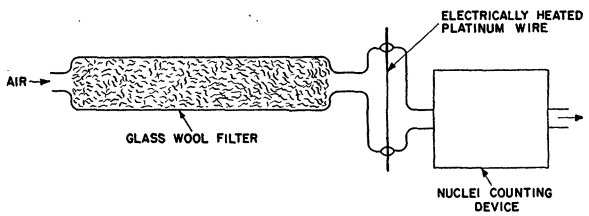

FIG. 1.

the filament ceased producing any nuclei, and the air entering the measuring device became nuclei-free.

It was observed that when small amounts of gaseous halogen or halogen-containing substances such as Cl_2, I_2, Br_2, and CCl_4 were present in the air being drawn through the filter the heated platinum filament produced large numbers of nuclei. The sensitivity of the apparatus to CCl_4 was found to approach that of the commercial halogen leak detector which operates on the positive ion emission of a heated platinum filament.

A possible explanation for this phenomenon is as follows. At 500° C the vapor pressure of platinum is so low that an insufficient concentration of atoms is introduced into the air to condense and form condensation nuclei. The halogen or halogen-containing gases in the air, which pass freely through the glass wool filter, react with the hot platinum surface. The resulting compounds, although relatively nonvolatile at room temperature, have a higher vapor pressure than the platinum and at 500° C are vaporized in sufficient concentration to condense upon mixing with air at room temperature to form large numbers of nuclei.

It is reasonable to suppose that other systems can be devised in which small concentrations of certain gaseous materials will result in the production of large numbers of nuclei from certain nonvolatile substances maintained at an elevated temperature.

The fact that nuclei having masses of the order of 10^{-17} g are readily detectable in concentrations as low as 10/ml suggests that very sensitive analytical techniques based on nuclei detection are feasible.

References

1. VONNEGUT, B. *Proc. Natl. Air Pollution Symposium. 1st Symposium*, Pasadena, Calif. (Nov. 1949).
2. AITKEN, J. *Proc. Roy. Soc. Edinburgh*, **18**, (1890–91).

Manuscript received July 10, 1952.

Analysis of Dose-Response in Relation to Mechanism of Pulmonary Tumor Induction in Mice

W. E. Heston and M. A. Schneiderman[1]

National Cancer Institute,
National Institutes of Health,
U. S. Public Health Service, Bethesda, Maryland

It is almost universally assumed that the transformation of cells to malignancy involves some change in the cell. The nature of this change remains one of the basic questions in cancer research. Berenblum and Shubik (*1*) have postulated a two-phase process, the initiative phase and the promoting phase, a concept that has been supported by others. Blum (*2*) has suggested that in the induction of skin tumors by ultraviolet irradiation there is progressive acceleration of growth by successive doses. This suggests that there may be successive changes in the cell.

Much consideration has been given to the somatic mutation theory of carcinogenesis proposed by Von Hansemann and later by Boveri, and recently vigorously supported by Strong (*3*) and others. Although the number of changes might not necessarily be limited to one, the present concept of this theory would tend to locate the change or changes in the nucleus, presumably as gene changes.

Interest in the somatic mutation hypothesis recently has been strengthened by the general search for a positive correlation between mutagenic and carcinogenic capacities of chemicals. An over-all positive correlation has not been observed, but isolated experiments testing related compounds under standardized conditions have presented positive correlations that in themselves suggest that possibly the change to malignancy is basically genic. Tests in this laboratory (*4, 5*) on the induction of pulmonary tumors in strain A mice with mustard compounds have shown that both the nitrogen mustard, methyl-bis (2-chloroethyl) amine hydrochloride, and sulfur mustard, bis (2-chloroethyl) sulfide, which Auerbach (*6*) and others have shown to be strong mutagens, were also potent carcinogens, whereas mustard oil, ethyl iso-thiocyanate, which was found to be a very weak mutagen, did not significantly increase the number of lung tumors.

In an analysis of the number of papillomas observed in mice painted repeatedly with Benzpyrene, Charles and Luce-Clausen (*7*) demonstrated a linear relationship when the square root of the number of papillomas was plotted against time, an expression of dose. This suggested the necessary occurrence of two separate events, or mutations, in the cell for the induction of a papilloma, the requirement if a recessive mutation were involved.

From our experience with pulmonary tumors in mice, it seemed desirable to analyze the pulmonary tumor response to graded doses of a carcinogen to ascertain whether here also would be found a parabolic curve indicating more than one change, or a straight line, as could be expected if only one change were necessary for a cell to give rise to a tumor. Certain outstanding advantages are offered by this type of tumor: (1) the many nodules appearing on the surface of the lungs afford a quantitative measure of response; and (2) a single dose of the carcinogen, even of very small amount, gives a measurable response. Thus, repeated doses such as were encountered in the studies of papillomas and Blum's radiation studies could be avoided.

[1] With the technical assistance of W. D. Levillain.

Five groups of strain A mice approximately 2 months old were injected intravenously, respectively, with .1, .2, .3, .4, and .5 mg 1:2:5:6-dibenzanthracene in colloidal dispersion in .5 ml distilled water/mouse.[2] A sixth group was injected with .5 ml distilled water as controls. The sexes were approximately equally divided in all groups, and the mice were individually identified. They were kept in plastic cages, 8 mice to the cage, fed Derwood pellets, and given an unlimited supply of tap water. Six months after the injection all animals were killed, their fresh lungs were examined with the aid of the dissecting microscope, and the number of tumors appearing on the surface of the lungs of each animal was recorded.

The average number of tumors for each dosage group is listed in Table 1. Sexes are combined, since no sex difference was observed.

TABLE 1

PULMONARY TUMORS IN STRAIN A MICE INJECTED INTRAVENOUSLY WITH 1:2:5:6-DIBENZANTHRACENE

Dose (mg)	No. animals	Av no. nodules	SE of av no.
0.	55	.29	.033
.1	51	8.08	.542
.2	50	18.25	1.225
.3	44	30.02	1.663
.4	50	38.64	1.923
.5	46	53.37	2.166

Subject to certain limitations, the tumor-dose relationship is linear. The limitations require that one postulate the operation of two conflicting endogenous factors. The first of these is a tumor-increasing factor, the genetic susceptibility of the strain. Some animals that have received no dibenzanthracene at all will develop tumors. This means that a dose of zero is effectively zero plus this small factor, designated as G, and other doses are not .1 mg, .2 mg, etc., but rather $.1+G$, $.2+G$, etc. Operating in the other direction, there appears to be a factor reducing the effective amount of the carcinogen. Perhaps a small amount is lost in the circulation prior to reaching the lungs, or in some other way a small amount is eliminated or made ineffective. This represents a sort of "threshold" dose. Calling this factor R, one finally would have as the effective dose, the reported dose $+(G-R)$.

If one fits a least-squares straight line to the data, excluding the response at the reported zero dose, one gets for the constants of the equation

y = average number of tumors $= a + bx$
$a = -2.86$
$b = 108.35$

This implies that at zero dose there should be expected an average of -2.86 tumors per animal. However, we are not really interested in fitting $y = a + bx$, but rather in fitting $y = a' + b[x + (G-R)]$. If we can do this we can make an estimate of the combined factor $(G-R) = k$. At the zero reported dose the average number of tumors per animal from the experimental data was .29. This is a'. Then we can write, setting $x = 0$, for the zero dose:

$$-2.86 = .29 + 108.35\ (0 + k).$$

Then $k = (G-R) = -.0293$, and the equation is: average number of tumors $= .29 + 108.35$ (dose $- .0293$).

This equation fits the data very well, as shown in Fig. 1. A test of accuracy of fit (χ^2) gives a nonsignificant deviation from linearity; that is, there is no statistical evidence that the linear hypothesis is inadequate to explain the results.

Postulating a "threshold" dose implies that for doses less than .03 mg dibenzanthracene, there should be no greater response than at a zero dose. Of course one cannot expect this sort of sharp break to occur. The value .03 (that is, .0293) is subject to error, and, in addition, individual animals could be expected to have individual abilities to "dispose" of some small amount of the carcinogen. What might be expected is that the portion of the curve between 0 and .1 would have a smooth form, concave upward, rather than a sharp breaking form (Fig. 1).

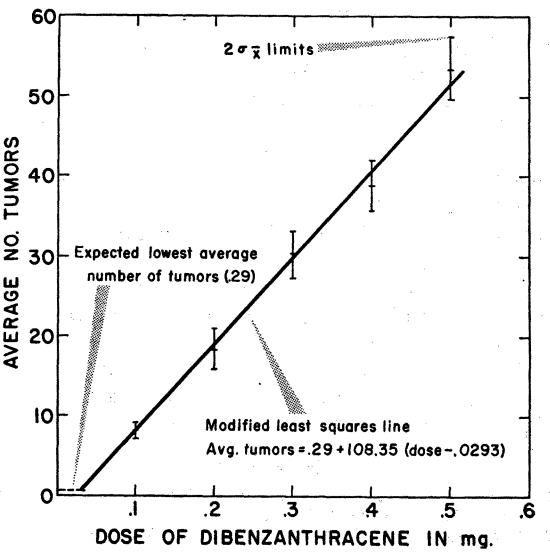

FIG. 1.

An attempt was made to fit the data to the two-action hypothesis set forth by Charles and Luce-Clausen (7), which implies that the square root of the number of tumors is proportional to the dose. It was barely possible to fit a least squares straight line that was not outside the 2 × standard error of mean limits. χ^2 is 5.405, which with two degrees of freedom, gives a probability of so large a deviation from the fitted line (due to chance alone) of between .10 and .05. The two-action hypothesis leads to a positive constant to be added to the dose rather than the negative constant found for the single-action hypothesis. This

[2] This dispersion was prepared by Joanne Hollcroft.

constant was higher than could be accounted for on the basis of the incidence of spontaneous tumors in this strain. The hypothesis implies an average of more than 3 tumors at the zero dose, whereas the observed average was .29. This increased response is even more difficult to explain than the decreased response shown for the single-action hypothesis. The two-action hypothesis implies that the curve is concave upward, which would give a rapidly increasing number of tumors at the larger doses. Instead, at the higher doses the curve probably would be found to flatten out. Since there is also a time element involved if the actual response curve is sigmoid, observing the tumors earlier might give an upward curve, whereas later observations might give a downward curve. This requires further investigation.

Data on pulmonary tumors induced in mice with methylcholanthrene by Shimkin and McClelland (*8*) were examined to see if the linear relationship between the number of nodules and the dose was also present in their experiment. Such a linear relationship could not be established, there being significant departure from linearity when the χ^2 test was used. However, there were not as many animals per group in their experiment as in the groups reported herein, and the technique used in counting was not the same for the two experiments. In their experiment the nodules were counted in lungs that previously had been fixed in Tellyesniczky's fluid, and apparently the counting was done without the aid of the dissecting microscope. Their data did not fit a parabolic curve such as reported by Charles and Luce-Clausen either.

It was possible, however, to estimate the "net disposal" constant (k) for Shimkin and McClelland's data such as was estimated from our own experiment. Their data were in three series, with observations made after 8, 13, and 18 weeks. For these series, k was .047 mg, .040 mg, and .047 mg methylcholanthrene, respectively. These constants are more nearly alike than would have been expected from the variance of k which is implied by the mathematics.

Although the data of Shimkin and McClelland do not support, they do not oppose our own data, which with the analysis thereof would fit the postulate that the induction of pulmonary tumors in the mouse is the result of a single change in the cell. If this were a genic change it would be assumed to be a dominant mutation.

References

1. Berenblum, I., and Shubik, P. *Brit. J. Cancer,* **3**, 109 (1949).
2. Blum, H. F. *J. Natl. Cancer Inst.,* **11**, 463 (1950).
3. Strong, L. C. *Proc. 8th Intern. Congr. Genet.,* Hereditas, Suppl., **486** (1949).
4. Heston, W. E. *J. Natl. Cancer Inst.,* **10**, 125 (1949).
5. *Ibid.,* **11**, 415 (1950).
6. Auerbach, C. *Nature,* **157**, 302 (1946).
7. Charles, D. R., and Luce-Clausen, E. M. *Cancer Research,* **2**, 261 (1942).
8. Shimkin, M. B., and McClelland, J. N. *J. Natl. Cancer Inst.,* **10**, 597 (1949).

Manuscript received July 21, 1952.

Ascorbic Acid and the Oxidation of Tyrosine

Bert N. La Du, Jr., and David M. Greenberg

Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, New York, Section on Chemical Pharmacology, National Heart Institute, National Institutes of Health, USPHS, Bethesda, Maryland, and *Division of Biochemistry, University of California School of Medicine, Berkeley*

Many lines of evidence have suggested that ascorbic acid is involved in the biological oxidation of tyrosine. Studies with rat liver homogenate preparations (*1*) and acetone powder extracts (*2*) have indicated that the effect of ascorbic acid in the conversion of tyrosine to acetoacetic acid is primarily upon the first oxidative step of this metabolic pathway, the oxidation of *p*-hydroxyphenylpyruvic acid. Recently it has been proposed that ascorbic acid is a cofactor for the enzyme catalyzing this oxidative step (*3*). In the present communication, evidence will be presented that ascorbic acid may have a less specific role and can be replaced by a number of structurally unrelated compounds that are susceptible to oxido-reduction.

In experiments with extracts of liver acetone powder of rat, rabbit, and dog, it has been reported that tyrosine, *p*-hydroxyphenylpyruvic acid, 2,5-dihydroxyphenylpyruvic acid, and homogentisic acid oxidized enzymatically to acetoacetic acid (*2, 4*). The addition of ascorbic acid to this preparation increases the oxidation of tyrosine or *p*-hydroxyphenylpyruvic acid but has no effect upon the oxidation of 2,5-dihydroxyphenylpyruvic acid and homogentisic acid (*2*). The effect of ascorbic acid on the oxidation of *p*-hydroxyphenylpyruvic acid can be studied by using either *p*-hydroxyphenylpyruvic acid as the substrate or by using tyrosine with α-ketoglutarate to generate *p*-hydroxyphenylpyruvic acid via the tyrosine transaminase system present in the extract. The effect of ascorbic acid was established by measuring the rate of disappearance of substrate and by following the reaction manometrically in the Warburg apparatus. When 10 μM of L-tyrosine was used as the substrate with the liver extract preparation, the addition of ascorbic acid increased the oxidation of tyrosine only when α-ketoglutarate was also present, as shown in Fig. 1. A concentration of 0.001 *M* (4 μM) ascorbic acid was found to be sufficient to produce a maximal stimulatory effect upon the oxidation. Further increase in the concentration of ascorbic acid did not increase the initial rate of oxygen uptake, nor did it alter the products of the reaction. Suboptimal concentrations of ascorbic acid resulted in less tyrosine being converted to acetoacetic acid.

The preparation of the acetone powder removes part of the ascorbic acid orginally present in the tissue. The residual amount remaining in the crude powder extract is sufficient, however, to maintain appreciable oxidative activity. Dialysis of the crude