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A NEW MODEL OF THE MECHANISM OF INDUCTION OF PULMONARY TUMORS IN MICE

1. Introduction. In the paper* we discuss a one-branching model as defined in [6] but it differs from that one with regard to the postulated nature of the feeding function and its variation from one animal to the next. The new element is the hypothesis that the number of urethan-sensitive lung cells may change after injection of urethan.

The application of the model to a simultaneous analysis of data from references [7], [13] and [15] leads to a number of new factual findings. Among others, that with a single injection of a dose of urethan up to 0.5 mg/gm of body weight, the average number of lung tumors is proportional to the estimated internal exposure as defined in (14), whereas with a dose of 1.0 mg/gm the corresponding average number of tumors is systematically smaller than in proportion to the estimated internal exposure. It is found that the younger the mice the larger is the deviation. Moreover, the analysis of the data suggests that after the injection of a single dose of urethan the number of urethan-sensitive cells first decreases and subsequently increases before it stabilizes at the initial level. This provides a heuristic explanation to the well-established empirical fact [13] that fractionation of a single dose into two equal subdoses may in some cases lead to an increase and in others to a decrease in the ultimate number of lung tumors.

Two experiments are indicated that may offer a promise for verifying empirically the conjectures regarding the suggested changes in the number of urethan-sensitive cells after a single dose of urethan.

2. Basic assumptions. In the last 15 years many authors have been concerned with the question as to whether carcinogenesis in general, and particularly urethan carcinogenesis, is a one-stage or a multistage phenom-

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enon. A substantial amount of experiments have been performed partly with the hope to distinguish between these two models. The most elaborate up to the present statistical analysis has been presented by Neyman and Scott [9] in 1967. They concluded that the experimental findings favor the two-stage model and contradict the one-stage model. However, recent experimental findings (see [12] and [14]) dictate modifications and generalizations of some of the assumptions which underly their conclusion; in particular, the assumption that the number of normal lung cells which can undergo a mutation-like change and initiate the tumorigenic process are always constant whatever be the time pattern of administering the urethan.

The model considered in this paper is a generalization of the one-stage mutation model. The generalization includes, among others, the possibility that the number of the lung cells which can initiate a pulmonary tumor may change after administering the urethan. It is conjectured that the number of those cells first decreases and then increases before reaching the original level. It is shown that the suggested model, called *one-branching*, is qualitatively consistent with the empirical data available today. In the last section two experiments are outlined which offer a promise for verifying some predictions of the one-branching model.

In this paper we develop the ideas first formulated in [7].

The model described in the paper is considered with reference to experiments consisting of exposures of experimental animals to action of urethan administered in varying doses and, subsequently, in counting the number of tumors on the surface of the lungs. The administered doses are measured in milligrams of urethan per gram of body weight (mg/gm) of the mouse injected.

The adopted assumptions underlying the chance mechanism of urethan carcinogenesis are the following:

(i) A tumor originates from a mutation-like change in a single normal lung cell.

(ii) Whatever be the dose schedule, the number of initial events occurring in the lungs of one experimental animal is a random variable with a Poisson distribution.

(iii) Under the same treatment conditions the Poisson distribution of the number of initial events in one animal need not be the same as that in another.

(iv) A single initial event can result in at most one tumor.

(v) All clones initiated by mutation-like changes in the normal lung cells are mutually independent.

The mechanism of carcinogenesis such as described by hypotheses (i)-(v) will be called *one-branching* [6].

The one-branching mechanism so defined is a generalization of the one-stage mutation mechanism considered by Neyman and Scott [9]. In order to obtain the latter, hypotheses (i)-(v) must be supplemented by some detailed additional assumptions regarding what happens after the initial event; among others, that following an initial event in a particular cell this cell turns directly into a cancer cell.

The assumptions regarding the mechanism of occurrence of the initial events will be described in the next section.

3. Feeding function. Consider an experimental animal which is exposed to a total dose D of mg/gm of urethan administered as a single dose at time $t_0 = 0$ or in some s fractions, each of D/s mg/gm at times, say, $t_0 = 0, t_1, \ldots, t_{s-1}$. Throughout the paper we assume the origin on the time scale to be the moment when the first dose is injected.

Let $Q = Q(D; s; t_0, \ldots, t_{s-1})$ represent the time pattern of administering the carcinogen. Let $x(t|Q)$ represent the amount of urethan in the animal's body that remained unexhaled at time $t > 0$. Let $a = a(t|Q)$ stand for the number of those lung cells at time t in which urethan can induce initial tumorigenic changes (or, for short, the number of cells that are in the carcinogen-sensitive part of the cell life cycle), given the time pattern Q of administering the urethan. Here we take into account the possibility suggested by some authors (see [7] and [16]) that the number of the carcinogen-sensitive cells may change after the injection of urethan.

Finally, let

$$f(t|Q) = ea(t|Q)x(t|Q), \tag{1}$$

where e is a non-negative constant.

It is assumed that the probability that in any time interval $[t, t+h)$, with $h > 0$, a single mutation will occur in the lungs of the animal considered is equal to $f(t|Q)h + o(h)$, irrespective of the number of earlier initial events. Also it is assumed that the probability of two or more initial events in the time interval $[t, t+h)$ is $o(h)$.

This implies that the total number of initial events in the lungs of the animal considered occurring in any time in interval (t_1, t_2) is a Poisson variable with expectation

$$\int_{t_1}^{t_2} f(t|Q)\,dt.$$

The function $f = f(t|Q)$ defined by (1) is called the *feeding function* [9]. The coefficient e characterizes the sensitivity to urethan of the population of animals considered.

Remark 1. It is not known whether urethan or some metabolite of urethan is the active carcinogen. If it were not urethan, then $x(t|Q)$

should stand for the active carcinogen. In that case it is necessary to replace "urethan" by the "active carcinogen" throughout the paper.

4. Distribution of the number of visible tumors. Hypotheses (i)-(v) supplemented by the additional assumptions regarding the feeding function (1) imply the formula for the generating function $G(s|T, Q)$ corresponding to the distribution of the number of visible tumors $y(T|Q)$ at any preassigned time $T > 0$, given the dose schedule Q.

In fact, for a particular animal the number of visible tumors at any time $T > 0$ is a Poisson random variable with expectation

$$\lambda(T|Q) = e \int_0^T q(T-t|Q)\, a(t|Q)\, x(t|Q)\, dt, \tag{2}$$

where $q(T-t|Q)$ is the probability that a single initial event occurring in the animal lungs at time $t > 0$ will lead to a visible tumor at time $T > t$ provided that urethan is administered according to the time pattern Q. The expected value $\lambda(T|Q)$ for one animal need not be the same as that for another animal and it is treated as a random variable $\lambda \geqslant 0$ with an unspecified distribution function, say, $F(\lambda|T, Q)$, non-degenerate at zero.

Using this notation leads to

$$G(s|T, Q) = \int_0^\infty \exp\{\lambda(s-1)\}\, dF(\lambda|T, Q), \tag{3}$$

which is the generating function corresponding to a mixture of Poisson distributions. It follows from (3) that the expected value of $y(T|Q)$ is

$$\mathrm{E}y(T|Q) = \int_0^\infty dF(\lambda|T, Q),$$

which can be written in the form of

$$\mathrm{E}y(T|Q) = e\mathrm{E} \int_0^T q(T-t|Q)\, a(t|Q)\, x(t|Q)\, dt, \tag{4}$$

where the expectation on the right-hand side is taken with respect to the population of the animals considered. For example, this can be a given strain of mice.

5. Some implications of the one-branching model of carcinogenesis. Unless stated the contrary, we assume throughout the section that the random variables $q = q(T|Q)$, $a = a(T|Q)$ and $x = x(T|Q)$ are independent. Under this assumption we derive approximate formulae for the relations of the expected number of visible tumors to the expected number of ini-

tial events and to the expected internal exposure for two particular time patterns, namely $Q_1 = Q(D; 1; 0)$ and $Q_2 = Q(2D; 2; 0, t_1)$.

If urethan is eliminated from the bodies of mice before time T at which the tumors are counted, say, it is eliminated already at time $\tau_0 < T$, then it follows from formula (4) that $\mathrm{E}y(T|Q_1)$ is approximately equal to $\mathrm{E}q(T|Q_1)\,\mathrm{E}i(D)$, i.e.

$$\mathrm{E}y(T|Q_1) \simeq \mathrm{E}q(T|Q_1)\mathrm{E}i(D), \tag{5}$$

where

$$i(D) = e\int_0^{\tau_0} a(t|Q_1)x(t|Q_1)\,dt.$$

Note that $I(D) = \mathrm{E}i(D)$, where the expectation is taken with respect to the population of the animals considered, is the expected number of initial events given that at time $t_0 = 0$ a single dose of D mg/gm of urethan is injected.

Clearly, the larger T and the smaller τ_0, the better the right-hand side of (5) approximates the left-hand side of (5). Moreover, if the elimination of urethan is rapid, i.e. if τ_0 is small, then instead of (5) we may write

$$\mathrm{E}y(T|Q_1) \simeq cJ(D), \tag{6}$$

where

$$J(D) = \mathrm{E}\int_0^{\tau_0} x(t|Q_1)\,dt, \tag{7}$$

while $c = e\mathrm{E}q(T|Q_1)\mathrm{E}a(0|Q_1)$. The quantity $\int_0^{\tau_0} x(t|Q_1)\,dt$ is called the *total internal exposure* to urethan of the particular animal to which the function $x(t|Q_1)$ is attached, while $J(D)$ stands for the corresponding expected value, given the experimental conditions.

In summary, we can state the following proposition:

PROPOSITION 1. *Within the framework of the adopted assumptions, if T is large, then the expected number of visible tumors at time T resulting from the application of a single dose of urethan is proportional to the expected number of initial events. Moreover, if the elimination of urethan from the bodies of mice is rapid, then the expected number of visible tumors at time T resulting from a single injection of urethan is proportional to the expected internal exposure.*

Now we give the corresponding formulae for the time pattern Q_2.

If urethan from the first injection is eliminated before time τ_0, $0 < \tau_0 < t_1$, and if urethan from the second injection is eliminated before

time τ_1, $t_1 < \tau_1 < T$, then, in view of (4), we can write

$$\mathrm{E}y(T|Q_2) = e\Big[\mathrm{E}\int_0^{\tau_0} q(T-t|Q_1)a(t|Q_1)x(t|Q_1)\,dt + \int_{t_1}^{\tau_1} q(T-t|Q_1)a(t|Q_2)x(t|Q_2)\,dt\Big].$$

Consequently, if $q(T-t|Q) \simeq q(T|Q)$ for $t \in (0, \tau_1)$ and if τ_0 and τ_1 are small, while T is large, then we have the approximate formula

$$\mathrm{E}y(T|Q_2) \simeq c\big[A_1 J(D) + A_2\big(J(2D, t_1) - J(D)\big)\big], \tag{8}$$

where $c = e\mathrm{E}q(T|Q_1)$, $A_1 = \mathrm{E}a(0|Q_1)$, and $A_2 = \mathrm{E}a(t_1|Q_2)$, while $J(D)$ is given by (7) and

$$J(2D, t_1) = \mathrm{E}\int_0^{\tau_1} x(t|Q_2)\,dt,$$

which is the expected value of the total internal exposure resulting from applying the dose D twice, t_1 days apart, given the experimental conditions.

If $J(D) = J(2D, t_1) - J(D)$ or, equivalently, if $J(2D, t_1) = 2J(D)$, then we say that the internal exposures are *additive*.

Under the assumption of additivity it follows from (8) that

$$\mathrm{E}y(T|Q_2) \simeq c(A_1 + A_2)J(D). \tag{9}$$

If the elimination of urethan from the bodies of mice is rapid, if T is large, and if the internal exposures are additive, then in view of (6) and (9) we can state Propositions 2 and 3.

Let

$$A_1 = \mathrm{E}a(0|Q_1) = \mathrm{E}a(0|Q_3) \quad \text{and} \quad A_2 = \mathrm{E}a(t_1|Q_2),$$

where

$$Q_1 = Q(D; 1; 0), \quad Q_2 = Q(2D; 2; 0, t_1) \quad \text{and} \quad Q_3 = Q(2D; 1; 0).$$

PROPOSITION 2. *Let* $\mathrm{E}y(T|Q_1)$ *and* $\mathrm{E}y(T|Q_2)$ *be the expected numbers of tumors visible at time* $T > 0$ *and resulting from a single dose* D *at time* $t = 0$ *and from two doses* D *at times* $t = 0$ *and* $t = t_1$, *respectively. Then, within the framework of the adopted assumptions, the following relations hold*:

$$\begin{aligned} &\mathrm{E}y(T|Q_2) > 2\mathrm{E}y(T|Q_1) && \text{if } A_1 < A_2,\\ &\mathrm{E}y(T|Q_2) = 2\mathrm{E}y(T|Q_1) && \text{if } A_1 = A_2,\\ &\mathrm{E}y(T|Q_2) < 2\mathrm{E}y(T|Q_1) && \text{if } A_1 > A_2. \end{aligned}$$

PROPOSITION 3. *Let* $\mathrm{E}y(T|Q_2)$ *and* $\mathrm{E}y(T|Q_3)$ *be the expected numbers of tumors visible at time* $T > 0$ *and resulting from two doses* D *at times* $t = 0$ *and* $t = t_1$ *and from a single dose* $2D$ *at time* $t = 0$, *respectively. Let* $\varepsilon = J(2D)/J(D) - 1$. *Then, within the framework of the adopted assumptions, the following relations are true*:

$$\mathrm{E}y(T|Q_2) > \mathrm{E}y(T|Q_3) \quad \text{if } A_1(1+\varepsilon) < A_2,$$
$$\mathrm{E}y(T|Q_2) = \mathrm{E}y(T|Q_3) \quad \text{if } A_1(1+\varepsilon) = A_2,$$
$$\mathrm{E}y(T|Q_2) < \mathrm{E}y(T|Q_3) \quad \text{if } A_1(1+\varepsilon) > A_2.$$

Proposition 3 shows that the effect of fractionation of a single dose into two equal subdoses may depend, in addition to A_2/A_1, also on $\varepsilon = J(2D)/J(D) - 1$.

In the model of urethan carcinogenesis presented above there are a number of unspecified elements. In the following sections we investigate what additional assumptions to adopt regarding these unspecified elements to guarantee the consistency of the model with empirical data. This, in turn, leads us to a number of hypotheses that can be tested by further investigations. All these speculations are limited to experiments consisting of exposures of mice to urethan administered in one or two doses only.

In the following section, we discuss the question as to whether $q(T|Q_1)$ does or does not vary from one animal to the next.

6. Variation of $q(T|Q_1)$. If urethan is eliminated from the bodies of mice before time $\tau_0 < T$, then (2) reduces to

$$\lambda(T|Q_1) = e\int_0^{\tau_0} q(T-t|Q_1)\,a(t|Q_1)\,x(t|Q_1)\,dt.$$

In this section we assume that every realization $q(T-t|Q_1)$ satisfies the condition $q(T-t|Q_1) = q(T|Q_1)$ if $0 < t < \tau_0$ and if T is sufficiently large, say $T \geqslant T_0$.

Then we have

$$\lambda(T|Q_1) = eq(T|Q_1)\int_0^{\tau_0} a(t|Q_1)\,x(t|Q_1)\,dt. \tag{10}$$

Since the joint distribution of (a, x) is clearly independent of T, therefore, we can conclude that the distribution of $q(T|Q_1)$ is non-degenerate for $T \in (\alpha, \beta)$ if $C = C(T|Q_1) = \mathrm{Var}\lambda/\mathrm{E}^2\lambda$ depends explicitly on T in (α, β).

We now describe a criterion for the verification of the hypothesis H_0 asserting that $\mathrm{Var}\lambda/\mathrm{E}^2\lambda$ does not depend on T in (α, β) against the alternative hypothesis H_1 that $\mathrm{Var}\lambda/\mathrm{E}^2\lambda$ does depend explicitly on T in (α, β).

Clearly, in view of the above, the rejection of the null hypothesis H_0 allows us to assert that the distribution of $q(T|Q_1)$ is non-degenerate for $T \in (\alpha, \beta)$ provided $\alpha > T_0$.

The criterion is based on the fact that under the null hypothesis the expression

$$\frac{\mathrm{Var}\, y(T|Q_1) - \mathrm{E}\, y(T|Q_1)}{\mathrm{E}^2 y(T|Q_1)} \tag{11}$$

does not depend on time whereas under the alternative hypothesis it does explicitly depend. Indeed, this is a consequence of the formula

$$\frac{\mathrm{Var}\, y(T|Q_1) - \mathrm{E}\, y(T|Q_1)}{\mathrm{E}^2 y(T|Q_1)} = \frac{\mathrm{Var}\, \lambda}{\mathrm{E}^2 \lambda} \tag{12}$$

which, in view of (10), is easily derived from (3).

To verify the null hypothesis we suggest to estimate C for various times $T > T_0$ and to inspect whether or not a time trend is observed.

As the estimator of C we take the expression

$$\hat{C} = \frac{N(S^2 - \bar{y})}{\bar{y}(N\bar{y} - 1)},$$

where $\bar{y}$ is the group mean, S^2 the unbiased group variance, and N the size of the group.

Remark 2. The distribution of $\hat{C}$ depends on the unknown probability distribution function of λ. In case λ has a degenerate distribution the expected value of $\hat{C}$ is zero, and in case λ has a gamma distribution the expected value of $\hat{C}$ is $C - C^2/(N + C)$. It might be interesting to formalize this test procedure and to investigate the power by Monte Carlo methods.

We used data from reference [16], and estimated C for 48 experimental groups each counting 27 or 28 female A/Jax mice. The groups embrace all combinations of two dose schedules (0.5 and 1.0 mg/gm of urethan), three ages of animals at time of injection (4.5, 6.5 and 8.5 weeks) and eight times of sacrifice after injection (11, 12, 15, 16, 19, 20, 23 and 24 weeks).

The calculated values of C for these 48 experimental groups are presented in Tables 1 and 2. Since there appears to be no noticable differences in the values of the estimates among the 3-age groups, we estimated C by their arithmetic mean at the various times T of sacrifice after injection. Figs. 1 and 2 show these estimates plotted against time after injection corresponding to 0.5 and 1.0 mg/gm of urethan, respectively. The curves are drawn by hand.

TABLE 1. Estimated values of C_1

Age at injection (weeks)	Weeks between urethan injection and sacrifice							
	11	12	15	16	19	20	23	24
4.5	.097	.141	.097	.108	.077	.061	.027	.011
6.5	.065	.063	.129	.063	.004	.027	.041	.015
8.5	.156	.169	.031	.056	.072	.001	.007	−.013
Mean value	.106	.126	.086	.076	.051	.030	.025	.004

Dose 0.5 mg/gm of urethan. Data from [16].

TABLE 2. Estimated values of C_2

Age at injection (weeks)	Weeks between urethan injection and sacrifice							
	11	12	15	16	19	20	23	24
4.5	.058	.012	.040	.019	.033	.002	.015	.036
6.5	.031	.059	.016	.010	.019	.009	.014	.017
8.5	.006	.034	.019	.011	.013	.028	.017	.010
Mean value	.032	.035	.025	.013	.022	.013	.015	.021

Dose 1.0 mg/gm of urethan. Data from [16].

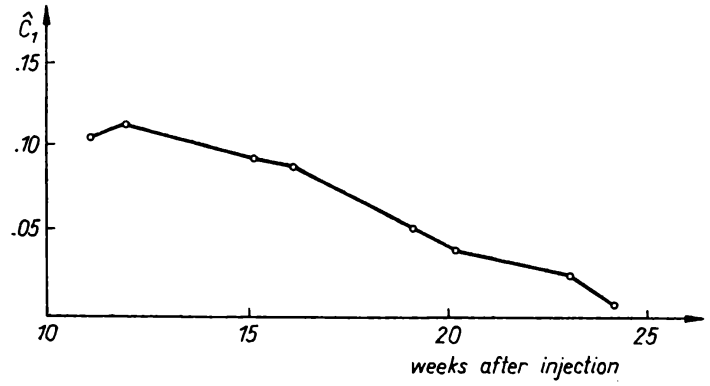

Fig. 1. Estimated values of C_1
Dose 0.5 mg/gm of urethan. Data from [16]

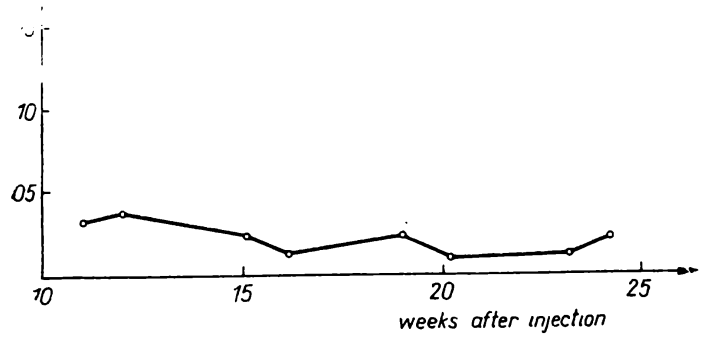

Fig. 2. Estimated values of C_2
Dose 1.0 mg/gm of urethan. Data from [16]

The inspection of Figs. 1 and 2 suggests strongly two effects:

(i) C_1 is a decreasing function of T in the time interval 11 to 24 weeks,

(ii) C_2 is smaller than C_1 for T in the time interval 11 to 20 weeks.

Henceforth, in view of these findings, we shall treat $q(T|Q_1)$ as a non-degenerate random variable with a probability distribution function that, in addition to T, depends upon the amount D of the injected urethan.

Remark 3. Expression (11) seems to be considered for the first time by Polissar and Shimkin [10] and described by them as the coefficient of variation in susceptibility to pulmonary tumors. Our findings contradict the findings of Polissar and Shimkin that the coefficient of variation in susceptibility is a constant independent upon the conditions of the experiment.

Remark 4. The interpretation of the differences between the curves exhibited in Figs. 1 and 2 is not straightforward. It depends on what is assumed on the joint distribution of q, a and x. If we assume that q and (a, x) are independent random variables, then the following interpretation is possible. First note that under the additional assumption the expression (12) may be rewritten as

$$C(T|Q_1) = \frac{\operatorname{Var} q}{\mathrm{E}^2 q}\cdot\frac{\operatorname{Var} i}{\mathrm{E}^2 i} + \frac{\operatorname{Var} q}{\mathrm{E}^2 q} + \frac{\operatorname{Var} i}{\mathrm{E}^2 i}, \qquad \text{where } i = i(D).$$

If it were true that $\operatorname{Var} i(0.5)/\mathrm{E}^2 i(0.5)$ and $\operatorname{Var} i(1.0)/\mathrm{E}^2 i(1.0)$ were approximately equal, then a confrontation of the above-given formula with Figs. 1 and 2 would lead to the conclusion that

$$\mathrm{E}\, q(T|Q_2) > \mathrm{E}\, q(T|Q_1) \quad \text{if } 11 < T < T_0$$

and/or

$$\operatorname{Var} q(T|Q_2) < \operatorname{Var} q(T|Q_1) \quad \text{if } 11 < T < T_0,$$

where $11 < T_0 < 24$ (weeks), while $Q_1 = Q(0.5; 1; 0)$ and $Q_2 = Q(1.0; 1; 0)$.

7. Conjecture regarding $a(t|Q_1)$. In all the models of carcinogenesis we have seen discussed in the literature the feeding function is assumed to be direct proportional to the amount of urethan in the animal body. In particular, this hypothesis is presumed by Neyman and Scott [9]. In our considerations we shall admit the possibility that the quantity $a(t|Q)$ appearing in (1) may depend upon t and Q. Unfortunately at present an explicit formula cannot be given. Although most probably $a(t|Q)$ varies from animal to animal in a random fashion over any population of experimental mice, we shall treat henceforth $a(t|Q)$ as a non-random quantity. However, even with these simplifying assumptions $a(t|Q)$ can still not be estimated on the base of the data available at present. To

estimate $a(t|Q)$ we need the internal exposures resulting from single and multiple injections at various ages which are not known today. In this situation it seems to be justified to make some conjectures regarding the function $a(t|Q)$.

The conjectures presented in this paper were inspired by a possibility suggested by a number of biologists (see [12], [16], and also [7]) that urethan initially depresses and then increases the number of cells in the lungs which are in the DNA synthesis. If this be true, and if, in fact, lung cells are most sensitive to carcinogen at a particular part of the cell-life cycle, then one can expect at the time of the second injection a different number of those cells which are capable to originate a tumor that at the time of the first injection depending upon spacing between injection [7]. We conjecture that, whatever be the amount of the injected urethan, $a(t|Q_1)$ is a cosine-like function somewhat as exhibited in Fig. 3. As we

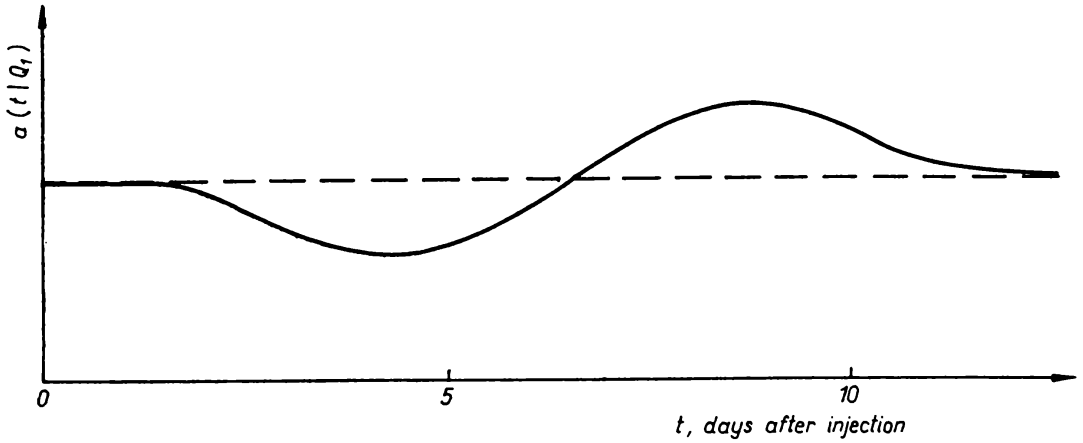

Fig. 3. Hypothetical fluctuations of the number of carcinogen-sensitive lung cells after injection of urethan

shall show in the following sections this conjecture provides a heuristic explanation to most of the experimental findings that have been reported in a number of independent experiments by Shimkin et al. [13], White [14], and White et al. [15] and [16]. Nevertheless it ought to be viewed with a substantial dose of scepticism. Additional experimental evidence is needed to clarify the situation.

No conjectures will be made here with regard to $a(t|Q)$ for other time patterns Q. Intuitively it is clear that the depressions and the increases which one expects to follow after successive injections of urethan may have a rather complicated form. It is also hoped that further experiments will provide the needed information to estimate $a(t|Q)$ for arbitrary Q's.

Remark 5. It would be more realistic to assume that, given a dose schedule Q, to every phase of the cell cycle there corresponds a probabil-

ity that the cell will change its properties (perhaps, caused by contact with carcinogenic compounds) and start the tumorigenic process. The postulated simplification was accepted in view of its mathematical simplicity. Also it is difficult to visualize a method of estimating the probabilities in question.

Remark 6. Note that the mathematical developments will not change if $a(t|Q)$ is treated simply as the coefficient in the postulated relation (1).

8. Estimation of internal exposures. In this section we analyze data resulting from exposures of animals to urethan administered in single doses only.

If it is assumed that, say, for $T > T_0$, the expected value $\mathrm{E}q(T|Q_1)$ does not depend upon the administered dose D of urethan, then the expected numbers of initial events

$$I(D_i) = e\mathrm{E}\int_0^\infty a(t|Q_{1i})x(t|Q_{1i})\,dt, \qquad i = 1, \ldots, m,$$

where $Q_{1i} = Q(D_i; 1; 0)$, can be estimated (up to a scale factor only). In view of (5) the expected numbers of initial events $I(D_1), \ldots, I(D_m)$ can be estimated by $\bar{y}_1, \ldots, \bar{y}_m$, where $\bar{y}_i$, $i = 1, \ldots, m$, is the number of tumors per mouse resulting from the application of dose D_i, provided that all animals are sacrificed at the same time $T > T_0$ after injection.

If, for every i, animals injected with dose D_i are sacrificed, say, at $T_1, \ldots, T_n$ days after injection, where $T_i > T_0$, $i = 1, \ldots, n$, then it is natural, in view of (5), to take as the estimates of $I(D_1), \ldots, I(D_m)$ those numbers $u_1, \ldots, u_m$, respectively, that minimize the expression

$$\min_{(v_1,\ldots,v_n)} \sum_{i=1}^{m}\sum_{j=1}^{n} (\bar{y}_{ij} - u_i v_j)^2, \tag{13}$$

where $\bar{y}_{ij}$, $i = 1, \ldots, m$, $j = 1, \ldots, n$, is the mean number of tumors per mouse resulting from the application of dose D_i and counted at time T_j.

In order to find the minimizers of (13) we need to differentiate the sum of squares with respect to $u_1, \ldots, u_m$, $v_1, \ldots, v_n$. This leads to the following system of non-linear equations

$$Yu = \Big(\sum_{i=1}^{m} u_i^2\Big) v, \qquad Y'v = \Big(\sum_{j=1}^{n} v_j^2\Big) u,$$

where $u = (u_1, \ldots, u_m)'$ and $v = (v_1, \ldots, v_n)'$, while $Y = (\bar{y}_{ij})$. Eliminating $v_1, \ldots, v_m$ leads to

$$Y'Yu = \Big(\sum_{i=1}^{m} u_i^2 \sum_{j=1}^{n} v_j^2\Big) u \quad \text{or} \quad (Y'Y - \gamma I)u = 0,$$

where I stands for the identity matrix.

Clearly, the estimates of $I(D_1), \ldots, I(D_m)$ we are looking for are the coordinates $(u_1, u_2, \ldots, u_m)$ of the eigenvector corresponding to the largest characteristic root of $|Y'Y - \gamma I| = 0$. In case all $\bar{y}$'s are positive, the estimates of $I(D_1), \ldots, I(D_m)$ so obtained are all of the same sign and, obviously, it can be selected to be the plus sign. Further results regarding this procedure of estimation will be reported elsewhere (see also [1] and [4]).

If the elimination of urethan from the animal bodies is rapid so that formula (6) is applicable, then, in view of (5) and (6), the expected number of initial events is approximately proportional to the expected internal exposure. In consequence, the estimates calculated as described above are estimates (again up to a scale factor only) of the expected internal exposures $J(D_1), \ldots, J(D_m)$.

Remark 7. Analysis of data from [16] indicates that T_0 must be larger than $T_1 = 15$ (weeks). This is concluded from the fact that for $T = 11$ and 12 (weeks) the ratio $\bar{y}(T|Q_2)/\bar{y}(T|Q_1)$, where $Q_1 = Q(0.5; 1; 0)$ and $Q_2 = Q(1.0; 0; 1)$, is systematically larger than it is for $T \geqslant 15$ (weeks). This finding contradicts a conjecture of Arley [2] that once a tumor has been initiated the growth rate and other observable characteristics of any tumor are independent both of the nature and the quantity of the applied carcinogen.

Remark 8. Since it is likely that $\mathrm{E}q(T|Q)$ is a non-decreasing function of T and that $\lim \mathrm{E}q(T|Q_1) = \lim \mathrm{E}q(T|Q_2)$ as T converges to infinity, while Q_1 and Q_2 are defined as in Remark 7, one is willing in view of the findings mentioned in Remark 7 to conjecture that $\mathrm{E}q(T|Q_1) < \mathrm{E}q(T|Q_2)$ for $11 < T < 16$ (weeks). In fact, this coincides with the conclusion reached in Remark 4. Biologically, the inequality conjectured is plausible. For example, there might be a defence mechanism which suppresses the growth rate of tumors for a period of time while they are small, whereas the activity of the defence mechanism lessens when the administered dose of urethan increases. In connection with this it might be interesting to mention a conclusion reached by Ribacchi and Giraldo [11] that transformed cells with high antigenic responsiveness either do not become or need a longer time to become macroscopically visible, while cells with a low antigenic responsiveness need a shorter time.

Using the procedure described here we calculated six sets of estimates, each of them corresponding to different experiments. They are presented in Table 3. The first set was calculated by using data reported by Shimkin et al. [13], the next four — by using counts of White [15] and [16], and the sixth set presented in Table 3 — by using unpublished counts of M. White. These mice were A/Jax, from 10 to 12 weeks of age at the time of the application and were sacrificed at 20, 24 and 28 weeks after injection.

TABLE 3

	Number of initial events (estimates)						Internal exposure (estimates)
Experiment	1	2	3	4	5	6	7
Strain of mice	A/J	A/Jax	A/Jax	A/Jax	A/Jackson	A/Jax	A/Jax
Number of mice	110	120	120	120	200	120	80
Age (weeks) at injection / Dose (mg/gm)	4-6	4.5	6.5	8.5	8.5-10.5	10-12	10-11
.125					.23	.21	.20
.250	.46				.40	.45	.44
.500	1.00	1.00	1.00	1.00	1.00	1.00	1.00
.750						1.82	1.73
1.000	1.80	2.07	2.26	2.38	2.43	2.51	2.46

Data used in experiment 1 are from [11], in experiments 2, 3 and 4 — from [16], in experiment 5 — from [15], in experiment 6 — from [7]. The last column gives the estimated internal exposure (mg-hrs/g; 24 hrs.) from [14].

Injections of single doses of 0.25, 0.5, 0.75 or 1.0 mg/gm of urethan were given. The number of tumors counted are presented in Table 4.

In the last column of Table 3 the experimental values of internal exposures obtained by White [14] from direct measurements of the catabolized and excreted urethan are presented. In each case, the estimate corresponding to 0.5 mg/gm of urethan has been set equal to 1.0. With such a normalization, the sets of estimates are easily compared with each other.

TABLE 4. Mean numbers of tumors per mouse

Dose (mg/gm) (given as one injection)	Time of sacrifice (weeks after injection)		
	20	24	28
.25	4.9	5.8	6.3
.50	10.0	12.8	14.6
.75	19.9	22.4	25.7
1.00	25.5	33.3	31.4

Data from [7].

Table 3 shows that estimates of the corresponding expected numbers of the initial events in experiments 5 and 6 are close to each other despite the fact that in experiment 5 a dose of 1.0 mg/gm yielded approximately 21 tumors per mouse whereas in experiment 6 a dose of 1.0 mg/gm yielded approximately 33 tumors per mouse.

At the first glance, one would be tempted to explain this agreement by postulating that the sensivity of the mice changes from one experiment to the next, and this would be in agreement with formula (2). However, as we shall see later, data of tumors resulting from two applications indicate that within the framework of the above-adopted assumptions this explanation must be rejected. Another explanation, which does not contradict the data, is as follows. It is based on two assumptions which we hope to be, at least approximately, satisfied. The first assumption is that the relation of the integral $I(D)$ to dose D is of the form $I(D) = mD^n$, where m can vary from one experiment to the next, while n is a constant dependent possibly upon the age of the animals. Table 5

TABLE 5. Number of initial events (up to a scale factor). Comparison of observation and least square fit

Number of initial events				
Experiment	5		6	
Dose (mg/gm) (given as one injection)	observed	computed $m = 2.28$ $n = 1.15$	observed	computed $m = 2.40$ $n = 1.20$
.125	.23	.20	.20	.19
.250	.40	.46	.44	.45
.500	1.00	1.02	1.00	1.04
.750			1.73	1.70
1.000	2.43	2.28	2.46	2.41

Least square fits of $I(D) = mD^n$ to data from [7] and [15]. Data used in experiment 5 are from [15] and in experiment 6 – from [7].

shows how well $I(D) = mD^n$ fits to the data of experiments 5 and 6. The second assumption is that the carcinogenicity of the unit dose of urethan can vary from one experiment to the next. This might be due to errors in measurements and/or due to different carcinogenicities of the urethan which was used. If this be true, then, whatever be $0 < \varkappa_1 < \ldots < \varkappa_m < 1$, it follows that the two sets $I(\varkappa_1 D_A), \ldots, I(\varkappa_m D_A)$ and $I(\varkappa_1 D_B), \ldots, I(\varkappa_m D_B)$ differ by a scale factor only. Here D_A and D_B stand for the unit doses in experiments 5 and 6, respectively.

We adopt this heuristic explanation in further considerations.

Before we proceed to the discussion of the implications of Table 3 we quote some experimental findings from the literature. White [14]

reported that there seems to be a linear relation of the number of tumors to the internal exposure for small doses. The linearity was reconfirmed by Klonecki and White [7] (see also [8]) in two additional independent experiments. White [14] reported that if the dose is large, then there are less tumors than expected under proportionality to the internal exposure.

Remark 9. It is interesting to note that the internal exposures reported by White [14] (see also [5]) are approximately equal to the corresponding estimated expected numbers of initial events. For mice that are 8, or more, weeks old, the best fit gives ethyl (-1-^{14}C) (carbamate) corresponding to 24 hours. However, contrary to what one expects, the internal exposures corresponding to 48 hours differ more from the estimated expected numbers of initial events than the ones corresponding to 24 hours. This might be due to random fluctuations, since each estimate corresponding to 48 hours is based on one experiment (4 mice),

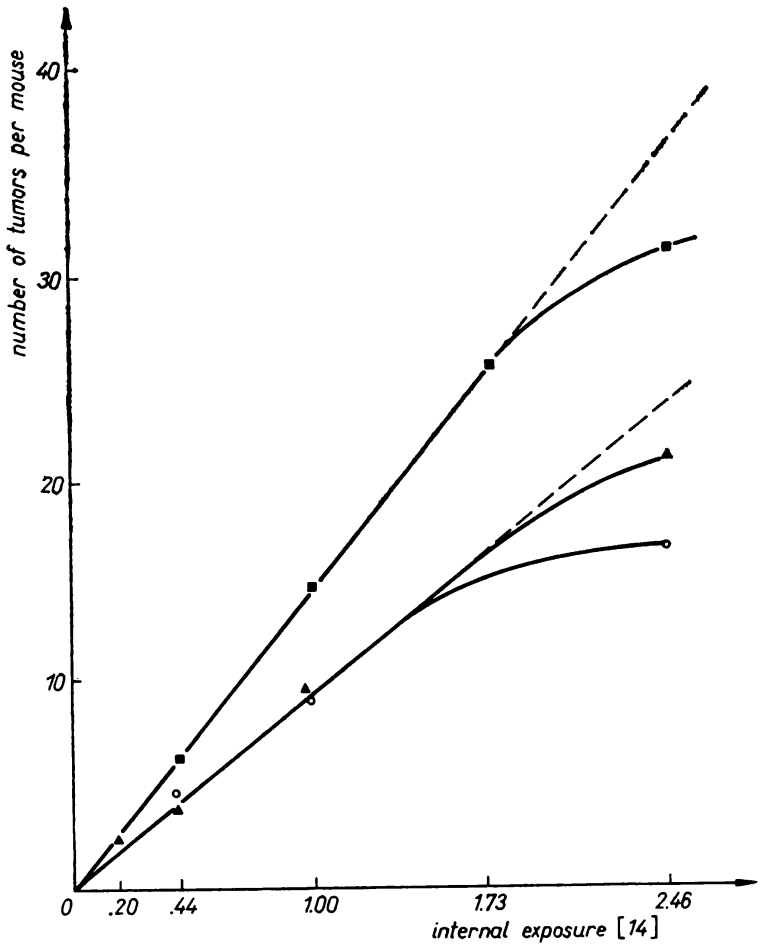

Fig. 4

Data: ■ – White [7], ▲ – White et al. [15], ○ – Shimkin et al. [13]

whereas each estimate corresponding to 24 hours is based on four or five experiments. In case this would be confirmed in more extensive experiments, is it an indication that the active carcinogen is a component not appearing in the animal bodies after 24 hours following the injection? Are the components into which carbon enters after 24 hours not carcinogenic?

Table 3 confirms that, for all age groups considered and for doses up to 0.5 mg/gm of urethan, the number of tumors is linearly related to the internal exposures which are presented in the last column of this table. Table 3 also confirms that for a dose of 1.0 mg/gm of urethan there are less tumors than it would be expected under proportionality for mice from 4 to 10.5 weeks of age at injection (see Fig. 4). Moreover, Table 3 shows that the younger the mice the larger are these deviations (relative to the expected number of tumors yielded by 0.5 mg/gm of urethan) from that what ought to be expected under proportionality. The relation of this deviation against age is exhibited in Fig. 5. The curve is drawn by hand.

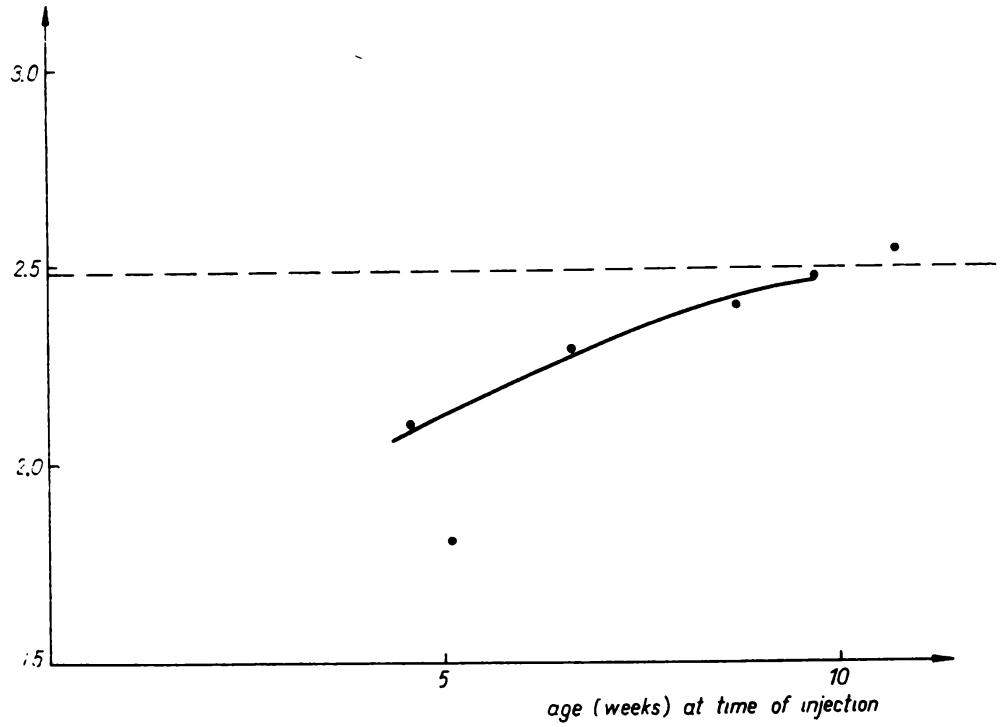

Fig. 5. Estimated proportion of the expected number of initial events induced by 1.0 mg/gm of urethan to the expected number of initial events induced by .5 mg/gm of urethan

Data from [7], [13], [15] and [16]

Remark 10. If it were not for experiment 6, one would be tempted to extend the curve in Fig. 5 to the right and to think about the horizontal line at level 2.46 as its asymptote, which is the value of the internal expo-

sure corresponding to 1.0 mg/gm of urethan as reported in [14]. It is hoped that further experimentation will provide the needed information regarding both the internal exposure and the number of tumors for mice 12 or more weeks old at the time of injection.

If animals of ages from 4.5 to 12 weeks eliminate urethan at the same rate, then within the framework of the adopted assumptions the explanation of the findings of Table 3 is straightforward. The model becomes consistent with the findings if we assume that $a(t|Q_1)$ depends, in addition to t and D, upon age, somewhat as exhibited in Fig. 6. If, however, animals of different ages eliminate urethan at different rates, then the functional relations are expected to be more complex.

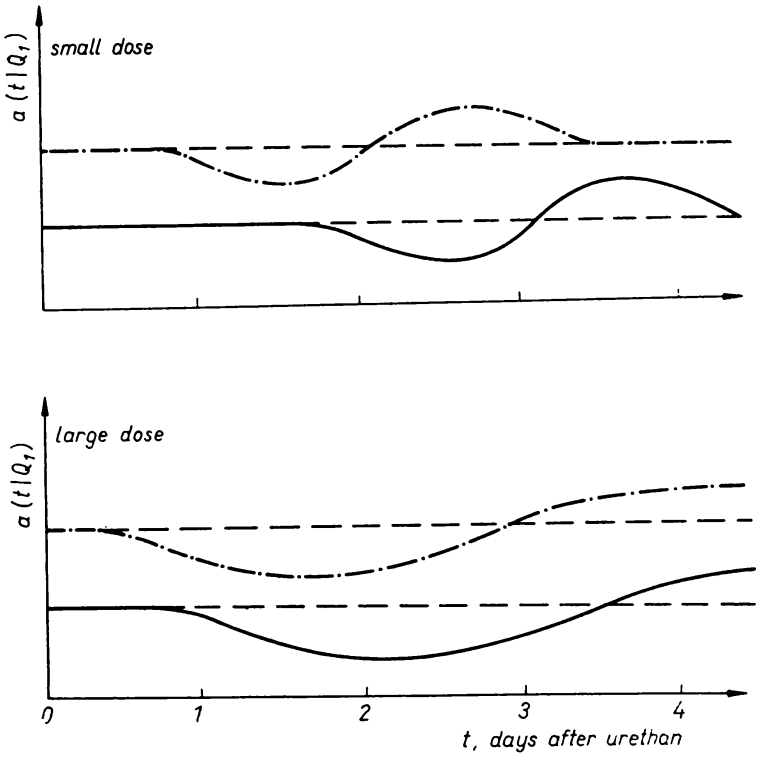

Fig. 6. Hypothetical fluctuations of the number of carcinogen-sensitive lung cells after injection of urethan
-·-·-·- young animals, ——— adult animals

Clearly, this explanation ought to be treated only tentatively. It is hoped, however, that these hypothetical interpretations will be tested by further investigations.

9. Confrontation of the model with data resulting from two injections. The intention of this section is to show that within the framework of the adopted assumptions the one-branching model of carcinogenesis is consistent with counts of tumors resulting from two successive injections. In particular, we want to show that with an appropriate selection of

functions $a(t|Q_1)$ as described in Section 7 a heuristic explanation can be provided to the experimental findings now available.

The data from a number of independent experiments are exhibited in Fig. 7. Every point represents one particular experiment. The first coordinate gives the number of visible tumors per mouse resulting from dose D mg/gm of urethan given in one injection, and the second coordi-

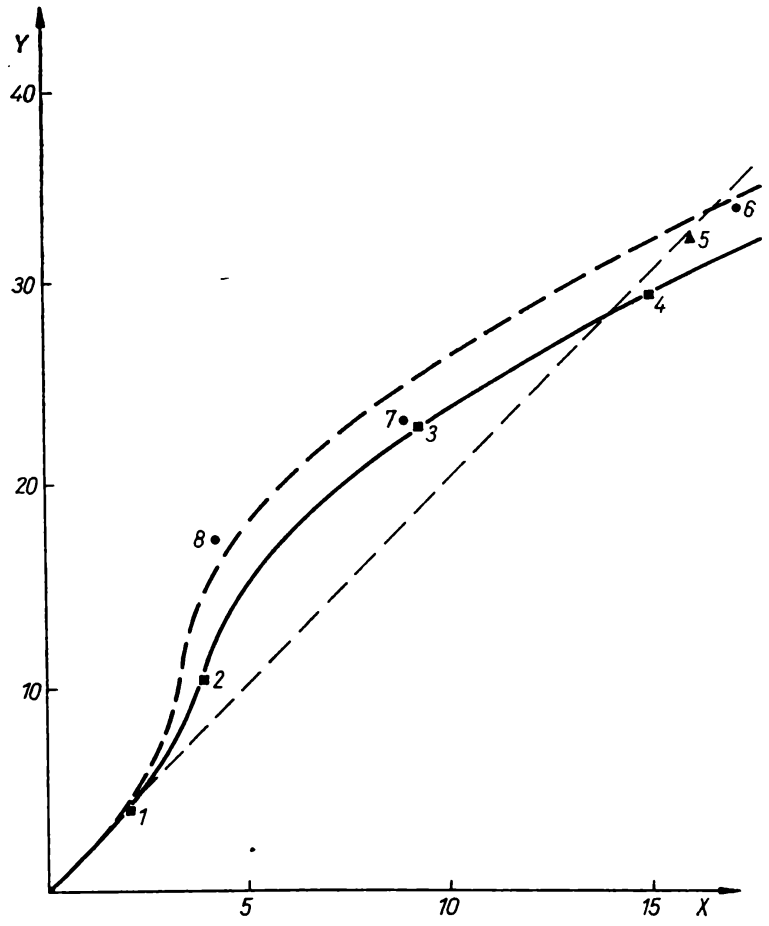

Fig. 7. X — number of tumors per mouse resulting from D mg/gm of urethan given as one injection; Y — number of tumors per mouse resulting from $2D$ mg/gm of urethan given in two injections 6 or 7 days apart

Data: *1, 2, 3* — White et al. [15], *4, 5, 6* — White et al. [16], *7, 8* — Shimkin et al. [13]. Age at the first injection: ● — 4-5 weeks, ▲ — 6.5 weeks, ■ — 8.5-10.5 weeks

nate gives the number of visible tumors per mouse resulting from dose $2D$ mg/gm of urethan given in two injections 6 or 7 days apart. The time of sacrifice after injection varies from one experiment to the next but it is always above 22 weeks. For example, the point (9.1; 23.4) represents the experiment reported by Shimkin et al. [13] in which 0.5 mg/gm urethan yielded 9.1 tumors, and 1.0 mg/gm of urethan injected in two subdoses 6 days apart yielded 23.4 tumors.

A glance at Fig. 7 suggests the following. Let $Q_1 = Q(D; 1; 0)$ and $Q_2 = Q(2D; 2; 0, t_1)$, where $t_1 > 0$. Assume that $t_1 = 6$ (days) or $t_1 = 7$ (days) and that $T > 22$ (weeks). Then, for every age group, there exists a number y_0 such that if $\mathrm{E}y(T|Q_1) \leqslant y_0$, then

$$\mathrm{E}y(T|Q_2) \geqslant 2\mathrm{E}y(T|Q_1),$$

and if $\mathrm{E}y(T|Q_1) \geqslant y_0$, then

$$\mathrm{E}y(T|Q_2) \leqslant 2\mathrm{E}y(T|Q_1).$$

Moreover, the younger the mice the larger is y_0.

Clearly, these suggestions should be verified in further experimentation. However, it is interesting that they lead to a conjecture that functions $a(t|Q_1)$ may depend upon dose D somewhat as exhibited in Fig. 8.

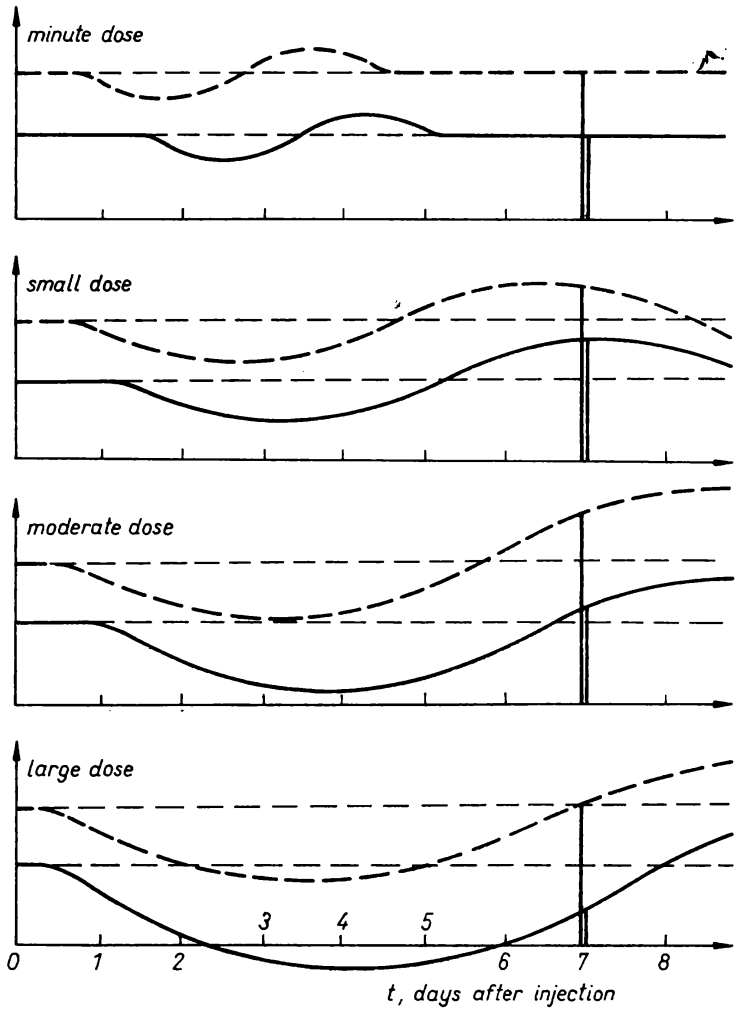

Fig. 8. Hypothetical fluctuations of the number of carcinogen-sensitive lung cells after injection of urethan

– – – young animals, ——— adult animals

These plots harmonize qualitatively with Fig. 7 as well as with the assertions of Proposition 2 and the conjectures regarding $a(t|Q_1)$ stated in Section 7. However, in addition to the consequences that harmonize with the empirical findings, Fig. 8 suggests a number of hypotheses that can be tested in further experimentation. Two of them will be formulated in the next section.

10. Hypotheses implied by the one-branching model of carcinogenesis. One of the consequences of the above-given speculations, not yet observed empirically, is a change of the negative effect of fractionation to a positive one when the time interval between injections is gradually increased. Up to date, all data we have seen reported are from experiments in which subdoses were injected 2, 6, 7 and more days apart, and with such a spacing this effect could not be noticed. As it is seen from Fig. 8 one can expect to observe the predicted changes under the following experimental conditions.

If the individual dose D is selected so that $\mathrm{E}y(T|Q_1) \simeq 9$ at $T = 24$ weeks after the first injection, then the applications of the urethan should be made 3, 5, 7, 9 and 11 days apart. The mice should be of the A/Jax strain, 4-6 weeks old at the time of the first injection, and sacrificed 22-28 weeks after the first injection. According to the predictions, the relation of the number of tumors per mouse to the time interval between injections should be convex with the maximum at spacing of 6-7 days, independently of the time at which the tumors are counted.

An experiment with parameters as described above should duplicate the Gubareff experiment (see Shimkin et al. [13]) in which a substantive increase in the number of tumors due to division of a single dose into two equal subdoses was noted.

In connection with this it might be interesting to mention that up to now the effect observed by Gubareff could not be duplicated despite the fact that a number of experiments especially designed to reproduce it has been performed [16]. The theory developed in this paper indicates that some of those experiments may have failed, since the potency of the injected dose was too large. Moreover, the theory indicates that in order to observe an increase in the number of tumors due to fractionation of a dose of such a carcinogenicity as the one reported in [16] the time interval between injections should be greater than 6 days, perhaps about 12 days.

Remark 11. In case these predictions are not confirmed, this may be an indication that the internal exposures are not additive for the proposed spacings and/or the period of $a(t|Q_1)$ is not as conjectured (see Fig. 8).

A promise for verifying the crucial conjectures regarding $a(t|Q_1)$ offer experiments of the kind as reported by Shimkin et al. [12] and con-

sisting of exposures of experimental animals to urethan and to some antimetabolic compounds that block the cell cycle in different phases. If there is a carcinogen-sensitive phase in the life cycle of lung cells, then the ultimate number of tumors should depend upon whether first urethan or first the compound that blocks the cell cycle is administered.

Remark 12. Mathematical models of real phenomena are based on simplifying assumptions. In our case this may be a serious threat, since the true mechanism of urethan carcinogenesis is beyond doubt a tremendously complex phenomenon. At present, however, a mathematical model of carcinogenesis may be a helpful guide for further experimentation. With reference to the model described in this paper, it was our guiding thought to suggest hypothetical interpretation of the data that can be tested by further investigation.

It is hoped that the present paper will be followed by further experimentation on urethan carcinogenesis, and that on the base of the new experimental evidence new mathematical models of urethan carcinogenesis, better than the ones known to date, will be constructed.

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NOWY MODEL MATEMATYCZNY MECHANIZMU POWSTAWANIA GUZÓW RAKOWYCH WYWOŁANYCH URETANEM W PŁUCACH MYSZY

STRESZCZENIE

Przedstawiony w pracy model mechanizmu powstawania guzów rakowych w płucach myszy jest jednogałązkowy. Istotnie nowym elementem, jaki w tej pracy został wprowadzony, jest uwzględnienie możliwości, że liczba komórek, które zdolne są do zapoczątkowania procesu powstawania guza, może się zmieniać po podaniu środka rakotwórczego. W poprzednich pracach, poświęconych temu problemowi, zakładano, że liczba tych komórek jest niezmienna w czasie.

Za pomocą zaproponowanego modelu analizuje się w pracy wyniki pewnych opublikowanych doświadczeń. Analiza ta prowadzi do wykrycia kilku nowych regularności w dostępnym w literaturze materiale eksperymentalnym, m. in. do stwierdzenia, że w przypadku pojedynczych doz poniżej 0.5 mg/gm uretanu średnia liczba

raków jest proporcjonalna do tzw. wewnętrznej ekspozycji na uretan, a dla doz powyżej 1.0 mg/gm uretanu obserwuje się systematycznie mniej guzów niż wynikałoby to z proporcjonalności do wewnętrznej ekspozycji. Ponadto analiza wyników eksperymentalnych prowadzi do wniosku, że po wprowadzeniu uretanu do organizmu liczba komórek zdolnych do mutacji początkowo maleje, a zanim ustabilizuje się ponownie na poziomie wyjściowym — wyraźnie wzrasta powyżej tego poziomu. To stwierdzenie tłumaczy znany z wielu obserwacji fakt, że dzielenie jednej dozy na dwie może prowadzić zarówno do zwiększenia, jak i do zmniejszenia liczby guzów rakowych powstałych w płucach myszy.