THEORETICAL ANALYSIS OF CARCINOGENESIS

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1. Introduction

Several comprehensive reviews of our total experimental knowledge of carcinogenesis have been published recently: J. Huxley [1], E. Boyland [2], L. C. Strong [3], G. E. W. Wolstenholme and M. O'Connor [4], A. Levan [5]. In another paper we plan to give a more detailed discussion of the experimental material on carcinogenesis and its interpretation (R. Eker and N. Arley [6]). We shall, therefore, here limit ourselves to giving a review of a stochastic theory of carcinogenesis worked out previously by S. Iversen and N. Arley [7] to [14].

From the total experimental knowledge now available, and especially from the results of the chromosome studies of the last few years [5], more and more evidence has been brought forward which tends to indicate that in all forms of neoplasia, whether spontaneous or induced by chemicals, irradiation, virus, or disturbance of cell environment as in tissue culture and in the Oppenheimer effect, *the key step*, a necessary although not necessarily a sufficient step, *is an irreversible change in the genetic apparatus of the somatic cells.* All alternative hypotheses, especially the virus hypothesis, seem to reduce on closer investigation to being special cases of a genetic interpretation of the neoplastic phenomenon (J. Schultz [15]). Although the basic mechanisms of the two fundamental biological phenomena of cell division and cell differentiation are still unknown, even in normal organisms (D. Mazia [16], J. Huxley [1]), great progress has in recent years taken place in our understanding of a third fundamental biological phenomenon, namely the structure, function, and replication of DNA (M. F. Perutz [17], M. B. Hoagland, [18], L. A. Bliumenfel'd [19], H. Gay [20]). Also, our knowledge of the structure of the chromosomes has progressed (A. E. Mirsky [21], J. H. Taylor [22]).

Thus, more specifically, the picture of the key step now emerging is one of somatic mutation by *cytogenetical loss engendered by interference with the synthesis and function of the DNA of the chromosomes and leading, presumably, to the deletion of growth-controlling and/or regulatory proteins* (A. Haddow [23]), as well as to the loss of some, or all, of the tissue-specific antigens (H. N. Green [24], [25]). Once this necessary, irreversible step has taken place, the population of these

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cells and the clones they give rise to become by continued genotype variation a genetic mosaic of competing cell clones showing great plasticity in permitting cellular adaptations, both spontaneously as in cancer progression and induced as when leading to resistance to chemotherapy and even irradiation, through selection away from life as part of an ordered multicellular organization, toward life analogous to that of a parasitic, autonomous microorganism [5].

We shall here consider only carcinogenesis experimentally induced by external agents. For practical reasons we have introduced the convenient name of *cancer control centers*, or simply *control centers*, for those cellular, genetic entities which undergo the irreversible changes, often called *somatic mutations*, in the key step mentioned, without which neoplasia cannot arise. As just stated, more and more experimental evidence indicates that these control centers are located in the chromosomes as are the genetic "control centers," the genes, and, more specifically, that they are, like the genes, DNA molecules or parts of DNA molecules, and that finally the changes consist of atomic rearrangements and/or substitutions in these molecules, just as is now generally thought to be the case for mutations in the germ cells (M. F. Perutz [17], M. B. Hoagland [18], H. Gay [20], A. E. Mirsky [21], G. W. Beadle [26]).

This is just the essential idea of the so-called hit- or target-theory in its modern general form (N. Arley and H. Skov [27]) that all cells contain *essential molecules*, each cell containing only a small number of each kind, which must remain intact if the cell is to retain its capacity for multiplication, but not for metabolism and respiration since these two processes, although controlled by the gene molecules, are chemical processes between macroscopic numbers of molecules (P. Howard-Flanders [28]). *Thus it is becoming more and more probable that all the fundamental secrets of biology are to be found at the molecular level.*

In figure 1 we show the general scheme we have thus obtained so far, of the sequence of events taking place in radiobiology, carcinogenesis, and similar phenomena where macromolecular control centers seem to play an essential role.

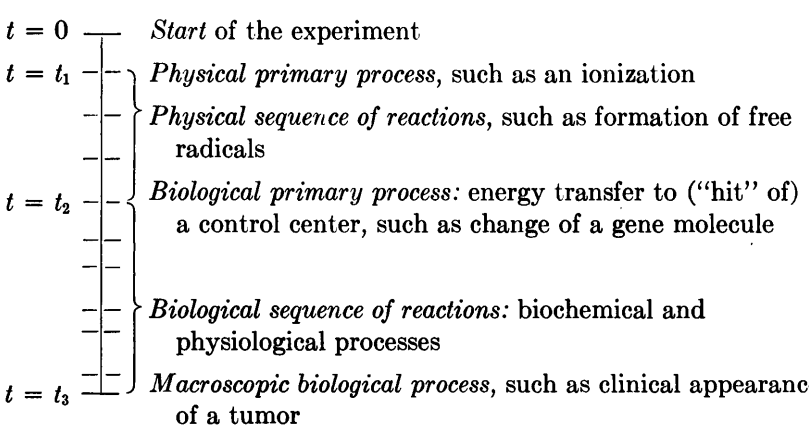

FIGURE 1

Strong support for these ideas of the importance of events on the molecular level for the final macroscopic biological processes has recently been obtained: for the first time it has been demonstrated experimentally (V. M. Ingram [29]) that a specific human disease, sickle-cell anemia, is caused, although in a way still unknown, by a mutation of one single gene and, furthermore, that the disease consists of one specific atomic substitution in the normal hemoglobin molecule, one of its ca 300 amino acids, a glutamic acid, being replaced by another amino acid, valine. It thus seems to be a fruitful working hypothesis that many other diseases, both somatic and psychic, and especially perhaps also both benign and malignant tumors, may have a similar etiology to sickle-cell anemia: to be due to a single gene mutation, that is, a specific atomic substitution or rearrangement in a specific DNA molecule, and to consist of a specific atomic substitution in a specific molecule of biological importance.

To initiate the changes in the control centers that lead to induced neoplasia, action from external agents is necessary. The common physical feature of such actions is the *transfer of energy* such as takes place in collisions between molecules entering into chemical reactions, in irradiation processes such as ionization, excitation, and decay recoil, and so on. The changes leading to spontaneous neoplasia are presumably due to energy transfers in the action of internal chemical agents such as hormones, to thermal energy fluctuations, or to other inter- and intramolecular energy transfers (I. A. Vladimirov and S. V. Konev [30], T. Henriksen and A. Pihl [31], T. Brustad [32]).

Now these basic energy transfers, often for simplicity spoken of as "hits," can take place in four, and only four, different ways (N. Arley and H. Skov [27] and the literature quoted there).

On the one hand, either the energy transfer from the agent to the control center may be *direct*, as in ionization, excitation, and decay recoil processes, leading among other things to the breaking of chemical bonds, which processes may take place directly in the DNA molecule of the control center. Or the energy transfer from the agent to the control center may be *indirect*, as via *free radicals*, or secondary products of such, produced for example by ionization of water molecules close to the DNA molecules of the control centers (W. Gordy [33]).

On the other hand, either one energy transfer from the agent to the control center is able—but need not necessarily be sufficient—to induce neoplasia, analogously to what seems to be the case for instance with radiation-induced gene (or point) mutations. This case is often for simplicity spoken of as a *one-hit* phenomenon. Or more than one energy transfer from the agent to the control center is required as a necessary, but not necessarily a sufficient, condition to induce neoplasia. In this case one often speaks of a *multi-hit* phenomenon. In particular we may have a *two-hit* phenomenon such as seems to be the case with radiation-induced chromosome mutations.

Thus all in all we have the four possibilities, and no other:

(1) Direct one-hit initiation of neoplasia.

(2) Indirect one-hit initiation of neoplasia.
(3) Direct multi-hit initiation of neoplasia.
(4) Indirect multi-hit initiation of neoplasia.

It is, of course, ultimately only possible by direct experimentation to distinguish between these four possibilities. However, for technical reasons hitherto it has not been possible to observe directly the transition of one single somatic cell from its normal to its neoplastic state. It may be hoped that by the technique of single mammalian cell culture (T. T. Puck [34]) such experiments may soon be made possible. Until then, one has to apply statistical analyses, working out for each of the four possibilities mentioned and from plausible assumptions regarding the physicochemical mechanisms of the energy transfers, the consequences in the form of quantitative relationships between the causal and stochastic variables which may be observed with present experimental technique. By comparison with the corresponding relationships observed experimentally one may hope to elucidate the basic mechanisms of carcinogenesis. We shall describe here this program for the model of direct one-hit initiation of neoplasia. Work on the indirect one-hit model is in progress in connection with our Institute's research program for electron spin resonance studies of free radicals and their function in radiobiology and carcinogenesis; it will be described elsewhere. Direct multi-hit models are described in the contributions to this Symposium by P. Armitage and R. Doll [35], H. Blum [36], W. R. Bryan [37], J. E. Dunn, Jr. [38], J. Neyman and E. L. Scott [39], H. G. Tucker [40], W. A. O'N. Waugh [41].

A comprehensive review of the experimental material available, the various stochastic models, comparison of the models with experiments, and conclusions of such comparisons regarding the basic mechanisms of carcinogenesis and their further study is planned in another paper (R. Eker and N. Arley [6]).

2. Theory of the direct one-hit model

2.1. *The experimentally observable quantities.* Statistical experiments on carcinogenesis have the following form: at the time $t = 0$ a sample of n_0 animals, being as homogeneous as is technically possible with respect to genotype and phenotype (age, sex, and so on), is exposed to a carcinogenic agent of given quality and quantity (concentration, dose rate, and so on). For each value of the time t, that is, as a rule in practice at discrete and equidistant times, the *absolute incidence* $n_{\text{eff}}(t)$ is measured, that is, the number of animals in the sample showing at the time t at least one tumor. Hereby also the *apparent induction* time t_{app} of each animal and for a given type of tumor is measured, or the time between the moment of application of the carcinogenic agent and the moment of first observability of the induced tumor. This time is a stochastic variable and the *relative incidence*

$$F(t) = \frac{n_{\text{eff}}(t)}{n_0} \tag{1}$$

thus gives the experimental accumulated distribution of the induction time. If by n_0^* we denote the number of animals in the sample that at $t = \infty$, which is in practice at the end of the experiment, have shown at least one tumor, the *final relative incidence* is given by

$$F(\infty) = \frac{n_0^*}{n_0}. \tag{2}$$

In practice there is the complication that an experiment may be finished, as by the natural death or death from other causes of the test animals, at a time when the theoretical value of $F(t)$, that is, its average value $\bar{F}(t)$, has not yet reached its limit $\bar{F}(\infty)$.

From (1) one calculates the experimental values of the *average induction time* and of its variance,

$$\bar{t}_{\text{app}}(\text{exp}) = \frac{1}{n_0^*}(t_1 + t_2 + \cdots + t_{n_0^*}), \tag{3}$$

$$\sigma^2_{\text{app}}(\text{exp}) = \frac{1}{n_0^*}[(t_1 - \bar{t})^2 + \cdots + (t_{n_0^*} - \bar{t})^2], \tag{4}$$

where t_i is the induction time of test animal number i among the n_0^* animals which do show at least one tumor.

Also the experimental value of the stochastic variable which is the number of tumors $M_i(t)$ on animal number i at the time t is measured and the average number of tumors

$$\bar{M}(t)(\text{exp}) = \frac{1}{n_0}[M_1(t) + \cdots + M_{n_0}(t)]. \tag{5}$$

To elucidate from such measurements the basic mechanisms of carcinogenesis one first has to deduce the theoretical functions corresponding to the experimental functions (1) to (5) as functions also of the quality and quantity of the carcinogenic agent.

2.2. *Basic assumptions of the direct one-hit model.* According to the discussion in the introduction, present knowledge seems to indicate that both for *molecular energy transfers* such as take place when neoplasia is initiated by chemical carcinogens, carcinogenic viruses, and incorporated radioisotopes (giving rise to short-range α-rays, β-rays, γ-rays, and their decay recoils), and for *radiation energy transfers* such as take place when neoplasia is initiated by external irradiation by long-range charged particles, neutrons, or photons, the following sequence of events takes place after application of the carcinogenic agent at the time $t = 0$ (compare also S. Fiala et al. [42] and T. T. Puck [43]).

(a) The molecules, particles, or photons applied reach the cell nuclei after having penetrated the cell membranes, cytoplasms, and nuclear membranes.

(b) The molecules, particles, or photons applied react at a certain time, which we have denoted the *excitation time*, t_{ex}, with the DNA molecules of the control centers, irreversibly damaging their normal structure (as by bond breakage, atomic rearrangements, or deletions), thus interfering with their normal func-

tions, especially in protein synthesis, and thereby liberating the mutated somatic cells from the growth-controlling and regulatory influences from the other cells of the organism.

(c) Once such a mutation has taken place in a somatic cell, it may give rise to a clone of cells which can, but need not in all cases, develop through continued genotype variation, adaptation, and selection to the formation of a visually or palpably detectable tumor after a certain *growth time* t_{gr}, which is measured from the moment of excitation of step (b).

We stress that present knowledge seems to indicate that the carcinogenic viruses in principle act by this same sequence of events (S. E. Luria [44]). For example, it has recently been found (J. H. Tjio and G. Östergren [45]) that also in tumors initiated by certain carcinogenic viruses the key step is changes in the genetic apparatus of the somatic cells, the result of which changes is directly observable in changes in their chromosome structure. Furthermore, it has recently been found (DiMayorca et al. [46], S. E. Stewart [47]) that the DNA extracted from SE polyoma virus can produce cancer when injected. These findings are in agreement with the present general feeling that more and more evidence seems to show that in all virus multiplications the key step is that the nucleic acids of the virus particle enter the nucleus of the host cell, and presumably the chromosomes themselves, and interact with the DNA molecules of the cell. All evidence indicates that gene control and virus control over cellular functions are two aspects of the same genetic mechanism. This concept removes, as Luria remarks, any a priori incompatibility between a viral and a genetic theory of cancer etiology. It reduces the interpretation of virus-induced cancer to that of the control of cellular development and differentiation by genetic elements capable, either intrinsically or by association with other specialized genetic units, of assuming an infectively transmissible form (S. E. Luria [44]).

From this sequence of events there follows the important conclusion that the directly observable stochastic variable which is the apparent induction time t_{app} of the induced tumor is the sum of the two stochastically independent variables, the excitation time, or true induction time, t_{ex} of step (b) and the growth time t_{gr} of step (c),

$$t_{app} = t_{ex} + t_{gr}. \tag{6}$$

Experimentally, neither t_{ex} nor t_{gr} can as yet be observed directly, but such observations will presumably soon be technically possible by the technique of single mammalian cell culture (T. T. Puck [34]). Theoretically, only the distribution of the stochastic variable t_{ex} can be estimated with fair reliability from our present knowledge of the mechanisms of basic energy transfers, whereas the theoretical estimation of the distribution of the growth time t_{gr} demands a detailed knowledge of the development of cell clones which we do not yet possess, but which we also hope will soon be available from the study of single mammalian cell culture. Furthermore, even if we assume, as we do here in the direct one-hit model, that the continued growth of the cell clones from the primarily mutated

somatic cells, which seems to be of a multistage type, being formed by continued genotype variation, adaptation, and selection (A. Levan [5]), is *independent* of how the primary somatic mutation is initiated, that is, is independent of both the quality and quantity of the applied carcinogenic agent, it seems to be a highly complicated mathematical problem to deduce theoretically for the growth of such clones reliable predictions in such a form that they may by compared with experiments (S. Iversen and N. Arley [7], Appendix III, D. G. Kendall [48], and J. Neyman [49]). To make an approximative short cut to such predictions we have, therefore, tentatively assumed that the growth time is normally distributed about a mean value T_{gr} with dispersion σ_{gr}. When more experimental knowledge is available, or when analytical or numerical solutions of the equations of suitable stochastic models for the growth of cell clones become available, such knowledge can easily be taken into account in the following theoretical analysis.

2.3. *The main problem: the distribution of the excitation time.* We are left with the main problem of deducing the theoretical distribution of the excitation time t_{ex}. Due to the different mechanisms of energy transfers we here have to take into account the separate forms and mechanisms of action of the various carcinogenic agents. Furthermore, even in a first approximation to a realistic description we have to take into account the following phenomena: (1) *elimination* of the carcinogenic agent as by diffusion, other chemical reactions, or radioactive decay; (2) *adaptation*, or induced resistance, of the somatic cells to the carcinogenic power of the agent; (3) *toxicity* of the carcinogenic agent, killing or inactivating some of the somatic cells; (4) *growth*, or multiplication, in the case of carcinogenic viruses.

2.3.1. *Molecular energy transfers.* According to the theory of chemical reactions, basic molecular energy transfers take place in bimolecular reactions, and the probability $\lambda\, dt$ of the excitation process of step (b) taking place in one test animal in the time interval dt at the time t after application is given by

$$\lambda(t)\, dt = \kappa(t)c(t)c_c(t)\, dt, \tag{7}$$

where $\lambda(t)$ is the reaction velocity, $c(t)$ the instantaneous, local concentration of the carcinogenic agent, $c_c(t)$ the instantaneous concentration of control centers, and $\kappa(t)$ the reaction velocity for unit concentrations, which depends on the absolute temperature T and the activation energy E of the reaction through the Arrhenius factor

$$\kappa = \kappa' \exp\left(-\frac{E}{kT}\right), \tag{8}$$

where k is Boltzmann's constant.

(1) *Elimination effect.* Experimentally very little is as yet known of both the space and time distribution of applied carcinogenic agents after application, both in the organism as a whole and within a single cell. This applies even in the simplest case of neoplasia induced on mouse skin by painting with carcinogenic hydrocarbons (L. Klinken-Rasmussen [50], C. Heidelberger [51], K. Borum

[52]). To obtain more direct experimental knowledge of this phenomenon is, therefore, one of the most urgent tasks of experimental carcinogenesis. As a first approximation we have assumed first that the time duration of step (a) is negligible compared to the time duration of step (b) and next that the local and instantaneous concentration of the carcinogenic agent, at the site of the control centers and at time t, is proportional with a *reduction proportionality factor* r to the applied concentration c_0 at the time $t = 0$. Under these assumptions the local time variation of $c(t)$ in (7) is determined by the local elimination processes mentioned above. Since these processes are all uni- or bimolecular reactions, it follows that $c(t)$ decreases exponentially in time after step (a) has taken place (S. Iversen and N. Arley [7], N. Arley and S. Iversen [8])

$$c(t) = c_0 r \exp(-\alpha_e t), \tag{9}$$

where r is the reduction factor of step (a), c_0 the initially applied concentration of the carcinogenic agent, and α_e, the *elimination constant*, measures the rate of local elimination of the agent. Thus $1/\alpha_e$ is the *mean elimination time*. The elimination effect may be counterbalanced by repeated applications of the agent.

(2) *Adaptation effect.* Experiments show that both single cells and whole organisms have various defense mechanisms for counterattacking invasions by chemical agents, ionizing radiations, and so on, thereby adapting themselves to new conditions. Thus broken chromosomes may reunite in the original way; antibodies, adaptive enzymes, and physiological recovery and restoration factors may be produced; protective scavenger molecules may inactivate free radicals; blocking effects and steric hindrances may be built up; inter- and intramolecular transfers of ionization electrons and excitation energies may take place, and so forth. The most characteristic feature of such defense mechanisms is that they often do not set in with full strength immediately after the attack, but are built up in time gradually. In N. Arley and S. Iversen [8] and S. Iversen and N. Arley [9] (compare also H. A. Blair [53] and H. P. Yockey [54]), it is shown that this adaptation effect may be described as a decrease of the reaction velocity κ in (7) with time and that as a first approximation this decrease may be written

$$\kappa = \kappa_0 \exp(-\alpha_a t), \tag{10}$$

where $\kappa_0 = \kappa(t = 0)$ and α_a, the *adaptation constant*, measures the degree of adaptation. Thus $1/\alpha_a$ is the mean *adaptation time*. The adaptation constant will, presumably, decrease with increasing concentration of the carcinogenic agent for strong concentrations and strongly interfering agents such as ionizing radiations, because in these cases the defense mechanisms themselves may be expected to be interfered with and weakened. To obtain more detailed experimental knowledge of the adaptation phenomenon is another urgent task of experimental carcinogenesis.

(3) *Toxicity or lethal effect.* Experiments show that carcinogenic agents kill or inactivate the reduplication of some of the cells when the concentration is sufficiently great. Further experiments show that this toxicity effect takes place practically instantaneously, during a time interval which is short compared with

both the mean excitation time of step (b) and with the mean elimination time of the agent. As discussed in N. Arley and S. Iversen [8], the toxicity effect may, therefore, in first approximation be described as an instantaneous decrease of the concentration of the control centers in (7), varying exponentially with the initial concentration of the applied agent, or

$$c_c = c_c' \exp(-\mu c_0), \tag{11}$$

where μ, the *toxicity constant*, measures the degree of toxicity. Since step (b) has a very small probability, the concentration of the control centers will be practically constant in time, and c_c is, therefore, in first approximation time independent.

(4) *Growth effect.* In the case where the applied carcinogenic agent is a virus we have to take its multiplication into account. Experiments show that the increase in virus concentration due to the multiplication is exponential with time, so long as such effects as saturation, antibody production, and so forth can be neglected

$$c(t) = c_0 \exp(\alpha_g t), \tag{12}$$

where α_g, the *growth constant* of the carcinogenic virus, measures the rate of virus multiplication.

Taking all four effects into account in expression (7) for the reaction velocity of step (b) we thus have as a first approximation

$$\begin{aligned} \lambda(t)\,dt &= \kappa_0 c_0 r c_c' \exp[-\mu c_0] \exp[(\alpha_g - \alpha_e - \alpha_a)t]\,dt \\ &= \lambda_0 \exp(\alpha t)\,dt, \end{aligned} \tag{13}$$

where

$$\lambda_0 = k \exp(-\mu c_0) c_0, \qquad k = \kappa_0 r c_c', \qquad \alpha = \alpha_g - \alpha_e - \alpha_a. \tag{14}$$

The constant k we have denoted the *specific carcinogenicity constant* since for given species of test animal, genotype, phenotype, tissue, and type of tumor, k gives an observable measure of the carcinogenic potency of the agent, which has a direct physical interpretation on the molecular level. Thus, in principle, measured values of k may be correlated with other observable properties of the carcinogenic agents to elucidate the still unsolved question of what properties distinguish a carcinogenic agent from a noncarcinogenic agent. Unfortunately, however, in practice even inbred test animals show such large fluctuations that at present the specific carcinogenicity constant cannot be measured sufficiently accurately for such correlations to be of more than qualitative value.

From (13) it follows (see S. Iversen and N. Arley [7], [9]) that the probability $P(t)$ that the excitation step (b) has not taken place for a given animal up to the time t is given by

$$P(t) = \exp\left[-\int_0 \lambda(t')\,dt'\right] = \exp\left\{-\frac{\lambda_0}{\alpha}[\exp(\alpha t) - 1]\right\}, \tag{15}$$

and that the differential distribution $\varphi_{ex}(t)\,dt$ of the excitation time of step (b) is given by

$$(16) \qquad \varphi_{ex}(t)\,dt = P(t)\lambda(t)\,dt = \exp\left\{-\frac{\lambda_0}{\alpha}\left[\exp(\alpha t) - 1\right]\right\}\lambda_0 \exp(\alpha t)\,dt.$$

From (16) and (6), together with our tentative assumption above that the growth time t_{gr} is normally distributed with mean T_{gr} and dispersion σ_{gr}, we may deduce by trivial but tedious calculations all the theoretical quantities needed for comparison with the experimentally observed quantities: incidence, average induction time, induction time variance, and average number of tumors per animal, defined in formulas (1) to (5). It should be stressed that for such theoretical calculations to give a realistic description of nature, which may guide the planning of new experiments, it is necessary to keep an operational viewpoint by taking into account in each step how the corresponding experimental relative frequencies are actually measured.

In S. Iversen and N. Arley ([7] and [9]) this program has been carried out and in those cases where solutions are not obtainable in analytical form, the results have been extensively tabulated numerically and compared with some experiments, the numerical values of the five parameters k, α, μ, T_{gr}, and σ_{gr} being determined graphically to give best fit. Also such a comparison has been carried out by L. Klinken-Rasmussen [50]. A comprehensive review is being planned in R. Eker and N. Arley [6], as already mentioned.

In some experiments (for example, see L. Klinken-Rasmussen [50], I. Berenblum and P. Shubik [55], I. Berenblum [56], S. Iversen, J. Engelbreth-Holm, and O. Noring [57], K. Setälä [58]), two different agents are applied successively, one being a carcinogenic hydrocarbon in suboptimal concentration, that is, such low concentration that the probability of its inducing a neoplasia is too low to be observable in the usually used small samples of test animals, the other being a noncarcinogenic substance such as croton oil which does not induce neoplasia when applied alone. In some of these cases it is found that the tumor incidence is augmented when the noncarcinogenic agent is applied *after* the suboptimal carcinogenic agent, but not when the order of application is inverted. The suboptimal carcinogen is, therefore, denoted by Berenblum an *initiator*, and the noncarcinogen, a *promoter*.

These findings may be fitted into the above scheme of the sequence of events, (a), (b), (c), in several ways, according to whether the promoter is thought to act in step (a), step (b), or step (c).

Hypothesis 1. *The promoter acts in step* (a). This hypothesis has been put forward by W. Gordy [33], who points out that the role of agents such as croton oil, which are not themselves carcinogenic but which augment the effects of other carcinogenic agents, may be that of facilitating the entrance of carcinogenic free radicals into the cell nuclei. The experimental basis for this hypothesis is the fact that electron spin resonance studies have shown the existence of free radicals in many chemical agents known or suspected to cause cancer, and in many biochemical substances such as proteins when irradiated by ionizing radiations. Furthermore, such studies have shown that free radicals can become trapped and stabilized so as to persist for long periods during which they can be trans-

ported from place to place within an organism. The effect of a promoter on this hypothesis is, therefore, to increase the effective concentration of the carcinogen, or, mathematically, to replace the reduction factor r in (9) by a function which increases with increasing concentration of the promoter. It will be seen that Gordy's hypothesis works with an *indirect* model of carcinogenesis, assuming the carcinogenic agents to act indirectly via secondary products such as free radicals. Experiments are actually known which indicate that such indirect mechanisms of energy transfers may also play a role besides the direct transfer mechanisms in carcinogenesis (see for example H. S. Kaplan [59]). As mentioned before, we are presently investigating stochastic models of such indirect initiation of neoplasia in connection with our Institute's electron spin resonance studies of free radicals (T. Henriksen and A. Pihl [31]).

Hypothesis 2. *The promoter acts in step* (b). The stochastic model of this hypothesis has been worked out by N. Arley and S. Iversen [10] and is based on the following physical ideas. At room temperature, the activation energy of the excitation process of step (b), E in (8), may be so high for the initiating agent, that is, the reaction velocity λ in (7) may be so small, that the probability that a tumor will be initiated is too small to be observed for suboptimal concentrations of the initiator. The activation energy E may be still higher for the promoting agent so that the probability that a tumor will be initiated is too small to be observed even for the highest concentrations of the promoter. However, by suitable reactions between the DNA molecules of the control centers and the molecules of the initiator some of the former may go over into a new state, for which the activation energy for an excitation to the neoplastic state in a subsequent reaction with a molecule of the promoter is somewhat lower than for the state before the reaction with the molecules of the initiator. Actually, from the exponential form of the Arrhenius factor (8) we see that even a very small change in the activation energy is sufficient to change the reaction velocity λ by several powers of ten; a change of E from 1.8 eV to 0.9 eV will at room temperature, for which $kT = 0.03$ eV, increase the reaction velocity by a factor 10^{13}. Also it is known from the kinetics of many chemical reactions of organic molecules that changes in other atomic groups of a given molecule than that atomic group participating in a given chemical reaction of that molecule may to a high degree influence the activation energy and thus the reaction velocity of that reaction.

Thus in our case a small change in the activation energy brought about by the initiator in some of the control centers may be sufficient to increase to an observable extent the probability that the promoter will further excite them into the neoplastic state leading to a tumor. The inverse effect has also been observed experimentally, that is, the carcinogenic potency of a given chemical agent may be lowered by first applying a second chemical agent (compare S. Iversen and N. Arley [7], p. 791; K. Setälä [58], p. 227).

In most such experiments the applied concentration (c_{01}) of the first applied agent, the initiator, is varied, while that (c_{02}) of the secondly applied agent, the promoter, is not. Using the above theory and terminology, and disregarding for

simplicity the toxicity effect, we adopt the following notation. [Compare formulas (7) and (13) to (15).]

M_0: the number of control centers on one test animal within the region treated,

M_1: the number among the M_0 control centers which have been excited to a new state by the initiator,

M_2: the number among the M_1 control centers which have been excited to the neoplastic state by the promoter,

$\kappa_1 c_1\, dt = \kappa_{01} c_{01} \exp(-\alpha_1 t)\, dt$: the probability that one of the M_0 control centers is excited in the time interval dt by the initiator; $\alpha_1 = \alpha_{1e} + \alpha_{1a}$,

$p_1 = \exp\{-[\kappa_{01} c_{01}/\alpha_1][1 - \exp(-\alpha_1 t)]\}$: the probability that one of the M_0 control centers is *not* yet excited at the time t,

$\kappa_2 c_2\, dt = \kappa_{02} c_{02} \exp(-\alpha_2 t)\, dt$: the probability that one of the M_1 control centers is excited in dt by the promoter; $\alpha_2 = \alpha_{2e} + \alpha_{2a}$,

$p_2 = \exp\{-[\kappa_{02} c_{02}/\alpha_2][1 - \exp(-\alpha_2 t)]\}$: the probability that one of the M_1 control centers is *not* yet excited at the time t.

Assuming, furthermore, for simplicity as a first approximation that the mean excitation time for the promoter is long compared with that for the initiator, as seems to be substantiated by the findings of I. Berenblum ([56], p. 66), we have, since the excitations of different control centers are stochastically independent, the result that the stochastic variables M_1 and M_2 are both Bernoulli distributed:

$$P\{M_1\} = \binom{M_0}{M_1}(1 - p_1)^{M_1} p_1^{M_0 - M_1}, \qquad \bar{M}_1 = M_0(1 - p_1), \tag{17}$$

$$P\{M_2 | M_1\} = \binom{M_1}{M_2}(1 - p_2)^{M_2} p_2^{M_1 - M_2}, \qquad \bar{M}_2 = M_1(1 - p_2). \tag{18}$$

From here on the theoretical quantities corresponding to the experimentally measured quantities—the final relative incidence, the average induction time, and the average number of tumors per test animal—can be deduced by trivial but tedious calculations and compared quantitatively with observations. This program is carried out in N. Arley and S. Iversen [10] (see also S. Iversen, J. Engelbreth-Holm, and O. Noring [57], L. Klinken-Rasmussen [50]). The analysis shows that the experimental findings can actually be interpreted on the hypothesis that the promoter acts in step (b). However, this fact does not, of course, prove this hypothesis to be correct and the two other hypotheses to be wrong.

Hypothesis 3. *The promoter acts in step* (c). The growth process of step (c) seems to be a highly complicated process formed by continued genotype variation, adaptation, and selection, and thus showing high plasticity in its interaction with the environment (A. Levan [5]). The possibility cannot, therefore, be excluded that the promoter may act by changing both the direction and intensity of the selection pressure. However, so far as we know, no stochastic model for this case has yet been worked out or is in process of being worked out. Since both the growth process is biologically complicated in itself, and the mathematics of realistic stochastic models of neoplastic growth are not easily tractable

with respect to numerical results, it seems less probable that this hypothesis can be elucidated by theoretical analysis.

More experiments aiming at studying the role of promoting agents in each of the steps (a), (b), (c) separately are, therefore, highly needed.

2.3.2. *Radiation energy transfers.* According to the theory of photo- and radiochemical reactions, basic radiation energy transfers take place in electronic excitations and ionizations plus recoils in the case of neutrons. The probability $\lambda\, dt$ of the excitation process of step (b) taking place in one test animal in the time interval dt at the time t after the start of the irradiation is given by an expression like (7),

$$\lambda(t)\, dt = \kappa(t)\delta_l(t)c_c(t)\, dt, \tag{19}$$

where λ is the reaction velocity, $c_c(t)$ the instantaneous concentration of control centers, $\delta_l(t)$ the local instantaneous radiation intensity, or dose rate, which is proportional to the number of photons or particles crossing unit area per unit time in the applied beam, and $\kappa(t)$ a proportionality factor, the reaction velocity for unit concentration and unit dose rate.

In the case of radiation energy transfers there is, of course, no elimination effect, so $\alpha_e = 0$ in (9), and

$$\delta_l(t) = \delta(t)r, \tag{20}$$

where $\delta(t)$ is the instantaneous applied dose rate and r is a reduction proportionality factor which measures the absorption of the applied beam during its passage through the tissues of the test animal. Also we have, of course, no growth effect, so $\alpha_g = 0$ in (12). However, for radiation energy transfers we may also have both an adaptation effect described by (10) with an adaptation constant α_a which will, presumably, decrease with increasing values of the applied dose rate, and a toxicity effect described by (11) with c_0 replaced by $\delta(0)$. So instead of formulas (13) and (14), for radiation energy transfers we have

$$\begin{aligned}\lambda(t)\, dt &= \kappa_0\delta(t)rc_c' \exp\left[-\mu\delta(0)\right] \exp\left(-\alpha_a t\right) dt \\ &= \lambda_0 \exp\left(-\alpha t\right) dt,\end{aligned} \tag{21}$$

$$\lambda_0 = k \exp\left[-\mu\delta(0)\right]\delta(t), \qquad k = \kappa_0 r c_c', \qquad \alpha = \alpha_a. \tag{22}$$

In actual experiments the irradiation of the test animals is carried out in various ways.

(1) The irradiation may be continuous with constant dose rate δ throughout the remaining lifetime of the test animals.

(2) The irradiation may be discontinuous throughout the remaining lifetime of the test animals, constant total doses $D = \delta\tau$ being given with constant dose rate δ in exposure intervals of constant length τ at constant time intervals t_i, $i = 1, 2, \cdots$, between successive irradiations.

(3) The irradiation may in both cases, continuous and discontinuous irradiations, be stopped at a time t_1 before the death of the test animals. In particular, a single dose may be applied, so that $t_1 = \tau$.

From (21) and (6), together with our tentative assumption introduced above that the growth time t_{gr} is normally distributed with mean T_{gr} and dispersion σ_{gr}, we may in each of the three cases (1) to (3) deduce by trivial but tedious calculations all the theoretical quantities needed for comparison with the experimentally observed quantities. In N. Arley and S. Iversen [11] this program has been carried out and the detailed results compared with the very extensive observations of H. F. Blum et al. [60] and [61] for ultraviolet irradiation of mice carried out according to both scheme (2) and scheme (3). In case (2) all the observations can be interpreted (with $\alpha_a = \mu = 0$) according to our direct one-hit model; in case (3) the comparison between theory and experiments indicates that also indirect energy transfers—for example via free radicals as suggested by W. Gordy [33] (see above)—may play a role for neoplasia induced by ultraviolet irradiation. More detailed experiments are, therefore, urgently needed to test the role of indirect energy transfers directly. This may, perhaps, be technically feasible by a combination of the electron spin resonance technique (W. Gordy [33]) with the technique of mammalian single cell culture (T. T. Puck [34]).

The theoretical quantities worked out in N. Arley and S. Iversen [11] are equally comparable with experimental observations of neoplasia induced by ionizing radiations from external sources. In the case of ionizing radiations from internally administered radioisotopes the radioactivity is carried along with molecular carriers from which the energy transfers take place and this case is, therefore, theoretically best described as a case of molecular energy transfers; that is, by expression (13) for the reaction velocity λ. However, in both cases only few quantitative experiments have been published as yet (see the complete list in table 1 in R. H. Mole [62]) which satisfy the following conditions necessary for comparison with any theoretical model:

(a) The over-all incidence of tumors was determined at two or more dose levels.

(b) One dose produced no effect, that is, no increase above the control incidence.

(c) Another dose produced a substantial frequency of tumors.

Of the experiments listed by Mole none gives such extensive observations as those on ultraviolet carcinogenesis (H. F. Blum et al. [60], [61]). Consequently a detailed comparison between theory and experiments regarding neoplasia induced by ionizing radiation seems not yet possible.

Finally we shall make a qualitative remark regarding a much discussed question, *the question whether the curve giving the dose-response relationship is linear or curved for neoplasia induced by ionizing radiations* (see for example, A. M. Brues [63] and R. H. Mole [62]). The reason why this question has become so important is that its solution is highly critical for the estimation of the radiation hazards to the total world population from the fallout from atomic weapon tests as well as from the increasing medical and industrial applications of atomic energy and ionizing radiation. As already pointed out by R. M. Mole ([62], p. 188), "It is probably too naive to expect a linear dose-response relation for

carcinogenesis by radiation even if the mutation hypothesis were true, for radiation is known to kill cells," and a result of this toxicity effect, discussed above, is that "the dose-response relation might be quite complex." Actually, as shown in N. Arley and R. Eker [64], expression (21) for the reaction velocity of the process in step (b) may lead to a dose-response curve of exactly the same form, having a maximum for a certain dose and then decreasing with increasing dose, as that observed by S. Lindsay, G. D. Potter, and L. L. Chaikoff [65] for thyroid tumors in rats given a single injection of I-131 (reproduced as figure 1 in R. H. Mole [62]).

Since the adaptation constant presumably decreases with increasing values of dose rate, still more complex dose-response relationships may follow from the direct one-hit model. For example, the simple assumption that the adaptation constant α_a in (10) is inversely proportional to the dose rate δ will lead to a dose-response relation of the sigmoid type (N. Arley and R. Eker [64]) which may simulate the existence of a threshold dose below which carcinogenesis apparently does not occur, simply because the probability of radiation carcinogenesis for small doses is for such a dose-response relationship too small to be observable in the small samples of test animals usually used in experiments on radiation carcinogenesis.

More detailed experimental investigations elucidating both the toxicity effect and the adaptation effect are, therefore, necessary before reliable conclusions can be drawn from the direct one-hit model of radiation-induced carcinogenesis regarding the form of the dose-response relationship.

3. Summary

A review is given of the present experimental evidence regarding the basic physicochemical events which take place when neoplasia is induced by carcinogenic agents. Next, the various possibilities for a theoretical description of induced carcinogenesis are discussed. A description is given of the assumptions which are necessary for the deduction from a realistic stochastic model of the theoretical quantities needed for a detailed numerical comparison with the experimentally observed quantities such as incidence and induction time. The carrying out of such a program is described for the direct one-hit model of induced carcinogenesis. Finally, the question of the linearity or nonlinearity of the theoretically expected dose-response relationship of radiation-induced carcinogenesis is discussed, and it is shown that more experimental information about the toxicity and adaptation effects is needed before the question can be decided.

We fully agree with the conclusion [66] that "In spite of the vast sums of money and of human effort devoted to cancer research in recent times, there is still no sign that man has yet succeeded in penetrating to a deeper understanding of the etiology of the disease, its early diagnosis by serological methods, or its effective treatment by means of chemotherapeutic agents. The same methods of diag-

nosis and treatment known half a century ago still remain—albeit in greatly refined and perfected form—the only weapons available in the fight against malignancy."

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