MICROBIAL GROWTH - -Elements of Microbial Nutrition

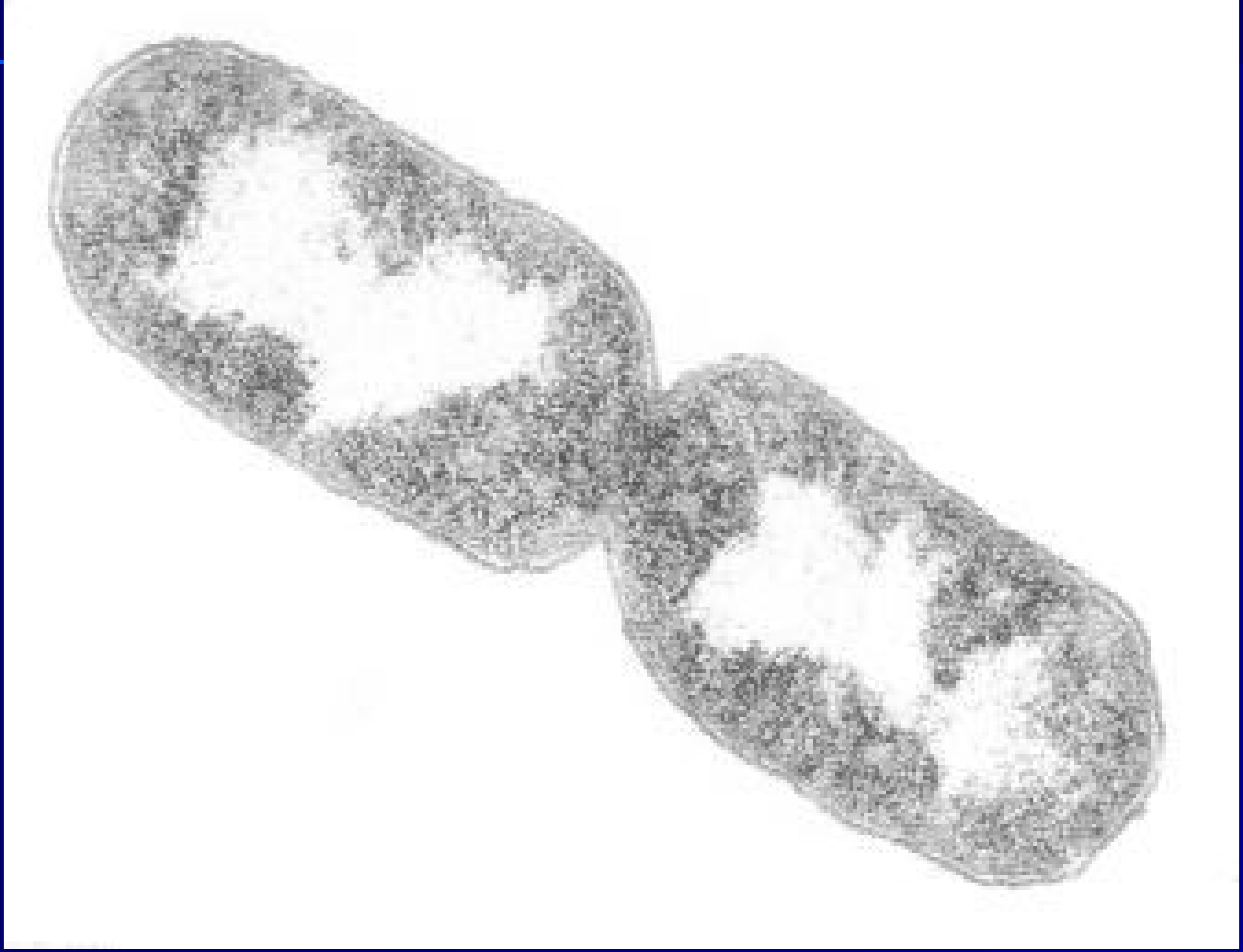
Bacterial Cell Division

- New cells are formed by cell fission
- Cells do not grow in size – they double their cytoplasmic contents and membrane
- They synthesize essential molecules needed for their metabolic processes

Prokaryote vs. Eukaryote

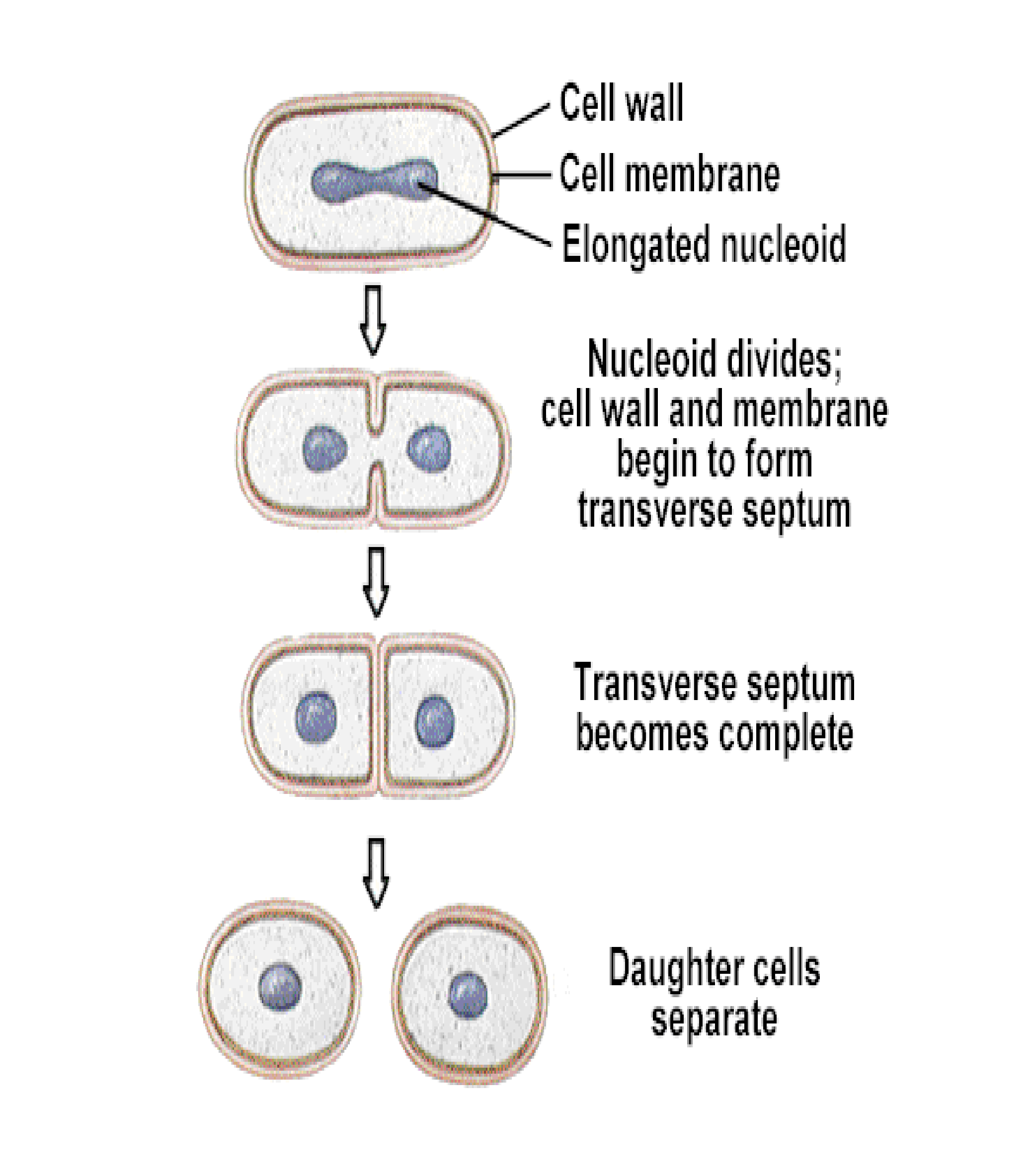
- Prokaryote cells do not go through the cell cycle like eukaryote cells
- They divide by fission
- In some species there is some linkage which forms tetrads, sarcinae, and even staphylococci

Binary Fission in Bacteria

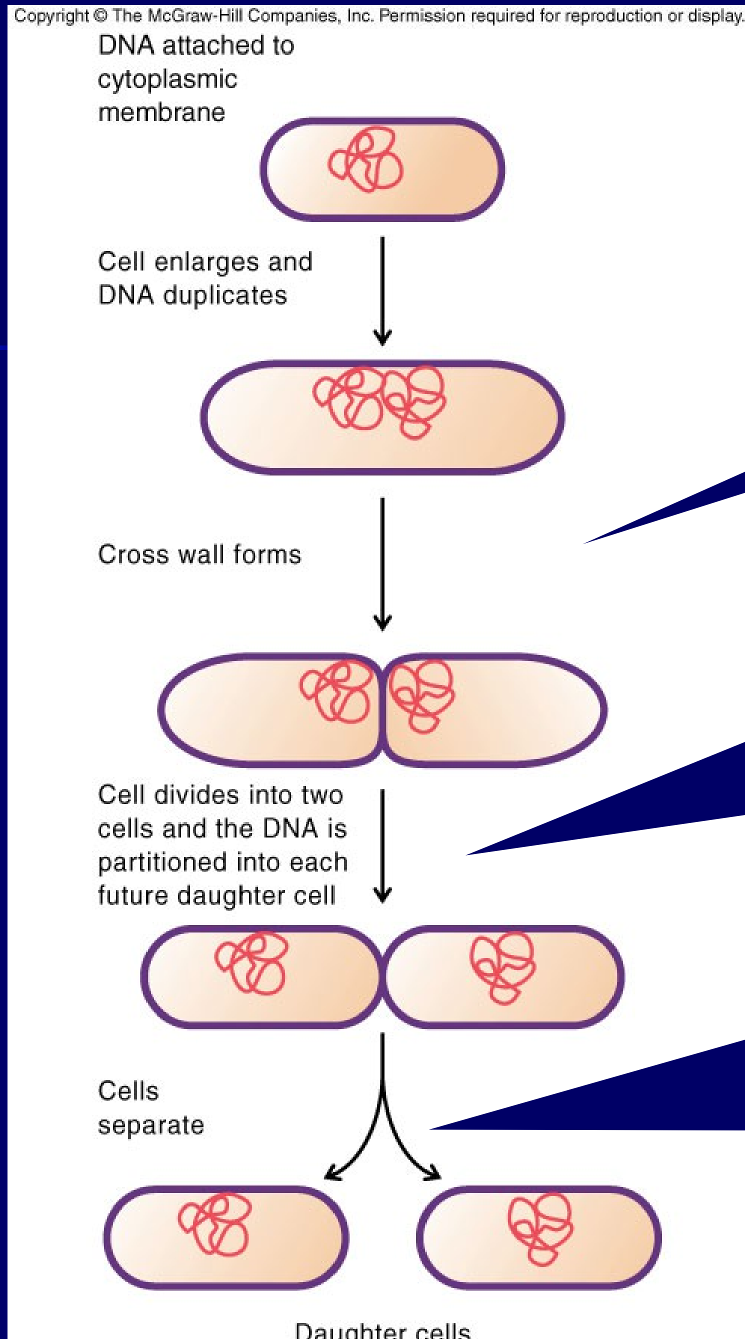


Binary Division

- 1 to 2 to 4 to 8 to



Binary Fission

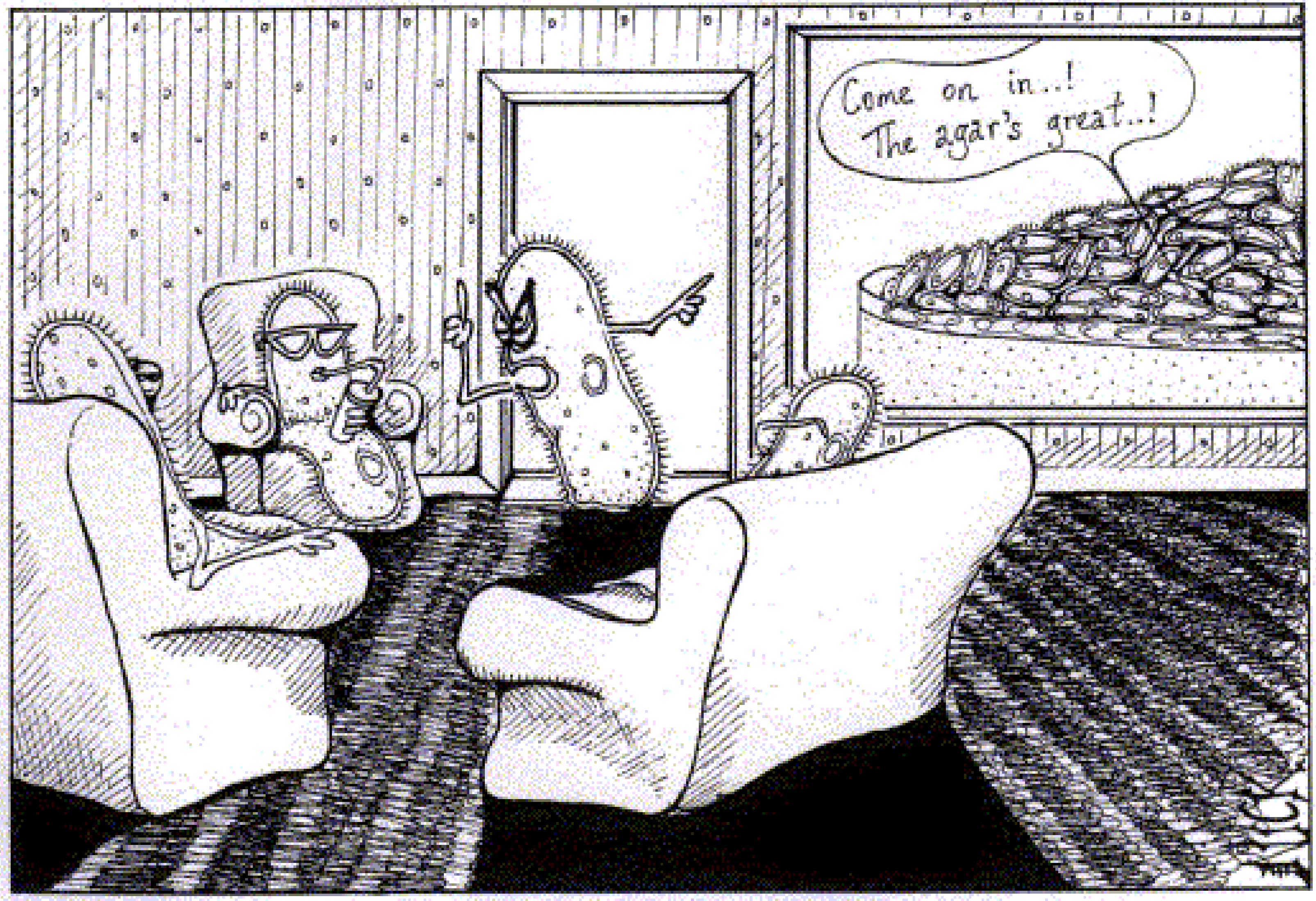


This is how most bacteria undergo cell division (how they replicate).

The interval, division to division, is called the Generation or Doubling Time.

Note that not all daughter cells fully separate after division, e.g. streptococci, etc.

- **Growth in the microbial world usually refers to an increase in the numbers of individuals; that is, an increase in the population size with each cell carrying the identical genetic instructions of the parent cell.**
- **Asexual reproduction is a process to maintain genetic constancy while increasing cell numbers.**
- In eucaryotic microbes, an elaborate interaction of microtubules and proteins with chromosomes allows for the precise events of mitosis and cytokinesis.
- Prokaryotes can accomplish the same thing without the microtubular involvement.



" I wish you'd learn to put the lid on your Petri Dish, Harry..!! We came here with four kids and now it looks like we've got twenty million !! "

- At the onset we need to grasp that
- 1- Microbial Growth refers to the NUMBER of cells, not the size of the cells
- Microbial Growth is defined as an increase in cell number rather than size.
- Bacteria- divide by binary fission- a mother cell doubles in size and then divides into two equal daughter cells.
- 2- The requirements for Growth are both
  - Physical
  - Chemical

- **Most Prokaryotes Reproduce by Binary Fission**
- Binary fission produces genetically identical daughter cells.
- **Most prokaryotes reproduce by an asexual process called binary fission, which usually occurs after a period of growth in which the cell doubles in mass.**
- At this time, the chromosome (DNA) replicates and the two DNA molecules separate.

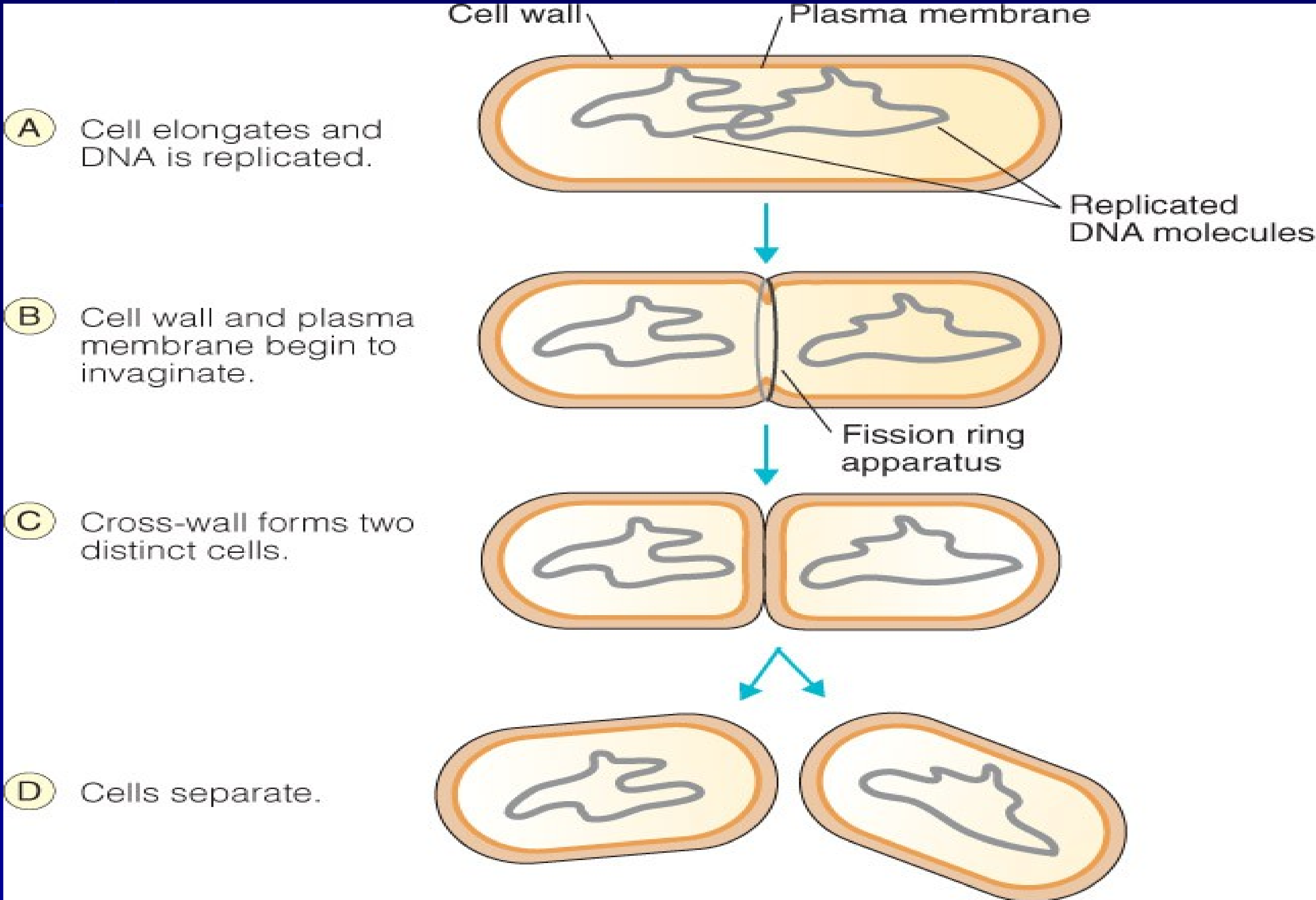
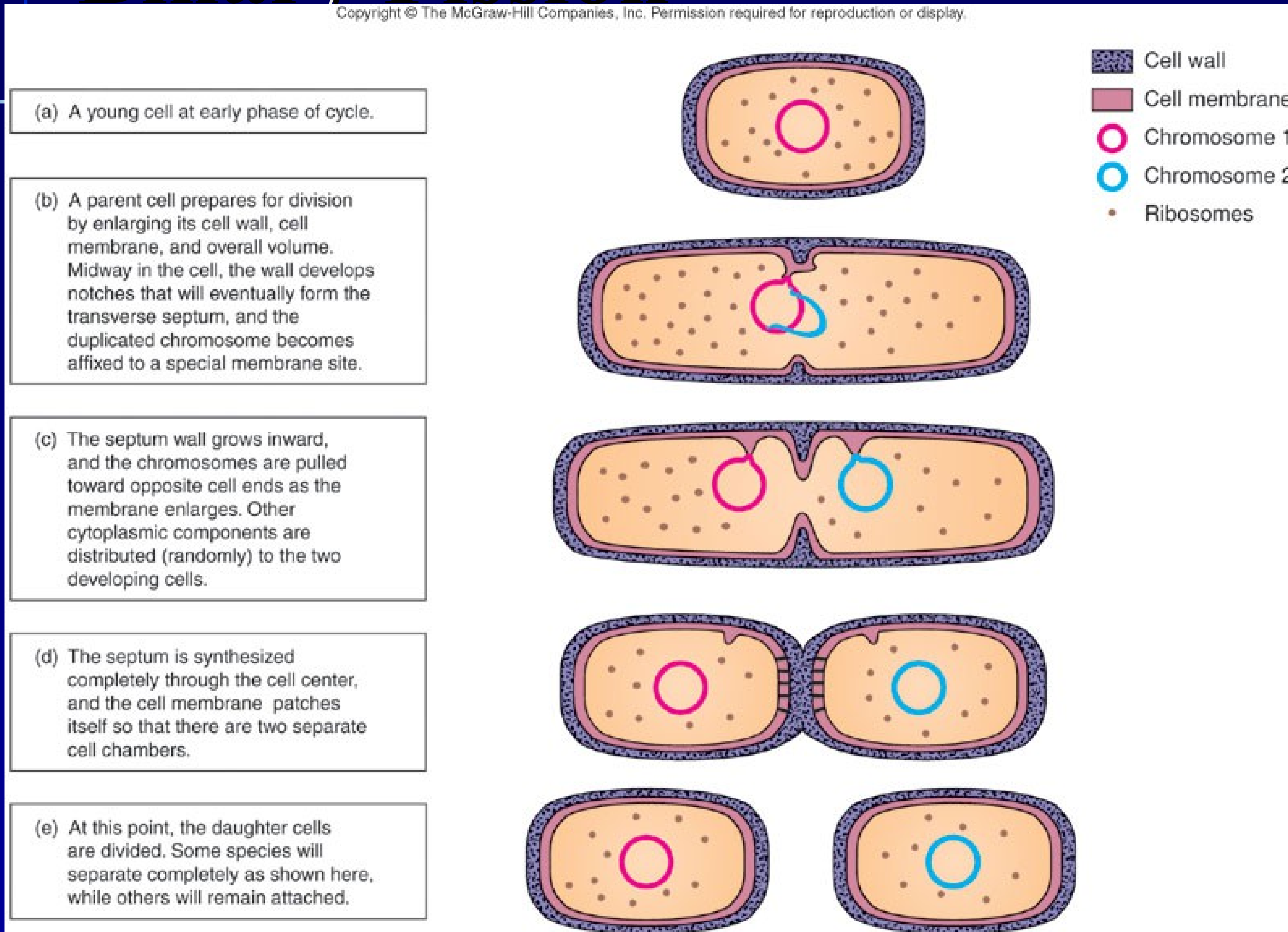
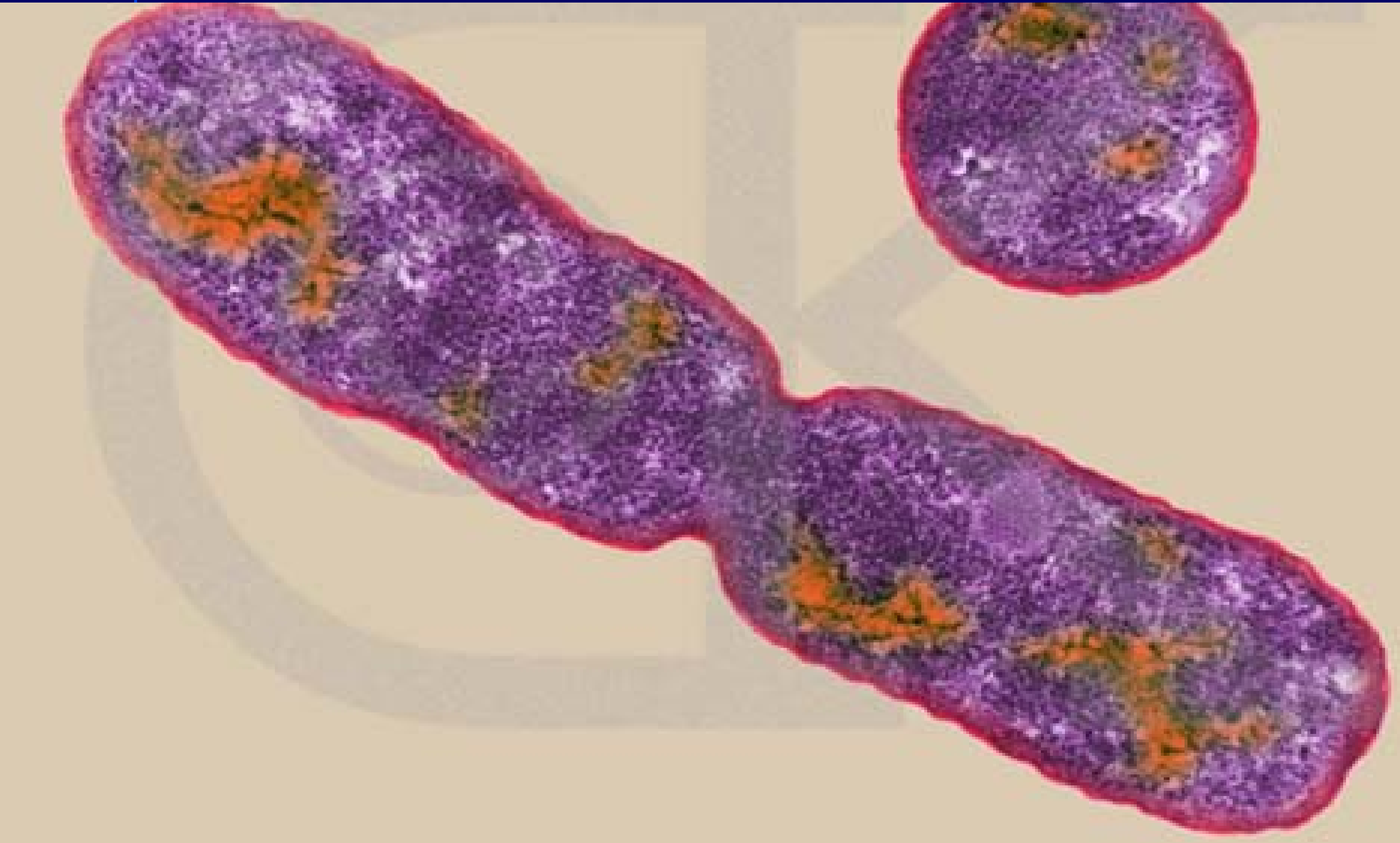


Figure 5.2a, page 139

Binary Fission

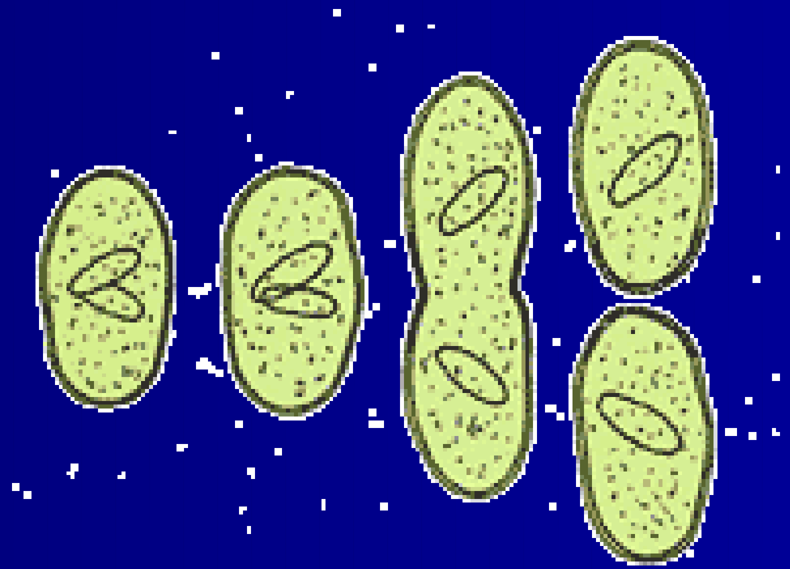


Binary Fission

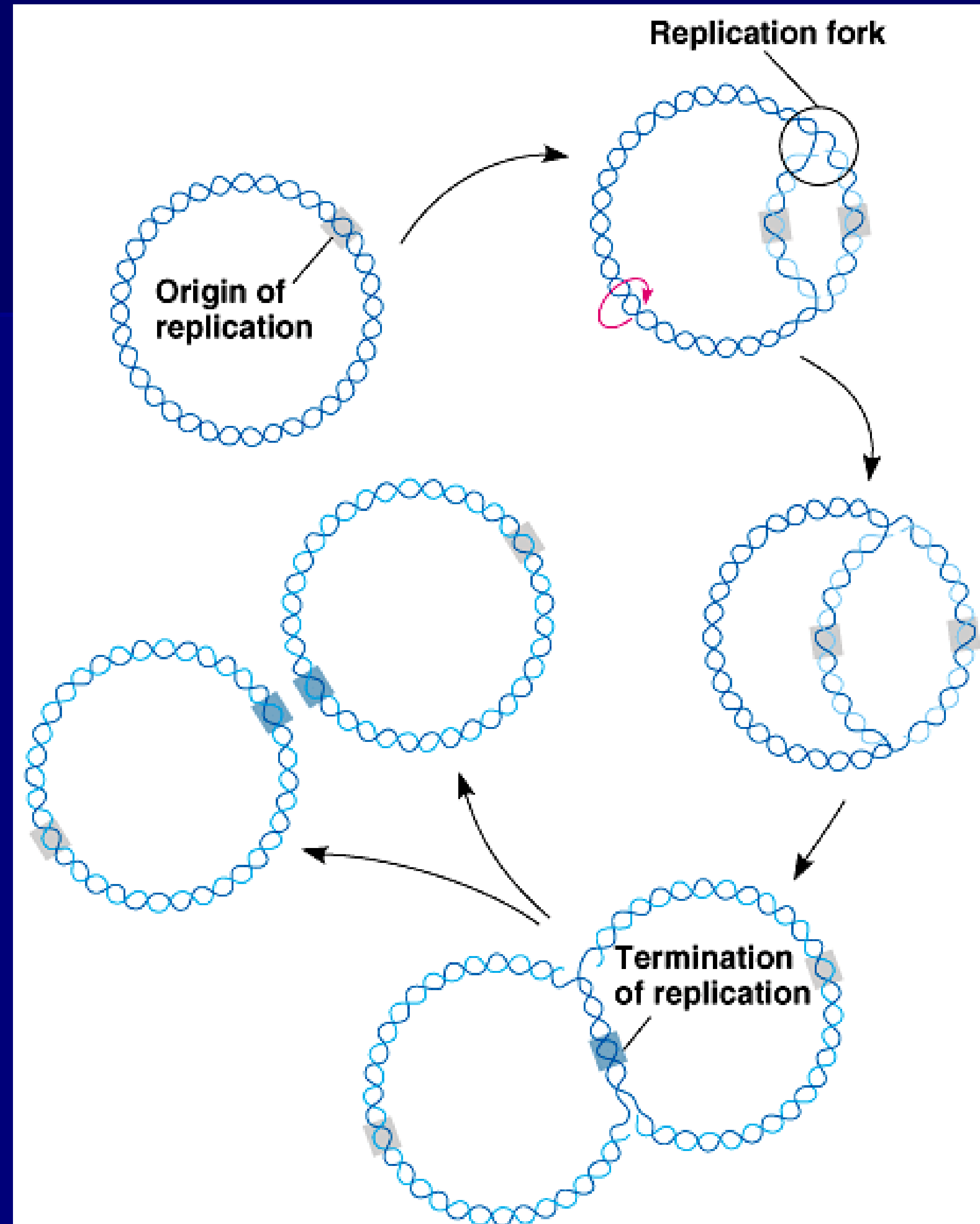


Partitioning

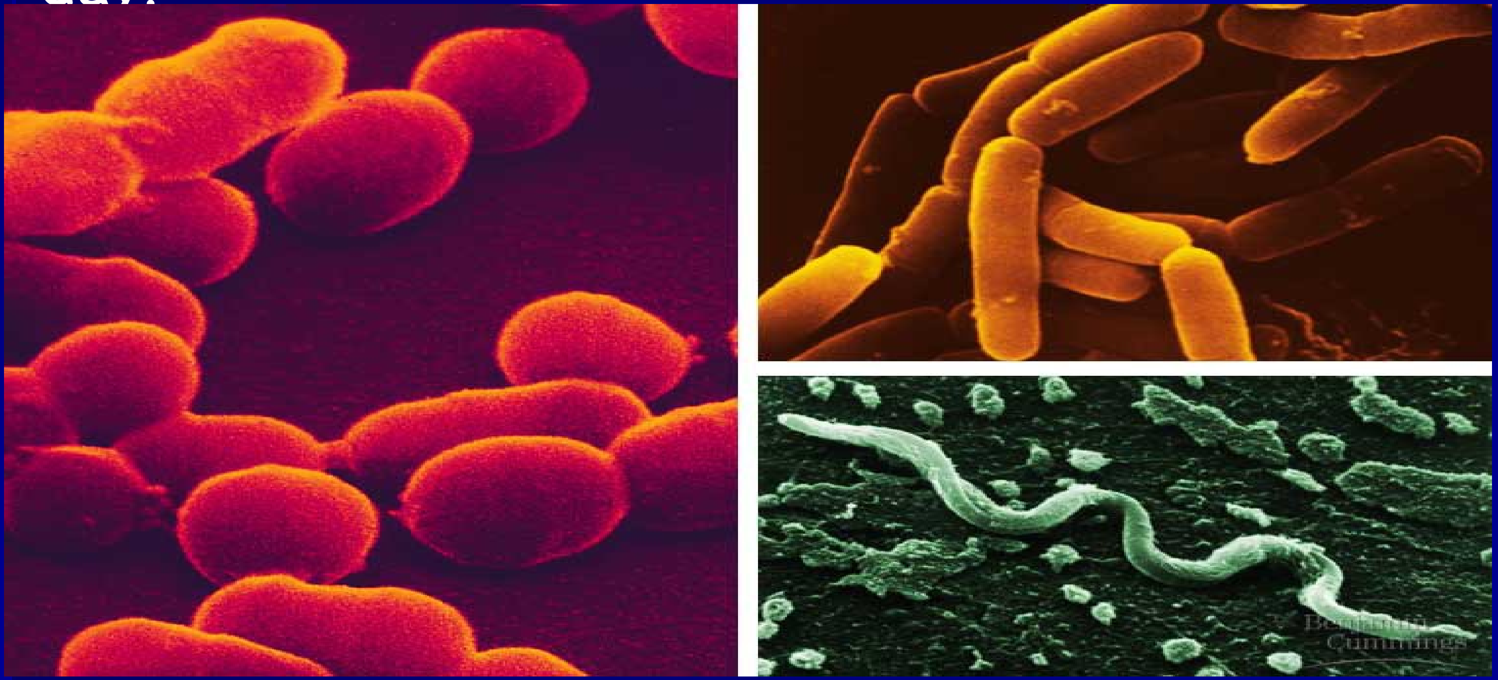
- Prior to cell division, bacteria copy their DNA(replicate their DNA)
- They then partition the DNA by constructing a cell wall between the two molecules of DNA
- This insures that the new cell receives a copy of the chromosome
- The division or partitioning of chromosomes is more difficult in those organisms that have more than one chromosome



- Bacterial cells divide by binary fission.
- This is preceded by replication of the bacterial chromosome from a single origin of replication.
- Plasmids replicate in a similar way but independent of the bacterial chromosome.



- Through binary fission, most of the bacteria in a colony are genetically identical to the parent cell.
  - However, the spontaneous mutation rate of *E. coli* is 1×10^{-7} mutations per gene per cell division.
  - This will produce about 2,000 bacteria in the human colon that have a mutation in that gene per day.



Staphylococcus aureus Division

- Chromosome segregation in prokaryotes is not well understood
- **Unlike eukaryotes, prokaryotes lack a mitotic spindle to separate replicated chromosomes.**
- The segregation process in prokaryotes involves specialized chromosomal-associated proteins but there is no clear picture describing how most of these proteins work to ensure accurate chromosome segregation.
- **In any event, cell fission at mid cell involves, cytokinesis, an inward pinching of the cell envelope (cell membrane and cell wall) to separate the mother cell into two genetically identical daughter cells.**
- The tubulin homolog found in prokaryotic cells is part of the fission ring apparatus that organizes invagination of the cell envelope.

- Cytokinesis is an inward pinching of the cell membrane and cell wall to separate the cell into two genetically identical cells.
- Cytokinesis occurs in two different ways in the *Bacteria.*
- **In gram-negative cells, like *Escherichia coli,* division occurs by the constriction of the cell envelope, followed by cell separation**.
- **In gram-positive cells, such as *Bacillus licheniformis,* constriction allows a newly synthesized wall to form a septum between daughter cells.**
- Cell separation then occurs by dissolution of the material in the septum.

Growth

- Increase in cellular constituents that may result in:
  - increase in cell number
    - when microorganisms reproduce by budding or binary fission
  - increase in cell size
    - coenocytic microorganisms have nuclear divisions that are not accompanied by cell divisions. Fungi have a syncytium and their nuclei are not separated.
- Microbiologists usually study population growth rather than growth of individual cells

- Let's look at the growth of bacterial populations in a little more detail.
- **A Bacterial Growth Curve Illustrates the Dynamics of Growth**
- **Bacterial population growth goes through four phases.**

The Growth Curve

- Observed when microorganisms are cultivated in batch culture
  - culture incubated in a closed vessel with a single batch of medium
- Usually plotted as logarithm of cell number versus time
- Usually has four distinct phases

- Whether several bacterial cells infect the human respiratory tract or are transferred to a tube of fresh growth medium in the laboratory, **four distinct phases of growth occur:**
- **the lag phase;**
- **the logarithmic phase or log phase;**
- **the stationary phase; and**
- **the decline phase or death phase**

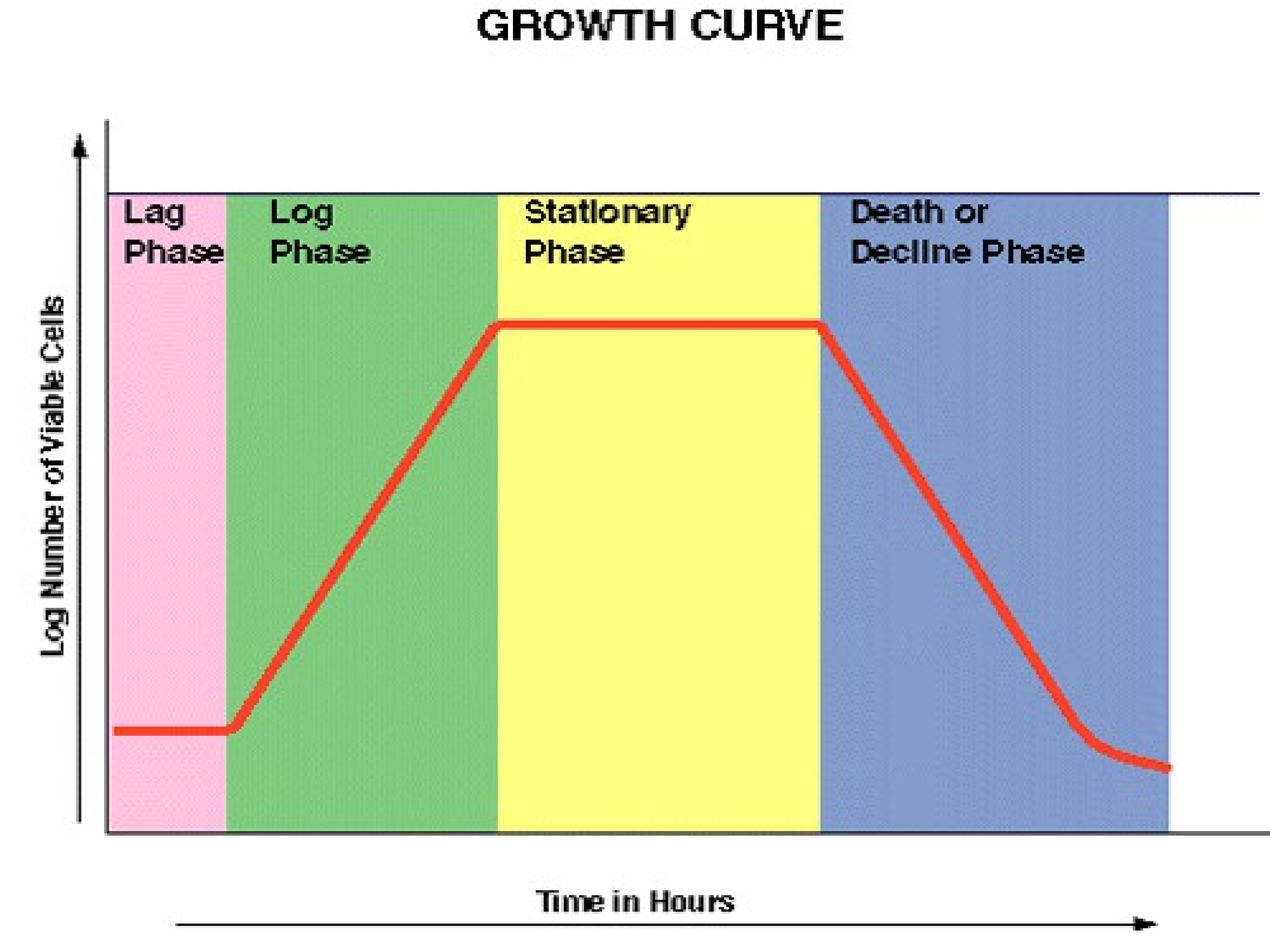
Phase 1. Lag Phase

- **The lag phase** encompasses the first portion of the curve.
- Bacteria are first introduced into an environment or media
- Bacteria are “checking out” or adapting to their surroundings (or culture media)
- Cells are very active metabolically and may increase in size, but
- There is little cell division going on so the number of cells changes very little
- 1 hour to several days

- During the lag phase, no cell divisions occur. Rather, the bacteria are adapting to their new environment
- In the respiratory tract, scavenging white blood cells may engulf and destroy some of the cells: in growth media, some cells may die from the shock of transfer or the inability to adapt to the new environment.
- The actual length of lag phase depends on the metabolic activity of the remaining cells.
- They must grow in size, store nutrients, and synthesize enzymes in preparation for binary fission.

Lag Phase

- Cell synthesizing new components
  - to replenish spent materials
  - to adapt to new medium or other conditions
- varies in length
  - in some cases can be very short or even absent


GROWTH CURVE
Lag Phase
Log Phase
Stationary Phase
Death or Decline Phase
Log Number of Viable Cells
Time in Hours

The Population Growth Curve

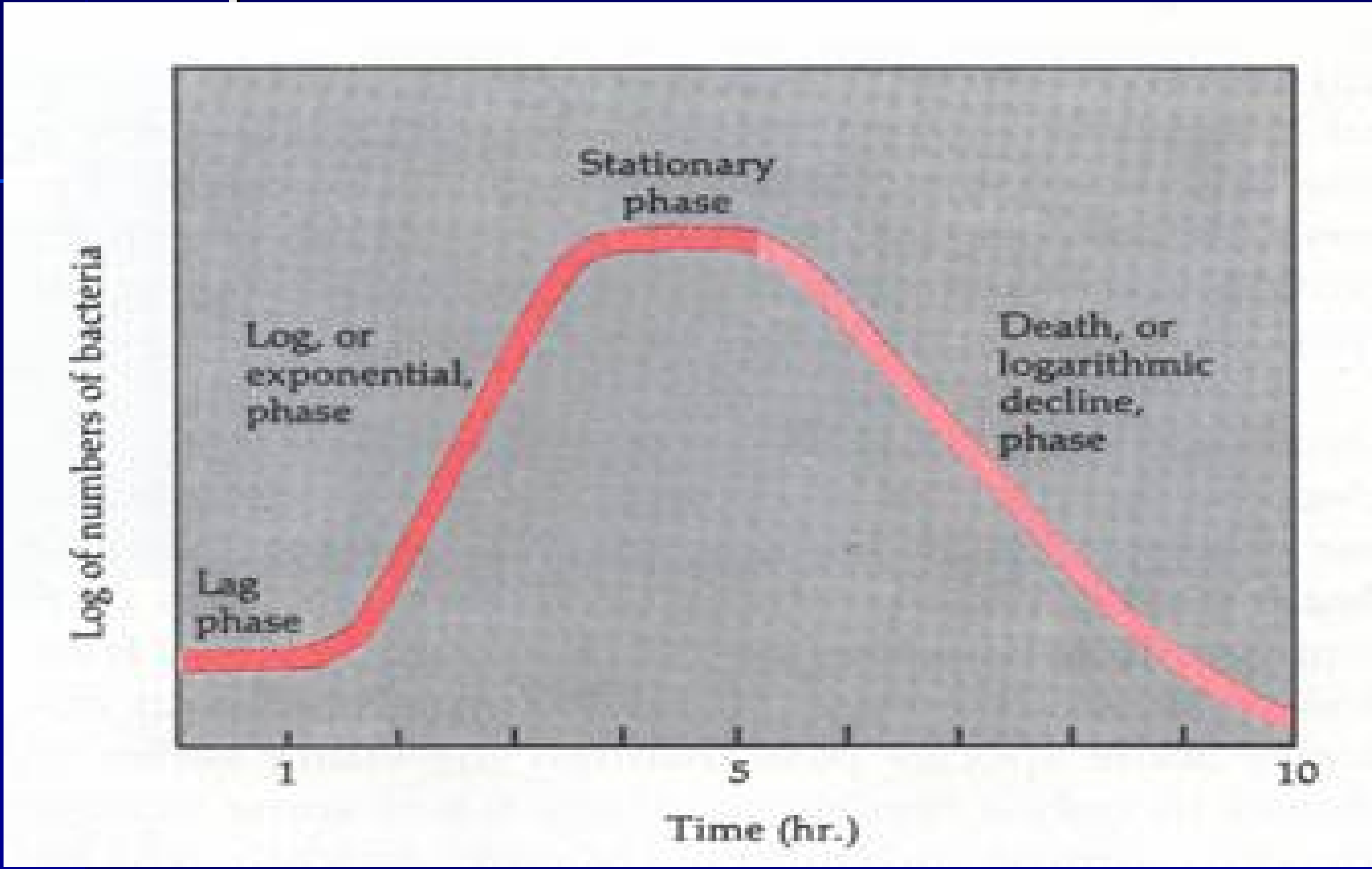
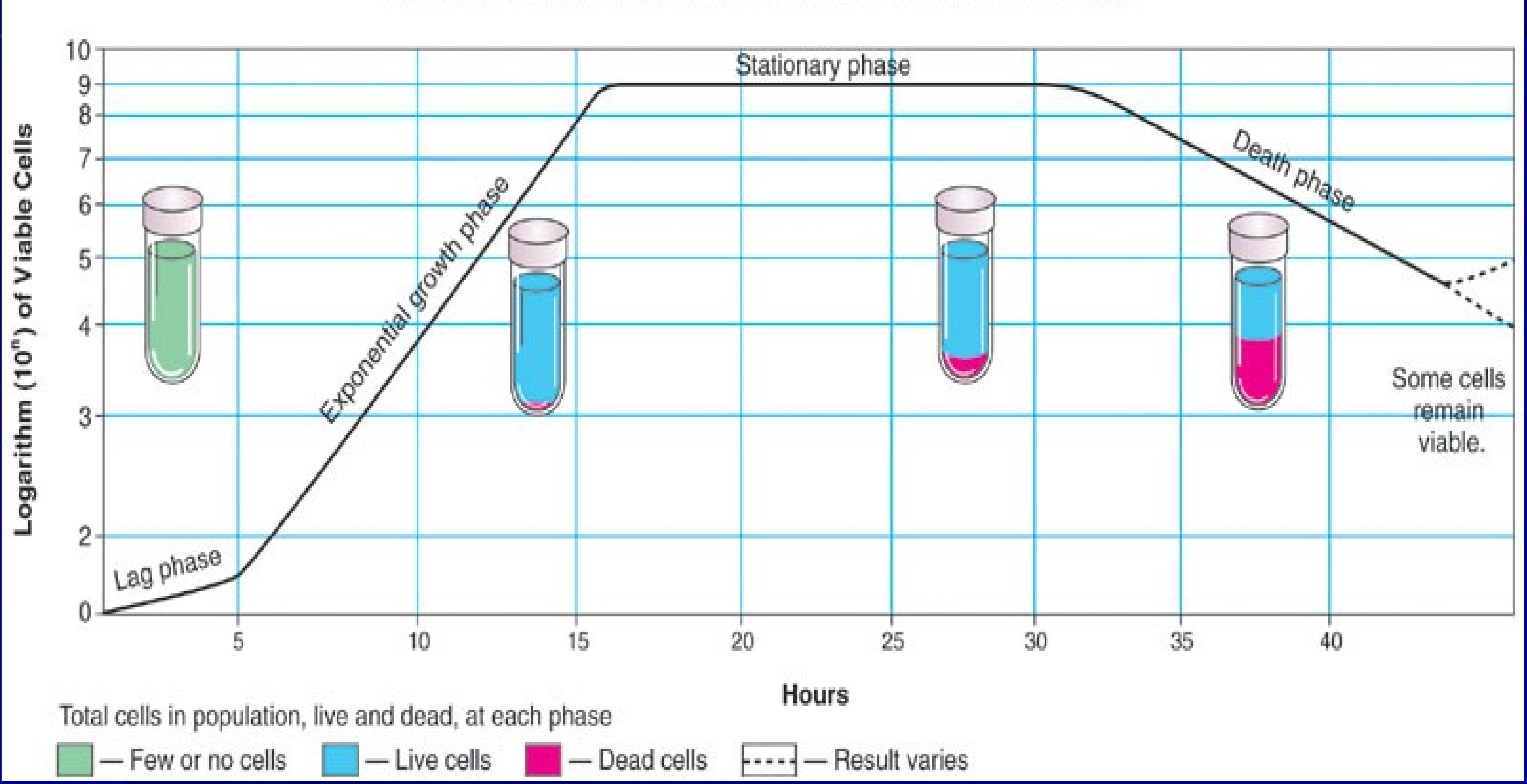


Image: Pearson Education Inc. (2004) publishing as Benjamin Cummings

Growth curve



Phase 2. Log Phase

- The population then enters an active stage of growth called the **logarithmic phase or log phase).**
- This is the exponential growth described above for E. *coli*.
- **In the log phase all cells are undergoing binary fission,** and the generation time is dependent on the species and environmental conditions present.
- As each generation time passes, the number of cells doubles and the graph rises in a straight line on a logarithmic scale.

- **In humans, disease symptoms usually develop during the log phase because the bacterial cells cause tissue damage.**
- Coughing or fever may occur, and fluid may enter the lungs sacs are damaged.
- **If the bacterial cells produce toxins, tissue destruction may become apparent.**
- During the log phase in our broth tube medium becomes cloudy (turbid) due to increasing cell numbers.
- **If plated on solid medium, bacterial growth will be so vigorous that visible colonies appear and each colony may consist of millions of cells.**
- **Vulnerability to antibiotics is also highest at this active stage of growth because many antibiotics affect actively metabolizing cells.**

Phase 2. Log Phase

- Rapid cell growth (exponential growth)
- Cells divide exponentially , and the population doubles every generation.
- This is the phase of most rapid growth
- During this phase, microbes are sensitive to adverse conditions, including
  - antibiotics
  - anti-microbial agents

Exponential or Log Phase

- Rate of growth is constant
- Population is most uniform in terms of chemical and physical properties during this phase

Table 6.1 An Example of Exponential Growth

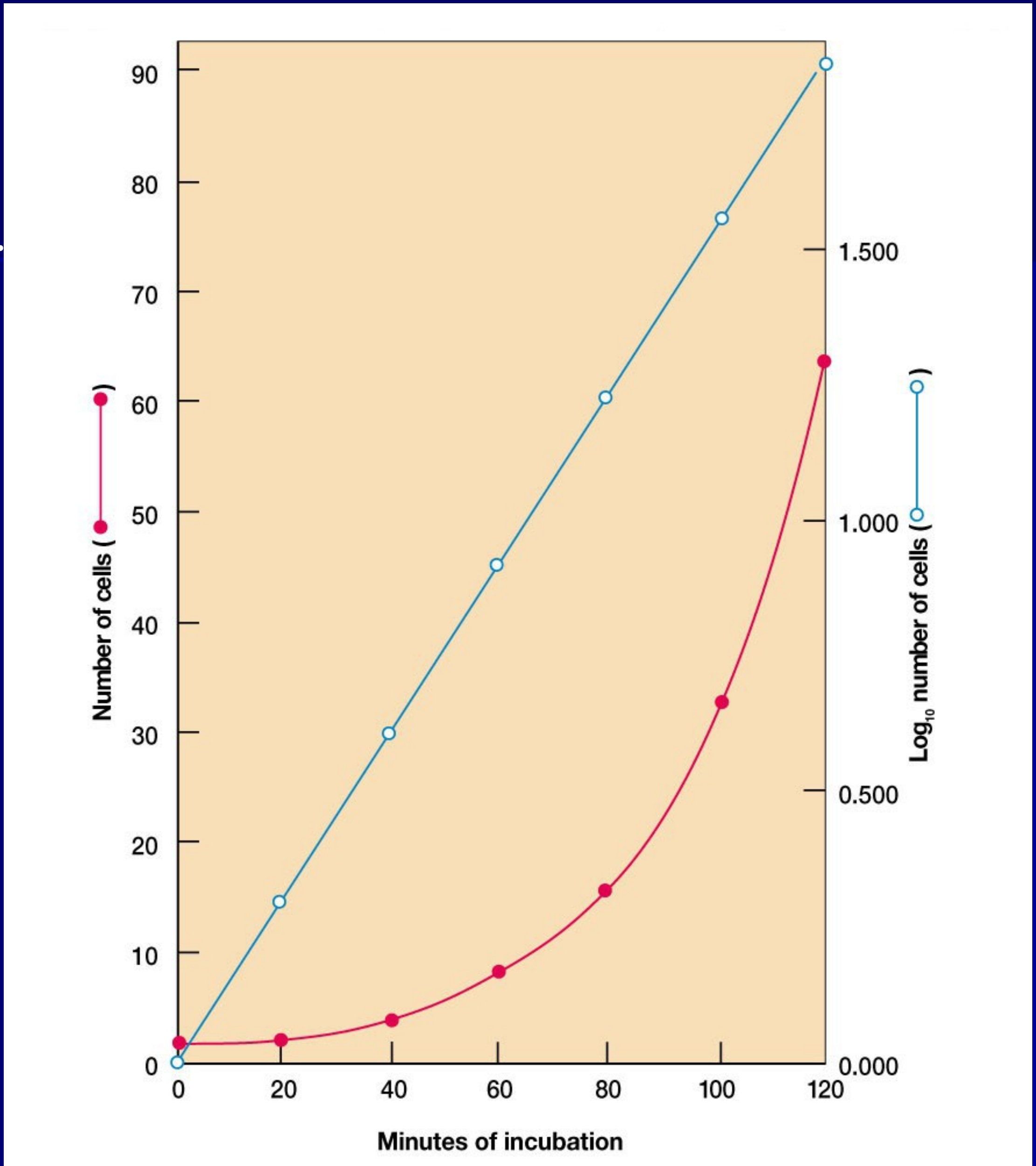
Time[a]	Division Number	2^n	Population ($N_0 \times 2^n$)	$\log_{10} N_t$
0	0	$2^0 = 1$	1	0.000
20	1	$2^1 = 2$	2	0.301
40	2	$2^2 = 4$	4	0.602
60	3	$2^3 = 8$	8	0.903
80	4	$2^4 = 16$	16	1.204
100	5	$2^5 = 32$	32	1.505
120	6	$2^6 = 64$	64	1.806

[a]The hypothetical culture begins with one cell having a 20-minute generation time.

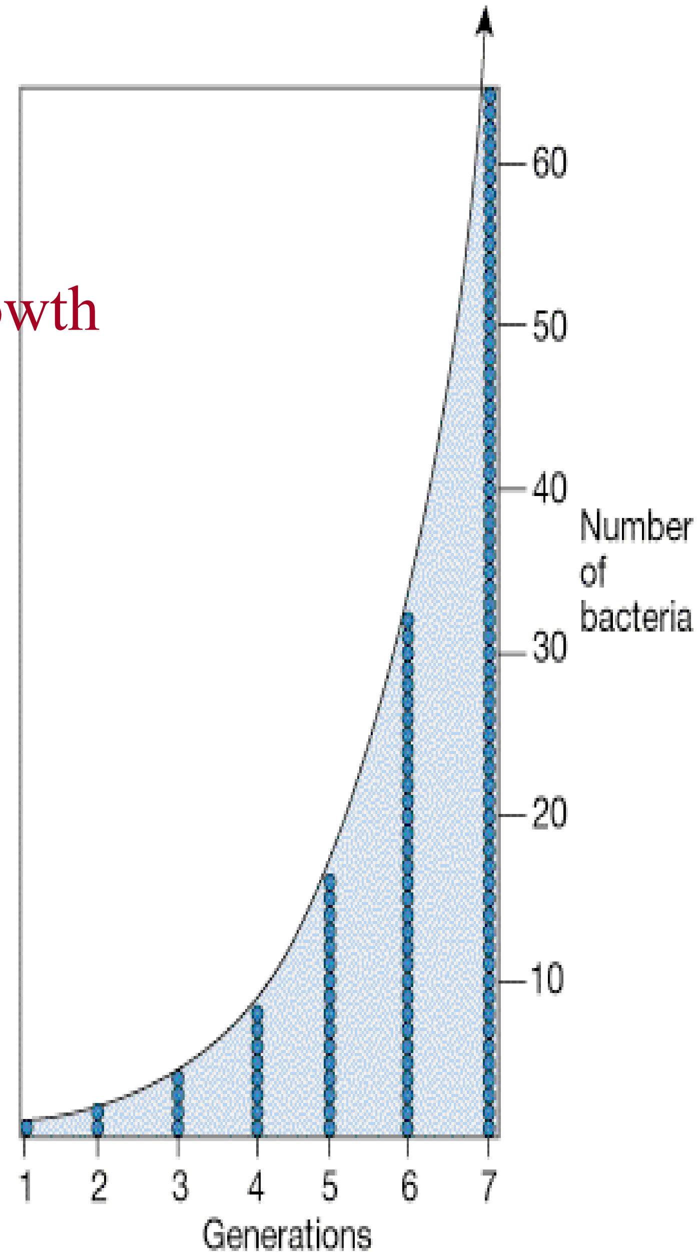
cells are dividing and doubling in number at regular interval

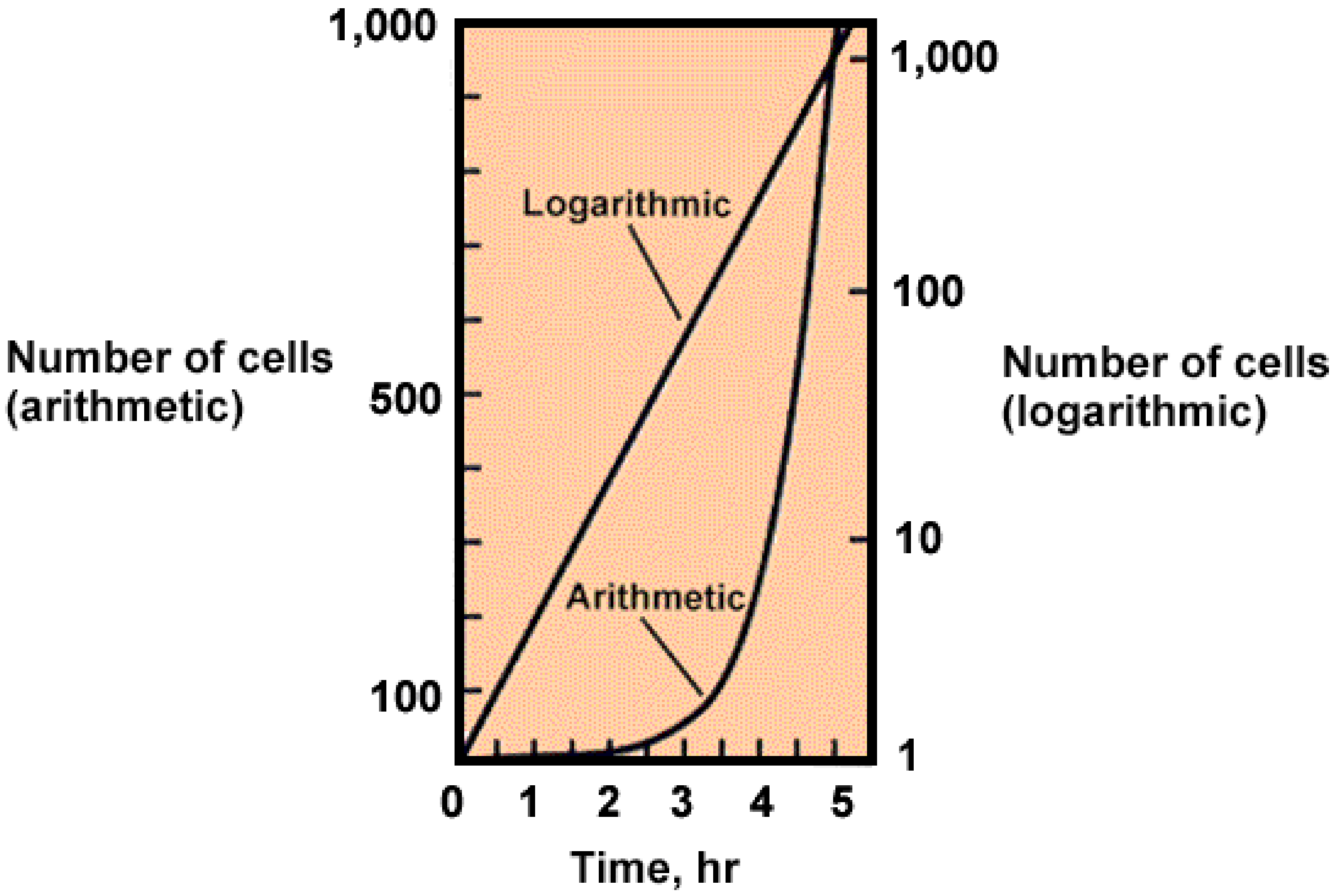
Each individual cell divides at a slightly different time

Curve rises smoothly rather than as discrete steps



Log Growth





Number of cells (arithmetic)
1,000
500
100
Logarithmic
Arithmetic
1,000
100
10
1
Number of cells (logarithmic)
0
1
2
3
4
5
Time, hr

Balanced growth

- During log phase, cells exhibit balanced growth
  - cellular constituents manufactured at constant rates relative to each other

Unbalanced growth

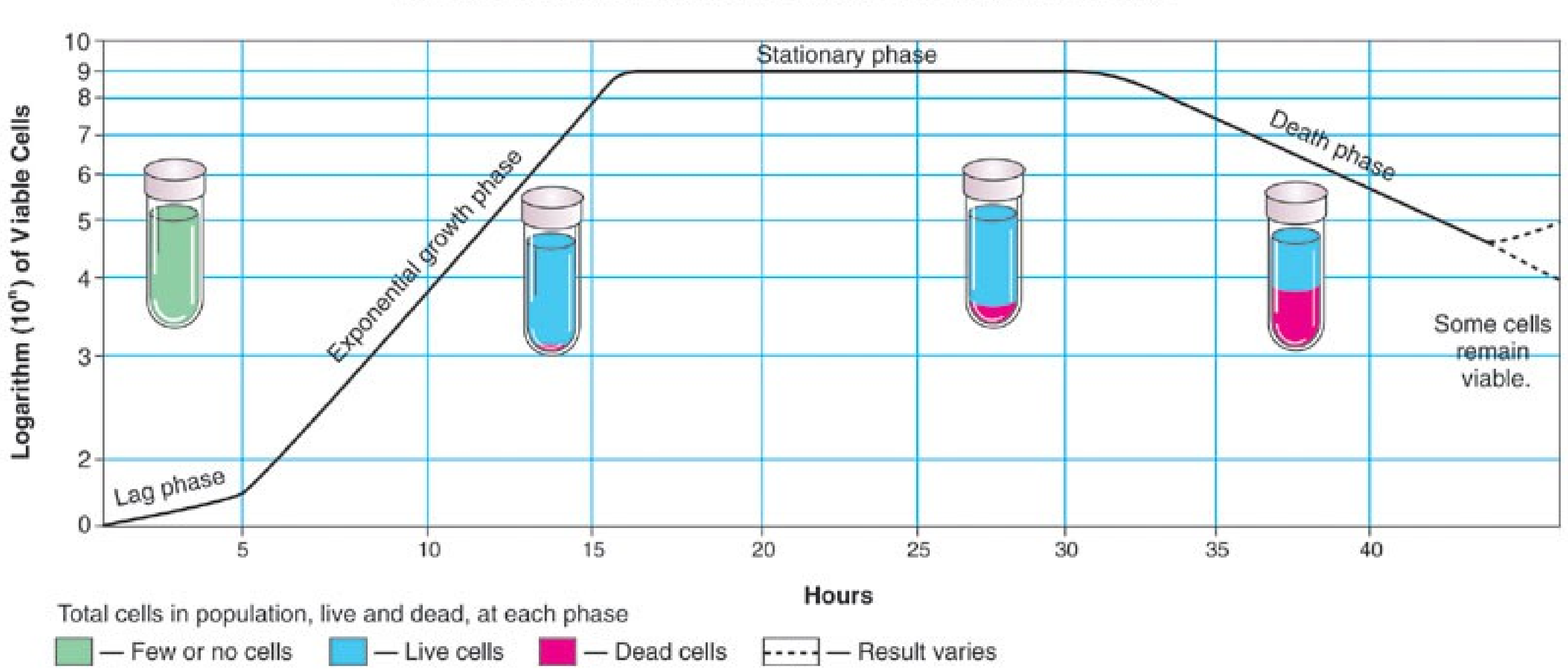
- Rates of synthesis of cell components vary relative to each other
- Occurs under a variety of conditions
  - change in nutrient levels
    - shift-up (poor medium to rich medium)
    - shift-down (rich medium to poor medium)
  - change in environmental conditions

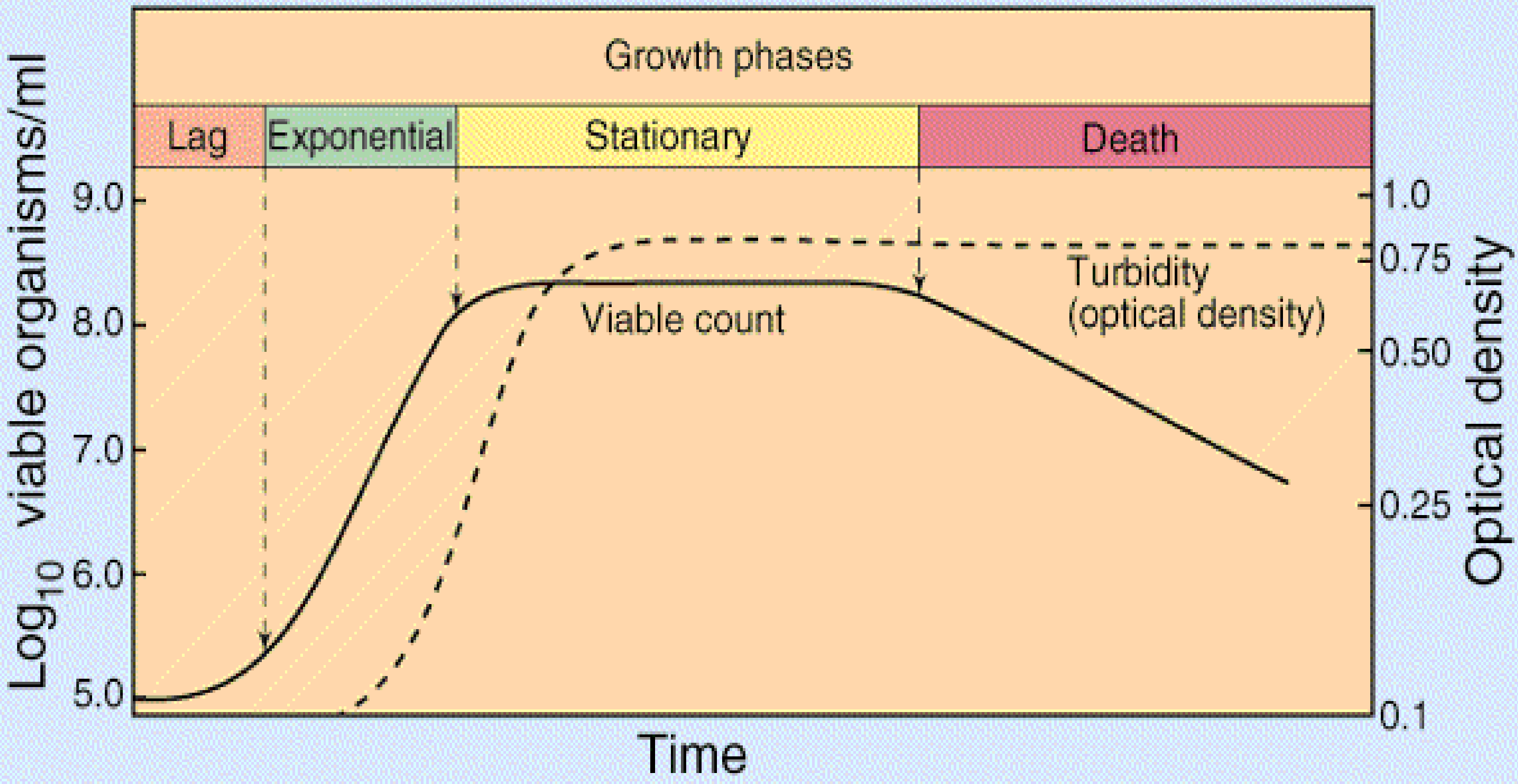
Phase 3. Stationary Phase

- After some days (in an infection) or hours (in a culture tube), the vigor of the population changes and, as the reproductive and death rates equalize, the population enters a plateau, called **the stationary phase**.

Growth curve

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.




Growth phases
Lag
Exponential
Stationary
Death
9.0
8.0
7.0
6.0
5.0
Log10 viable organisms/ml
1.0
0.75
0.50
0.25
0.1
Optical density
Turbidity
(optical density)
Viable count
Time

- E.g. In the respiratory tract, antibodies from the immune system are attacking the bacterial cells, and phagocytosis by white blood cells adds to their destruction.
- In the culture tube, available nutrients become scarce and waste products accumulate.
- Factors such as oxygen also may be in short supply. This limitation of nutrients and buildup of waste materials leads to the death of many cells.

Phase 3. Stationary Phase

- Death rate = rate of reproduction; i.e new cells produced = cell death rate.
- Cells begin to encounter environmental stress
  - lack of nutrients
  - lack of water
  - not enough space
  - metabolic wastes
  - oxygen
  - pH

Endospores would form now

Stationary Phase

- total number of viable cells remains constant
  - may occur because metabolically active cells stop reproducing
  - may occur because reproductive rate is balanced by death rate

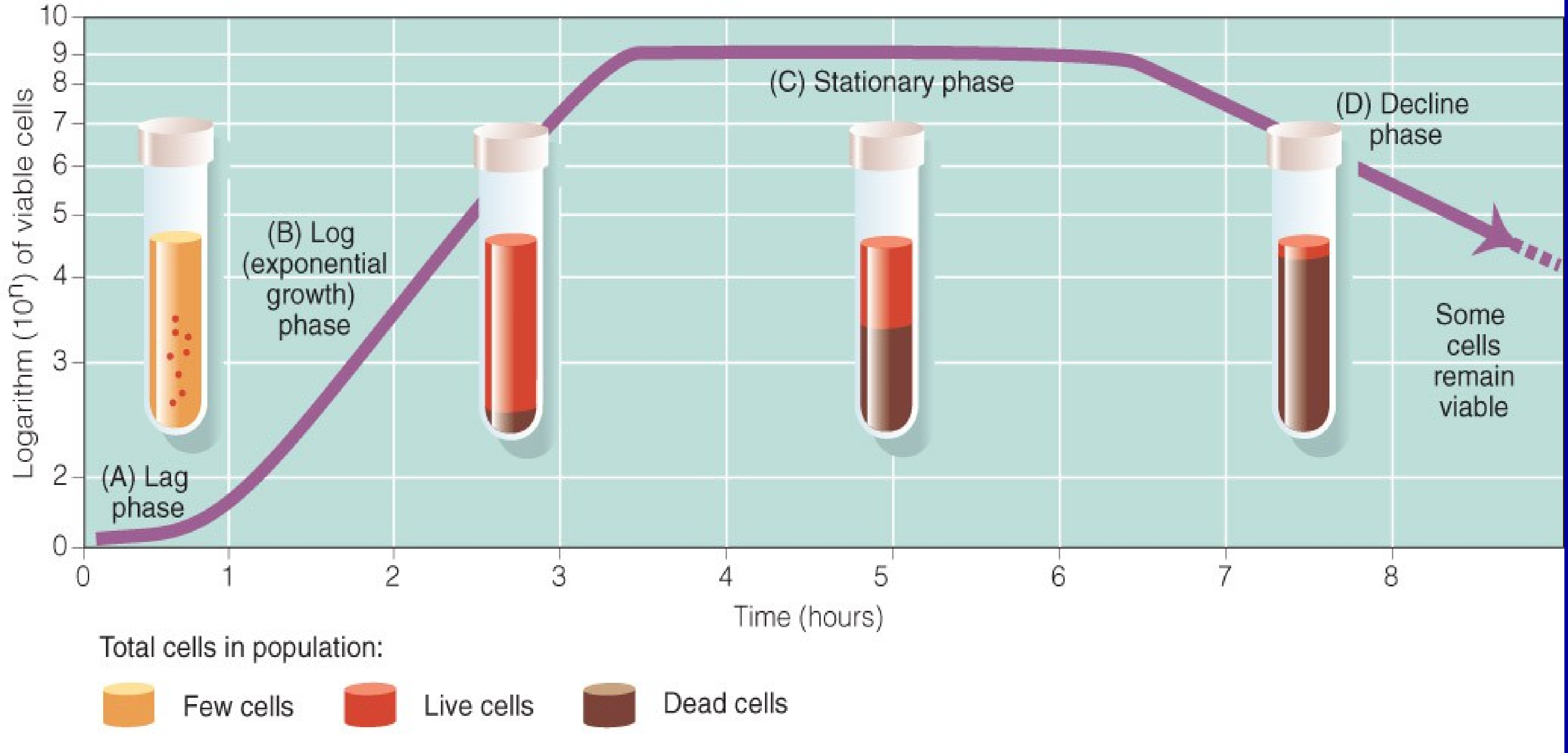
Possible reasons for entry into stationary phase

- nutrient limitation
- limited oxygen availability
- toxic waste accumulation
- critical population density reached

Starvation responses

- morphological changes
  – endospore formation
- decrease in size, protoplast shrinkage, and nucleoid condensation
- production of starvation proteins
- long-term survival
- increased virulence

- A typical bacterial growth curve for a population illustrates the events occurring over time.
- When reproductive and death rates equalize, the population enters the stationary phase.
- The accumulation of waste products and scarcity of resources causes the population to enter the decline (exponential death) phase

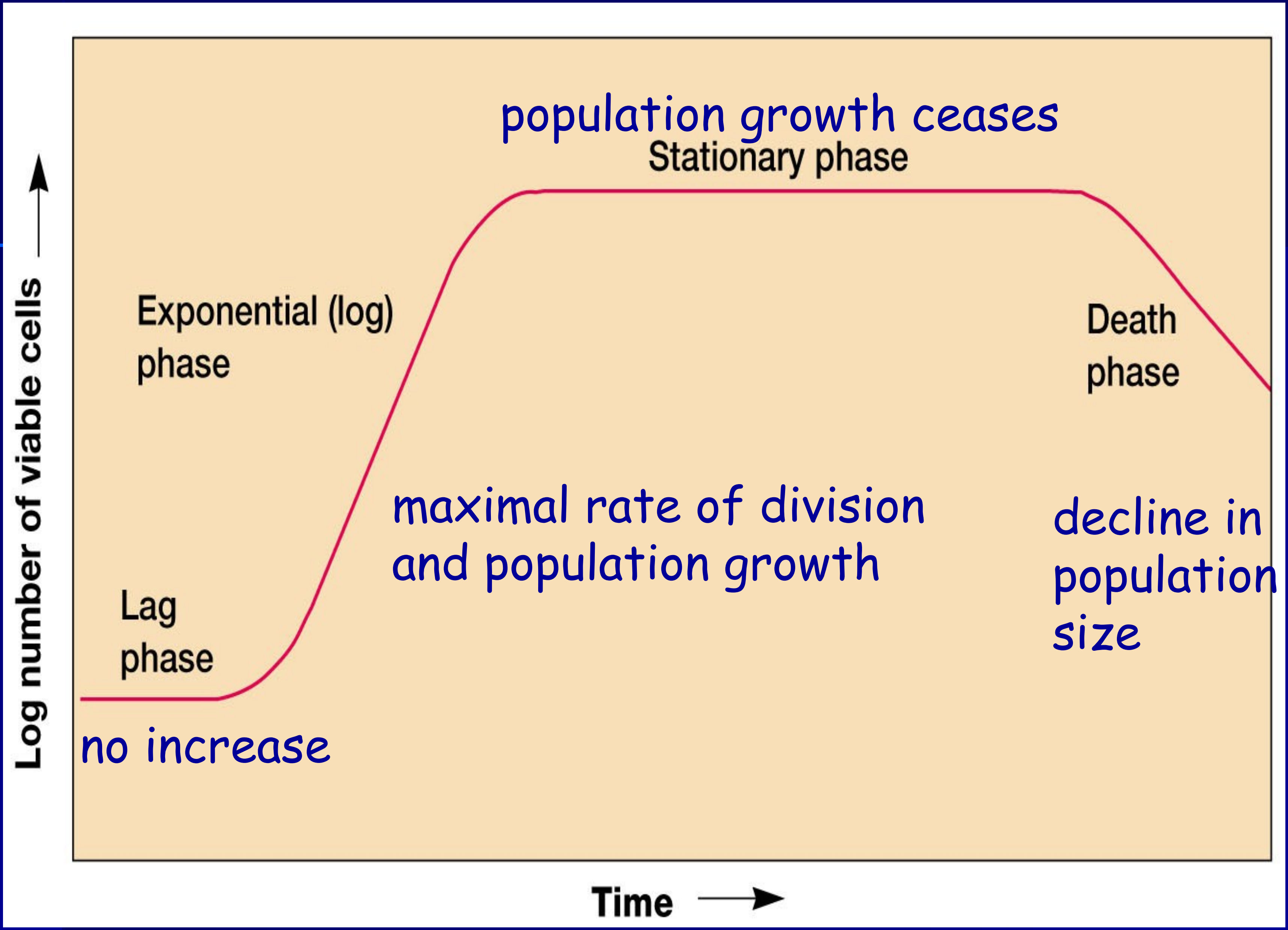


Phase 4. Decline or Death Phase

- Death rate > rate of reproduction and the number of the live cells decrease logarithmically….
- Due to limiting factors in the environment such as …….
- **Lack of food, water or nutrients**
- **space**
- **accumulation of metabolic wastes**
- **lack of oxygen**
- **changes in pH**
- **Temperature**

- **The environment or growth medium is now unable to support growth.**

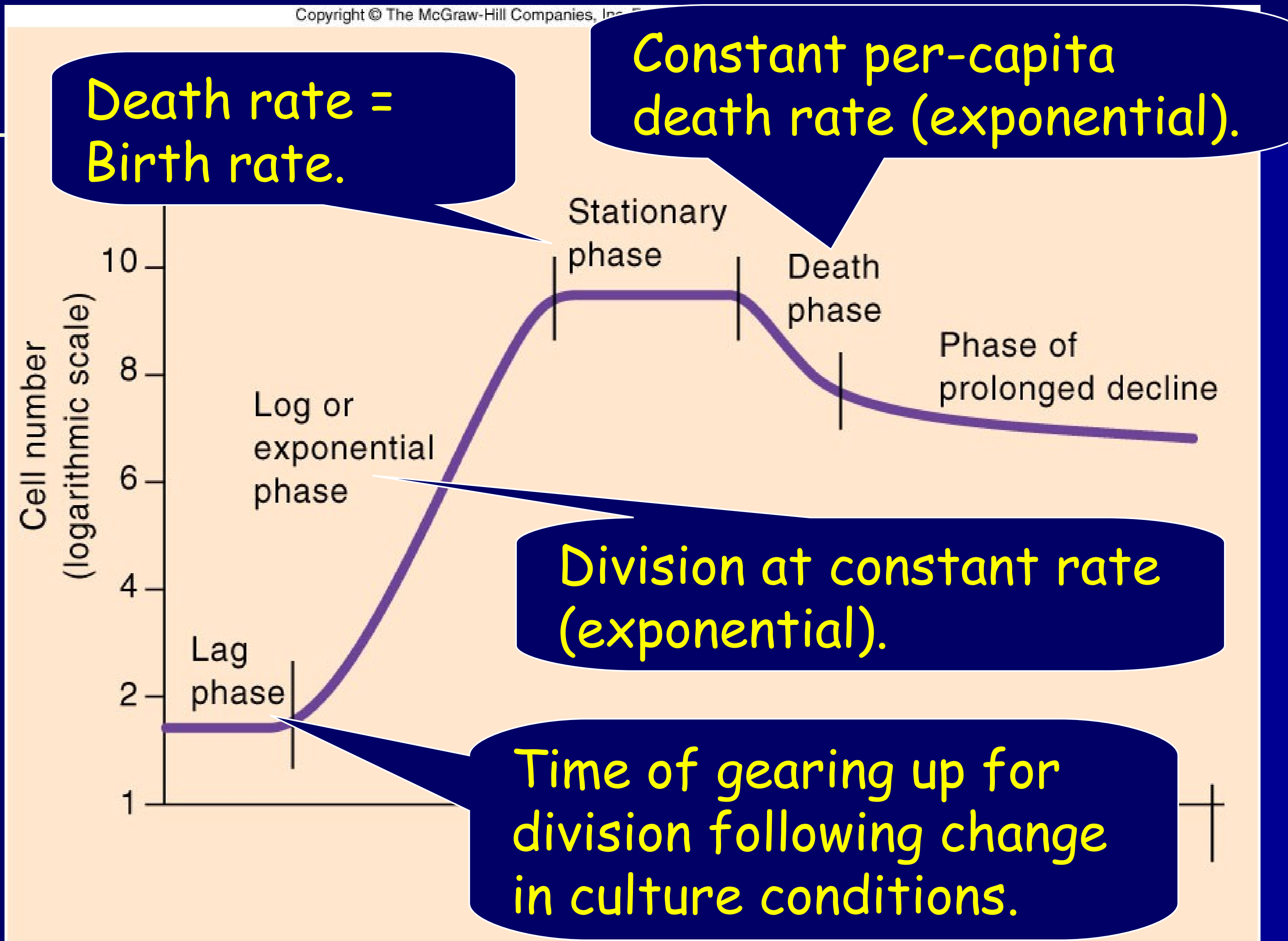
- If nutrients in the external environment remain limited or the quantities become exceeding low, the population enters a **decline phase (or exponential death phase).**
- Now the number of **dying cells** far exceeds the number of new cells formed.
- A bacterial glycocalyx may forestall death by acting as a buffer to the environment, and flagella may enable organisms to move to a new location.
- For many species, though, the history of the population ends with the death of the last cell.
- If we monitor the progression of human diseases caused by microbes ,we will see a similar curve for the stages of a disease.
- For **some bacterial species, especially soil bacteria, they can escape cell death by forming endospores.**


population growth ceases
Stationary phase
Exponential (log)
phase
Death
phase
maximal rate of division
and population growth
decline in
population
size
Lag
phase
no increase
Log number of viable cells
Time

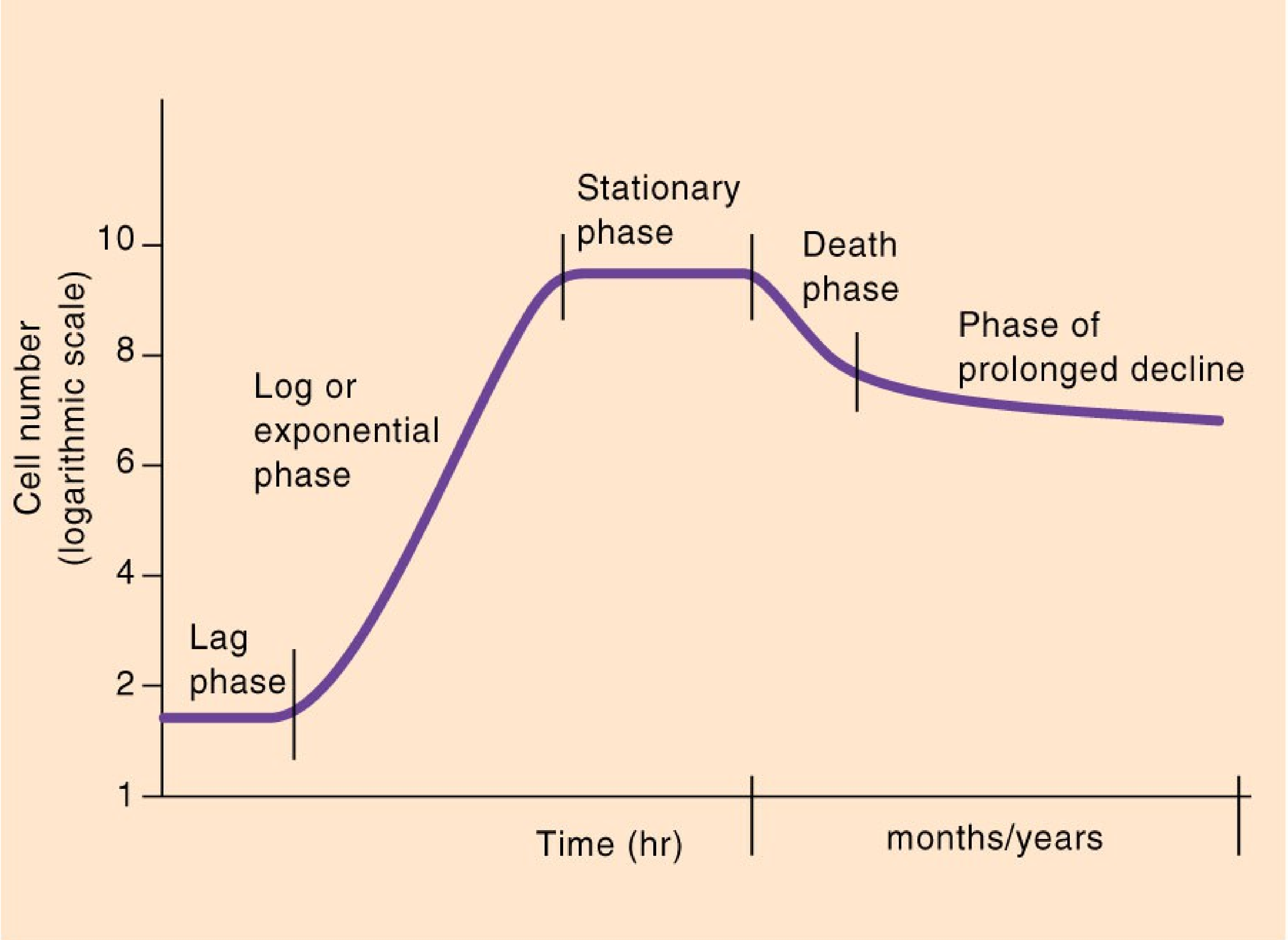
Phase 4. Death Phase

- cells dying, usually at exponential rate
- death
  - irreversible loss of ability to reproduce
- in some cases, death rate slows due to accumulation of resistant cells

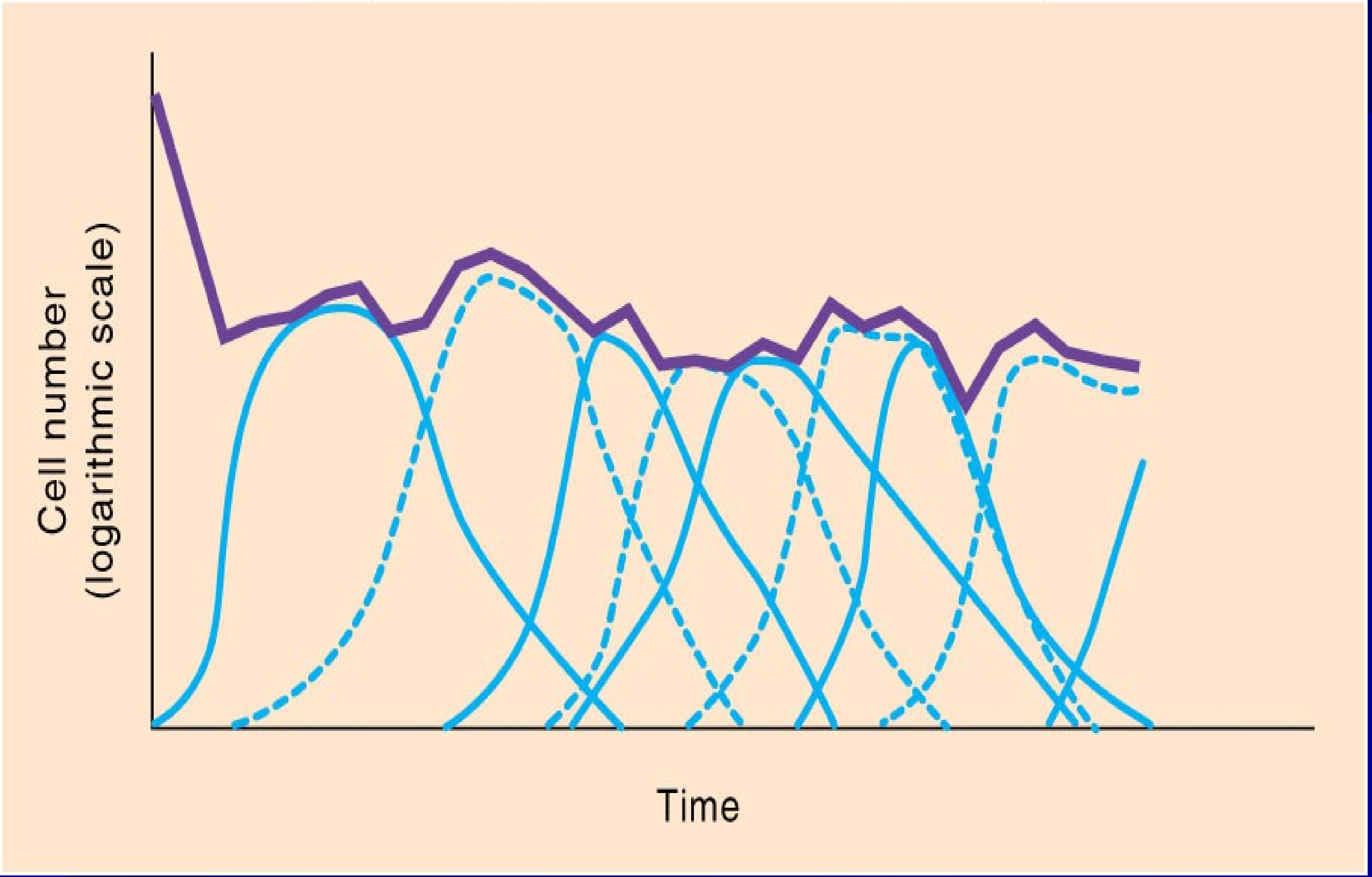
Growth Curve



Growth Curve



Phase of Prolonged Decline



- **REVIEW:- Growth curve**

1. **Lag phase** – "flat" period of adjustment, enlargement; little growth
2. **Exponential growth or log phase** – a period of maximum growth will continue as long as cells have adequate nutrients & a favorable environment
3. **Stationary phase** – rate of cell growth equals rate of cell death cause by depleted nutrients & O_2, excretion of organic acids & pollutants
4. **Death or decline phase** – as limiting factors intensify, cells die exponentially in their own wastes

Phases of Growth

- Lag
  - Adapt to nutrients
- Log
  - Active growth
- Stationary
  - Death = Growth rate
- Death
  - Nutrients consumed
  - pH too low (why?)
- Optimize curves in production

The Mathematics of Growth

- Generation (doubling) time
  - time required for the population to double in size
- Mean growth rate constant
  - number of generations per unit time
  - usually expressed as generations per hour

The Generation Time

- The generation time for most species is between twenty minutes and 24 hours.
- Some organisms take a longer time to go through the lag phase
- Some organisms due to their characteristics like *Mycobacterium tuberculosis* grow slowly due to the cell wall

Synchronous Growth

- Cells doubling or dividing every 20 minutes

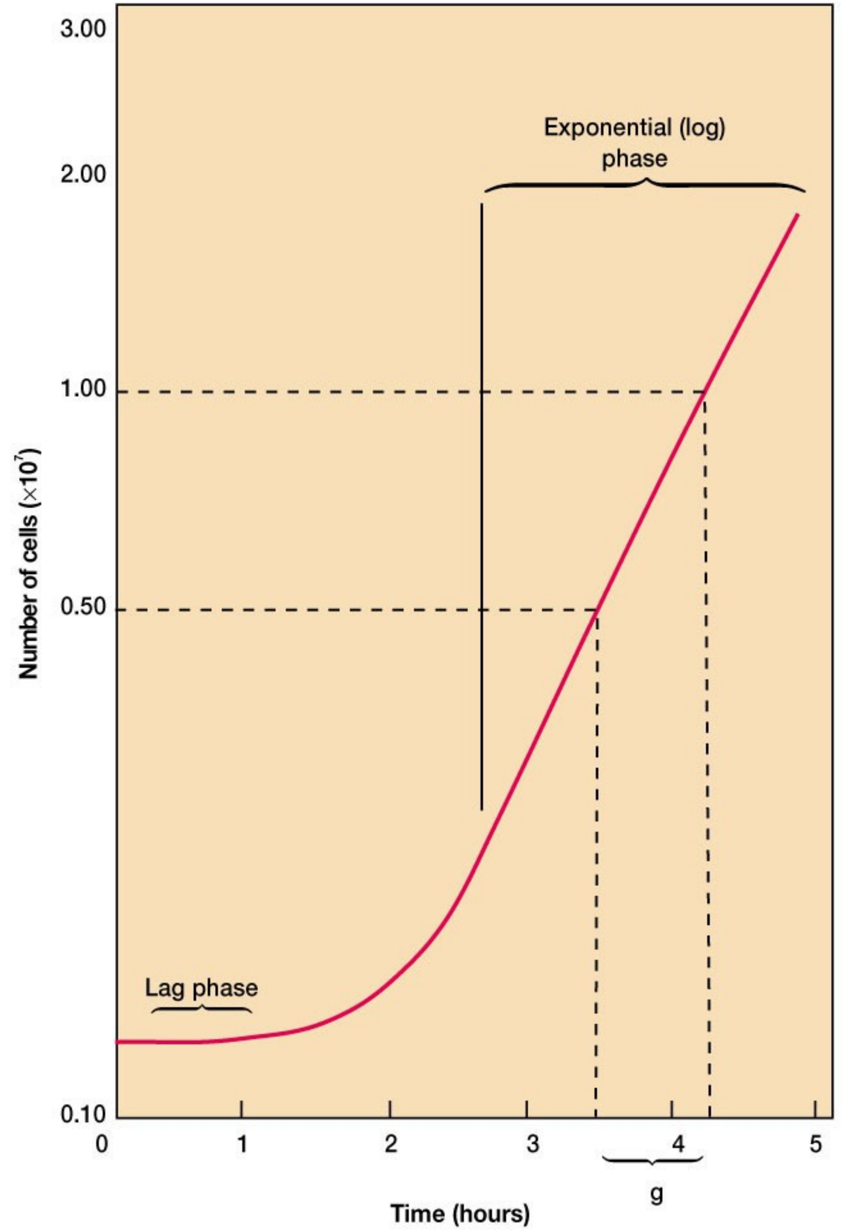

3.00
2.00
1.00
0.50
0.10
Number of cells (×10^7)
Exponential (log) phase
Lag phase
0
1
2
3
4
5
g
Time (hours)

Table 6.2 Generation Times for Selected Microorganisms

Microorganism	Temperature (°C)	Generation Time (Hours)
Bacteria		
Beneckea natriegens	37	0.16
Escherichia coli	40	0.35
Bacillus subtilis	40	0.43
Staphylococcus aureus	37	0.47
Pseudomonas aeruginosa	37	0.58
Clostridium botulinum	37	0.58
Rhodospirillum rubrum	25	4.6–5.3
Anabaena cylindrica	25	10.6
Mycobacterium tuberculosis	37	≈12
Treponema pallidum	37	33
Algae		
Scenedesmus quadricauda	25	5.9
Chlorella pyrenoidosa	25	7.75
Asterionella formosa	20	9.6
Euglena gracilis	25	10.9
Ceratium tripos	20	82.8
Protozoa		
Tetrahymena geleii	24	2.2–4.2
Leishmania donovani	26	10–12
Paramecium caudatum	26	10.4
Acanthamoeba castellanii	30	11–12
Giardia lamblia	37	18
Fungi		
Saccharomyces cerevisiae	30	2
Monilinia fraa	25	30

IMPORTANCE OF GENERATION TIMES

- **Prokaryotes Reproduce Asexually**
- **Prokaryotes vary in their generation times.**

- The interval of time between successive binary fissions of a cell or population of cells is known as **the generation time (or doubling time).**
- Under optimal conditions, some prokaryotes have a very fast generation time; for others, it is much slower.

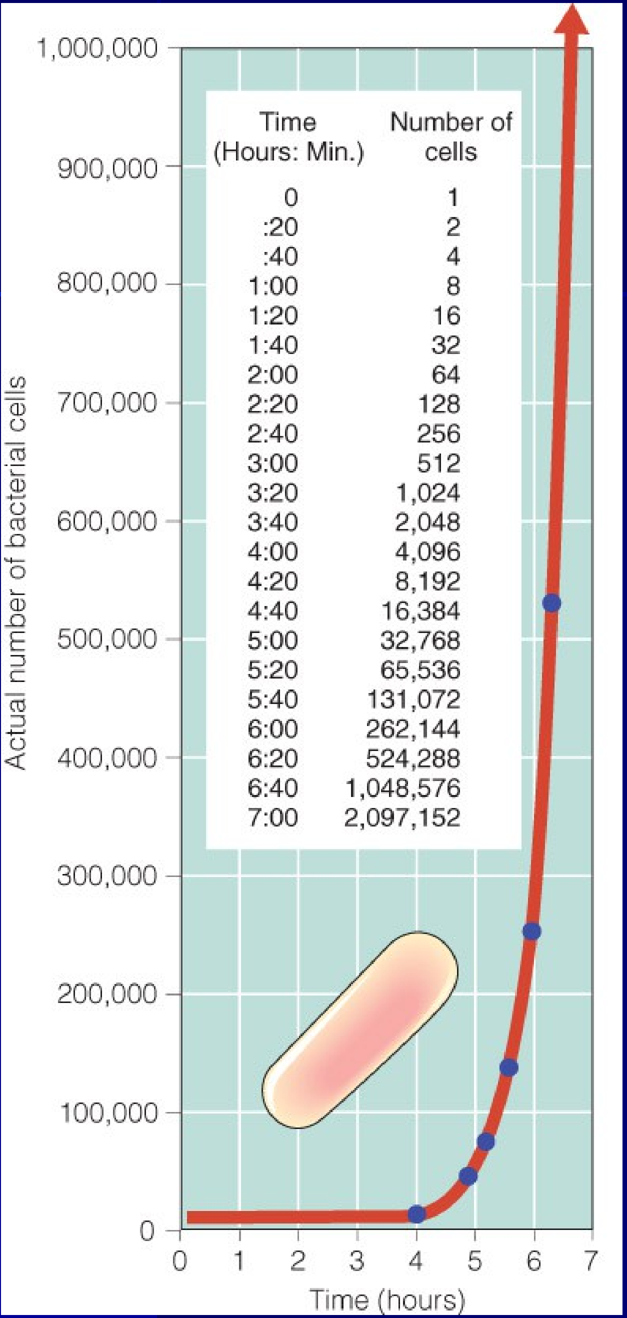
- For example, the optimal generation time for *Staphylococcus aureus* is about 30 minutes; for *Mycobacterium tuberculosis,* the agent of tuberculosis, it is approximately 15 hours; and for the syphilis spirochete, *Treponema pallidum,* it is a long 33 hours.

- One enterprising mathematician calculated that if *E. coli* binary fissions were to continue at their optimal generation time (15 minutes) for 36 hours, the bacterial cells would cover the surface of the Earth!

- Thankfully, this will not occur because of the limitation of nutrients and the loss of ideal physical factors required for growth. The majority of the bacterial cells would starve to death or die in their own waste.

- **The generation time is useful in determining the amount of time that passes before disease symptoms appear in an infected individual; faster division times often mean a shorter incubation period for a disease.**
- Suppose you eat an undercooked hamburger contaminated with the pathogen *E. coli* O157:H7, which has one of the shortest generation times- just 20 minutes under optimal conditions.

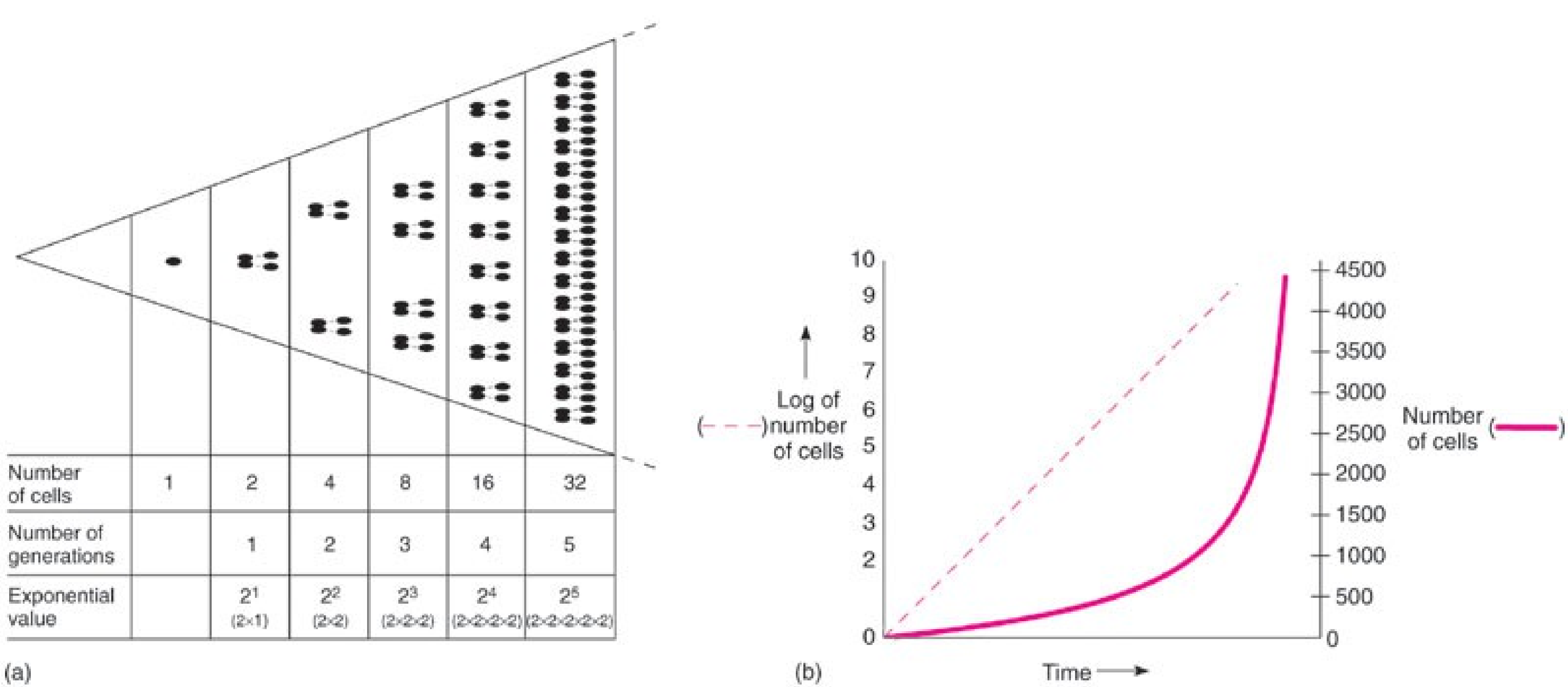
- **If you ingested one cell (more likely several hundred at least) at 8:00 PM this evening, two would be present by 8:20, four by 8:40, and eight by 9:00.**
- **You would have over 4,000 by midnight.**
- **By 3:00 AM, there would be over 2 million.**
- Depending on the response of the immune system, it is quite likely that sometime during the night you would know you have food poisoning.



Time (Hours: Min.)	Number of cells
0	1
:20	2
:40	4
1:00	8
1:20	16
1:40	32
2:00	64
2:20	128
2:40	256
3:00	512
3:20	1,024
3:40	2,048
4:00	4,096
4:20	8,192
4:40	16,384
5:00	32,768
5:20	65,536
5:40	131,072
6:00	262,144
6:20	524,288
6:40	1,048,576
7:00	2,097,152

- Prokaryotes Reproduce Asexually
  - **The generation (or doubling) time is the interval of time between successive binary fissions**
  - **In pathogens, a shorter doubling time means a shorter incubation period of disease**

Population growth



Bacterial Growth - increase in the # of cells

- **Binary Fission**

- Generation Time (Doubling Time)
  - **time required for a cell to divide**
  - **most about 1 Hr. To 3 Hrs.**
    - *E. coli* - 20 minutes
    - *Mycobacterium tuberculosis* - 24 Hrs.

Binary Fission - unchecked

- E. coli - generation time of 20 min.
- 20 generations (about 7 hrs.)
  - 1 million cells
- 30 generations (about 10 hrs.)
  - 1 billion cells
- 72 generations (about 24 hrs.)
  - 1 x 10^{21}
    - 1,000,000,000,000,000,000,000 cells

Growth of Microbial Populations

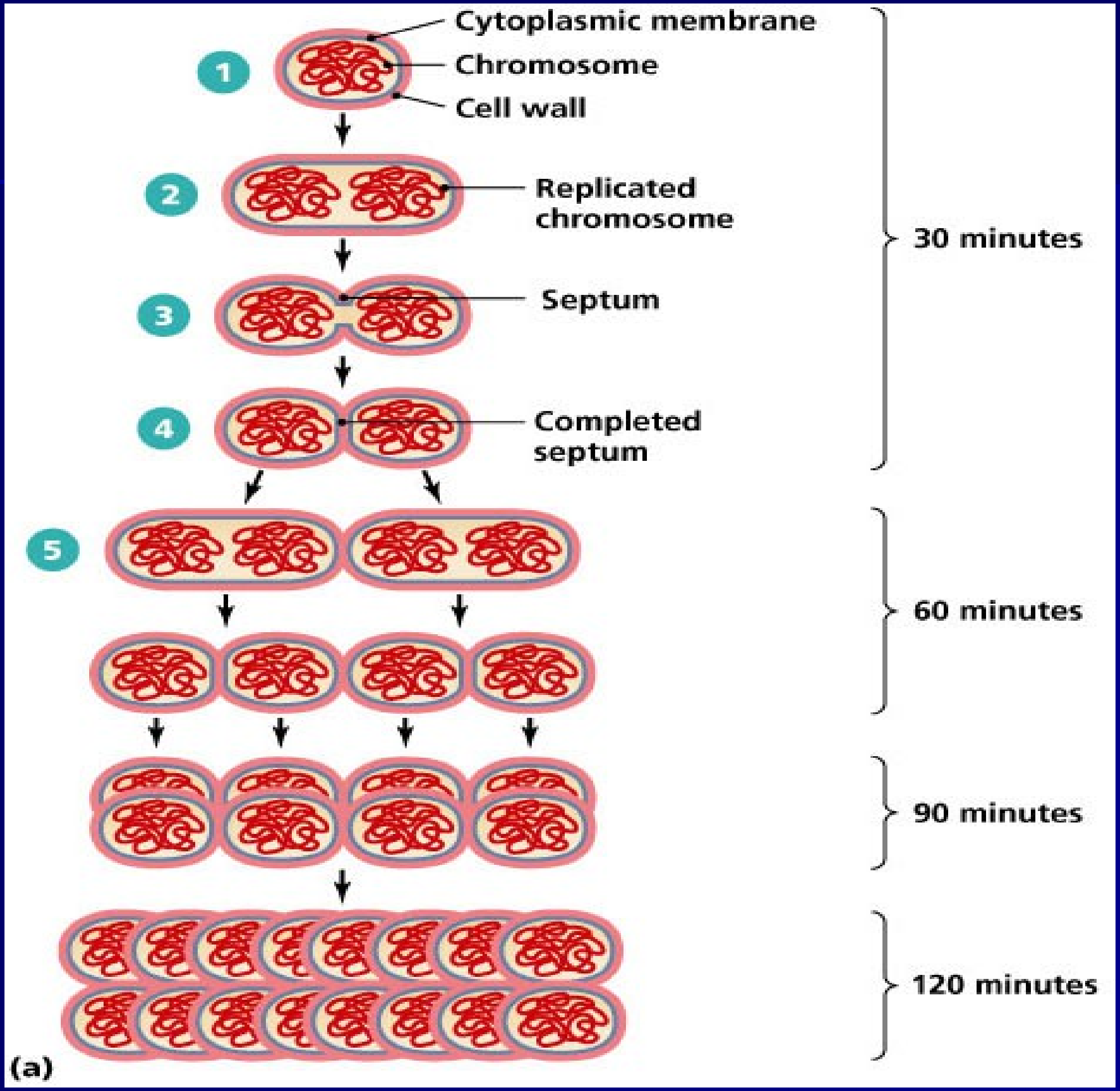
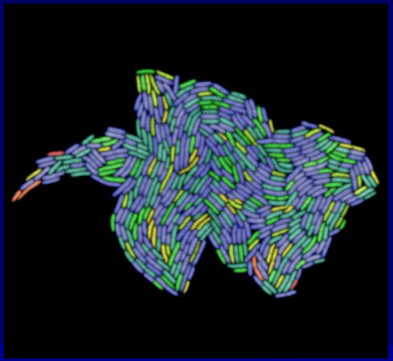


Image: Pearson Education Inc. (2004) publishing as Benjamin Cummings

Increase in Cell Count From Binary Fission

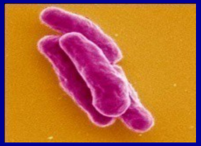


Generation Number	Cell Count
0	1
1	2
2	4
3	8
4	16
5	32
10	1,024
20	1,048,576

View: http://www.cellsalive.com/qtmovs/ecoli_mov.htm

Image: **GlowingColonyEColi** : A false-colored image from fluorescence microscopy of a growing colony of E coli cells. "Aging and Death in E. coli" (2005) PLoS Biol 3(2): e58 doi:10.1371/journal.pbio.0030058.

Generation Time Under Optimal Conditions *(at 37°C)*

Organism	Generation Time *(min)*	
Bacillus cereus	28	
Escherichia coli	12.5	
Staphylococcus aureus *(causes many infections: toxic shock syndrome one example)*	27-30	
Mycobacterium tuberculosis *(agent of Tuberculosis)*	792 – 932	
Treponema pallidum *(agent of Syphilis)*	1,980	

Review Prokaryotic Growth- a summary

- A Bacterial Growth Curve Illustrates the Dynamics of Growth
  - During the lag phase, no cell division occurs while bacteria adapt to their new environment
  - Exponential growth of the population occurs during the logarithmic (log) phase
  - **Human disease symptoms usually develop during the log phase**
  - When reproductive and death rates equalize, the population enters the stationary phase
  - The accumulation of waste products and scarcity of resources causes the population to enter the decline (exponential death) phase

- A stressed cell undergoes asymmetrical cell division, creating a small prespore and larger mother cell
- The prespore contains:
  - Cytoplasm
  - DNA
  - dipicolinic acid, which stabilizes proteins and DNA
- The mother cell matures the prespore into an endospore, then disintegrates, freeing the spore
- Endospores:
  - are resistant to desiccation, heat
  - undergo very few chemical reactions