For students of cellular proliferation, the most important event in the life of a cell has always been mitosis, in its classic stages from prophase to telophase (248). However, in recent years it has been found that the long interval between 2 successive mitoses, called the interphase, included a series of metabolic events that are of importance in the over-all process of cell division. The orderly sequence of these metabolic activities, from midpoint of mitosis to midpoint of a successive mitosis, constitutes what is called the cell cycle. The object of this review is to present a general outline of the cell cycle, to describe the sequence of events leading from one mitotic division to the next, and to understand the way in which knowledge of these events helps to clarify some of the problems related to kinetics of cellular proliferation and tumor growth.

THE CELL CYCLE

Our knowledge of the cell cycle has been obtained mostly by the use of high-resolution autoradiography (20, 63). The interested reader is referred to the literature for a detailed description of the technic of high-resolution autoradiography (14, 61, 62, 107, 124, 140, 169, 225), and of the various radioactive precursors that are most frequently used to investigate the metabolic activities of cells (3, 4, 6, 7, 13, 14, 44, 61, 62, 64, 74, 131, 167, 194, 216, 218, 247, 256). The most commonly used precursor is tritium-

[Page continued on the following page]
labeled thymidine (thymidine-\(^3\)H), a deoxynucleoside which is incorporated exclusively into DNA (69, 200). Since thymidine and its degradation products are soluble in ordinary fixatives, in which DNA is insoluble, any radioactivity found in fixed tissues or cells after exposure to radioactive thymidine is due to newly synthesized DNA (5, 215), a conclusion confirmed by histochemical studies (4, 14). In addition, thymidine, when injected into intact animals, is either promptly incorporated into DNA or catabolized to nonutilizable products (189, 255). The time during which injected thymidine is available for incorporation into DNA is about 30 min (119, 135), so that for all practical purposes, an in vivo exposure to thymidine results in pulse labeling. The cell cycle was discovered by Howard and Pelc (104). Numerous modifications have been introduced since their original experiment, but the basic model remains the same. Let us suppose an asynchronous population of cells, such as the lining epithelium in the crypts of the small intestine of mice. When thymidine-\(^3\)H is injected into mice, cells that are synthesizing DNA at the time of injection take up thymidine and become labeled. An autoradiograph of a section of small intestine, 30 min after injection of thymidine, discloses 3 types of epithelial cells in the crypts: cells in mitosis (all unlabeled), unlabeled interphase cells, and labeled interphase cells. The 1st bit of information we have obtained is that DNA is synthesized during interphase and not during mitosis, since all cells in mitosis are unlabeled. But because of the short period of time during which thymidine is available, and the fact that DNA is stable (21, 97, 116, 150, 202), we can now follow the fate of cells that were in DNA synthesis at the time of injection by taking samples at different intervals after injection of thymidine. For 30 min or so after exposure to thymidine-\(^3\)H all mitoses are unlabeled, indicating that there is a measurable period before mitosis during which cells do not synthesize DNA. After 30 min, the percentage of labeled mitoses increases and the interval between administration of thymidine and the time at which 50% of mitoses are labeled gives the duration of the premitotic phase, called the G\(_2\) phase (104, 128) during which no DNA is synthesized. As more and more cells that were in DNA synthesis at the time of thymidine injection enter mitosis, the percentage of labeled mitoses increases until 100% of the mitoses are labeled (Chart 1). After a while, the percentage of labeled mitoses decreases. The interval between the two 50% points on the ascending and descending limbs of the curve representing percentage of labeled mitoses (Chart 1) gives the duration of the phase during which DNA is synthesized, called the S phase (104, 128, 197, 198). The length of the entire cell cycle can be estimated on the basis of the time elapsing between 2 successive peaks of labeled mitoses (70, 219). In Chart 1, the interval between the midpoints of the 1st and 2nd peaks is 12 hr and the length of the cell cycle is then estimated at 12 hr. The duration of G\(_2\) is 1 hr, and the length of S phase, 7 hr. Since mitoses usually last less than 1 hr (71, 99, 139, 250) another phase must be postulated to complete the 12 hr of the cell cycle. The existence of this phase is also indicated by the decrease in percentage of labeled mitoses after the 8th hr, which suggests a period, preceding DNA synthesis, during which no DNA is synthesized. The duration of this period, called the G\(_1\) phase, can be ob-

**Chart 1.**—Percentage of labeled mitoses in the lining epithelium of the crypts of the small intestine of mice at various intervals after a single injection of \(^3\)H-thymidine (see explanation in the text). Reproduced, with permission, from the paper by Lesher et al. (137). The mice were injected at 0 time with 30 \(\mu\)g of thymidine-\(^3\)H. In this particular experiment, the cell cycle was studied in the intestine of chronically irradiated animals. One of these groups was chosen for the present illustration, because the amount of variation at the various points was remarkably low. However, the curve for normal mice was essentially similar.
I, is defined here as the percentage of cells labeled within a
entering and leaving a compartment: e.g., $t_8$ is the length of cell
division (13). In the above notation:

$$I = \frac{100 N_s}{N_i}$$

(A)

tained by subtracting from the entire length of the cell
cycle the duration of $G_2 + S + M$ mitosis.

The cell cycle (104, 128, 197) may then be defined as the
interval between midpoint of mitosis in the parent cell
and midpoint of the subsequent mitosis in the daughter
cell (Chart 2). If one of the sister cells is destined not to
divide again, it leaves the cell cycle and becomes a non-
dividing cell that may eventually die or persist for the
entire life of the organism without dividing (Chart 2).

The lining epithelium of the small intestine can be used
in other tissues (181). DNA synthesis and cell division occur only in the
crypts (136, 198). From the crypts, cells migrate to the villus,
where they become nondividing cells, destined to die without any further mitotic
division.

THE 4 PHASES OF THE CELL CYCLE

$S$ phase.—We have seen that DNA synthesis takes place in
a discrete period of interphase, called the S phase (104,
236, 246). There are several ways of determining the
average length of the $S$ phase, $T_s$. The most commonly
used is the one described above in Chart 1 (70). Another
way of determining $T_s$ is by continuous labeling (251). If
cells are constantly exposed to tritiated thymidine, as in
infusion technics (180) or in tissue cultures (228), the
grain count per cell, that is, the autoradiographic intensity
of the label, increases for a time equal to $T_s$ and then
reaches a plateau. A variant of this approach, described
by Stanners and Till in tissue cultures (228), has been
applied in vivo by Koburg (123). $T_s$ can also be calculated
by exposing cells to 2 different pulses, one of thymidine-
$^{14}$C and the other of thymidine-$^3$H (16, 186, 252). Cells
labeled with thymidine-$^{14}$C only can be distinguished from
cells labeled with thymidine-$^3$H only or with both isotopes
by 2-emulsion autoradiography (17), and the length of the
$S$ phase can be calculated from appropriate equations
(252). Although each of these methods is useful in
particular situations, the one described in Chart 1 must
still be considered the most accurate and the method of
choice whenever feasible (197).

The length of the $S$ phase in mammalian cells is often
very close to 8 hr (42, 159, 175, 228, 254). However, the
number of exceptions to this rule has been rapidly increasing
in the past few years. Certain near-tetraploid ascites
tumors of mouse have a $T_s$ of 11–12 hr (8, 55, 102), and
other instances of $T_s$ of unusual length have been described
in cells of germinal centers of rat spleen, 4.5 hr (66); in dog
myeloblasts, 5 hr (179); in epithelial cells of human colon,
9 hr (145); in transplantable mammary tumors of mouse,
10 hr (159); in epithelial cells of mouse stomach, 13.5
hr (253); in mouse spermatogonia, 14 hr (163); in alveolar
cells of mouse mammary gland, 20 hr (33); and in epithelial
cells of mouse ear epidermis, 30 hr (220). $T_s$ may even
vary in the same tissue under different conditions, as in
the intestinal epithelium of mice, where $T_s$ in chronically
irradiated mice is 1 hr shorter than in controls (137). In
Ehrlich ascites cells $T_s$ is shorter in diploid than in near-
tetraploid lines (55). Actually, in the same hypotetraploid
line, both $T_s$ and $T_m$ may vary with the sex of the
recipient mouse. In the Ehrlich tumor described by
Baserga and co-workers, $T_s$ and $T_m$ last, respectively, 11
and 18 hr in male mice (8) and 17 and 36 hr in females (9).
Thus, the original assumption of the constancy of the $S$
phase in mammalian cells is no longer tenable.

DNA synthesis is only one of several synthetic activities
that take place during the $S$ phase. Cells synthesize
RNA throughout the entire interphase, as evidenced by
the fact that nearly all interphase cells are labeled by a
brief exposure to a radioactive RNA precursor (94, 237).
In the ciliated protozoan $Euplotes$, Prescott has shown that
dNA and RNA syntheses are mutually exclusive, as
evidenced by the lack of uptake of RNA precursors in
cells in DNA synthesis and in other cells in interphase (6).
Thus, the original assumption of the constancy of the $S$
phase in mammalian cells is no longer tenable.

The rate of protein synthesis, as measured by uptake of
labeled amino acids, is increased during the $S$ phase. This
was shown directly in protozoa by Prescott (192), in
onion root meristems by Olszewksa (173), and in mam-
malian cells by Baserga (7) and Baserga and Kisielewski
(13). This conclusion is also supported by the findings of
Bloch and Godman (29) and De (54) on histone synthesis
and by those of Lipkin et al. (144) that the uptake of radio-
active leucine by the small intestine is higher in crypt cells than in villus cells, where no DNA synthesis occurs. However, DNA and protein synthesis may proceed independently from each other, as shown by Maaloe (147) in bacteria, and by Rueckert and Mueller (207) and Rusconi (208) in mammalian cells.

Postsynthetic phase.—In this phase, the G2 phase, cells synthesize RNA and proteins but not DNA (6, 7, 94, 170, 192, 237). The length of the postsynthetic phase is fairly constant in mammalian cells, ranging from $1/4$ to $1/2$ hr (67, 175, 197, 228). There are exceptions, like cells of the ear epidermis of mouse (220), pre-enamel cells of the developing enamel organ in teeth of young rabbits, in which the G2 phase lasts from 6 to 9 hr (229), some near-tetraploid ascites tumors of mouse with a G2 phase of about 6 hr (8, 55), and mouse spermatogonia, that have a variable G2 time (163).

Mitosis.—The duration of mitosis has been estimated to vary in different tissues from 30 min to 2.5 hr (71, 99, 139, 201, 250). It may be mentioned briefly that, during mitosis, protein synthesis reaches a minimum (7, 13) and RNA synthesis is limited to early prophase and late telophase (51, 194). The end result of mitosis is reduction of the amount of DNA per cell to the diploid value (236) characteristic of the species (30).

Postmitotic phase.—At completion of mitosis, the cell enters the G1 phase. Of the 4 phases of the cell cycle, this is the most variable in length (67, 159, 232). It is very short, practically undetectable by ordinary methods, in the slime mold Physarum polycephalum (170), in the sea urchin embryo (153), in Ehrlich ascites cells growing in the peritoneal cavity of male mice (8), and in pre-enamel cells of young rabbits (229), while in other mammalian cells it may last from a few hours to several months (181). It is also the phase in which most cells with a long cycle time stop for an indefinite time as evidenced by the following findings: (a) in tissue cultures the S and G2 phases are constant and variations in length of the cell cycle depend upon variations in length of the G1 period (224, 238); (b) in adrenal gland of rats, the length of the cell cycle varies from 80 hr in cells of the glomerulosa to about 250 hr in those of the outer fasciculata. Since the length of S, G2, and mitosis appears to be the same, the phase that varies in length must be the G1 (67); (c) in regenerating liver, the curve for percentage of hepatocytes in DNA synthesis at different intervals after partial hepatectomy precedes by about 6–8 hr, both in initial elevation and peak incidence, the curve for mitotic index (87, 138). There are, however, exceptions. Gelfant (75) was the first to suggest that some cells of mouse ear epidermis may be arrested, not in G1, but in the G2 phase, from which they entered mitosis after a suitable stimulation. Cameron and Cleffmann, taking advantage of the fact that cell proliferation, depressed by starvation, can be stimulated by refeeding (133, 164), have determined variations in mitotic and thymidine indices of starved chickens with time after refeeding. These authors were able to show that practically all epithelial cells of the duodenum were resting in G1, while a sizable portion of esophageal cells were in G2, from which they proceeded directly to mitosis (43). Starkey (229) also found that, in rabbit teeth, enamel is elaborated by cells that have synthesized DNA without proceeding to mitosis, and a similar situation has been reported by Owen and MacPherson (174) in rabbit osteocytes. These cells arrested in the G2 phase must contain at least twice the amount of DNA of interphase diploid cells, that is, they are polyploid cells, a condition that is frequently found in liver cells and other tissues of rodents (39, 105, 235, 236) and is the rule in myocardial cells of man (209). Despite these exceptions, in most instances, cells rest in the G1 period of the cycle (165).

Cellular sites of nucleic acid and protein syntheses.—There is general agreement that DNA is synthesized in the nucleus, with the exception of DNA viruses, which can replicate in the cytoplasm (112), and possibly of the DNA fraction located in mitochondria (76). It is also agreed that proteins are synthesized in the nucleus as well as in the cytoplasm (14, 172, 247), but there is still some uncertainty as to the sites of synthesis of RNA. This subject has been reviewed recently by Graham and Rake (84). Briefly, we can say that the bulk of evidence indicates that RNA is synthesized in the nucleus, from which it migrates to the cytoplasm (83, 223). A large portion of RNA has a high turnover rate and breaks down rapidly after being synthesized (95). Soluble RNA and ribosomal RNA are also synthesized in the nucleus (40, 45, 78), ribosomal RNA actually in the nucleolus (36, 154, 183, 184), and then transferred, at least in part, to the cytoplasm (223).

THE STEADY STATE SYSTEM

Many tissues of the adult animal are in a steady state in respect to the total number of cells. This means that not only does the total number of cells remain constant, but the number of cells in each compartment also remains constant. A good example of a steady state system is the lining epithelium of the mucosa of the small intestine (132, 198). The epithelial cells that line the intestinal mucosa belong to 2 distinct compartments: the crypt and the villus. The crypt is where cell division occurs (28) and it is called the progenitor compartment. The villus is the functional compartment, where cells do not divide. While cells from the crypt migrate to the villus, cells almost never return from the functional into the progenitor compartment (198). Each time a cell in the crypt divides into 2 daughter cells, another cell migrates from the crypt into the villus. Similarly, in the villus, for every cell entering it from the crypt, another cell leaves at the tip and is shed into the intestinal lumen, where it dies (132, 136, 198). If the number of cells remains constant, it follows that 1 of the 2 cells produced by cell division is destined to die without dividing again. It would be tempting to speculate that, of 2 sister cells produced by each division, 1 divides again and the other migrates to the villus and dies. This is not necessarily true and it is possible that, in some instances, both sister cells divide again, while in other instances both sister cells leave the crypt without any further division. Leblond (130) inclines to the opinion that, at least in esophagus and intestinal epithelium, the decision as to whether a proliferating cell will differentiate or continue to divide takes place during interphase and depends on local environment.

The various parameters of cell division and migration in...
the lining epithelium of the intestine have been described in detail, in mouse by Quastler and Sherman (198) and Lesher et al. (136), in rat by Leblond and Stevens (132), and in man by Lipkin et al. (145), and are substantially similar in various species. Analogous systems are found elsewhere in the body, for instance in squamous epithelium lining the skin and mucosa of internal cavities (182).

Steady state systems like bone (113) and bone marrow (32, 177, 180) are rather more complicated, but they are also characterized by the fact that the total number of cells remains constant, with cell production balanced by cell loss. In the rat, several cell populations, like those of the kidney, pancreas, or skeletal muscle, that in man are considered in a steady state system because the weights of the organs plateau in adult life (199), are in fact slowly expanding until 1 year of age (60). During this time, the only tissue that shows no increase in cell number is cerebellum (60). After 1 year of age, however, these expanding cell populations also become steady state systems (130).

**LENGTH OF CELL CYCLE**

We have defined \( T_c \) as the mean transit time around the entire cell cycle, that is, the time elapsed between the midpoints of 2 successive mitoses. Sisken and Kinosita (224) have determined the length of the cell cycle of individual cells in tissue culture by time-lapse microcinematography. These authors followed individual cells in a colony of human amnion cells from one anaphase to the next and found \( T_c = 21.6 \text{ hr} \pm 0.5 \). In vivo measurements of individual cells are not available yet, and thus, \( T_c \) usually gives the mean transit time for a cell population without any knowledge of individual variations. Variations in length of the cell cycle by individual cells may make \( T_c \) rather meaningless. For instance, if a population of cells consists of 2 distinct sub-populations, one with a long cell cycle and a second one with a short cell cycle, the mean cycle time \( T_c \) may mean very little. Such populations have been described in the forestomach of mice by Wolfsberg (253) and in macrophages of pulmonary alveoli of mice by Shorter et al. (222). Even in a more homogeneous population, individual cycle times of cells may vary greatly about the mean although the direct measurements of Sisken and Kinosita (224) indicate small variations, a possibility confirmed by the findings that the length of \( T_c \) may be measured by the interval between 2 successive peaks of labeled mitoses (70), and that in bacteria the cycle time has a normal distribution (120). However, in mammary tumors of mice, the 2nd and 3rd waves of labeled mitoses are lower than the 1st peak, and the cells have completely desynchronized by the 4th generation. Together with the damped 2nd wave of labeled mitoses described by Johnson in a transplantable mouse sarcoma (108), they indicate a considerable amount of variance in cycle times of individual cells, at least in some populations of cells. However, until methods become available for determining \( T_c \) of individual cells in vivo, one should not reject as meaningless the measurements that can be obtained by the methods to be described below.

The classic methods for studying some of the parameters of cell proliferation have been based on the use of colchicine (24, 129, 231), and a combined method for determining \( T_c \) using both thymidine-\(^{3}H\) and colchicine, has been introduced recently by Van't Hof and Ying (244) and applied to root meristems of *Pisum sativum*. Among the methods using only high-resolution autoradiography and labeled DNA precursors, the one illustrated in Chart 1 probably represents the most straightforward way of determining \( T_c \). Unfortunately, it is not always feasible because, as mentioned above, some cell populations desynchronize in 1 generation and the 2nd wave of labeled mitoses fails to materialize (108). The possibility exists that failure of the 2nd wave of labeled mitoses to materialize may be due to a statistical error inherent in a small number of observations. For instance, in his original description of 28 mammary tumors of C3H mice, Mendelsohn (155) could not detect a 2nd wave of labeled mitoses, but when an additional 92 tumors were investigated, he was able to recognize that the 1st wave of labeled mitoses was followed by 2 other recognizable and equally spaced waves (158). It is therefore conceivable that the method described by Fry et al. (70) may turn out to have a wider application than presently allowed.

Another way of determining \( T_c \) is based on the fact that \( T_c \) can be determined independently in an accurate way (see above), and that the relation between \( T_c \) and \( T_e \) can be expressed by the equation:

\[
N_e / N_r = T_e / T_c
\]

where \( N_r \) is the number of cells labeled by a pulse-exposure to thymidine-\(^{3}H\) and \( N_e \) is the total number of cells in the population. This equation is valid in an ideal system (197), but, in practice, it can be used in only a few selected instances. Apart from the fact that \( N_r \) may undergo diurnal fluctuations (68, 185), a serious shortcoming of Equation B is that it requires the assumption that all cells in the population participate in the proliferative process. Actually, most proliferative compartments contain admixtures of cells which are not going to reproduce. The ratio \( N_e / N_r \) will then be less than the ratio \( N_r / N_p \), where \( N_p \) is the total number of cells in the proliferative cycle (157). The result is an overestimate of the length of the cell cycle. For instance, \( T_c \) in the epithelial cells lining the small intestine of mice is 7 hr. The ratio \( N_e / N_r \) is about 0.065 if determined on all epithelial cells lining the intestinal epithelium, that is, epithelial cells of the crypts as well as non-dividing cells of the villi. If only crypt epithelial cells are counted, the ratio is 0.45. By rearranging and substituting in Equation B, one would obtain 2 values of \( T_c \) of 107 and 15 hr, respectively, a substantial difference. When using Equation B it is therefore important to know \( N_p \), defined by Mendelsohn (155, 157) as the Growth Fraction, or the fraction of cells in the total population that participate in the proliferative process. Since \( N_p = N_e / p \) (where \( p \) is the fraction of proliferating cells), the problem is how to determine \( p \) in a cell population in vivo. One way around it is to obtain \( T_e \) from the equation:

\[
T_e = T_c \cdot M^*/M
\]

where \( M^* \) is the number of labeled mitoses and \( M \) the total number of mitoses (157). The equation requires an
independent determination of $T_c$, followed by a determination of $M*/M$ after the labeled cells have desynchronized. A difficulty with this method is that if one waits too long for cells to become properly desynchronized, the concomitant dilution of the label makes recognition of labeled cells increasingly uncertain. On the other hand, if cells desynchronize rapidly, the $T_c$ obtained will represent a mean to which individual cells confirm very poorly (157). An alternative is to determine the fraction of proliferating cells, $p$, from the number of cells that are labeled by continuous exposure to thymidine-$^3$H provided only the progenitor compartment is taken into consideration. In tissue cultures, $p$ can be determined by incubating cells with thymidine-$^3$H and observing the number of cells that become labeled over an extended period of time (228). An in vivo modification was applied by Baserga et al. (15) to Ehrlich ascites cells growing in lungs of mice. Tumor-bearing animals were injected every 4 hr with thymidine-$^3$H, and since all cells were found to be labeled after 6 injections, it was concluded that all cells participated in the proliferative process, that is $p = 1$. Continuous incubation or repeated injections can be replaced by continuous infusion of thymidine-$^3$H (156). When the latter method was used in mammary tumors of C3H mice, a number of morphologically intact tumor cells remained unlabeled even after infusions as long as 10 days. This indicated that $p$ in these tumors was considerably below 1. Precise measurements showed $p = 0.18$ (158).

Another method for determining $T_c$ is based on the dilution of the label. In a population of labeled cells in which all cells divide, the specific activity of DNA decreases in direct proportion to the number of cell divisions (150, 202). In autoradiographs, the decrease in specific activity of DNA can be followed by determining the mean grain count of cells pulse-labeled with thymidine-$^3$H. Using this principle, Baserga et al. (18) have proposed a method based on the distribution of grain counts that gives at the same time $p$ and $T_c$. The method requires 2 determinations of the mean and distribution of the grain counts of a cell population at different intervals after a single injection of thymidine-$^3$H. The 1st determination must be taken after the labeled cells have undergone the 1st division following exposure to thymidine. From the grain count mean and variance at time $t$, and at time $t + \Delta t$, it is possible to obtain the proportion of cells that have not divided, $a$, that have divided once, $b$, and that have divided twice, $c$, in the interval $\Delta t$ (18).

The length of the cell cycle, $T_c$, of the proliferating subpopulation is then given by:

$$T_c = \frac{\Delta t}{1 + c}$$  \hspace{1cm} (D)

The method requires that the interval $\Delta t$ be $> T_c < 2T_c$. It gives a good first approximation of both $p$ (in this case, $1 - a$) and $T_c$, but it again requires a rather rapid desynchronization of labeled cells. A more elaborate statistical method has been proposed recently by Tyler and Baserga (241), but it would go beyond the purpose of the present discussion.

In conclusion, while there are several methods that can be used to determine $T_c$, each of them has limitations that restrict its use to selected situations. In practice it is often difficult to differentiate nonproliferating cells from cells with a very long cycle, the $G_0$ cells of Patt and Quastler (181). For instance, there are cells with a long cell cycle which are still capable of DNA synthesis and which, under proper conditions may shorten the length of the cycle. A remarkable example of this kind is the liver, ordinarily a rather quiescent tissue with a low thymidine index (11, 58, 162, 221), which can markedly increase its capacity for DNA synthesis during the regenerative phase that follows partial hepatectomy (37, 38, 87, 138). Epithelial cells of the anterior surface of the crystalline lens (92), basal cells of the epidermis (98), smooth muscle cells of arteries and arterioles (50), regenerating skeletal muscle cells (27, 120), and kidney cells (100) are also cells known to respond to an appropriate stimulus with an increase in the number of cells synthesizing DNA and a shortening of the cycle time. Other cells, like keratinizing epithelial cells of the upper layer of the epidermis, various neurons, cells of the nervous layer of the retina, and polymorphonuclear leukocytes are not known to be capable of taking up thymidine, at least in the adult mouse (58, 162, 221), and should be considered as cells that have left the cycle.

Because of the limitations mentioned above, most of the measurements of $T_c$ reported in the literature must be considered with caution. A large majority of these measurements are simply based on determinations of $I$ and $T_c$ and the use of equation B, with its attendant inconveniences. In only a few instances has $T_c$ actually been determined, either by the method of Fry et al. (70) or by using $N_e$ to modify equation B. Some of these measurements and estimates in man and mice are summarized in Table 1. The figures given for human tissues are all approximate estimates but in mice we have some measurements that can be accepted with greater confidence. For instance, the length of the cell cycle in the epithelium of the small intestine has been determined by the method of Fry et al. (70), and that of the Ehrlich ascites tumor by using Equation B, since, in this tumor in the exponential phase, $p = 1$ (146). It will be noted that the length of the cell cycle is shorter in the non-growing normal adult tissue than in the rapidly growing Ehrlich tumor.

**KINETICS OF TUMOR GROWTH**

The simplest equation that describes the growth of a tumor is of the exponential type $y = b^t$, and it seems to fit best some ascites tumors. Patt and Blackford (178) used it to describe the 1st week of growth of Krebs ascites tumor, Maruyama (150) fitted it to the growth of LSA ascites tumor, and Baserga (9) found an exponential pattern of growth in Ehrlich ascites tumor growing in the peritoneal cavity of mice, regardless of the method used to estimate growth, e.g., number of tumor cells, dry weight of tumor cells, or total amount of tumor DNA. In all these cases, however, the curves flatten after the 1st week or so of growth, usually when the total number of tumor cells per mouse reaches a figure of 500-600 million cells (9, 178). Probably for this reason, Klein and Revesz (121) thought that the growth curve of Ehrlich ascites carcinoma was better expressed in terms of the cube roots of tumor cell...
numbers. If a generalization must be drawn from the growth curves proposed for ascites tumors, it is perhaps the conclusion reached by Patt and Blackford (178) that growth curves proposed for ascites tumors, it is perhaps the initial stages grow exponentially, exponential equations are of limited value in describing the actual growth of a solid tumor (157). Mendelsohn (158) has reiterated that solid tumors, like mammary tumors of mice and human tumors, do not conform to an exponential pattern of growth, and that different equations must be used to describe the growth pattern of tumors in which the nonproliferating subpopulation of tumor cells constitutes a substantial part of the total cell population. A plausible model for such a mixed population is the stochastic model of cell proliferation and differentiation proposed by Till et al. (239), based on a "birth-and-death" process, for details of which we must refer the reader to the original paper. The situation is further complicated by the fact that a tumor is not a closed system in which there is no cell loss from the compartment. To begin with, necrosis, that is, cell death, is a frequent occurrence, especially in solid tumors. In addition to cell loss because of necrosis, of individual cells or of entire portions of the tumor, cell loss may occur because of migration of cells out of the main proliferative compartment. Metastases are instances of cell migration, although the number of metastasizing cells is usually negligible (47, 257) and secondary colonies established by tumor cell emboli can be considered as independent compartments. More important is migration of cells in surrounding tissues, which is of such magnitude in some ascites tumors as to produce an actual decrease in total number of cells in late stages of growth, although some cell proliferation is still taking place (9, 59).

The growth pattern of a tumor is important not only in any attempt to understand how a tumor grows, but also because the kinetics of the system require corrections in the equations that we have found useful for measuring $T_\alpha$ in a steady state system. For the purpose of the present discussion, we shall limit ourselves to corrections necessary in case of exponential growth. Although this is not always correct, as mentioned above, we should remember that the error may be of little value in comparative studies. For instance, Reiskin and Mendelsohn (201), using an equation valid for linear growth, estimated the cell cycle time of induced epithelial tumors of the hamster pouch at 20.7 hr, and assuming an exponential growth at 17.5 hr. The difference may seem substantial but it becomes much less impressive when it is compared to the cell cycle time of the normal counterpart, the squamous epithelium of the hamster pouch, estimated at 142 hr.

Several corrections have been proposed in the case of exponentially growing populations (59, 108, 135), most of which are useful in particular situations. We have found convenient, in studying the Ehrlich ascites tumor, to adopt the correction proposed by Johnson (108), the general statement of which is expressed in the equation:

$$\frac{dN}{dt} = \ln 2 \cdot N \quad (F)$$

Since both $T_\alpha$ and $N$ can be determined experimentally, $dN/dt$ can be obtained by substitution. If $T_\alpha$ is unknown, $dN/dt$ can be calculated from the equation given by Stanners and Till (228).

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Estimated length of cycle</th>
<th>Reference no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAN:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermis</td>
<td>1.5 mo</td>
<td>110</td>
</tr>
<tr>
<td>Polychromatophil normoblasts</td>
<td>15-18 hr</td>
<td>179</td>
</tr>
<tr>
<td>Epithelium of large intestine</td>
<td>25 hr</td>
<td>145</td>
</tr>
<tr>
<td>Epithelium of large intestine</td>
<td>53 hr</td>
<td>46</td>
</tr>
<tr>
<td>Carcinoma of colon, metastases</td>
<td>27 hr</td>
<td>10</td>
</tr>
<tr>
<td>Leukemic blast cells</td>
<td>50-80 hr</td>
<td>118</td>
</tr>
<tr>
<td>Carcinoma of stomach</td>
<td>66 hr</td>
<td>10</td>
</tr>
<tr>
<td>Carcinoma of colon, metastasis</td>
<td>75 hr</td>
<td>10</td>
</tr>
<tr>
<td>Multiple myeloma cells</td>
<td>2-6 days</td>
<td>117</td>
</tr>
<tr>
<td>Carcinoma of liver</td>
<td>10 days</td>
<td>10</td>
</tr>
<tr>
<td>Glioblastoma multiforme</td>
<td>1 mo</td>
<td>109</td>
</tr>
<tr>
<td>Ovarian cystadenoma</td>
<td>1.5 mo</td>
<td>110</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>1.5 mo</td>
<td>110</td>
</tr>
<tr>
<td>Carcinoma of breast</td>
<td>3 mo</td>
<td>110</td>
</tr>
<tr>
<td>Leiomyoma of spermatic cord</td>
<td>5.5 mo</td>
<td>110</td>
</tr>
<tr>
<td><strong>MOUSE:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium of small bowel</td>
<td>11.6 hr</td>
<td>136</td>
</tr>
<tr>
<td>Epithelium of epidermis</td>
<td>150 hr</td>
<td>57</td>
</tr>
<tr>
<td>Bronchial epithelium</td>
<td>18 days</td>
<td>122</td>
</tr>
<tr>
<td>Trophoblast cells, 12th-day placenta</td>
<td>15 hr</td>
<td>42</td>
</tr>
<tr>
<td>Ehrlich ascites tumor</td>
<td>18 hr</td>
<td>8</td>
</tr>
<tr>
<td>Skin cancer</td>
<td>32 hr</td>
<td>57</td>
</tr>
<tr>
<td>Melanoma (transplanted)</td>
<td>2.5 days</td>
<td>26</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>1-3.5 days</td>
<td>159</td>
</tr>
</tbody>
</table>

These manipulations are valid in an exponentially growing population, but since only ascites tumors in their initial stages grow exponentially, exponential equations are of limited value in describing the actual growth of a solid tumor (157). Mendelsohn (158) has reiterated that solid tumors, like mammary tumors of mice and human tumors, do not conform to an exponential pattern of growth, and that different equations must be used to describe the growth pattern of tumors in which the nonproliferating subpopulation of tumor cells constitutes a substantial part of the total cell population. A plausible model for such a mixed population is the stochastic model of cell proliferation and differentiation proposed by Till et al. (239), based on a "birth-and-death" process, for details of which we must refer the reader to the original paper. The situation is further complicated by the fact that a tumor is not a closed system in which there is no cell loss from the compartment. To begin with, necrosis, that is, cell death, is a frequent occurrence, especially in solid tumors. In addition to cell loss because of necrosis, of individual cells or of entire portions of the tumor, cell loss may occur because of migration of cells out of the main proliferative compartment. Metastases are instances of cell migration, although the number of metastasizing cells is usually negligible (47, 257) and secondary colonies established by tumor cell emboli can be considered as independent compartments. More important is migration of cells in surrounding tissues, which is of such magnitude in some ascites tumors as to produce an actual decrease in total number of cells in late stages of growth, although some cell proliferation is still taking place (9, 59).

The growth pattern of a tumor is important not only in any attempt to understand how a tumor grows, but also because the kinetics of the system require corrections in the equations that we have found useful for measuring $T_\alpha$ in a steady state system. For the purpose of the present discussion, we shall limit ourselves to corrections necessary in case of exponential growth. Although this is not always correct, as mentioned above, we should remember that the error may be of little value in comparative studies. For instance, Reiskin and Mendelsohn (201), using an equation valid for linear growth, estimated the cell cycle time of induced epithelial tumors of the hamster pouch at 20.7 hr, and assuming an exponential growth at 17.5 hr. The difference may seem substantial but it becomes much less impressive when it is compared to the cell cycle time of the normal counterpart, the squamous epithelium of the hamster pouch, estimated at 142 hr.

Several corrections have been proposed in the case of exponentially growing populations (59, 108, 135), most of which are useful in particular situations. We have found convenient, in studying the Ehrlich ascites tumor, to adopt the correction proposed by Johnson (108), the general statement of which is expressed in the equation:
work of Killmann et al. (118) who stated "... the general concept of acute leukemia as a disorder of rapid proliferation process but involves other parameters of normal cells (10, 56, 148, 214), and that the growth of a tumor is not due exclusively to an acceleration of the proliferative pool and the amount of cell loss through normal mouse liver and in hepatomas, but in the former exception of cells of the pyloric mucosa and of the crypts of the small intestine (23). Baserga and co-workers, on the basis of the dilution of the label, indicated that some normal cells proliferated at a faster rate than tumor cells, in both mice (12) and man (10), and Killmann et al. (118), investigating in vivo the cell cycle of human leukemic cells, found that the estimated cycle time in leukemic blast cells was equal to or longer than the cycle time of their normal counterparts, that is the cells of the adult animal from which the tumors originated, by rigorous kinetic methods. In addition to the comparison reported above by Reiskin and Mendelsohn (201), Dörner et al. (57) compared cycle times of mouse epidermis and chemically induced skin carcinomas and found cycle times, respectively, of 32 and 150 hr. On the other hand, Post et al. (187, 188), comparing cycle times of rat liver cells and chemically induced hepatoma cells, found that normal cells had a shorter cycle time, 21.5 hr, against 31 hr for hepatoma cells. Other comparisons between tumor and normal tissues have been based on the use of daily mitotic index or thymidine index. Bertalanffy and Lau (25) found that daily mitotic rates of certain rat tumors were higher than daily mitotic rates of most normal tissues, with the exception of cells of the pyloric mucosa and of the crypts of the small intestine (23). Baserga and co-workers, on the basis of the dilution of the label, indicated that some normal cells proliferated at a faster rate than tumor cells, in both mice (12) and man (10), and Killmann et al. (118), investigating in vivo the cell cycle of human leukemic cells, found that the estimated cycle time in leukemic blast cells was equal to or longer than the cycle time of normal neutrophil precursors. Although it may be possible that in many instances tumor cells proliferate faster than their normal counterparts, the concept is gaining acceptance that tumor cells do not necessarily proliferate faster than normal cells (10, 56, 148, 214), and that the growth of a tumor is not due exclusively to an acceleration of the proliferative process but involves other parameters of cellular kinetics, such as the fraction of cells participating in the proliferative pool and the amount of cell loss through death or migration. This concept, expressed in 1962 by Baserga and co-workers, has found confirmation in the work of Killmann et al. (118) who stated "... the general concept of acute leukemia as a disorder of rapid proliferation... should be discarded as a general concept of the nature of leukemia," and, more recently, in the findings of Post and Hoffman (187, 188). The picture may be more difficult to comprehend than the traditional uncomplicated picture of tumor cells proliferating at full speed, but it is probably closer to the truth and opens new horizons for a proper design of chemotherapeutic or radiotherapeutic procedures. CONTROL OF DNA BIOSYNTHESIS

An increased production of cells, caused by either shortened length of the cell cycle or increased size of the proliferating pool, ultimately means an alteration in the control of cell division. In previous sections, we have seen that a cell that has synthesized DNA is generally a cell that has already made the decision to divide. Control of cell division (233, 234) then becomes control of the initiation of DNA biosynthesis. Factors that may intervene in temporal regulation of DNA biosynthesis (127) are conveniently grouped in: (a) factors that may control DNA synthesis through the availability of DNA precursors and related enzymes; (b) the physical state of the DNA molecule; and (c) regulatory mechanisms related to the synthesis of RNA and proteins.

DNA synthesis in vitro requires primer DNA, the 4-component deoxynucleotides, and the enzyme, DNA polymerase (125), and it is reasonable to assume that the availability of precursors and enzyme is a necessary condition for the synthesis of new DNA. However, it may well be that both precursors and enzymes are manufactured in response to a message issued by the cell after it has taken the decision to synthesize DNA. Several kinds of evidence suggests that the concentration of DNA precursors and related enzymes may be determinant in the initiation of DNA synthesis: (a) deoxynucleotides, although present in small amounts in tissues of the adult animal (210), are found in increased amounts in proliferating tissues, like regenerating liver (212) and tumor tissue (206, 211); (b) pyrimidines, purines, and nucleotides decrease the helix-coil transition temperature of thymus DNA, that is, they favor the transition of the DNA molecule from the 2-stranded helical form to the single-stranded coiled form (240); (c) the concentration of enzymes concerned with the phosphorylation of thymidine and the polymerization of deoxynucleotides, such as thymidine kinase, thymidylate synthetase, and DNA polymerase, increases in proliferating tissues (53, 86, 106, 171). However, in regenerating liver, the activity of these enzymes reaches a maximum after the rate of DNA synthesis has begun to decline (31, 101). Similarly, in cultures of L cells, DNA polymerase activity increases when DNA synthesis is inhibited and decreases when DNA synthesis is allowed to proceed (82). An interesting alternative has been suggested by Kielley (115), who found that thymidylate kinase activity was about equal in normal mouse liver and in hepatomas, but in the former the activity was particle-bound and did not become evident until after disruption of the cellular constituents. If one had to evaluate the present evidence on the concentration of enzymes and precursors in relation to DNA synthesis, one would be tempted to conclude that while these
are necessary for the **continuation** of DNA synthesis (232), their role in the **initiation** of DNA synthesis is still somewhat uncertain (190, 191).

The physical state of the DNA molecule may also be important in temporal control of DNA synthesis. For instance, *in vitro* heat-denatured DNA, that is, single-stranded DNA (160), is often a better primer than native DNA (19, 134, 166), and in nondividing bacteria 90% of the DNA is nonfunctional when used as primer *in vitro* (81). Rolfe (204) has shown that the native helical configuration of DNA is altered some time prior to replication, and that part of this altered DNA is associated with proteins. It is difficult to separate this nascent DNA from the associated protein (22, 91). Autoradiographic studies of *Escherichia coli* DNA have confirmed that the DNA molecule, in replicating, unwinds into 2 strands (41). These findings suggest that native, resting DNA must be converted to primer DNA before DNA biosynthesis may begin. However, a biologic system capable of effecting this conversion *in vivo* has not been identified, although it has been suggested that such a role could be attributed to the enzyme DNase (134, 153) or possibly another nuclease (203).

The 3rd group of factors that may be of importance in initiation of DNA biosynthesis deals with the role of RNA and proteins, in particular with the possibility that the initiation of DNA biosynthesis may be preceded by the synthesis of specific RNA and proteins (191, 232). Such a possibility is suggested by the following evidence: (a) the rate of RNA synthesis is higher in mammary glands of mice stimulated by pregnancy than in resting mammary glands (1), and higher in liver of weaning rodents than of adults (226); (b) when liver cells begin to proliferate after partial hepatectomy, there is a sudden increase in the rate of RNA synthesis (38, 242). Amounts of actinomycin D that have no effect upon the normal rate of RNA synthesis completely suppress the rate increase that follows partial hepatectomy and produce a delay in the initiation of DNA synthesis, which usually begins 12–18 hr after operation (72); (c) Lieberman et al. (141, 142) have described changes in the pattern of RNA synthesis in rabbit kidney cortex cells cultured directly from the animal. After explantation, these cells undergo a lag period of 32 hr before DNA synthesis begins. In the 1st stage of this lag period, from 0 to 12 hr, there is increased formation of stable ribosomal RNA. In the 2nd stage, from 12 to 22 hr, there is a sharp increase in the rate of formation of nuclear RNA. This nuclear RNA has properties analogous to messenger RNA of bacteria, and its inhibition by actinomycin D results in complete blocking of DNA synthesis. In the 3rd stage, from 22 to 32 hr, the rate of RNA synthesis remains constant at its new elevated level, and addition of actinomycin D does not result in inhibition of the subsequent DNA synthetic period. In the same system, the concentration of such enzymes as thymidine kinase and DNA polymerase increases only at the time DNA synthesis begins; (d) Giudice and Novelli (80) found that the rise in DNA polymerase that follows partial hepatectomy is inhibited by actinomycin D only when the antibiotic is given 3–8 hr after surgery, while puromycin (79), an inhibitor of protein synthesis, is most effective 13–19 hr after hepatectomy; (e) a similar sequence of events, leading from RNA synthesis to synthesis of thymidine kinase to synthesis of DNA, has been described by Hotta and Stern (103) in microspores of Lilium.

These findings are compatible with a model in which initiation of DNA biosynthesis involves DNA itself and is mediated through RNA and specific enzymes. The model is based on generally accepted concepts that protein synthesis in cells takes place on ribosomes (77, 168, 249), and that the sequence of amino acids, which is responsible for the specificity of proteins, is determined by a sequence of bases in a particular kind of RNA molecule (151, 205). In bacterial cells, this RNA molecule has been identified with short-lived messenger RNA (34, 88) but in mammalian cells there is evidence that at least some of the RNA templates are stable (85, 95, 223). The sequence of bases in template RNA is, in turn, determined by a complementary sequence of bases in one of the DNA strands (89, 93, 149, 227, 245). However, at any time, only a very small portion of the DNA molecule is genetically active, that is, only a small segment of the DNA strand is coding template RNA for the building of specific proteins (2, 243). Thus in the adult organism most of the DNA molecule is genetically inactive (33) and a majority of genes are repressed (65). However, repressed genes may become active and code RNA templates for the coding of specific proteins (52). This situation is apparently true in enzyme induction in bacteria. Exposure of a bacterial culture to a β-galactosidase inducer causes an increased synthesis of a messenger RNA that hybridizes with DNA containing a gene for β-galactosidase (96). This is interpreted as indicating that an important function of the inducer is to inactivate a repressor at the level of transcription of the coding message from DNA to RNA. However, the possibility has not been ruled out that regulation of enzyme synthesis may occur at the level of translation of the message from RNA to protein (230).

As shown by Schwartz et al. (217) and by Lieberman et al. (72, 141, 142), inhibition of RNA synthesis a few hr before initiation of DNA synthesis does in fact inhibit subsequent DNA synthesis, while inhibitors of protein synthesis are effective in preventing initiation of DNA synthesis when administered immediately before replication. On this basis, the sequence of events in initiation of DNA biosynthesis could be compared to enzyme induction in bacteria (114) and higher animals (176) or to the mechanism of action of certain hormones (90), and could be summarized as follows: an environmental stimulus, perhaps a critical concentration of deoxynucleotides (48) or a slight modification in the physical state of the DNA molecule, may inactivate the repressor of a genetically inactive portion of DNA. The inactivation of the repressor would cause the synthesis of a template RNA which, in turn, would code the enzymes involved in the separation of the DNA strands and the polymerization of the component deoxynucleotides. In this context, it may be of interest to quote Swann’s conclusions (234) of a few years ago: "Differentiation during development is brought about by a process known as induction, the essence of which is that a short-lived stimulus of some sort produces a relatively
long-lasting effect on the pattern of synthesis. A number of lines of evidence suggest that stimulation of differentiated cells to divide is in fact an inductive process. . . ."

CONCLUSIONS

In 1955, in a 12-page section dealing with cell division, Cowdry (49) was able to condense our knowledge of cell interphase in a little less than 6 lines. Since then, the amount of information on metabolic activities of the interphase cell has reached such volume that a review of this kind can only touch the most important points. There are still many uncertainties, but one is justified in saying that the life cycle of cells has been mapped out in its broad outline, and that the details are being filled in rapidly. Our knowledge of the cell cycle has had considerable influence on 4 major areas of investigation: kinetics of cellular proliferation, control of DNA biosynthesis, life span of cells, and cell differentiation. The last 2 areas have not been touched in the present review, but are closely interrelated with cellular kinetics and cell division. For the student of cancer, it may be of interest to evaluate the impact exerted on our approach to the investigation of cancer by knowledge of the cell cycle and by permanent tagging of cells with DNA precursors. It is reasonable to say that the kinetics of cellular proliferation, in normal as well as in tumorous tissues, have been made more truly quantitative and closer to the Pythagorean ideal that "the principles of mathematics are also the principles of things as they are" (196). At the same time, emphasis has been shifted from the control of cell division to the control of DNA biosynthesis, a shift that has required and will continue to require considerable reorientation in theoretical concepts and in experimental designs.

REFERENCES

33. Bonner, J., Huang, R. C., and Gilden, R. V. Chromosomally

Downloaded from cancerres.aacrjournals.org on April 19, 2017. © 1965 American Association for Cancer Research.
592 Cancer Research  Vol. 25, June 1965


The Relationship of the Cell Cycle to Tumor Growth and Control of Cell Division: A Review

Renato Baserga


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/25/5_Part_1/581

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.