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GENETIC ASPECTS OF CARCINOGENESIS

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Abstract—Genetic alterations are considered as one of the primary causes of neoplastic growth. Genetic evidence for this notion derives from a coincidence of mutagenicity and carcinogenicity for many agents. Exceptions to this coincidence are frequent but mostly due to physiological difference between the organisms used for the carcinogenicity and the mutagenicity test. A genetic hypothesis of cancer has to comprise mutation of both nuclear and cytoplasmic genetic determinants. Pleiotropic effects of mutations could be explained by effects on the accuracy of the translational system of protein biosynthesis. A very common objection against a mutation hypothesis of cancer derives from the apparent recessiveness of most mutant alleles in relation to their wildtype alleles. This view is valid only for a superficial phenotypic level. If however, mutant and wildtype allele interactions are investigated at the level of gene products, e.g. enzymes, it can be shown that many mutant alleles produce a gene product which is directly different from wildtype or which forms a different active enzyme in a hybrid protein together with wildtype protein subunits. In addition to mutation there are two more processes leading to somatic variegation. Mitotic crossing over leads to homozygosis of detrimental genes the effects of which are only weak in the presence of wildtype alleles. Mitotic gene conversion is a process that can formally be described as directed mutation. Alleles differing at more than one site generate via gene conversion predetermined new types of alleles at frequencies much higher than by mutation. There are cellular repair systems eliminating DNA damage. Inhibition of these leads to increased genetic instabilities. Considering all these facts makes a genetic hypothesis of cancer much less contradictory.

TUMOROUS growth is caused by the failure of certain cells to obey the regulatory mechanisms of an organism. This escape from regulatory control is a rather stable property of such cells because they behave as tumor cells even after many transfers through other host individuals. This condition is highly suggestive that the processes which transform normal cells to tumor cells are of a genetic nature.

The suggestion that genetic processes can lead to neoplastic transformation does not mean that there are no other mechanisms conceivable to explain carcinogenesis. The genetic hypothesis of cancer is considered to be one of several working hypotheses and the present report is intended to provide evidence for its validity.

Direct evidence for the genetic hypothesis of carcinogenesis is difficult to obtain for the simple reason that carcinogenesis occurs in somatic cells of multicellular organisms—cells which are not amenable to direct genetic analysis. Carcinogenesis is a problem of somatic genetics. The direct approach would be to isolate a tumor cell, cross it to a non-tumorous cell, induce meiosis, and see whether cancer alleles segregate from the cross. Such a procedure is not feasible yet. Evidence for the genetic nature of tumorous transformation is available at a more indirect level. It has been observed that species hybrids in many genera of plants[1] and animals[2] may bear tumors. Analysis of such hybrid tumors has shown that they are under genetic control. Obviously, there are discrepancies in gene action in such hybrids, and genes have been located which cause neoplastic growth when combined with the genetic background of another

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species. It is conceivable, therefore, that mutation could alter a gene in a way that would cause such discrepancies even in a normal non-hybrid individual.

Mutagenesis

It has been observed that cancer can be induced by many agents and, moreover, that some carcinogens react with DNA. This has been demonstrated for potent carcinogens like nitrosamines and nitrosamides by Magee and co-workers[3] or like hydrocarbons.[4] Reaction with DNA is also typical for mutagens.[5] Hence, there is chemical evidence which suggests the involvement of mutation in carcinogenesis. Such a concept leads to the prediction that mutagens are carcinogens and vice versa.

There are three major obstacles in establishing a correlation between the carcinogenic and the mutagenic action of an agent. Carcinogenesis is studied in vertebrates, mostly in mammals. These organisms are not very suitable for investigating mutagenesis. Therefore, the comparison of the mutagenic and the carcinogenic activity of an agent is usually based on results obtained in different test organisms. Test organisms for mutagicity are usually micro-organisms, Drosophila or plants. The first difficulty arises because many carcinogens are not active as such but they have to be converted by metabolic processes to an active form—the proximate carcinogen, e.g. nitrosamines are potent carcinogens in mammals where they are activated but they are not mutagenic in microorganisms[6] where no activation occurs. Second, some potent carcinogens like hydrocarbons are very poorly soluble in water and not very reactive. It is conceivable that due to the low doses and slow reactions, these agents induce genetic damage at such a low rate that cellular repair processes can neutralize the effects in one organism but not in another. Finally, there are relatively good mutagens like sulfate and sulfonate esters which are not very potent carcinogens. In such a case it could be argued that some organisms are very sensitive against those agents at the non-genetic level, whereas others are more sensitive at the genetic level. In the first case, the agent appears to be mainly toxic, whereas in the second case it appears to be also mutagenic. Some well known mutagens which have been found to be carcinogenic are shown in Table 1. Considering the difficulties of comparison, there appears to be quite an obvious correlation between these two properties.

As pointed out already, carcinogenesis is a problem of somatic genetics. The major problem of somatic genetics is the lack of methods for analysis of genetic alterations in cells not belonging to the germ line. Fortunately, however, organisms are available which are amenable to genetic analysis and, at the same time, can be considered as models of somatic cells. The yeast *Saccharomyces cerevisiae* is such an organism. There is a stable haploid and a stable diploid phase in which vegetative cells can grow indefinitely. Any haploid cell can act as a gamete when brought into contact with another haploid cell of opposite mating type. Any diploid cell can behave as a germ line cell and perform meiosis under well controlled conditions. Hence, these cells are readily amenable to genetic analysis and genetic alterations induced in haploid or diploid cells can be easily identified.

Mutagenesis has to be studied in haploid rather than in diploid cells because in a diploid cell each gene is present in duplicate so that many mutations would remain undetected. The mutational process can be subdivided into two parts. The first part is the chemical reaction of the mutagen with DNA. This part of the process is well understood for several mutagens and carcinogens.[5] The next sequence of reactions

TABLE 1. EXAMPLES FOR THE CARCINOGENICITY OF WELL KNOWN AND POTENT MUTAGENS

Mutagen	Carcinogenicity	Mutagenicity
dimethyl-nitrosamine	7	8
diethyl-nitrosamine	9	8
methyl-vinyl-nitrosamine	10	8
methyl-butylnitrosamine	10	8
N-methyl-*N'*-nitrosopiperazine	10	8
N-methyl-*N*-nitrosourea	11	12
N-methyl-*N*-nitrosourethane	11	13
1-methyl-3-nitro-1-nitrosoguanidine	14	15
N-methyl-*N*-nitrosoacetamide	16	17
N-ethyl-*N*-nitrosourea	18	19
N-butyl-*N*-nitrosourea	14	20
1-nitroso-imidazolidone-2	14	12
diazomethane	21	22
dimethylsulphate	23	24
methylmethanesulfonate	25	26
diethylsulphate	25	24
β-propiolactone	27	28
ethylenimine	29	13
triethylene melamine	30	31
1,2:3,4-diepoxybutane	32	33
1,2-dimethyl-hydrazine	34	35
nitrogen mustards	37	36
sulfur mustards	38	39
propane sultons	40	41

The numbers refer to the corresponding references.

involves the transformation of reacted DNA into mutated DNA which means new sequences of nucleobases. These reactions are summarised as mutation fixation. Finally the new genetic information must be expressed so that a cell with a new phenotype is formed. The problem of mutation fixation is not yet well understood in eukaryotes, particularly with chemical mutagens. It has been shown that fixation of a carcinogen induced mutation in yeast is a process readily separable from the induction phase, i.e. the period of reaction of the carcinogen with DNA.[42] It is now becoming evident that certain metabolic activities of the repair type are required to establish a new genotype after DNA has been altered due to chemical attack.[43, 44]

In considerations of mutagenesis in relation to carcinogenesis the main problem concerns the functional properties of carcinogen induced mutants. Are carcinogen induced mutations gross alterations changing the sequence of many base pairs in DNA or are there very mild effects only involving the alteration of a single base pair? Genetic evidence is available to show that mutations induced with carcinogens and other chemical mutagens are of the type affecting single base pairs only.[45–47] The same conclusion has been arrived at for spontaneous mutation in eukaryotes.[48] Consequently, the majority of carcinogen induced and spontaneous mutations are only slight alterations and not gross deletions.

The base pair sequence of a structural gene codes for the amino acid sequence of an enzyme. How does a single base pair substitution affect the properties of such an

enzyme? There are thought to be two types of base substitutions according to the effect exerted at the protein level. There are missense mutations which change one amino acid code word into another one so that the polypeptide chain coded by such a mutated gene shows an amino acid replacement. A gene product of normal size is still formed. Comparative analysis of proteins like hemoglobin have shown that there are many positions in the amino acid sequence which tolerate more than one amino acid without a concomitant complete inactivation of function. However, amino acid replacements have been found to be the cause of abnormal properties of such hemoglobins.[49] The complete loss of function of hemoglobin would be fatal rather than pathological.

The other type of simple base pair substitution mutants are nonsense mutations. At least in bacteriophage and bacteria, there exist codons which are not code words for amino acids. They do not make sense in the determination of the amino acid sequences. Many amino acid code words can be converted to such nonsense codons by simple base pair substitution. The result of such a mutation on the protein would be termination of the polypeptide chain formation at the mutant site.[50, 51] There is evidence for the existence of the nonsense type of mutation in eukaryotes.[52] It is obvious that such nonsense mutations prevent completely the formation of a functional protein. In addition to simple base pair substitutions there are other types of mutation viz., insertion or deletion of base pairs. During translation of the genetic message into amino acid sequences, insertion or deletion of a base pair will shift the reading frame so that starting from the mutant site the genetic information will be misread, giving rise to frame shift mutation.[53] Evidence for the existence of this type of mutation in eukaryotes has been put forward by Magni.[54] Finally, there is the classic type of deletion which is visible at the microscopial level or else detectable by more sophisticated genetic methods.[55] This last type of mutation may lead to a significant loss of genetic information, and consequently may be lethal in the homozygous condition rather than causing abnormalities. According to present knowledge, the missense type of mutation is the most frequent among carcinogen induced and possibly also among spontaneous mutations in eukaryotes.

The problem of diploidy

Tumors are observed in diploid organisms; therefore any genetic theory of carcinogenesis has to be adapted to the genetic conditions in the diploid cell. The salient feature of a diploid cell is that all genetic, autosomal information is present in duplicate. There is some evidence to show that this double amount of genetic information is by far redundant and that half the dose of a gene is enough to allow the proper function of the cell.[56] This means that a mutation which merely inactivates one of the two alleles does not have any effect on the phenotype. In this case the wildtype allele is said to be dominant over the recessive mutant allele, and this situation is realized for most combinations of active wildtype and inactive mutant alleles. This concept of dominance and recessiveness is based on the conclusive consideration of the phenotypically redundant formation of gene products by the active allele. However, the gene products of different alleles can interact to form hybrid proteins the properties of which are different from the two types of homogeneous proteins. Therefore, the interaction of two alleles cannot be considered only under quantitative but also under qualitative aspects, i.e. expression of new properties.

Many enzymes are composed of identical subunits or monomers, such enzymes are called aggregate or multimeric enzymes. In a heterozygous cell carrying two allelic forms of a structural gene two types of monomers are formed. The different monomers not only form homogeneous multimers or hybrid proteins. The hybrid proteins can have properties not expressed by either of the homogeneous type of monomers.[57] This situation is quite dramatically realized in the phenomenon of intragenic or interallelic complementation. Two mutant alleles of a structural gene are unable to code for a functional enzyme protein so that both mutant alleles cause, in the haploid or in the homozygous diploid state, a requirement for a nutrilite. When the same two alleles are brought together to form a diploid heteroallelic cell, the nutritional requirement is alleviated. This phenomenon has been observed in many organisms and for many gene loci. The explanation of this phenomenon was put forward in 1958 by Catcheside and Overton.[58]

Interallelic complementation is a good criterion to test the functional properties of carcinogen induced mutations. In the case of mutants induced in yeast with the carcinogen 1-nitrosoimidazolidone-2,[14] Nashed[59] found that all of 120 mutants were of the complementing type. Malling and de Serres[60] using the carcinogen 1-methyl-3-nitro-1-nitrosoguanidine[14] were so impressed by the high ratio of complementation among mutants induced by this carcinogen in *Neurospora* that they suggested that this effect might be typical for efficient carcinogens. These observations confirm again that most carcinogen induced mutations are of the missense type. Hybrid enzyme formation is observed also in mutant × wildtype heterozygotes. We have induced mutants of the gene locus is_1 in *Saccharomyces cerevisiae* with the carcinogen 1-nitroso-imidazolidone-2. Altogether 35 mutants were induced all of which required isoleucine for growth and were free of threonine dehydratase. These mutants were crossed to the wildtype and threonine dehydratase from the resulting heterozygotes was assayed. The formation of hybrid enzymes could be demonstrated for many heterozygotes. The most notable cases were those where a feedback resistant hybrid enzyme was formed.[61] Hybrid enzymes with reduced substrate affinities were found relatively frequently, and this even with mutant alleles which according to the general classification in the interallelic complementation test would have been classified as generating none or only a strongly disordered gene product.[62] In all these cases, alleles coding for catalytically inactive enzyme caused the formation of hybrid enzymes with altered properties. There was no complete recessiveness of the mutant alleles or phenotypic masking of their presence by the wildtype allele. A general objection to the mutation hypothesis of cancer is based on the apparent recessiveness of mutant versus wildtype alleles. This objection should be considered with considerable reserve.[63]

The hybrid protein concept does not only apply to enzymes. The membrane systems of the cell contain both lipids and proteins. The membrane bound protein can be considered as belonging to huge multimeric aggregates of the same extent as the membranes. The insertion of a mutant protein into such a huge aggregate would occur also if the cell is a mutant × wildtype heterozygote at one of the corresponding loci, and this could have marked consequences. This idea put forward by Wallach[64] places emphasis on the role of mutation in diploid cells and points to the major importance of the functional properties of carcinogen induced mutations. Most carcinogen induced mutations are functional in a manner to show hybrid enzyme formation.

Mutations affecting translation of the genetic message

The base sequence and consequently the genetic information of a cell is transcribed from DNA to messenger RNA. This species of RNA takes the genetic message to the protein synthesizing sites where the base sequence of mRNA is translated into the amino acid sequence of the polypeptide chain. Three major components are involved in this translational process: ribosomes, transfer RNA and amino acid activating-enzymes. Mutations in structural genes coding for these components are known to lead to the incorporation of wrong amino acids into the newly formed polypeptide chain. Such mutations are called suppressor mutations. There are two types of suppressors: nonsense suppressors[65] and missense suppressors.[66, 67] Nonsense suppressors cause the insertion of an amino acid in response to a codon site of mRNA occupied by a code word which does not code for an amino acid in a normal cell. Missense suppressors cause, at a low degree of efficiency the insertion of an amino acid into the growing polypeptide chain at a position normally occupied by a different amino acid. This erroneous incorporation can occur at any gene of the entire genome provided there is the one specific codon the misreading of which is caused by the missense suppressor. The frequency of misreading a particular code word could be very low but this misreading would cause the formation of abnormal varieties of numerous proteins of structural, catalytic or regulatory functions. The mutation producing a missense suppressor could therefore have a pleiotropic effect, i.e. it would cause many different alterations. The possibility is quite attractive for a genetic theory of cancer but there is not much experimental evidence as yet.

Cytoplasmatic mutation

Most of the genetic information in a eukaryote cell is located in the nucleus but there is also genetic material to be found in organelles such as plastids or mitochondria.[68] Consequently, there should be mutation also at the cytoplasmic level. In respect to carcinogenesis mitochondrial mutation is most relevant because mitochondria are the carriers of the energy supplying cellular machinery. It is not very easy to study mitochondrial mutation because of the large numbers of mitochondria present in a cell. Nevertheless, many carcinogens have been found to mutate mitochondria in yeast, e.g. *N*-nitro-methylurethane, 1-nitrosoimidazolidone,[69] 1-methyl-3-nitro-1-nitrosoguanidine[70] or anthracene derivatives.[71] More sophisticated mutation systems could be established on the basis of mitochondrial resistance to certain antibiotics.[72] The green alga *Chlamydomonas* might provide promising systems for cytoplasmic mutation.[73] Extrakaryotic, mitochondrial genetic material shows differences from chromosomal material in its responses to mutagens.[69] The important role of mitochondria in cellular metabolism emphasises the possible importance of mitochondrial mutation in carcinogenesis.[74]

Mitotic reciprocal recombination and gene conversion

The genotype of a multicellular organism is established after the fusion of the two haploid gamete nuclei during the process of zygote formation. The genetic identity of all descendant cells is insured by the accuracy and fidelity of DNA replication and mitotic nuclear division. Consequently all cells of a multicellular organism should be genetically identical. Variation is caused only by mutation if the two gametes are

genetically identical for all genes excepting those involved in sexual differentiation. However, mutation is not the only process to cause genetic variation in the cells descending from a *heterozygous* zygote nucleus. In mitotic cells, homologous chromosomes make localized contact on rare occasions and this contact results in two exchange processes. One is reciprocal recombination or crossing over which causes homozygosis for long segments of chromosomes distal to the site of interaction[75]—the other process is a non-reciprocal interaction which is called gene conversion, inter-allelic recombination or non-reciprocal recombination. It differs from mitotic, reciprocal crossing over in several ways. Crossing over always leads to two reciprocal products—gene conversion produces only one "recombinant" type.[76, 77] Moreover, gene conversion does not lead to the rearrangement of large chromosomal segments, the rearrangements concern chromosomal regions usually much smaller than the size of a gene. Therefore, gene conversion is also called interallelic recombination. In mitotic or somatic cells these two processes are called mitotic crossing over and mitotic gene conversion. Mitotic gene conversion is relatively frequently associated with a crossing over event in the vicinity. This coincidence is rather variable with different gene loci and organisms.

Mitotic crossing over leads to homozygosis and consequently to the expression of recessive alleles which could be responsible for neoplastic growth. Mitotic recombination can be investigated in cells with appropriate marker arrangements in a way described by C. Stern in 1936.[75] Several mutagens and carcinogens have been found to induce mitotic recombination (see Table 2). The list presented is not very long since the unequivocal demonstration of the induction of mitotic crossing over is rather laborious. If an individual is heterozygous for recessive genes causing tumorous growth, mitotic crossing over could generate the genetic condition for tumor initiation. In this case, however, a genetic pre-disposition is a necessary condition.

TABLE 2. INDUCTION OF MITOTIC CROSSING OVER AND MITOTIC GENE CONVERSION WITH SOME CARCINOGENS

Chemicals	Carcinogenicity	Mitotic crossing over	Mitotic gene conversion
1-methyl-3-nitro-1-nitrosoguanidine	14	78	79
N-methyl-*N*-nitrosourea	11		80
N-methyl-*N*-nitrosourethane	11	78	79
methylmethanesulfonate	25		80
methyltoluenesulfonate	25		81
N-ethyl-*N*-nitrosourea	18		81
diethylsulphate	25	78	79
1-nitroso-imidazolidone-2	14	78	79
1,2-dimethylhydrazine	34	82	
ethylenimine	29	83	81
diepoxybutane	32		81
N-acetoxy-2-acetyl-aminofluorene (proximal carcinogen)	84		81
p-(*N*-acetoxy-*N*-acetyl-amino)-trans-stilbene (proximal carcinogen)	85		81
9,10-dimethyl-benz(a)anthracene	86		87

The numbers refer to the corresponding references.

Mitotic gene conversion can also lead to simple homozygosis by transferring the genetic information of one allele to replace the other allele on the homologous chromosome. The frequency of spontaneous mitotic gene conversion is of the order of 1 per 10^5 cell divisions whereas mitotic crossing over occurs spontaneously at frequencies between 1 per 10^3 to 10^4 cell divisions. This ratio holds also true for induced frequencies at moderate doses of the inducing agent.

The relevance of mitotic gene conversion does not reside in the creation of homozygosis in mutant × wildtype heterozygotes. It is known that many series of multiple alleles occur in human populations. In such cases alleles can be combined in a diploid nucleus which do not differ at one site only, as in the case of simple heterozygosis, but at two or more sites. Gene conversion brings about the transfer of small pieces of genetic material between strictly homologous regions of chromosomes. This can be easily demonstrated with two mutant alleles of different origins. In our experiments we have used mutant alleles that caused nutritional requirements in the haploid or else in the homozygous or homoallelic diploid state. Moreover, these alleles were very stable in the haploid or in the homoallelic diploid condition and they produced prototrophic cells at very low frequencies. The stability of such alleles was drastically reduced, and numerous cells arose without nutritional requirement from a heteroallelic diploid carrying two different mutant alleles instead of only one. This instability could be drastically increased up to 1000-fold by treating cells with carcinogens. Numerous carcinogens were found to enhance this heteroallelic instability or mitotic gene conversion. All mutagens that were available to us were found to induce mitotic gene conversion provided that they were biologically active in yeast[79, 80] (Table 2).

The biological importance of mitotic gene conversion resides in two main features. First, there is the instability created in heteroallelic cells and second, there is the strong directedness of the newly created allele. This directedness of the newly formed active alleles obtained from heteroallelic diploids after treatment with the carcinogen *N*-nitroso-*N*-methylurethane was tested using an isoleucine requiring heteroallelic diploid of the type is_{1-1}/is_{1-2}: Convertants not requiring isoleucine for growth were analysed genetically,[79] and the haploid segregants carrying the active alleles were tested for the properties of their threonine dehydratases. Threonine dehydratase is the enzyme coded for by the is_1-locus. Altogether 23 convertants were analysed and all of them were found to produce an enzyme indistinguishable from wildtype.[88] The same accuracy of conversional information transfer has been found in other systems.[89, 90] This means that mitotic gene conversion can be considered equivalent to directed mutation. Mitotic gene conversion causes the formation of well defined new alleles starting with a given pair of heteroalleles. This situation provides a model for a genetic predisposition in carcinogenesis in which certain pairs of heteroalleles generate alleles which might cause tumorous growth. This process can be induced by genetically active agents.

Interference with cellular systems repairing genetic damage

It has been demonstrated that there are cellular mechanisms which repair damage to the genetic material. This was found to be true for chemically induced damage,[91] and it occurs in mammalian cells.[92] These repair activities can be inhibited so that cells become very sensitive to genetic alterations. A well known example is the caffeine effect in bacteria.[93] A similar situation was created in yeast cells which had lost the

ability to perform oxidative metabolism. Energy supply in such cells is derived from anaerobic glycolysis exclusively. In the absence of glucose, the cells showed an increased frequency of mitotic gene conversion and sensitivity to subacute doses of carcinogens was enhanced as well. Under these conditions energy requiring processes were considered to be blocked, and the observed increase in genetic alterations was ascribed to the inefficiency of the repair systems.[80, 87]

These observations suggest that genetic alterations and carcinogenesis are not only induced by agents reacting directly with DNA. Agents interfering with repair processes may also be effective.

CONCLUSION AND SUMMARY

There is a good correlation between carcinogenicity and genetic activity of many chemicals. Several genetic mechanisms can create the conditions necessary for carcinogenesis including mutation of chromosomal and cytoplasmic genetic material, mitotic crossing over and mitotic gene conversion. Genetic alterations and, according to the concept reported here, carcinogenesis may not only be induced by agents reacting directly with DNA but also by agents interfering with cellular repair processes. Objections to a genetic hypothesis of cancer should not, therefore, be based on the observation that some carcinogens do not react with DNA. A further objection to the genetic hypothesis of cancer is the recessiveness of induced mutations as compared to wildtype. Carcinogen induced mutations allow the formation of gene products which can interact with the gene products of the corresponding wildtype alleles to form hybrid enzymes with new properties. Mitotic recombination and gene conversion lead to intraindividual genetic variegation under defined genetic preconditions, a situation providing a genetic model to explain a hereditary disposition to carcinogenesis.

REFERENCES

1. M. R. AHUJA, *Quart. Rev. Biol.* **40**, 329 (1965).
2. F. ANDERS, *Experientia* **23**, 1 (1967).
3. P. N. MAGEE and E. FARBER, *Biochem. J.* **83**, 114 (1962).
4. P. BROOKES and P. D. LAWLEY, *Nature, Lond.* **202**, 781 (1964).
5. P. D. LAWLEY, *Prog. Nucleic Acid Res. Molecular Biol.* **5**, 89 (1966).
6. H. MARQUARDT, F. K. ZIMMERMANN and R. SCHWAIER, *Z. Vererbungsl.* **95**, 82 (1964).
7. P. N. MAGEE and J. M. BARNES, *Br. J. Cancer* **10**, 114 (1956).
8. L. PASTERNAK, *Arzneimittel. Forsch.* **14**, 802 (1964).
9. D. SCHMÄHL, R. PREUSSMANN and H. HAMPERL, *Naturwissenschaften* **47**, 89 (1960).
10. H. DRUCKREY, Z. PREUSSMANN, D. SCHMÄHL and M. MÜLLER, *Naturwissenschaften* **48**, 134 (1961).
11. H. DRUCKREY, R. PREUSSMANN, D. SCHMÄHL and M. MÜLLER, *Naturwissenschaften* **48**, 165 (1961).
12. H. MARQUARDT, F. K. ZIMMERMANN and R. SCHWAIER, *Naturwissenschaften* **50**, 625 (1963).
13. J. A. RAPOPORT, *Dokl. Akad. Nauk. USSR* **59**, 1183 (1948).
14. H. DRUCKREY, R. PREUSSMANN, S. IVANKOVIĆ, B. T. SO, C. H. SCHMIDT and J. BÜCHELER, *Z. Krebsforsch.* **68**, 87 (1966).
15. J. D. MANDELL and J. GREENBERG, *Biochem. biophys. Res. Commun.* **3**, 575 (1960).
16. H. DRUCKREY, R. PREUSSMANN, S. IVANKOVIĆ, D. SCHMÄHL, J. AFKHAM, G. BLUM, H. D. MENNEL, M. MÜLLER, P. PETROPOULOS and H. SCHNEIDER, *Z. Krebsforsch.* **69**, 103 (1967).
17. F. K. ZIMMERMANN and R. SCHWAIER, *Z. Vererbungsl.* **94**, 261 (1963).
18. H. DRUCKREY, S. IVANKOVIĆ and R. PREUSSMANN, *Nature, Lond.* **210**, 1378 (1966).
19. J. A. RAPOPORT, *Dokl. Akad. Nauk. USSR* **146**, 1418 (1962).
20. T. GICHNER and J. VELEMINSKÝ, *Mutat. Res.* **4**, 207 (1967).
21. R. SCHOENTAL and P. N. MAGEE, *Br. J. Cancer* **16**, 92 (1962).
22. K. A. JENSEN, G. KØLMARK and M. WESTERGAARD, *Hereditas* **35**, 521 (1949).
23. H. DRUCKREY, R. PREUSSMANN, N. NASHED and S. IVANKOVIĆ, *Z. Krebsforsch.* **68**, 103 (1966).

24. J. A. RAPOPORT, *Dokl. Akad. Nauk* **12**, 12 (1947).
25. P. F. SWANN and P. N. MAGEE, *Biochem. J.* **110**, 39 (1968).
25a. PREUSSMANN, personal commun.
26. O. G. FAHMY and M. J. FAHMY, *Nature, Lond.* **180**, 31 (1957).
27. F. DICKENS and H. E. H. JONES, *Br. J. Cancer* **15**, 85 (1961).
28. H. H. SMITH and A. M. SRB, *Science* **114**, 490 (1951).
29. A. L. WALPOLE, *Ann. N.Y. Acad. Sci.* **68**, 750 (1958).
30. J. A. HENDRY, F. L. ROSE and A. L. WALPOLE, *Br. J. Pharmac.* **6**, 201 (1951).
31. M. J. BIRD, *J. Genet.* **50**, 480 (1952).
32. A. HADDOW, *Br. Med. Bull.* **14**, 79 (1958).
33. H. G. KØLMARK and M. WESTERGARD, *Hereditas* **39**, 209 (1953).
34. H. DRUCKREY, R. PREUSSMANN, F. MATZKIES and S. IVANKOVIĆ, *Naturwissenschaften* **54**, 285 (1967).
35. F. LINGENS, *Z. Naturforsch.* **19b**, 151 (1964).
36. C. AUERBACH and J. M. ROBSON, *Proc. Roy. Soc. Edin.* **B62**, 284 (1947).
37. A. HADDOW, in *The Physiopathology of Cancer, Lond.* p. 605 (1958).
38. W. E. HASTON, *J. natn. Cancer Inst.* **10**, 125 (1949).
39. C. AUERBACH, *Proc. Roy. Soc. Edin.* **B62**, 211 (1946).
40. H. DRUCKREY, H. KRUSE and R. PREUSSMANN, *Naturwissenschaften* **55**, 449 (1958).
41. H. HESLOT, *Erwin-Bauer-Gedachtnis-Vorlesungen, II.* Abh. dt. Akad. Wiss., Berl. (Med) **1**, 193 (1962).
42. F. K. ZIMMERMANN, R. SCHWAIER and U.v. LAER. *Z. Vererbungsl.* **98**, 152 (1966).
43. E. CERDA-OLMEDO and P. C. HANAWALT, *Mutat. Res.* **4**, 369 (1967).
44. C. AUERBACH and D. RAMSAY, *Molec. gen. Genetics* **103**, 72 (1968).
45. H. V. MALLING, *Mutat. Res.* **2**, 320 (1965).
46. H. V. MALLING and J. F. DE SERRES, *Mutat. Res.* **4**, 425 (1967).
47. H. V. MALLING and J. F. DE SERRES, *Mutat. Res.* **6**, 181 (1968).
48. N. LOPRIENO, S. BONATTI, A. ABBONDANDOLO and R. GUGLIEMINETTI, *Molec. gen. Genetics* **104**, 40 (1969).
49. G. BRAUNITZER, *Naturwissenschaften* **54**, 407 (1967).
50. A. S. SARABHAI, A. O. W. STRETTON, S. BRENNER and A. BOLLE, *Nature, Lond.* **201**, 13 (1964).
51. A. V. FOWLER and I. ZABIN, *Science* **154**, 1027 (1966).
52. T. R. MANNEY, *Genetics* **60**, 719 (1968).
53. F. H. C. CRICK, L. BARNETT, S. BRENNER and J. WATTS-TOBIN, *Nature, Lond.* **192**, 1227 (1961).
54. G. E. MAGNI, *J. Cell. Comp. Physiol.* **64**, 165 (1964).
55. F. J. DE SERRES, *Genetics* **58**, 69 (1968).
56. U. REICHERT, *Zbl. Bacteriol., Org.* **205**, 63 (1967).
57. J. R. S. FINCHAM, *Genetic Complementation*, New York (1966).
58. D. G. CATCHESIDE and A. OVERTON, *Cold. Spring. Harb. Symp. Quant. Biol.* **23**, 137 (1958).
59. N. NASHED, *Molec. gen. Genetics* **102**, 348 (1968).
60. H. V. MALLING and F. J. DE SERRES, *Genetics* **60**, 201 (1968).
61. F. K. ZIMMERMANN and E. GUNDELACH, *Molec. gen. Genetics* **103**, 348 (1969).
62. F. K. ZIMMERMANN, I. SCHMIEDT and A. M. A. TEN BERGE, *Molec. gen. Genetics* **104**, (1969).
63. F. K. ZIMMERMANN, *Arzneimittel. Forsch.* **19**, 1046 (1969).
64. D. F. H. WALLACH, *Proc. natn. Acad. Sci. U.S.A.* **61**, 868 (1968).
65. A. GAREN, *Science* **160**, 149 (1968).
66. J. CARBON, P. BERG and C. YANOFSKY, *Proc. natn. Acad. Sci. U.S.A.* **56**, 764 (1966).
67. N. K. GUPTA and H. G. KHORANA, *Proc. natn. Acad. Sci. U.S.A.* **56**, 772 (1966).
68. R. HAGEMANN, *Fortschritte Botanik* **30**, 225 (1968).
69. R. SCHWAIER, N. NASHED and F. K. ZIMMERMANN, *Molec. gen. Genetics* **102**, 290 (1968).
70. K. NORDSTROM, *J. gen. Microbiol.* **48**, 277 (1967).
71. B. O. GILBERG, G. ZETTERBERG and G. S. SWANBECK, *Nature, Lond.* **214**, 415 (1967).
72. D. Y. THOMAS and D. WILKIE, *Biochem. biophys. Res. Commun.* **30**, 368 (1968).
73. C. G. ARNOLD and O. SCHIMMER, *Molec. Gen. Genetics* **107**, 281 (1970).
74. A. GRAFFI, *Zbl. Gynak.* **28**, 945 (1968).
75. C. STERN, *Genetics* **21**, 625 (1936).
76. H. WINKLER, *Die Konversion der gene*, Jena (1930).
77. H. ROMAN, *Cold Spring Harb. Symp. Quant. Biol.* **21**, 175 (1956).
78. F. K. ZIMMERMANN, R. SCHWAIER and U. v. LAER, *Z. Vererbungsl.* **98**, 230 (1966).
79. F. K. ZIMMERMANN and R. SCHWAIER, *Molec. gen. Genetics* **100**, 63 (1967).
80. F. K. ZIMMERMANN, *Molec. gen. Genetics* **103**, 11 (1968b).
81. F. K. ZIMMERMANN, unpublished.

82. F. K. Zimmermann and R. Schwaier, *Naturwissenschaften* **54**, 251 (1967).
83. F. K. Zimmermann and U. v. Laer, *Mutat. Res.* **4**, 377 (1967).
84. R. H. Wilson, F. de Eds and A. J. Cox, *Cancer Res.* **1**, 595 (1941).
85. H. Druckrey, D. Schmähl and R. Mecke, *Naturwissenschaften* **42**, 128 (1955).
86. C. Huggins, C. L. Grand and E. P. Brillantes, *Nature, Lond.* **189**, 204 (1961).
87. F. K. Zimmermann, *Z. Krebsforsch.* **72**, 65 (1969).
88. F. K. Zimmermann, *Molec. gen. Genetics* **101**, 171 (1968a).
89. S. Fogel and R. K. Mortimer, *Proc. natn. Acad. Sci. U.S.A.* **62**, 96 (1969).
90. L. Volterra and G. Morpurgo, *Mutat. Res.* **7**, 155 (1969).
91. B. S. Strauss, in *Current Topics in Microbiology* **44**, 1 (1968).
92. J. J. Roberts, A. R. Crathorn and T. P. Brent, *Nature, Lond.* **218**, 970 (1968).
93. J. N. Doneson and D. M. Shankel, *J. Bact.* **87**, 61 (1964).