STATISTICAL ASPECT OF THE PROBLEM OF CARCINOGENESIS

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1. Introduction

The present paper is intimately related to the two preceding papers [1], [2], and is the outcome of collaboration which was broader than a mere reference would indicate. It is appropriate, then, for us to begin with acknowledging our indebtedness to the authors of the other papers, particularly to Michael B. Shimkin. Our familiarity with the problem and, more specifically, our knowledge of cancer as a biological phenomenon (which we readily admit to be of negligible extent) originate from conversations that one of us had with Shimkin, and from reading his papers written jointly with Milton Polissar. Subsequently, in the process of planning and execution of the experiments described in the two earlier papers, the present authors benefited greatly from discussion with our biological colleagues on both sides of this continent, in Philadelphia with Shimkin, and in Berkeley with White, Grendon, and Jones.

2. Different scales of study

A study of any natural phenomenon may be conducted on a variety of levels, or scales. The choice of the scale contributes considerably to the nature of questions asked and to the general character of answers attempted. In particular, in the currently conducted studies of carcinogenesis there are discernible scales that might be classified roughly as follows.

(1) *Somatic scale.* This label, the appropriateness of which we are not prepared to defend, is used to describe the studies conducted on the broadest possible level, concerned with age specific death rates from cancer, with the problem of cancer and smoking, and so forth. The characteristic feature of this scale is the absence of a closely considered mechanism which originates cancer.

(2) *Clone and cell scale.* Under this heading we include studies, like the present, in which the happenings within cells or within clones of cells, the happenings that are observed or hypothesized, are coming under explicit consideration.

(3) *Molecular biology,* which, of course, is the finest scale.

As we see it, the purposes of studies, both empirical and statistical, conducted

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on different levels are frequently different. It seems to us that most of the studies on a very broad level are characterized by practical purposes, not to say, necessities. It is, then, not so much the desire to understand the true mechanism of the given phenomenon as the need for guidance in some practical situations that is the prime motive of the study. In our understanding, this is the case in the computation of age specific death rates from cancer. As a result, when some postulates are adopted regarding the possible mechanism behind the computed death rates, little effort is expended on an empirical verification as to whether the hypothetical postulates have any empirical counterparts. The important point is to have realistic death rates. These are obtained, essentially, through fitting an interpolatory formula.

With the studies conducted on a finer scale, the situation is different. Here the primary purpose is to find something about the actual mechanism of the phenomenon and a step by step comparison between the postulates and reality is essential. The underlying formulas refer to entities that, hopefully, are identifiable in the empirical material and reflect hypothetical properties that these entities are expected to possess. The set of hypotheses determining these formulas is described as a structural model of the phenomenon. Ordinarily, each structural model will involve some interpolatory elements, perhaps some unspecified functions or at least constants that must be estimated from the observations. For example, in Newtonian mechanics the square in the inverse square law is an interpolatory element.

The three levels of the phenomenon of carcinogenesis just enumerated are obviously interconnected. However, there are wide gaps between them and it must be obvious that the actual happenings on one particular level, say the cellular, will be found consistent with a great variety of hypotheses as to the mechanisms operating on the molecular level. Also, because of the multiplicity of factors intervening between any two given levels, it seems to us quite probable that a given mechanism on a specified level does not determine uniquely the happenings on the next, less refined, level. Thus, it seems to us that a comprehensive picture of the phenomenon of cancer can be obtained through a combination or harmonization of findings on several levels on which experimental or observational studies are or may be conducted. However, any effort at a harmonization of findings at different levels must be preceded by careful studies conducted on each particular level treated in isolation.

The present paper is limited to the cellular-clone level. Broadly, the purpose of the paper is to construct a model of carcinogenesis, representing a combination of several birth and death stochastic processes, such that the consequences of the model agree, at least qualitatively, with certain empirical findings. The model constructed postulates several different categories of cells and clones, and involves several parameters and, even, unspecified functions. The degree to which the model may be considered realistic depends on further experiments. In particular, this degree depends on the possibility of identifying real cells and real clones that correspond to those postulated. Further, it is important to have

independent new experiments leading to direct estimates of the unspecified parameters and unspecified functions involved in the model. Hopefully, with such independent estimates, the consequences of the model will harmonize with the relevant empirical findings. If not, the differences may suggest changes in the model bringing it closer to the real mechanism of carcinogenesis.

3. Some early stochastic models of carcinogenesis

A review of the theoretical statistical studies of carcinogenesis was given at the Fourth Berkeley Symposium by Armitage and Doll [3]. From this review we learn that the credit for the first attempt to construct a probabilistic model of carcinogenesis on the cellular-clone level is due to Arley and Iversen [4], who, from 1950 on, published a series of papers fitting their model to the results of a number of experiments. The second attempt in the same direction is due to Armitage and Doll [5] (1957). Our own efforts are concerned with the developments of the ideas formulated by these authors and with the choice between two basic assumptions in which the two models differ radically.

The Arley and Iversen model is based on the one stage mutation hypothesis. Briefly: a contact (or "hit") between an element of the carcinogen and a normal cell causes this cell to "mutate." This event is followed by a variable "induction period," the length of which is assumed to have a specified distribution. After the induction period a tumor is identifiable. The observable variables are the number of tumors and the induction time. The experiments for which Arley and Iversen found excellent fit of their theory are characterized by the dose of the carcinogen as an experimental factor, this dose being administered following some fixed time pattern. This is an important point. The experiments of Harold Blum [6] included series where the time pattern of ultraviolet irradiation was changed. Arley and Iversen found that the dose-response relation in any one series characterized by a fixed time pattern could be fitted excellently by their model. However, the constants of the model computed for one time pattern would not fit the data resulting from another time pattern. Thus, as readily admitted by Arley and Iversen, the mechanism of carcinogenesis involves an element not included in their model.

In considering alternative possibilities we make a conceptual distinction not specifically considered by Armitage and Doll [3]. The distinction is between *multihit* and *multistage* mechanisms. The term multihit is used to describe the mechanism in which a cancer initiating mutation requires not just one hit but a certain minimum number $k > 1$ hits *on the same cell.* The term multistage mutation mechanism is used to describe a mechanism involving several successive mutations, *each generating a clone of mutant cells.* Thus, the first mutation (whether induced by a single hit or by $k > 1$ successive hits on the same cell) leads to a *benign* clone of cells described as first order mutants. Each of the first order mutant cells is subject to the risk of a second mutation. This second mutation generates a growth of what are called second order mutants. If second

order mutant cells are cancer cells, the mechanism is a two stage mutation mechanism. However, it is conceivable that the growth of second order mutants is again benign, with each cell being exposed to the risk of another mutation leading to a growth of third order mutant cells, and so forth.

Each of the contemplated successive mutations may or may not depend upon a carcinogenic factor (the same or a new factor, perhaps cosmic radiation). Also, each of these mutations may well require its own minimum number of hits. Thus, we contemplate a double hierarchy of hypothetical mechanisms of carcinogenesis, determined by the number of stages (that is the number of successive mutations each generating a specific growth of cells) and by the number of hits of the carcinogen required to produce each particular mutation.

It is natural to begin by studying the simplest possible models. The original Arley and Iversen model is a one hit, one stage mutation model. The subject of studies of Nordling [7] and by Stocks [8], whom we quote after Armitage and Doll [3], is a k hit, one stage mutation mechanism. In the present study we consider alternatively a one hit, one stage and one hit, two stage model. Both were studied earlier by Arley and Iversen and by Armitage and Doll. Our work incorporates a number of structural elements, some involved in earlier studies [9], [10], [11].

As mentioned, the first one hit, two stage mutation model of carcinogenesis is due to Armitage and Doll (1957). This model is characterized by a deterministic assumption that the clone of first order mutants grows exponentially. In the framework of the present study this assumption is not tenable because it would imply that the first order mutant clone is "malignant" and, in due course, would kill the animal. The roots of our ideas are elsewhere.

A series of papers by Shimkin and Polissar, appearing since 1955 [12], are the only papers known to the present authors in which the possibility of a multistage mechanism of carcinogenesis was investigated seriously. This appears to be the case even though Shimkin and Polissar never expressed this idea explicitly. As described briefly in Shimkin's paper [1], a persistent effort was made to see what, if any, changes occur in the cell population of mice's lungs following an injection of urethane. It was noted that some kind of modified cells do appear, that their number begins by growing, reaches a maximum and then declines. Tumor nodules appear concurrently but with a very noticeable delay.

The question arises: (i) are these somehow modified cells real precursors of cancer, that is, first order mutants, each subject to the risk of a second mutation like event turning it into cancer? Or, alternatively, (ii) should one suppose that the hypercellularity observed by Shimkin and Polissar is a phenomenon occurring, so to speak, in parallel with carcinogenesis, but having no connection with it?

This is precisely the question that has preoccupied us over the last several years. Limited as this question may appear, we were and still are interested in the subquestion: how can (i) and (ii) be answered authoritatively?

The first possibility which occurred to us had to be abandoned for observational reasons. This possibility is connected with the expectation that, if the Shimkin and Polissar modified cells are really predecessors of cancer tumors, then there should be a calculable theoretical correlation between the number of tumors and the number of the Shimkin and Polissar cells, a correlation that could be compared with the empirical correlation obtained from actual counts. With this motivation, Klonecki developed [13] a method of numerical calculation of the joint distribution considered. Unfortunately, the original counts of modified cells, on the one hand, and of tumors on the other, were made independently from each other. Thus, the empirical data contain information on marginal distributions of the two variables of interest, not on their joint distribution. Also discussions revealed that the counting of the supposed first order mutants is difficult and we could not induce our biological friends to attempt it. As a result, other possible avenues had to be investigated, all based on counts of tumor nodules only.

4. Basic assumptions

The models considered below are all based on the following assumptions.

4.1. *Action of carcinogen.* As postulated by Arley and Iversen, the action of a carcinogen on cells of a given tissue consists in randomly distributed "hits" on particular cells. Each normal cell hit by the carcinogen undergoes a mutation like change which is the initial event in the process of carcinogenesis. For an experiment beginning at time $t = 0$ we visualize a function $f(t)$, nonnegative for all $t \geqq 0$, with its integral from zero to infinity equal to one, and a positive number D. The specific assumption is that to any time interval $[t, t + \tau)$ with $\tau > 0$, there corresponds a probability

$$Df(t)\tau + o(\tau) \tag{4.1}$$

that in $[t, t + \tau)$ there will be exactly one normal cell of the tissue "hit" by the carcinogen, irrespective of the number of cells hit earlier. Also it is assumed that the probability of two or more hits in time $[t, t + \tau)$ is $o(\tau)$.

As is well known, the above hypothesis implies that the total number of cells hit in any time interval (t_1, t_2) is a Poisson variable with expectation given by

$$D \int_{t_1}^{t_2} f(t)\, dt. \tag{4.2}$$

Thus, D is the expectation of the total number of mutations produced by the carcinogen used in the experiment. Ordinarily it is assumed that this number is proportional to the dose of carcinogen administered. For this reason the constant D will be described as the total dose of the carcinogen. The function f, determining the time pattern of the application of the carcinogen will be called alternatively the "time pattern function" or the "feeding function."

In the above form, the hypothesis about the action of the carcinogen is fairly general and is not likely to be seriously questioned. This situation changes just

as soon as one tries to interpret the total dose and the time pattern function in terms of a particular experiment. In order to clarify this point consider two mice M_1 and M_2. Assume that the first mouse is given just one injection of urethane at $t = 0$, amounting to 1 milligram per gram of body weight (1 mg/gm BW). Assume next that the second mouse M_2 is given two injections of urethane, the first 0.5 mg/gm body weight at $t = 0$ and the second, also amounting to 0.5 mg/gm body weight, at some later time t_1. The question arises as to whether the two mice receive the same total dose D of the carcinogen. One point is that, if M_2 is fairly young and t_1 fairly large, the body of M_2 must have grown between $t = 0$ and t_1. Thus, the total amount of carcinogen administered at t_1 would be larger than that administered at $t = 0$ and it is not immediately clear that the expected number of fresh hits on normal cells in the lungs of M_2 resulting from the second injection must be equal to that resulting from the first.

This one point of doubt is reinforced by the following. It is known [14] that with very young mice the speed of elimination of urethane is slower than with older mice. Thus, if mouse M_2 used in the above hypothetical experiment is relatively young, then the carcinogenic action of the urethane administered at $t = 0$ is likely to be greater than that administered at time t_1 even if the initial concentration of urethane in the blood stream in the two cases is the same.

The relationship of the conclusions reached to the results of some particular experiments, in which amounts of the carcinogen actually administered and the time pattern of actual administration are varied in some specified way, is a separate question subject to hypothetical judgment and, hopefully, to separate empirical verification. The following theoretical developments are based only on the hypothesis of the action of the carcinogen as stated above. The purpose of this theory is to deduce effects on the ultimate yield of cancer tumors to be expected from specified changes in the value of D and, separately, in the time pattern function f.

4.2. *Nature of cellular growth.* The growth of a clone of abnormal cells, whether benign or malignant, originates from a single mutant cell and its development represents a realization of a birth and death stochastic process, independent of other similar processes.

This is the basic "structural" assumption. It is accompanied by another, of an interpolatory character, namely: the birth and death processes representing the growths of abnormal clones are time homogeneous, so that the unit time rates of births and of deaths, λ and μ, respectively, are absolute constants. Also it is assumed that benign growths correspond to subcritical and malignant growths to supercritical processes, with $\lambda < \mu$ in the first case and $\lambda > \mu$ in the second.

In terms of the above two basic assumptions, the Arley and Iversen one stage mutation theory of carcinogenesis amounts to the assumption that the mutation of the normal cell, which is caused by the initial hits of the carcinogen, results in clones of malignant cells, that is, realizations of supercritical birth and death processes. The two stage mutation theory amounts to the assumption

that the initial hits of the carcinogen result in benign clones of cells, each of which is exposed to the risk of a second mutation like change. It is only these second mutations that lead to cancer clones.

Before studying the consequences of these two alternative possibilities, it will be useful to reproduce certain known formulas relating to birth and death processes, most of them deduced by D. G. Kendall and presented systematically by Harris [15].

Let $P_n(t)$ stand for the probability that a noncritical clone of cells (that is, a birth and death process with $\lambda \neq \mu$), originating at time $t = 0$ from a single cell, will have exactly n live cells at time t. The formula for $P_n(t)$ may be written conveniently in terms of two functions of time, say

$$\psi(t) = \frac{q^2 e^{-qt}}{\lambda(1 - e^{-qt})(\mu - \lambda e^{-qt})}, \tag{4.3}$$

and

$$R(t) = \frac{\lambda(1 - e^{-qt})}{\mu - \lambda e^{-qt}} = 1 - \frac{q}{\mu - \lambda e^{-qt}} < 1, \tag{4.4}$$

with $q = \mu - \lambda$. Namely, we have

$$P_0(t) = \frac{1 - R(t)[1 + \psi(t)]}{1 - R(t)} \tag{4.5}$$

and, for $n > 0$,

$$P_n(t) = \psi(t)R^n(t). \tag{4.6}$$

It will be seen that, whether the process is subcritical ($q > 0$) or supercritical ($q < 0$), the two functions ψ and R are never negative and are monotone in t, the first decreasing and tending to zero, and the second increasing. For a subcritical clone we have

$$\lim_{t \to \infty} R(t) = \lambda/\mu < 1. \tag{4.7}$$

For the supercritical clone

$$\lim_{t \to \infty} R(t) = 1. \tag{4.8}$$

The above notation is convenient for use with reference to both subcritical and supercritical clones. However, in some cases when it is desired to emphasize the malignant character of a growth of cells, the corresponding unit time rates of birth and death will be denoted by capital letters Λ and M with $\Lambda > M$.

5. Probability that an isolated tumor will be counted

The experimental data considered in this paper all refer to counts of cancer tumors either on the surface or within the lungs of mice. Obviously, a tumor composed of only a small number of cells may easily be overlooked. Therefore, in order to be able to compare the theory with the results of observation, it

will be necessary to consider the probabilities, say π_n, that a tumor composed of exactly n cells will be counted. The dependence of π_n on $n \geqq 1$ is not known, but it is plausible that, as n grows, π_n never decreases and eventually tends to unity. Assuming this to be the case, we shall write, for $n \geqq 1$,

$$\pi_n = \sum_{i=0}^{n-1} a_i, \tag{5.1}$$

where the numbers a_i are arbitrary but nonnegative with $\sum_0^\infty a_i = 1$. Also, we shall introduce the generating function of these numbers, say,

$$g(u) = \sum_{i=0}^{\infty} a_i u^i. \tag{5.2}$$

Obviously $g(u)$ is a strictly increasing function of its argument, with $g(1) = 1$.

In the early period of study of the two stage mutation theory of carcinogenesis, an interpolatory formula $\pi_n = 1 - \rho^n$ with $0 < \rho < 1$ was considered. Actually, this formula was adopted in his study by D. G. Kendall [10]. For this particular choice,

$$g(u) = \frac{1 - \rho}{1 - \rho u}. \tag{5.3}$$

With a great variety of experimental conditions, ranging from the use of microscope in the search for minute cancer tumors in thin slices of the lungs, to naked eye inspection of the surface of the lungs, to search for vaguely identifiable hyperplastic foci, it is difficult to foresee what kind of interpolatory formula for π_n might be adequate and, if (5.3) is found not to be sufficiently flexible, some other function might be used. One possibility is the familiar negative binomial

$$g(u) = (1 + \vartheta - \vartheta u)^{-\alpha} \tag{5.4}$$

with $\alpha, \vartheta > 0$.

The choice of an appropriate function $g(u)$ is important in studies concerned with sequences of counts of tumors made at varying times since the beginning of the experiment, occasionally quite early times. The present study is concerned principally with the ultimate numbers of tumors, under varying conditions, counted at a reasonably distant time T. As will be shown below, the expectation of this ultimate number of tumors is independent of the exact form of the function $g(u)$. Therefore, it will be left unspecified.

Now let $\Pi(t)$ denote the probability that an isolated clone of cells of age t (that is, a clone generated by a single cell t units of time ago) will be counted. We have

$$\Pi(t) = \sum_{n=1}^{\infty} P_n(t)\pi_n = \psi(t)\, R(t) \sum_{n=1}^{\infty} R^{n-1}(t) \sum_{k=0}^{n-1} a_k. \tag{5.5}$$

On changing the order of summation, this yields

$$\Pi(t) = \psi R \sum_{k=0}^{\infty} a_k \sum_{n=k}^{\infty} R^n \tag{5.6}$$

$$= \frac{\psi R}{1 - R} \sum_{k=0}^{\infty} a_k R^k = \frac{\psi R}{1 - R} g(R),$$

where, for the sake of compactness, the argument t of the functions ψ and R is omitted. This formula for $\Pi(t)$ can be used for benign as well as for malignant tumors. In particular, for malignant tumors we have

$$\Pi(t) = \frac{(\Lambda - \mathrm{M})e^{(\Lambda-\mathrm{M})t}}{\Lambda e^{(\Lambda-\mathrm{M})t} - \mathrm{M}} g[R(t)]. \tag{5.7}$$

Because of the properties of $R(t)$ established earlier, it is seen that, as $t \to \infty$, the probability $\Pi(t)$ tends to $1 - \mathrm{M}/\Lambda$, which is known to be the probability that a supercritical clone will grow without limit. Tumors of this kind will be called killer tumors.

6. Probability that a double tumor will be counted

In this section we consider the possibility that the mutation like change (of any given order, the first or the second, and so forth) resulting from the hit of a specified cell C_0 does not manifest itself in this cell but only in its two daughter cells, C_{11} and C_{12}, and their progeny. This makes it necessary to introduce special terms, namely, the primary nth order mutant to designate the cell C_0 and the secondary nth order mutants, C_{11} and C_{12}. If the mutation considered is cancer creating then the two daughter cells, C_{11} and C_{12}, will generate two independent malignant clones, which, because of their proximity, could hardly be counted as separate tumors. It will be convenient to use the term double tumor to describe the combination of the two.

In connection with the possibility that the tumors counted are in reality double tumors, it will be necessary for us to consider the probability, say $\Pi^*(t)$, that a double tumor resulting at $t = 0$ from the division of a primary mutant, will be counted at time $t \geqq 0$.

The evaluation of $\Pi^*(t)$ follows the lines of the preceding section. We have

$$\Pi^*(t) = 2P_0(t)\Pi(t) + \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} P_m(t)P_n(t)\pi_{m+n}. \tag{6.1}$$

Using (4.6) and (5.11) and omitting the argument t, the double sum in the right side can be written as

$$\psi^2 R^2 \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} R^{m+n-2} \sum_{k=0}^{m+n-1} a_k, \tag{6.2}$$

or in the form

$$\psi^2R^2\sum_{s=2}^{\infty}(s-1)R^{S-2}\sum_{k=0}^{s-1}a_k$$

$$=\psi^2R^2\sum_{k=0}^{\infty}a_k\sum_{s=k+1}^{\infty}(s-1)R^{S-2} \tag{6.3}$$

$$=\psi^2R^2\sum_{k=0}^{\infty}a_k\frac{d}{dR}\frac{R^k}{1-R}$$

$$=\psi^2R^2\sum_{k=0}^{\infty}a_k\left[\frac{kR^{k-1}}{1-R}+\frac{R^k}{(1-R)^2}\right].$$

Finally, this yields

$$\Pi^*(t)=2P_0(t)\Pi(t)+\left(\frac{\psi R}{1-R}\right)^2[(1-R)g'(R)+g(R)], \tag{6.4}$$

where g' stands for the derivative of the function g. In order to evaluate the limit of $\Pi^*(t)$ as t is increased, we refer to figure 1. Here the continuous curve

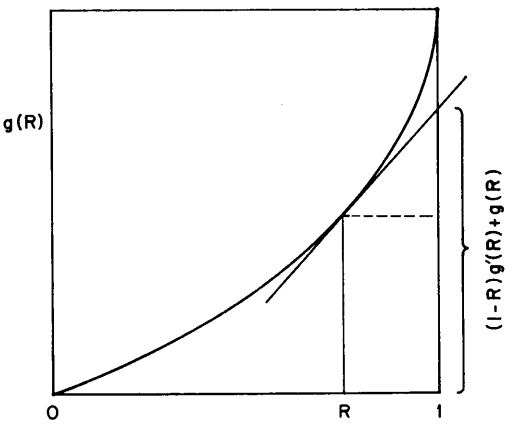

FIGURE 1

Interpretation of the expression $(1-R)g'(R)+g(R)$.

represents the graph of the function g. Because of the convexity of the function g, with $g(1)=1$, it is seen that the term in square brackets increases with R and tends to unity, irrespective of the details of the nature of g. Using the expressions for $P_0(t)$, $R(t)$ and $\psi(t)$ it is easily found that

$$\lim_{t\to\infty}\Pi^*(t)=2\frac{M}{\Lambda}\left(1-\frac{M}{\Lambda}\right)+\left(1-\frac{M}{\Lambda}\right)^2 \tag{6.5}$$

$$=1-\left(\frac{M}{\Lambda}\right)^2.$$

7. Expected number of killer tumors under the one stage mutation theory

In this section we consider two slightly different versions of the one stage mutation theory of carcinogenesis. For each, we evaluate the limit of the expectation, say $\zeta(T)$, of the random variable $Z(T)$ defined as the number of tumors counted at time T, generated at some time between $t = 0$ and $t = T$. It will be seen that, no matter what the time pattern function may be, the limit of $\zeta(T)$ as $T \to \infty$ is always the same. It is proportional to the total dose D with the coefficient of proportionality depending upon the rates of birth and death of the relevant cells. The practical conclusion from these results is that, if in a reliable experiment, the counts of tumors performed at a rather distant time T are either not proportional to D or depend upon the time pattern in which the same dose of carcinogen is administered, then either our basic hypotheses are not realistic or the number of stages in the mutation theory must be more than one.

The two versions of the one stage mutation mechanism are as follows. First we consider version A assuming that the hit on a normal cell turns this cell into a malignant cell with rates of birth and death equal to Λ and M with $\Lambda > \mathrm{M}$. Second we consider the possibility B, envisioned in section 6, that the mutation of a cell C_0 induced by a hit at time $t = 0$, manifests itself not in C_0 but in each of the two daughter cells C_{11} and C_{12}. Here, then it will be necessary to assume that, following a hit at $t = 0$, the cell C_0 continues to function normally, with probabilities $\beta\tau + o(\tau)$ and $\gamma(\tau) + o(\tau)$ that in the time interval $[t, t + \tau)$ it will either divide into C_{11} and C_{12}, or will die. Then, in the case of division, the two daughter cells C_{11} and C_{12} will jointly generate what we called a double tumor. At time T this double tumor may or may not be counted.

Version A. For arbitrary $0 < t \leqq T$ denote by $Z(t, T)$ the number of tumors generated in $[0, t)$ and counted at T. Let

$$P_n(t) = P\{Z(t, T) = n\}. \tag{7.1}$$

Following the usual procedure, we write

$$\begin{aligned} P_n(t + \tau) = {} & P_{n-1}(t)Df(t)\Pi(T - t)\tau \\ & + P_n(t)[1 - Df(t)\Pi(T - t)\tau] + o(\tau). \end{aligned} \tag{7.2}$$

By a familiar reasoning, this equation implies that $Z(t, T)$ is a Poisson variable with expectation, say,

$$\zeta(t, T) = D\int_0^t f(x)\Pi(T - x)\,dx. \tag{7.3}$$

In particular, putting $t = T$, we have

$$\zeta(T) = D\int_0^T f(x)\Pi(T - x)\,dx. \tag{7.4}$$

Using the fact that f is nonnegative and that, its integral from zero to infinity is equal to one, and also using (5.5) it is easily found that

$$\lim_{T\to\infty} \zeta(T) = D(1 - \mathrm{M}/\Lambda) = \zeta(+\infty), \quad \text{say.} \tag{7.5}$$

Version B. In studying version B it will be necessary to consider simultaneously two random variables $Y(t)$, the number of normal cells that were hit before time $t > 0$, still alive at t and not yet divided (these cells are the primary mutants), and $Z^*(t, T)$, defined as the number of double tumors generated some time before the moment t and counted at $T \geqq t$. Let

$$P_{m,n}(t) = P\{Y(t) = m, \quad Z(t, T) = n\}. \tag{7.6}$$

Proceeding as formerly, we have for $\tau > 0$,

$$\begin{aligned} P_{m,n}(t + \tau) = {} & P_{m-1,n}(t)Df(t)\tau \\ & + P_{m+1,n}(t)(m + 1)\{\beta[1 - \Pi^*(T - t)] + \gamma\}\tau \\ & + P_{m+1,n-1}(t)(m + 1)\beta\Pi^*(T - t)\tau \\ & + P_{m,n}(t)\{1 - Df(t)\tau - m(\beta + \gamma)\tau\} + o(t). \end{aligned} \tag{7.7}$$

Let $G(u, v, t)$ stand for the joint probability generating function of the two variables $Y(t)$ and $Z(t, T)$. The letter G with subscripts t or u will denote the partial derivatives of $G(u, v, t)$ with respect to t or u. Applying the usual procedure to equation (7.7), and omitting the obvious arguments of the various functions, we find

$$G_t = (u - 1)DfG + \{(1 - u)(\beta + \gamma) + (v - 1)\beta\Pi^*(T - t)\}G_u. \tag{7.8}$$

This partial differential equation can be solved. However, since we are interested only in the expectation of $Z^*(t, T)$, we omit the process of solution. Differentiating (7.8) with respect to u, setting $u = v = 1$, denoting the expectation of $Y(t)$ by $\eta(t)$, we obtain

$$\eta'(t) = Df(t) - \Delta\eta(t), \tag{7.9}$$

where $\Delta = \beta + \gamma$ and the prime indicates differentiation with respect to t. Also, differentiating (7.8) with respect to v and setting $u = v = 1$, we have

$$\zeta'(t, T) = \beta\Pi^*(T - t)\eta(t). \tag{7.10}$$

The last two equations yield

$$\zeta(t, T) = D\beta \int_0^t e^{\Delta x} f(x) \int_x^t e^{-\Delta y}\Pi^*(T - y)\, dy\, dx. \tag{7.11}$$

After substituting $t = T$, this formula gives the expectation $\zeta(T)$ which is the subject of our interest. Noticing that the factor of $f(x)$ is bounded by

$$\frac{1 - e^{-\Delta(T-x)}}{\Delta} < \frac{1}{\Delta} \tag{7.12}$$

it is easily found that, as $T \to \infty$, we have

$$\lim_{T\to\infty} \zeta(T) = D\frac{\beta}{\Delta}[1 - (\mathrm{M}/\Lambda)^2] = \zeta(+\infty), \tag{7.13}$$

which completes the proof of the assertion stated at the outset.

In an effort to use the assertions just proved to obtain empirical evidence either in favor of or against the one stage mutation theory, a difficulty must

be anticipated. This is the problem of deciding how large the value of T must be in order to represent a "rather distant time" since the beginning of the experiment so that the averages of counts of tumors could be reliably considered as empirical counterparts of the limits (7.5) or (7.13). Presumably, the solution lies in arranging the experiment so that sacrifices of animals and counts of tumors are made for a substantially long sequence of times since the beginning of the experiment. Then the plot of the average count of tumors against time is likely to indicate an approach to an asymptote and, even, the actual asymptotic value of the mean count. The conclusions (7.5) and (7.13) apply to these asymptotic values.

There is another circumstance that must be borne in mind in studies of the above kind. As is well known, lung tumors in mice occur from time to time even if these mice are not intentionally exposed to the action of a carcinogen. Thus, it is reasonable to suppose that, if a dose D of carcinogen is intentionally administered to mice, the total dose of carcinogen to which the mice react is not D but somewhat more, say $D_0 + D$, where D_0 may represent the effect of "environmental carcinogen," perhaps radiation. Therefore, on the one stage mutation theory, the average number of "killer tumors" must be expected to be proportional not to D but to $D_0 + D$. In effect, this means that the counts of tumors made at a reasonable time T plotted against D should align themselves along a straight line with a nonnegative intercept, not necessarily equal to zero. This intercept would be equal to D_0 multiplied by the factor of D in (7.5) or in (7.13), depending upon the version of the theory that is closer to reality.

8. Some empirical results

At this time it is appropriate to reproduce some empirical results illustrating certain points of the above theory and leading to the theoretical developments given in subsequent sections. It will be seen that, if one admits that in the experiments described the amount of urethane injected at any time, in mg/gm body weight of mice is proportional to what was described as the dose D of carcinogen, then the experimental results contradict the one stage mutation theory. In fact, the contradiction is on two counts: the average number of killer tumors per mouse, corresponding to a fixed time pattern, is not a linear function of D and, when D is fixed, the average number of killer tumors depends upon the time pattern of administering the fixed dose D. The theory developed below is intended to examine whether the empirical results quoted in the present section are consistent with a version of the two step mutation mechanism.

8.1. *Shimkin and Polissar data.* Table I and figure 2 represent the experimental results of Shimkin and Polissar [12]. For purposes of the present section, only the last column of the table is needed. This gives the estimated average number $\hat{\xi}(T)$ of tumor nodules in the lungs of mice all given the same dose of urethane 1 mg/gm BW, and sacrificed at varying times after the injec-

TABLE I

COUNTS OF CELLS, OF HYPERPLASTIC FOCI, AND OF TUMORS IN LUNGS OF MICE
After Shimkin and Polissar [12].

Days after Urethane	*Estimated Mean Number of:* Cells per Square (106.3 sq. micra)	Presumed First Mutants per Square	Foci per Lung	Tumors per Lung
0	0.73	0.00	—	—
1	0.85	0.12	—	—
3	0.92	0.19	—	—
7	1.11	0.38	—	—
14	1.02	0.29	294	—
21	1.35	0.62	450	—
28	1.57	0.84	390	15.5
38	—	—	610	—
49	1.33	0.60	450	37.3
84	1.20	0.47	260	34.8
105	—	—	200	35.2
133	—	—	83	35.7

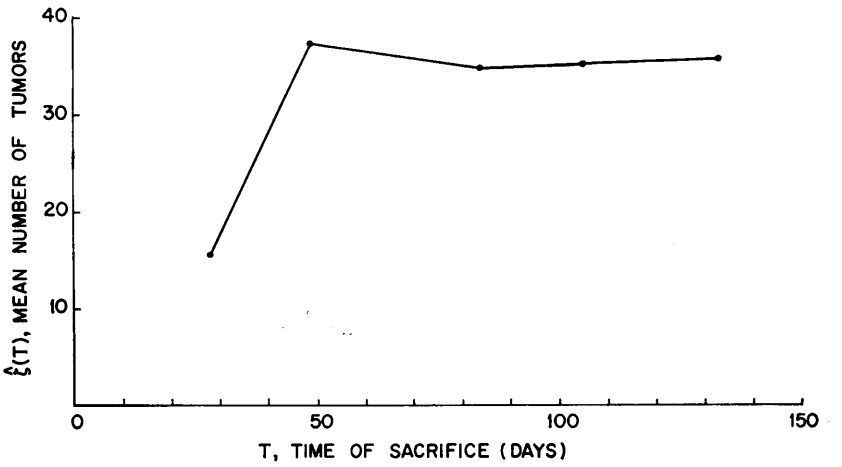

FIGURE 2

Estimated mean number of tumor nodules per lung.
Each mouse received same dose of urethane 1 mg/gm BW,
sacrificed at varying times T after injection.
Data from Shimkin and Polissar [7].

tion specified in the first column (other columns of table I will be used later). Figure 2 shows a plot of $\hat{\xi}(T)$ against T. It is seen that, while the unavoidable random fluctuation of $\hat{\xi}(T)$ are quite noticeable, the convergence of $\hat{\xi}(T)$ to an

asymptotic value is pronounced. Inspection of figure 2 suggests strongly that, if more than one series of mice were available, each series with a different dose D of urethane administered in a single injection, then counts of tumors made after some 20 weeks might reasonably be considered as empirical counterparts of $\zeta(+\infty)$, and used for the verification of the one stage mutation hypothesis. Naturally, this would apply to the kind of mice and to the method of counting tumors used by Shimkin and Polissar (microscopic examination of slices of the lungs). With a different method of counting and/or a different experimenter, the probabilities π_n might well be different, leading to the requirement of a different time period T. In particular, this applies to counting tumors through a naked eye inspection of the surface of the lungs. In this case, in order that a tumor be counted, it must consist of a very large number of cells and, as a consequence, the convergence of $\zeta(T)$ to its asymptotic value must be much slower than in the experiment of Shimkin and Polissar.

8.2. *Three experiments of Gubareff.* The results of three experiments performed by Gubareff in consultation with Shimkin are given in their joint paper [1]. The carcinogen used was urethane administered in several different time patterns and with varying total doses. Figure 3 illustrates all the results of the three experiments that refer to the same total dose of urethane, namely, 1 mg/gm BW. The Roman numerals I, II and III refer to the particular experiments. The subscripts 1, 2, or 12 indicate the number of subdoses in which

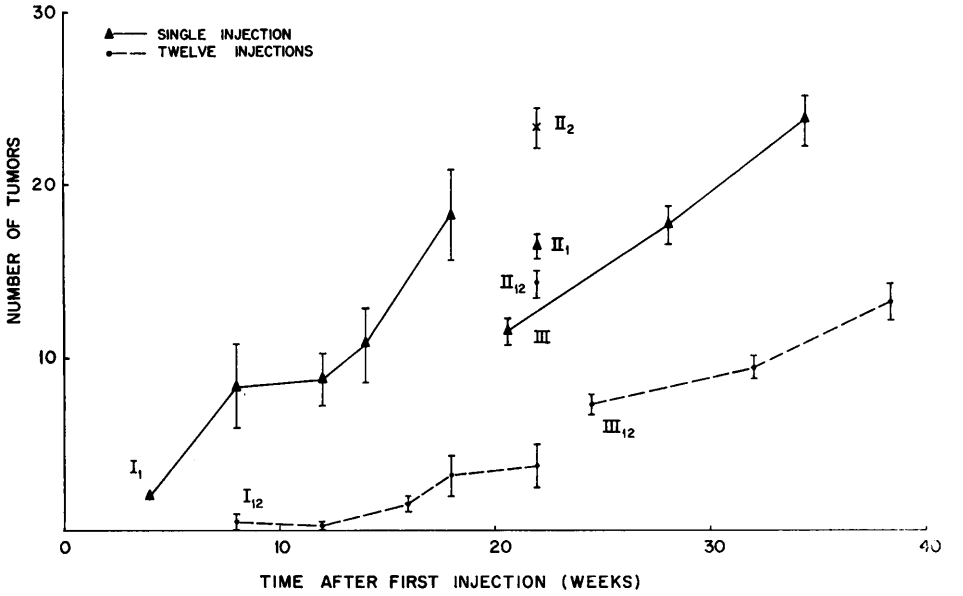

FIGURE 3

Counts of tumors on the surface of the lung in three experiments by Gubareff (see [1]).

the total dose was administered. As indicated the two subdoses, of 0.5 mg/gm BW each, were administered six days apart. The twelve subdoses were injected over four weeks, on Mondays, Wednesdays and Fridays, approximately two days apart. In experiments I and III only two time patterns were used and mice were sacrificed at varying times. Figure 3 illustrates the dependence of the average number of tumors on time after urethane. In experiment II all the mice were sacrificed at the same time, 22 weeks after urethane started.

Gubareff's counts of tumors were restricted to the surface of the lungs and were made by naked eye. Presumably because of this technique the counts of tumors are not stabilized even after 30 weeks.

Experiments I and III complement each other very nicely even though I was a small pretrial involving only a few mice. They strongly suggest that with many subdoses administered at relatively short intervals the ultimate number of lung tumors per mouse must be substantially less than that following a single injection of the same total dose of urethane.

Experiment II does not appear to be consistent with the other two. However, even in experiment II the twelve subdoses resulted in a smaller number of

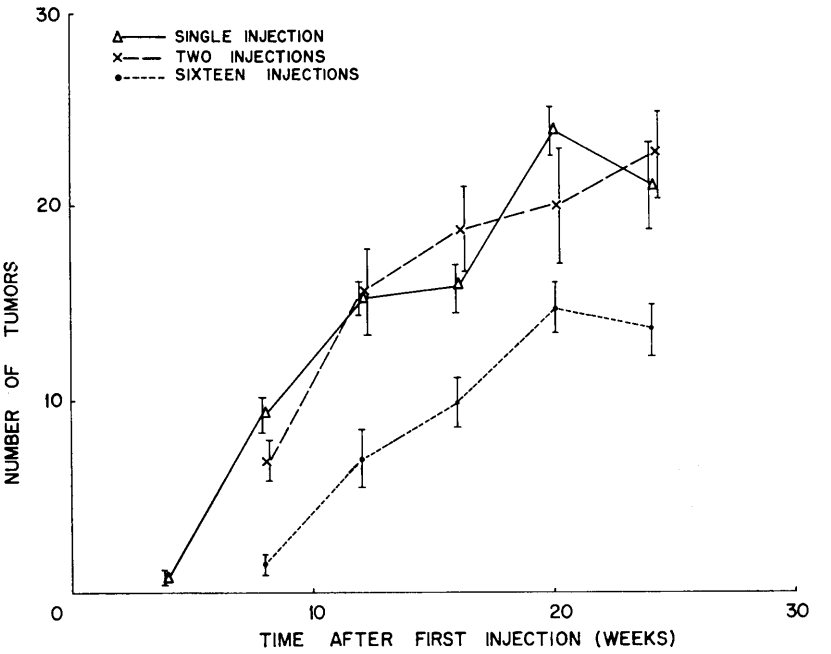

FIGURE 4

Counts of tumors on the surface of the lung
in mice receiving dose 1 mg/gm BW;
Three time patterns: single injection, two injections week apart,
and sixteen injections two days apart (after White [2]).

tumors than did a single dose. The interesting feature is an increase in the number of tumors resulting from the division of the total dose into two subdoses administered six days apart. This increase is quite large and occurs not only with the basic dose of 1 mg/gm BW, but also when the basic dose is half as large (see table II in [1]).

8.3. *Experiment of White, Grendon and Jones.* Out of many tabulations given in [2], we reproduce only one, illustrating the effect of the time pattern in administering the same basic dose of urethane, namely, 1 mg/gm BW. These results, giving the numbers of tumors counted on the surface of the lungs, are illustrated in figures 4 and 5.

The time patterns considered are three: single injection; two injections one week apart; and 16 injections two days apart. A glance at figure 4 indicates that White's experiment confirms the existence of one of the two effects noted in Gubareff's experiment, but not the other. One mg/gm BW administered in small subdoses over a month produced a substantially smaller crop of tumors than the same dose injected at once. On the other hand, there is no noticeable difference between two subdoses injected one week apart and a single injection. White's mice were adult females averaging some 23 gm initial weight. Gubareff's mice were younger and of both sexes, weighing between 11 gm and 17 gm at

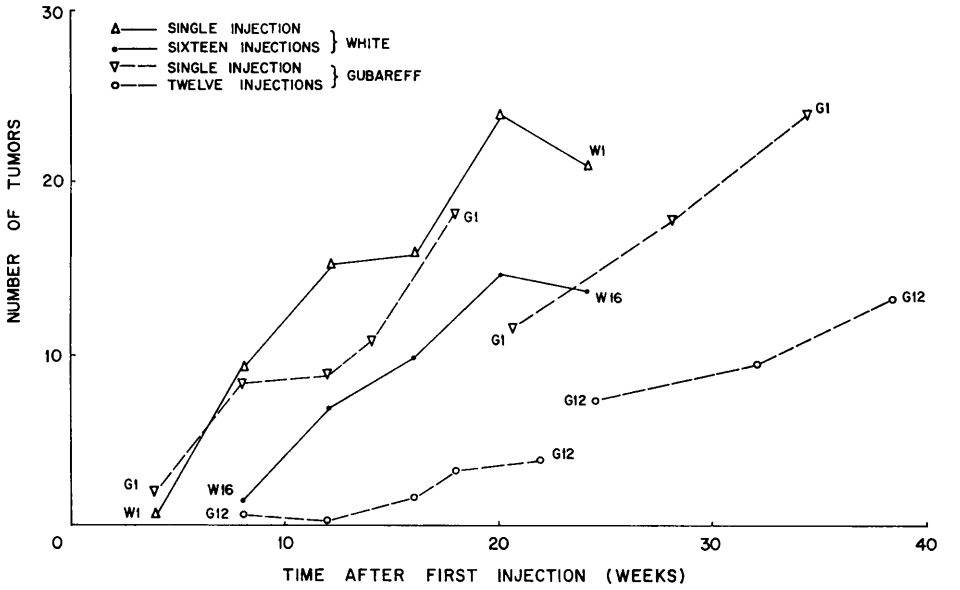

FIGURE 5

Comparison of the counts by Gubareff and by White.
Total dose of urethane, 1 mg/gm BW.
Two time patterns: single injection and
fine fractionation over a month.

the start of the experiment. Can this explain the difference in the results of dividing the basic dose into two subdoses?

Figure 5 combines the results of Gubareff's experiments I and III with those of White referring to the same basic dose of urethane, 1 mg/gm BW, and to two similar time patterns, single injection and many small subdoses administered at short time intervals. It is seen that, while supporting each other qualitatively, the two sets of data differ considerably in numerical values. In neither case has the final count leveled to its asymptotic value. However, White's counts at 24 weeks seem to foreshadow this approach. Also, her counts are generally much higher than Gubareff's. Is this a difference in mice and/or environment or a difference in technique of counting?

8.4. *Experiments at the Fels Research Institute.* Two more experiments are described in [1], listed as experiments IV and V, both performed by Mrs. Dianne Marzi and Mr. Ronald Wieder.

Experiment IV, somewhat smaller than that of Miss White, dealt with mice of the same strain and approximately the same age. Its results are in perfect agreement with those of Miss White. The subdivision of the basic dose into a large number, namely ten, equal subdoses of the urethane leads to a decrease in tumors counted at approximately 20 weeks after the first injection. On the other hand, the division of the basic dose of urethane into two subdoses administered one week apart has no noticeable effect. Here again the reaction of adult mice appears to be different from that of the young mice used in experiment II by Gubareff.

The purpose of experiment V was to investigate one effect suggested by Gubareff's experiment II, namely, the increase in tumors due to the subdivision of the basic dose of urethane into two equal subdoses. Only one basic dose was used in experiment V, 1 mg/gm BW. The interval between subdoses was varied: 1; 2; 4; and 7 weeks. The effect was studied separately for two considerably different ages of mice, 16 to 18 gm at the start of the experiment and 23 to 25 gm. Observations were made separately for males and for females. All mice were sacrificed at 20 weeks after the only or the second injection of urethane.

This very interesting experiment suggested a number of effects: (a) adult females behaved just as they did in the White experiment, with no apparent effect of subdivision of the basic dose; (b) young females indicated two substantial increases in tumors corresponding to the intervals between subdoses of 1 and of 7 weeks; (c) with adult males the 7 week interval between the two subdoses doubled the number of tumors obtained with a single injection; (d) young males responded spottily and indistinctly.

Unfortunately, the mice used in experiment V were not of the same strain as those in all the other experiments. They were Swiss mice characterized by a very strong variability of response to urethane. Thus, in experiment V the coefficient of variation of the number of tumors is 63 per cent, compared to only 22 per cent in experiment IV. In other words, in conditions at the Fels Research Institute, in order that experiment V with Swiss mice yield the same

precision as experiment IV with strain A mice, the number of Swiss mice would have to be multiplied by a factor of about nine. In consequence of this variability, the results of experiment V are very blurred. Because of the obvious interest in the possibility of increasing the number of tumors by a subdivision of the basic dose into two doses administered at an appropriate interval, perhaps depending on the age and the sex of the mice, it appears desirable to repeat experiment V using more homogeneous mice.

In summary, the experiments conducted seem to have established one effect of fractionation quite firmly. This is a decrease in the number of tumors counted up to some 30 weeks after urethane, due to a subdivision of the basic dose into a relatively large number of subdoses administered over a long period of time. This effect was observed in all the experiments in which the particular time pattern was studied, whether with young mice or with adults, to wit in the three experiments of Gubareff, in the experiment of White, and in experiment IV of Marzi and Wieder. In addition, there is a suggestion of another effect: increase in the number of tumors counted some 20 weeks after urethane due to the subdivision of the basic dose into two subdoses administered one week apart. This effect appears distinct in Gubareff's experiment II performed with young mice of both sexes. On the other hand, the adult females used by White show no such effect. A hypothetical mechanism, labeled the two stage mutation model, consistent with both of the above effects, perhaps age and sex dependent, is described in the following sections.

9. Confrontation of the one stage mutation theory with empirical findings

The answer to the question whether the one stage mutation theory is or is not consistent with the available empirical findings depends very much on the attitude one wishes to adopt towards the results described in section 8. The experiments of Gubareff and of White do suggest that the subdivision of a single dose of urethane into a large number of small subdoses administered at short time intervals decreases the crop of tumors considerably. This is obvious for counts made up to 34 weeks in one case and up to 24 weeks in the other. However, it is not quite clear that the decrease will persist if the counts are made much later, when they approach stabilization. If one accepts that this is probably the case, then we have a contradiction with one stage mutation theory.

Another apparent contradiction is to be noted, again depending upon the possibility of using the available data to estimate the asymptotic counts of tumors. Using White's counts of tumors resulting from single injections of increasing doses of urethane, Table II was constructed. It lists the ultimate actual counts of tumors, made after 24 weeks, and also the estimated asymptotic values of these counts, against the total dose of the urethane administered in a single injection. Figure 6 gives the corresponding plots. It is seen that the indicated relation of tumor to dose is far from linear. Indeed this relation is fitted excellently by a parabola. Referring to section 7 it is seen that these

TABLE II

ASYMPTOTIC VALUE OF MEAN NUMBER OF TUMORS PER MOUSE FOR VARYING DOSE D GIVEN AS ONE INJECTION
COMPARISON OF OBSERVATION AND LEAST SQUARES FIT
Data of White, Grendon, and Jones [2].

Dose D (mg/gm BW)	Last Observed Value (24 weeks)		Estimated Asymptotic Value	
	Observed	Computed	Observed	Computed
0.0625	1.2	1.07	1.4	1.40
0.125	2.0	1.99	2.3	2.19
0.250	3.8	4.10	4	4.20
0.500	9.5	9.31	10	9.90
1.000	23.7	23.73	28	28.01

findings appear to contradict the deductions from one stage mutation mechanism.

Finally, if the results of Gubareff's experiment II, indicating an increase in tumors due to the division of a single dose into two subdoses are accepted as

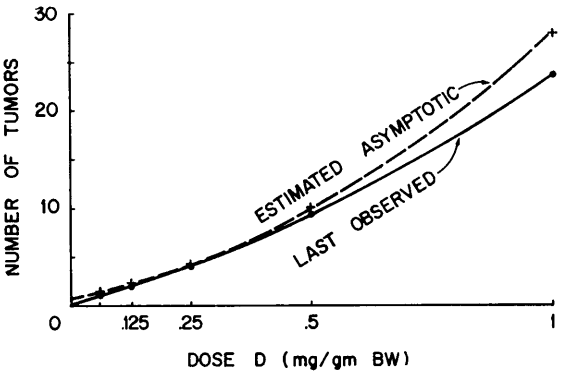

FIGURE 6

Ultimate tumor counts versus dose.
Single injection (Data from White [2]).
Solid curve: last observed number of tumors (24 weeks) fitted by least squares;
Dashed curve: estimated asymptotic value.

applying to asymptotic values of the counts, this would be the third point of contradiction.

Tentative as the above interpretations of the experimental results are, it appears justifiable to investigate whether they are consistent with a version of the two stage mutation mechanism. This is done in the following sections.

10. Revision of the original two stage mutation model of carcinogenesis

In the original version of the two stage mutation mechanism of carcinogenesis [9], it was assumed that a hit on a normal cell turns this cell immediately into a first order mutant subject to three time independent risks: the risk of division at rate λ; of death at rate $\mu > \lambda$; and of second order mutation at rate ν. In addition, all cells and clones involved were assumed mutually independent. Apart from second order mutants, all the cells descending from the original first order mutants were assumed to have the same properties, forming a subcritical birth and death process "with emigration." The second order mutant cells were considered cancer cells, each generating a supercritical birth and death process.

In his reexamination of this model, D. G. Kendall [10], suggested that second order mutations might also be induced by hits of the carcinogen. If these hits are governed by a dose D of carcinogen administered with the time pattern f, then, with the above notation, ν should be replaced by the product $\nu Df(t)$.

In the present revision of the two stage mutation mechanism we will generalize this assumption somewhat by setting

$$\nu = \nu_0 + \nu_1 Df(t), \tag{10.1}$$

where ν_0 and ν_1 are nonnegative constants not both equal to zero.

The adoption of formula (10.1) represents no more than a natural generalization of the models considered earlier. However, the empirical findings described in section 8 dictate a change in the model which goes a little farther.

Denote by $Y(t)$ the number of first order mutant cells alive at time $t > 0$. We shall consider the expectation of $Y(t)$ within the framework of the original model generalized by assuming (10.1). The system of random variables $Y(t)$ is what is sometimes called a birth and death process "with immigration" at time dependent rate $Df(t)$. The rate of birth is λ and the rate of death is $\mu + \nu_0 + \nu_1 Df(t)$. For the sake of compactness, now let $q = \mu + \nu_0 - \lambda$ and

$$F(x) = \int_0^x f(t)\, dt. \tag{10.2}$$

Let $\eta(t)$ stand for the expectation of $Y(t)$. Easy and familiar calculations yield

$$\eta(t) = De^{-qt-\nu_1 DF(t)} \int_0^t f(x) e^{qx+\nu_1 DF(x)}\, dx. \tag{10.3}$$

This formula is valid for any time pattern function f. Our present interest in this formula is motivated by experimental result of Shimkin and Polissar and by the tentative assumption that the hypercellularity they observed following a single injection of urethane represents the growth of first order mutants. As will be seen from the third column of table I, the average number of these mutants begins to grow immediately after the injection, reaches a maximum at about 28 days, and then declines. The question arises as to whether this behavior is consistent with formula (10.3).

Obviously, formula (10.3) depends on the properties of the time pattern

function f. With reference to injections of urethane it is empirically established that within a very short time, certainly within 24 hours, all the urethane injected is eliminated from the bodies of the mice. Barring the possibility that the carcinogenic effect is due not to urethane itself, but to some other chemical originating from the urethane, the above finding implies that the time pattern function $f(t)$ is equal to zero for all $t > t_0$ where t_0 may be as small as one day. If this be so, then for $t > t_0$, formula (10.3) reduces to

$$\eta(t) = De^{-qt-\nu_1 D}\int_0^{t_0} f(x)e^{qx+\nu_1 DF(x)}\,dx, \qquad t > t_0 \tag{10.4}$$

implying that, for $t > t_0$, the average number of first order mutants must decrease in proportion to a negative exponential. It follows that, if the hypercellularity observed by Shimkin and Polissar is indeed due to first order mutants —predecessors of cancer, then the original model is inconsistent with the observation and must somehow be revised. Our choice is the assumption already discussed in section 6 in a different connection.

We assume that a normal cell, say C_0, hit by the carcinogen changes into what we call a primary first order mutant subject to two risks only: the risk of division at a constant rate β, and the risk of death at a constant rate $\gamma = \Delta - \beta$. Thus, the primary first order mutant is not subject to the risk of secondary mutation. However, if and when C_0 divides, the two daughter cells, say C_{11} and C_{12}, called secondary first order mutants, have properties different from those of C_0. Namely, C_{11} and C_{12} each generate an independent clone of cells identical with C_{11} and C_{12}, exposed to three risks: of division at a constant rate λ; of death at a constant rate $\mu > \lambda$; and of secondary mutation at a possibly carcinogen dependent rate $\nu = \nu_0 + \nu_1 Df(t)$. The rates β and γ of the division and of death of the primary first order mutants might be those of normal cells. However, presumably both λ and μ are much larger.

The postulated distinction between primary and secondary first order mutants is suggested by the fact that mutations in irradiated flies manifest themselves not in these same flies but in their progeny and further descendants.

In summary, the revised hypothetical two stage mutation mechanism of carcinogenesis is as follows:

(a) Normal cells are hit by carcinogen at an instantaneous rate $Df(t)$, starting at $t = 0$.

(b) Each normal cell that is hit turns into a primary first order mutant subject to risks of division and of death at constant rates β and γ, respectively.

(c) Each of the daughter cells resulting from a division of a primary first order mutant, called secondary first order mutant, generates a subcritical birth and death clone with emigration, independent of all others, with rates λ and $\mu > \lambda$ of birth and death, and with rate $\nu = \nu_0 + \nu_1 Df(t)$ of secondary mutation.

(d) Each secondary mutant is a cancer cell, generating a supercritical birth and death clone of identical cells, with constant rates Λ and $M < \Lambda$, respectively.

The above hypotheses are supplemented by the usual assumptions of independence of particular cells and clones.

Within this model, we let $X(t)$, $Y(t)$, and $Z(t, T)$ denote three random variables defined as follows:

$X(t)$ is the number of primary first order mutants alive at time $t \geqq 0$;

$Y(t)$ is the number of secondary first order mutants alive at t;

$Z(t, T)$ denotes the number of second order mutant clones generated before time $t > 0$ and counted at a subsequent time $T \geqq t$. Also, for $t = T$ we shall write $Z(T, T) = Z(T)$.

In the next section we study the distribution of the variables enumerated and, in particular, the expectations $EX(t) = \xi(t)$, $EY(t) = \eta(t)$ and $EZ(t, T) = \zeta(t, T)$. Here again $\zeta(T, T)$ will be written simply as $\zeta(T)$. In particular, it will be seen that, with an appropriate adjustment of the various rates, the behavior of $\eta(t)$ is consistent with the gradual increase and the subsequent decrease of hypercellularity observed by Shimkin and Polissar [12].

11. Some implications of the revised one hit, two stage mutation model of carcinogenesis

For $t > 0$, let

$$P_{\ell,m,n}(t) = P\{X(t) = \ell, Y(t) = m, Z(t, T) = n\}. \tag{11.1}$$

Proceeding in the customary manner, we now deduce a relation between the probability $P_{\ell,m,n}(t + \tau)$, with $\tau > 0$, and similar probabilities relating to time t. For the sake of compactness, the arguments t are omitted. We have

$$\begin{aligned} P_{\ell,m,n}(t + \tau) &= P_{\ell-1,m,n}Df\tau + (\ell + 1)P_{\ell+1,m-2,n}\beta\tau + (\ell + 1)P_{\ell+1,m,n}\gamma\tau \\ &\quad + (m - 1)P_{\ell,m-1,n}\lambda\tau \\ &\quad + (m + 1)P_{\ell,m+1,n}\{\mu + \nu[1 - \pi(T - t)]\}\tau \\ &\quad + (m + 1)P_{\ell,m+1,n-1}\nu\Pi(T - t)\tau \\ &\quad + P_{\ell,m,n}\{1 - [Df + \ell\Delta + m(\lambda + \mu + \nu)]\tau\} + o(\tau). \end{aligned} \tag{11.2}$$

This equation leads to a partial differential equation for the probability generating function $G = G(u, v, w, t)$ of the three variables considered. Using subscripts to denote partial derivatives, we have

$$\begin{aligned} G_t &= (u - 1)DfG + (\beta v^2 + \gamma - \Delta u)G_u \\ &\quad + [(\lambda v - \mu - \nu)(v - 1) + \nu\Pi(T - t)(w - 1)]G_v. \end{aligned} \tag{11.3}$$

Differentiating this equation in turn with respect to u, v and w and equating these arguments to unity, we obtain a system of three ordinary differential equations for the unknown functions $\xi(t)$, $\eta(t)$ and $\zeta(t, T)$,

$$\begin{aligned} \xi'(t) &= Df - \Delta\xi(t), \\ \eta'(t) &= 2\beta\xi(t) - (q + \nu_1 Df)\eta(t), \\ \zeta'(t, T) &= (\nu_0 + \nu_1 Df)\Pi(T - t)\eta(t), \end{aligned} \tag{11.4}$$

where $q = \mu + \nu_0 - \lambda$ and the primes designate differentiation with respect to t. Solutions of the system (11.4) are easily obtained yielding

$$\xi(t) = De^{-\Delta t} \int_0^t e^{\Delta x} f(x)\, dx, \tag{11.5}$$

$$\eta(t) = 2\beta e^{-qt-\nu_1 DF(t)} \int_0^t e^{qx+\nu_1 DF(x)} \xi(x)\, dx, \tag{11.6}$$

where $F(t) = \int_0^t f(x)\, dx$, and

$$\zeta(t, T) = \nu_0 \int_0^t \Pi(T - x)\eta(x)\, dx + \nu_1 D \int_0^t f(x)\Pi(T - x)\eta(x)\, dx. \tag{11.7}$$

12. Qualitative consistency of the revised one hit, two stage mutation model of carcinogenesis with the hypercellularity observed by Shimkin and Polissar

Before proceeding any further it is necessary to verify whether the distinction between the primary and the secondary first order mutants, introduced in the above revision of two stage mutation model, provides the possibility of adjustment to the behavior of hypercellularity noticed by Shimkin and Polissar. Following a single injection of urethane, this hypercellularity grows, reaches a maximum at about four weeks, and then declines to zero. The question is whether, with an appropriate time pattern function f and with appropriately adjusted constant parameters Δ, q, and ν_1, the function $\eta(t)$ of formula (11.6) will behave in the manner indicated.

In order to answer this question we use a modification of the method of section 10 that indicated the necessity of a revision of the original two stage mutation model. Namely, we shall assume that the time pattern function $f(t)$ vanishes for all t exceeding a limit t_0. Next, after some transformation of formula (11.6), we shall make t_0 tend to zero. The limit so obtained will represent an approximation to $\eta(t)$ in cases where the carcinogen injected at $t = 0$ is rapidly eliminated from the body of the experimental animal.

Using (11.5) and (11.6), and assuming $t > t_0$, we have

$$\eta(t) = 2\beta De^{-qt}\Big\{\int_0^{t_0} e^{(q-\Delta)x-\nu_1 D[1-F(x)]} \int_0^x e^{\Delta u} f(u)\, du\, dx + \int_{t_0}^t e^{(q-\Delta)x} \int_0^{t_0} e^{\Delta u} f(u)\, du\, dx\Big\}. \tag{12.1}$$

As $t_0 \to 0$, the first term in curly brackets tends to zero. The limit of the second term is

$$\int_0^t e^{(q-\Delta)x}\, dx = \frac{e^{(q-\Delta)t} - 1}{q - \Delta}, \tag{12.2}$$

and it follows that

$$\lim_{t_0 \to 0} \eta(t) = 2\beta D \frac{e^{-\Delta t} - e^{-qt}}{q - \Delta} = \eta_0(t), \tag{12.3}$$

say. It is easy to see that, with appropriate choice of Δ and q, the qualitative behavior of the function $\eta_0(t)$ is the same as that of the hypercellularity investigated by Shimkin and Polissar. In fact, $\eta_0(t)$ vanishes at $t = 0$ and at infinity and has a unique maximum at

$$t^* = \frac{\log q - \log \Delta}{q - \Delta}, \tag{12.4}$$

which may be just as close to zero or just as large as desired. Thus, qualitatively at least, the consequences of the revised model agree with the changes in hypercellularity observed by Shimkin and Polissar.

13. Two general properties of the expected number of tumors counted at time T

In this section we turn to formula (11.7) evaluated at $t = T$, giving the expectation of the number of tumors induced by the experimental carcinogen counted at time T. We prove two interesting properties of this function. First we give two easy lemmas.

LEMMA 1. *Within the framework of the revised two stage mutation model, if* $\nu_1 = 0$ *then*

$$\int_0^\infty \eta(t)\, dt = \frac{2\beta D}{q\Delta}. \tag{13.1}$$

Under conditions of the lemma, the formula for $\eta(t)$ can be written in the form

$$\begin{aligned} \eta(t) &= 2\beta D e^{-qt} \int_0^t f(x) e^{\Delta x} \int_x^t e^{(q-\Delta)u}\, du \\ &= 2\beta D e^{-qt} \int_0^t f(x) e^{\Delta x} \frac{e^{(q-\Delta)t} - e^{(q-\Delta)x}}{q - \Delta}\, du \\ &= \frac{2\beta D}{q - \Delta} \left\{ e^{-\Delta t} \int_0^t f(x) e^{\Delta x}\, dx - e^{-qt} \int_0^t f(x) e^{qx}\, dx \right\}. \end{aligned} \tag{13.2}$$

However, for any positive number A,

$$\int_0^\infty e^{-At} \int_0^t f(x) e^{Ax}\, dx = \frac{1}{A} \int_0^\infty f(x)\, dx = \frac{1}{A}. \tag{13.3}$$

It follows that, whatever the time pattern function f,

$$\int_0^\infty \eta(t)\, dt = \frac{2\beta D}{q - A} \left(\frac{1}{\Delta} - \frac{1}{q} \right) = \frac{2\beta D}{q\Delta}. \tag{13.4}$$

LEMMA 2. *Within the framework of the revised two stage mutation model, the integral of* $\eta(t)$ *taken from zero to infinity is always finite.*

The assertion of lemma 2 follows from the obvious fact that, with $\nu_1 > 0$, the value of $\eta(t)$ is always less than that corresponding to $\nu_1 = 0$.

THEOREM 1. *Within the framework of the revised two stage mutation model of carcinogenesis, the ultimate number of killer tumors has the expression*

$$\zeta = \lim_{T \to +\infty} \zeta(T) = \left\{ \nu_0 \int_0^\infty \eta(t)\, dt + \nu_1 D \int_0^\infty f(t)\eta(t)\, dt \right\} \left(1 - \frac{M}{\Lambda} \right). \tag{13.5}$$

This theorem is a simple consequence of lemma 2.

THEOREM 2. *Within the framework of the revised two stage mutation model of*

carcinogenesis, if $\nu_1 = 0$, *so that the rate of the second order mutation does not depend upon the presence of the carcinogen that caused the first order mutation, then the ultimate number of killer tumors does not depend upon the time pattern function and is proportional to the total dose D of the carcinogen.*

$$\zeta = \nu_0 \left(1 - \frac{M}{\Lambda}\right) \frac{2\beta D}{q\Delta}. \tag{13.6}$$

Theorem 2 is a simple consequence of lemma 1 and formula (13.5).

As a result of theorem 2, if figure 6 is accepted as an indication that the ultimate number of killer tumors is not a linear function of D, then this is evidence not only against the one stage mutation mechanism, but also against the hypothesis that $\nu_1 = 0$. Similarly, the apparent decrease in the ultimate number of killer tumors due to fractionation of the basic dose D into a large number of subdoses administered every second day or so, is also evidence in favor of the assumption that $\nu_1 > 0$. In fact, any dependence of the ultimate number of killer tumors on the time pattern, in which the carcinogen is administered, is evidence that $\nu_1 > 0$.

REMARK. One of the conclusions reached in [11] is contrary to theorem 2. This conclusion is a consequence of an error. We are indebted to Dr. Witold Klonecki for pointing out this error to us.

14. Source of parabolic like dependence of the expected ultimate number of killer tumors on the total dose of carcinogen

The result of substituting (11.6) into (11.7) and, then, of (11.5) into the results of the first substitution yields

$$\begin{aligned}(14.1)\quad &\zeta(t, T) \\ &= \nu_0 2\beta D \int_0^t \Pi(T - x) e^{-qx - \nu_1 DF(x)} \int_0^x e^{(q-\Delta)u + \nu_1 DF(u)} \int_0^u e^{\Delta v} f(v)\, dv\, du\, dx \\ &\quad + \nu_1 2\beta D^2 \int_0^t \Pi(T - x) f(x) e^{-qx - \nu_1 DF(x)} \int_0^x e^{(q-\Delta)u + \nu_1 DF(u)} \int_0^u e^{\Delta v} f(v)\, dv\, du\, dx.\end{aligned}$$

The subject of discussion in this section is the result of substituting $T = +\infty$ into (14.1). This yields the expected number of killer tumors generated between the beginning of the experiment and some preassigned time t. We have

$$\zeta(t, +\infty) = AI(t, D)D + BJ(t, D)D^2 \tag{14.2}$$

with

$$\begin{aligned}A &= 2\beta\nu_0 \left(1 - \frac{M}{\Lambda}\right), \\ B &= 2\beta\nu_1 \left(1 - \frac{M}{\Lambda}\right), \qquad (14.3) \\ I(t, D) &= \int_0^t e^{-qx - \nu_1 DF(x)} \int_0^x e^{(q-\Delta)u + \nu_1 DF(u)} \int_0^u e^{\Delta v} f(v)\, dv\, du\, dx, \\ J(t, D) &= \int_0^t f(x) e^{-qx - \nu_1 DF(x)} \int_0^x e^{(q-\Delta)u + \nu_1 DF(u)} \int_0^u e^{\Delta v} f(v)\, dv\, du\, dx.\end{aligned}$$

It is plausible that, with appropriate values of the constants involved, the dependence on D of the integrals $I(t, D)$ and $J(t, D)$ is only slight. If this be the case, then formula (14.2) indicates that, with a fixed t and with varying D, the expected number of killer tumors generated before t will depend on D in a manner close to parabolic. This, then, is a heuristic explanation of the parabolic change in White's counts of tumors generated by single injections of varying doses of urethane as illustrated in figure 6. However, the same conclusion applies to any fixed time pattern in the application of the carcinogen as is noticeable in figure 2 in paper [2].

15. Numerical illustration of the various functions involved in the revised two stage mutation model of carcinogenesis

We are indebted to Mrs. Jeanne Lovasich of the Statistical Laboratory for programming and for performing the calculations that led to the plots exhibited in figures 7 and 8. The purpose of these graphs is to illustrate the several functions involved in the model, namely, the time pattern function $f(t)$, the expected number $\xi(t)$ of primary first order mutants, the expected number $\eta(t)$ of secondary first order mutants, and the expected number $\zeta(t, +\infty)$ of killer tumors generated before time t.

In figure 7 only two time pattern functions are considered. The upper panel corresponds to a single injection of urethane, supposed to be eliminated from the bodies of the mice exponentially at a unit time rate α. The lower panel is intended to represent an experiment with the same total dose of urethane administered in two equal subdoses one week apart. Here again it is assumed that the elimination of urethane follows the same exponential law. The intention is to show that, with appropriately selected values of the constants involved, the number of killer tumors generated by two equal subdoses can either be smaller than or larger than that obtained by administering the same amount of urethane in a single injection, depending on the time when the second subdose is administered.

Figure 7 exhibits all the functions involved, the upper panel corresponding to a single injection and the lower to two injections. The assumed values of ν_0 and ν_1 are very unequal, the first being small and the second very large. Since the carcinogen is eliminated very quickly, and the production of secondary first order mutants requires some time, the bulk of the tumors resulting from a single injection are produced at the slow rate ν_0. With two injections, each amounting to half of the total dose, the initial number of primary and of secondary first order mutants is about half of what is obtained by a single injection. This also applies to the initial number of tumors. However, immediately after the second injection, administered at the time when the number η of expected secondary first order mutants is already substantial, the formation of tumors receives a large boost due to the presence of the carcinogen and the high value of ν_1. As a result, the value of ζ increases rapidly and overshoots that correspond-

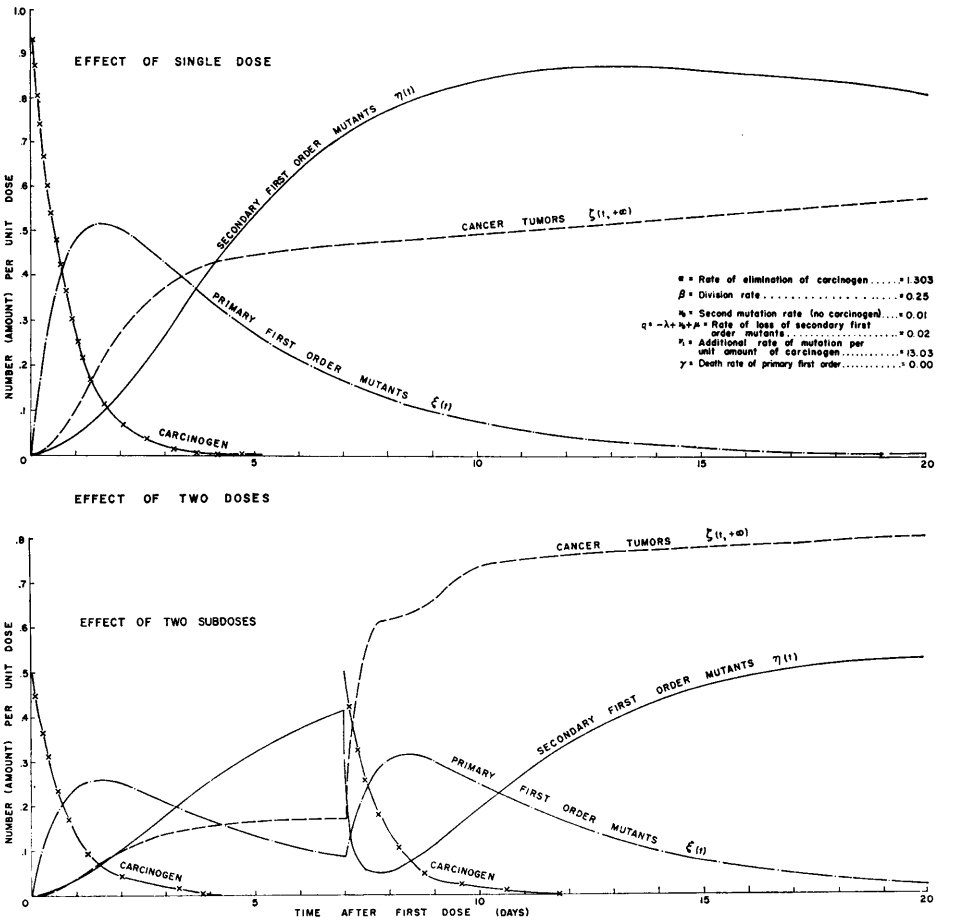

FIGURE 7

Comparison of effects of single injection and two injections. Expectations $\xi(t)$, $\eta(t)$, and $\zeta(t)$ of the number of primary and of secondary first order mutants and of killer tumors, respectively.

ing to a single injection. It is intuitively clear that, if ν_1 were substantially smaller or if the second injection were applied at a time when the number η of secondary first order mutants is small, then two injections of a half dose each would result in a decrease in the number of tumors.

The possibility of either an increase or a decrease in killer tumors by fractionation is illustrated in figure 8, which shows two sets of three curves, corresponding to three different time patterns in the application of the same total dose of carcinogen: a single injection, two injections and sixteen injections administered at short time intervals, somewhat as in the experiment of White.

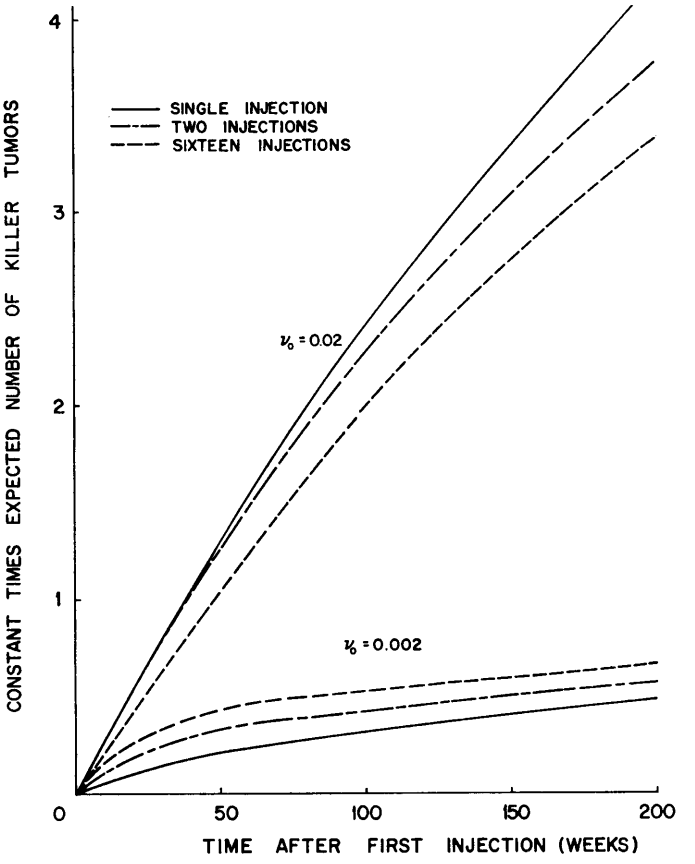

FIGURE 8

Change in expected number of tumors resulting from change in parameter ν_0 from 0.02 to 0.002;

$\alpha = 1.30$, $\beta = 0.25$, $\gamma = 0.00$, $q = 0.004$, $\nu_1 D = 1.00$.

Three time patterns
Solid curve: Single injection of dose D,
Long dash curve: Two injections of $D/2$, week apart;
Short dash curve: Sixteen injections of $D/16$, two days apart.

It is seen that a change in values of one of the constants involved leads to an interchange in the location of the curves indicating the expected number of killer tumors.

16. Concluding remarks

The results obtained may be summarized as follows.

16.1. *Revision of the original two stage model.* It was shown that the implica-

tions of the original two stage mutation model of carcinogenesis are not consistent with the behavior of hyperplasia observed by Shimkin and Polissar. The modification adopted to remove this difficulty consists in the assumption that the first order mutation induced by a hit of the carcinogen manifests itself not in the cell that incurred the hit, but in its two daughter cells and in their descendants. Another modification of the original model was adopted. This consists in the assumption that the rate of second order mutations, leading to cancer cells, may be written as $\nu = \nu_0 + \nu_1 Df(t)$, where ν_0 and ν_1 are adjustable nonnegative constants not both equal to zero, and $Df(t)$ stands for the rate of hits by the carcinogen at time t.

16.2. *Cases where the total expected number of killer tumors is independent of the time patterns of the application of the carcinogen.* The one stage mutation mechanism of carcinogenesis (actually two slightly different versions of this mechanism) and also the two stage mutation mechanism, with the additional assumption that $\nu_1 = 0$, imply that the expected ultimate number of killer tumors is a linear function of the total dose D of the carcinogen applied in the experiment, and that it is independent of the time pattern $f(t)$ in which this dose is administered.

16.3. *Cases where the expected number of killer tumors depends upon the time pattern of the application of the carcinogen.* Numerical calculations, performed on the two stage mechanism with $\nu_1 > 0$, show that the fractionation of the basic dose D of the carcinogen can decrease, and also that it can increase, the expected number of tumors, depending upon the values of the various parameters involved in the model, which may depend on the age of the experimental animals.

16.4. *Reference to experimental data.* With a degree of interpretation, and within the framework of the basic assumptions (see section 4), the experimental data favor the two stage mechanism with $\nu_1 > 0$. In fact, the experiments by Gubareff and by White strongly suggest that fractionation of the total dose of urethane into many subdoses decreases the ultimate yield of tumors considerably. Also, in White's experiment the estimated ultimate yield of tumors plotted against the total dose of urethane indicates a parabolic rather than a linear relation. Finally, Gubareff's experiment II performed with young mice indicates an increase in tumors due to the division of the basic dose of urethane into two subdoses administered six days apart. (However, White's experiment with adult mice shows no such effect.) Within the framework of our basic assumptions these effects are possible only on the two stage mutation model with $\nu_1 > 0$. In other words, these effects are possible only if the first cancer cells result from a urethane induced mutation occurring not in a normal cell but in a cell of a predecessor benign growth, also induced by urethane.

16.5. *Qualifications.* The above tentative conclusions are subject to a number of qualifications of which we emphasize the following.

(i) The interpretation of experimental results depends upon the assumption that a single injection of a dose D mg/gm body weight of urethane produces

the same number of hits as s successive injections, each of (D/s) mg/gm BW. The process of elimination of the urethane from the bodies of mice does not seem to have been fully investigated and there is no certainty that the above assumption is realistic.

(ii) With the possible exception of the experiment of Shimkin and Polissar, the counts of tumors are not the "ultimate" counts corresponding to the asymptotic values. Thus, suggestive as Gubareff's and White's experiments are, it is not impossible that counts of tumors made some 50 or 60 weeks after urethane would have shown no difference between a single and a fractionated injection of the same total dose of the carcinogen.

In this connection it is appropriate to mention that a prolonged duration of the experiment may produce new difficulties. For example, tumors formed deep in the lungs might become visible on the surface.

16.6. *Possibility of validating the model.* The validation of the revised two stage mutation model of carcinogenesis depends upon the possibility of identifying the several different kinds of cells postulated and on independent experiments leading to direct estimates of the unspecified functions $f(t)$ and π_n, and of the several constants involved. The validation would consist in substituting the estimates into the relevant formulas and in verifying whether the results agree with the actual counts of tumors.

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