Mathematical Modeling of Comparative Initiation/Promotion Skin Paint Studies of B6C3F$_1$ Mice and Swiss CD-1 Mice.

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Abstract

In this study we mathematically describe a cancer initiation/continuous promotion mechanism and estimate the related biological parameters. The data on Swiss CD-1 and B6C3F$_1$ mice was collected by NTP(Design A, 1994). These mice were initiated with DMBA and then promoted with TPA on a weekly basis. By varying the dosage of DMBA and the type of mice, we analyze four different subsets of the original data. This study identifies a working model to describe the mutation of normal cells to papillomas, then the final mutation of papillomas into carcinomas for each of the subsets. Our model assumes that there are multiple stages from initiation to papilloma. For each stage of the mutation, we assume any single cell will either mutate or not. Therefore, the underlying probability distribution of the number of papillomas at the initiated stage is binomial. For similar reasons, at the final stage after promotion, the probability distribution of the number of carcinomas is also binomial. We try to ascertain a general model, which would account for the data from all four groups. Finally, we compare the cell birth rate for the papilloma model between two strains of mice for the same dosage of DMBA. We also compare the birth rates for different dosage of DMBA within each strain of mice.

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1 Introduction

An initiation/continuous promotion study typically involves a single sub-threshold application of a carcinogen substance, followed by repeated applications of a non-carcinogen substance. This type of study is usually conducted on mice because they are a far more responsive model for skin initiation/promotion studies than other rodent species. At the same time, not all strains of mice are equally sensitive to the initiation/promotion protocol [2].

The data for this paper have been obtained from a one-year study conducted by NTP (National Toxicology Program, Study Design A in 1994). In that study, groups of 30 male and 30 female mice were administered 7,12-dimethylbenz(a) anthracene (DMBA) as an initiator treatment in the first week of the 52-week study period, followed by weekly application of 12-O-tetradecanoyl-phorbol-13-acetate(TPA) as a promoter treatment for the remaining 51 weeks. Different doses of DMBA in combination with different doses of TPA were used for three different strains of mice. For the purpose of our study, however, we use only the data on two different strains (Swiss CD-1 and B6C3F$_1$) of mice. We compare the sensitivity of Swiss CD-1 and B6C3F$_1$ mice strains in terms of the number of papillomas. We also compare different doses of DMBA (2.5 and 25.0μg). Each group has the same repeated typical application of TPA (5 μg) Therefore, in our study, we have the following four groups:

Swiss CD-1	DMBA:	2.5μg	TPA:	5 μg
Swiss CD-1	DMBA:	25.0μg	TPA:	5 μg
B6C3F$_1$	DMBA:	2.5μg	TPA:	5 μg
B6C3F$_1$	DMBA:	25.0μg	TPA:	5 μg

Consistency of the data was maintained by the standard method of recording clinical observations, whereby the appearance and progression of any tumor development on the skin were recorded. When a skin tumor first appeared, it was considered a tissue mass, until it became at least 1 mm in diameter and had been present for 14 days. Then, the tissue mass was considered a papilloma. Furthermore, when a papilloma became necrotic in appearance and was attached to the underlying tissue, it was recorded as a carcinoma. In addition, microscopic evaluations were carried out to confirm the state of carcinoma.

2 The Model

The design of our model is two-fold, incorporating growth of papilloma and then carcinoma. First, we focus on modeling the growth of a papilloma (see Figure 1) and then substitute the papilloma model into the overall model of the probablility of a normal cell forming a carcinoma. In Figure 2, the papilloma stage is represented by "Initiated Cells." The meanings of the parameters of the model are explained below.

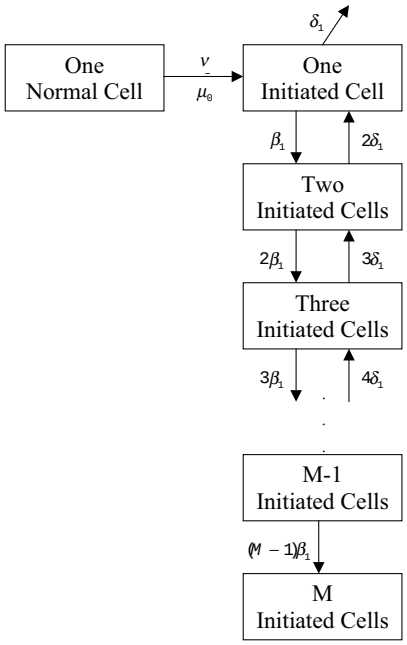

Figure 1: Model of a Normal Cell Generating a Papilloma.

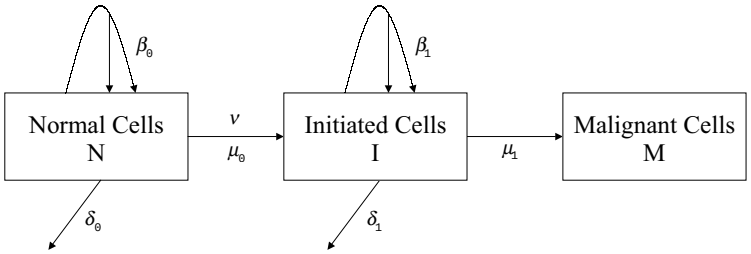

Figure 2: Model of a Normal Cell Generating a Carcinoma.

β_0: the birth rate of a normal cell (set equal to 0)
δ_0: the death rate of a normal cell (set equal to 0)
β_1: the birth rate of an initiated cell
δ_1: the death rate of an initiated cell
μ_0: the mutation rate of a cell from the normal to initiated state
μ_1: the mutation rate of a cell from the initiated state to malignant state
ν: the instantaneous mutation rate from the normal to initiated state
m: the number of the normal cells at the beginning for each animal
M: the minimal number of initiated cells needed to comprise a detectable papilloma

Let I_i represent the ith initiated stage; i.e., the stage in papilloma development with i initiated cells present, and let $Q_{iM}(s,t)$ represent the probability that a unit at stage I_i at time $t-s$ will not reach the stage I_M before the time t.

We now describe mathematically the model for the papilloma data (see Figure 1). We make the following assumptions:

Assumption 1: Initiated cells follow a linear birth-death process with constant rates.

Assumption 2: The minimal number of initiated cells, M, is large enough that we may ignore stages after I_M.

Assumption 3: One normal cell yields at most one papilloma.

Under these assumptions the papilloma stage of our two-stage model can be described by the following system of differential equations with initial conditions (see Appendix for derivations):

$$\begin{aligned}
\frac{dQ_{0M}(s,t)}{ds} &= -Q_{0M}(s,t)\mu_0 + Q_{1M}(s,t)\mu_0 && (1)\\
\frac{dQ_{1M}(s,t)}{ds} &= Q_{2M}(s,t)\beta_1 - Q_{1M}(s,t)(\beta_1+\delta_1) + \delta_1 && (2)\\
\frac{dQ_{iM}(s,t)}{ds} &= iQ_{i+1,M}(s,t)\beta_1 - iQ_{iM}(s,t)(\beta_1+\delta_1) + iQ_{i-1,M}(s,t)\delta_1 && (3)\\
Q_{MM}(s,t) &= 0, \qquad \forall s,t && (4)\\
Q_{iM}(0,t) &= 1, \qquad i = 0,1,\ldots,M-1. && (5)
\end{aligned}$$

The system (2) – (5) can be solved analytically [8], and the solution can be written as

$$Q_{1M}(s,t) = \begin{cases} 1 - \dfrac{(\beta_1-\delta_1)\beta_1^{M-1}\left(1-e^{-(\beta_1-\delta_1)s}\right)^{M-1}}{\left[\beta_1 - \delta_1 e^{-(\beta_1-\delta_1)s}\right]^M}, & \delta_1 \neq \beta_1 \\ 1 - \dfrac{(\beta_1 s)^{M-1}}{(1+\beta_1 s)^M}, & \delta_1 = \beta_1 \end{cases}$$

Now we use equation (1) to solve for $Q_{0M}(s,t)$.

Figure 1 illustrates that, when starting from m normal cells, the number of papillomas has a binomial distribution since each normal cell can either evolve into a papilloma or not. Now consider the incidence of papilloma over time.

$$\begin{aligned}
P_{NP}(t) &\doteq P(\text{1 normal cell reaching stage } I_M \text{ (papilloma) before } t, \text{ starting at time 0})\\
&= P(\text{1 normal cell} \to \text{papilloma before } t \,|\, \text{no mutation at } t=0)\\
&\qquad \times P(\text{no mutation at time 0})\\
&+ P(\text{1 normal cell} \to \text{papilloma before } t \,|\, \text{mutation at } t=0) \times P(\text{mutation at time 0})\\
&= [1-Q_{0M}(t,t)](1-\nu) + [1-Q_{1M}(t,t)]\nu
\end{aligned}$$

For a particular group and a particular mouse, let $X(t)$ be the number of papilloma before t, starting with m normal cells at time 0. Then

$$P[X(t)=x] = \binom{m}{x}(P_{NP}(t))^x(1-P_{NP}(t))^{m-x}, \qquad x = 0,1,2,...,m,$$

and

$$\mathrm{E}[X(t)] = mP_{NP}(t) = m([1 - Q_{0M}(t,t)](1-\nu) + [1 - Q_{1M}(t,t)]\nu).$$

For the carcinoma incidence analysis, we have to treat the two-stage model as one system (see Figure 2). To describe the system, two ordinary differential equations are needed. Let $P_{02}(s,t)$ denote the probability of one normal cell not reaching carcinoma before time t, starting at time $t-s$ and $P_{12}(s,t)$ the probability of one initiated cell not reaching carcinoma before time t, starting at time $t-s$. Then

$$\begin{aligned}
\frac{dP_{02}(s,t)}{ds} &= \beta_0 P_{02}(s,t)^2 + \delta_0 + \mu_0 P_{12}(s,t) - (\beta_0 + \delta_0 + \mu_0) P_{02}(s,t) \\
\frac{dP_{12}(s,t)}{ds} &= \beta_1 P_{12}(s,t)^2 + \delta_1 + \mu_1 P_{22}(s,t) - (\beta_1 + \delta_1 + \mu_1) P_{12}(s,t)
\end{aligned}$$

Similarly to the papilloma stage, we have several conditions:

$$\beta_0 = \delta_0 = 0, \quad P_{02}(0,t) = P_{12}(0,t) = 1, \quad P_{22}(s,t) = 0.$$

After simplification, we obtain

$$\begin{aligned}
\frac{dP_{02}(s,t)}{ds} &= \mu_0 P_{12}(s,t) - \mu_0 P_{02}(s,t) \\
\frac{dP_{12}(s,t)}{ds} &= \beta_1 P_{12}(s,t)^2 + \delta_1 - (\beta_1 + \delta_1 + \mu_1) P_{12}(s,t).
\end{aligned}$$

For a certain group, a certain mouse, consider the random variable $Y(t)$ define by $Y(t) = 1$, if carcinoma is detected before time t starting from m normal cells at time 0; $Y(t) = 0$, otherwise. By the definition of $P_{12}(t,t), P_{02}(t,t)$ and ν, we know that the probability of one normal cell not reaching carcinoma before time t, starting from time 0 is $\nu P_{12}(t,t) + (1-\nu)P_{02}(t,t)$. So $P[Y(t)=1] = 1 - [\nu P_{12}(t,t) + (1-\nu)P_{02}(t,t)]^m$, since we assume m normal cells act independently.

We now use the method of maximum likelihood to derive estimators for the parameters of our model.

1. The Papilloma Stage

Let x_{ijk} represent the number of papillomas for the $i-$th animal in the $j-$th experimental group at the time k. The likelihood function for the number of papillomas can then be expressed as

$$L_1 = \prod_i \prod_j \prod_k P\left[X(t) = x_{ijk}\right]. \tag{6}$$

Taking the natural logarithm of (6) yields

$$\ln L_1 = \sum_i \sum_j \sum_k \ln \left[\binom{m}{x_{ijk}} P_{NP}^{x_{ijk}} (1 - P_{NP})^{(m - x_{ijk})} \right]$$

Since we are maximizing L_1, or equivalently $\ln(L_1)$, with respect to P_{NP}, the constant term can be ignored, leaving

$$\sum_i \sum_j \sum_k [x_{ijk} \ln P_{NP} + (m - x_{ijk}) \log(1 - P_{NP})].$$

2. The Carcinoma Stage

Let y_{jk} represent the number of malignant tumors in the $j-$th experimental group at the time k. The likelihood function is then defined as

$$L_2 = \prod_j \prod_k P\left[Y(t) = y_{jk}\right]$$

having corresponding natural logarithm

$$\ln L_2 = \sum_j \sum_k \ln P\left[Y(t) = y_{jk}\right]$$

To attain estimators $\{\hat{\beta}_1, \hat{\delta}_1, \hat{\mu}_0, \hat{\mu}_1, \hat{\nu}\}$, the function $\ln L_1 + \ln L_2$ is maximized over all possible values of $\{\beta_1, \delta_1, \mu_0, \mu_1, \nu\}$.

After achieving the optimally estimated parameters, we can calculate the incidence of papilloma $P_{NP}(t)$, expected number of papilloma $E[X(t)]$, the incidence of carcinoma $1 - P_{02}(t,t)^m$, etc. Then we can compare the difference of all these values among different groups.(i.e. different initiators, promotors, doses, strains, etc.)

3. Likelihood Ratio Test

The likelihood ratio test statistic is used for testing the null-hypothesis $H_0 : \theta \in \Theta_0$ versus $H_1 : \theta \in \Theta_0^c$. The corresponding statistic is

$$\lambda(x) = \frac{L(\hat{\theta_0} \mid x)}{L(\hat{\theta} \mid x)}$$

where x is the data, $\hat{\theta}_0 = \hat{\theta}_0(x)$ is obtained by maximizing $L(\theta|x)$ over the parameter subspace Θ_0 and $\hat{\theta} = \hat{\theta}(x)$ is obtained by maximizing $L(\theta|x)$ over the whole parameter space Θ.

The asymtotic distribution of the statistic $-2\log\lambda(x)$ is a χ^2 distribution with degrees of freedom being the difference in number of parameters of the two hypotheses.[1].

We apply this theory to test the hypothesis that the cell birth rates between the two strains of mice in our study are equal. Thus for our problem

$$H_0 : \beta_1^s = \beta_1^b, \quad H_1 : H_0 \text{ is not true,}$$

where the superscript s stands for Swiss CD-1 and b for B6C3F$_1$. These hypotheses yield $\Theta_0 = \{\beta_1, \delta_1^s, \mu_0^s, \nu^s, \delta_1^b, \mu_0^b, \nu^b\}$, and $\Theta = \{\beta_1^s, \delta_1^s, \mu_0^s, \nu^s, \beta_1^b, \delta_1^b, \mu_0^b, \nu^b\}$, and therefore

$$\log\lambda(x) = \left[\log L(\hat{\theta_0}|x) - \log L(\hat{\theta}|x)\right].$$

The value of $-2\log\lambda(x)$ is compared with $\chi^2(1)$. At 0.05 level of significance, the null hypothesis will be rejected if $-2\log\lambda(x) > \chi^2(1) = 3.84$. In this case, the conclusion will be that the birth rates of initiated cells are not same for the two different strains of mice. The results are summarized in Section 3.

3 Results

The method of maximum likelihood method was used to obtain expressions for estimators of the biological parameters of the initiation/promotion model of skin cancer. The initial values for the parameters were obtained from the work of Kopp-Schneider and C.J. Portier [4]. In their work, they found that the cell-cycle time of an initiated cell with promotion is 20 hours. Since our data are the numbers of papillomas per week for each mouse, the initial values for the biological parameters of an initiated cell with promotion translate into:

$\beta_0 = 10$ births/week
$\delta_0 = 10$ deaths/week
$\mu_0 = 1$ mutation/week
$\nu_1 =$ probability of instantaneous mutation
$\beta_1 = 10$ births/week
$\delta_1 = 10$ deaths/week
$\mu_1 = 1$ mutation/week

Table 1: B6C3F$_1$ Mice.

	2.5 μg DMBA	25.0 μg DMBA
$\hat{\mu}_0^b$	$1.22 * 10^{-6}$	$9.5402 * 10^{-4}$
$\hat{\beta}_1^b$	3.933	3.9089
$\hat{\delta}_1^b$	3.9463	3.9779
$\hat{\nu}_1^b$	$3.5584 * 10^{-4}$	$8.0428 * 10^{-3}$

Table 2: Swiss CD-1 Mice.

	2.5 μg DMBA	25.0 μg DMBA
$\hat{\mu}_0^s$	0.0713	0.0275
$\hat{\beta}_1^s$	5.922	4.1881
$\hat{\delta}_1^s$	5.2961	4.2696
$\hat{\nu}_1^s$	0.1015	0.8059

In our model, we considered the number of normal cells to be $m = 12 * 10^6$ and number of initiated cells needed to form a visible papilloma to be $M = 387$ [3]. Maximum likelihood estimates for the parameters related to B6C3F$_1$ and Swiss CD-1 mice are presented in Tables 1 and 2 respectively.

Analysis of the Results

1. Figures 3 and 4 depict the graphs of the empirical average (observed) and expected numbers of papillomas (under the model) for B6C3F$_1$ mice with DMBA dosage of 2.5 and 25.0 μg respectively. For DMBA dosage of 2.5 μg, the fit appears to be reasonably good after the 27-th week (Fig. 3). The poor fit in the early stages of the study may

be due to the assumption of zero birth and death rate of normal cells in our model. The fit appears to be quite good for DMBA dosage of 25.0 μg (Fig. 4).

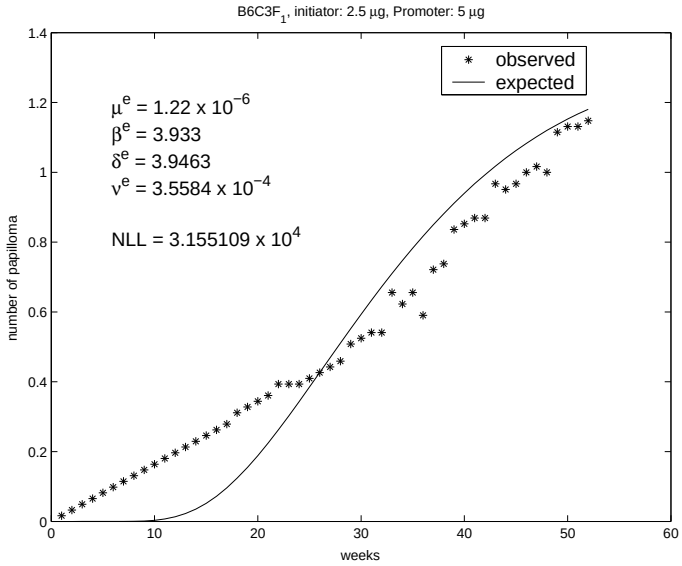

Figure 3: Model fit to the papilloma count for B6C3F$_1$ mice initiated with 2.5 μg DMBA.

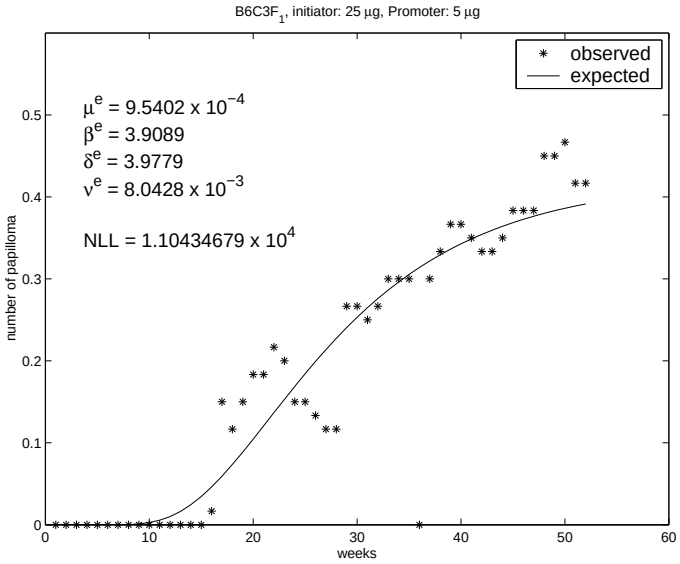

Figure 4: Model fit to the papilloma count for B6C3F$_1$ mice initiated with 25.0 μg DMBA.

2. Figures 5 and 6 depict the graphs of the empirical average and expected numbers of papillomas (under our model) for Swiss CD-1 mice with DMBA dosage of 2.5 and 25.0 μg respectively. The fit appears to be quite good for both DMBA dosages of 2.5 μg (Fig. 5) and 25.0 μg (Fig. 6).

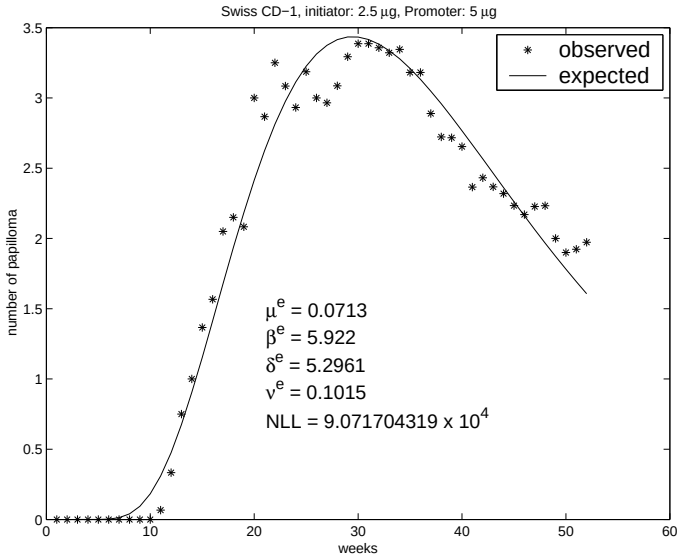

Figure 5: Model fit to the papilloma count for Swiss CD-1 mice initiated with 2.5 μg DMBA.

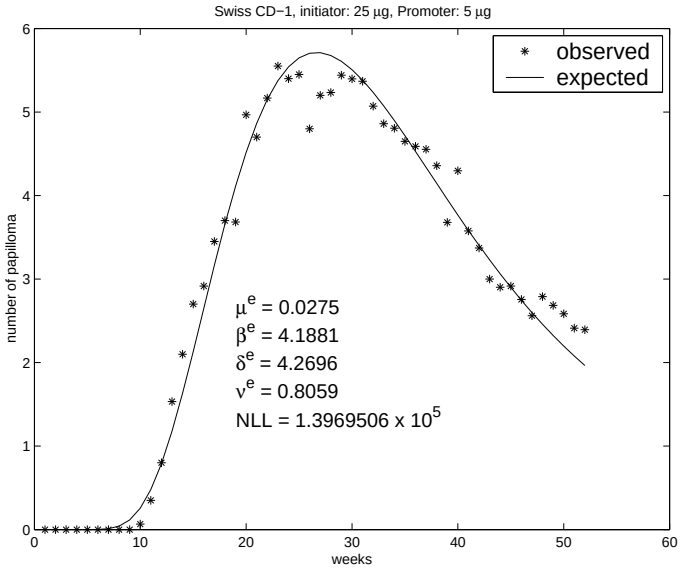

Figure 6: Model fit to the papilloma count for Swiss CD-1 mice initiated with 25.0 μg DMBA.

3. In order to find out whether the two different strains of mice are significantly different with respect to their birth rates, we use the likelihood ratio test statistic [1] to test the following hypotheses:

H_0: Swiss CD-1 and B6C3F$_1$ mice have equal birth rates.
H_1: Swiss CD-1 and B6C3F$_1$ mice have unequal birth rates.

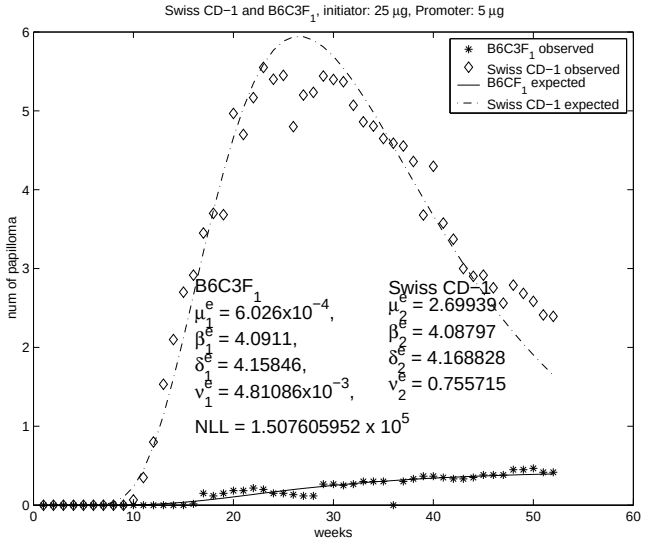

Figure 7: Model fit to the papilloma count for Swiss CD-1 and B6C3F$_1$ mice assuming different birth rates (both groups were initiated by 25.0 μg DMBA).

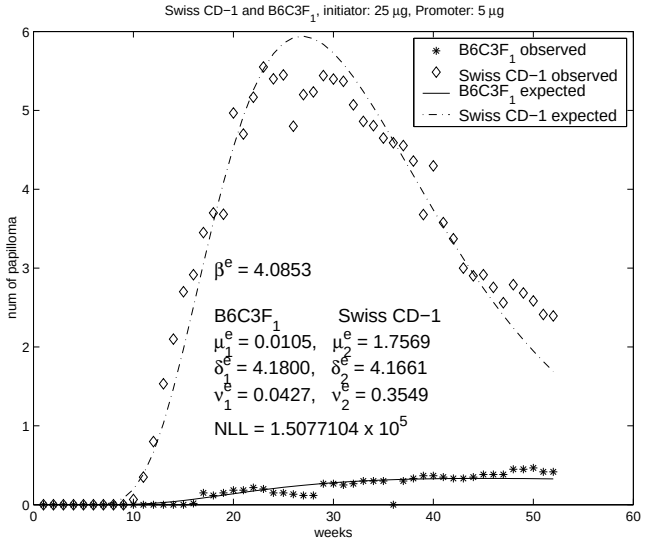

Figure 8: Model fit to the papilloma count for Swiss CD-1 and B6C3F$_1$ mice assuming equal birth rates (both groups were initiated by 25.0 μg DMBA).

From Figures 7 and 8, the difference in birth rates is apparent. Moreover the difference in likelihoods is 10.448 ($1.5077104 \times 10^5 - 1.507605952 \times 10^5$), is significant having a p-value of 0.0012. Therefore, the null hypothesis is rejected. We can conclude that the Swiss CD-1 and B6C3F$_1$ mice have unequal birth rates.

4. To find out whether different levels of initiation dosage have any effect on the same strain of mice, we test the following hypotheses

   H_0: Equal birth rates for DMBA dosages of 2.5 and 25.0 μg in Swiss CD-1 mice
   H_1: Unequal birth rates for DMBA dosages of 2.5 and 25.0 μg in Swiss CD-1 mice

   The observed difference in likelihoods under two hypotheses is 55.5981 ($2.30463021 \times 10^5 - 2.3040742297 \times 10^5$), which is significant having a p-value that is less than 0.0001. Therefore, we reject the H_0 and conclude that different initiator dosages produce different birth rates for the Swiss CD-1 mice.

4 Discussion

In this study we identify a general model which describes the mutation of normal cells to papilloma. We studied two different strains of mice under two separate initiator dosages. We present 4 different working models for these four cases. The underlying model is the same for all four cases based on the binomial distribution likelihood function. It should be noted that in all of these four cases the promotor and its dosage is the same (TPA 5 μg). The estimated values for the parameters tested significantly different for all four cases. Specifically we have tested for the equality of birth rate between two different strains of mice, both strains were initiated with 25 μg of DMBA. It is observed that Swiss CD-1 and B6C3F$_1$ mice have different birth rates even when the initiator dosage is the same. We also try to determine whether initiator dosage affects birth rates. In particular for Swiss CD-1 mice, we performed the test for the equality of birth rates under two different initiator dosages. Birth rates were found to be unequal for Swiss CD-1 mice for 2.5 and 25 μg DMBA.

The carcinoma data available to us were in the form of a set of summary statistics, giving insufficient information about carcinoma to conduct a meaningful maximum likelihood analysis. Therefore our likelihood function is only based on papilloma data. Once we get relevant carcinoma data, the likelihood function can be directly applied to the data, since we have derived the incidence of the carcinoma in our model. One advantage of our model is that we assume the numbers of papilloma and carcinoma have binomial distributions instead of Poisson distributions. This allows us to describe the process more accurately. Based on our model, it will be very easy to expand to a multi-stage model using a multinomial distribution. This approach should fit the data better, since it takes into account the different stages a normal cell goes through to reach the stage of papilloma. From the biological viewpoint, since there always exists some uncertainty, one may try to describe the process with a system of stochastic differential equations or with a continuous time Markov chain.

Overall, it appears that our model works quite well in the present setting and can be applied to a more general situation. However, it may be of interest to add more parame-

ters in the model in order to account for inherent biological complexities, such as cellular interactions, regression of papillomas etc.

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A Appendices

The system of ordinary differential equations derived in this section represents the probability of a normal cell forming a papilloma. Correspondingly, this derivation does not include the malignant stage. The derivation of the ordinary differential equations for the probabilities of two-stage mutation of normal cells into malignant cells has been done previously by Marjo V. Smith and Christopher J. Portier [8]. We apply this tecnique to the probability of a normal cell forming a papilloma. The difference is that we consider forming a papilloma to be a multistage process (as described in Section 2) and thus the result of our derivation is a system of differential equations rather than a single equation. As done by Smith and Portier, we will assume that cells act independently and set the normal-cell birth rate (β_0) and death rate (δ_0) to zero (because the size of the initial sample remains constant). In the notation introduced in Section 2, the following events may happen over a time interval $[t-s-\Delta s, t-s]$:

1. A normal cell mutates with probability $\Delta s\,\mu_0$.
2. A normal cell does not change with probability $1-\Delta s(\beta_0+\delta_0+\mu_0)$.
3. An initiated cell replicates with probability $\Delta s\beta_1$.
4. An initiated cell dies with probability $\Delta s\delta_1$.
5. An initiated cell does not change with probability $1-\Delta s(\beta_1+\delta_1)$.

We first derive the equations for two special cases, $Q_{0M}(s,t)$ and $Q_{1M}(s,t)$, followed by the general case, $Q_{iM}(s,t), i=2,3,\ldots,M-1$.

A.1 The Equation for $Q_{0M}(s,t)$

Following [8], there are only four events that may happen to a single normal cell over the interval $[t-s-\Delta s, t-s]$:

- nothing may happen, so there is still one normal cell at the time $t-s$;
- the normal cell may replicate, so that there are two normal cells at time $t-s$;
- the normal cell may die, so the probability of no papilloma is 1;
- the normal cell may mutate, so the stage I_1 is achieved.

Thus we have:

$$\begin{aligned} Q_{0M}(s+\Delta s,t) &= P(\text{no papilloma is visible at } t \,|\, \text{one normal cell } I_0 \text{ at } t-s-\Delta s) \\ &= P(\text{no papilloma is visible at } t \,|\, \text{one normal cell } I_0 \text{ at } t-s) \\ &\quad \times P(\text{one normal cell } I_0 \text{ at } t-s \,|\, \text{one normal cell } I_0 \text{ at } t-s-\Delta s) \\ &\quad + P(\text{no papilloma is visible at } t \,|\, \text{two normal cells } I_0 \text{ at } t-s) \end{aligned}$$

$$
\begin{aligned}
&\times P(\text{two normal cells } I_0 \text{ at } t-s \,|\, \text{one normal cell } I_0 \text{ at } t-s-\Delta s)\\
&+P(\text{no papilloma is visible at } t \,|\, \text{no normal cells } I_0 \text{ at } t-s)\\
&\times P(\text{no normal cells } I_0 \text{ at } t-s \,|\, \text{one normal cell } I_0 \text{ at } t-s-\Delta s)\\
&+P(\text{no papilloma is visible at } t \,|\, \text{one initiated cell } I_1 \text{ at } t-s)\\
&\times P(\text{one initiated cell } I_1 \text{ at } t-s \,|\, \text{one normal cell } I_0 \text{ at } t-s-\Delta s)\\
= \;& Q_{0M}(s,t)[1-\Delta s(\beta_0+\delta_0+\mu_0)]\\
&+(Q_{0M}(s,t))^2\Delta s\beta_0+\Delta\delta_0\cdot 1+Q_{1M}(s,t)\Delta s\,\mu_0
\end{aligned}
$$

Subtracting $Q_{0M}(s,t)$ from both sides, dividing by Δs, and taking the limit as $\Delta s \to 0$, we obtain

$$\frac{dQ_{0M}(s,t)}{ds} = (Q_{0M}(s,t))^2\beta_0 - Q_{0M}(s,t)(\beta_0+\delta_0+\mu_0)+\delta_0+Q_{1M}(s,t)\mu_0. \tag{7}$$

Since $\beta_0 = \delta_0 = 0$, equation 7 becomes

$$\frac{dQ_{0M}(s,t)}{ds} = -Q_{0M}(s,t)\mu_0 + Q_{1M}(s,t)\mu_0. \tag{8}$$

A.2 The equations for $Q_{1M}(s,t)$

In this case, there are only three events that may happen to a single initiated cell at the stage I_1 over the interval $[t-s-\Delta s, t-s]$:

- nothing may happen, so there is still one initiated cell at the time $t-s$;
- the initiated cell may replicate, so that there are two initiated cells at time $t-s$;
- the initiated cell may die, so the probability of no papilloma is 1.

Thus, we have:

$$
\begin{aligned}
Q_{1M}(s+\Delta s,t) &= P(\text{no papilloma is visible at } t \,|\, I_1 \text{ at } t-s-\Delta s)\\
&= P(\text{no papilloma is visible at } t \,|\, I_1 \text{ at } t-s)\\
&\qquad \times P(I_1 \text{ at } t-s \,|\, I_1 \text{ at } t-s-\Delta s)\\
&\qquad +P(\text{no papilloma is visible at } t \,|\, I_2 \text{ at } t-s)\\
&\qquad \times P(I_2 \text{ at } t-s \,|\, I_1 \text{ at } t-s-\Delta s)\\
&\qquad +P(\text{no papilloma is visible at } t \,|\, \text{no cells } I_1 \text{ at } t-s)\\
&\qquad \times P(\text{no cells } I_1 \text{ at } t-s \,|\, I_1 \text{ at } t-s-\Delta s)\\
&= Q_{1M}(s,t)(1-\Delta s(\beta_1+\delta_1)) + Q_{2M}(s,t)\Delta s\beta_1 + \Delta s\delta_1\cdot 1.
\end{aligned}
$$

Subtracting $Q_{1M}(s,t)$ from both sides, dividing by Δs, and taking the limit as $\Delta s \to 0$, we obtain:

$$\frac{dQ_{1M}(s,t)}{ds} = Q_{2M}(s,t)\beta_1 - Q_{1M}(s,t)(\beta_1+\delta_1)+\delta_1. \tag{9}$$

A.3 The Equations for $Q_{iM}(s,t),\ i = 2, 3, ..., M-1$

In this case, there are only three events that may happen to the initiated cells at the stage I_i over the interval $[t-s-\Delta s, t-s]$:

- nothing may happen, so there are still i initiated cells at the time $t-s$;
- any one of the i initiated cells may replicate, so that there are $i+1$ initiated cells at the time $t-s$ and the stage I_{i+1} is achieved;
- any one of the i initiated cell may die, so that there are $i-1$ initiated cells at the time $t-s$ and the process returns to the stage I_{i-1}.

Thus, we have:

$$\begin{aligned} Q_{iM}(s+\Delta s, t) &= Q_{iM}(s,t)(1 - i\,\Delta s(\beta_1 + \delta_1)) \\ &\quad + iQ_{i+1,M}(s,t)\Delta s\beta_1 + iQ_{i-1,M}(s,t)\Delta s\,\delta_1. \end{aligned}$$

Subtracting $Q_{iM}(s,t)$ from both sides, dividing by Δs, and taking the limit as $\Delta s \to 0$, we obtain:

$$\frac{dQ_{iM}(s,t)}{ds} = iQ_{i+1,M}(s,t)\beta_1 - iQ_{iM}(s,t)(\beta_1 + \delta_1) + iQ_{i-1,M}(s,t)\delta_1. \tag{10}$$

Finally, to complete the system (8)-(10), we need initial conditions for $Q_{iM}(s,t)$. Since we consider the papilloma stage irreversible, we have

$$Q_{MM}(s,t) = 0, \quad \text{for all } s,t,$$

and, by definition,

$$Q_{iM}(0,t) = 1, \quad i = 0, 1, \ldots, M-1.$$