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THEORY OF THE MITOTIC INDEX AND ITS APPLICATION TO TISSUE GROWTH MEASUREMENT

JOSEPH G. HOFFMAN*
RADIOBIOLOGY SECTION OF THE UNIVERSITY OF CALIFORNIA
LOS ALAMOS SCIENTIFIC LABORATORY†
LOS ALAMOS, NEW MEXICO

Analysis is made to show that the mitotic index is simply proportional to the ratio of the duration of mitosis (T) to the intermitotic time only under special conditions. In the case of exponential growth of cell population the simple proportionality holds if the product of T and the growth constant is small. For power law (t^n) growth of cell population the simple proportionality holds only when a steady state of growth has existed for at least ten intermitotic periods. The simple proportionality does not apply in conditions of transient growth.

The mitotic index, m/N, for tissues in which mitosis is occurring is defined as the ratio of the number of cells, m, seen in mitosis to the total number of cells, N, examined. The mitotic index has been used to determine the duration in time of the process of division (Hoffman, 1947; Gray, 1931; Laughlin, 1919; Wright, 1925) on the assumption that it is directly proportional to the ratio of the duration of mitosis to the total time between mitoses. In the following analysis we shall show that the direct proportionality holds true only under special conditions.

Designating the mitosis duration as T and the total intermitotic time as L, then $m/N = T/L$ when the value of N is constant with time, i.e., as many cells are being removed as are being produced. Contrasted with this case of the stationary population there are the cases in which the *cell numbers* are known to increase exponentially with time as in transplantable mouse tumors (Hoffman et al, 1943; Reinhard et al, 1945) and in regenerating liver (Brues and Marble, 1937). The purpose of the following analysis is to show how the

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*Now at Roswell Park Memorial Institute, Buffalo, New York.

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mitotic index depends on the parameters of the life cycle (Figure 1) of the cell and on the rate of growth of the cell population. Case I is concerned with the tissue in which exponential growth of cell numbers occurs, while Case II deals with the general case in which the cell number increases as the *n*th power of the time.

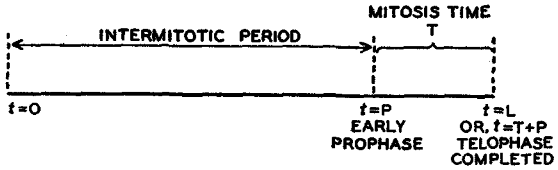

FIGURE 1. Parameters in the life cycle of a single cell which undergoes division. The time between the complete formation of daughter cells is L. When T is small, L is approximately equal to P, the intermitotic time.

Case I. *The number of cells in the tissue mass increases exponentially with time.*

Suppose that no cells are removed. Examples of this case are given in Table I. The number of cells present is then $N_0 e^{\lambda t}$ where t is the time in days, N_0 the initial number of cells present, and λ is the characteristic exponential constant.

TABLE I

Tissues in which Cell Numbers Increase Exponentially with Time

TYPE OF TISSUE	λ	REFERENCE
Transplantable mouse adenocarcinoma	0.37 days^{-1}	1, 2
Ascite Tumor cells in mice	0.6 "	3
Transplantable mouse tumor	0.7 "	4
Regenerating liver tissue (rats)	1.3 " *	5

*Maximum, initial value of λ.

1. J. G. Hoffman et al, 1943.
2. M. C. Reinhard et al, 1945.
3. H. Lettre, 1943.
4. S. Konsuloff, 1933.
5. A. M. Brues and B. B. Marble, 1937.

Table I shows the order of magnitude of the values of λ as measured by the volume rate of growth. It is assumed that the cell volumes of the tissues concerned are restricted to fixed limits. Therefore, the value of λ applies to the growth of cell numbers as well as of the tissue volume.

The analysis of the mitotic index is carried out as follows: Suppose that the time schedule in the life of a tissue cell is given by Figure 1. The newly formed cell grows for a time, P, then undergoes mitosis for a time, T; the total time between the appearance of the daughter cells being L. At a time, t, let there be N cells present. The number, m, of cells in mitosis has an age between P and $P + T$. All of these cells must have been born at a time between $t—L$ and $t—P$ or $t—L + T$. The rate of births at any time is $2(dN/dt)$ for binary fission. Therefore, the value of m is given by:

$$m = 2N_0\{e^{\lambda(t-L+T)} - e^{(t-L)}\};$$

or (1)

$$m = 2e^{\lambda(t-L)}N_0(e^{\lambda T} - 1).$$

The mitotic index is m/N and thus becomes:

$$m/N = 2e^{-\lambda L}(e^{\lambda T} - 1).$$

But, $e^{-L\lambda} = 1/2$, since L is the number doubling time for the cells. Therefore

$$m/N = (e^{\lambda T} - 1). \tag{2}$$

Since λT is usually small, this can be approximated by

$$m/N = \lambda T, \quad \text{since} \quad e^{\lambda T} = 1 + \lambda T, \tag{3}$$

and if L is the doubling time, the growth constant, λ, is related to L by:

$$\lambda = \ln 2/L,$$

and the mitotic index becomes

$$m/N = 0.693\ T/L. \tag{4}$$

The essential feature in the expressions (3) and (4) is that the index m/N is proportional to the ratio of the mitosis time, T, and the doubling time, L. And in this case, the value of λ is a measure of the volume rate of growth as well as of the rate of production of cell numbers. Equation (2) shows that if λT is large the mitotic index is no longer simply related to the ratio T/L. However, equation (2) permits an estimation of T once m/N and λ are known.

Case II: *The number of cells in the population increases as* t^n.

The analysis of the case is carried out similar to that given in Case I. The number of cells is given by

$$N = N_0(at^n + 1); \tag{5}$$

the doubling time, t_2, is given by $N/N_0 = 2$, or

$$t_2 = \sqrt[n]{1/a} = L\,. \tag{6}$$

The mitotic index for the general case can be shown to be:

$$\frac{m}{N} = \frac{(t-L+T)^n - (t-L)^n}{t^n - (t-L)^n}\,. \tag{7}$$

By expanding both terms of the numerator and the second term of the denominator it is easily seen that for very large values of t we have

$$m/N = aT\,. \tag{8}$$

Discussion.

An outstanding feature in the foregoing discussion of the mitotic index is the ratio T/L. Few data exist concerning the duration of mitosis, T, which is the datum required if the mitotic index is to be used for determining L. Turning to quantitative data on exponential growth of a tissue mass we shall estimate the duration of mitosis, T, for the Ascite tumor cells in mice from the mitosis data as reported by H. Lettre (1943). He gives values of the cell number doubling time, L, as 25.7, 27.7 and 28.8 hours. From his Table I, we find three values of the mitotic index: 1.5, 1.0, and 1.1%. To compute T we shall use the mitotic index or (mi) $= 1\%$ and $L = 25.7$ hours; $T =$ (mi) $\times L/0.693 = .01 \times 25.7/0.693 = 0.4$ hour or 24 min. This is the lowest value of T to be derived from Lettre's data. Lettre estimates that $T = 15$ min. from the rate of build-up of mitoses in the tumor due to colchicine poisoning.

The transplantable mammary adenocarcinoma in mice described by J. G. Hoffman et al (1943) and M. C. Reinhard et al (1945) lends itself to quantitative estimates for the mitosis time on the basis of the mitotic index. The values found (unpublished data) for the index were: (mi) $= 0.3$ and 0.5%. The growth constant (Hoffman, et al, 1943) was $\lambda = 0.37$ per day. Hence, $T = 0.004/0.37 = 0.0108$ days, or 15.5 minutes, where we have taken 0.4% as the average value of the mitotic index.

A. M. Brues and B. B. Marble (1937) in their study of the exponential growth of regenerating liver estimate an average time duration of mitosis of 49 minutes. On the other hand, in human scalp epidermis, where it is reasonably assumed that growth is not exponential, J. M. Thuringer (1928) estimated the mitosis duration as being from 15 to 30 minutes.

The analyses given in Cases I and II indicate the nature of the variables which may enter into an interpretation of the mitotic index. For example, in early squamous cell carcinoma of the cervix or of epidermis, where the mitotic index averages 4% and is sometimes as high as 10%, it is first necessary to determine the possibility of T being very long (greater than 1 hour). If T is very long then the mitotic index is correspondingly increased and does not necessarily indicate a high rate of growth. Next it is necessary to know if any of the cells in the preparation were near a region in which they could be removed by differentiation into another cell form or by direct lysis. If neither of these possibilities exists it may be assumed that the population was growing exponentially. If there is evidence that cells might be removed by these processes it is necessary to estimate by which power law of the time, t, the cell population is increasing (or indeed decreasing, depending on the tissue region seen). A similar problem in estimating cell removal rates occurs in the use of the mitotic index to measure growth in tissues under hormonal stimulation such as in the proliferation induced by estrogens.

Summary:

1. It is shown that for exponential growth of a cell population the mitotic index is simply proportional to the ratio of the duration time of mitosis to the intermitotic time only when λT is small. For power law (t^n) growth of a cell population the mitotic index is simply proportional to the ratio of the duration time of mitosis to the intermitotic time only when the population has grown for times long compared with the intermitotic time.
2. For exponential growth of tumors the experimental data are used to compute the time duration of mitosis.
3. Special cases in which the mitotic index cannot be applied due to transient conditions of growth stimulation are discussed.

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