COMPARABLE MODELS FOR CARCINOGENESIS BY ULTRAVIOLET LIGHT AND BY CHEMICAL AGENTS

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1. Introduction

Although there have been many experimental studies of carcinogenesis, using a variety of agents, relatively few have been carried out in such a way as to make them useful for quantitative study. No doubt, in some cases, additional data exist which have not been fully reported because of limitations of means of publication or other reasons. A recent request from the group under which this Symposium has been organized, has called to my attention incompleteness in reporting of my own data on induction of cancer by ultraviolet light, and I have therefore made them available in some detail in mimeographed form. Requests for this material may be addressed to me at the Department of Biology, Princeton University, Princeton, New Jersey. But even with the best planned studies the kinds of data that can be obtained are severely limited by the available means of experiment.

Since the individual cancer cell cannot be distinguished from the cells of the tissue of origin, the moment of emergence of a cancer cannot be directly established; cancers are recognized only after they have grown to masses composed of large numbers of cells. The principal measurement feasible in experimental studies is the time elapsed between the application of a carcinogenic agent and the appearance of a tumor of grossly observable volume. This interval I refer to as the *development time.*

Clearly the development time must be occupied, at least in part, by growth of the tumor. In order to extrapolate back from the terminal volume to the moment of origin of the cancer it would be necessary to know the character of the growth curve. Yet various hypotheses regarding the origin of cancer have tacitly contained this extrapolation without reference to a growth function, and without quantitative support. Some quantitative models also appear to have

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been constructed without specific attention to the character of the growth function.

The model for carcinogenesis by ultraviolet light, which is discussed in this paper, has perhaps two merits: (1) it is based on growth of the tumor, and (2) it achieves good agreement with the various aspects of the existing data. Its author is well aware that the smooth growth curve presented by this deterministic model does not accurately describe the intimate events of cancer development, but it is thought to describe the cumulative effect of these events on the growth of the cancer mass.

It is not possible in this brief space to present the model in complete detail, nor its justification in terms of what is known about the cancer process. These things have been discussed at length in journal articles and in a monograph, to which frequent reference will be made in the course of the paper. It is hoped that the critical reader will consult these references before arriving at a final evaluation of the merits of the model.

A model for carcinogenesis by chemical agents, which is based on the same fundamental concept as that for ultraviolet carcinogenesis is also sketched in this paper. While still in incomplete form, the model seems to account plausibly for some of the published experimental data which seem on first consideration to be quite incompatible.

2. Carcinogenesis by ultraviolet light

It is possible to produce cancers of the skin of mice or rats by repeated doses of ultraviolet light, with incidences up to 100 per cent, and with very good reproducibility if uniform experimental conditions are maintained. It has been possible to work out a model that fits the data from such experiments, and seems to shed some light on the nature of the carcinogenic process. It would require more space than is available here to describe in detail the steps by which I arrived at the model, so I shall discuss only salient points to show that the data support the model (for a detailed account see [1] to [5]).

Since tumors grow by the proliferation, or replication, of their component cells, the rate of increase in volume is always proportional to the volume of the tumor at the moment. There is no appreciable change in the volume of the cells themselves, and since these are very small compared to the tumor volume we observe, we may write

$$\frac{dV}{dt} = f(t)V = \text{rate of growth}, \tag{1}$$

where V is the volume, t the time, and $f(t)$ some function of time. If the rate of proliferation is constant, $f(t)$ is a constant. But much of the cancer problem centers around the mechanism of proliferation, and we may emphasize this by rearranging (1) to read

$$\frac{dV}{V\,dt} = f(t) = \text{rate of proliferation}. \tag{2}$$

The basic idea in our model is that the rate of proliferation is accelerated by each successive dose of radiation in amount proportional to the magnitude of the dose. If we deal with many closely spaced doses we may think of the acceleration as occurring smoothly, and write

$$\frac{dV}{V\,dt} = kDn = kD\frac{t}{i}, \tag{3}$$

where V is the volume, k is a constant, D is the dose; n is the number of doses, which is equal to t/i where i is the interval between doses.

Integrating, we obtain

$$\log V = \frac{kDt^2}{2i} + C. \tag{4}$$

This should describe the growth of a single clone or a fixed number of clones.

But in order to describe the data we find it necessary to assume that new groups of clones are added in the course of the tumor development, so we write

$$\log V = \frac{\varphi(t)kDt^2}{2i} + C, \tag{5}$$

where $\varphi(t)$ describes the addition of clones as t increases. We do not know $\varphi(t)$ definitely, since we cannot follow directly the course of growth during the development time.

But for the terminal condition we may write

$$\log V_d - \log V_c = \frac{bkDt_d^2}{2i}, \tag{6}$$

where V_d is the volume at the end of the development time t_d, V_c is the initial volume at the time of the first dose, which we assume to be constant, and b corresponds to the value of $\varphi(t)$ at time t_d.

The experiments on which the model is based were carried out at the National Cancer Institute, beginning in the year 1940. They were begun in collaboration with Dr. H. G. Grady and Dr. J. S. Kirby-Smith. They are described in a series of articles in the Journal of the National Cancer Institute between 1940 and 1944; the principal ones being cited in [1] to [4] and more completely in [5]. Because of the war they were terminated three years later, and have not been resumed. One would feel happier if some points were better established, but since all the data fit well with the model it seems that some conclusions can be drawn with considerable confidence.

Within the limits that are inevitably placed on such experiments, these were well controlled. One sex only of a genetically homogeneous strain of mice (strain A) was used. The animals were subjected to monitored doses of ultraviolet radiation, repeated at regular intervals, the dose being always very short compared to the interval between doses. In most cases, the doses were continued until a tumor of approximately 60 mm^3 appeared on one or other ear of the mouse (that is, $V_d = 60$ mm^3); the time from the first dose to the appearance of such a tumor

was taken as the development time, t_d. The animals could not be held in fixed position with respect to the light source, and this may have contributed considerably to the wide variance of t_d observed within a population of otherwise identically treated mice. The data follow a regular distribution, however, as is shown in figure 1. Here are represented the results of four of our experiments each involving about 50 mice, in which the dosage was different for each experiment. It is noted that by plotting the logarithm of t_d against the percentage incidence of mice with tumors, the points for a given experiment are described

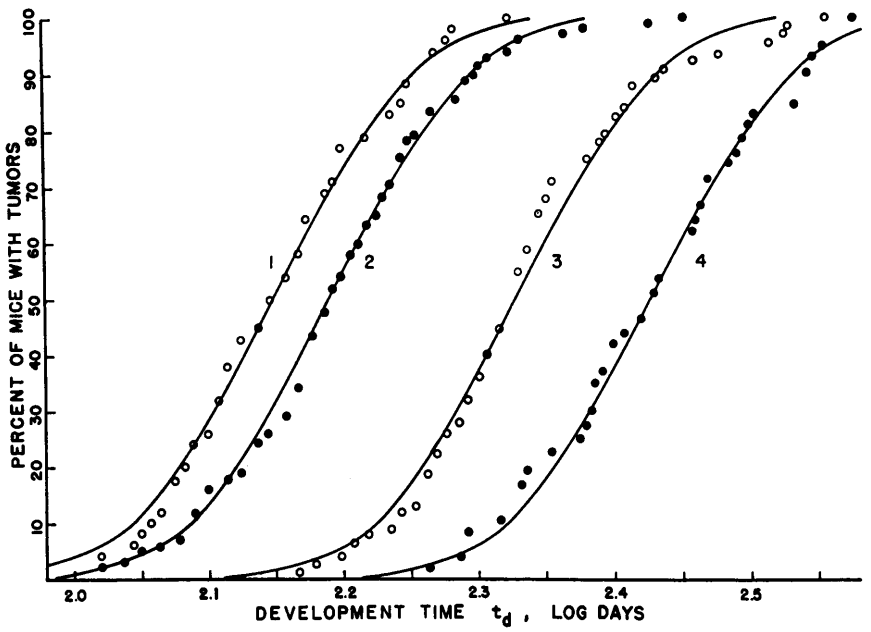

FIGURE 1

Data from four experiments in which mice were dosed with ultraviolet light at regular intervals until the time of appearance of tumors of the ear. Reproduced from [5] with the permission of Princeton University Press.

by the integral of a normal distribution, as represented by the drawn curves. The curves represent the same variance, that is, changing the dosage—either the size of the individual doses, the interval between the doses, or the dose rate, simply moves the curve along the abscissa. This is demonstrated more clearly in figure 2, where data from several experiments are brought to a common mean on the abscissa by multiplying by an appropriate factor.

From a quantitative point of view the most notable characteristic of a cancer is that it grows faster than the tissues from which it derives—indeed, this faster growth is the basis of all our quantitative measurements. There must, then, be an acceleration of the rate of proliferation of the cancer cells, on the average, to

a point above that of the normal tissue cells. A priori, one might think of this acceleration as occurring either rapidly or gradually; but the character of the development times versus incidence curves seems to argue against this. Suppose, for example, that the development time were made up of a period of "induction" followed by a period of growth; proportionality between these two periods is suggested by the curves, and this seems difficult to account for ([5], pp. 216–220).

On the other hand, the constant acceleration assumed in our model would fit

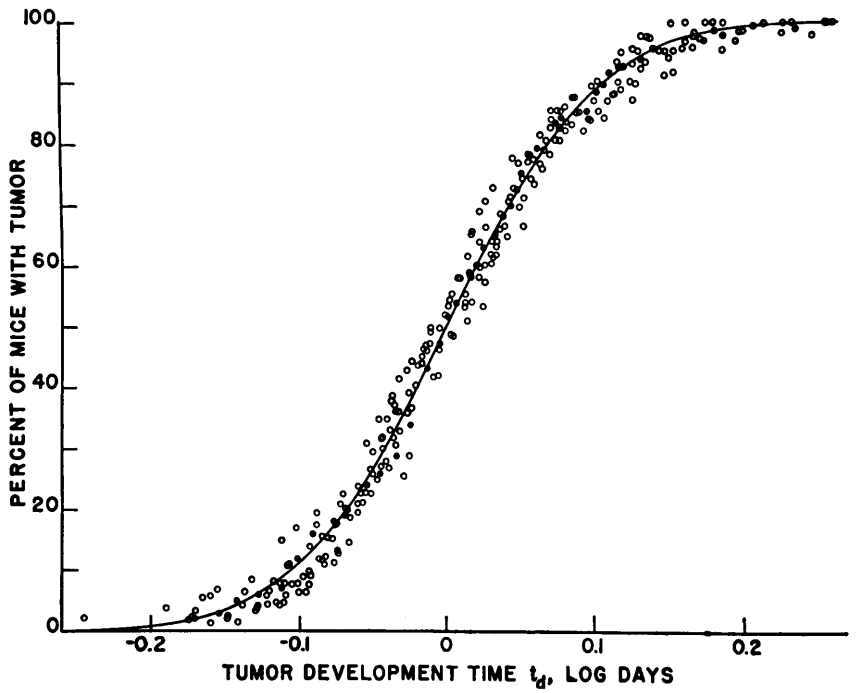

FIGURE 2

The data from the experiments described in figure 1 together with those from eight other experiments (dosed on a total of 676 mice) brought to a common mean on the abscissa. From [1].

well enough with the observed distribution. If one assumes that the initial volume V_c from which the cancer starts is the same in all cases, then, in experiments such as those described in figure 1 where D and i are held constant, the variance of t_d must be ascribed to the variance of b and k. That is, collecting constants in (6) we may write

$$bkt_d^2 = (\text{constant})_{D,i}. \tag{7}$$

The coefficients b and k, which describe two aspects of the effectiveness of D in producing growth of the cancer, have different values for each incidence level (see [5], pp. 239–242).

If a series of experiments is carried out in which i is held constant, but D varied, we may write for a given incidence level

$$dt_d^2 = (\text{constant})_{b,k,i}. \tag{8}$$

Figure 3 shows that this relationship was followed, within limits, for a series of experiments in which doses were applied five times per week, that is, with an average interval of 7/5 days. The data plotted is for the 50 per cent incidence level. It is seen that up to a certain dose, the expected curve is followed well, but

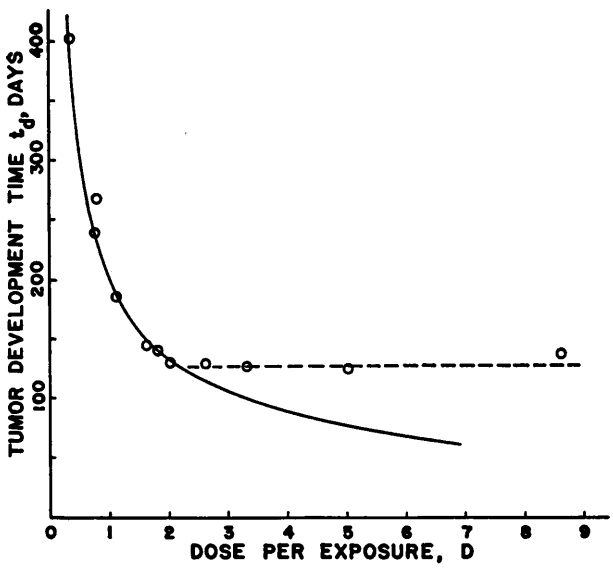

FIGURE 3

Relationship between tumor development time and dose per exposure. Data are for the 50 per cent incidence level. Reproduced from [5] with permission of Princeton University Press.

that above this dose there is no further decrease in t_d with increase in dose. This critical dose may correspond to a limit of the rate of proliferation that can be achieved by the cells. There are a few points for two other intervals, one day and seven days, and as is seen in figure 4, these may be described by curves similar to those for the 7/5-days interval.

The assumption of linearity of photochemical change with dose, which is tacit in the above argument, can be valid only within limits. For a first-order photochemical reaction we may write

$$\frac{P}{P_0} = e^{-\gamma D}, \tag{9}$$

where P_0 is the initial amount of material that can enter into photochemical reaction, and P is the amount remaining unchanged after the dose D is applied;

γ is a constant (not strictly the quantum yield). The amount of this material, P_r, that has been altered photochemically under these conditions is given by

$$P_r = P_0 - P = P_0(1 - e^{-\gamma D}). \tag{10}$$

For small amounts of photochemical change,

$$(1 - e^{-\gamma D}) \cong \gamma D \tag{11}$$

and

$$P_r \cong P_0 \gamma D. \tag{12}$$

Since P_0 and γ are constant, the amount of photochemical change is very nearly linear with dose for low doses.

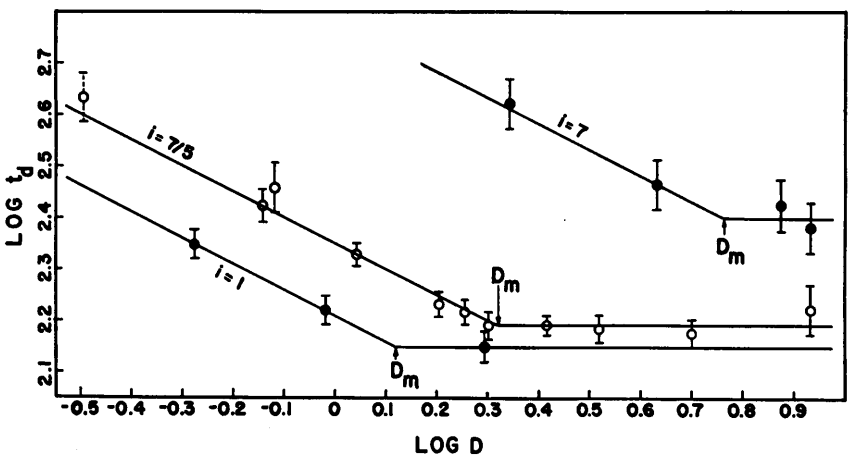

FIGURE 4

Relationship between tumor development time and dose, for three intervals between doses: 1 day, 7/5 days, and 7 days. Data are for the 50 per cent incidence level. The slope of the curves is $-1/2$ on the log-log plotting, as predicted by the model.

Recent studies in this laboratory dealing with the hyperplasia produced by single doses of ultraviolet light in the ear skin of the same strain of mice [6], [7] show that such linearity holds over a considerable range of doses, including, insofar as comparison can be made, that range within which the $t_d^2 D$ relationship holds. It may be that the leveling off of the curves in figures 3 and 4 results in part from the departure from linearity at higher doses, but this is not clearly established.

Although the hyperplasia induced by single doses is almost certainly not related directly to the carcinogenesis produced by repeated doses, there is much reason to think that the same or parallel photochemical reactions are concerned.

The effect of interval (dose being held constant) differs slightly from the prediction of equation (6), requiring the following modification

$$\log V_d - \log V_c = \frac{kDbt_d^2}{2i(1 - 0.55/i)}. \tag{13}$$

The correction seems to be accounted for by a difference in population of the tumors, which are of essentially two types. The ratio of these types (carcinoma/sarcoma) varies with i in a manner that fits well with the effect of i indicated in (13). The explanation involves the idea that there is a slight recovery from the carcinogenic process and that this recovery is greater for one type of tumor (carcinoma) than for the other ([5], pp. 251–253).

Without more complete data one would certainly hesitate to accept the Dt_d^2 relationship as adequately established. But the general success of the idea of

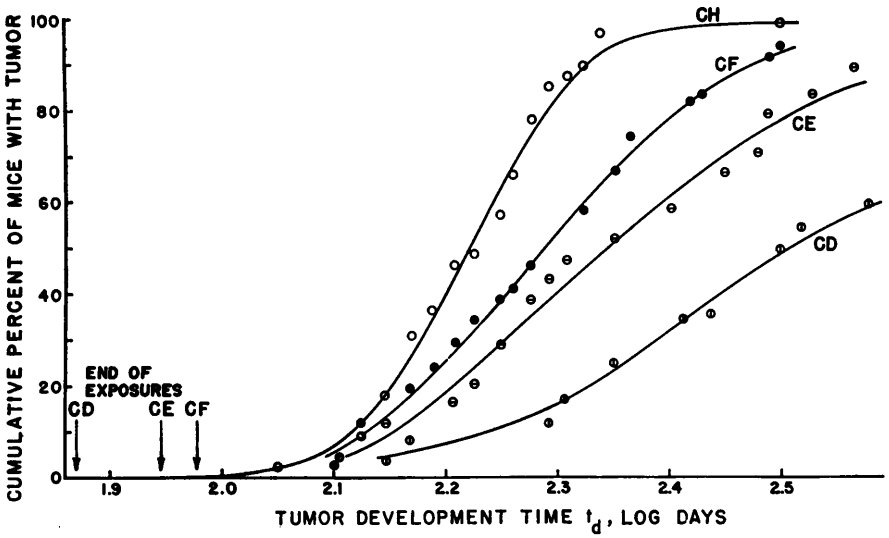

FIGURE 5

Delay of appearance of tumors as a result of discontinued dosage. In series *CH*, the dosage was continued until the tumors appeared. In the other series the dosage was discontinued at the points indicated by the arrows. From [1].

constantly accelerated proliferation with constant dosage is supported by data from another type of experiment which represents an attempt to probe into what is happening during the development of the cancer. This was done by discontinuing the dosage before the cancer appeared. This kind of experiment has not often been performed, but should be useful in getting at the course of events that go on during the development time. Needless to say, successful models must account for the data from such experiments. It is seen in figure 5 that although the dosage was discontinued, tumors eventually appeared, but later than when dosage was continued up to the time of their appearance. The earlier the dosage was stopped the later the tumors appeared. This is what might be expected if growth of the tumors was constantly accelerated during the period of dosage but

growth continued after the end of the dosage at a constant rate which had been established at the end of the period of acceleration.

The experiments with discontinued dosage are described by the equation

$$\log V_d - \log V_1 = \frac{kDt_1t_2}{i} = \frac{bkD(t_d - t_1)^2}{2i}, \tag{14}$$

where V_1 is the volume at the end of the period of dosage t_1, and t_2 is the time from the end of t_1 to the appearance of the tumor. The first term on the right side of equation (14) describes the growth at a constant rate established at the end of t_1, that is, with no further acceleration and no further addition of clones. The last term describes the growth of the tumor if acceleration took place during the period $(t_d - t_1)$ (see [5], pp. 223–250).

The agreement of the data with equation (14) is shown in figure 6, where the straight line is the relationship predicted.

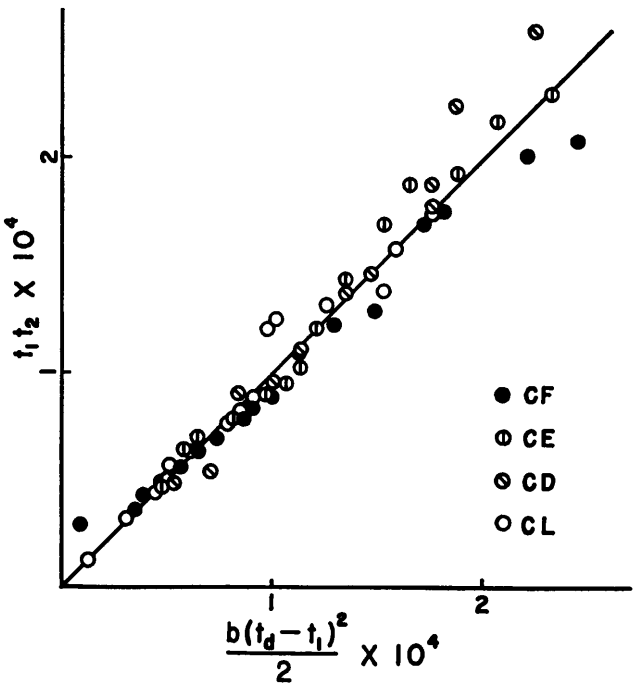

FIGURE 6

Plotting of the data from the experiment described in figure 5, according to equation (14). The straight line is the predicted relationship. From [2].

There is still another type of experiment in which the dosage was continued for a brief period, then discontinued, then continued again, and subsequently discontinued. A variety of dosage periods was used. Figure 7 shows the agreement obtained. The straight line is that predicted if it is assumed that the

process is irreversible, that is, that there is no opposed or "recovery" process. There is a small systematic variation from prediction, which may represent a slight degree of such recovery ([5], pp. 230–242).

Another bit of evidence has already been mentioned, which suggests a recovery process, equation (13), and still another may be added, that reciprocity between dose rate and time breaks down at low intensities of ultraviolet light ([6], p. 194). If such recovery occurs it is clear that there should be some lower

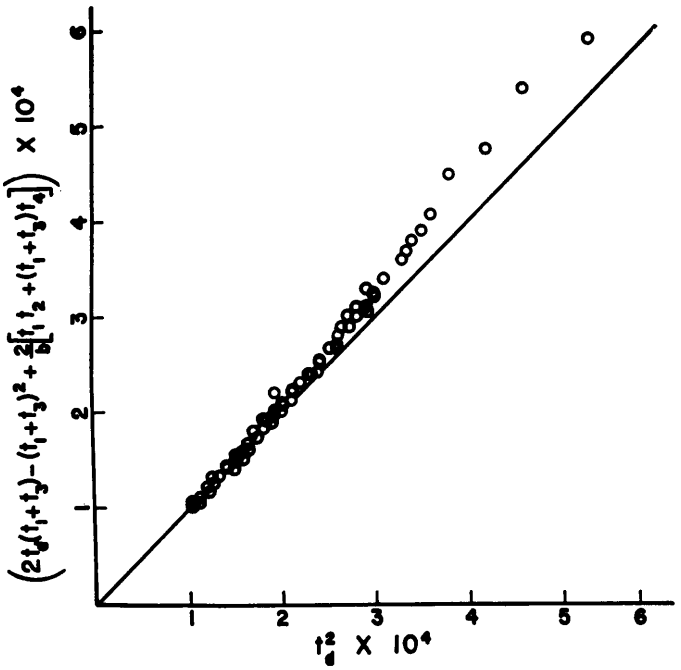

FIGURE 7

Data from an experiment with interrupted dosage. The straight line is the predicted relationship if complete irreversibility is assumed (see [5], pp. 236–238). From [2].

limit for the dose that will produce cancers, that is, there should be a "threshold," but the data suggest that the threshold is so low that it can be generally disregarded. Moreover, the evidence for reversibility is open to other explanations (for example, see [5], footnote on p. 196 and pp. 238 and 239).

It seems that our model agrees reasonably well with the data for induction of cancers with ultraviolet light, and that certain general conclusions may be drawn: the cancer process is continuous and cumulative; it is effectively irreversible, and effectively nonthreshold. I will discuss some of the implications of these conclusions below, but I wish first to attempt to apply the same kind of analysis to induction of cancers by chemical agents.

3. Carcinogenesis by chemical agents

The basic concept for the following model is the same as that for the ultraviolet model. That is, it is assumed that the rate of proliferation of cancer cells is proportional to the effective dose of the inducing agent: in the case of ultraviolet light the rate is proportional to the amount of radiation absorbed; in the case of a chemical carcinogen the rate is proportional to the amount of the chemical reacting in the tissue. The equations are quite different for the two models because the immediate effect of ultraviolet light ceases with the termination of the radiation, whereas the chemical agent is only gradually removed from the tissue.

3.1. *For single doses of carcinogen.* If a single dose of a chemical carcinogen is applied it may be expected that the concentration of the agent in the tissue at the moment will be given by

$$c = c_0 e^{-at}, \tag{15}$$

where c_0 is the initial concentration (corresponding to the dose applied), c is the concentration at time t after introducing the carcinogen, and a is a constant which should be characteristic of the specific carcinogen, of the tissue under consideration, and of the manner of application. In at least one case [8] such a logarithmic die-away curve has been observed in studies of the disappearance of the carcinogenic agent.

The amount of carcinogen that has disappeared at time t is given by

$$c = c_0(1 - e^{-at}). \tag{16}$$

Only a fraction of the agent would be expected to react with the tissue to induce carcinogenesis, the remainder being lost by excretion or through other channels. The carcinogenic fraction, R, should be described by

$$R = rc_0(1 - e^{-at}) \tag{17}$$

where r describes the proportionality between carcinogen reacting and carcinogen lost.

Assuming that the rate of proliferation of the cancer cells is proportional to the reacting carcinogen,

$$\frac{dV}{V\,dt} = sR, \tag{18}$$

where s is a constant.

Substituting from (7),

$$\frac{dV}{V\,dt} = src_0 - src_0e^{-at}. \tag{19}$$

This indicates that the rate of proliferation rises rapidly at first and then falls off toward a constant value.

Integrating (19),

$$\log V = src_0 t + src_0 \frac{e^{-at}}{a} + C. \tag{20}$$

If, as in the model for ultraviolet light, we assume that growth starts from an initial volume V_0, and reaches the volume V_d at time t_d, then

$$\log V_d - \log V_0 = src_0 t_d + src_0 \frac{e^{-at_d}}{a} - \frac{src_0}{a}. \tag{21}$$

3.2. *For repeated doses of carcinogen.* In certain experiments described in the literature, doses of carcinogen have been given at regular intervals. These offer the best material for comparison with the model for ultraviolet light.

Let us consider the expected relationships when doses of the carcinogen are given at regular intervals, i. The concentration of reactive carcinogen during the first interval is given by

$$c_1 = c_0 e^{-ai}. \tag{22}$$

And the carcinogen reacting during this period, which we represent by R_1, is

$$R_1 = rc_0(1 - e^{-ai}). \tag{23}$$

The growth of the tumor during this first interval is described by

$$\log V_1 - \log V_0 = src_0 i + src_0 \frac{e^{-ai}}{a} - \frac{src_0}{a}. \tag{24}$$

For the second interval we start with the residue of carcinogen left from the first dose, c_1, plus an additional dose c_0. Thus

$$c_2 = (c_0 + c_1)e^{-ai}, \tag{25}$$

where c_2 is the concentration following the second dose. And

$$R_2 = r(c_1 + c_0 - c_2), \tag{26}$$

where R_2 is the carcinogen reacting during the second interval.

Substituting from (22) and (25) and summing,

$$R_2 = rc_0 - rc_0 e^{-2ai}. \tag{27}$$

The growth of the tumor during the second interval is given by

$$\log V_2 - \log V_1 = src_0 i + \frac{src_0 e^{-2ai}}{2a} - \frac{src_0}{2a}. \tag{28}$$

The growth of the tumor during the first two intervals is then described by summing (24) and (28)

$$\log V_2 - \log V_0 = 2src_0 i + \frac{src_0 e^{-ai}}{a} + \frac{src_0 e^{-2ai}}{2a} - \frac{src_0}{a} - \frac{src_0}{2a}. \tag{29}$$

For a series of n intervals

$$\log V_n - \log V_0 = src_0 ni + \left(\frac{src_0 e^{-ai}}{a} + \frac{src_0 e^{-2ai}}{2a} + \cdots + \frac{src_0 e^{-nai}}{na}\right) - \left(\frac{src_0}{a} + \frac{src_0}{2a} + \cdots + \frac{src_0}{na}\right). \tag{30}$$

Since the doses are given at regular intervals

$$t = ni \tag{31}$$

and we may substitute in (30), rearrange, and write for the condition V_d, t_d

$$\log V_d - \log V_0 = src_0t_d + src_0\left\{\left[\frac{1}{ae^{ai}} + \frac{1}{2ae^{2ai}} + \cdots + \frac{1}{nae^{nai}}\right] - \left[\frac{1}{a} + \frac{1}{2a} + \cdots + \frac{1}{na}\right]\right\}. \tag{32}$$

The series within the brackets in (32) permits a variety of conditions according to the values of s, r, and a. We may attempt, by making some approximations, to find whether the model could account for such apparently diverse results as are indicated in tables I and II, where we note close reciprocity between dose

TABLE I

DOSE-TIME RELATIONSHIPS FOR CARCINOGENESIS

From Druckrey [9]. Liver tumors produced in rats by ingested 4-Dimethylaminoazobenzol given daily (baked in bread).

c_0 (mg. per day)	t_d (average time to 50% tumors)	c_0t_d	Number of Rats
1	700	700	169
3	350	1050	70
5	190	950	70
6	167	1002	145
10	95	950	30
20	52	1040	15
30	34	1020	30

TABLE II

DOSE-TIME RELATIONSHIPS FOR CARCINOGENESIS

Data from Crabtree [10]. Skin tumors produced in mice by painting with 3,4-benzpyrene dissolved in ether containing two per cent liquid paraffin.

c_0 (per cent) Applied Twice Weekly	t_d from Graphical Mean	c_0t_d	Number of Mice
0.3	100	30.0	60
0.1	117	11.7	30
0.03	142	4.3	30

and development time in one case, but far from this in the other.

As in the ultraviolet model, we may assume that the initial and terminal volumes (V_0 and V_d, respectively) are the same in all experiments carried out in a

given manner. If the terms s, r, and a are constant at a given incidence level and the interval between doses is held constant, we may write

$$c_0 t_d + c_0\{\cdots\cdots\} = (\text{constant})_{s,r,a,i}. \tag{33}$$

Let us see how this model fits some of the published data. It is conceivable that with a very high value of a, and with i reasonably small, the second term in (33) could be negligible as compared to the first. In this case we should expect to find approximate reciprocity between t_d and the dose of carcinogen, that is,

$$c_0 t_d \fallingdotseq (\text{constant})_{s,r,a,i}. \tag{34}$$

Table I shows that this condition is quite well fulfilled by the data of Druckrey [9].

On the other hand, data from experiments by Crabtree [10], from which are taken those in table II, do not show this reciprocity, so we may assume that the second term of (33) cannot be neglected in this case. We note that the members of the series in this term become progressively less important as the number of doses increases, so that sooner or later the sum of the series becomes approximately constant. That is, we may write as an approximation

$$c_0 t_d + c_0 M \fallingdotseq (\text{constant})_{s,r,a,i}, \tag{35}$$

where M is approximately constant. With i held constant, as was done in Crabtree's experiments, the number of doses which must be applied before M may be treated as constant depends upon the value of a; the smaller is a, the greater the number of doses and hence of t_d.

Crabtree's data, which are quite complete for the three doses he studied, are plotted in figure 8; t_d against percentage incidence. They do not appear, on first examination, to follow a simple relationship, the situation not being improved by plotting the logarithm of t_d, as was the case with ultraviolet light carcinogenesis. In applying equation (35) to these data, I have assumed that r, s, and a are distributed independently with incidence level. In this case it should be possible to compare the three sets of values for c_0 and t_d by means of the following equation derivable from (35)

$$M = \frac{c_0' t_d' - c_0'' t_d''}{c_0' - c_0''}, \tag{36}$$

where c_0' and c_0'' are the two doses to be compared, and t_d' and t_d'' the corresponding values for the development times. The latter are obtained from figure 8 (as indicated there).

Three values for M, thus calculated, are given for several incidence levels in column 4 of table III. The values of M for a given incidence level do not differ too greatly, but there is some systematic drift. This drift is in the direction that might be expected if our assumption, that approximate constancy of M had been reached in all cases, does not hold. That is, the calculated values of M are lowest, and presumably more nearly correct, when cases are compared in which the values of t_d, and hence the number of doses, are highest.

The values of M obtained for comparable pairs of values increase systematically with incidence level. The value of M should reflect inversely the value of a; thus greater value of M corresponds to longer development time, that is, less effectiveness of the carcinogen. By substituting the values of M from table

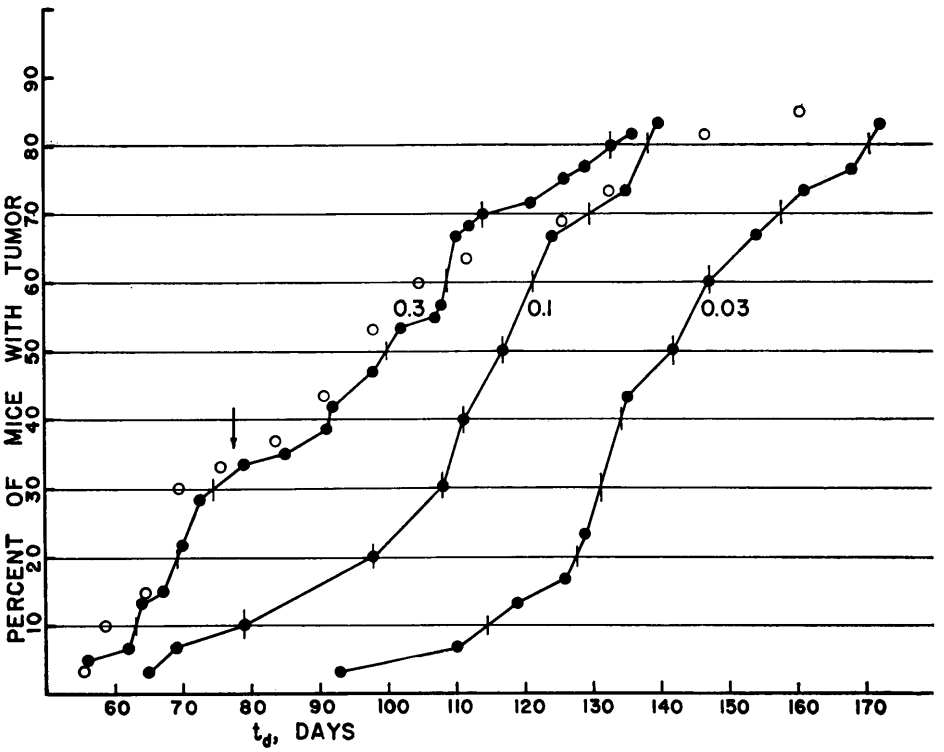

FIGURE 8

Data from Crabtree [10] for skin tumors produced by painting with 3,4-benzpyrene. Three concentrations of the carcinogen were used: 0.3, 0.1, and 0.03 per cent. The solid disks are from the experiments in which doses of carcinogen were applied twice weekly until tumors appeared. The open circles are points for an experiment in which doses of 0.3 per cent were applied twice weekly for seven weeks only. The values of t_d used in tables 2 and 3 were obtained as indicated by the short vertical lines intercepting the horizontal lines representing incidence level.

III in (35) we obtain values that should correspond to $1/sr$ (log V_d − log V_0); these are given in column 5 of table III. Obviously these values cannot be too meaningful since they reflect the deviations of M from constancy, but they are approximately the same, and do not display any clear systematic drift. Since V_0 and V_d are constant, the above values should reflect any variance in s and M. Hence it is indicated that the variances of s and r are not great, and that the

TABLE III

CALCULATIONS BASED ON THE MODEL, USING DATA OF CRABTREE [10]
M determined for pairs of values in the following order for each per cent incidence: $c_0 = 0.3, 0.03$; $c_0 = 0.3, 0.1$; $c_0 = 0.1, 0.03$.

Per cent Incidence	c_0	t_d	M	$c_0t_d + c_0M$
10	0.3	63	−58.4	1.4
10	0.1	79	−55.1	2.4
	0.03	105	−67.6	1.1
	0.3	69	−62.5	1.9
20	0.1	98	−54.4	4.4
	0.03	127	−85.0	1.3
	0.3	75	−68.7	1.9
30	0.1	108	−58.4	5.0
	0.03	131	−97.8	1.0
	0.3	91	−86.2	1.4
40	0.1	111	−81.2	3.0
	0.03	134	−101.0	1.0
	0.3	100	−95.5	1.35
50	0.1	117	−90.7	2.6
	0.03	142	−106.0	1.1
	0.3	109	−105.0	1.2
60	0.1	121	−103.0	1.8
	0.03	147	−109.8	1.4
	0.3	114	−109.8	1.3
70	0.1	130	−106.0	2.4
	0.03	157	−118.0	1.2
	0.3	133	−129.0	1.2
80	0.1	138	−130.2	0.8
	0.03	170	−124.0	1.4

variance of t_d with incidence level is due chiefly to variance in the exponent a.

If dosage stops after a time t_1 before the tumor appears, we may write

$$\log V_d - \log V_0 = src_0t_1 + src_0\{\cdots\cdots\} + src_0t_2 + \frac{1}{ae^{at_2}} - \frac{src_0}{a}, \tag{37}$$

where n_1 is the number of doses given in time t_1, and t_2 is the time from the end of t_1 until the tumor appears. If the conditions were such that the sum of the series could be regarded as constant, the last term should be negligible; and since the sum of t_1 and t_2 is t_d, (37) should reduce to the same form as (35). That is, after enough doses are given for constancy to be assumed, further dosage should not appreciably affect t_d, but if dosage ends before this point, t_d will be lengthened. In one experiment, Crabtree stopped dosage at the end of seven weeks; in this

case, as indicated in figure 8, there was a progressive increase in t_d at higher incidence levels. This would indicate that approximate constancy of M had not been reached for the higher incidence levels, at the end of seven weeks. This accords with the drift in the values of M which is seen in table III.

Considered as a first approximation the agreement between the model and the data of Crabtree seems not too bad. From the direction of the deviations from prediction, it seems that a more refined treatment, which I have not been able to carry out as yet, may be even more successful. There are more published data to be explored, but I doubt that any are much more complete of their kind, that is, for repeated dosage, than are those of Crabtree. At the moment, from the two examples I have been able to examine (Crabtree's and Druckrey's) it seems hopeful that the model I have used may find general application. On the other hand, modification or amplification may be necessary, or the model may have to be discarded altogether.

Note added in proof. Having now computed the partial sums of series in (32), it becomes clear that the model will not fit, without modification, the data of Crabtree. This would not seem, however, reason for changing the following paragraph.

But whatever its limitations may be, I think the model helps to point out certain factors that need to be taken into account in any successful explanation of carcinogenesis. In the first place, the model takes into consideration the fact that the principal quantitative aspect reflected in the measurements obtainable in such experiments is an acceleration of proliferation of some clones of tissue cells above the normal, and that our problem is, essentially, to trace the course of the proliferation of these clones. Secondly, the model takes into consideration the "die-away" character of the effect of a chemical carcinogen after it is introduced into the tissue. The basic idea, that acceleration of proliferation rate is proportional to the effectiveness of the carcinogenic agent, is the same as that which was successful in describing the data for ultraviolet carcinogenesis. This does not, of course, constitute proof of either model, but may give comfort to those who feel intuitively that there must be a common denominator at some level in carcinogenesis by all agents.

4. Discussion

A glimpse of mechanism. The clones of cells that make up a cancer proliferate faster than do the clones of corresponding tissue cells from which the cancer originates; but this does not constitute a basic difference between normal and cancer cells—the former may for brief periods proliferate at even higher rates than the latter. For the tumors induced by ultraviolet light, growth measurements indicate a cell division about every six days [11]. In transient hyperplasia induced by single doses of ultraviolet light, cell division occurred as often as once per day within certain clones [7]. A real difference is that the increased rate of proliferation of cancer cells is inherited, as is shown by transplantation

experiments and by metastasis. That is, the cancer cells differ from normal cells in some facet of their genetic pattern having to do with replication, so that the cancer cells proliferate at higher rates under comparable environmental conditions.

As generally studied at the cellular level, changes in genetic pattern are saltatory, there being no gradual transition. But our studies of ultraviolet carcinogenesis indicate that the changes involved there are gradual and cumulative; this can hardly be due to accumulation of clones from cells that have jumped abruptly to a faster rate of proliferation. Clones which suddenly adopted rates of proliferation comparable to the terminal growth rates observed in these cancers would very quickly dominate tumor growth, so that further doses of radiation would have little effect after a relatively short time, and this is not compatible with evidence from experiments with discontinued dosages such as that described in figure 5 ([5], pp. 220–223). It appears that the change from the normal to the cancer cell is a quantitative, cumulative change rather than an abrupt qualitative one, which would accord with our inability to find, as yet, consistent qualitative differences between normal and cancer cells.

I do not think that such a gradual change in cell heredity is necessarily in disaccord with modern ideas of biochemical genetics. These place the locus of genetic pattern in the structure of nucleic acid molecules, which serve as templates for the replication of the parts of the cell, including the templates themselves. We may expect the differences between normal and cancer cells—differences which escape our means of direct chemical analysis—to be in some way related to nucleic acid templates. It seems possible that the gradual change in proliferation rate which takes place in the cancer cells could result from an accumulation of templates that determine the rate of proliferation of these cells, or, alternatively, a gradual unmasking of templates although the inheritance would seem more difficult to explain in the latter case.

An extrapolation based on the model for ultraviolet carcinogenesis is of interest in this regard. Using growth rates measured terminally (that is, in the neighborhood of t_d) an initial volume (V_c) is arrived at, which is several orders of magnitude less than the volume of one of the tissue cells concerned. It may be reasoned that the basic units, the course of whose replication the model describes, are not cells but much smaller intracellular particles. These particles would be replicated by the cell, at the same time governing the rate of replication of the cells, and hence of the particles themselves. These hypothetical particles I have called "tems," for brevity and because it seems that if they exist they must in some way act as templates. A very rough estimation suggests that the tems could be nucleic acid molecules or fractions thereof ([6], pp. 255–281).

How the tems accumulate in the cell is a question which it may not be profitable to debate at this time. I have formulated a hypothesis, mainly for descriptive purposes, which assumes that there is an exchange of tems between moribund and viable cells that results in an accumulation in the latter. This should be a rather haphazard business, and suggests that the growth relationships predicted

by the models are not smooth, but are to be regarded as statistical functions ([5], pp. 266–280). I am not really happy with this mechanism I have suggested, but find it useful for descriptive purposes while I am looking for a better one. I have not introduced this discussion of mechanism in order to present one to which I would hold strongly, but to indicate some of the characteristics of the process of cancer induction, and some of the problems it presents.

5. Thresholds and tolerance levels

Probably all biological processes have "thresholds," that is, there is a limit of dose below which a given agent elicits no response. This is to be expected because of the ability of living systems to set up repair or recovery processes which oppose changes that may be brought about by extraneous agents. But the determination of such thresholds may be difficult, and it would seem that the threshold assigned may sometimes represent only a lower limit of feasible measurement, rather than an inherent biological limit. Extrapolation to thresholds would seem generally more reliable than direct measurement, but only, of course, if the shape of the dose-response relationship is known over a considerable range.

I have already pointed out three particulars of evidence that suggest recovery, and hence a threshold for carcinogenesis by ultraviolet light, although none of these do I consider to be definitively established. Nevertheless, I hope that no one will quote me as saying that no threshold exists. On the other hand, I would be happy to be quoted to the effect that I do not see how thresholds for carcinogenesis can be effectively determined without greater knowledge of the cancer process than we now possess.

In the first place, when we consider dose response relationships for carcinogenesis we need to remember that the response we measure has its basis in a change in rate of proliferation of cells. This rate we cannot follow directly, our only index being a single point on the curve. Extrapolation can only be reliable if the underlying rate law is well understood. In the case of ultraviolet light carcinogenesis we have a model that appears to predict something about the rate curve with reasonable accuracy, but I would not attempt to use this to determine a threshold, even though I have considerable confidence in the model and the data it describes. Above all, I would not attempt to set a tolerance level for this carcinogenic agent for human skin on the basis of my experiments on mice.

It seems hardly necessary to point out the difficulties of direct determination of thresholds, or of assessing the relative carcinogenicity of different agents. Even with the microscope, the tumors we can detect with assurance are composed of very large numbers of cells, so obviously they have already been growing for some time. Thus we can never be sure how many animals in a population have cancers we do not detect even at the time of death. The incidence of cancers we measure, whether observed grossly or microscopically, must differ according to the time at which we choose to make our observation—whether a time is elected arbitrarily, as is not uncommon, or we wait until the animals die from cancers

or other causes. This seems clear from figures 1 and 8. We cannot determine zero incidence, which would correspond to a threshold dose, in this way.

6. Irreversibility and accumulation

It seems clear from the ultraviolet studies that the effect of successive doses is cumulative, and this means over-all irreversibility even though, as has been pointed out, there is probably a minor degree of recovery. The reciprocity between dose and development time found by Druckrey [9], also indicates irreversibility, as he points out, and accumulation of effect; that the conditions of Druckrey's experiments may make it a special case, does not diminish the argument. The hereditary nature of the rapid proliferation of clones of cancer cells also indicates an effective irreversibility, although not in itself indicating accumulation of effect at the cellular level. Thus, although there are, no doubt, factors tending to oppose or reverse the cancer process—and we should not forget that regression of cancers sometimes occurs—it seems that carcinogenesis may be considered as essentially, or effectively, irreversible and cumulative.

The concept that the characteristics of cancer cells are hereditary, and at the same time cumulative, may seem difficult to harmonize with the saltatory nature of mutation that classical genetics indicates. Perhaps I should point out that the differentiation of cells in the course of development of the multicellular organisms is likewise not easy to explain in terms of classical ideas of mutation. Both here and in cancer it may be necessary to think in terms of genetic pattern at the molecular rather than at the cellular level. At any rate I feel reasonably sure as regards cancer that the idea of cumulative effect will be harmonized in the end with modern ideas of genetics.

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