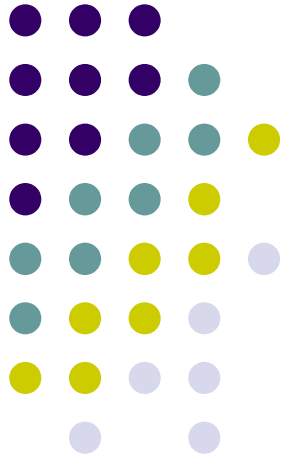
Curso de Radiobiología
UDELAR
Facultad de Ciencias
Unidad de Física Médica

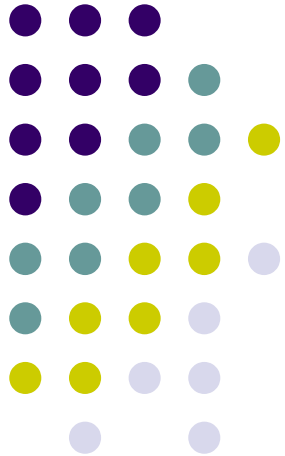
Dr. Eduardo Francisco Larrinaga Cortina



4R. Repoblación

Créditos:

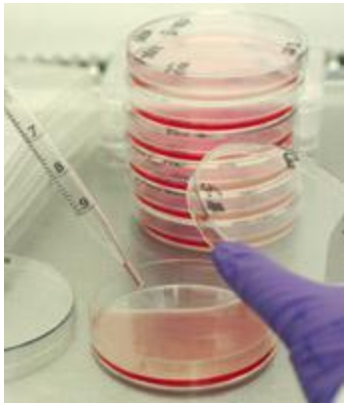
Dr. Jerry Battista



R3 Re-Population

J. Battista

"Cell replication in vitro is a far cry from tumour growth in vivo"



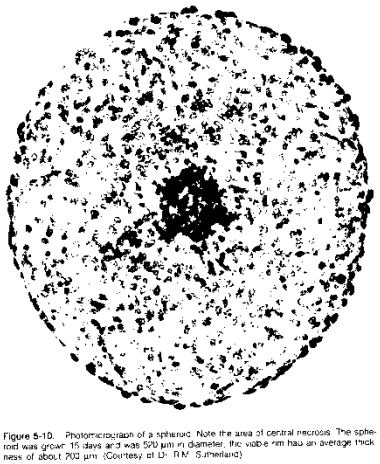
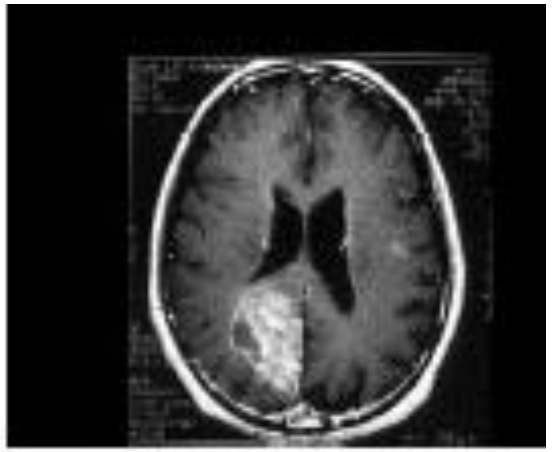


Figure 5-10. Photomicrograph of a spheroid. Note the area of central necrosis. The spheroid was grown 15 days and was 570 μm in diameter, the viable rim has an average thickness of about 703 μm. (Courtesy of Dr. R.M. Sutherland)



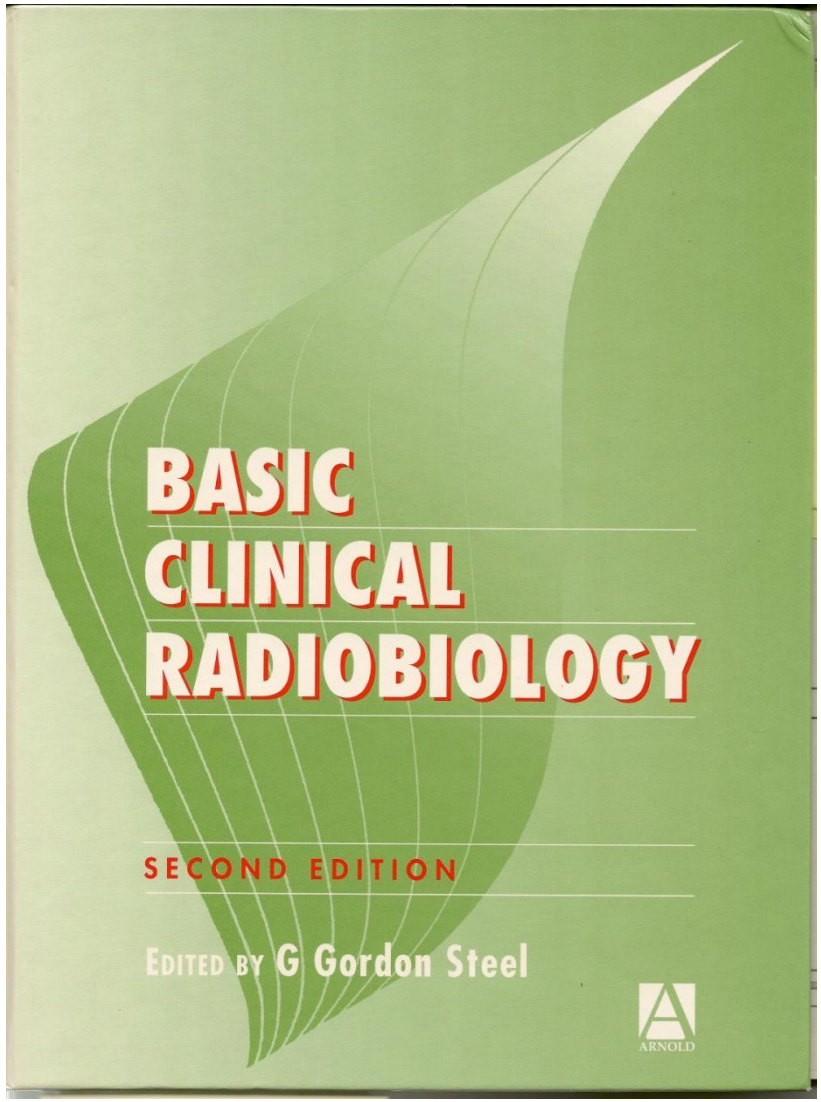
As you go to a bigger mass, growth rate goes down

http://upload.wikimedia.org/wikipedia/commons/thumb/a/ae/SMirC-cry.svg/320px-SMirC-cry.svg.png

http://info.cancerresearchuk.org/images/gpimages/384547

http://www.scielo.br/img/revistas/babt/v50nspe/a09fig02.jpg

Cell Number and Tumour Size



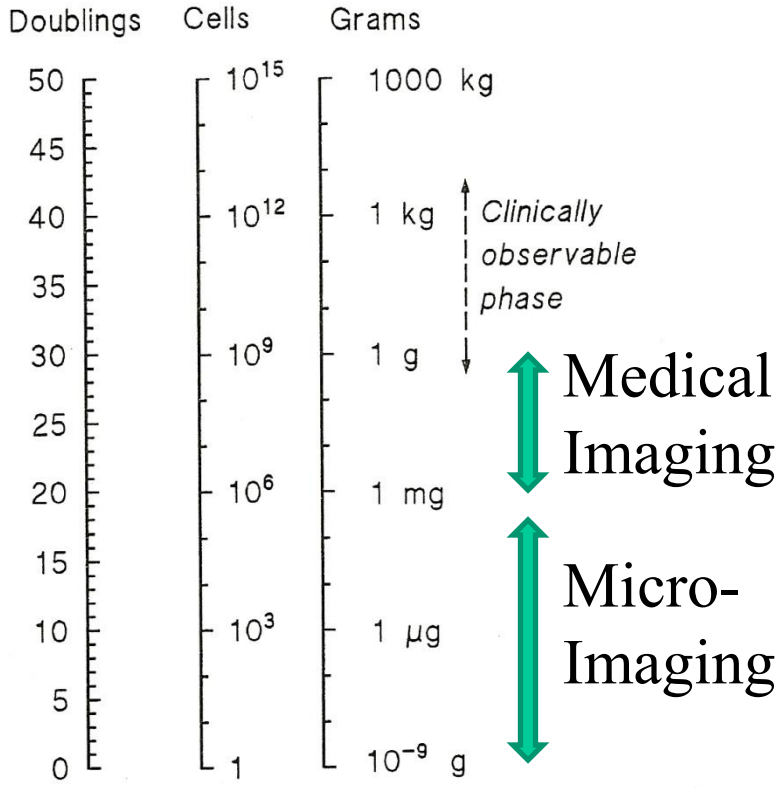
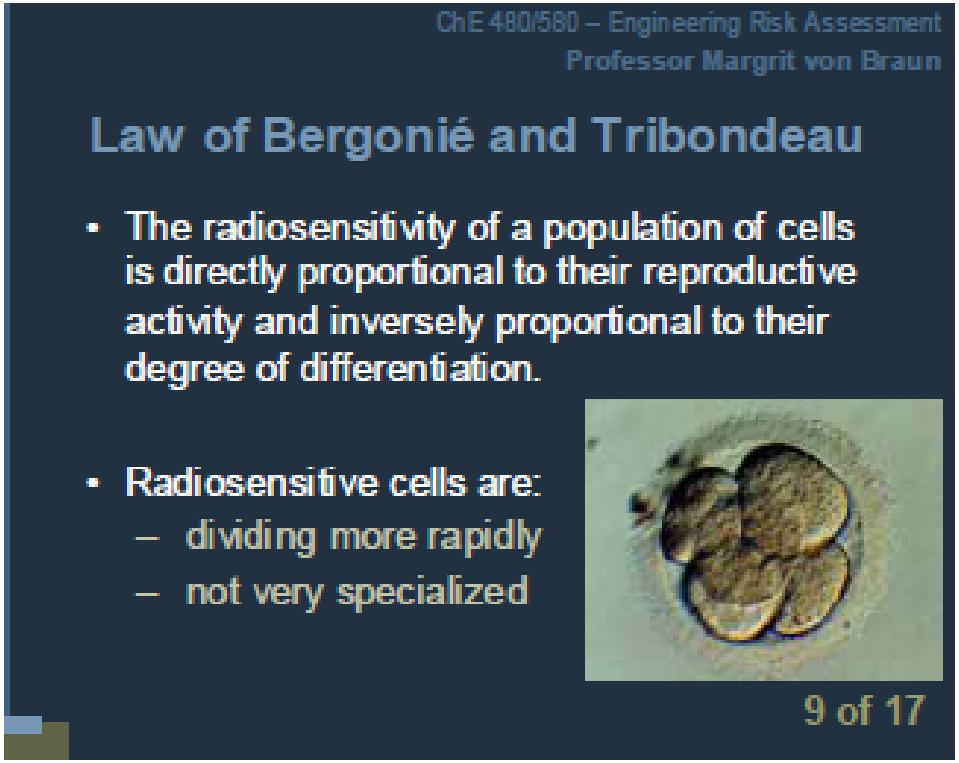


Figure 2.1 The relationship between the weight of a tumour, the number of cells it contains (assuming 10^9 per gram) and the number of doublings from a single cell.

What Matters Most in Tumour Response

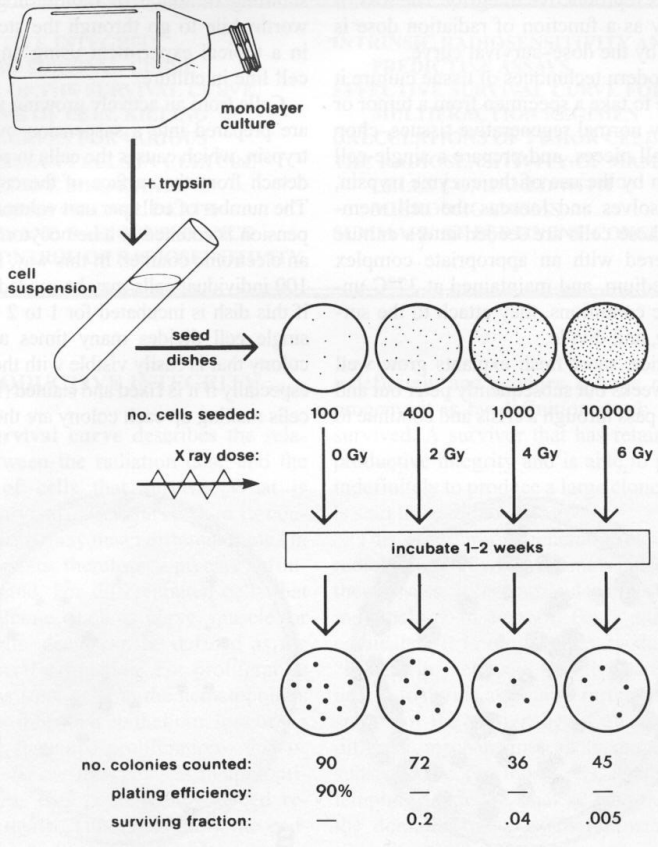
(Predictive Assays, Chapter 23)

- Intrinsic Cell Radiosensitivity ($SF_{?Gy}$)
  - "Law" of Bergonie & Tribondeau
- Oxygen Levels (hypoxia)
- Tumour Growth Rate ($T_{doubling}$)



In-Vitro Models

Good experimental system
Well- behaved cells
Controlled environment (e.g. O_2)
Artificial conditions (e.g. no micro-vasculature) !
Not easily extrapolated to *in-vivo* situations



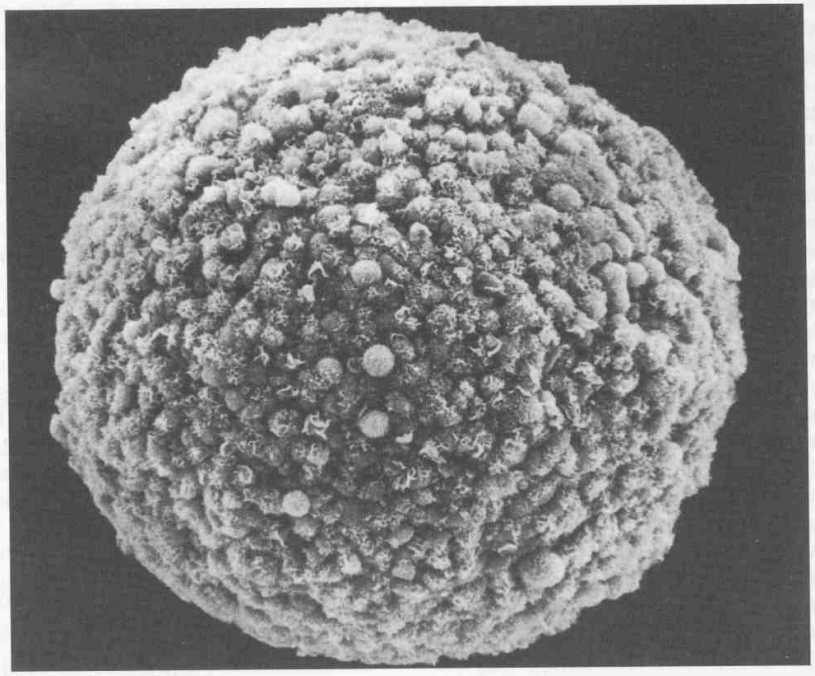
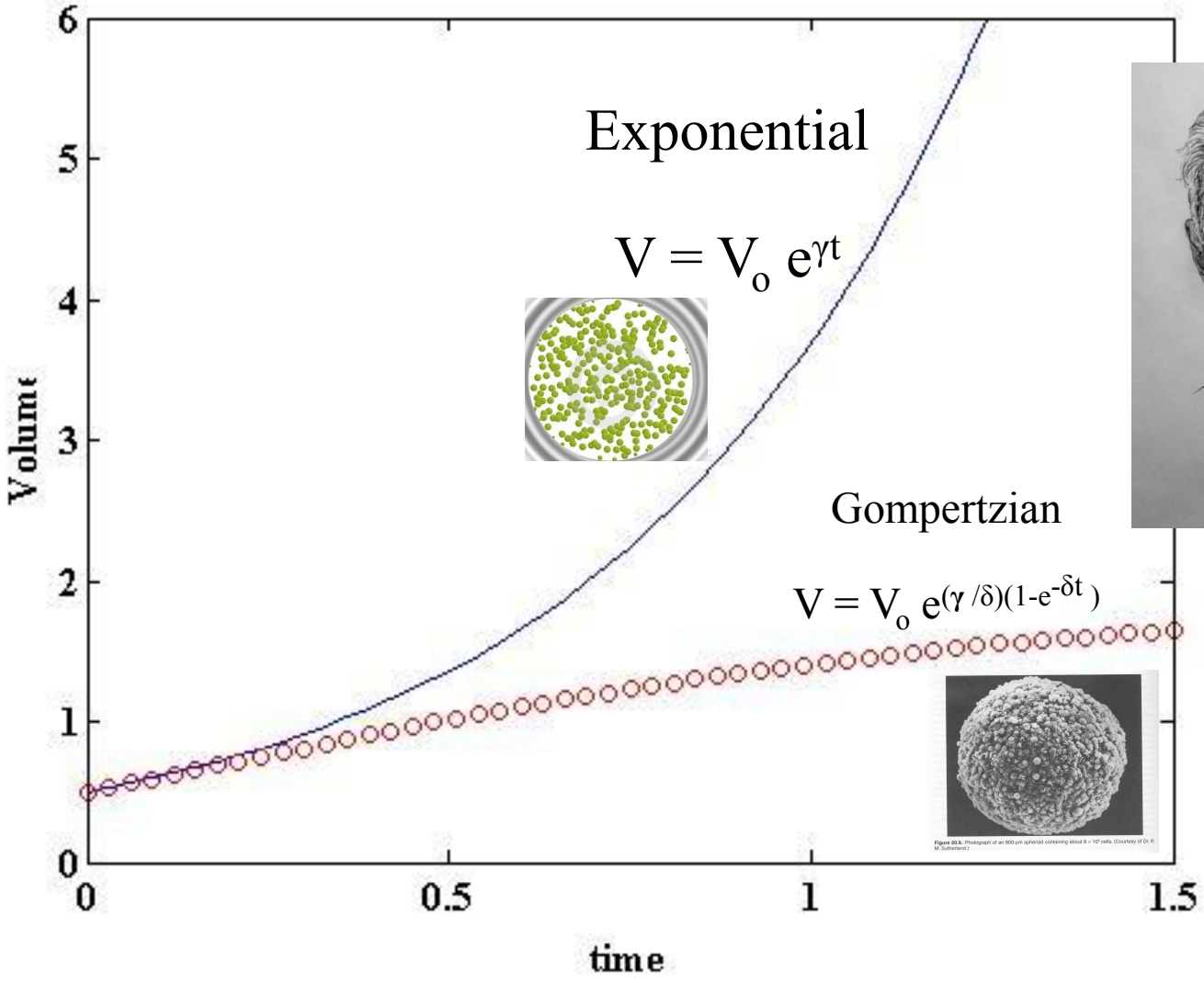
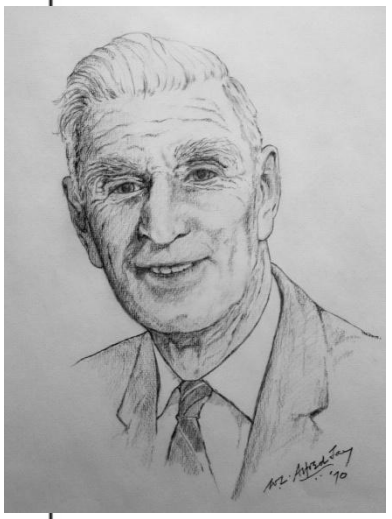


Figure 20.9. Photograph of an 800-μm spheroid containing about 8×10^4 cells. (Courtesy of Dr. R. M. Sutherland.)

Spheroid Growth already deviates from Exponential Growth

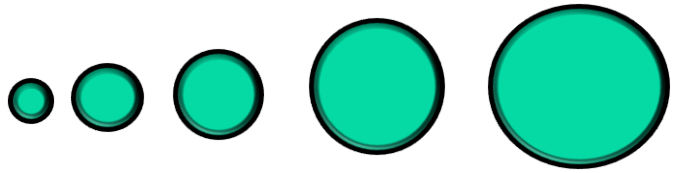




AC Burton

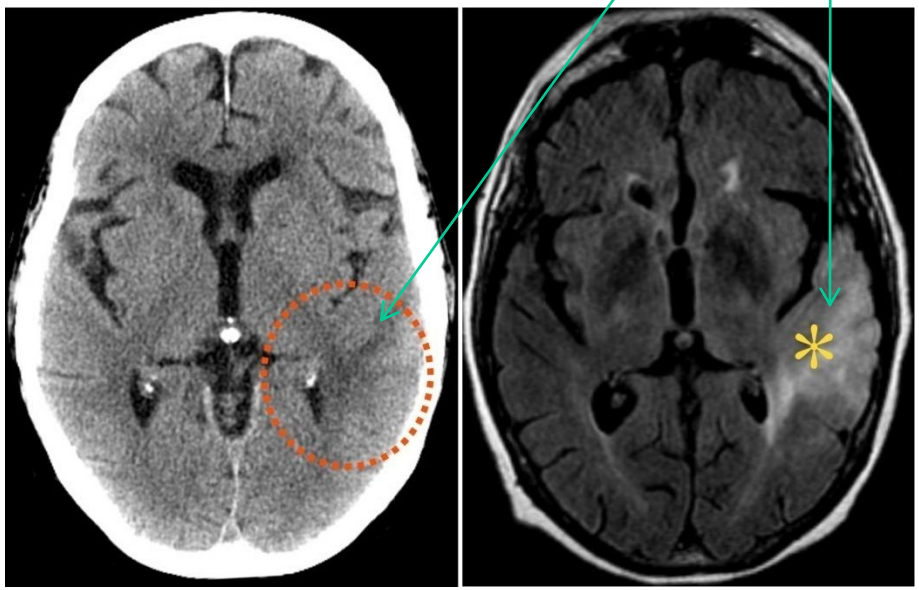
http://thumbs.dreamstime.com/thumb_357/1232842467247iAu.jpg

In-Vivo Growth Assays



Measure tumour volume by:

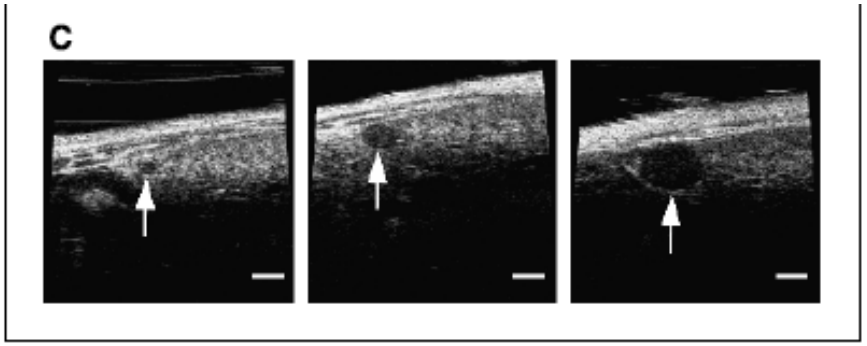
- Mechanical Means – palpation, calipers, Archimedes
- Imaging Techniques (Ultrasound, CT, MRI etc.)
- Surrogate markers (e.g. blood PSA, growth enzymes)



Three-dimensional High-Frequency Ultrasound Imaging for Longitudinal Evaluation of Liver Metastases in Preclinical Models

Kevin C. Graham,[1,4] Lauren A. Wirtzfeld,[2,5] Lisa T. MacKenzie,[1] Carl O. Postenka,[4] Alan C. Groom,[1] Ian C. MacDonald,[1] Aaron Fenster,[1,2,5] James C. Lacefield,[1,2,3,5] and Ann F. Chambers[1,4]

Departments of [1]Medical Biophysics, [2]Biomedical Engineering Graduate Program, and [3]Electrical and Computer Engineering, The University of Western Ontario; [4]London Regional Cancer Program; and [5]Robarts Research Institute, London, Ontario, Canada



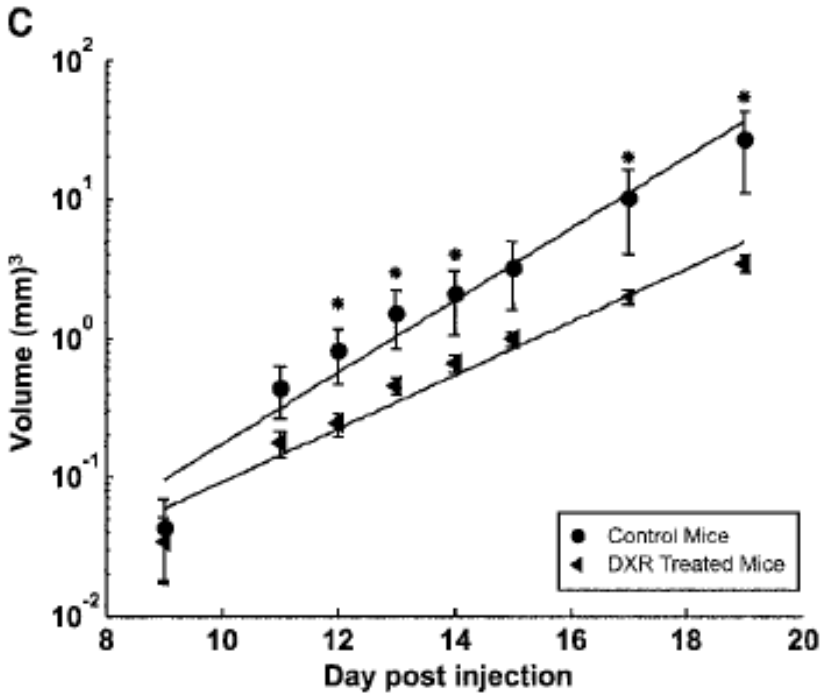
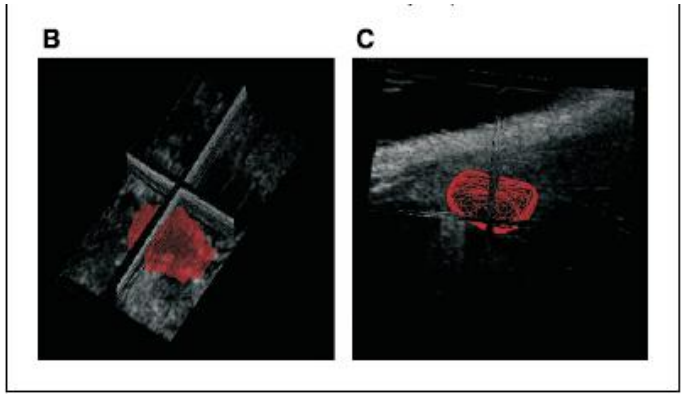


Figure 2. Tracking the growth of individual liver metastases by noninvasive ultrasound imaging. *A*, growth curves of B16F1 liver metastases plotted on a semilogarithmic scale. *B*, growth curves of HT-29 and MDA-MB-435/HAL liver metastases plotted on a semilogarithmic scale. *C*, representative two-dimensional ultrasound images of B16F1-E. Sizes of B16F1-E (maximum diameter → segmented volume) are 0.50 mm → 0.06 mm^3 (day 10), 1.07 mm → 0.61 mm^3 (day 14), and 2.09 mm → 3.79 mm^3 (day 18). Bar on the ultrasound images, 1.00 mm.



Primary Human Lung Tumours

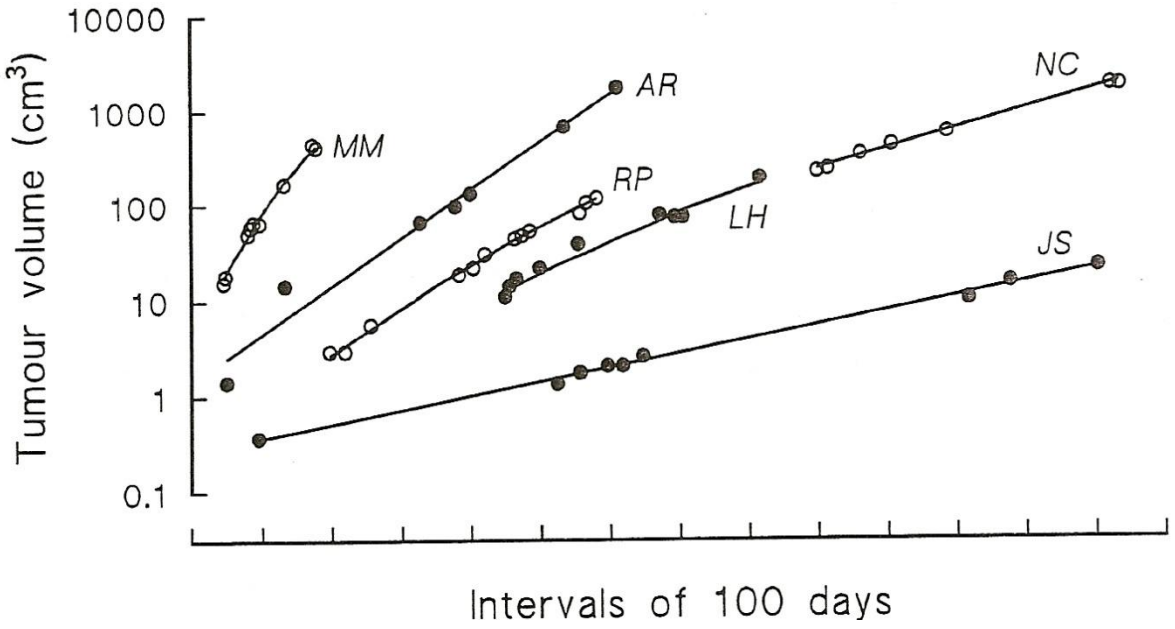


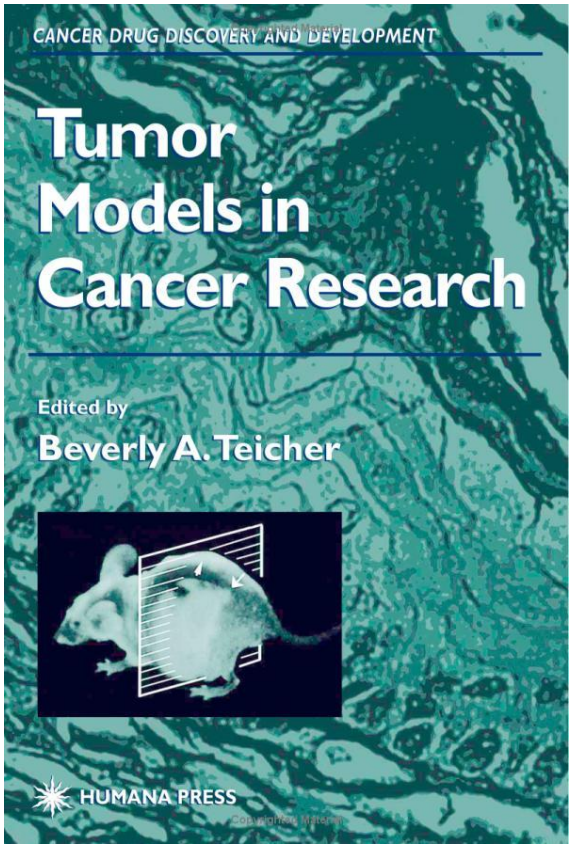
Figure 2.4 Growth curves for primary human lung tumours. Data of Schwartz, redrawn by Steel (1977), with permission.

Steel 1997

Tumour Growth Delay Assay

(following therapy)

Traditional way to measure effectiveness or radiotherapy



T_{AB} = Time to grow back from Size "A" to Size "B"

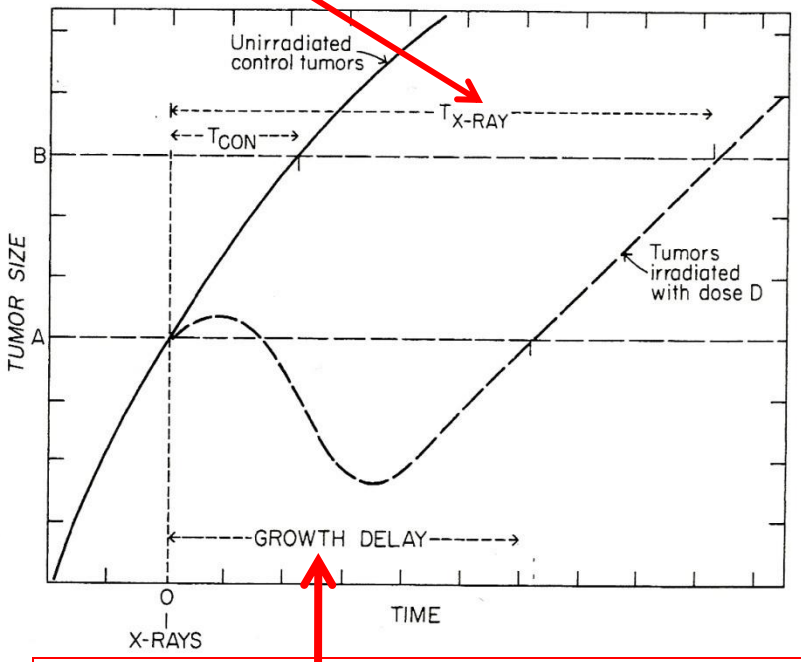


FIGURE 20.1 The pattern of response of a tumor to a dose of x-rays. The size of the tumor, either the mean diameter or the volume, is plotted as a function of time after irradiation. Two different indices of tumor responses have been used by different investigators. Growth delay represents the time after irradiation that it takes for the tumor to regrow to the size at the time of irradiation. Alternatively, the index of radiation damage may be the time taken for the tumor to grow from a specified size A at the time of irradiation to some specified larger size B. Typically, this may be from 9 to 25 mm in diameter for rat tumors. This quantity is shown as T_{CON} for unirradiated control animals and $T_{X\text{-}RAY}$ for tumors irradiated with a dose (D) of x-rays. Either index of tumor response may be plotted as a function of radiation dose.

T_{delay} = Time to re-grow to initial size at start of treatment

Tumour Cure Assay – TCD_{50}

- Animal Study
- Mimics clinical trial
- Groups of animals exposed to various dose fractionation schemes
- Score the % of animals in which local control of tumour growth was achieved

- TCD_{50} = Dose that controls 50% of tumours

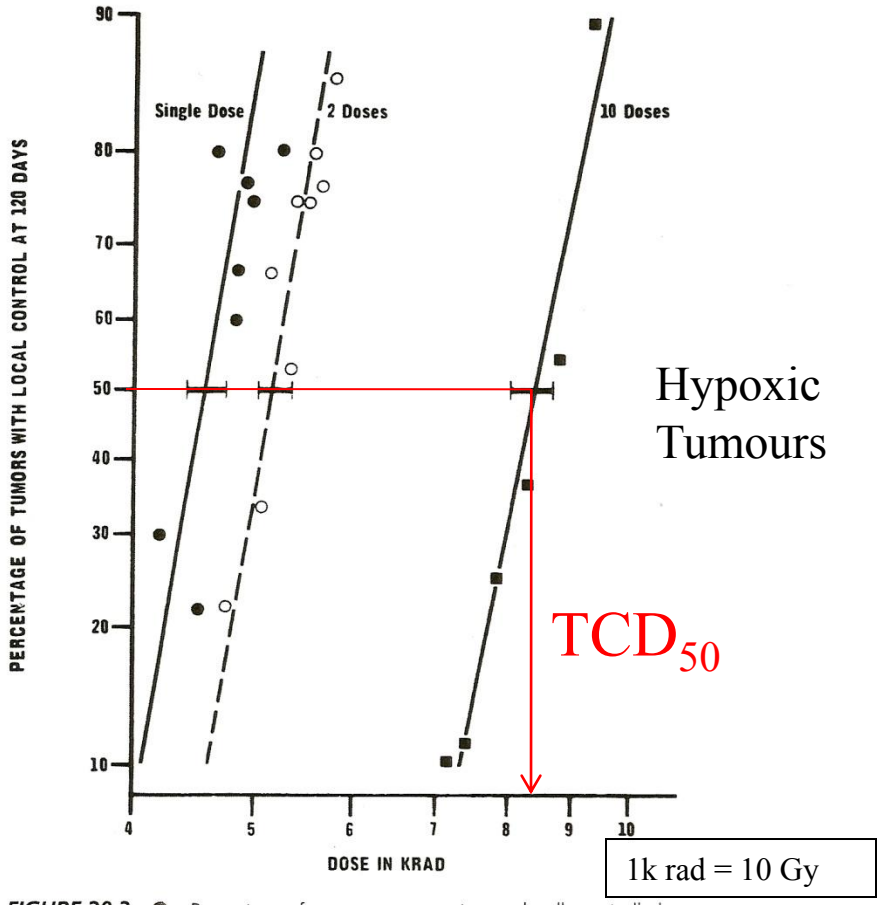
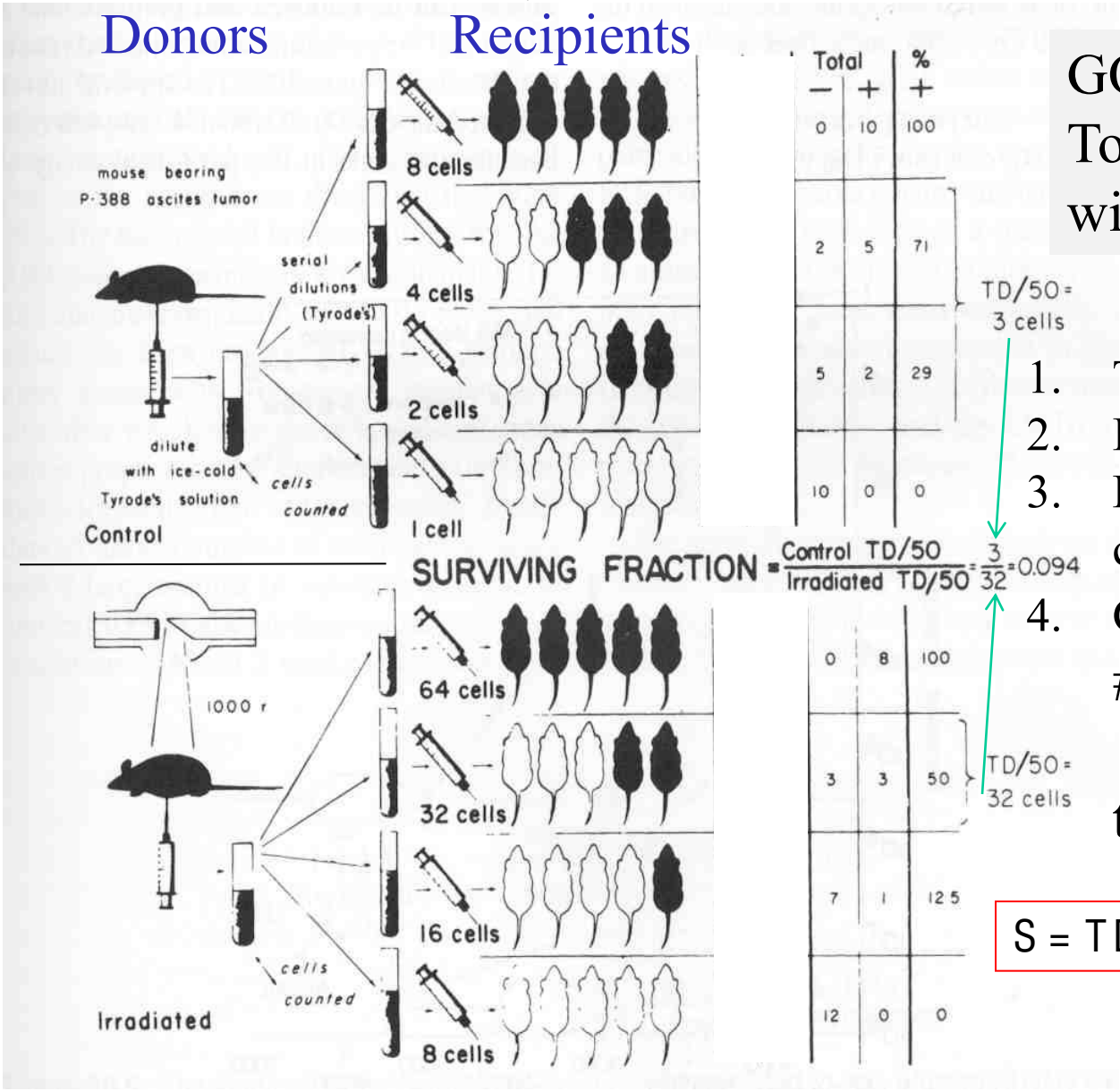


FIGURE 20.3 Percentage of mouse mammary tumors locally controlled as a function of x-ray dose, for single exposures and for two different fractionation patterns. The tumors were isotransplants derived from a spontaneous mammary carcinoma in a C_3H mouse. The transplantation was made into the outer portion of the ear with 4×10^4 viable cells. The tumors were treated when they reached a diameter of 2 mm (i.e., a volume of about 4 mm^3). (From Suit H, Wette R: Radiation dose fractionation and tumor control probability. *Radiat Res* 29:267–281, 1966, with permission.)

Tumour Dilution Assay - TD_{50}

(not to be confused with other TD !)



GOAL:
To measure Cell Survival with *in-vivo* conditions

1. Treat donor cells *in vivo*
2. Extract cells from donor
3. Dilute and transplant # of cells in recipients
4. Calculate TD_{50} = # of cells that yields 50% transplant "takes"

$S = TD_{50}$ (control) / TD_{50} (treated)

Lung Colony Assay

- Similar to TD50 transplantation method but uses solid tumours
- Grow and irradiate tumour *in vivo* in donor
- Extract cells and inject into identical recipient animal
- Wait and count lung colonies formed
- Number of lung colonies in recipient "tracks" with cell survival in donor tumour

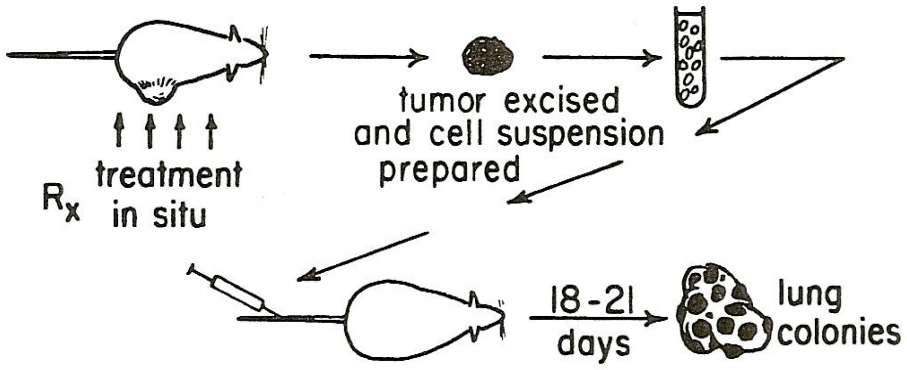


FIGURE 20.6 The lung colony assay system. The tumor is irradiated *in situ*, after which it is excised and made into a single-cell suspension. A known number of cells are then injected intravenously into recipient animals. About 3 weeks later, the recipient animals are sacrificed and the colonies that have formed in the lungs are counted. The number of lung colonies is a measure of the number of surviving clonogenic cells in the injected suspension. (From Hill RP, Bush RS: The effect of continuous or fractionated irradiation on a murine sarcoma. *Br J Radiol* 46:167–174, 1973, with permission.)

Note : Xenografts (human tumours grown in immuno-deficient "nude" mice) are also possible. See Page 355-356

"In Vivo - In Vitro" Assay

- Treat *in vivo* and measure cell survival *in vitro*
- Replaces the tedium, expense, and variability of "recipient"

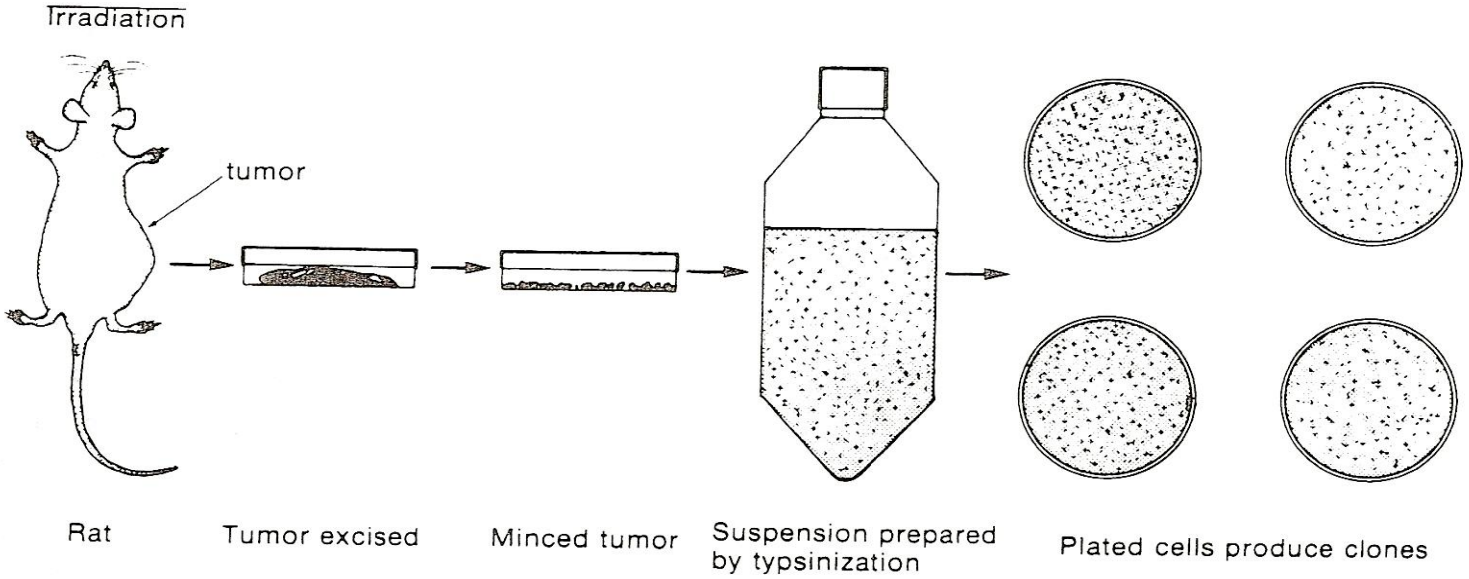
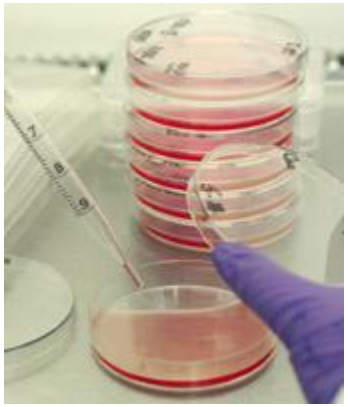


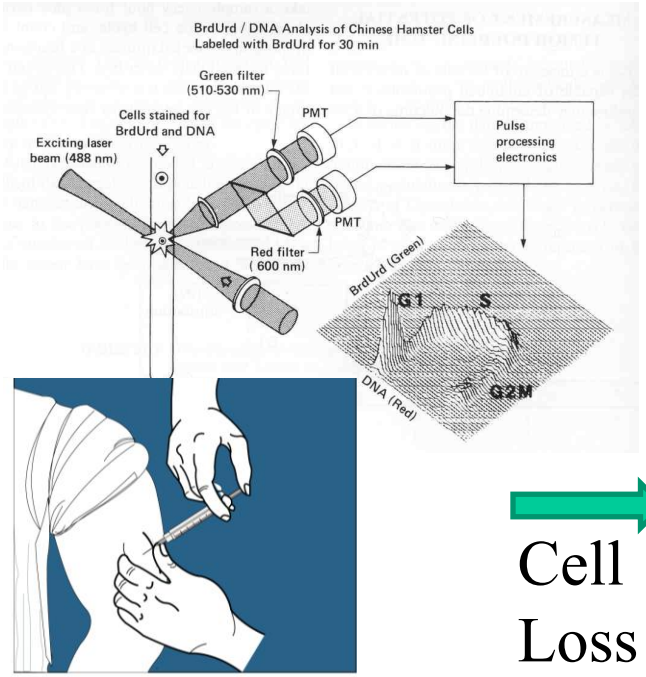
FIGURE 20.7 The principle of the *in vivo/in vitro* assay system using the rhabdomyosarcoma in the rat. The solid tumor in the animal can be removed and the tumor cells assayed for colony formation in petri dishes. This cell line can be transferred back and forth between the animal and the petri dish. (Courtesy of Drs. G.W. Barendsen and J.J. Broerse.)

3 Types of "Repopulation" Times

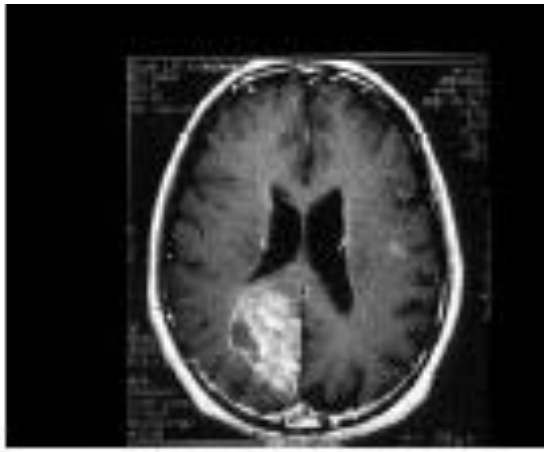


In Vitro

Growth Fraction (GF)



Cell Loss Φ



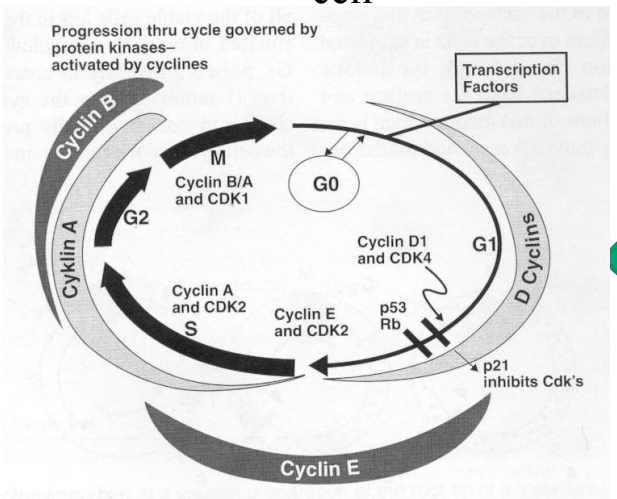
In Vivo

$T_{cell} < T_{pot} < T_{doubling}$

http://www.lifeinthefastlane.ca/wp-content/uploads/2008/05/body_builder_7sfw.gif

Cell Equilibrium Model

T_{cell}

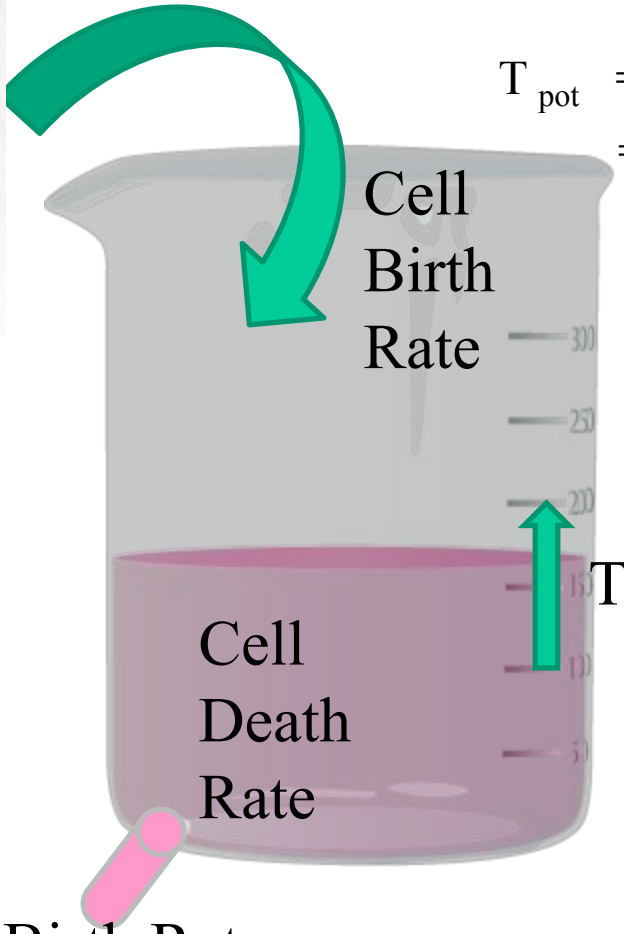


Growth Fraction (GF in %)

Tpot is doubling time if there is no cell loss

T_{pot} = ln2 /mitotic rate constant

$= (T_c/\ln 2) \ln [(100 + GF)/GF]$



T_d Doubling Time

Cell Loss Factor Φ

= Cell Death Rate/Cell Birth Rate

$= 1 - (T_{pot}/T_d) = (T_d - T_{pot})/T_d = \Delta T/T_d$

0 if $T_d = T_{pot}$
(Death Rate = 0)

1.0 if $T_d >> T_{pot}$
Birth Rate = Death Rate

Time after Time

More *in vivo*

Cell Cycle Time (T_c) – *in vitro*

Idealized conditions

With Growth Fraction and NO Cell Loss, we get

Potential Doubling Time (T_{pot}) – *in vivo/vitro*

Based on flow cytometry (FACS)

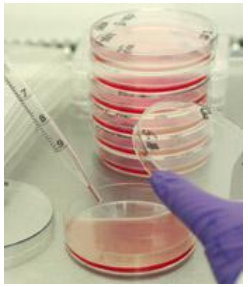
Called T_{pot} by English radiobiologists !

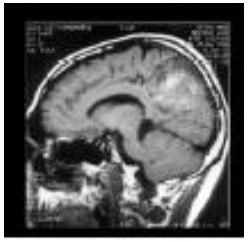
With Growth Fraction and Cell Loss, we get

Tumour Doubling Time (T_d)– *in vivo*

Tumour volume is measured (e.g. Imaging)

Longer Duration





Cell Cycle Times

TABLE 21.4

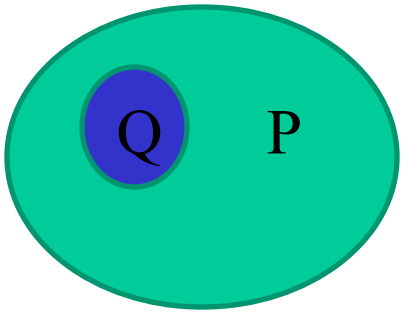
Individual Values for the Duration of the Cell Cycle (T_C) in Human Solid Tumors of Various Histologic Types

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

[a] Measured by the mean grain count halving time.

From Tubiana M, Malaise E: Growth rate and cell kinetics in human tumors: Some prognostic and therapeutic implications. In Symington T, Carter RL (eds): *Scientific Foundations of Oncology*, pp 126–136. Chicago, Year Book Medical Publishers, 1976, with permission.

Growth Fraction



$$GF = P/(P+Q)$$

where:

P Proliferating Cells (cycling and undergoing mitosis)

Q Quiescent Cells (stalled in G_o-phase, necrotic, hypoxic…)

TABLE 21.2

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

Potential Doubling Time (T_{pot})

Used to study kinetic of human tumours *in vivo*

Method:

1. Inject tracer amount of (unlabelled) BudR
2. Wait a time less than estimated S-duration of cell cycle (T_s) Typically 6 hours
3. Take a biopsy sample from the tumour
4. Add a "Green" fluorescent marker for BUdR to label cells that were in S-phase at time of step 1
5. Add a "Red" fluorescent marker for current DNA content, independent of cell cycle phase
6. Extract cells to a "single-file"suspension of individual cells
7. Analyze by Flow Cytometry (aka FACS)

Flow Cytometry

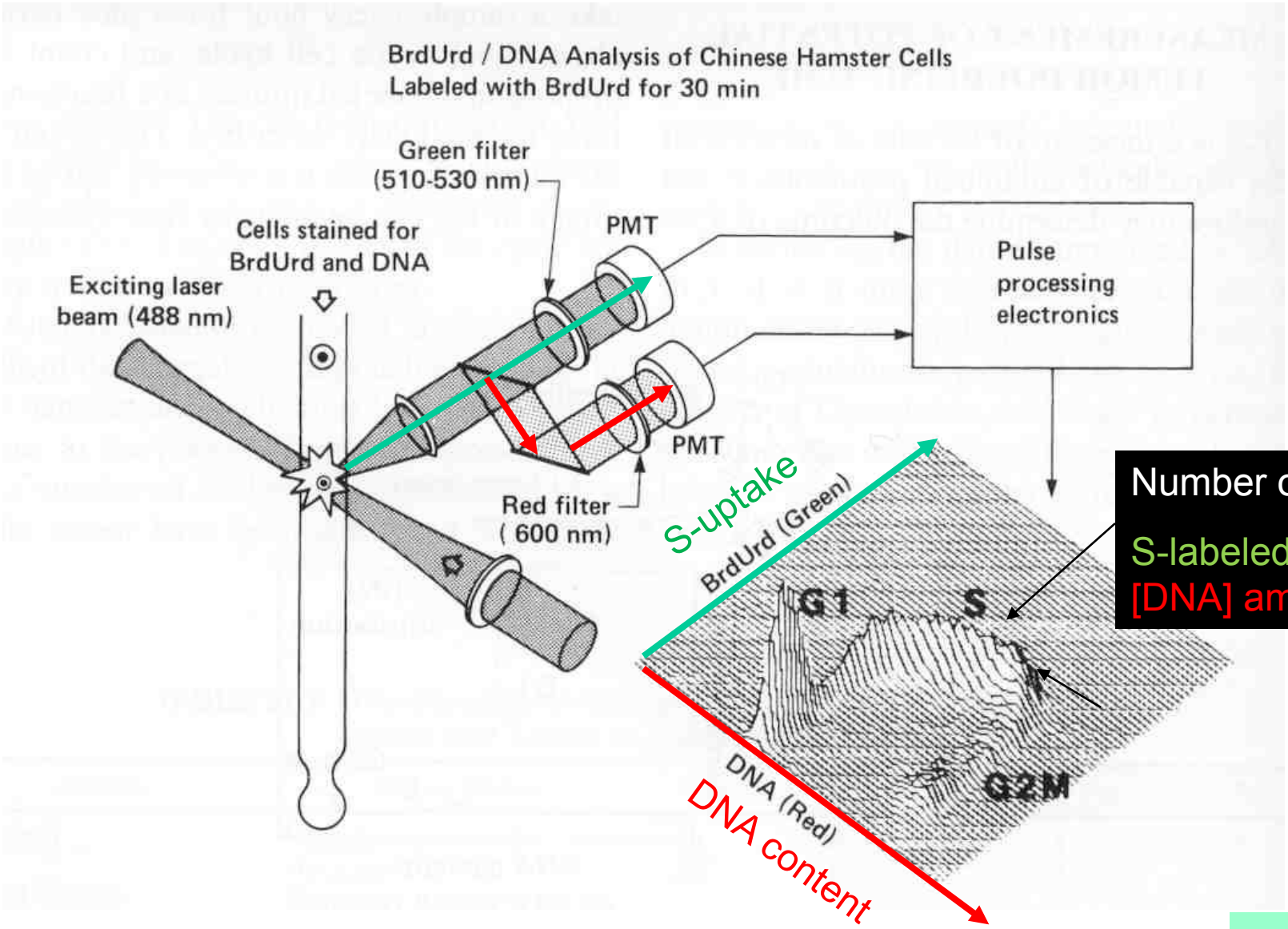


Fig 21.9

Cytometry 14:527–534 (1993)

Determining T_{pot} in Heterogeneous Systems: A New Approach Illustrated With Multicell Spheroids[1]

Ralph E. Durand

B.C. Cancer Research Centre, Vancouver, British Columbia V5Z 1L3, Canada

Received for publication September 10, 1992; accepted December 22, 1992

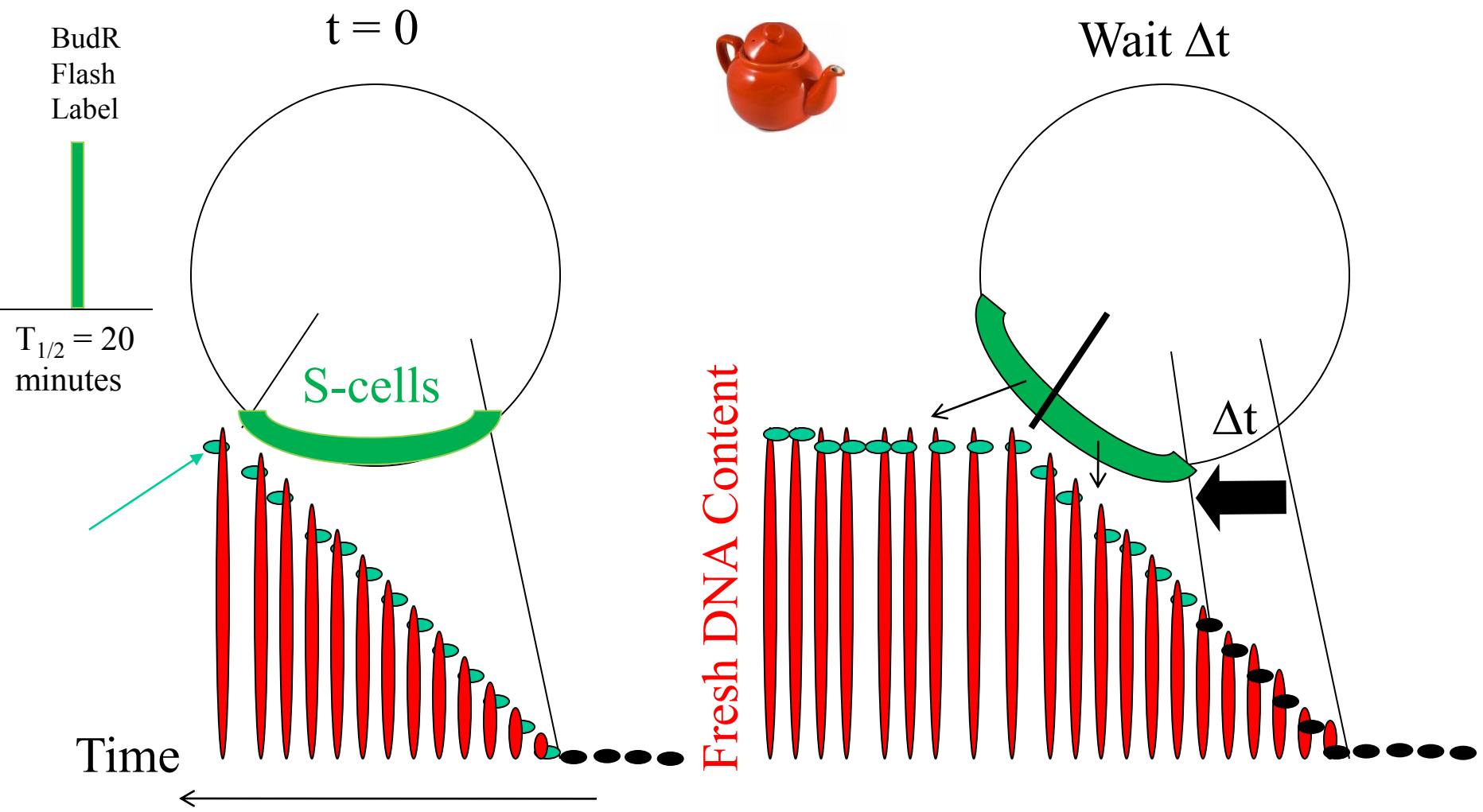
Staining Method applied to specimen Δt after BudR injection:

"Intact bromodeoxyuridine (BrdUrd) labelled cells recovered from the spheroids were fixed in ethanol, hydrolyzed in HCl, reacted with fluorescein-conjugated anti-BrdUrd antibody (purchased from Becton Dickinson, Oxnard, CA), washed, and resuspended in 5 pg/ml propidium iodide (PI) for analysis.'

T_{pot} Basics

Green (BUdR- Ab) tags S-cells at t = o

Red (propidium iodide) tags the mass of manufactured DNA



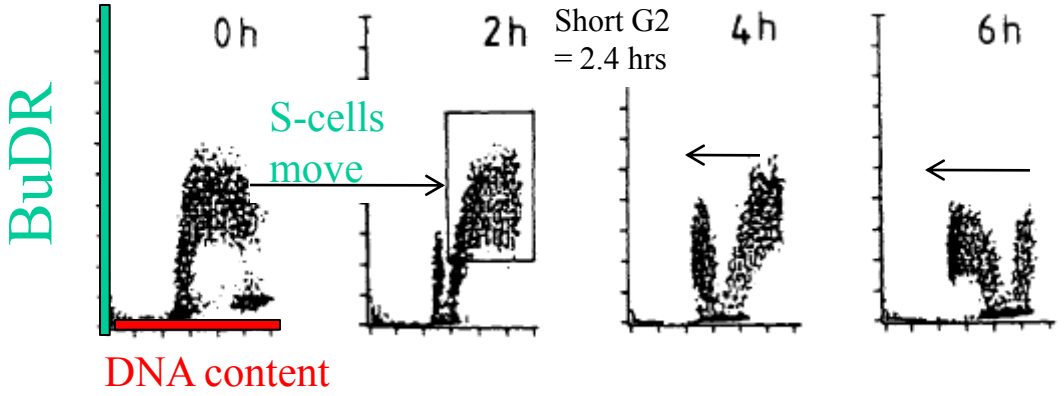
Cytometry 6:620–626 (1985)

A Method to Measure the Duration of DNA Synthesis and the Potential Doubling Time From a Single Sample

A.C. Begg[1], N.J. McNally, D.C. Shrieve[2], and H. Kärcher[3]

Gray Laboratory of the Cancer Research Campaign, Mount Vernon Hospital, Northwood, Middlesex HA6 2RN, United Kingdom

Received for publication February 28, 1985; accepted July 19, 1985



Calculation of S-Duration (T_s)

RM: average red signal due to fresh DNA

Method 1 - Relative Movement (RM) (Begg et al. 1985)Δ

$$T_s = \frac{0.5}{RM - 0.5} \times \Delta t\,, \qquad RM = \frac{F_L - F_{G1}}{F_{GM} - F_{G1}},$$

where F_L is the mean red fluorescence of the green-labeled cells and F_{G1} and F_{GM} are the mean red fluorescence valus of G1 and G2+M cells, respectively. At time zero (immediately after labelling), F_L will be approximately half-way between the G1 and G2 values, i.e., RM will be approximately 0.5. With time, F_L will approach the fluorescence of G2 cells and RM will approach unity.

Method 2 - % Still in S (Durand 1993)

Determine population fraction (f) of BudR-labeled "S" cells still in S after a wait time, Δt. These S cells have variable DNA conent:

G1 cell DNA < DNA content < G2 cell DNA).

$$T_s = \Delta t / (1.00 - f)$$

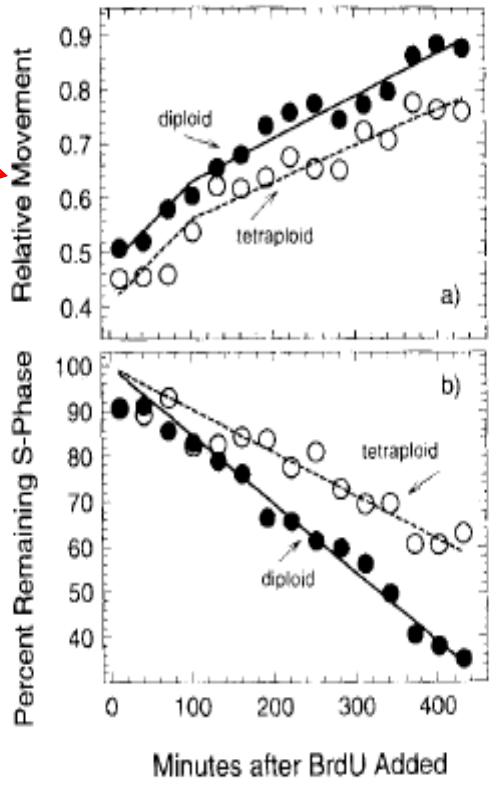
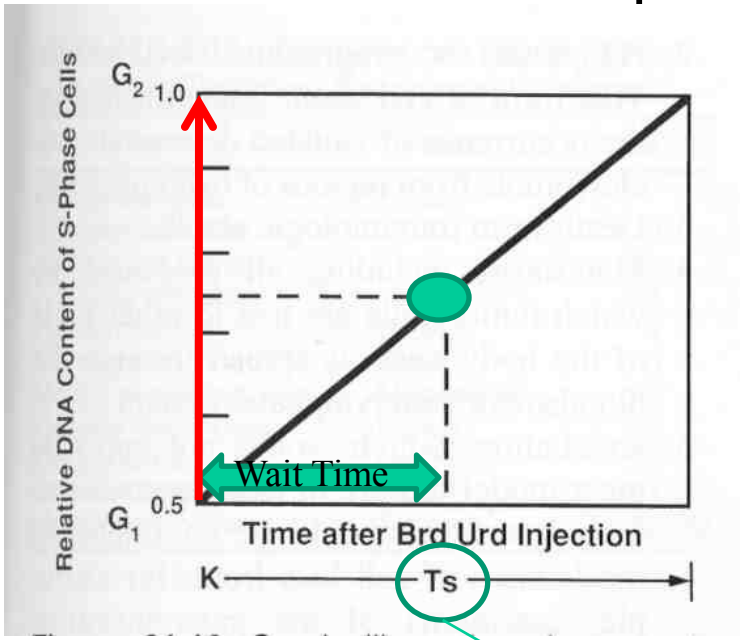


FIG. 5. A comparison of the observed "relative movement" (a) or "%S-phase" (b) for labelled cells, expressed as a function of the time of assay. Note the linearity of the data in b (see text for futher discussion).

Calculating T_{pot}



T_S and LI from Flow Cytometer

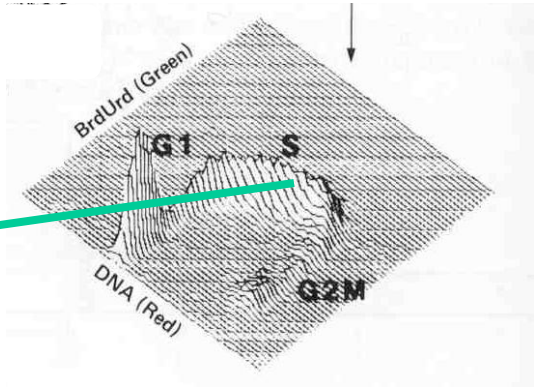
$T_{pot} = \lambda T_S / LI$

where :

λ= 0.67 to 1.00

T_s – duration of S

LI – fraction of cells in S -phase



Cytometry 14:527–534 (1993)

Determining T_{pot} in Heterogeneous Systems: A New Approach Illustrated With Multicell Spheroids[1]

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Received for publication September 10, 1992;

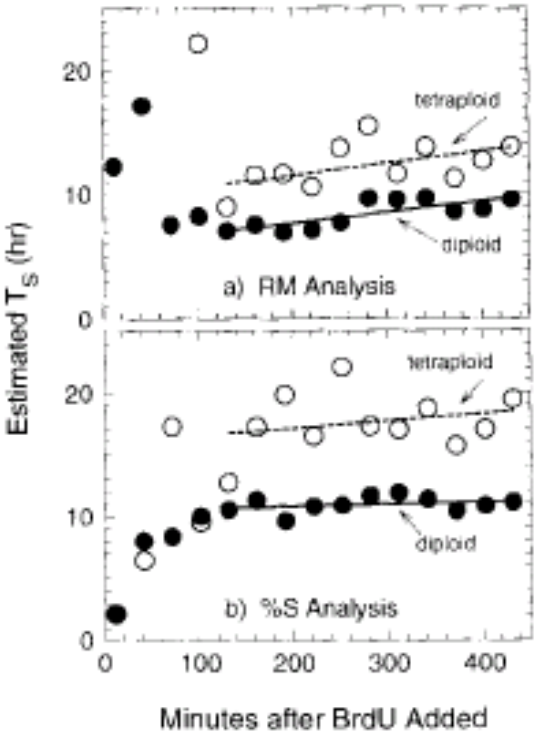


FIG. 6. Estimates of S-phase duration for the cycling diploid and tetraploid cells, based on "relative movement" (**a**) or "percent S-phase" (**b**). Note that the best-fit curves for data acquired 2 or more h after labelling were shallower in panel b, indicating less dependence of the T_s measurement on the time the assay was performed (see text).

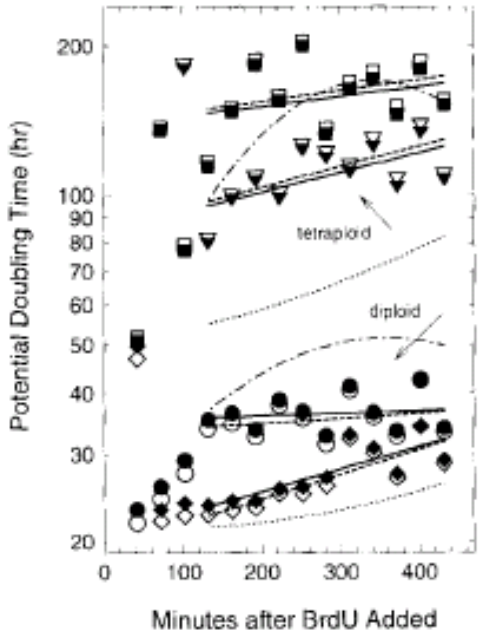


FIG. 4. A compilation of several different estimates of T_{pot}, all expressed as a function of the time of assay relative to beginning BrdUrd administration. Diamonds and triangles resulted from the "relative movement" model, whereas the circles and squares reflect the new "%S-phase" approach. Open symbols show an assigned value of $\lambda = 0.7$; the closed symbols were derived with a more objective correction for the age distribution of the cells (see text). The regression lines show linear best-fits to data acquired more than 2 h post-labelling; note that the new technique resulted in higher values for T_{pot} that tended to be more constant with time. The curves show the range of T_{pot} values that could result from the relative movement analysis depending upon the way the 4N cells are classified (see text).

Comments on T_{pot} Estimates

Issues of Uncertainty:

- Biopsy is only a sample of all the tumour cells
- Cell Age distribution assumed
  - Cell Loss effects ?
- Cell "velocity" around the cycle (variable T_{cell})
- DNA manufacturing linear with time ?
- Cytometer noise, sensitivity/specificity
  - e.g. detection thresholds
- Non-diploid cells in tumours
- Validation
  - *versus* other *in-vitro* method
  - predictive of tumour response/control ?

References

Begg AC, McNally NJ, Shrieve DC, Karcher H: A method to measure the duration of DNA synthesis and the potential doubling time from a single sample. Cytometry 6:620-626, 1985.

White RA, Terry NHA, Meistrich ML: New methods for calculating kinetic properties of cells in vitro using pulse labelling with bromodeoxyuridine. Cell Tissue Kinet 23561-573, 1990.

White RA, Terry NHA, Meistrich ML, Calkins DP: Improved method for computing potential doubling time from flow cytometric data. Cytometry 11:314-317, 1990.

Time after Time

More *in vivo*

Cell Cycle Time (T_c) – *in vitro*

Idealized conditions

With Growth Fraction and NO Cell Loss, we get

Potential Doubling Time (T_{pot}) – *in vivo/vitro*

Based on flow cytometry (FACS)

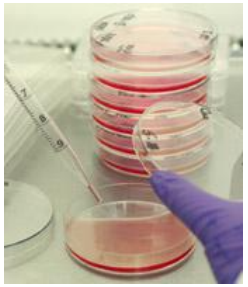
Called T_{pot} by English radiobiologists !

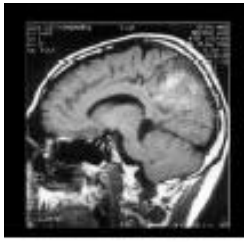
With Growth Fraction and Cell Loss, we get

Tumour Doubling Time (T_d)– *in vivo*

Tumour volume is measured (e.g. Imaging)

Longer Duration





Cell Loss Factor Φ

Cell losses due to:

- Cell death including apoptosis
- Immunologic attack
- Metastasis escape and migration

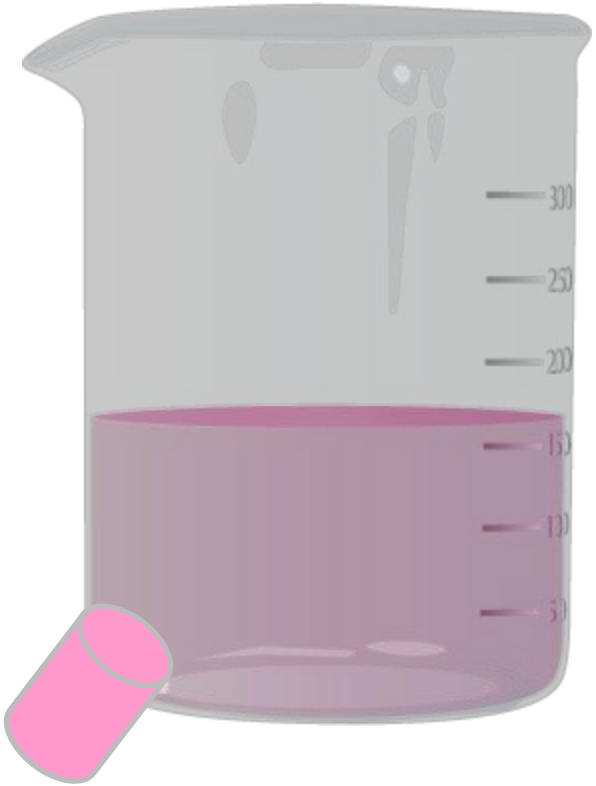


TABLE 21-3

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

Volume Doubling Times

(Lung Metastases)

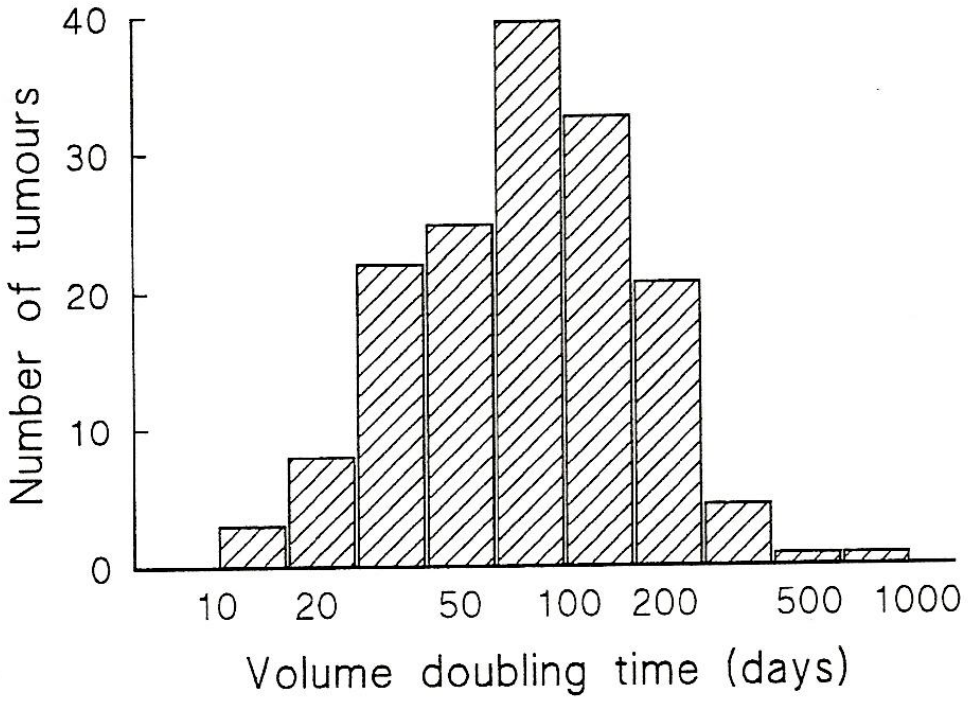
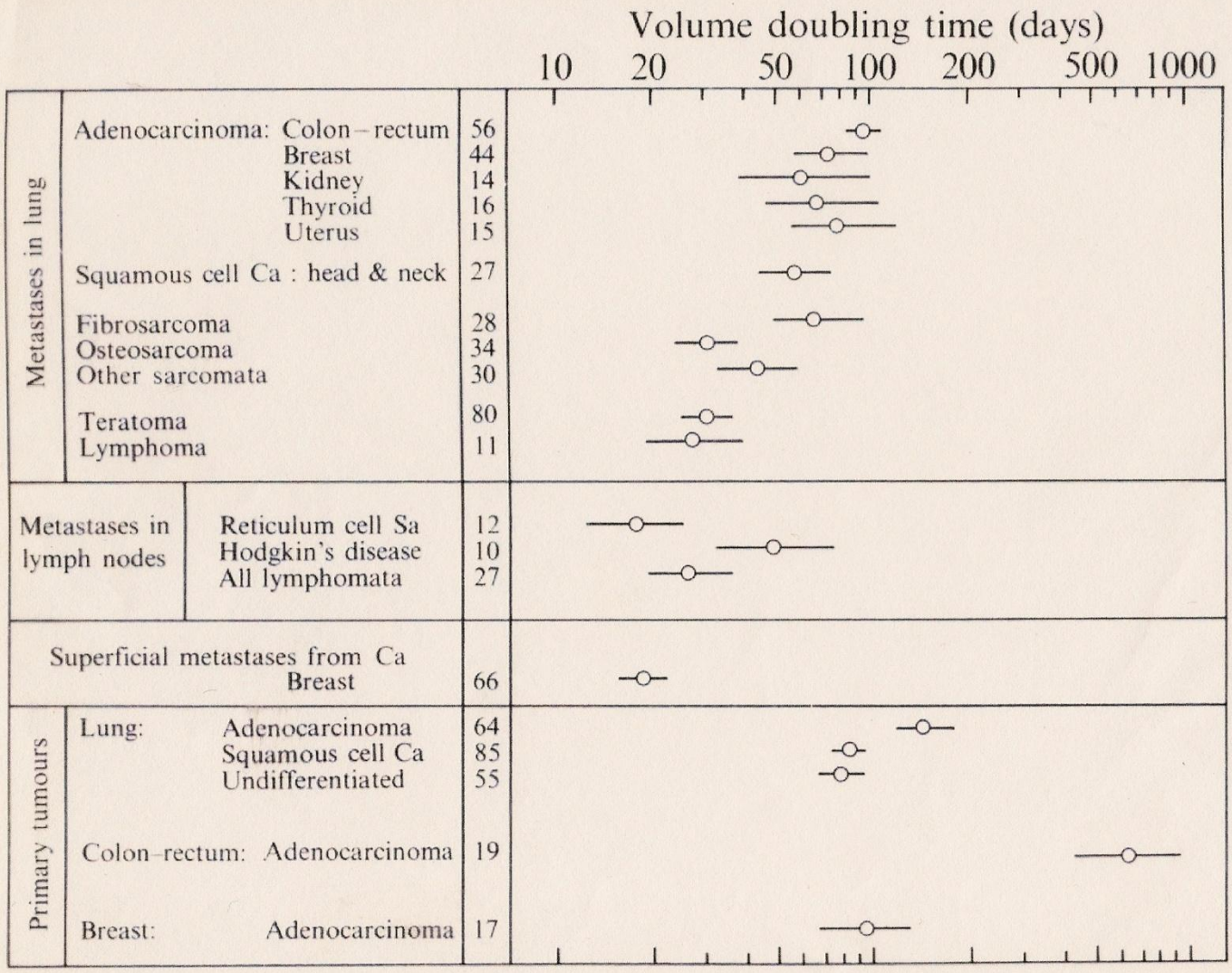


Figure 2.5 The distribution of volume doubling times for 159 lung metastases of adenocarcinoma from various primary sites. From Steel (1977) with permission.

Volume Doubling Times - Human Tumours



Steel, 1993

See Hall's Table 21.5

Figure 2.6 A summary of data on the volume doubling times of human tumours. The points show geometric mean values, with standard errors. From Steel (1977) with permision.

Summary of Results

Table 3.1 Kinetic parameters of a typical human tumour

Cell cycle time (~2 d)	Potential doubling time (~5 d)	Volume doubling time (~60 d)
Growth fraction (~40%)		
Cell loss (~90%)		

Table 3.2 Cell loss calculations for human tumours

	Thymidine labelling index (%) *(median and range)*	**Volume doubling time (days)** *(median and range)*	**T_{pot} (days)**	**Cell loss factor (%)**
Colorectal carcinoma	15 (10 – 22)	90 (60 – 170)	3.1	96
Squamous cell carcinoma of head and neck	6.9 (5 – 17)	45 (33 – 150)	6.8	85
Undifferentiated bronchial carcinoma	19 (8 – 23)	90 (40 – 160)	2.5	97
Melanoma	3.3	52 (20 – 150)	14	73
Sarcoma	2.0 (0.3 – 6)	39 (16 – 78)	23	40
Lymphoma	3.0 (0.4 – 13)	22 (15 – 70)	16	29
Childhood tumours	13 (10 – 25)	20	3.6	82

From Steel (1977)

Summary on R4

R3, not R4

- *In vivo* conditions are very different from *in vitro* conditions for tumour growth
- GF and Φ factors (< 100 %) in tumours work to our advantage in slowing the growth of most tumours
- There is evidence that radiation causes accelerated proliferation after a delay time ($T_{kickoff}$ in BED equations)
- Higher Dose Rate or Dose per Fraction is better for tumour control to achieve:

Tumour cell killing rate > Cell repopulation rate

- BUT… Normal Tissues also need to repopulate !

Summary of Differential Effects on Tumour and Normal Tissues

4Rs	Tumour Cells	Normal Cells
Repair		
Re-Assortment		
Re-Population		
Re-Oxygenation		

Balance is affected by Dose and Dose Rate/Fractionation