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On the Problem that some known Carcinogens do not appear to be Mutagens in Short-term Tests

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Abstract: An explanation is sought as to why certain known carcinogens do not appear to be mutagens. It is hypothesized that an intermediary substance and not the suspected carcinogen itself, is the actual carcinogen. A model for the mechanism of carcinogenesis is constructed utilizing forms of DNA damage and repair as known at present. When equations are obtained for the carcinogenic response via this intermediary substance, it is found that these equations can explain not only the commonly observed response patterns, but also certain peculiarities in response which have been observed in some experiments. An experiment to estimate the ratios of the parameters in the model could show if indeed this is the mechanism that occurs, and possibly identify the type of damage that leads to carcinogenesis.

Key Words and Phrases: Mutagens, Metabolite carcinogens, mechanism of cancer, Carcinogenesis.

1. Introduction

Some carcinogens that are tested for mutagenic properties do not appear to be mutagens. For example, the chemical "urethane" is known to cause tumors in rodents but has not given positive results in tests for mutagenicity. One explanation may be that these substances cause mutations in a way that is different from the process of forming mutations in, for example, the Salmonella used in the Ames[1] test described in Ames.[2] Another could be that they cause mutations so severe that the colonies cannot grow. The first problem can be solved by developing strains of organisms that would be sensitive to the new substance, and the second problem could be studied by examining if indeed the organisms are severely damaged. This examination can be done perhaps by introducing a known mutagen to see if revertants occur again.

Still another explanation comes from the fact that rat liver is sometimes used in the Salmonella test for metabolic activation. It could be that some metabolite other than rat liver might activate the mutation causing substance. The explanation of interest here is that the substance in its original form is not the actual mutagen. The rat liver metabolic activation is an example of this. Perhaps the substance in the presence of some other substance turns into a product which is a mutagen. Or the substance itself may do nothing, but it may turn into mutagens other substances which are usually harmless.

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Another unusual feature that has recently been noticed is that the carcinogenic response is linear at very low doses, becomes a quadratic curve as the dose rises, switches suddenly to a linear form as it rises further, and then becomes a second order curve again before the response curve finally stops rising because the substance becomes toxic at higher doses.

The process described in what follows can be equally well applied to mutagenicity and carcinogenicity by substituting the appropriate words (for example, "mutagen" for "carcinogen" and "colonies" for "tumors"). The model used is an extension of that used in the paper.[2]

2. The Formation of the Actual Carcinogen

We shall call the substance under study S1 and the actual carcinogen S2. First, a supply of S1 has to be provided. Then, either the presence of S1 causes another substance to transform into S2, or it is S1 itself that transforms into S2 because of the presence of some other substance. The substance S1 itself, in its original form is not a carcinogen. The formation of S2 will depend on the availability of S1. The more there is of S1, the more of S2 will be formed. We shall also assume that there is a process of repair or elimination. The units of S2 could be made harmless (repaired) by some other agents or eliminated by either physical removal or transformation into another substance. We shall be interested in the amount of S2 available at time, say, t. It is this quantity and not the amount of S1 which will be the "actual dose" of carcinogen.

Assumptions

(i) The supply of S1 is made available at an instantaneous rate of $Df(t)$, where D is the dose used and $f(t)$ is some function which vanishes for $t<0$ and as t tends to infinity while its integral from zero to infinity is equal to unity. (2.1)

(ii) In a small time period h, the presence of S1 causes a unit of S2 to be formed with probability $vDf(t)h$ where v is a constant. (2.2)

(iii) In the time period h, a unit of S2 can be repaired or eliminated with probability ph. ..... (2.3)

(iv) The probability that any combination of the events in (ii) and (iii) would occur in the period h is of small order in h. (2.4)

Let

$p_n(t)$ = P[There are exactly n units of S2 at time t]. (2.5)

These assumptions lead to the equation

$$P_k(t+h) = P_{k-1}(t)vDf(t)h + P_{k+1}(t)(K+1)\rho h + P_k(t)[1 - h(vDf(t) + k\rho)] + o(h). \tag{2.6}$$

Multiplying by s^k, summing over k and letting h tend to zero, we have in the limit an equation in the probability generating function.

$$\frac{\delta}{\delta t}G(s, t) = (s - 1)\, vDf(t)\, G(s, t) + (1 - s)\rho\frac{\delta}{\delta s}G(s, t). \tag{2.7}$$

If we denote the expected value of the number of units of S2 at time t as R(t) and its derivative with respect to t by $R^1(t)$, we obtain the following equation by partially differentiating (2.7) with respect to s and then putting s = 1.

$$R^1(t) = vDf(t) - \rho R(t) \tag{2.8}$$

which is easily solved to yield

$$R(t) = e^{-\rho t}\int_0^t e^{\rho x} vDf(x)dx \tag{2.9}$$

This equation gives the expected amount of the actual carcinogenic substance S2 which will be available at some time t after the substance S1 is first supplied. Since this is the actual carcinogen, we will need it in the next section as something similar to an "actual dose" of carcinogen.

3. Method of Carcinogenesis

We shall assume that cancer cells are caused by mutations or some other sort of damage that occurs in normal cells. What is likely is that the DNA in a normal cell is slightly altered, slight enough not to kill it, but altered enough for it to pass on wrong information and somehow create mutant cells that form growths. If these growths become large enough, they can be observed as tumors. What causes the DNA damage could be radiation, carcinogens and perhaps some other malevolent phenomena. Here, we shall be interested in the case where it is the carcinogens that cause the damage.

The DNA damage can be caused in several ways. In the presence of the carcinogen a normal cell could be damaged just the right amount to transform it into a mutant cancer cell. But it could also be that the damage is too slight for it to become a cancer cell and that it changes to an intermediate stage which we shall call a primary cell. After a while, the primary cell could be repaired by the normal repair mechanisms of the body and revert back to a normal cell. It could also die or divide into two daughter cells. In division, either of two things could occur. On the one hand, the two daughter cells could have the same mutations as the mother cell and so be two primary cells. On the other hand, it has been found that there exists a repair mechanism which

could operate during cell division, and if this occurs, they turn into two normal daughter cells. If the possibility exists that one daughter cell turns out normal while the other remains a primary cell, then this leaves the number of primary cells unchanged, and as far as the number of mutant cells are concerned, nothing has happened. Finally, the primary cell could undergo a second damage. If damaged too much it will die, but if the damage is of just the sufficient amount then it will turn into a cancer cell. Cancer cells could also divide or die, but if tumors are to be formed they should be undergoing a supercritical growth process.

This may be the mechanism behind the process of carcinogenesis as it appears at present. We shall attempt to form a model that will conform with this mechanism and see if the consequences of the model conform with the results of observations.

Assumptions

The DNA damage is caused by the presence of the actual carcinogen. Hence, it appears reasonable to assume that the chances of any such damage being caused are proportional to the amount of the actual carcinogen present at the time. This amount can be approximated by its expected value, R(t), (2.9). In view of this, we shall base our model on the following assumptions.

In a small space of time h,

(i) a normal cell could be damaged and turn into a cancer cell with probability $K_1 R(t)h$ (3.1)

(ii) a normal cell could be damaged and turn into a primary cell with probability $K_0 R(t)h$ (3.2)

(iii) a primary cell could be damaged and turn into a cancer cell with probability $K_2 R(t)h$ (3.3)

(iv) a primary cell can divide unrepaired into two primary cells with probability λh (3.4)

(v) a primary cell could be repaired, die or be repaired in the process of division with probability μh (3.5)

(vi) a cancer cell can divide with probability Λh (3.6)

(vii) a cancer cell can die with probability Mh (3.7)

(viii) the probability that a combination of the above events will occur is of small order in h. (3.8)

In the above K_0, k_1, k_2, λ, μ, Λ and M are constants.

We shall also assume that the cancer cells form a supercritical growth process and that the probability that a cancer cell starting at time t will have grown to be a tumor and is observable at time T is given by $\pi(T - t)$ as calculated in the paper by Neyman and Scott (1967). The result we require is that when T tends to infinity.

$$\pi(T - t) \rightarrow (1 - \frac{M}{\Lambda}). \tag{3.9}$$

Suppose that

$Y(t)$ = number of primary cells at time t;

$Z(t, T)$ = number of tumors counted at time T, which were formed at time t;

E_Y = expected value of $Y(t)$;

E_Z = expected value of $Z(t, T)$;

$P_{m,n}(t) = P[Y(t) = m, Z(t, T) = n]$; and

$G(u,v,t)$ = Probability Generating Function of $Y(t)$ and $Z(t, T)$.

By considering a small time interval $[t, t + h]$ and the events that can occur in it, we get the following equation.

$$\begin{aligned} P_{m,n}(t + h) = {} & P_{m,n-1}(t)K_1R(t)h\pi(T - t) + P_{m,n}(t)K_1R(t)h[1 - \pi(T - t)] \\ & + P_{m-1,n}(t)K_0R(t)h \\ & + P_{m+1,n-1}(t)K_2R(t)h(m + 1)\pi(T - t) \\ & + P_{m+1,n}(t)K_2R(t)h(m + 1)[1 - \pi(T - t)] \\ & + P_{m-1,n}(t)(m - 1)\lambda h + P_{m+1,n}(t)(m + 1)\mu h \\ & + P_{m,n}(t)[1 - h(K_0R(t) + K_1R(t) + (\lambda + \mu + K_2R(t)(m)] \\ & + o(h) \end{aligned} \tag{3.10}$$

Multiplying this by $u^m v^n$, summing over m and n, and taking the limit as h tends to zero, we get an equation in the probability generating function.

$$\frac{\partial}{\partial t} G(u,v,t) = [(u - 1) K_0 + (v - 1)\Pi(T - t) K_1 + K_1] R(t) G(u,v,t)$$
$$+ [(u - 1)(\lambda u - \mu - K_2 R(t) + \Pi(T - t) K_2 (v - 1) R(t)] \frac{\partial}{\partial t} G(u,v,t) \quad (3.11)$$

What we now need is an expression for the expected number of tumors. This will probably depend on the number of primary cells; hence, we seek equations in both E_z and E_y. We do this by differentiating (3.11) in turn with respect to u and v, and then by setting both u and v to unity.

$$\frac{d}{dt} E_y = K_0 R(t) + [\lambda - \mu + (K_1 - K_2) R(t)] E_y. \quad (3.12)$$

$$\frac{d}{dt} E_z = \Pi(T - t) K_1 R(t) + \Pi(T - t) K_2 R(t) E_y + K_1 R(t) E_z. \quad (3.13)$$

Equation (3.12) can be solved through the multiplication by an integrating factor without much difficulty to yield,

$$E_y(t) = e^{g(t)} \int_0^t K_0 R(y) e^{-gy} dy \quad (3.14)$$

where $$g(x) = \int_0^x [\lambda - \mu + (k_1 - K_2) R(w)] dw.$$

To proceed further we need to postulate the nature of the function f(t). In most cases, S1 is administered in the form of an injection or some other form which is given suddenly at one dose. Especially in the case of urethane it has been found that it is then speedily removed from the body. Under these circumstances we choose f(t) to be the impulse function described in the appendix (A.1). Other cases of interest could be studied using some other appropriate functions as f(t). Then, using the value of R(y) (2.9), and the result of integration of impulse functions (A.2.2), we have

$$E_y(t) = e^{h(t)} \int_0^t K_0 \nu D e^{-h(y)} e^{-\rho y} dy \quad (3.15)$$

where $$h(x) = (\lambda - \mu)x - (K_1 - K_2) \nu D(e^{-\rho x} - 1)/\rho.$$

We would like to know what occurs after the mechanism has had sufficient time to function, which means we have to examine the behavior as t tends to infinity.

CASE 1. When $\lambda > \mu$

We first consider the case when $\lambda - \mu$ is positive, so that the probability of a primary cell dividing unrepaired is greater than the probability of a primary cell being eliminated without forming a cancer cell. It can be shown that the integral in (3.15) is bounded from below, away from zero, and that its multiplying factor tends to infinity as t tends to infinity. Hence,

$$E_Y(t) \to \infty \text{ as } t \to \infty \text{ when } \lambda > \mu. \tag{3.16}$$

In this case, there being an unlimited supply of primary cells so long as K_2 is non-zero, there will be an infinite number of tumors as T increases. The relationship with the dose may be obscured by the fact that there are too many tumors, if the dose is large.

CASE 2. When $\lambda < \mu$

We next consider the case when $\lambda - \mu$ is negative. If $\rho > \mu - \lambda$ the non-negative integral in (3.15) is bounded above by unity while the multiplying factor tends to zero, as t tends to infinity. If $\rho < \mu - \lambda$, it can be shown that (3.15) which is non-negative is then bounded above by an expression that tends to zero as t tends to infinity. Thus,

$$E_Y(t) \to 0 \text{ as } t \to \infty \text{ when } \lambda < \mu \tag{3.17}$$

This is almost equivalent to the case where the primary cells take no part in the process of carcinogenesis since they die off too fast. If they do take no part, we can approximate this process by having $K_0 = 0$ in case 3.

However, if they do contribute a little and (3.17) still holds true, we cannot simplify the equations any further.

CASE 3. When $\lambda = \mu$

Although it is unlikely that the case $\lambda = \mu$ will hold exactly, it is reasonable to assume that λ and μ could be approximately equal.

Now we can use (A.3.1) and integrate to obtain

$$E_Y(t) = \frac{k_0}{k_1 - k_2}\left[e^{\frac{\upsilon}{\rho}D(k_1 - k_2)(1-e^{-\rho t})} - 1\right]. \tag{3.18}$$

In the limit

$$\lim_{t\to\infty} E_Y(t) = \frac{k_0}{k_1 - k_2}\left[e^{\frac{\upsilon}{\rho}D(k_1 - k_2)} - 1\right] \tag{3.19}$$

which means that for small doses D, (3.19) is approximately

$$\frac{k_0 \upsilon}{\rho} D + \frac{k_0 \upsilon^2 (k_1 - k_2)}{2\rho^2} D^2 \tag{3.20}$$

We are interested in $E_Y(t)$ only because we need it to obtain $E_z(t.)$ Returning to $E_z(t)$, we can solve (3.13) too through the use of integrating factors and get

$$E_z(t) = e^{r(t)} \int_0^t e^{-r(y)} R(y)\, \pi(T - y)\, [K_1 + K_2 E_Y(y)]dy \tag{3.21}$$

where $r(x) = K_1 \int_0^x R(w)dw$.

Letting T tend to infinity, π becomes $(1 - \frac{M}{\Lambda})$, and using the impulse function as f, we get

$$E_z(t) = \upsilon D(1 - \frac{M}{\Lambda})\, e^{-r(t)} \int_0^t e^{r(y)}\, e^{-\rho y}\, [K_1 + K_2 E_Y(y)]dy \tag{3.22}$$

where $r(x)$ is now $\frac{k_1 \upsilon D}{\rho}(e^{-\rho x} - 1)$ and $E_y(y)$ is as given in (3.18).

The integral can be calculated using the result(A.3.1) several times, which will finally yield

$$E_z(t) = (1 - \frac{M}{\Lambda})\, [e(K_1, t) - 1 + \frac{k_0 k_2}{(k_1 - k_2)} [e(K_1, t) \frac{1 - e(-k_2, t)}{k_2} + \frac{e(k_1, t) - 1}{k_1}]] \tag{3.23}$$

where $e(K, t) = e^{\frac{K \upsilon D}{\rho}(1 - e^{-pt})}$

From this result we get the behavior in the limit:

$$\lim_{t \to \infty} E_z(t) = (1 - \frac{M}{\Lambda})\, [e(K_1) - 1 + \frac{k_0 k_2}{k_1 - k_2} [e(K_1) \frac{1 - e(-k_2)}{k_2} + \frac{e(k_1) - 1}{k_1}]] \tag{3.24}$$

where $e(K) = e^{\frac{K \upsilon D}{\rho}}$

For small doses D, this is approximately

$$(1 - \frac{M}{\Lambda})\, [\frac{k_1 \upsilon}{\rho} D + \frac{k_1^2 \upsilon^2}{2\rho^2} D^2 + \frac{k_0 k_1}{k_1 - k_2} [\frac{2\upsilon}{\rho} D + \frac{(3k_1 - k_2)\upsilon^2}{2\rho^2} D^2]]. \tag{3.25}$$

4. Resulting Consequences of the Model

At first glance equation (3.25) is a quadratic in the dose D. When the dose is small, the second order in D will be negligible and it would be a linear response. This quadratic or linear approximation would fit most commonly observed response curves. However,

this is not all, the co-efficients of D are not necessarily constants: K_0, K_1 and K_2 while being constant for a particular dose or dose range, could take different values in certain dose ranges.

Recalling the definitions of K_0, K_1, K_2, we see that K_1 represents forming cancer cells in one stage and, K_0 and K_2 represent forming them in two stages - first K_0 to form the primary cell and then K_2 to form the cancer cell. When the dose is very small the chances are that it is very unlikely that a second damage could occur to the same cell. In other words, K_2 will be very small or negligible when compared to K_0 or K_1. As the dose gets larger, K_2 becomes a substantial quantity, even of the order of K_1 but still is very likely to be smaller than K_1.

In view of this, equation (3.25) has some surprising implications. At first, K_2 is approximately zero so the terms with K_2 drop out. For very small doses, D^2 is negligible, and hence we have only the first term. Thus, at small doses D, we have an approximately linear curve which, as the dose gets a little larger, becomes a quadratic since the second term becomes prominent. At some point, K_2 becomes no longer negligible and since there is a factor of $K_1 - K_2$ in the denominator which are of the same order, $\left(\frac{0}{k_1 - k_2}\right)$ becomes a large quantity and the first two terms are masked. The curve becomes linear again. Next, the second D^2 term becomes prominent and we have a second order curve once more.

FIGURE 1

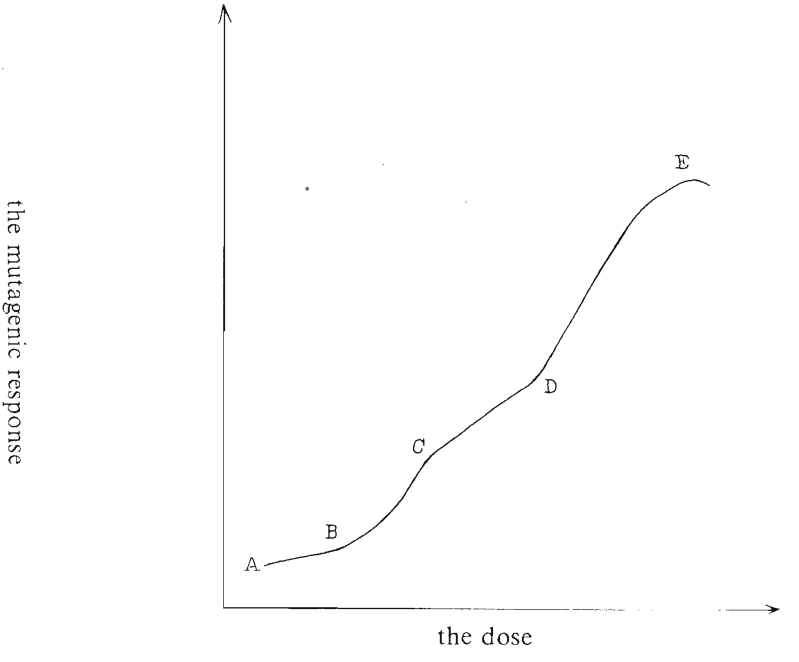

Figure – 1. This figure shows an example of the described phenomenon of a linear curve switching to a quadratic twice over in the mutagenic response. The figure is not to scale in order to show the four segments clearly. AB and CD are the linear segments, while the dose becomes toxic near E. (Produced here with the kind permission of Dr. Kendric C. Smith.)

This is exactly what some experiments (e.g., Sargentini and Smith 1979) have shown: a linear curve smoothly becoming a quadratic, a sudden switch to linearity with a steeper gradient than the previous linear segment, and once again a quadratic before finally the dose becomes lethal (see Figure 1).

From A to C the dose is too small for a second damage to occur to the same cell, thus K_2 is negligible. From C onwards.

$\frac{k_0 k_2}{k_1 - k_2}$ is comparatively large because K_2 becomes non-zero and

K_1 is of the same order of magnitude as K_2. Hence, there are two segments (AC and CE) where approximately linear curves (AB and CD respectively) switch to quadratics as the dose increases, before the dose finally becomes toxic around E. (See equation 3.25).

At least in the shape of the curve, the observations seem to agree remarkably with the model. If one assumes the first two terms can be neglected once K_2 comes into prominence, the relative ratios of the K's can be estimated from the experimental data (all having a multiplicative factor of $\frac{v}{\rho}$, only the ratios can be obtained). This assumption may not be justified, since being masked by other factors in experimental data does not imply that these terms can be totally neglected when it comes to estimation. However, some approximate values can be obtained.

To test the theory further, it is conceivable that an experiment can be performed to check the ratios of different degrees of DNA damage in a random sample of cells exposed to carcinogens. Would the ratios obtained by fitting equation (3.25) to experimental data bear any relation to them? If they do, this could even lead to the identification of what type of damage causes potential cancer tumors and what types are repairable.

When the assumptions of this model hold true, it is clear that the substance that should be tested in the short-term mutagenic tests should be the actual carcinogen. The original substance S1 should be examined in the environment that it is to be put to use, and all by-products that form from it should be carefully identified. The actual carcinogen S2 is not necessarily formed from S1, but could be a derivative of another

substance transformed in the presence of S1. Hence, it is important to carefully examine other substances in the usual environment in the proximity of the original substance, S1. Any changes in these substances in the proximity could well be a clue to the identity of the actual carcinogen.

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References

1. AMES, B. N., McCANN, J. & YAMASAKI, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutation Research*, **31**, 347-364.

2. NEYMAN, J. & SCOTT, E. L. (1967). Statistical aspect of the problem of carcinogenesis. In *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics*. L. Le Cam & J.Neyman (eds.) ,University of California Press, Berkeley & Los Angeles, **4**, 745-776.

3. SARGENTINI, N. J. & SMITH, K. C. (1979). Multiple, independent components of ultraviolet radiation mutagenesis in *Escherichia coli* K-12 *uvrB5*. *Journal of Bacteriology*, **140**, 436-444.

APPENDIX

A.1 The impulse function

Any function f satisfying

$$\int_0^{\infty} f(w) = 1 \text{ and } f(w) = 0 \text{ for all } w > 0 \qquad \text{(A.1.1)}$$

is an impulse function. It also satisfies the following equation for any function F which is continuous at zero.

$$\int_0^{\infty} f(w)F(w)dw = F(0) \qquad \text{(A.1.2)}$$

A.2

If f is any function such that $\int_0^\infty f(w)dw = 1$ then using a Laplace transform it can be shown that

$$\int_0^\infty R(x)dx = \frac{\upsilon D}{\rho} \tag{A.2.1}$$

where R(x) is given by (2.9). When f is the impulse function of A.3.1, then

$$\int_0^y R(x)dx = -\upsilon D \frac{e^{-\rho y} - 1}{\rho} \tag{A.2.2}$$

A.3

A result of integration:

$$\int_0^x e^{-at} Ae^{-at} \, dx = \frac{e^{A} - e^{Ae^{-at}}}{aA} \tag{A.3.1}$$