BACTERIAL GROWTH & PHYSIOLOGY

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Growth

- It is an increase in all the cell components, which ends in multiplication of cell leading to an increase in population.
- It involves - an increase in the size of the cell & an increase in the number of individual cells.
- Bacteria divide by binary fission.

Generation time

- Interval of time between two cell divisions
- OR
- The time required for a bacterium to give rise to 2 daughter cells under optimum conditions

- Also called population doubling time.

Bacteria grow exponentially

Time (h)	Total number of cells	Time (h)	Total number of cells
0	1	4	256 (2^8)
0.5	2	4.5	512 (2^9)
1	4	5	1,024 (2^{10})
1.5	8	5.5	2,048 (2^{11})
2	16	6	4,096 (2^{12})
2.5	32	.	.
3	64	.	.
3.5	128	10	1,048,576 (2^{19})

Figure 6-6a Brock Biology of Microorganisms 11/e

Most bacteria divide in a short amount of time and produce a large amount of bacteria – easier to represent these large numbers by logarithmic scales

Generation time

- Coliform bacilli like *E.coli* & other medically important bacteria – 20 mins
- Tubercle bacilli – 20 hrs
- Lepra bacilli – 20 days

Growth form in Laboratory

- Colony – formed by bacteria growing on solid media. (20-30 cell divisions)
- Each bacterial colony represents a clone of cells derived from a single parent cell.

- Turbidity – liquid media
- - 10^7-10^9 cells/ml

- Biofilm formation – thin spread over an inert surface.


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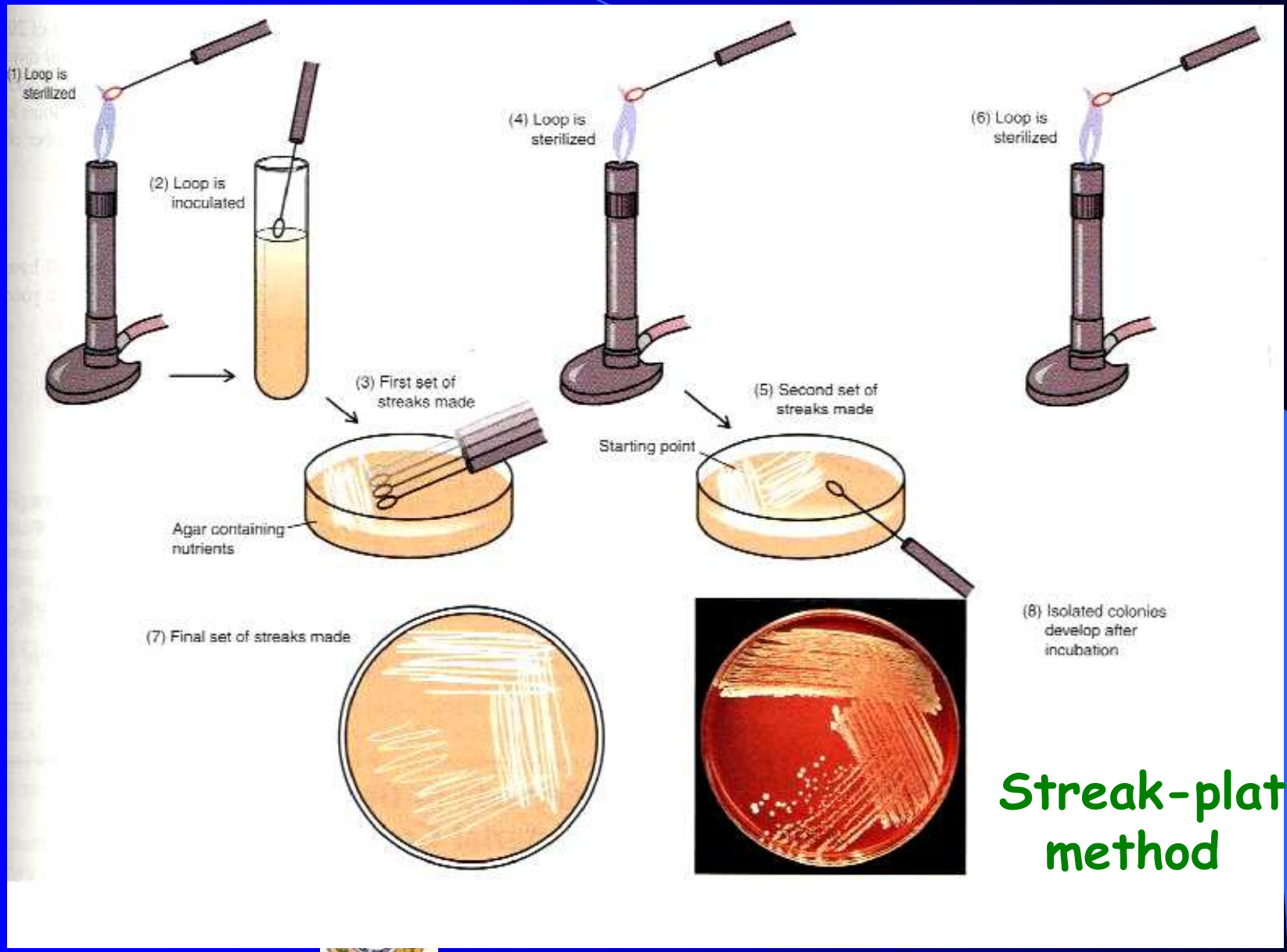


Streak-plate method

Bacterial counts

- Growth in numbers can be studied by bacterial counts.

- 2 methods – Total cell count
- - Viable cell count

Total Count

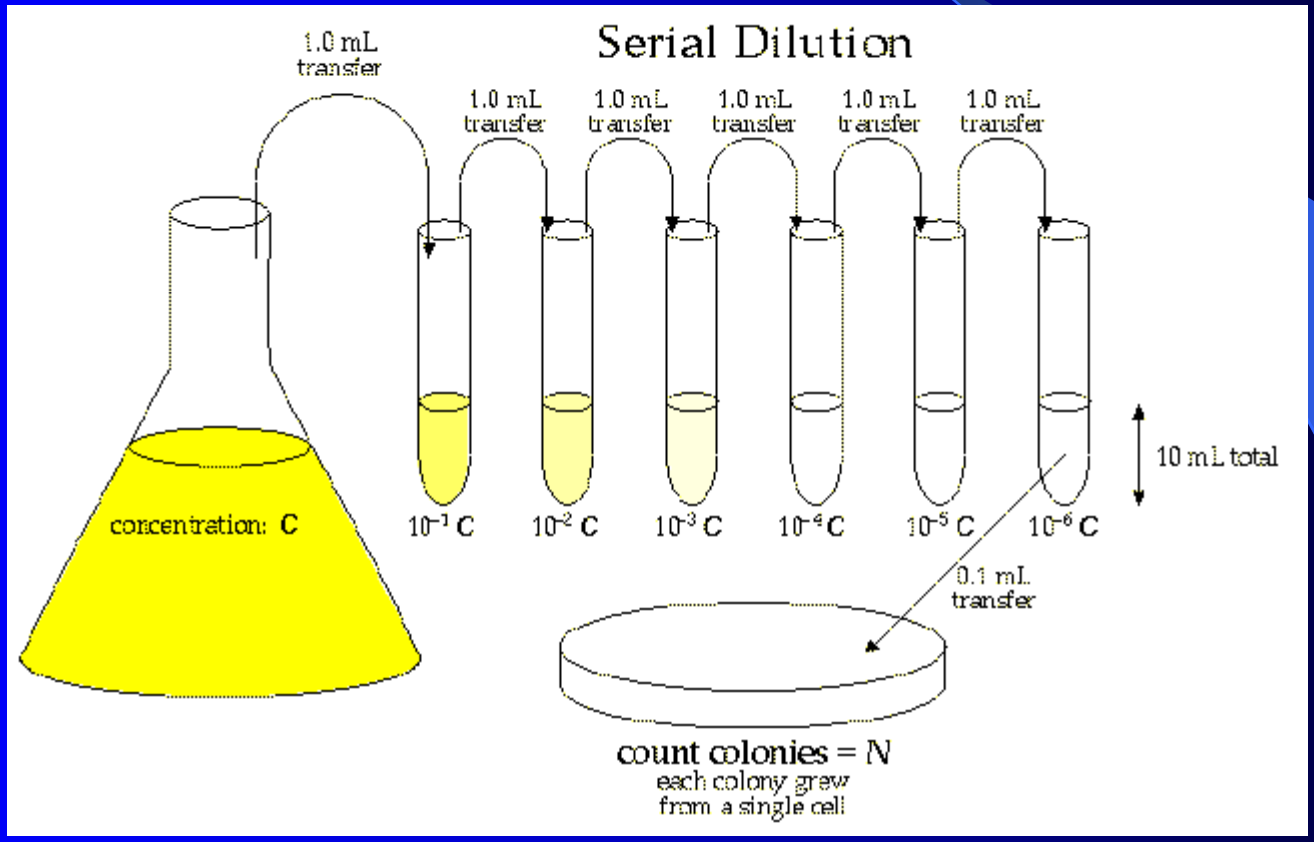
- Total number of cells in the sample – living + dead.
- Can be obtained by :
- Direct counting under microscope using counting chambers.
- Counting in an electronic device – Coulter counter.

Total Count

- Can be obtained by :
- Direct counting using stained smears - by spreading a known volume of culture over a measured area of slide.
- Opacity measurements using an absorptiometer/ nephalometer.
- Chemical assays of cell components.

Viable Cell Count

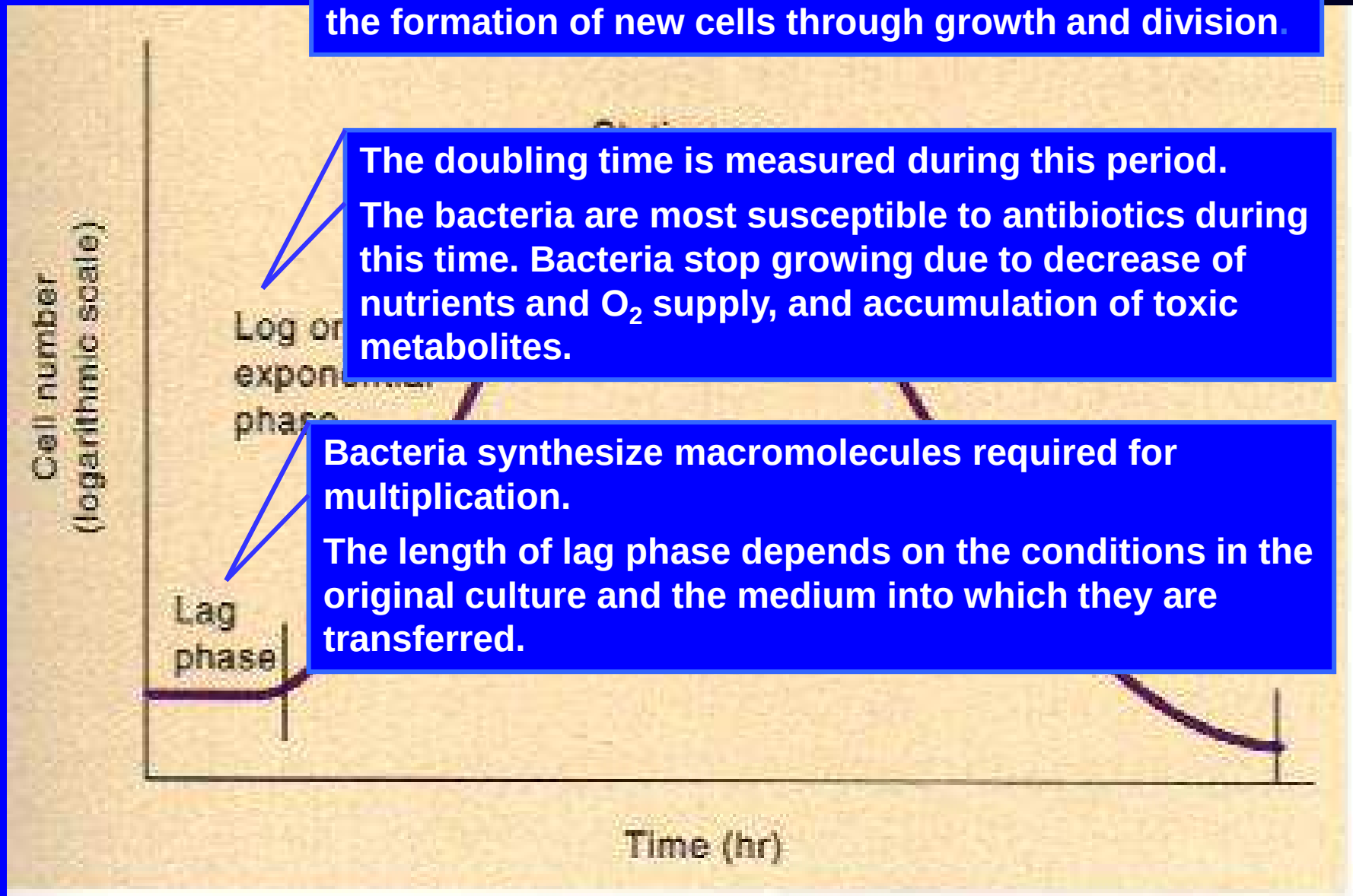
- Measures the number of living cells.
- Methods – Surface colony count
- Dilution method
- Plating method
- Number of colonies that develop after incubation gives an estimate of the viable count.


Serial Dilution
1.0 mL transfer
1.0 mL transfer
1.0 mL transfer
1.0 mL transfer
1.0 mL transfer
1.0 mL transfer
concentration: C
10 mL total
10^{-1} C
10^{-2} C
10^{-3} C
10^{-4} C
10^{-5} C
10^{-6} C
0.1 mL transfer
count colonies = N
each colony grew from a single cell

Bacterial Growth Curve

- When a bacterium is added to a suitable liquid medium & incubated, its growth follows a definite course.
- If bacteria counts are made at intervals after inoculation & plotted in relation to time, a growth curve is obtained.
- Shows 4 phases : Lag, Log or Exponential, Stationary & phase of Decline.

A balance between slow loss of cells through death and the formation of new cells through growth and division.



The doubling time is measured during this period.

The bacteria are most susceptible to antibiotics during this time. Bacteria stop growing due to decrease of nutrients and O_2 supply, and accumulation of toxic metabolites.

Bacteria synthesize macromolecules required for multiplication.

The length of lag phase depends on the conditions in the original culture and the medium into which they are transferred.

Phases of Growth Curve

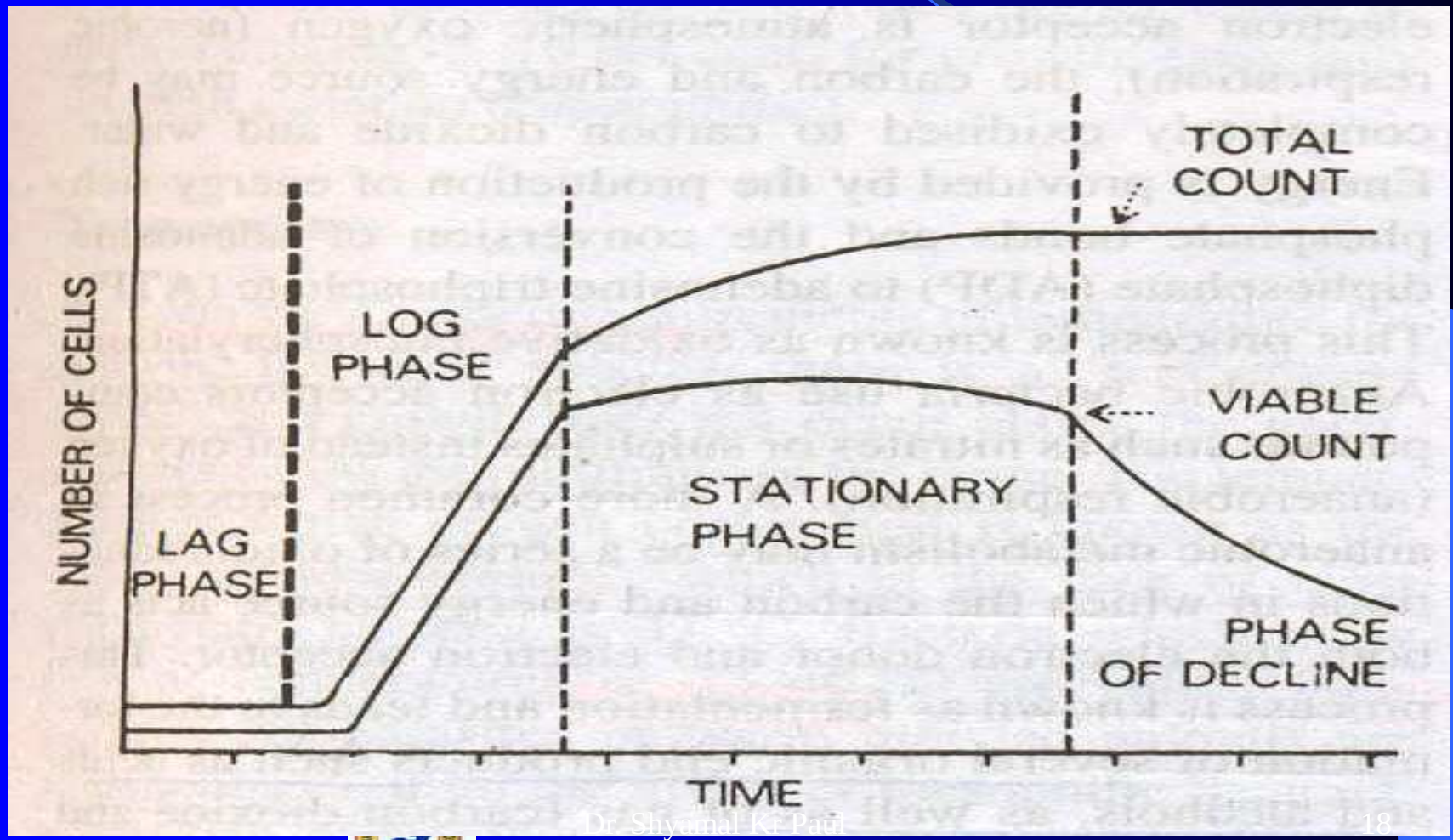
- Lag phase – No increase in number but there may be an increase in the size of the cell.
- Log OR Exponential phase – cells start dividing and their number increases exponentially.

Phases of Growth Curve

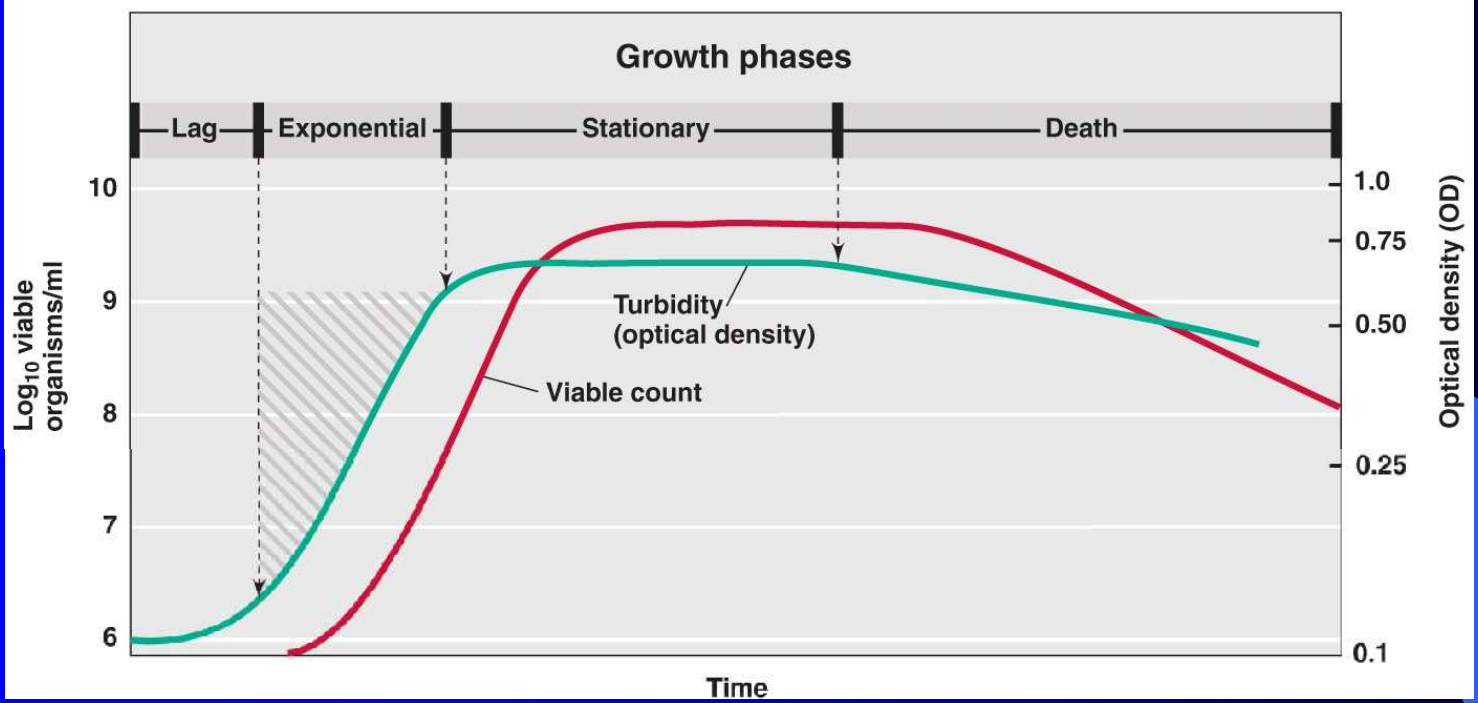
- Stationary phase – cell division stops due to depletion of nutrients & accumulation of toxic products.
- - equilibrium exists between dying cells and the newly formed cells, so viable count remains stationary

- Phase of Decline – population decreases due to the death of cells – autolytic enzymes.

Phases of Growth Curve



Growth Curve



Plot log cell concentration over time
Plot OD versus time for comparison here

Morphological & Physiological alterations during growth

- Lag phase – maximum cell size towards the end of lag phase.
- Log phase – smaller cells, stain uniformly
- Stationary phase – irregular staining, sporulation and production of exotoxins & antibiotics

Factors Affecting Bacterial Growth

- Temperature
- Atmosphere – O2 & CO2
- H-ion concentration
- Moisture & drying
- Osmotic effects
- Radiation
- Mechanical & sonic stress.

Temperature

- Vary in their temperature requirements.
- Temperature range – growth does not occur above the maximum or below the minimum.
- Optimum Temperature – growth occurs best, 37ºC for most pathogenic bacteria.

Temperature

- Mesophilic – grows best between 25ºC and 40ºC.
- e.g. most bacterial pathogens

- Psychrophilic (cold loving) – grows best below 20ºC
- e.g. Flavobacterium spps

- Thermophilic – grows best at high temp, 55- 58ºC
- e.g. Bacillus stereothermophilus

The Cardinal Temperatures

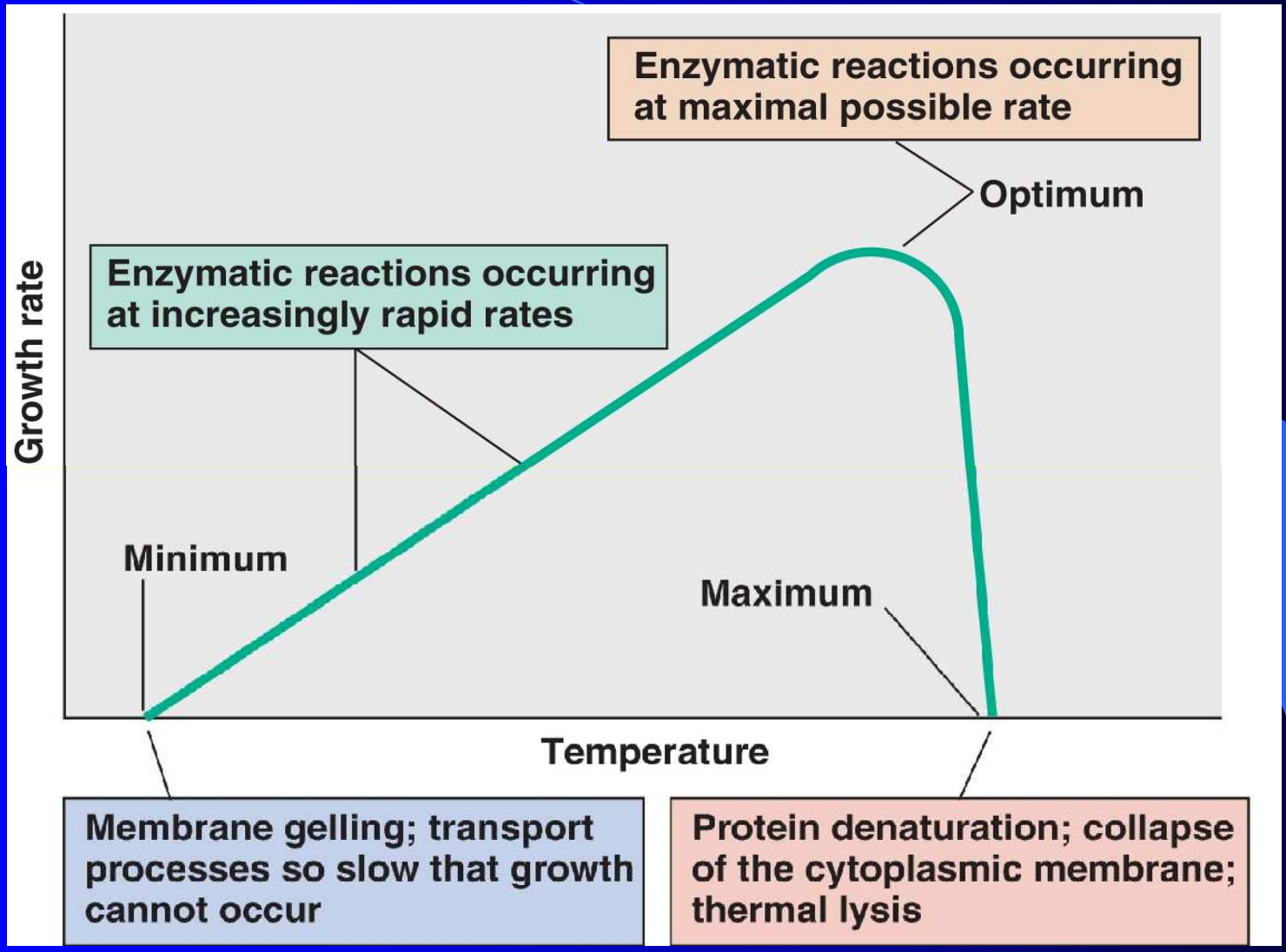
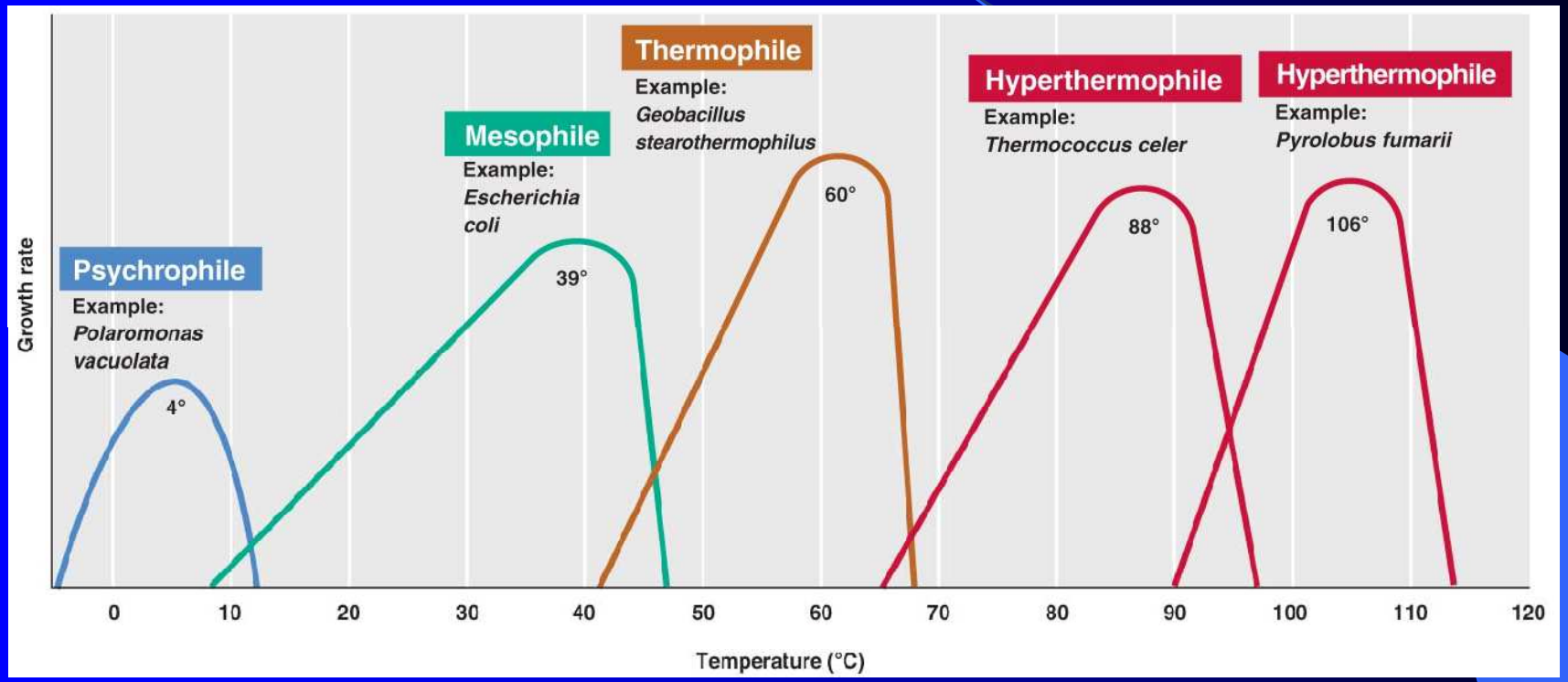


Figure 6.18

Temperature Requirements



Atmosphere

- Depending on the O2 requirement, bacteria are divided into :
- Strict (Obligate) Aerobes – require O2 for growth e.g. *Pseudomonas aeruginosa*
- Strict (Obligate) Anaerobes – grow in the absence of O2 & may even die on exposure to O2 e.g. *Bacteroides fragilis*
- Microaerophilic – grow best in the presence of low oxygen levels
- e.g. *Campylobacter* spp, *Helicobacter* spp

Atmosphere

- Facultative anaerobe – aerobic but can also grow in the absence of O2
  e.g. *Staphylococcus* spps

- Aerotolerant anaerobe – anaerobic, but tolerates exposure to O2 e.g. *Clostridium perfringens*

- Capnophilic organism – requires high CO2 levels e.g. *Neisseria* spps

Toxic Forms of Oxygen

- Products of O_2 metabolism → toxic
  - Singlet oxygen: O_2 boosted to a higher-energy state
  - Superoxide free radicals: O_2^-
  - Peroxide anion: O_2^{2-}
  - Hydroxyl radical (OH•)

Reactants		Products	
$O_2 + e^-$	→	O_2^-	Superoxide
$O_2^- + e^- + 2\,H^+$	→	H_2O_2	Hydrogen peroxide
$H_2O_2 + e^- + H^+$	→	$H_2O + OH\bullet$	Hydroxyl radical
$OH\bullet + e^- + H^+$	→	H_2O	Water

Outcome:

$$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O$$

Figure 6.29

Toxic Forms of Oxygen

- Organisms that use aerobic metabolism must detoxify these products
  - Catalase enzyme: $2\ H_2O_2 \rightarrow 2\ H_2O + O_2$
  - Peroxidase enzyme: $H_2O_2 \rightarrow 2\ H^+ + H_2O$
  - Superoxide dismutase enzyme: detoxifies O_2^- and $OH\bullet$
  - Obligate anaerobes lack these enzymes

H-ion Concentration

- pH range, optimum pH
- Neutral or slightly alkaline pH (7.2 – 7.6) – majority of pathogenic bacteria grow best.
- Lactobacilli – acidic pH
- *Vibrio cholerae* – alkaline pH

Moisture & Drying

- Water – essential ingredient of bacterial protoplasm. Hence drying is lethal to cells.

- Effect of drying varies :
- *T.pallidum* – highly sensitive
- *Staphylococci* sp– stand for months

- Spores – resistant to dessication, may survive for several decades.

Osmotic effects

- More tolerant to osmotic variation due to mechanical strength of their cell walls.

- Radiation
- X rays & gamma rays exposure – lethal

- Mechanical & Sonic Stress
- May be ruptured by mechanical stress.

Bacterial Nutrition

- Water constitutes 80% of the total weight of bacterial cells.
- Proteins, polysaccharides, lipids, nucleic acids, mucopeptides & low molecular weight compounds make up the remaining 20%.
- For growth & multiplication, the minimum nutritional requirements are water, a source of carbon, a source of nitrogen & some inorganic salts.

Classification of Bacteria Based on Nutritional Requirement

- Phototrophs – Bacteria which derive their energy from sunlight.
- Chemotrophs – Bacteria which derive energy from chemical reactions.
- Organotrophs : require organic sources of hydrogen
- Lithotrophs : require inorganic sources of hydrogen like NH3, H2S

Classification of Bacteria Based on Nutritional Requirement

- v Based on the utilization of carbon compounds, bacteria are classified as :

1. Autotrophs - can synthesise all their organic compounds by utilising atmospheric CO_2 & N_2. No medical importance.

2. Heterotrophs - unable to synthesise their own metabolites & depend on preformed organic compounds.

Growth Factors

- Some bacteria require certain organic compounds in minute quantities – Growth Factors OR Bacterial Vitamins.
- It can be :
- Essential – when growth does not occur in their absence.
- Accessory – when they enhance growth, without being absolutely necessary for it.

Growth Factors

- Identical with mammalian nutrition
- Vitamin B complex –
- thiamine
- riboflavine
- nicotinic acid
- pyridoxine
- folic acid &
- Vit.B 12