A NOTE ON PUCK AND STEFFEN'S EQUATIONS FOR THE LIFE CYCLE ANALYSIS OF MAMMALIAN CELLS

ERNESTO TRUCCO
From the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

ABSTRACT The equations given by Puck and Steffen (1963) are derived from the age density function of the population being studied. The results differ somewhat from those of Puck and Steffen, but, on the whole, their procedure and their conclusions in analyzing the life cycle by means of colcemide and tritiated thymidine remain valid.

A highly significant contribution to the life cycle analysis of mammalian cells cultured *in vitro* was made recently by Puck and coworkers (1962, 1963, 1964). These investigators used a blocking agent (colcemide) which prevents cells that enter the last stage of the cycle (mitosis) from continuing their normal cellular development, and thus from completing the cycle. The *collection function,* as defined and used by Puck, indicates the number of cells that are accumulating beyond the blocking point.

The equations derived by Puck and Steffen (1963) can be obtained from a formalism due to Von Foerster (1959) and, less explicitly, to other workers (*e.g.,* Kendall, pp. 231-233, 1949; Scherbaum and Rasch, 1957). The procedure consists in computing the *age density function,* $n(t, a)$, where a is cellular age and t denotes time (clock time). The meaning of $n(t, a)$ is explained by the statement that $n(t, a)da$ gives the number of cells in the population with ages between a and $a + da$ at time t. It can be shown that the function $n(t, a)$ is a solution of the partial differential equation

$$\frac{\partial n}{\partial t} + \frac{\partial n}{\partial a} = -\lambda \cdot n, \tag{1}$$

where λ, the *loss function,* measures the probability that a cell with age between a and $a + da$ will disappear from the population in the time interval from t to $t + dt$, for any cause whatever (cell division, cell death, or emigration). Details on equation (1) are given in Trucco (1965).

The solution of equation (1) is completely determined if, in addition to λ, we know the boundary values of $n(t, a)$ along the lines $t = 0$ and $a = 0$. We shall write $\alpha(t)$ for $n(t, 0)$, the "generalized birth rate" or *influx function,* and $\beta(a)$ for $n(0, a)$, the *initial age distribution.* It frequently happens that the loss function depends on age only: $\lambda = \theta(a)$. If, moreover, $\theta(a) \equiv 0$ in the range $0 \leq a < a_0$, where a_0 is a constant, the age density [for all values of $t > 0$ and for $0 \leq a < a_0$] will be a solution of the homogeneous equation

$$\frac{\partial n}{\partial t} + \frac{\partial n}{\partial a} = 0,$$

i.e, a function of $t - a$ only, and it must match the given boundary values. Thus, we have

$$n(t, a) = \left\{ \begin{matrix} \alpha(t - a) & \text{for} & t > a, \\ \beta(a - t) & \text{for} & a > t \end{matrix} \right\} \qquad [0 \leq a < a_0, t > 0]. \tag{2}$$

Here, the notation $\alpha(t - a)$ means that the function $\alpha(u)$ must be taken for the value of the argument $u = t - a$, and similarly for $\beta(a - t)$.

Following Puck, we shall assume that all the cells have identical generation time (or nearly so); this situation has been called *"equivivancy"* by Von Foerster (1959, p. 391). In the mathematical treatment outlined here, equivivancy can only be handled as a limiting case, namely when the distribution of cellular generation times becomes a Dirac delta function. For such an equivivant, non-synchronized population growing exponentially without losses from death or migration, the age density function is given by

$$n(t, a) = \left\{ \begin{matrix} 2kN_0e^{kt-ka} & \text{if} & 0 \leq a < D, \\ 0 & \text{if} & a > D, \end{matrix} \right\} \tag{3}$$

where N_0 is a constant, D is the generation time, and[1]

$$k = \frac{\ln 2}{D}, \tag{4}$$

so that

$$e^{-kD} = \tfrac{1}{2}. \tag{5}$$

Integrating the age density function, equation (3), over the variable a, between the limits 0 and D, we obtain the total number of cells in the population:

$$N(t) = N_0e^{kt} = N_0 \cdot 2^{t/D}. \tag{6}$$

[1] We denote natural logarithms by "ln," and logarithms to the base 10 by "log."

Similarly, the fraction of cells with ages between $D - M$ and D $(0 < M < D)$ is given by

$$\frac{1}{N(t)} \int_{a=D-M}^{D} n(t, a)\, da = e^{kM} - 1, \tag{7a}$$

and the fraction of cells with ages ranging from $a = 0$ to $a = D - M$ is equal to

$$\frac{1}{N(t)} \int_{a=0}^{D-M} n(t, a)\, da = 2 - e^{kM}. \tag{7b}$$

These expressions are, of course, well known. In one form or another they have been derived by[2] Hoffman (1947, 1949), Walker (1954), Scherbaum and Rasch (1957), Edwards *et al.* (1960), James (1960), Stanners and Till (1960), Smith and Dendy (1962), and perhaps also by Maruyama (1955), not available here, quoted by Zeuthen (1958). A few additional references on the same subject are given by Sisken and Morasca (1965), and there may well be others in the literature.

Suppose now that in a population characterized by equation (3) a blocking agent is introduced and begins to act instantaneously, exerting its effect on cells that reach age $A = D - M$ $(0 < A < D)$. Let $t = 0$ be the time at which the agent is administered. More precisely, then, cells with ages $\geq A$ at $t = 0$ are not affected, complete the cycle, and divide once, whereas all the cells reaching age A after time zero are prevented from doing so (*cf.* Fig. 1).

It is convenient, for our purposes, to subdivide the total population into two groups. Group I consists of the cells which had ages from A to D at time zero.

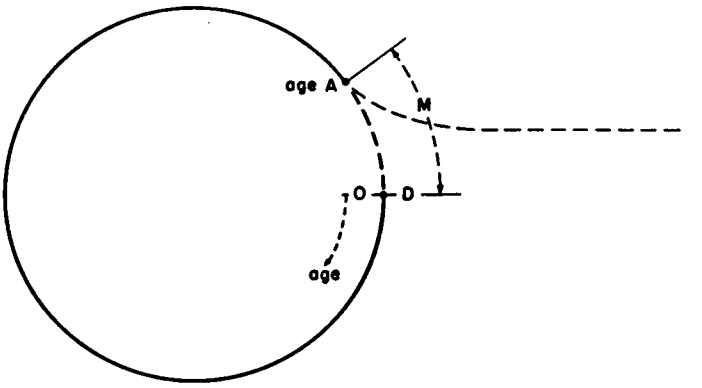

FIGURE 1 Interruption of normal life cycle by inhibitor acting on cells that reach age A (see text).

[2] As Edwards *et al.* (1960, pp. 276-277) point out, Crick's equation (cited by Hughes, 1952, p. 89) is incorrect, although approximately right if $M/D \ll 1$, in our notation. Actually, however, the error was made by Hughes, and not by Crick (*cf.* Smith and Dendy, 1962, p. 556).

Group II, on the other hand, includes those cells which were in the age range $0 \leq a < A$ at time zero, as well as all the daughter cells of group I. Thus, the maximum age reached in group I is D, and, clearly, this fraction of the population will be depleted at time $t = M$. Assuming that the effect of the blocking agent persists indefinitely, and that the cells do not disintegrate,[3] there will be no upper limit to the age[4] attained by cells of group II.

We now compute the age density function for the two groups.

In *group I* we have

$$\alpha_I(t) \equiv 0, \tag{8}$$

since there is no influx of cells after the cycle has been broken by the blocking agent. The initial age distribution, obtained from equation (3) with $t = 0$, is given by

$$\beta_I(a) = \begin{cases} 2kN_0e^{-ka} & \text{for} \quad A \leq a < D, \\ 0 \quad \text{for} \quad 0 \leq a < A \quad \text{or} \quad a > D. \end{cases} \tag{9}$$

The loss term, in the limiting case of equivivancy, is a singular function, equal to zero everywhere in the interval $0 \leq a < D$, and different from zero (actually tending to ∞) for $a > D$. Thus, using equation (2) and the fact that no cells in this group exceed age D, we obtain:

(*a*) if $0 \leq t < M$

$$n_I(t, a) = \begin{cases} 2kN_0e^{kt}e^{-ka} & \text{for} \quad t + A \leq a < D, \\ 0 \quad \text{for} \quad 0 \leq a < t + A \quad \text{or} \quad a > D; \end{cases} \tag{10}$$

(*b*) if $t > M$

$$n_I(t, a) \equiv 0. \tag{11}$$

The total number of cells in group I, $N_I(t) = \int_{a=A}^{D} n_I(t, a)da$, is given by

$$N_I(t) = \begin{cases} N_0(e^{kM} - e^{kt}) & \text{if} \quad 0 \leq t \leq M, \\ 0 \quad \text{if} \quad t \geq M. \end{cases} \tag{12}$$

The only influx for *group II* is provided by cells that have just divided in group I. Thus, the influx function, $\alpha_{II}(t)$, can be calculated from the equation

$$\alpha_{II}(t) = -2\frac{dN_I(t)}{dt} = \begin{cases} 2kN_0e^{kt} & \text{if} \quad 0 \leq t < M, \\ 0 \quad \text{if} \quad t > M. \end{cases} \tag{13}$$

---

[3] This is not the case, sometimes even over relatively short periods of time, see Puck, Sanders, and Petersen, 1964. A death term could be introduced in the equations, but is omitted here for simplicity.

[4] The word "age" is used in the sense of time elapsed since cell birth, and does not necessarily imply senescence or other changes usually associated with aging.

The initial age distribution, in this case, is given by

$$\beta_{II}(a) = \begin{cases} 2kN_0e^{-ka} & \text{if } 0 \leq a < A, \\ 0 & \text{if } a \geq A. \end{cases} \tag{14}$$

Finally, in group II there are no losses, and so we have from equation (2):

(*a*) if $0 \leq t < M$

$$n_{II}(t, a) = \begin{cases} 2kN_0e^{kt}e^{-ka} & \text{for } 0 \leq a < t + A, \\ 0 & \text{for } a \geq t + A; \end{cases} \tag{15}$$

(*b*) if $t > M$

$$n_{II}(t, a) = \begin{cases} 2kN_0e^{kt}e^{-ka} & \text{for } t - M < a < t + A, \\ 0 \quad \text{for } 0 \leq a < t - M \quad \text{or} \quad a \geq t + A. \end{cases} \tag{16}$$

From this we calculate the total number of cells in group II:

$$N_{II}(t) = \int_{a=0}^{t+A} n_{II}(t, a)\, da = \begin{cases} 2N_0e^{kt} - N_0e^{kM} & \text{if } 0 \leq t \leq M, \\ N_0e^{kM} & \text{if } t \geq M. \end{cases} \tag{17}$$

The number of cells in the whole population when the blocking agent is present thus becomes:

$$N(t) = N_I(t) + N_{II}(t) = \begin{cases} N_0e^{kt} & \text{if } 0 \leq t \leq M, \\ N_0e^{kM} = \text{constant} & \text{if } t \geq M. \end{cases} \tag{18}$$

Figs. 2 and 3 show the regions of the (t, a) plane in which the functions $n_I(t, a)$ and $n_{II}(t, a)$ are different from zero.

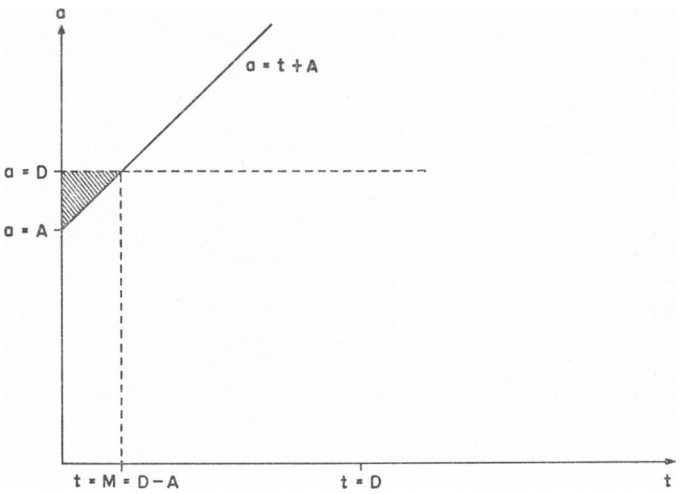

FIGURE 2 $n_I(t, a)$ differs from zero only in the shaded area.

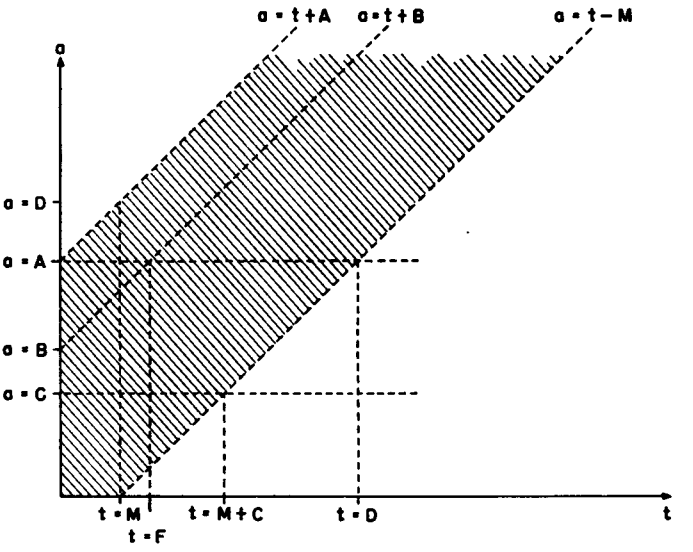

FIGURE 3 $n_{II}(t, a)$ differs from zero only in the shaded area.

Consider now a second value, B, of the age variable (see Fig. 4). Assume $0 < B < A < D$, and put

$$D - B = R, \tag{19}$$

$$A - B = R - M = F. \tag{20}$$

The blocking agent still acts on cells reaching age A, as described above. By inte-

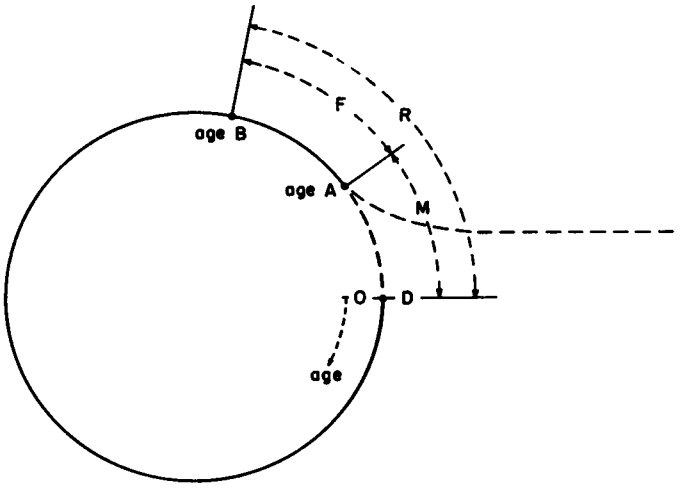

FIGURE 4 Diagram illustrating the derivation of equations (21) through (28).

grating the expressions for $n_{II}(t, a)$, equations (15) and (16), over the appropriate age ranges, we can compute

$N_{II}^{(A)}(t)$ = number of cells in group II with age $> A$,
$N_{II}^{(B)}(t)$ = number of cells in group II with age $> B$,
$N^{(A)}(t) = N_I(t) + N_{II}^{(A)}(t)$ = number of cells with age $> A$,
$N^{(B)}(t) = N_I(t) + N_{II}^{(B)}(t)$ = number of cells with age $> B$,

and

$\Delta N(t) = N^{(B)}(t) - N^{(A)}(t) = N_{II}^{(B)}(t) - N_{II}^{(A)}(t)$ = number of cells with ages between B and A.

The results, with $N(t)$ given by equation (18), are as follows.

$$N_{II}^{(A)}(t) = \begin{cases} N_0 e^{kM}(e^{kt} - 1) & \text{for } 0 \leq t \leq D, \\ N_0 e^{kM} & \text{for } t \geq D; \end{cases} \tag{21}$$

$$\frac{N^{(A)}(t)}{N(t)} = \begin{cases} e^{kM} - 1 & \text{for } 0 \leq t \leq M, \\ e^{kt} - 1 & \text{for } M \leq t \leq D, \\ 1 & \text{for } t \geq D; \end{cases} \tag{22}$$

$$N_{II}^{(B)}(t) = \begin{cases} N_0 e^{kM}[e^{k(t+F)} - 1] & \text{for } 0 \leq t \leq M + B = D - F, \\ N_0 e^{kM} & \text{for } t \geq M + B; \end{cases} \tag{23}$$

$$\frac{N^{(B)}(t)}{N(t)} = \begin{cases} e^{kB} - 1 & \text{for } 0 \leq t \leq M, \\ e^{k(t+F)} - 1 & \text{for } M \leq t \leq M + B = D - F \\ 1 & \text{for } t \geq M + B; \end{cases} \tag{24}$$

$$\frac{\Delta N(t)}{N(t)} = \begin{cases} e^{kM}(e^{kF} - 1) & \text{for } 0 \leq t \leq M, \\ e^{kt}(e^{kF} - 1) & \text{for } M \leq t \leq M + B = D - F, \\ 2 - e^{kt} & \text{for } D - F \leq t \leq D, \\ 0 & \text{for } t \geq D. \end{cases} \tag{25}$$

Equation (22) can be written in a form which comes closer to Puck and Steffen's notation:

$$\log\left[1 + \frac{N^{(A)}(t)}{N(t)}\right] = \begin{cases} \gamma \cdot \dfrac{M}{D} & \text{for } 0 \leq t \leq M, \quad (26a) \\ \gamma \cdot \dfrac{t}{D} & \text{for } M \leq t \leq D, \quad (26b) \\ \gamma & \text{for } t \geq D, \quad (26c) \end{cases}$$

with

$$\gamma = \ln 2 \times \log e = \log 2 \cong 0.301. \tag{27}$$

A special case of equation (24) is obtained by taking $A = D$, and therefore $M = 0, F = R$. This gives

$$\log\left[1 + \frac{N^{(B)}(t)}{N(t)}\right] = \begin{cases} \gamma \cdot \dfrac{(t+R)}{D} & \text{for } 0 \leq t \leq B, \quad (28a) \\ \gamma & \text{for } t \geq B, \quad (28b) \end{cases}$$

where again $\gamma \cong 0.301$.

Equation (26*b*) corresponds to Puck and Steffen's equation (4); they do not use it for values of t larger than D. Similarly, equation (28*a*) corresponds to equation (Appendix 7) of Puck and Steffen, which (in their notation) is valid only for $t \leq T - T(1)$. However, equations (Appendix 13 or 14) of Puck and Steffen, which are not used in their analysis, should be replaced by equation (25) of this note.

In describing the accumulation of mitotic figures,[5] equation (28) [with $R = F$ equal to the mitotic period] should be employed if the blocking agent acted at the end of mitosis; *i.e.*, at age D. However, Puck and Steffen find that equation (26), where now M represents the mitotic period, is more appropriate. This supports the hypothesis that the inhibitor acts only on cells which had not yet entered mitosis at time $t = 0$.

In addition to the blocking agent, Puck and his collaborators used tritiated thymidine (H^3T) as a radioactive label. To derive the equations for the percentage of labeled cells in the population, we make the following assumptions: (*a*) H^3T and the blocking agent are both added to the culture at time zero; (*b*) *all* the cells that are in S at any time > 0 became labeled; (*c*) cells do not lose their label.

We introduce the following notation[6] (Fig. 5):

C = duration of G_1 period = age at which synthesis period begins;
S = duration of synthesis period,
F = duration of G_2 period,
M = duration of mitosis[7]
B = age at which S period ends,
A = age at which G_2 period ends,

and

$$R = F + M = D - B.$$

After mitosis has been inhibited, the cells of group II can traverse the S period at most once. A cell with age $> t + B$ will have been beyond S at $t = 0$; on the other hand, cells with age $< C$ have not yet reached S.

[5] Taylor (1965) does not state quite clearly whether his equation (1) refers to the case of a block occurring at the beginning or at the end of mitosis.

[6] No confusion should arise from the fact that the same letter, S, is used to denote both the DNA synthesis period and its duration.

[7] Excluding telophase, which incorporated in G_1; *cf.* Puck and Steffen (1963, p. 382).

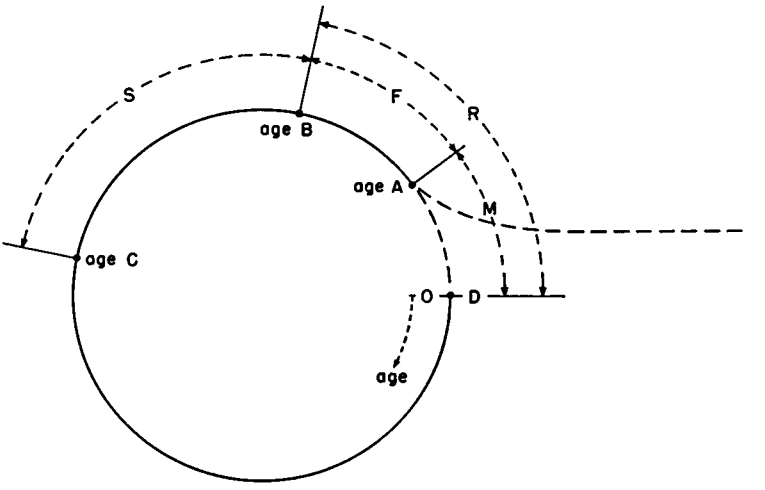

FIGURE 5 Cell life cycle, showing age ranges of G_1, S, G_2, and M (see text).

Thus, the labeled cells found at time t are those of group II with ages between C and $t + B$ (*cf.* Fig. 3). Therefore, the number of labeled cells, $L(t)$, is given by

$$L(t) = \int_{a=C}^{t+B} n_{II}(t, a)\, da. \tag{29}$$

Performing the integration one finds:

$$\frac{L(t)}{N(t)} = e^{kR}(e^{kS} - e^{-kt}) \quad \text{for} \quad 0 \leq t \leq M, \tag{30a}$$

$$\log\left[1 + \frac{L(t)}{\epsilon \cdot N(t)}\right] = \gamma \cdot \frac{(t + S)}{D} \quad \text{for} \quad M \leq t \leq M + C, \tag{30b}$$

$$\log\left[1 + \frac{L(t)}{\epsilon \cdot N(t)}\right] = \bar{\gamma} \cdot \left(1 - \frac{F}{D}\right) = \text{constant} \quad \text{for} \quad t \geq M + C, \tag{30c}$$

where $\gamma \cong 0.301$ and

$$\epsilon = 2^{F/D}. \tag{31}$$

Equation (30*b*) is the same as Puck and Steffen's equation (6). However, it is not valid for $t < M$ (or $t > M + C$). Fortunately, the mitotic time is short and, in fact, Puck and Steffen's Fig. 4 has no experimental points for $t < M$. It should also be remembered that the assumptions we have made on cellular uptake of label are probably incorrect for very small values of t.

In a similar manner the number of labeled mitoses can be calculated. These are the cells of group II with ages satisfying the condition $A < a < B + t$, so that, in particular, no labeled mitoses will appear before time $t = A - B = F$ (*cf.* Fig. 3). Let $\eta(t)$ denote the ratio *number of labeled mitoses/total number of cells* when both

H^3T and colcemide have been added to the culture at time zero. Also assume that the G_2 period is longer than the mitotic time: $F > M$. This is the case for the cell cultures studied by Puck, and it should be true of most cellular populations. Then we find that

$$\log\left[1 + \frac{\eta(t)}{\epsilon}\right] = \begin{cases} 0 & \text{for } 0 \leq t \leq F, \\ \gamma \cdot \dfrac{(t - F)}{D} & \text{for } F \leq t \leq D, \\ \gamma \cdot \left(1 - \dfrac{F}{D}\right) & \text{for } t \geq D; \end{cases} \tag{32}$$

here the quantities ε and γ have the same meaning as before.

Our result (32) differs from equation (2) in Puck, Sanders, and Petersen, 1964. It suggests that the appropriate[8] collection function for labeled mitoses is $\log [1 + \eta(t)/\varepsilon]$, rather than $\log [1 + \eta(t)]$ which is used by Puck *et al.* [1963, Fig. 3; 1964, Fig. 3].

One basic assumption which is made or implied in the equations developed by Puck, Stanners, Till, and others, is that there exists an unambiguous relation between the age of a cell and certain of its characteristics in which we are interested. This has been sufficiently illustrated in the present paper. If the correspondence between stage of life cycle and range of cellular age is perturbed; *e.g.*, by radiation, the concept of age becomes less useful and, in general, the equations cannot be applied without special precautions. Similar comments were made by Elkind and Sinclair (1965, pp. 191, 201).

Finally, if the assumption of equivivancy turns out to be inadequate, the Von Forester formalism can still be used, in principle, provided something is known about the distribution of cellular generation times. The equations, however, become very complicated.

This work was supported by the United States Atomic Energy Commission.

REFERENCES

EDWARDS, J. L., KOCH, A. L., YOUCIS, P., FREESE, H. L., LAITE, M. B., and DONALSON, J. T., 1960, *J. Biophysic. Biochem. Cytol.*, **7**, 273.

ELKIND, M. M., and SINCLAIR, W K., 1965, *in* Current Topics in Radiation Research, (M. Ebert and A. Howard, editors), Amsterdam, North-Holland Publishing Co., **1**, 165.

HOFFMAN, J. G., 1947, *Science*, **106**, 343.

HOFFMAN, J. G., 1949, *Bull. Math. Biophysics*, **11**, 139.

HUGHES, A., 1952, The Mitotic Cycle, New York Academic Press.

JAMES, T. W., 1960, *Ann. New York Acad. Sc.*, **90**, 550.

KENDALL, D. G., 1949, *J. Roy. Stat. Soc.*, **11B**, 230.

PUCK, T. T., 1962, *Animal Cell Newsletter*, **3**, 2.

[8] *i.e.*, that function which, in the ideal case of strict equivivancy, would give three segments of straight line when plotted *vs. t.*

PUCK, T. T., SANDERS, P., and PETERSEN, D., 1964, *Biophysic. J.*, **4**, 441.
PUCK, T. T., and STEFFEN, J., 1963, *Biophysic. J.*, **3**, 379.
SCHERBAUM, O., and RASCH, G., 1957, *Acta. Pathol. et Microbiol. Scand.*, **41**, 161.
SISKEN, J. E., and MORASCA, L., 1965, *J. Cell Biol.*, **25**, 179.
SMITH, C. L., and DENDY, P. P., 1962, *Nature*, **193**, 555.
STANNERS, C. P., and TILL, J. E., 1960, *Biochim. et Biophysica Acta.*, **37**, 406.
TAYLOR, E. W., 1965, *J. Cell Biol.*, **25**, 145.
TRUCCO, E., 1965, *Bull. Math. Biophys.*, in press.
VON FOERSTER, H., 1959, *in* The Kinetics of Cellular Proliferation, (F. Stohlman, Jr., editor), New York, Grune and Stratton Inc., 382.
WALKER, P. M. B., 1954, *J. Exp. Biol.*, **31**, 8.
ZEUTHEN, E., 1958, *Advances Biol. and Med. Physics*, **6**, 37.