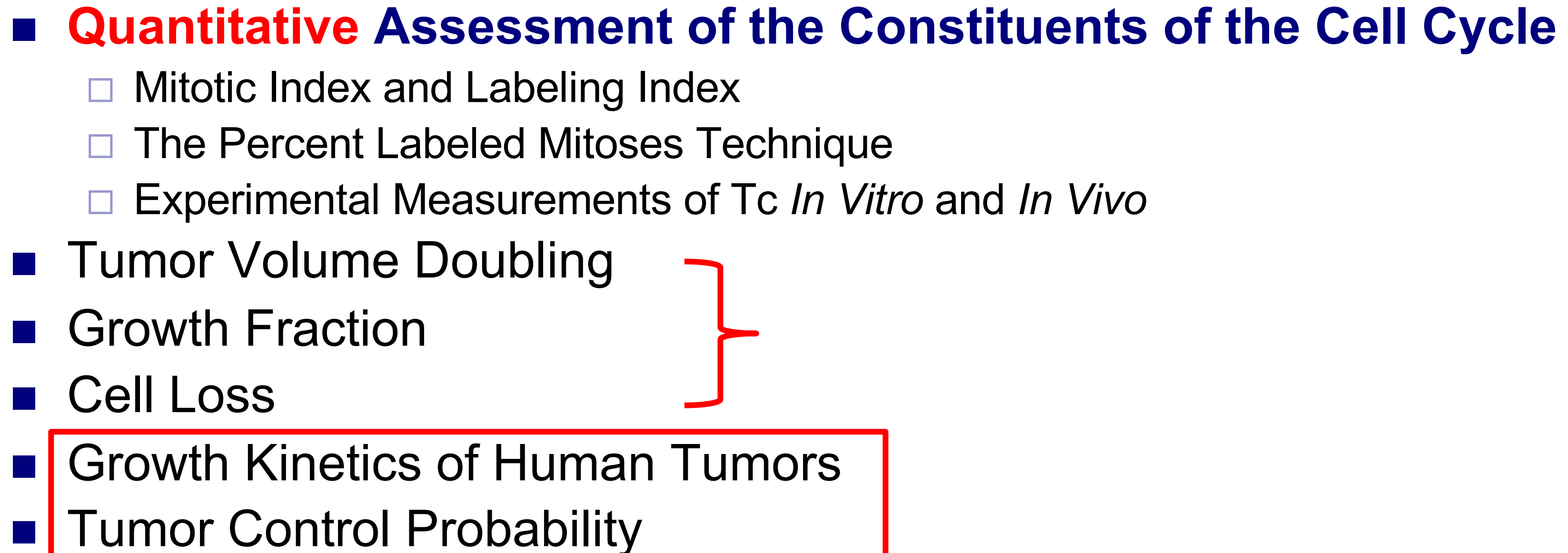
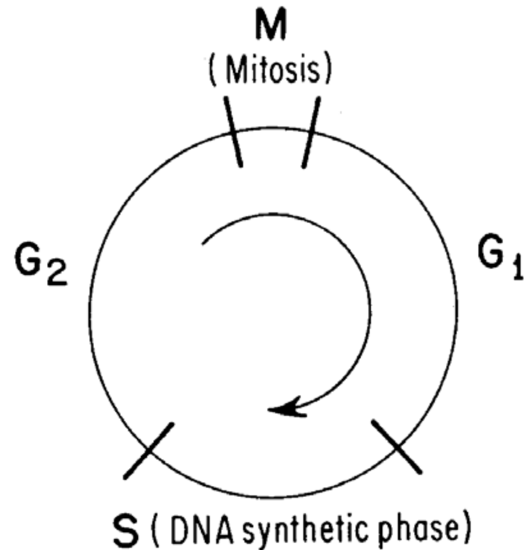
11/25/2024

Outline



Cell Cycle



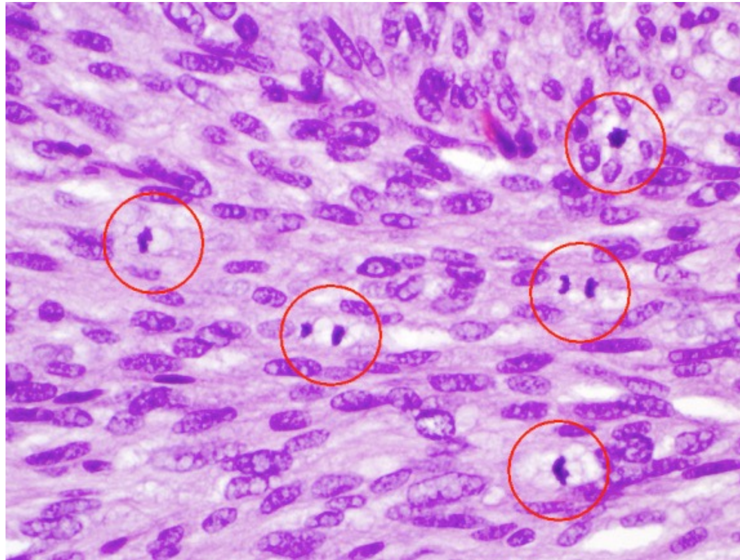
Mammalian cells replicate and increase in number by **mitosis**

Cell cycle in dividing mammalian cells are divided into 4 phases – mitosis (**M**) and DNA synthetic phases (**S**) are separated by 2 gaps (**G_1** and **G_2**)

The average interval between successive mitoses is called the **cell cycle time** or **mitotic-cycle time**

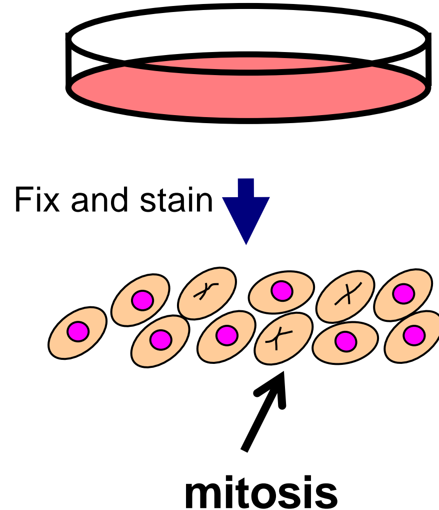
Goal – to obtain the length of each phase of the cell cycle

Cell Labeling Technique



Using conventional light microscope, the only event that can be distinguished is the process of **mitosis**

Mitotic Index

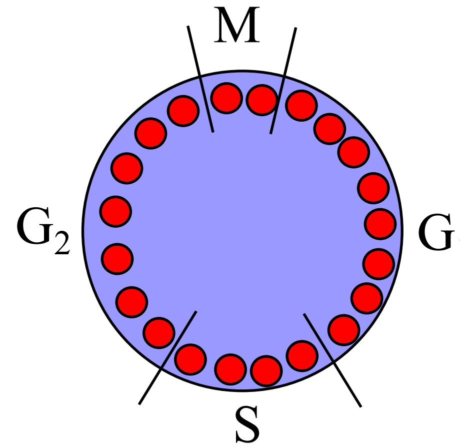


The proportion of cells in mitosis can be counted, this is known as the **mitotic index (MI)**

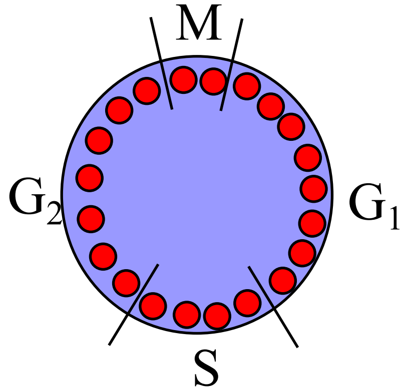
If we assume that all of the cells are dividing, and all cells have the same mitotic cycle then,

$$MI = T_M/T_C$$

T_M is the length of mitosis, T_C is the length of cell cycle

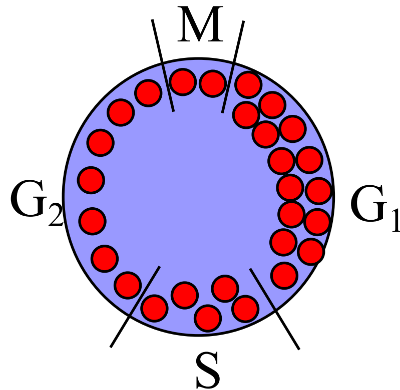


Mitotic Index



However, cells cannot be distributed uniformly in time around the cell cycle because they double during mitosis, therefore a correction factor, λ, is needed

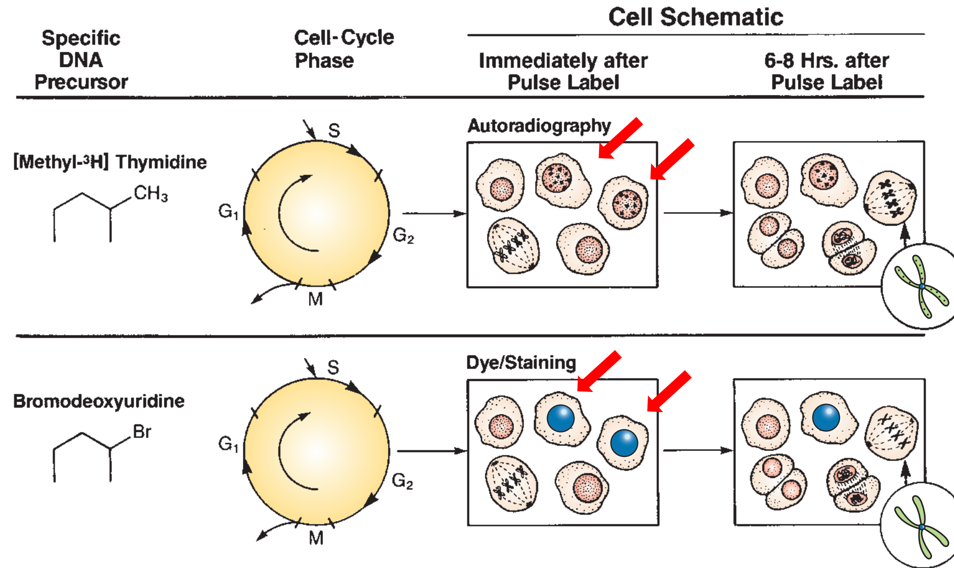
$$MI = \lambda T_M / T_C$$



The simplest assumption is that cells are distributed around the cycle exponentially in time, in which case $\lambda = 0.693$

λ is an unimportant correction factor

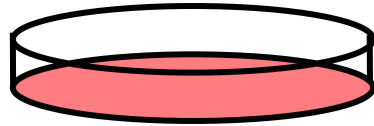
Cell Labeling Technique



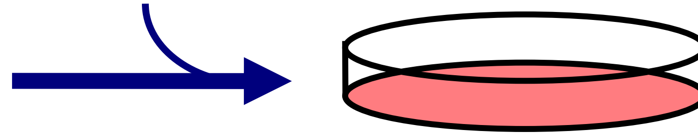
Cell labeling technique using either tritiated thymidine or BrdU allows us to follow the progression of cell cycle

Pulse label is only incorporated in cells undergoing DNA synthesis (**S phase**)

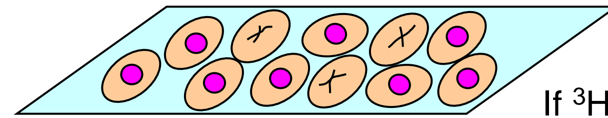
Labeling Index



Label with
^{3}H-TdR or BrdU for 20 mins



Fix

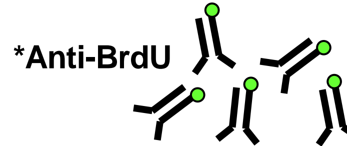


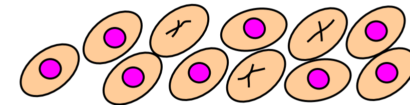
If BrdU labeled

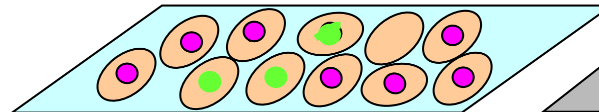
If ^{3}H-TdR labeled

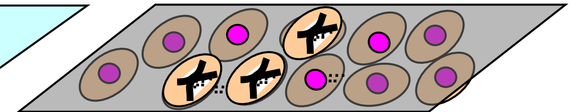
AR film

*Anti-BrdU









If cells are **flash-labeled** before fixing, proportion of cells in synthetic phase (S) can be counted, and we can define **labeling index (LI)**

$$LI = \lambda\ T_S/T_C$$

T_S is the length of DNA synthesis

MI and LI

Mitotic Index (MI) = $\lambda\ T_M/T_C$

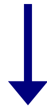
Labeling Index (LI) = $\lambda\ T_S/T_C$

Note that these are **ratios**

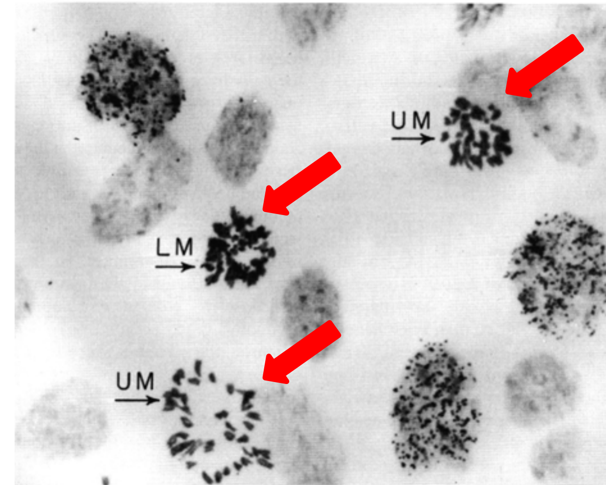
To obtain the **length of each phase**, we need to use the **percent labeled mitoses technique**

Percent Labeled Mitoses Technique

The entire cell population (*in vitro*) or specimen (*in vivo*) is flash labeled

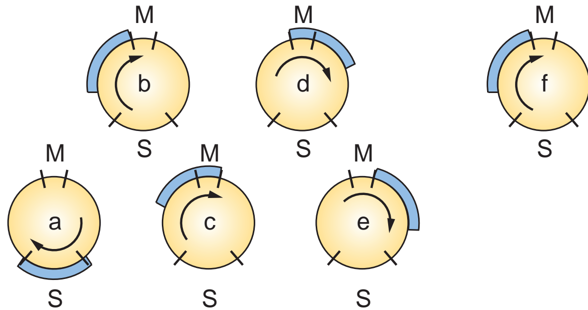


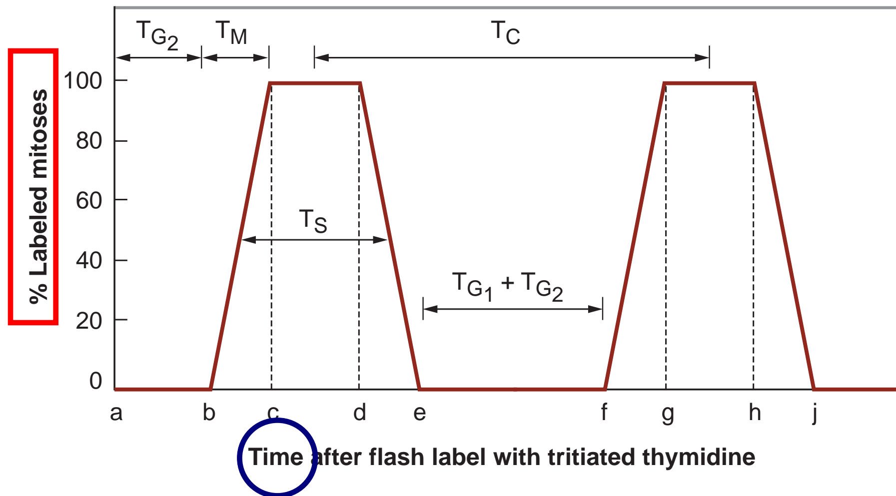
Collect samples ***each hour*** (for total time > T_C), fix, stain & count **% of mitotic cells that carry a radioactive label**



This is a very laborious process because only 1-2% of cells are in mitosis at any given time point, and only a fraction of these will be labeled

Percent Labeled Mitoses Curve



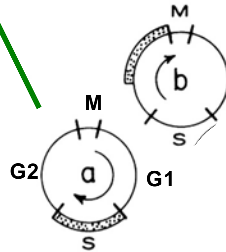


The movement of the labeled cells through the cell cycle is followed

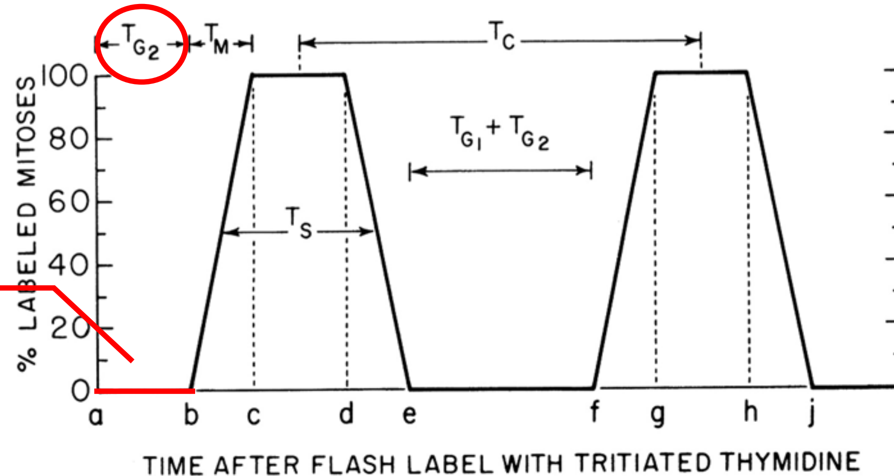
% of labeled mitoses is plotted as a function of time

Length of G_2

At time a, there will be no cells with labeled mitosis



The first labeled mitotic figure appears as the leading edge of the cohort of labeled cells reaches M

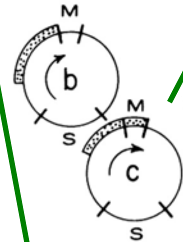


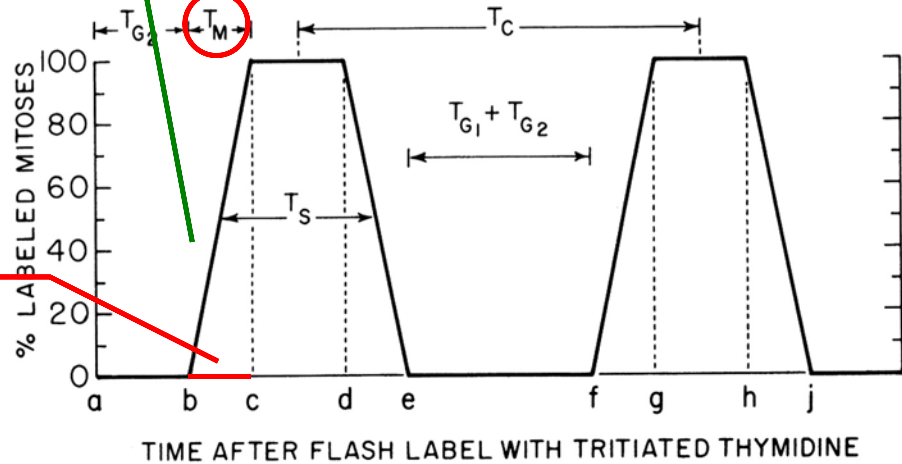
The length of time **ab** thus equals length of $\mathbf{G_2}$

Length of M

The % of mitotic figures labeled ↑ rapidly as the leading edge of the labeled cohort of cells passes thorough the M phase

When the leading edge reaches the end of the M phase, all mitotic figures are labeled (position c)





The length of **bc** thus equals length of **M**

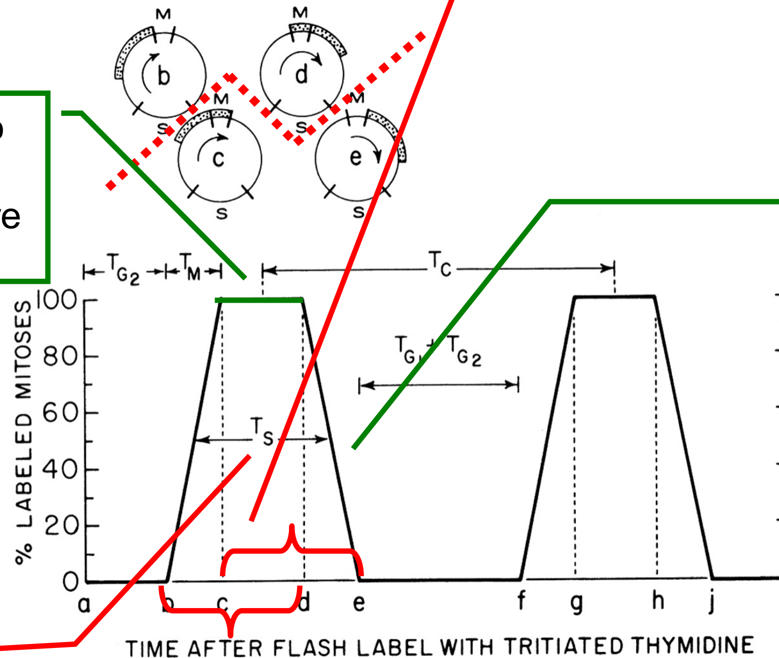
Length of S

The duration **bd** or **ce** is the length of DNA synthesis (**T_S**)

From time **c** to **d**, all of the mitotic cells are labeled

Once the trailing edge reaches the beginning of the mitosis (**d**), the % of labeled mitoses rapidly ↓ and becomes 0% when the trailing edge reaches the end of mitosis (**e**)

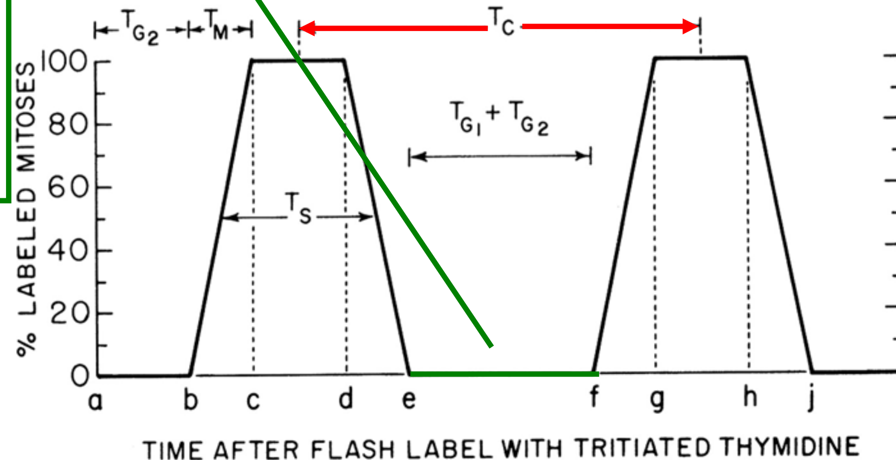
In practice, T_S usually is taken to be the **width of the curve at the 50% level**



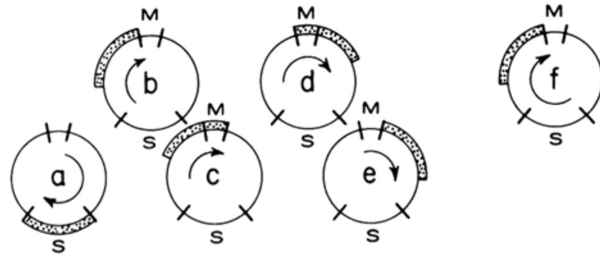
Length of T_C

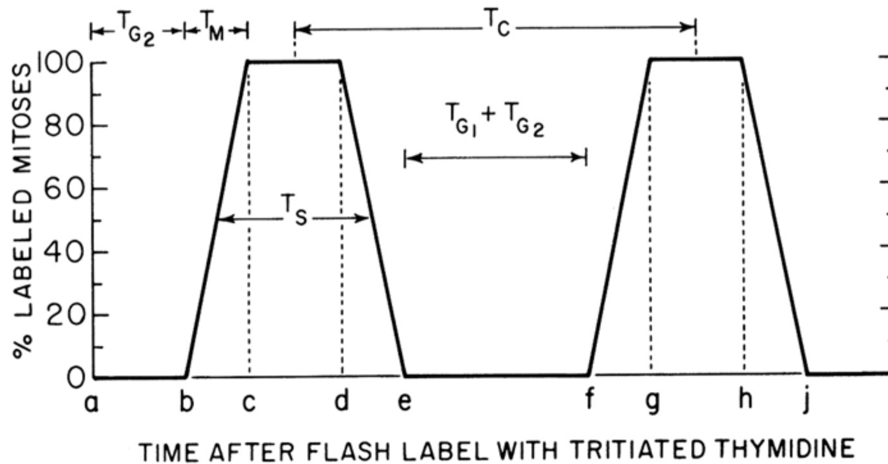
After the trailing edge moves through the end of mitosis (**e**), there is a long interval during which no labeled mitotic figures are seen until the labeled cohort of cells goes around the entire cycle and comes up to mitosis again (**f**)

The total cycle (T_C) is the distance b/w corresponding points on the first and 2nd wave (**bf**, **cg**, **dh**, or **ej**) or the **distance b/w the centers of the 2 peaks**



Length of G_1

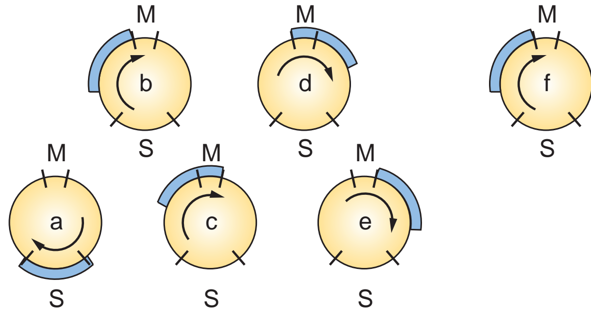


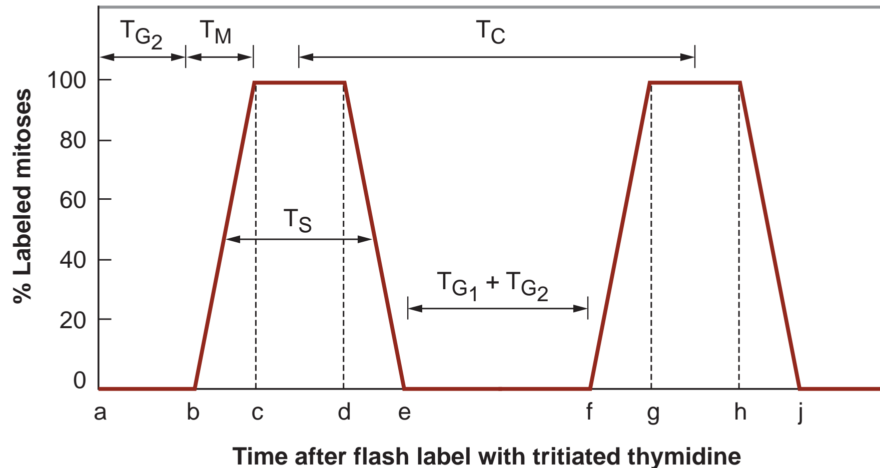


T_{G1} can be calculated by subtracting the sum of all the other phases of the cycle from the total cell cycle

$$T_{G1} = T_C - (T_S + T_{G2} + T_M)$$

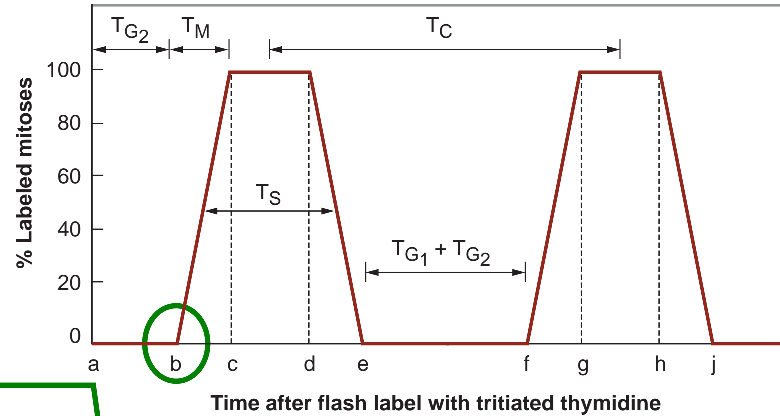
% Labeled Mitoses Summarized



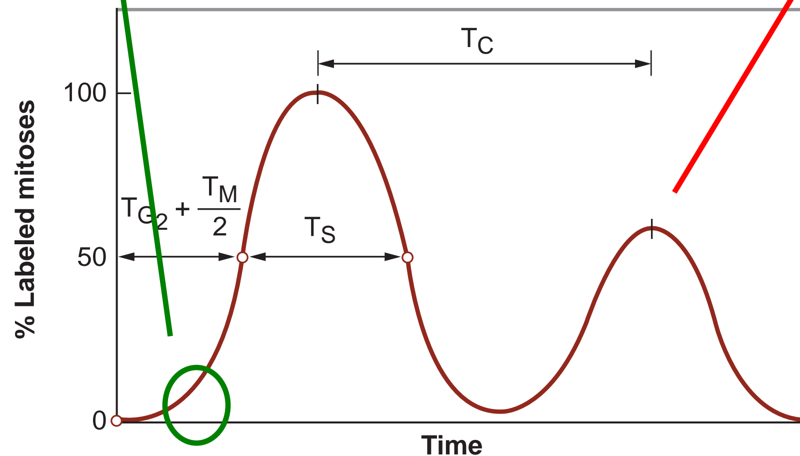


T_{G2} = ab
T_M = bc
T_S = bd or ce (or as in figure)
T_C = peak 1 – peak 2
$T_{G1} = T_C - (T_S + T_{G2} + T_M)$

% Labeled Mitoses in Practice



Points such as b and e are poorly defined

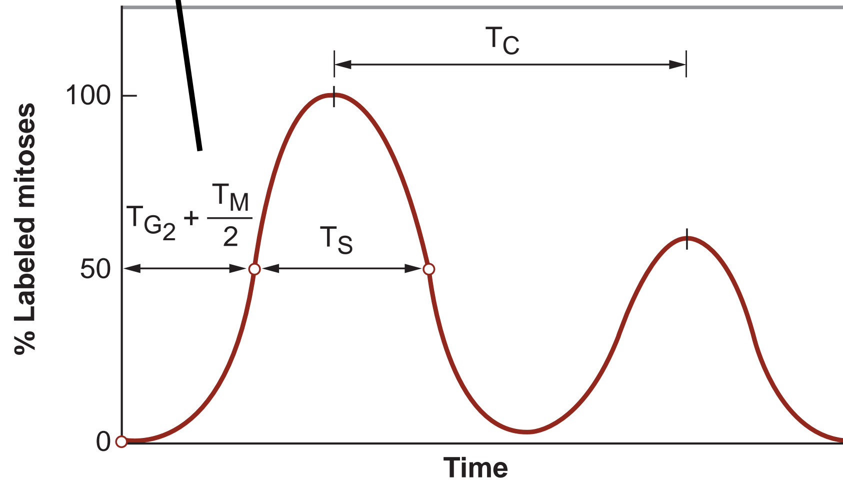


2nd wave has a smaller peak

% Labeled Mitoses in Practice

Only points discernable are the peaks and the 50% levels

3. T_{G2} may be calculated

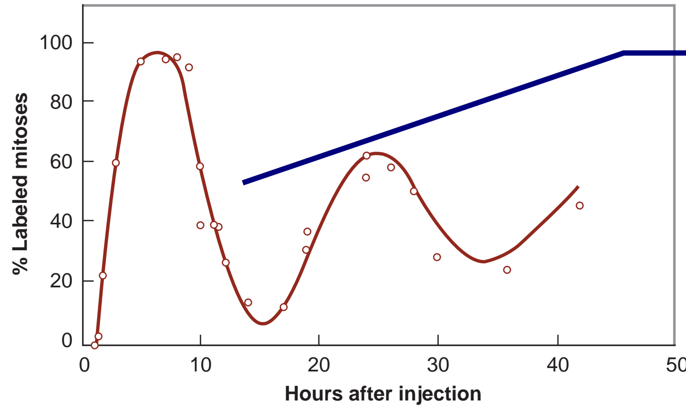


1. T_C and T_S are obtained the same way as discussed before

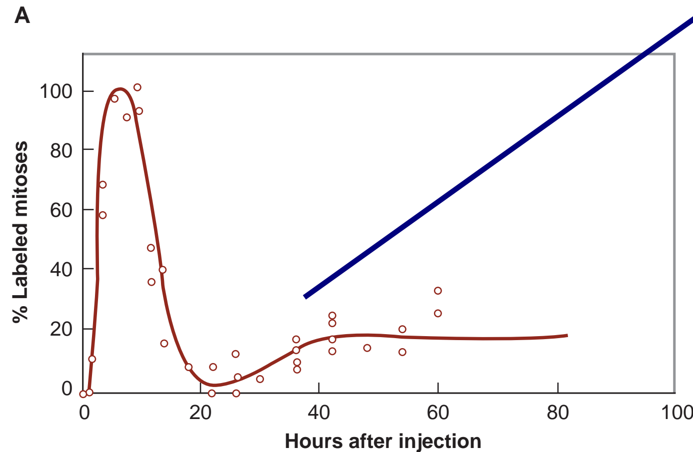
2. To obtain T_M, we have to determine MI from a separate experiment
$MI = \lambda\, T_M/T_C \rightarrow T_M = MI \times T_C/\lambda$

4. T_{G1} may be calculated by subtraction

Experimental Data – 2 Rat Sarcomas



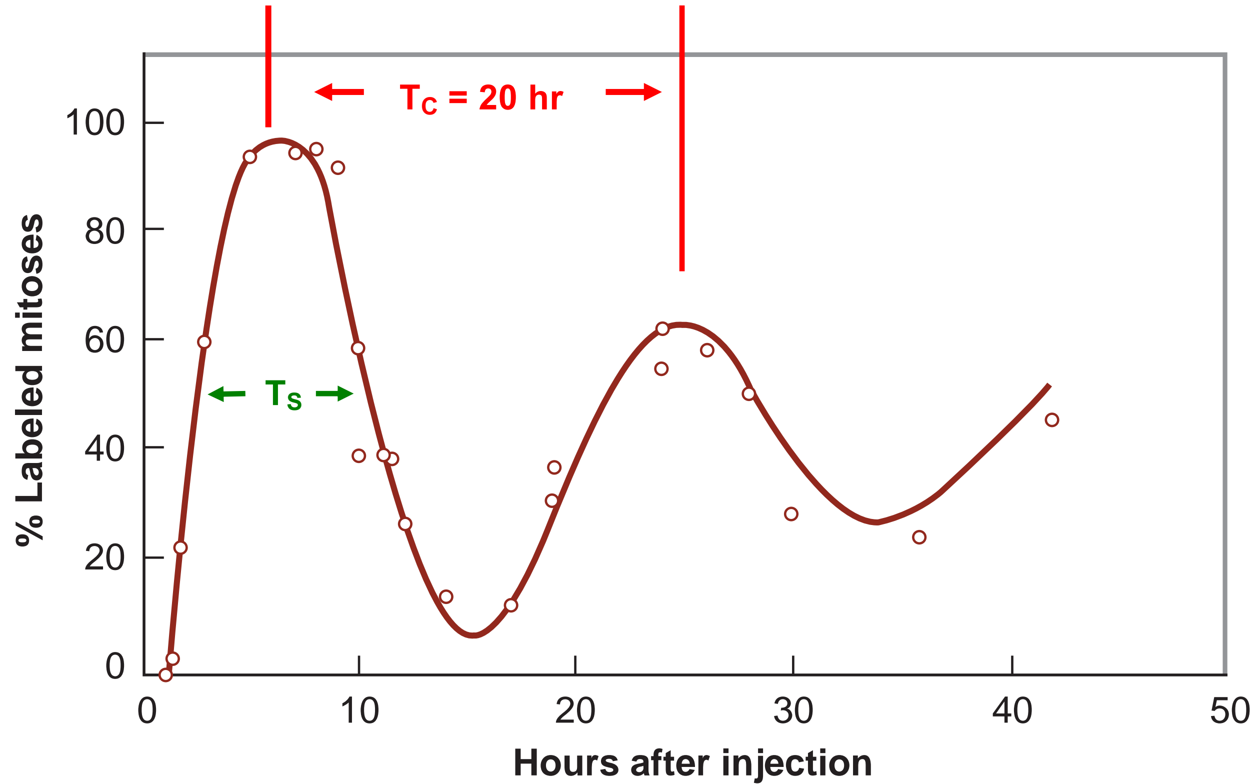
The first wave is not symmetric – the downswing is shallower than the upswing



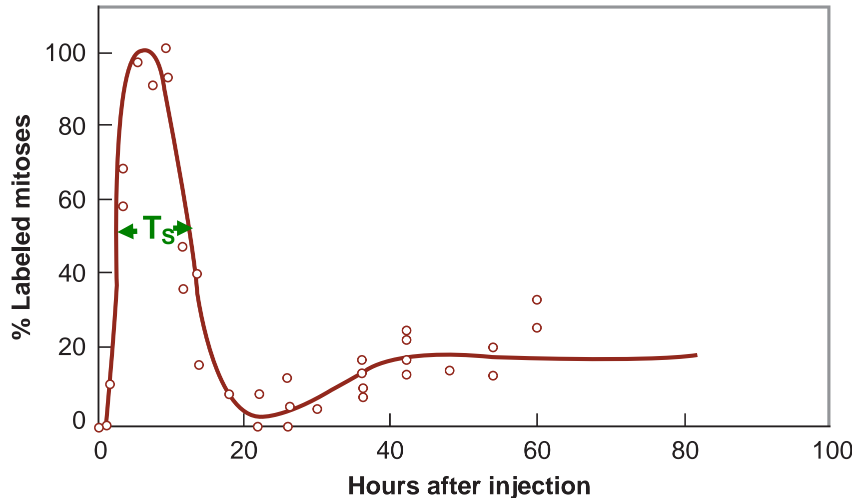
The 2nd wave is smaller, and in some instances, barely discernible

Both are due to the fact that the population is made up of cells with a wide range of cycle times

Experimental Data – Rat Sarcomas



Experimental Data – Rat Sarcoma



T_C cannot be simply obtained by measuring the distance b/w the 2 peaks

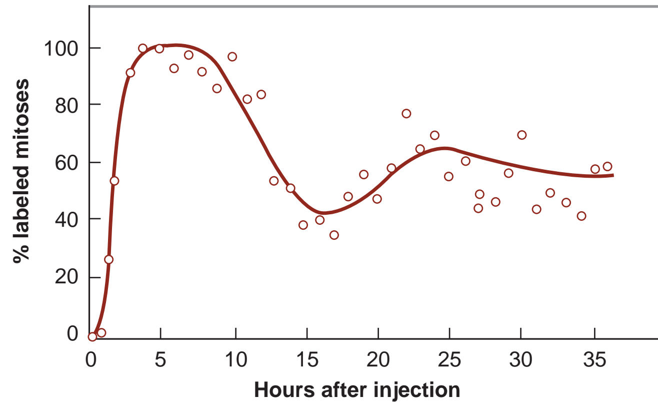
T_S, however, can still be read off the curve (~ 10 h)

If we know the labeling index, then, $LI = \lambda\, T_S/T_C \rightarrow$ **$T_C = \lambda\, T_S/LI$**

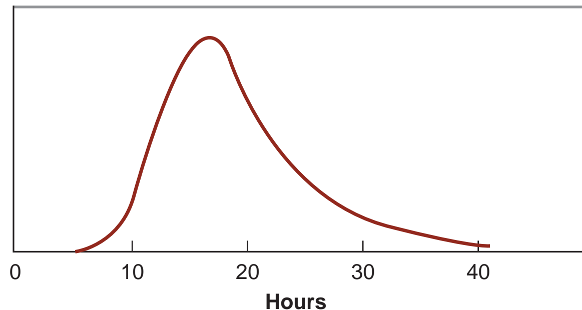
In this case, LI is ~ 3.6% → T_C (0.693 x 10) / (3.6/100) = 192.5 hours

The absence of a 2nd peak is a clue to the fact that there is a range of cell-cycle times for the cells of this population, so 192.5 hours is very much **an average value**

Experimental Data – EMT6 Mouse Tumor



The shallower downward swing and barely discernible 2nd peak indicates a wide range of cell cycle times for this tumor



Distribution of cell-cycle times estimated by computer analysis – varies from 10 to 40 hours, with a modal value of ~ 19 hours

Cell Cycle Time *In Vitro* and *In Vivo*

Authors	Cell or Tissue	T_C	T_S	T_M	T_{G_2}	T_{G_1}
Bedford	Hamster cells *in vitro*	10	6	1	1	2
	HeLa cells *in vitro*	23	8	1	3	11
Steel	Mammary tumors in the rat					
	BICR/M1	19	8	~1	2	8
	BICR/A2	63	10	~1	2	50
Quastler and Sherman	Mouse intestinal crypt	18.75	7.5	0.5	0.5–1.0	9.5
Brown and Berry	Hamster cheek pouch epithelium	120–15	28.6	1.0	1.9	108–140
	Chemically induced carcinoma in pouch	10.7	5.9	0.4	1.6	2.8

Note that in general, the malignant cells have a shorter T_C than their normal-tissue counterparts

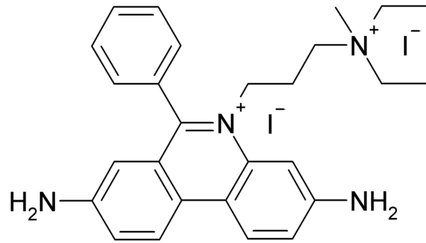
T_{G1} varies widely and is the major contributor to the widely different T_C values observed

Pulsed Flow Cytometry

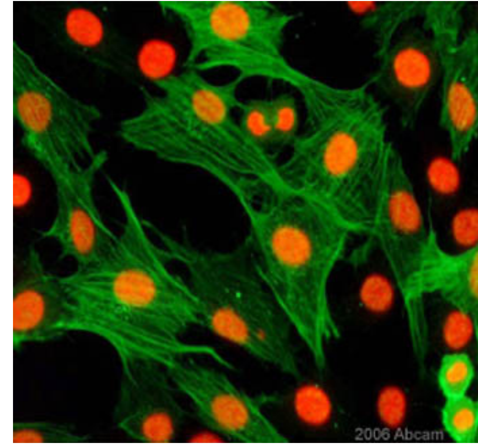
Classic autoradiography gives precise, meaningful data. However, they are laborious and so slow that information is never available quickly enough to act as a predictive assay to influence treatment options of an individual patient

During the past several decades, autoradiography has largely been replaced by **pulsed flow cytometry**, which provide data within a few days

Flow Cytometry



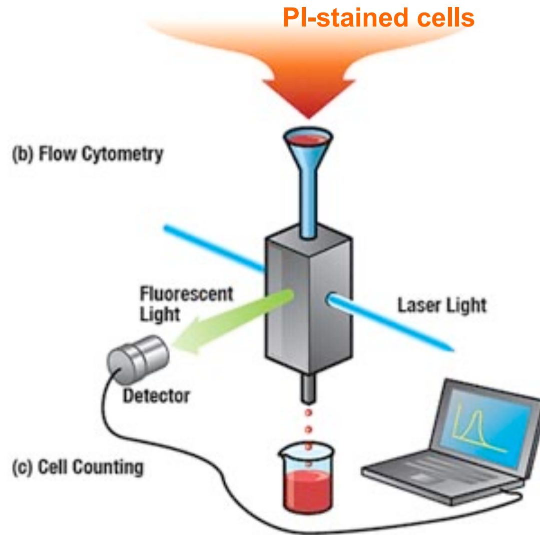
Propidium iodide (PI)

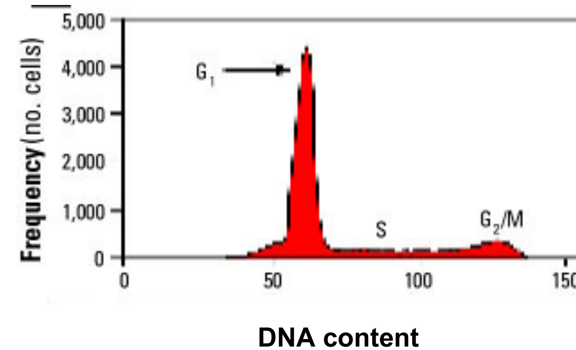


Propidium iodide is an **intercalating agent** and a **fluorescent molecule**

It binds to DNA by intercalating between the bases with ***little sequence preference***
→ amount of fluorescence is proportional to the **DNA content**

Flow Cytometry

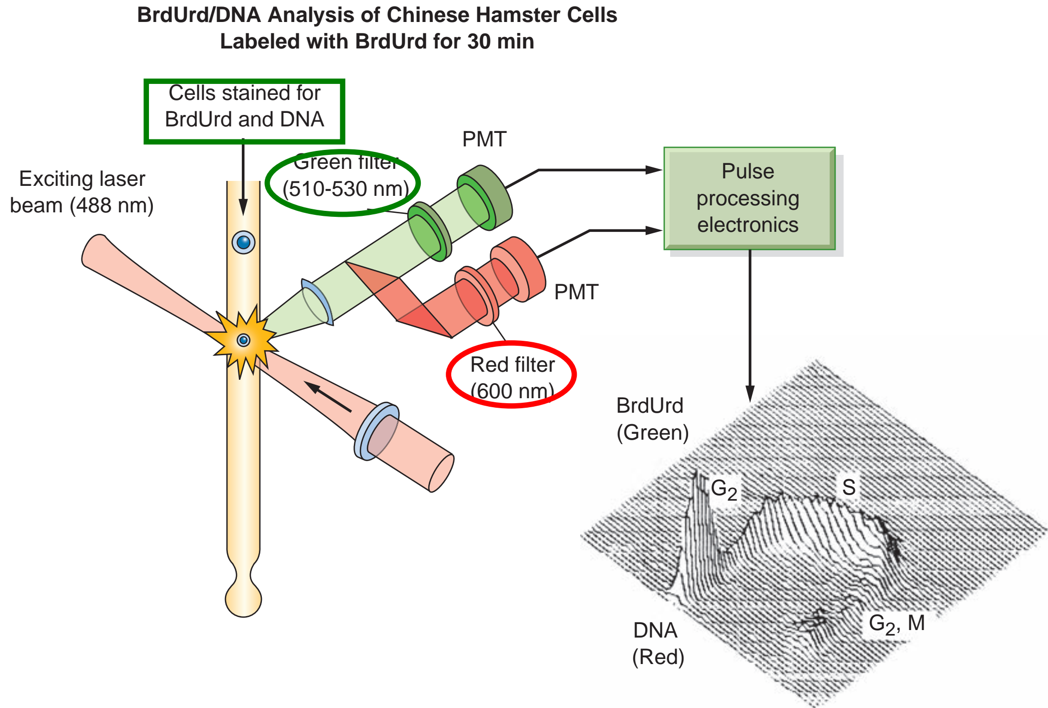




Suspensions of fluoresent-stained single cells flow one at a time through a light beam, with its wavelength adjusted to excite the fluorescent dye

Thousands of cells can be measured each second and the results accumulated to form a DNA distribution

Pulsed Flow Cytometry – Double Labeling

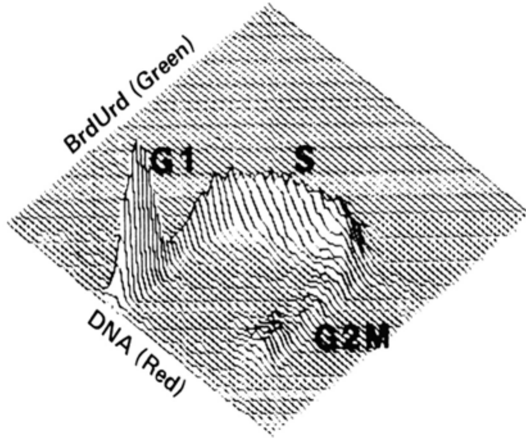


Cells labeled with **BrdUrd** are removed from the tumor in a biopsy specimen **4-8 h after labeling** and are detected with a fluorescent-labeled antibody (**green fluorescence**)

The biopsy specimen is treated with **PI** (**red fluorescence**)

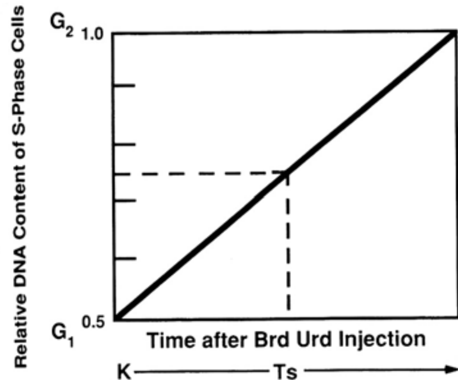
A 3D graph of labeled cells (green) vs. DNA content of cells (red) is generated

Pulsed Flow Cytometry – Double Labeling



Labeling Index (LI) = the proportion of cells that show green fluorescence

T_S can be calculated from the mean red fluorescence of S cells relative to G_1 and G_2 cells

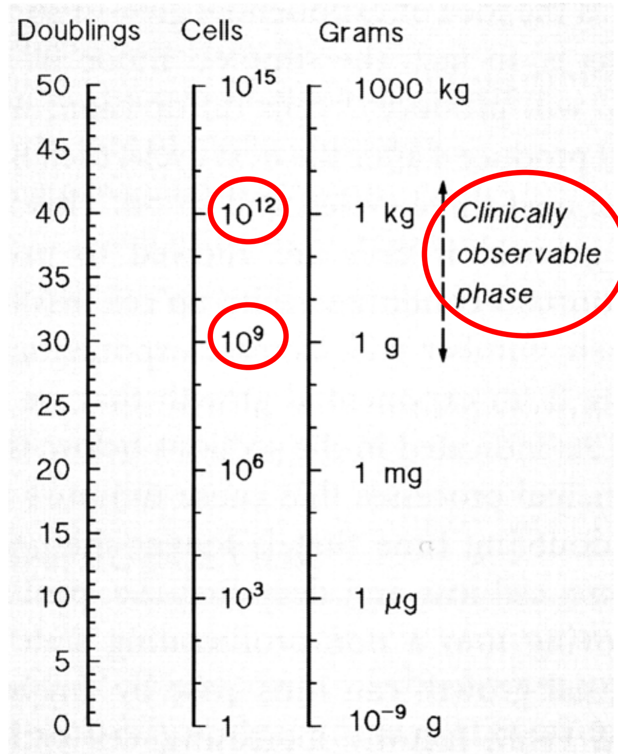


The method assumes that the red fluorescence of BrdU labeled cells (i.e., the DNA content of cells in S phase) increases linearly with time. If, for example, the biopsy specimen were obtained 6 hours after administration of BrdU, and the relative DNA content of cells labeled with BrDU (i..e, in S phase) were 0.75, midway between that characterstic of G1 and that of G2, Ts would be simply 12 hours.

Outline

- Quantitative Assessment of the Constituents of the Cell Cycle
  - Review of Cell Cycle
  - Mitotic Index and Labeling Index
  - The Percent Labeled Mitoses Technique
  - Experimental Measurements of Tc *In Vitro* and *In Vivo*
- **Tumor Volume Doubling**
- Growth Fraction
- Cell Loss
- Growth Kinetics of Human Tumors
- Tumor Control Probability

Tumor Weight and Cell Numbers



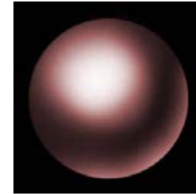
Assume 10^9 per gram

Tumor starts as **1** genetically altered cell

By the time a tumor becomes clinically detectable, it has already gone through 30 doubling times, and contains at least **10^9** cells

Tumor Volume and Doubling Time

$V = 4/3\ \pi r^3$



Question

A tumor has an initial diameter of 0.5 cm. In 100 days, tumor has grown to 4 cm. What is the tumor doubling time?

Answer

Diameter ↑ by a factor of 8 → volume ↑ by $8^3 = 512$-fold
$2^n = 512$ (n = # of doublings) → n = 9
9 doublings in 100 days → 1 doubling every 11 days
The tumor volume doubling time is ~ 11 days

In this calculation, we assumed no cell loss, and all tumor cells are growing

Tumor Growth Characteristics

Note that, volume doubling time ≠ cell cycle time, because tumor growth also depends on the fraction of tumor cells in cycle, as well as the fraction eliminated spontaneously

3 factors determine tumor growth (i.e., volume doubling)

1. Cell Cycle Time

2. Growth Fraction

3. Cell Loss Fraction

These are the parameters concerning the **growth kinetics of tumor**

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- Cell Loss
- Growth Kinetics of Human Tumors
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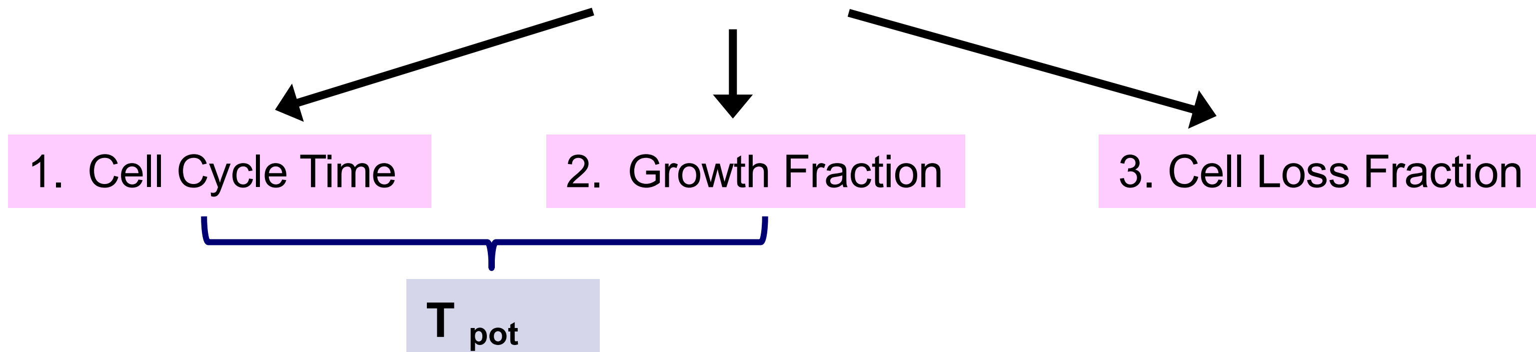
Growth Fraction

Not all of the tumor cells that are viable and capable of continued growth are actually proceeding through the cell cycle

Growth fraction is the ratio of the number of proliferating cells to the total number of cells

GF = P/(P+Q), where P = proliferating, Q = quiescent



Potential Tumor Doubling Time (T_{pot})

T_{pot} is the is defined as the cell doubling time ***without*** any cell loss

T_{pot} is the **average** doubling time of the cells capable of **continued proliferation**, thus it reflects the **cell cycle of individual cells** and the fraction of cells in active cycle (**growth fraction**), but ignores **cell loss** (to be discussed later)

Potential Tumor Doubling Time (T_{pot})

With pulsed flow cytometry determining T_S and LI, we can then estimate **T_{pot}**, the **potential tumor doubling time**

$$T_{pot} = \lambda \, T_s/LI$$

Reflects growth fraction

Relates to cell cycle length

Clinical relevance – tumors with a short T_{pot} may need to be treated with accelerated therapy, otherwise they will repopulate faster than they can be killed; T_{pot} has proved to be practical as a ***predictive assay***

Growth Fraction

Because not all cells in a tumor are in a growth fraction, potential doubling time (T_{pot}) is usually longer than the average cell cycle time (T_C) of dividing cells

$$T_{pot} = \lambda\, T_S / LI = 0.693 T_C / \ln(1+GF)$$

GF = 100%

$T_{pot} = 0.693\, T_C/\ln 2$
$T_{pot} = T_C$

GF = 0%

$T_{pot} = 0.693\, T_C/\ln 1$
$T_{pot} = \infty$

Thus, T_{pot} reflects the **cell cycle of individual cells** as well as the fraction of cells in active cycle (**growth fraction**) [We will look at cell loss in a minute]

Growth Fraction

Methods to Estimate Growth Fraction

1. **Pulsed labeling (then wait several cell generations)** – GF = fraction of cells labeled/fraction of mitoses labeled

2. **Continuous labeling** – tritiated thymidine is infused continuously for a time equal to the cell cycle minus the length of the S phase; the fraction of labeled cells then approximates to the growth fraction

3. **Ki-67 staining** – Ki-67 is an antibody that binds to nuclear antigen in cycling cells

GF in Solid Tumors in Experimental Animals

TABLE 22.2 Growth Fraction for Some Tumors in Experimental Animals

Tumor	Author	Growth Fraction, %
Primary mammary carcinoma in the mouse (G_3H)	Mendelsohn	35–77
Transplantable sarcoma in the rat (RIB_5)	Denekamp	55
Transplantable sarcoma in the rat (SSO)	Denekamp	47
Transplantable sarcoma in the rat (SSB_1)	Denekamp	39
Mammary carcinoma in the mouse (C_3H)	Denekamp	30
Chemistry induced carcinoma in the hamster cheek pouch	Brown	29

In general, for solid tumors in experimental animals, the GF ranges from **30%-50%**

Outline

- Quantitative Assessment of the Constituents of the Cell Cycle
  - Review of Cell Cycle
  - Mitotic Index and Labeling Index
  - The Percent Labeled Mitoses Technique
  - Experimental Measurements of Tc *In Vitro* and *In Vivo*
- Tumor Volume Doubling
- **Growth Fraction**
- Cell Loss
- Growth Kinetics of Human Tumors
- Tumor Control Probability

Cell Loss

We already discussed that potential doubling time (**T_{pot}**) is usually longer than the average cell cycle time (**T_C**) of dividing cells because not all cells in a tumor are in a growth fraction

$$T_{pot} > T_C$$

In most cases, tumor **grow much more slowly** (i.e., the observed doubling time **T_d**) than would be predicted from potential doubling time (i.e., **T_{pot}**)

$$T_d > T_{pot}$$

This difference is due to **cell loss**

Cell Loss

The **cell loss factor** is a measure of the rate of cell loss compared to the rate of new cell production

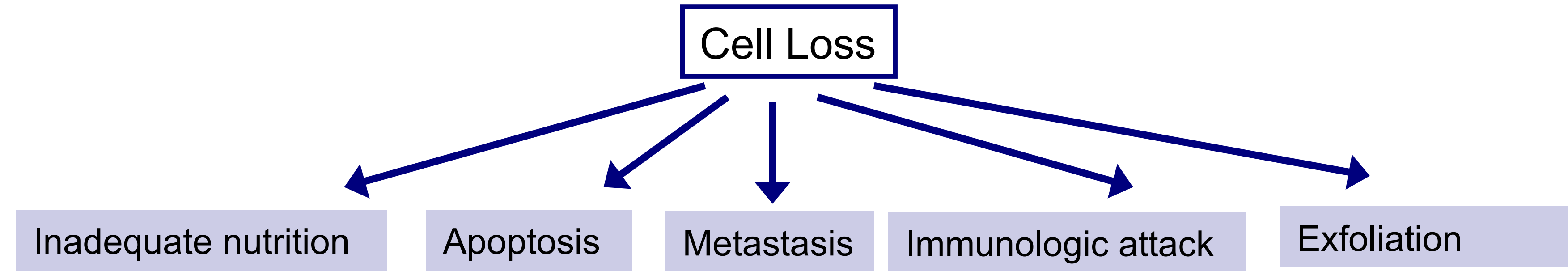
Cell loss factor (Φ) = $1 - T_{pot}/T_d$

T_{pot} is the potential tumor doubling time
T_d is the actual tumor doubling time

$\Phi = 0$ (i.e., no cell loss) $\rightarrow T_{pot} = T_d$
$\Phi = 1$ (i.e., cell loss balanced out cell growth) $\rightarrow T_d$ approaches ∞

The actual tumor doubling time can be determined if T_{pot} and Φ are known

Mechanism of Cell Loss



As the tumor outgrows its vascular system, some cells will have inadequate access of oxygen and other nutrients; they die, giving rise to necrotic zone

Cells are lost to other parts of the body

Could be important for carcinoma of the GI tract, in which the epithelium is renewed at a considerable rate

Cell Loss Factor in Experimental Animals

TABLE 22.3 Cell-Loss Factor (ϕ) for Some Tumors in Experimental Animals

Tumor	Author	ϕ, %
Mouse sarcoma	Frindel	
3-day-old tumor		0
7-day-old tumor		10
20-day-old tumor		55
Rat carcinoma	Steel	9
Rat sarcoma	Steel	0
Mouse carcinoma	Mendelsohn	69
Hamster carcinoma	Brown	75
Rat sarcoma	Hermens	26
Hamster carcinoma	Reiskin	81–93
Mouse carcinoma	Tannock	70–92

Φ varies from **0 to more than 90%**

Sarcomas tend to have low Φ

Carcinomas tend to have high Φ

The pattern correlates with the importance of apoptosis as a mode of cell death

Cell Loss Factor and Tumor Regression

Carcinoma

New cell production is stopped or reduced by radiation
Existing cells continue to be removed (high Φ)
} Tumor shrinks rapidly

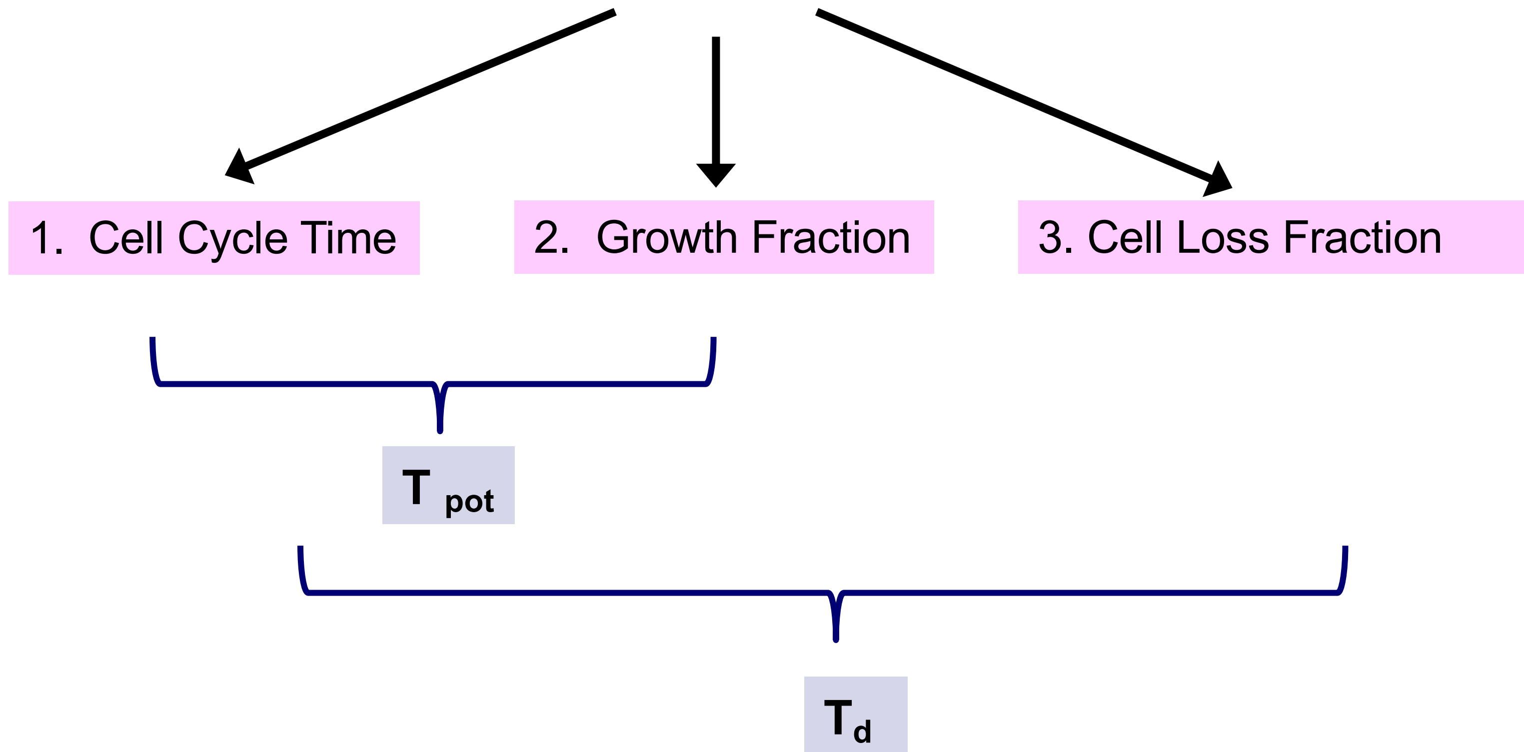
Sarcoma

New cell production is stopped or reduced by radiation
Existing cells are removed very slowly (low Φ)
} Tumor regresses slowly

In the short-term, carcinoma would be said to have "responded" to the radiation, whereas the sarcoma might be said to be unresponsive or resistant to radiation

In the long-term, the ultimate "cure rates" depends on the number of surviving clonogens and may well be identical for both

Tumor Growth Characteristics



Overall Growth Pattern of a Tumor

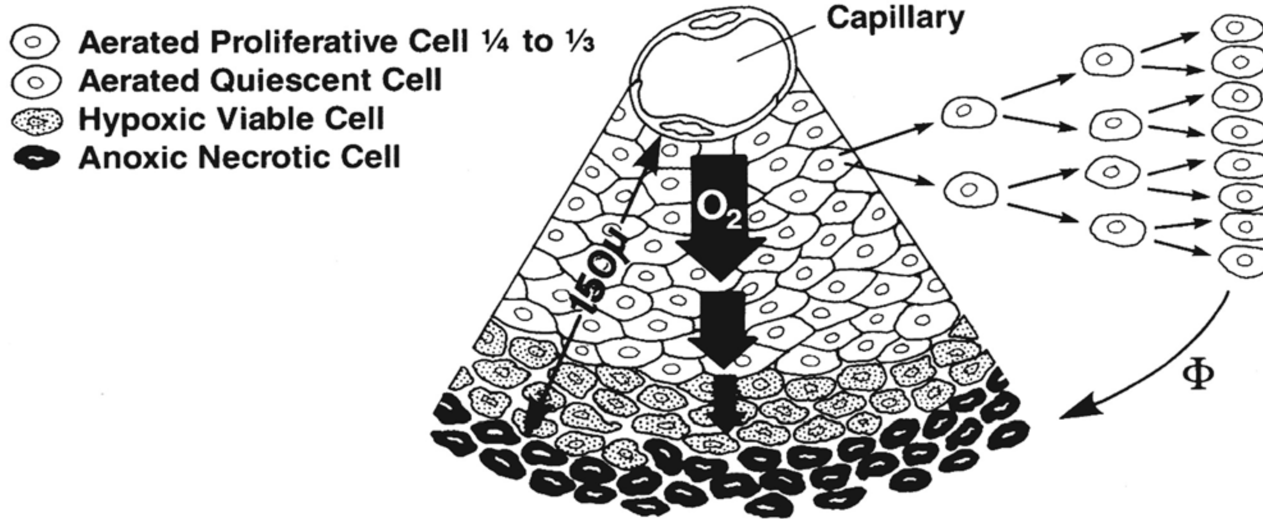
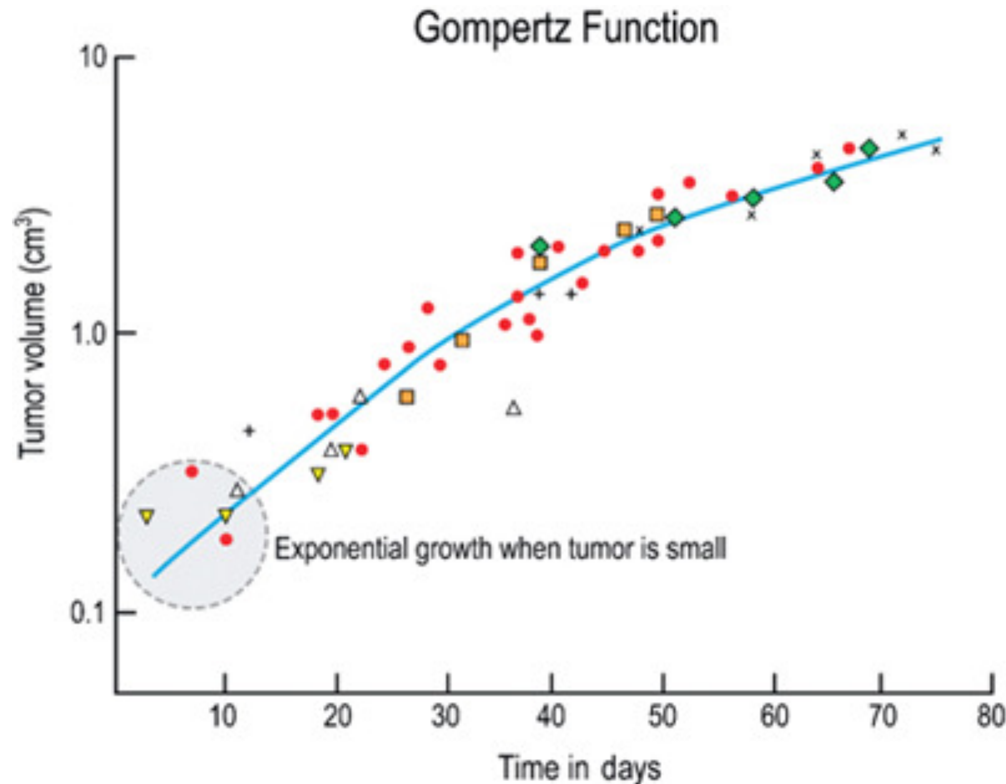


Figure 21.11. The overall pattern of the growth of a tumor. Clonogenic cells consist of proliferative (P) and quiescent (Q) cells. Quiescent cells can be recruited into the cell cycle as the tumor shrinks after treatment with radiation or a cytotoxic drug. In animal tumors the growth fraction is frequently 30 to 50%. Of the cells produced by division, many are lost, principally into necrotic areas of the tumor remote from the vasculature. The cell loss factor (Φ) varies from 0 to 100% and dominates the pattern of tumor growth. As the tumor outgrows its blood supply, some cells become hypoxic. This accounts for some of the quiescent cells that are out of cycle.

Typical Growth Curve – Gompertz Function



- When tumor is composed of only a few cells, it may grow exponentially
- When it gets larger, the growth rate slows as the supply of oxygen and nutrients are outgrown

Tumor Growth and Regression Patterns

Φ	GF	T_C	Tumor Behavior
Low	Low	Long	Slow growth Slow regression
High	Low	Long	Slow growth Rapid regression
Low	High	Short	Rapid growth Slow regression
High	High	Short	Rapid growth Rapid regression

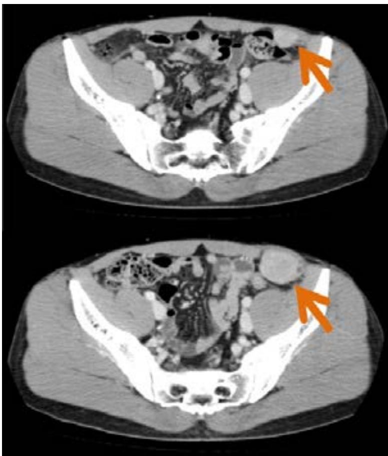
Outline

- Tumor Volume Doubling
- Cell Cycle Time
  - Review of Cell Cycle
  - Mitotic Index and Labeling Index
  - The Percent Labeled Mitoses Technique
  - Experimental Measurements of Tc *In Vitro* and *In Vivo*
  - Potential Tumor Doubling Time
- Growth Fraction
- Cell Loss
- **Growth Kinetics of Human Tumors**
- Tumor Control Probability

Doubling Time

Doubling Time is perhaps the easiest parameter to study

T_D of human tumors may be obtained by measurements from radiographs, or by direct measurements of skin tumors or metastases in soft tissues (non-invasive)



The T_D human tumors varies widely from patient to patient and is on the average very long; ***Tubiana and Malaise*** have estimated that the median value is about **2 months**

Doubling Time

Factors Affecting T_D

Poorly differentiated cancers generally grow more rapidly

Tumors of the same histologic type arising in **different patients** differ widely in growth rate (i.e, doubling time)

Metastases arising in the same patient tend to have similar rates of growth

Doubling Time

There is a correlation between **histologic type** and growth rate

Type	T_D days	LI %	GF %	Φ %	Radiosensitivity (Mean sterilization dose) Gy	Chemo-sensitivity
Embryonal Tumors	27	30	90	94	25-30	++
Lymphomas	29	29	90	94	35-40	++
Mesenchymal Sarcomas	41	4	11	68	85	--
Squamous Carcinomas	58	8	25	90	60-70	+
Adenocarcinomas	83	2	6	71	60-80	+/-

389 patients with pulmonary metastases (Tubiana & Malaise)

Study of Other Kinetic Paremeters

Studies of **human tumor cell population kinetics** raise both practical and ethical problems

Practical Issues

Percent labeled mitoses technique requires a large number of sequential samples to be taken → repeated biopsies is both uncomfortable and inconvenient

Ethical Issues

In vivo experiments require and injection of tritiated thymidine or BrdUrd intraperitoneally → possible genetic consequences

Nevertheless, a surprising # of human tumors have been studied in this way

Cell Cycle Time (T_C) and T_S (Human Tumor)

Authors	T_C, h
Frindel et al. (1968)	97, 51.5, 27.5, 48, 49.8
Bennington (1969)	15.5, 14.9
Young and de Vita (1970)	42, 82, 74
Shirakawa et al. (1970)	120, 144
Weinstein and Frost (1970)	217
Terz et al. (1971)	44.5, 31, 14, 25.5, 26
Peckham and Steel (1973)	59
Estevez et al. (1972)	37, 30, 48, 30, 38, 96, 48
Terz and Curutchet (1974)[a]	18, 19, 19.2, 120
Malaise et al. (unpublished data)[a]	24, 33, 48, 42
Muggia et al. (1972)	64
Bresciani et al. (1974)	82, 50, 67, 53, 58

Tubiana & Malaise, 1976

T_C

T_C ranged between 15 and 125 h
Modal value – **48 hr**

T_S

T_S ranged between 9.5 and 24 h
Modal value – **16 hr**

Mean duration of T_C is about **3x** of T_S

Growth Fraction

Methods of GF Measurement

1. Continuous labeling (all the actively proliferating cells should be labeled)
2. Estimate GF by assuming that proportions of cells in cell cycle is about 3x the labeling index (see previous $T_C = 3 \times T_S$)

General Conclusion

The GF is more variable in human tumors than in rodent tumors and correlates better with gross volume doubling time

Lymphoma and embryonic tumors – GF = 0.9

Adenocarcinomas – GF = 0.06

Growth Fraction

Type	T_D days	LI %	GF %	Φ %	Radiosensitivity (Mean sterilization dose) Gy	Chemo-sensitivity
Embryonal Tumors	27	30	90	94	25-30	++
Lymphomas	29	29	90	94	35-40	++
Mesenchymal Sarcomas	41	4	11	68	85	--
Squamous Carcinomas	58	8	25	90	60-70	+
Adenocarcinomas	83	2	6	71	60-80	+/-

Note that GF is estimated by LI x 3
Note also the correlation b/w GF and T_D

Cell Loss Factor (Φ)

Estimation of Φ

The cell loss factor is the most difficult to evaluate

It generally has been calculated by comparing the ***observed tumor volume-doubling time*** with the ***potential doubling time***

Φ – Tubiana and Malaise

Type	T_D days	LI %	GF %	Φ %	Radiosensitivity (Mean sterilization dose) Gy	Chemo-sensitivity
Embryonal Tumors	27	30	90	94	25-30	++
Lymphomas	29	29	90	94	35-40	++
Mesenchymal Sarcomas	41	4	11	68	85	--
Squamous Carcinomas	58	8	25	90	60-70	+
Adenocarcinomas	83	2	6	71	60-80	+/-

In general, the mean cell loss factor **exceeds 50%**

Note the correlation of cell loss factor with GF – in general, Φ is high for faster growing tumor and small for slow growing tumor

Φ – Steel’s Data

Steel has independently estimated the extent of cell loss in human tumors by comparing the potential doubling time with observed tumor growth rate

Authors	Site	Volume Doubling Time, d	Range, d
Breuer	Lung metastases	40	4–745
Collins et al.	Lung metastases	40	11–164
Collins	Lung metastases from colon or rectum	96	34–210
Garland	Primary bronchial carcinomas	105	27–480
Schwartz	Primary bronchial carcinomas	62	17–200
Spratt	Primary skeletal sarcomas	75	21–366

Based on the data from Steel GG. Cell loss from experimental tumors. *Cell Tissue Kinet*. 1968;1:193–207.

The average median doubling time is 66 days

The estimated cell loss factor is **77%**

Human Tumor Kinetics Summarized

There is a great disparity between the cell cycle time of individual dividing cells and the overall doubling time of the tumor

The mean T_C **is 48 h (range 1-5 days)**
The mean tumor doubling time T_D **is ~ 2 months (range 40-100 days)**

This discrepancy is largely accounted for by the cell loss factor, i.e., Φ is the most important factor determining the pattern of tumor growth

Law of Bergonié and Tribondeau

Law of Bergonié and Tribondeau (1906)

Tissue appear to be more *"radiosensitive"* if their cells are less differentiated, have a greater proliferative capacity and divide more rapidly

Human Tumor Kinetics and Radiosensitivity

Bergonie and Tribondeau established a relation between the rate of cell proliferation and the response to irradiation in **normal tissues**; it is conceivable that the law might also holds for **tumors**

The histologic groups of human tumors that have the most rapid mean growth rates and the highest GF and cell turnover rates are indeed those that are the most radiosensitive

Type	T_D days	LI %	GF %	Φ %	Radiosensitivity (Mean sterilization dose) Gy	Chemo-sensitivity
Embryonal Tumors	27	30	90	94	25-30	++
Lymphomas	29	29	90	94	35-40	++
Mesenchymal Sarcomas	41	4	11	68	85	--
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Human Tumor Kinetics and Chemosensitivity

Majority of the chemo agents acting on the cycling cells in S phase (nucleic acid analogues), therefore, cell cycle time is more relevant than actual tumor doubling time

Tumors with high labeling index can potentially be cured with chemotherapy alone

Tumors with a high cell loss factor appear to favor response to chemotherapy

Human Tumor Kinetics and Chemosensitivity

Type	T_D days	LI %	GF %	Φ %	Radiosensitivity (Mean sterilization dose) Gy	Chemo-sensitivity
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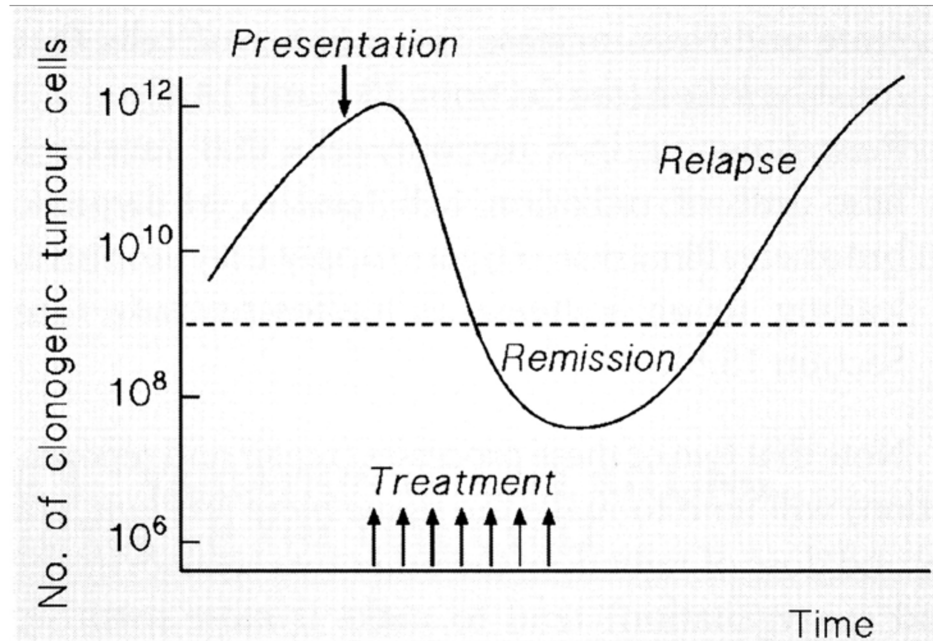
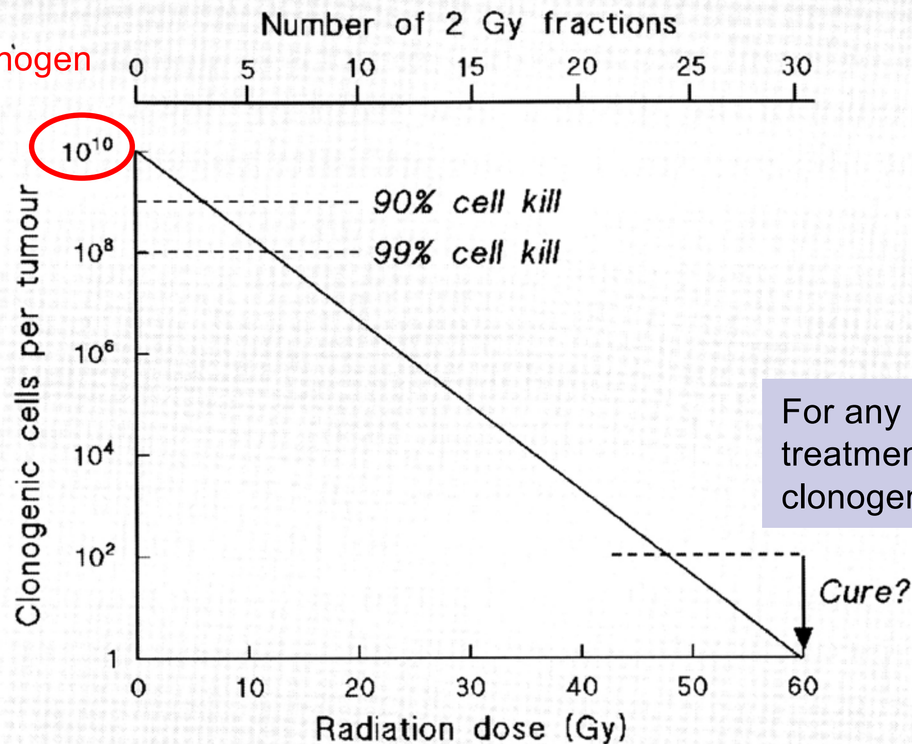
Time Course of Body Burden of Clonogenic Cells



Limit of detection

99% Tumor Kill Means Complete Failure

Initial # of clonogen



For any chance of cure, treatment has to reduce # of clonogen to at least < 1

Tumor Control Probability (TCP)

TCP is based on **Poisson statistics**

$$P = e^{-n}$$

where P is probability of cure, n is average number of surviving clonogenic tumor cells

TCD37 would leave about 1 cell/tumor
TCD90 would leave about 0.1 cells/tumor
TCD95 would leave about 0.05 cells/tumor
TCD99 would leave about 0.01 cells/tumor

For calculation, you may wish to review the examples given in Chapter 3

Review Questions

Problem 1

The diameter of a tumor was found to double in 18 days. Assuming that all of the cells in the tumor are proliferating and no cells are lost, the tumor cell doubling time is closest to:

A. 1 day

B. 3 days

C. 6 days

D. 12 days

E. 18 days

Volume (# of cells) increase 8-fold -> = 3 doublings (2^3). 18/3 = 6 days

Problem 2

Which of the following statements is TRUE concerning the cell cycle kinetics of human tumors?

A. The growth fraction of a tumor represents the proportion of cells capable for transplanting the tumor

B. Cell loss is often the major factor that determines the tumor volume doubling time

C. The growth rate generally increases with increasing tumor size

D. Volume doubling times are shorter than the value that would be predicted from the cell cycle time of individual cells longer

E. The volume doubling time is largely determined by the cell cycle time

Human Tumor Kinetics Summarized

There is a great disparity between the cell cycle time of individual dividing cells and the overall doubling time of the tumor

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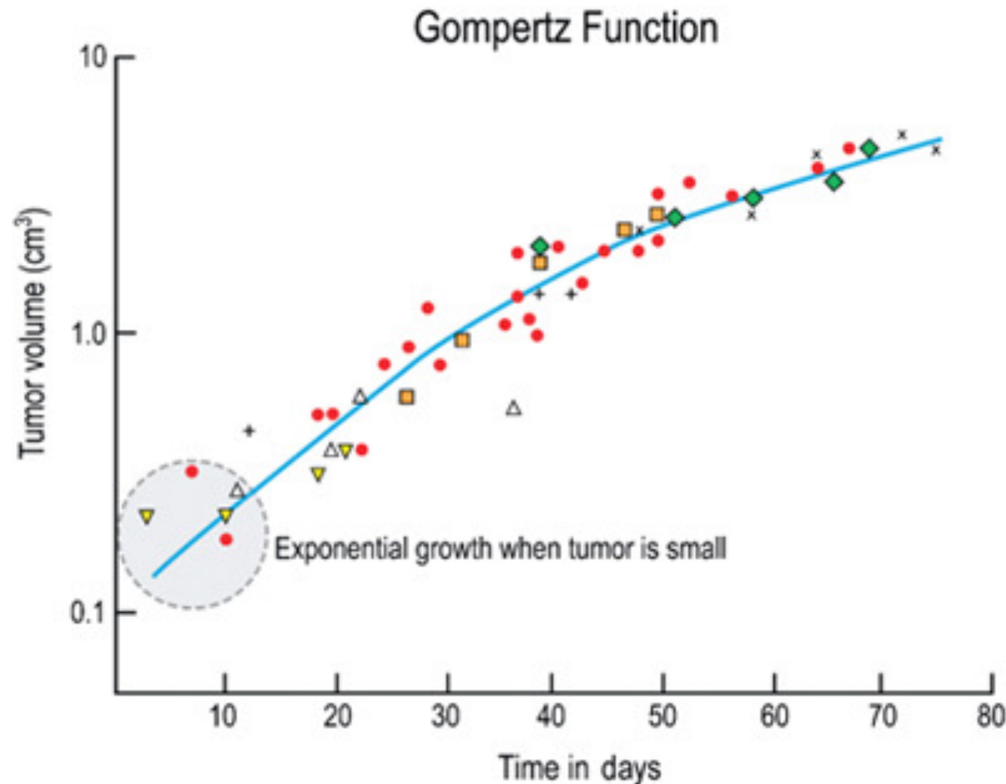
The mean tumor doubling time T_D **is ~ 2 months (range 40-100 days)**

This discrepancy is largely accounted for by the cell loss factor, i.e., Φ is the most important factor determining the pattern of tumor growth

Typical Growth Curve – Gompertz Function



- When tumor is composed of only a few cells, it may grow exponentially
- When it gets larger, the growth rate slows as the supply of oxygen and nutrients are outgrown

Problem 3

A tumor is characterized by a cell cycle time of 10 days, a growth fractions of 0.5 and a cell loss factor of 1.0. Assuming these kinetic parameters remain constant over a one-month period, how much would the tumor volume have increased during that time?

A. 2-fold
B. 3-fold
C. 4-fold
D. 5-fold
E. Remain the same

Cell loss factor of 1.0 means every for every new cell produced, there is a cell dying off.

Cell loss factor (Φ) = $1 - T_{pot}/T_d$

Problem 4

Medical Residents Only

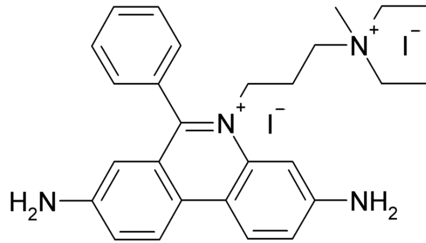
Which of the following flow cytometry methods or the combination of methods are used to estimate cell cycle distribution in mammalian cells?

A. Analysis of annexin V stained cells apoptosis
B. Analysis of cells treated with a high dose (2mM or more) thymidine
C. Analysis of propidium iodide-stained cells
D. Analysis of cells labeled with an H3 antibody
E. Analysis of cells pulsed-labeled with ^{3}H-thymidine

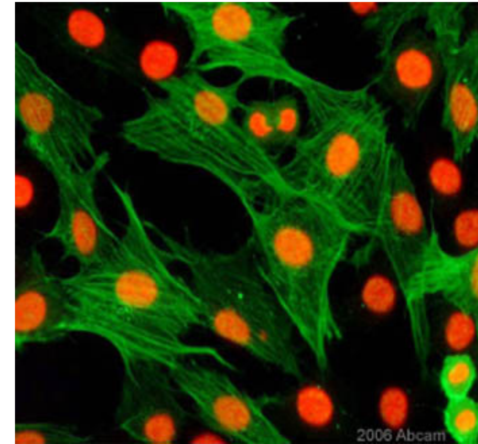
Only for cells in S-phase

Flow Cytometry

Medical Residents Only



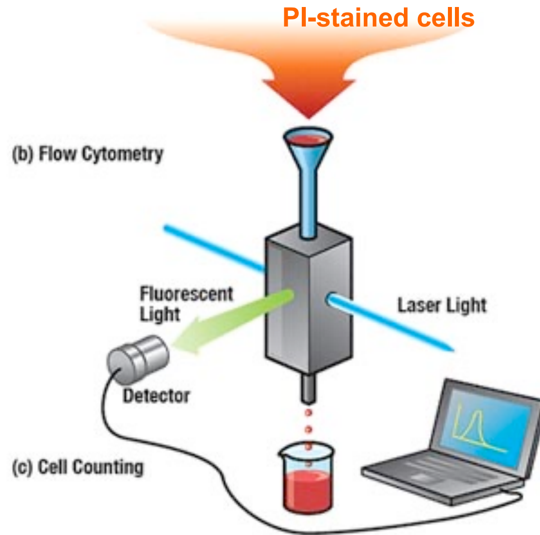
Propidium iodide (PI)

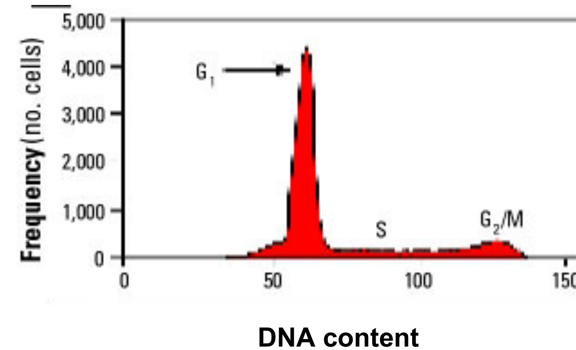


Propidium iodide is an **intercalating agent** and a **fluorescent molecule**

It binds to DNA by intercalating between the bases with ***little sequence preference***
→ amount of fluorescence is proportional to the **DNA content**

Flow Cytometry





Suspensions of fluoresent-stained single cells flow one at a time through a light beam, with its wavelength adjusted to excite the fluorescent dye

Thousands of cells can be measured each second and the results accumulated to form a DNA distribution

Problem 5

Two patients are diagnosed on the same day with tumors of approximately the same size. However, the T_{pot} for patient A's tumor was determined to be 5 days while the T_{pot} for patient B's tumor was calculated as 20 days. Assuming that there was no cell loss taking place and the tumor's growth fractions did not change, if treatment was delayed for 20 days, the ratio of the number of the tumors of patient A to patient B would have been approximately:

A. 16:1
B. 8:1
C. 1:1
D. 1:8
E. 1:16

$2^4/2 = 8$