	− 18
Mai	+ 7	+ 21	0	+ 22	+ 2	+ 6	+ 9	− 4
Juni	+ 14	+ 19	− 8	+ 40	− 11	+ 27	0	+ 7
Juli	+ 15	+ 16	− 6	+ 18	− 8	+ 15	− 8	+ 15
August	+ 23	+ 7	− 2	+ 14	0	+ 20	− 13	+ 10
September . .	+ 29	− 3	0	+ 10	+ 9	+ 12		
Oktober. . . .	+ 24	− 17	+ 4	− 6	+ 12	− 6		
November. . .	+ 23	− 21	+ 10	− 12	+ 16	− 5		
Dezember . . .	+ 18	− 13	+ 22	− 15	+ 22	− 10		